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Na^+-K^+ Exchange at the Xylem/Symplast Boundary

Its Significance in the Salt Sensitivity of Soybean

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We investigated the mechanism of Na^+ reabsorption in exchange for K^+ at the xylem/symplast boundary of soybean roots (*Glycine max* var Hodgson). The xylem vessels of excised roots were perfused with solutions of defined composition to discriminate between entry of ions into or reabsorption from the xylem vessels. In the presence of NaCl, the transport systems released K^+ into the xylem sap and reabsorbed Na^+. The Na^+-K^+ exchange was energized by proton-translocating ATPases, enhanced by external K^+ concentration, and dependent on the anion permeability. Evidence was presented for the operation of H^+/Na^+ and H^+/K^+ antiporters at the xylem/symplast interface.

The existence of electrogenic ion pumps working at the xylem/symplast interface, which provided the driving force for ion exchange, has been envisaged in pea hypocotyls (Okamoto et al., 1979) and *Plantago* roots (De Boer et al., 1983) and demonstrated in onion roots (Clarkson and Hanson, 1986) and *Vigna unguiculata* hypocotyls (De Boer et al., 1985; Mizuno et al., 1985) using techniques of perfusion of xylem vessels. For example, in the halophyte *Plantago maritima*, ion pumps mediate K^+ uptake from and Na^+ release into the xylem (De Boer and Prins, 1985). These processes are a general property of halophyte roots, which maintain high Na^+/K^+ ratios in the shoot. On the contrary, in Fabaceae (particularly in salt-sensitive species), it has been shown that any Na^+ that has crossed the root to the xylem may be removed from the xylem stream in exchange for K^+ (Läuchli, 1984), particularly in the proximal region of the root and the base of the stem.

In a previous paper (Durand and Lacan, 1994) we showed the effectiveness of the root in excluding Na^+ from the soybean (*Glycine max* L.) shoot. For a moderate range of sodium concentrations in the outer solution, Na^+ is removed from the xylem stream by surrounding tissues and subsequently excreted to the external medium. A low Na^+ concentration in the transpiration stream is important because salt sensitivity in soybean may be mainly dependent on the control of the Na^+ concentration in the xylem entering the leaf. It was possible to discriminate with the technique of perfusion (Clarkson et al., 1984) among the absorption of Na^+ from the external medium, the reabsorption of Na^+ from the transpiration stream, and the consequent release into the xylem. We have shown that even in the presence of mild salinity the access of Na^+ to the xylem stream of the root is relatively unrestricted, and the role of reabsorption of Na^+ released into the xylem may be of importance (Lacan and Durand, 1995). Thus, the purpose of this work was to examine the reabsorption of Na^+ from the xylem vessels and propose a model for the mechanisms of Na^+-K^+ exchange at the symplast/xylem interface.

MATERIALS AND METHODS

Material Preparation

Soybean (*Glycine max* L. Merr. var Hodgson) seeds were surface sterilized for 20 min in 3% (w/w) Ca^{2+} hypochlorite and then germinated at 27°C on filter paper moistened with deionized water for 2 d. Next, seedlings were transplanted to pots containing 10 L of aerated nutrient solution (20 plants per pot). The nutrient solution contained 6 mM NO_3^-, 1.4 mM SO_4^{2-}, 2 mM Ca^{2+}, 1.1 mM Mg^{2+}, 2.25 mM K^+, 1 mM NH_4^+, 0.9 mM $H_2PO_4^-$, 0.12 mM HPO_4^{2-}, FeK-EDTA (0.025 mM), and a micronutrient supplement, including Zn, B, Mn, Mo, Cu, and Co. The culture solution was changed the 2nd d after transplantation. Seedlings were used when they were 6 d old because roots were still unbranched at this stage.

Methods for severing roots, microdissection of the stele, and mounting in the perfusion apparatus were as described earlier (Lacan and Durand, 1995). Briefly, the perfusion apparatus described by Clarkson et al. (1984) consisted of a two-chambered, plexiglass vessel. The apical part of the root was in compartment A, which contained the perfusion solution. As the apical portion was excised, xylem vessels were in contact with the perfusion solution, which represented an artificial xylem sap. The major part of the root was in compartment B, which contained the uptake solution. The basal end of the root was fitted to a vacuum line via a coupling device and a tube for collecting perfusion solution (xylem exu-

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Abbreviations: An^-, anion; ΔpH, pH gradiant; FC, fusicoccin; FCCP, *p*-fluoromethoxycarbonyl cyanide phenylhydrazone; TRP, trans-root potential.

date). Seals between compartments A and B were made by injections of the paraffin wax-lanolin mixture. Perfusion and uptake solutions introduced in compartments A and B, respectively, were aerated during the experiments.

Experimental Solutions

Perfusion solution (compartment A) was usually buffered at pH 6.2 with 1 mM Mes-Tris and contained the following salts: 3 mM KCl, 0.3 mM KH_2PO_4, 0.3 mM $CaCl_2$, 0.3 mM $MgSO_4$, microelements, and FeK-EDTA as in the culture solution. Na^+ was added at a concentration of 37.5 mM (when NaCl or $NaNO_3$ was used) or 18.75 mM (when Na_2SO_4 was used). In some experiments, 12.5 mM K^+ was added as the chloride salt to NaCl or as the nitrate salt to $NaNO_3$. When sodium metavanadate was used, it was added to the perfusion solution to give a final concentration of 100 μM. At this concentration respiration was not inhibited. FCCP was dissolved in 80% ethanol to give a final concentration of 10 μM. FC was dissolved in 60% ethanol to give a final concentration of 10 μM. The final ethanol concentration did not exceed 2% (w/v). For anoxia experiments, roots were placed in an anaerobic environment that was created by bubbling N_2 gas through the experimental solutions.

Uptake solution (compartment B) contained 1 mM $CaSO_4$, KCl (1 or 12.5 mM), and the nontoxic fluorescent dye uranin (0.05% sodium fluorescein) to verify that water collected at the outflow came from compartment A via the xylem vessels and not by transfer via the cortical apoplast. Both solutions were supplemented with Glc (10 mM) and chloramphenicol (50 mg L^{-1}).

pH variations in xylem sap were followed using perfusion solutions without buffers adjusted at pH 6.8 (pH of the nutrient solution).

The effect of pH of the perfusion solution on Na^+ and K^+ transport was followed using perfusion solutions adjusted to different pHs with various buffers of different strengths (1 or 10 mM). For pH 4.0, citrate-KH_2PO_4 buffer was used. For pH 5.0, citrate-KH_2PO_4 or Mes-Tris were equally used. Mes-Tris was used to buffer perfusion solutions within the pH range 5.8 to 6.2. Solutions with pH 7.0 or 8.0 were prepared using Hepes-Tris.

Electrophysiology

TRP difference was measured between xylem sap and uptake solution. Roots were mounted in the perfusion apparatus as described earlier (Lacan and Durand, 1995). The reference electrode was put in uptake solution, which contained only 1 mM $CaSO_4$. The measuring electrode was periodically introduced into the outflow solution. All electrodes were Ag/AgCl type filled with 3 M KCl and were connected to an electrometer (model 646, Knick TÜV, Berlin, Germany). Several perfusion solutions adjusted to pH 6.2 with 1 mM Mes-Tris were used: basal perfusion solution, basal perfusion solution plus 100 μM vanadate, basal perfusion solution plus 100 μM vanadate plus NaCl (12.5 or 37.5 mM), and basal perfusion solution plus 100 μM vanadate plus KCl (12.5 or 37.5 mM). Roots were perfused successively with each of these solutions. Perfusion solutions were changed every hour.

pH Measurements and H^+ Transport

At the end of the experiment, solution in the collecting tube was removed and the pH of small volumes (25 μL minimum) of xylem sap was measured using a flat-surface glass combined electrode (model MI 410; Tacussel, Lyon, France) connected to a pH meter (Knick 646). Aliquots of perfusion solution were back titrated with 0.01 N HCl or 0.01 N NaOH to determine net H^+ equivalents needed to produce the observed adjustments of pH in the outflow solution.

Na^+ and K^+ Transport

Samples of the outflow solution in the collecting tube were withdrawn with a fine-tipped glass pipette and transferred to a tared vial and weighed. Na^+ and K^+ contents in xylem sap were assayed by flame photometry. The rate of the perfusion was maintained constant during the time of the experiments. Previously, it had been verified using ^{22}Na that the rate of Na release or reabsorption by tissues was constant during the experiments (Lacan and Durand, 1995). Accordingly, Na^+ and K^+ xylem transport were obtained from differences between the amounts of Na^+ and K^+ flowing into and out of the root and divided by the duration of the experimental period and fresh weight of the root tissue.

Anion Transport

NO_3^- amounts in the xylem exudate were measured in the experiments with 37.5 mM $NaNO_3$ or 37.5 mM $NaNO_3$ plus 12.5 mM KNO_3. NO_3^- amounts in the xylem exudate were determined on a high-performance liquid chromatograph equipped with a C 6000 column (Alltech, Deerfield, IL) 250 mm long and 4.6 mm in diameter using 0.01 M potassium hydrogenophtalate as the elution solvent and detected by indirect photometric chromatography at 298 nm. Cl^- amounts in the xylem exudate were measured in the experiments with 37.5 mM NaCl or 37.5 mM NaCl plus 12.5 mM KCl. Cl^- content was determined using a chloride titrator Tacussel-type TaCl. To verify the sensitivity of this method Cl^- content was also measured by HPLC. Cl^- transport values were obtained in the same manner as those for K^+ and Na^+ transport.

RESULTS

Effects of Regulators of Plasmalemma ATPase

After passage of the xylem sap through the root, the pH of the sap is adjusted to approximately 6.2 (Table I). This decrease of the pH of the perfusion solution (initially 6.8) indicates that there is an H^+ extrusion and consequently a ΔpH between the stelar cells and the xylem vessels. Furthermore, the addition of FC (an activator of H^+-ATPases)

Table I. *pH adjustments in solution perfusing through the xylem of soybean roots and the corresponding proton fluxes*

Unbuffered perfusion solutions were adjusted at pH 6.8 and contained 37.5 mM NaCl (Control), 37.5 mM NaCl plus 10 μM FC (+FC), 37.5 mM NaCl plus 100 μM vanadate (+Van), 37.5 mM NaCl in anoxia (Anoxia), or 37.5 mM NaCl plus 10 μM FCCP (+FCCP). pH of the xylem sap was measured at the end of the 3-h experiment. Aliquots of perfusion solutions were back titrated with HCl or NaOH to determine net transport of H^+. Uptake solution contained 1 mM KCl. Results are means ± SE of five experiments.

Transport	Experimental Conditions				
	Control	+FC	+Van	Anoxia	+FCCP
pH of the xylem sap	+6.2 ± 0.05	+5.5 ± 0.04	+6.7 ± 0.06	+6.77 ± 0.06	+6.25 ± 0.04
H^+ transport (μmol g^{-1} fresh wt h^{-1})	+5.5 ± 0.5	+9.4 ± 0.7	+1.2 ± 0.1	+0.8 ± 0.007	+5.1 ± 0.5

increased this excretion. By contrast, anoxia or the addition of vanadate, which is an inhibitor of H^+-ATPases, strongly inhibited the pH decrease, suggesting that ΔpH was caused by H^+ extrusion through the action of H^+-translocating ATPase. FCCP, a strong uncoupler, has no effect on the pH of the xylem sap.

The effects of plasmalemma ATPase regulators on K^+ transport to the xylem stream and Na^+ uptake from the xylem stream are reported in Table II. FC enhanced markedly both transports, whereas perfusion of vanadate and anoxia inhibited both K^+ and Na^+ transport, about 70% compared to the control. On the other hand, the perfusion of FCCP had no effect on K^+ and Na^+ transport.

Measurements of TRP

TRP was measured with various perfusion solutions to determine the electrical effects of Na^+, K^+, and Cl^- (Table III). When xylem vessels were perfused with basal solution, TRP between the xylem stream and uptake medium was −28 mV. When the xylem vessels were perfused with basal medium containing vanadate, TRP was +5 mV. This suggests that vanadate inhibited electrogenic H^+ pumps located at the plasmalemma of the cortical cells and at the xylem/symplast boundary. If 12.5 mM NaCl or KCl were added to the perfusion solution, it would be possible to evaluate the electrical effects (not energy linked) of Na^+, K^+, and Cl^- transport, since electrogenic pump activity was stopped. KCl and NaCl (12.5 mM) added to the perfusion solution, together with vanadate, had opposite effects on TRP. NaCl induced a depolarization (TRP was +16 mV), whereas KCl repolarized slowly (TRP was −3 mV). If we accept that electrical potential changes correspond to differences between the electrophoretic mobility of anions and cations, then the depolarization induced by NaCl in the xylem sap corresponds to a lower rate of transport for Na^+ than Cl^- into the symplast. By contrast, in the presence of KCl, the repolarization is due to a higher rate of transport for K^+ than Cl^-.

Implication of Anion Transport

Anion involvement in the regulation of Na^+-K^+ exchange was monitored (Table IV) by measuring Na^+ and K^+ transport in conditions that favor anion transport (increasing Cl^- concentration in the xylem sap) or do not (using the slowly permeating anion SO_4^{2-}). Results (Table II) proved that Cl^- and NO_3^- transport were equivalent to the difference between Na^+ and K^+ transport. Accordingly, in the other experiments, anion transport was estimated from the differences between Na^+ and K^+ transport.

When K^+ concentration in the uptake solution was low (1 mM), both Na^+ and K^+ transport were reduced in the presence of SO_4^{2-} compared to Cl^- or NO_3^-. In this situation (SO_4^{2-}), the charge imbalance of the K^+-Na^+ exchange was reduced (Table IV). By contrast, when the Cl^- or NO_3^- concentration in the perfusion solution increased (perfusion with NaCl plus KCl or $NaNO_3$ plus KNO_3), Na^+ transport was not modified, but K^+ release into xylem vessels was strongly diminished; therefore, charge equilibrium necessitated a large anion transport from the xylem to the surrounding cells.

When external K^+ was high (12.5 mM), Na^+ and K^+ transport were less dependent on the nature of accompa-

Table II. *Effects of ATPase regulators on Na^+ and K^+ transport*

Perfusion solution buffered with 1 mM Mes-Tris to pH 6.2 contained 37.5 mM NaCl (Control). Ten micromolar FC or 100 μM vanadate or 10 μM FCCP were added to this solution. Uptake medium contained 1 or 12.5 mM KCl. Both solutions were supplemented with 10 mM Glc and 50 mg L^{-1} chloramphenicol. Negative data correspond to a transport from xylem sap to surrounding cells. Results are means ± SE of five experiments.

Rate of Transport	Experimental Conditions				
	Control	+FC	+Vanadate	Anoxia	+FCCP
	μmol g^{-1} fresh wt h^{-1}				
1 mM K^+ (uptake medium)					
Na^+	−5.8 ± 0.4	−7.5 ± 0.5	−1.7 ± 0.1	−1.8 ± 0.1	−5.7 ± 0.5
K^+	+2.8 ± 0.1	+3.8 ± 0.2	+0.9 ± 0.05	+0.9 ± 0.06	+3 ± 0.2
12.5 mM K^+ (uptake medium)					
Na^+	−9.5 ± 0.5	−12.3 ± 0.5	−2.7 ± 0.1	−2.5 ± 0.1	−9.2 ± 0.6
K^+	+7.2 ± 0.5	+9.6 ± 0.6	+2.1 ± 0.2	+2 ± 0.1	+7.2 ± 0.6

Table III. *Effects of Na^+, K^+, and Cl^- on the TRP between xylem vessels and uptake solution*

The reference electrode was placed in uptake solution, which contained only 1 mM $CaSO_4$. The measuring electrode was periodically introduced into the outflow solution. Control, Basal perfusion solution. Van, Basal perfusion solution plus 100 μM vanadate. Na, Basal perfusion solution plus 100 μM vanadate plus 12.5 or 37.5 mM NaCl. K, Basal perfusion solution plus 100 μM vanadate plus 12.5 or 37.5 mM NaCl. Roots were perfused successively with each solution. Perfusion solutions were changed every hour. Results are means ± SE of six experiments.

	Perfusion Solution					
	Control	Van	Na		K	
			12.5 mM	37.5 mM	12.5 mM	37.5 mM
TRP	−28 ± 3	+5 ± 2	+16 ± 3	+17 ± 3	−3 ± 1	−5 ± 2

nying anions in the xylem sap: when K^+ was available for exchange with Na^+, the accompanying anion was less important for the reabsorption of Na^+. External K^+ reduced the SO_4^{2-} depressive effect on Na^+ and K^+ transport and the KCl depressive effect on K^+ release into xylem exudate.

Effect of pH

The effect of the pH of the perfusion solution on K^+ and Na^+ transport at the xylem/symplast boundary is presented in Figure 1. Both Na^+ and K^+ transport reflect their pH sensitivity, irrespective of the strength of the buffer (Fig. 1, A and B, weak buffer; C and D, strong buffer). Acid pH had a stimulating effect both on K^+ release into and Na^+ uptake from xylem stream. Increasing K^+ concentration in the uptake solution from 1 to 12.5 mM enhanced these transports. This stimulating effect of external K^+ was particularly significant when the pH in the perfusion solution was increased. For example, when the pH of the perfusion solution was 7.0 and the K^+ concentration in the uptake solution was 1 mM, Na^+ and K^+ transport were 6.5 and 2.9 μmol g^{-1} fresh weight h^{-1}, respectively; whereas when K^+ in the uptake solution was increased (12.5 mM), Na^+ transport was 9 μmol g^{-1} fresh weight h^{-1} and K^+ transport was 6.3 μmol g^{-1} fresh weight h^{-1}.

When the buffer strength of the perfusion solution was increased to 10 mM (Fig. 1, C and D), both Na^+ and K^+ transport were enhanced. Na^+-K^+ exchange reached a maximum rate at pH 5.0. Below this pH, increasing H^+ concentration in the perfusion solution did not enhance Na^+ and K^+ transports, suggesting the presence of H^+-saturable systems. It should be noted that K^+ transport was the most sensitive to pH changes, since K^+ transport was strongly decreased at pH 7.0. The ratio of Na^+ transport to K^+ transport (Fig. 1, A and B) was sensitive to pH changes when external K^+ concentration was low, since it was approximately 1.6 at pH 4.0 and reached 2 at pH 7.0, but it was almost independent of the pH when external K^+ was 12.5 mM.

DISCUSSION

The Na^+-K^+ exchange at the xylem/symplast boundary is the result of two transport systems operating in a parallel direction probably on the plasmalemma of the xylem parenchyma cells: one mediates the K^+ release into the xylem sap, and the other mediates the Na^+ removal from the xylem vessels. This lack of direct coupling between the two transports is supported by three experimental observations. First, it has never been possible to obtain a fixed stoichiometry between the K^+ and Na^+ transports (Lacan and Durand, 1995), the transport stoichiometry being usually variable when external K^+ has increased. Second, experimental observation is that the Na^+/K^+ ratio was highly pH sensitive (Fig. 1). Third, a supply of 12.5 mM K^+ in perfusion solution led to a decrease of K^+ transport but not of Na^+ transport (Table IV). However, Na^+ and K^+ transports seem to be strongly linked, since Na^+ in xylem sap enhanced K^+ release to the xylem vessels and increas-

Table IV. *Effects of accompanying anion in xylem sap on Na^+ and K^+ transport*

Perfusion solution adjusted to pH 6.2 with 1 mM Mes-Tris contained 37.5 mM NaCl or $NaNO_3$, 18.75 mM Na_2SO_4 or 37.5 mM NaCl plus 12.5 mM KCl, or 37.5 mM $NaNO_3$ plus 12.5 mM KNO_3. Uptake medium contained 1 or 12.5 mM KCl. Both solutions were supplemented with 10 mM Glc and 50 mg L^{-1} chloramphenicol. Negative data correspond to a transport from xylem sap to surrounding cells. Results are means ± SE of five experiments.

Transport	Experimental Conditions				
	NaCl	$NaNO_3$	Na_2SO_4	NaCl + KCl	$NaNO_3$ + KNO_3
	μmol g^{-1} fresh wt h^{-1}				
1 mM K^+ (uptake solution)					
Na^+	−5.7 ± 0.4	−5.6 ± 0.5	−2.7 ± 0.2	−5.95 ± 0.5	−6.1 ± 0.4
K^+	+2.75 ± 0.2	+2.8 ± 0.2	+1.85 ± 0.1	+1.1 ± 0.05	+1.2 ± 0.1
An^-	−2.9 ± 0.2	−2.7 ± 0.2	−0.85 ± 0.05	−4.85 ± 0.3	−4.9 ± 0.3
12.5 mM K^+ (uptake solution)					
Na^+	−9.3 ± 0.6	−9.45 ± 0.6	−8.0 ± 0.5	−9.7 ± 0.6	−9.8 ± 0.7
K^+	+7.0 ± 0.5	+6.9 ± 0.5	+6.8 ± 0.6	+6.7 ± 0.5	+6.7 ± 0.4
An^-	−2.2 ± 0.2	−2.4 ± 0.1	−1.2 ± 0.1	−3.0 ± 0.2	−3.1 ± 0.2

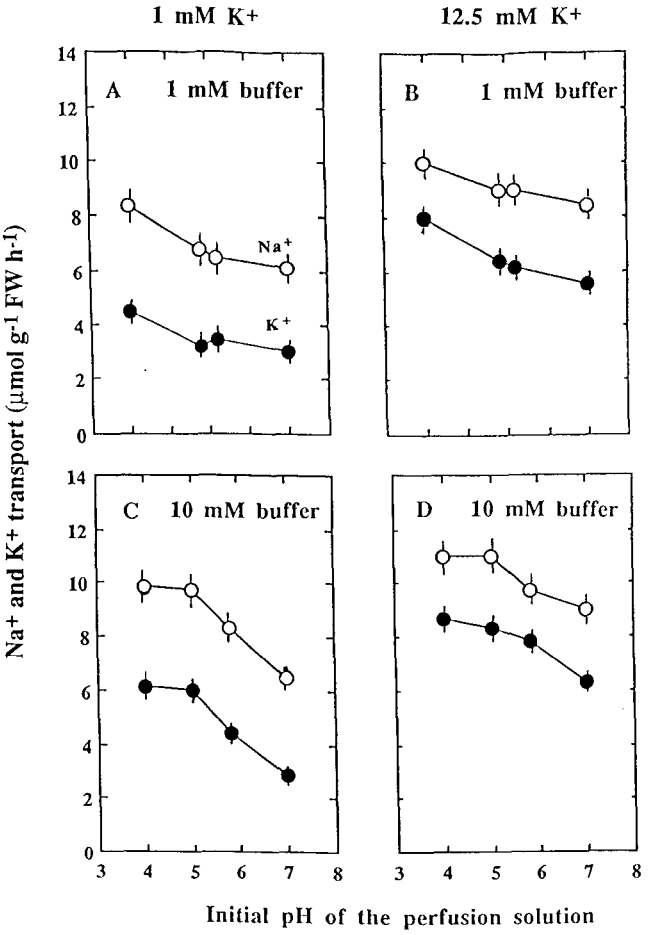

Figure 1. Effects of pH of the perfusion solution on Na^+ reabsorption from and K^+ release into the xylem sap. Perfusion solutions, which contained 37.5 mM NaCl, were buffered at pH 4.0 with citrate-KH_2PO_4, at pH 5.0 with citrate-KH_2PO_4 or Mes-Tris, at pH 5.8 and 6.2 with Mes-Tris, and at pH 7.0 or 8.0 with Hepes-Tris. The concentration of the buffer solutions was 1 mM (A and B) or 10 mM (C and D). Uptake solution contained 1 or 12.5 mM KCl. ○, Na^+ transport represents a transport from xylem sap to stele. ●, K^+ transport represents a transport from stele to xylem sap. Results are means of five experiments and 95% confidence limits. FW, Fresh weight.

ing external K^+ concentration enhanced the Na^+ uptake by the parenchyma cells (Lacan and Durand, 1995).

The adjustment of the xylem sap (initially at pH 6.8) to pH 6.2 after its passage through the xylem vessels suggests that there is a proton pump that provides energy for the ion exchange at the xylem/symplast boundary. Perfusion of FC, which enhanced H^+ transport (Table I) as well as the Na^+-K^+ exchange (Table II), confirmed this hypothesis. In the same way, vanadate in the perfusion solution or anoxia, which inhibits strongly both H^+ release in the xylem vessels and Na^+ and K^+ transports (Table II), strengthen this idea. The marked response of Na^+ and K^+ transport systems suggests that the proton pump provides energy for both transport systems via excreted protons and/or membrane potential (De Boer et al., 1983; Clarkson and Hanson, 1986). The lack of response of the FCCP treatment may be explained by a lack of response of xylem pumps (indeed, FCCP had no effect on the H^+ transport) as reported by De Boer and Prins (1985) and D.T. Clarkson (personal communication). In fact, these authors suggested that physical rather than physiological processes might explain this phenomenon: carbonyl cyanide *m*-chlorophenyl hydrazone (or in our experiments FCCP) might be adsorbed on vessel walls and not be able to reach operating sites.

The vanadate effect on TRP was dominated by a depolarization, since TRP increased from −28 to +5 mV. If vanadate inhibited only stelar proton pumps, we would observe a hyperpolarization. The depolarization observed signifies that proton pumps of cortical cells have also been inhibited when the vanadate was introduced into the xylem vessels. In the absence of measurement of a potential difference between outer apoplast and symplast, this did not allow us to identify vanadate-inhibited electrogenic systems at the xylem level. However, these results and the data in Tables I and II suggest that pumps both at the xylem/stelar interface and in the plasmalemma of the cortical cells had been inhibited and that the effect of the inhibition of xylem pumps was overridden by the simultaneous inhibition of pumps located in the cortex. We will admit as a conservative hypothesis that putative H^+ pumps at the xylem/stelar cells interface were inhibited. Measurements of TRP, when electrogenic pumps were inhibited, allowed us to determine the effects of the Na^+, K^+, and Cl^- transport (Table III). If we accept that electrical potential changes correspond to the difference between the electrophoretic mobility of anions and cations, Na^+ transport was less electrogenic than Cl^- transport, which is less electrogenic than K^+ transport. In the hypothesis of an electrically driven Na^+ uniport, as has been shown in salt-tolerant tobacco cells (Murata et al., 1994), the weakness of the electrical signal should be the result of a low Na^+ transport, and an increase in the NaCl concentration in the xylem sap would induce a major change in the electrical signal. Our results show that, among exchanges at the xylem/symplast boundary, Na^+ transports were always the most important and that TRP was not really affected by NaCl increase. Therefore, these results suggest that the mechanism of Na^+ transport from xylem sap to tissues may be an electroneutral process. Furthermore, the Na^+-K^+ exchange is limited by a factor linked to the anion transport (Table IV): this factor may be the electrical gradient or the pH gradient. It is unlikely that anions act on Na^+-K^+ exchange via the electrical gradient, because the same electrical effect on two systems working in opposite ways could not be observed except if both systems transported the same net charge (for example, uniport Na^+ and $2H^+/K^+$ antiport), but electrophysiological data show that Na^+ transport is probably an electroneutral process. Therefore, anions in the xylem sap modulate the Na^+ and K^+ transports via the pH gradient. This observation suggests that protons are involved in anion transport via a symport H^+-anion as has been proposed by Ullrich and Novacky (1990). These results also show that K^+ and anions had similar effects on Na^+ reabsorption but in opposite ways: external K^+ stimulates Na^+ transport out of the xylem, whereas internal anions (anions present in the xylem sap)

do the same. In conclusion, Na^+ and K^+ transports seem to be indirectly coupled via the osmotic component (ΔpH) rather than the electrical component of the proton motive force generated by the H^+-ATPase activity.

To evaluate the involvement of ΔpH in the regulation of Na^+ and K^+ transport processes, Na^+-K^+ exchange was followed as a function of the pH of the perfusion solution. It was possible to observe that Na^+ and K^+ transport were both enhanced when the pH of the xylem sap was decreased (Fig. 1, A and C) and were also enhanced when external K^+ concentration was increased (Fig. 1, B and D). Since the stimulating effect of external K^+ was more important when the pH of the perfusion solution was alkaline and K^+ transport was the most sensitive to pH changes of the perfusion solution, this suggests that the K^+ carrier directly uses the H^+ of the xylem sap.

From the results described above, a hypothetical model for the mechanism of the symplast/xylem Na^+-K^+ exchange can be proposed (Fig. 2): (a) The secretion of K^+ into the vessel lumen occurs via an H^+/K^+ antiport. (b) The uptake of Na^+ ions by xylem parenchyma cells also depends on the pH gradient, suggesting an H^+-Na^+ symport or an H^+/Na^+ antiport. However the hypothesis of an H^+-Na^+ symport may be discarded because such a system would be very electrogenic (not electrically possible) and could not explain the reversal of the direction of the Na^+ transports between xylem sap and tissues. It seems more reasonable to speculate that the reabsorption of Na^+ is the result of H^+/Na^+ antiport driven by Na^+ but limited by the availability of H^+ in the symplast. The highest cytoplasmic concentrations of Na^+ that permit H^+/Na^+ to work in the reabsorption way were determined. The free energy of the electroneutral exchange $1Na^+/1H^+$ is: $\Delta G = -RT\ln (Na^+)_{sap}(H^+)_{cyt}/(Na^+)_{cyt}(H^+)_{sap}$, where ΔG is free energy charge; R is the gas constant; T is the absolute temperature; $(Na^+)_{sap}$ and $(H^+)_{sap}$ are the Na^+ and H^+ concentrations, respectively, in xylem sap; $(H^+)_{cyt}$ and $(Na^+)_{cyt}$ are the H^+ and Na^+ concentrations, respectively, in the cytoplasm of stelar cells. At the thermodynamic equilibrium ($\Delta G = 0$), $(Na^+)_{sap}/(H^+)_{sap} = (Na^+)_{cyt}/(H^+)_{cyt}$. For example, with $pH_{cyt} = 7.5$, $pH_{sap} = 4$, and $(Na^+)_{sap} = 37.5$ mM (where pH_{cyt} and pH_{sap} are the pHs in cytoplasm of the stelar cells and xylem sap, respectively), Na^+/H^+ works at equilibrium for $(Na^+)_{cyt} = 12$ μM. When pH = 7.0 in the xylem sap, this equilibrium concentration is 12 mM. Therefore, this antiport system is very sensitive to the pH of the xylem sap. This implies that the stelar cells maintain low cytoplasmic Na^+ concentrations (less than 12 μM or 12 mM according to experimental conditions) to allow the H^+/Na^+ antiport to favor Na^+ reabsorption. The H^+/Na^+ antiport model permits us to explain the reversal of the direction of the Na^+ transport. In *Dunaliella salina*, a reversible H^+/Na^+ antiport acts as a regulator of cytoplasmic pH (Katz et al., 1991). In higher plants, the presence of a reversible H^+/Na^+ antiport has only been demonstrated in *Acer pseudoplatanus* cells (Mathieu et al., 1986). This antiport system involved in the extrusion of Na^+ from the cytoplasm to the external medium is also able to transport Na^+ in the cytoplasm and to pump out H^+ in some physiological circumstances (when the cytoplasm is acidified and the external concentration is high). Here, a reversible H^+/Na^+ antiport operating at the xylem/symplast boundary catalyzes either a transport of Na^+ from the xylem parenchyma cells to the xylem sap coupled to a transport of H^+ from the xylem sap to the xylem parenchyma cells and/or an uptake of Na^+ from the xylem sap to the stelar cells coupled to a transport of H^+ from the stelar cells to the xylem sap.

In conclusion, we hypothesize that transport systems that provide the release of K^+ to the xylem sap and Na^+ reabsorption by surrounding cells are linked by H^+ cycling. Na^+-K^+ exchange is the result of the parallel functioning of two antiports H^+/Na^+ and H^+/K^+. The H^+/Na^+ antiport mainly operates coupled to H^+/K^+ antiport but also to an H^+-An^- symport. This model explains that Na^+-K^+ exchange at the xylem/symplast boundary is enhanced by acid pH and external K^+ concentration and is dependent on anion permeability. The soybean has a capacity to remove xylem-borne Na^+ ions from the sap by an H^+/Na^+ antiporter driven by Na^+ concentration in the xylem sap. This removal from the transpiration stream, whose function is to limit Na^+ access to the leaves, might not be regarded as a criterion of salt tolerance because of its poor efficiency, and, in fact, may contribute to the salt sensitivity of soybean.

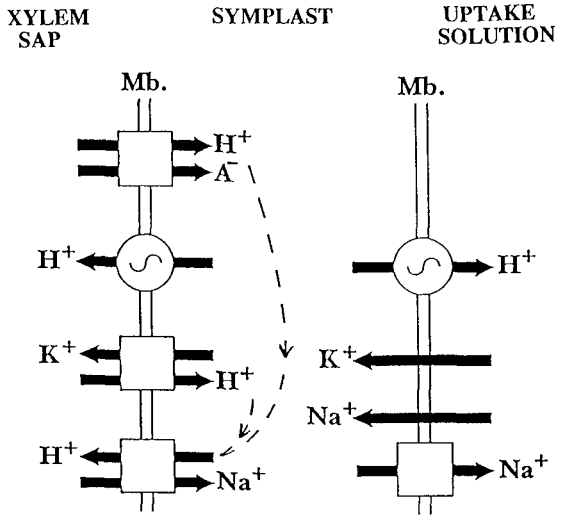

Figure 2. A hypothetical model to produce a basis for discussing the relationship between H^+ flux and K^+ exchange at the xylem/symplast interface. A proton pump (~) driven by ATP hydrolysis generates the proton motive force. K^+ and Na^+ transport are via H^+-K^+ and H^+-Na^+ antiport linked by an H^+ cycling. Anions transport is via an H^+-An^- symport. Mb., Plasma membrane; A^-, anion transport.

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