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Cs^+ Uptake in Subapical Maize Root Segments: Mechanism and Effects on H^+ Release, Transmembrane Electric Potential and Cell pH

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Subapical segments from maize (*Zea mays*) root took up Cs^+; at low external concentrations ($\leq$0.25 mM) the kinetic constants for the influx of this cation were similar to those for the uptake of Rb^+ and K^+ (K_m=26, 26 and 22 μM and V_{max}=3.62, 3.94 and 4.09 μmol h^{-1} (g FW)$^{-1}$, respectively). Competition experiments suggested that the three cations use the same transport system. At higher concentrations (>0.25 mM) the discrimination between Cs^+ and Rb^+ increased. At low external concentrations, the release of H^+ into the medium was promoted similarly by Cs^+, Rb^+ or K^+; at higher concentration (5 mM) the H^+ release was higher in the presence of K^+ than with Rb^+ or Cs^+. The transmembrane electrical potential difference (E_m) was depolarised when Cs^+, Rb^+ or K^+ were present in the incubation media; this depolarisation was greater in the presence of K^+ and was particularly evident at higher concentrations: the E_m was −85 mV, −105 mV and −119 mV in 5 mM K^+, Rb^+ or Cs^+, respectively. Tetraethylammonium-chloride, a K^+-channel blocker, strongly hyperpolarized E_m in the presence of K^+ but had little or no effect with Rb^+ or Cs^+, respectively. The influx of Cs^+ was accompanied by less dark fixation of CO_2 than was that of K^+ or Rb^+. These results are consistent with the activity of a system showing low selectivity between Cs^+, Rb^+ and K^+, operating primarily at low external concentrations, which possibly uses a cation-H^+ co-uptake mechanism, and of a channel-mediated system more selective for K^+, operating at higher concentrations; these systems differ in their involvement of H^+ transport activity and the metabolic pH-stat mechanism.

Key words: Cesium transport — Potassium transport — Uptake selectivity — *Zea mays*.

Interest in the uptake of Cs^+ into the cells of different organisms increased greatly after the Chernobyl nuclear accident in 1986. Nevertheless, only a few studies have examined the transport mechanisms involved in the uptake of Cs^+ by higher plants, unlike the situation for microbial and algae cells (Bossemeyer et al. 1989, Avery et al. 1993). The pioneering work by Epstein and Hager (1952), examining millimolar concentrations of cations, and the more recent results by Sheahan et al. (1993) at micromolar external concentrations, suggest that K^+, Rb^+ and Cs^+ enter the root cells of barley or *Arabidopsis*, respectively, via common uptake systems, probably those operating under normal physiological conditions for K^+.

Thermodynamic (Cocucci et al. 1976, Cheeseman and Hanson 1980, Maathuis and Sanders 1993) and kinetic (Epstein et al. 1963, Kochian and Lucas 1982, Kochian et al. 1985) considerations suggest that the uptake of K^+ by plant cells involves a high-affinity system predominating at low external concentration (< 1–0.3 mM) mediating the cation influx against its electrochemical potential (Maathuis and Sanders 1993, Gassmann and Schroeder 1994), and a low affinity system predominating at higher external cation concentrations. Several mechanisms have been proposed for the high-affinity system (Poole 1978, Rodriguez-Navarro et al. 1986, Kochian and Lucas 1989, Gibrat et al. 1990) but the low-affinity system is largely accepted to be a channel mechanism (Kochian et al. 1985, Schroeder and Fang 1991, Kourie and Goldsmith 1992, Gassman et al. 1993, Kochian and Lucas 1993, Gassmann and Schroeder 1994). It has also been proposed that K^+ uptake could be mediated by multistate entities having both carrier and channel-like properties at low and high K^+ external concentrations (Nissen 1991).

Voltage-clamp studies indicate that the inward-rectifying K^+ channels are characterized by a high selectivity for K^+ compared with Rb^+ and Cs^+. Moreover, in this experiments, Cs^+ was used as an inhibitor of the K^+ inward current and its inhibitory activity was concentration- and voltage-dependent (Kourie and Goldsmith 1992, Zanello and Barrantes 1992, Maathuis and Sanders 1995, Véry et al. 1995, Kourie 1996). The characteristics of selectivity and permeability of the inward-rectifying K^+-channel thus seem to exclude the possibility that Cs^+ crosses the plasmalemma of root cells through them; consequently, it is possible that Cs^+ mainly enters by the K^+ high-affinity transport system. From this hypothesis, because the K^+ high- and low-affinity systems use different mechanisms for transport, the E_m, the H^+ efflux and the related metabolic activities (e.g. those involved in the control of cellular pH) could change when roots are incubated in K^+, Rb^+ or Cs^+.

The present work aimed to analyse the selectivity of

Abbreviations: E_m, transmembrane electric potential difference; TEA^+, tetraethylammonium-chloride.

the transport systems for Cs^+, Rb^+ and K^+ in vivo, in maize root segments and to compare the effects of Cs^+ uptake on proton efflux, E_m and cell pH with those of K^+ and Rb^+ uptake.

Materials and Methods

Plant material and germination conditions—Maize (*Zea mays* L., cv. Dekalb XL 85) seeds were germinated in the dark for 2 days at 26°C on paper saturated with distilled water. Twenty-four hours prior to the experiments, seedlings were transferred to aerated 0.5 mM $CaSO_4$ solution and maintained in the dark at 26°C, after which the main roots were about 40 to 50 mm long. Subapical segments (6 mm long) were excised from the main roots between 2 and 8 mm from the tip (Marrè et al. 1982). The root segments were washed for 3 h in an aerated solution of 0.5 mM $CaSO_4$. Samples of 15 segments (about 100 mg FW), were further washed twice for 30 min in 10 ml of the same solution at 26°C and agitated at 80 oscillations min^{-1}, after which the segments had recovered completely from wounding (Cocucci and Sacchi 1993). In the following 3 h the segments absorbed oxygen at a constant rate (about 500 $\mu l\ h^{-1}$ $(g\ FW)^{-1}$) and the level of ATP (about 100 nmol $(g\ FW)^{-1}$) did not change significantly.

Influx of cations and H^+ release into the medium—Fifteen washed and preincubated subapical segments were incubated in 10 ml of a basal medium consisting of 0.5 mM $CaSO_4$, 0.1 mM MES-Ca (pH 6.0) containing Cs^+ or Rb^+ (as SO_4^{2-}) at 26°C and agitated at 80 oscillations min^{-1}. After incubation (15 and 30 min), the segments were washed twice at 4°C for 15 min with the corresponding media containing K^+ instead of Cs^+ or Rb^+. The segments were then rinsed twice in ice-cold distilled water, mineralised as described previously (Cocucci and Sacchi 1993) and the content of Cs^+ or Rb^+ determined by atomic absorption spectrophotometry (SpectrAA-20, Varian, Mulgrave, Victoria, Australia) using Cs_2SO_4 and Rb_2SO_4 solutions as standards. In the case of Rb^+, 100 mM K^+ was added as an ionisation suppressant. K^+ influx was evaluated as the sum of the net K^+ influx, assayed by the depletion of the cation in the incubation media, and the K^+ efflux, measured as described by Cocucci and Sacchi (1993). These values were very similar to the values of K^+ influx obtained using $^{86}Rb^+$ at very low concentrations as a K^+ tracer (Cocucci and Sacchi 1993). The kinetic constants and S.E. were calculated using Excel program for Windows (Microsoft) in 486 IBM compatible computer.

Competition between Cs^+, Rb^+ and K^+ for the uptake systems was studied by incubating the samples with different concentrations of Cs^+ or Rb^+, in the absence or presence of fixed concentrations of Rb^+ or K^+ for Cs^+, and of Cs^+ or K^+ for Rb^+. At the end incubation (15 and 30 min), the samples were washed twice in a ice-cold basal medium containing K^+ instead of Cs^+ or Rb^+. The amounts of Cs^+ and Rb^+ in the segments were measured in the same samples by atomic absorption spectrophotometry after mineralisation.

The H^+ release into the medium was determined in preincubated samples (15 segments) transferred into 10 ml of the basal medium containing 0.05, 0.5 or 5 mM K^+, Rb^+ or Cs^+ (as SO_4^{2-}), initially at pH 6.0, and incubated at 26°C under agitation. The pH values were measured with a Radiometer PHM-84 pH meter (Radiometer Copenhagen, Denmark) after 30 and 60 min of incubation. Back titration of the media of each sample was conducted as described by Lado et al. (1981) with a Radiometer TTT-80 titrator.

To avoid K^+ contamination, the glassware used for all the experiments was previously washed in 0.1 M HNO_3 and rinsed in double-distilled water; the pH of the incubation media was determined with a Radiometer PHM-84 equipped with a double-bridged reference electrode in which the outer chamber was filled with saturated NH_4NO_3.

Measurement of the transmembrane electric potential difference—The transmembrane electric potential difference (E_m) was measured conventionally as described by Cocucci et al. 1976. Briefly, four subapical segments, washed and preincubated, were placed in a Plexiglas cuvette (1.5 ml total volume) under a continuous flow (200 ml h^{-1}) of the thermoregulated (26°C) and aerated basal medium containing the monovalent cations as indicated, with or without 10 mM tetraethylammonium-chloride (TEA^+). A borosilicate glass micropipette (WPI Instruments, New Haven, Conn., U.S.A.) filled with 3 M KCl (pH 2.0) and with a resistance of 10–20 MΩ, was inserted perpendicular to the root axis at about 3 mm from the root tip. The E_m values were recorded with a high-impedance electrometer amplifier (WPI Instruments, model K5-700, New Haven, Conn., U.S.A.) in three different root cells in the 5th to 7th layer of cortical cells of each root segment. All experiments were repeated at least three times.

NMR methods—^{31}P-NMR spectra were obtained with a Bruker AMX 600 spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten-Forchheim, Germany) equipped with a X32 data system, running UX NMR software, version 920801. In vivo ^{31}P-NMR experiments were carried out by packing 150 subapical root segments, preincubated as described above, in a 10 mm diameter NMR tube (Wilmad Glass Co., Buena, NJ, U.S.A.) equipped with a perfusion system connected to a peristaltic pump in which the aerated, thermoregulated (26°C) basal medium, with or without 5 mM K^+, Rb^+ or Cs^+, flowed (8 ml min^{-1}). ^{31}P-NMR spectra were recorded at 242.9 MHz without lock, using a standard broad-band 10 mm probe and fast acquisition conditions (Kime et al. 1982) with a waltz-based broad-band proton decoupling and a spectral window of 16 kHz. The acquisition time was 0.386 s with a relaxation delay of 1 s and a pulse angle of 90°. Spectra were the results of 900 scans, corresponding to a total acquisition time of 20 min 48 s. Resonance identification was obtained according to Roberts et al. (1980) and Kime et al. (1982) and the cytoplasmic and vacuolar pH estimated from the chemical shift (δ) of inorganic phosphate (Pi) resonance. Standard titration curves relating δ to pH were constructed according to Roberts et al. (1981). Chemical shifts were measured in ppm relative to the signal of methylenediphosphonic acid (MDP; 33 mM, sealed in a coaxial capillary tube included with the sample), which resonates at 18.5 ppm relative to the signal of 85% H_3PO_4.

In vivo dark CO_2 fixation—Samples of 15 preincubated root segments were transferred into 50 ml flasks sealed with a rubber cap and incubated for 1 h at 26°C under agitation (80 oscillations min^{-1}) in 10 ml of basal medium with or without 5 mM K^+, Rb^+ or Cs^+; 37 kBq of $NaH^{14}CO_3$ (2.2 GBq $mmol^{-1}$) were injected into the flasks. At 1 h, the radioactive medium was drained quickly and the root segments blotted with a paper towel and homogenised with 1 ml of 0.1 M HNO_3. After bubbling with N_2, the homogenates were transferred into scintillation vials containing 10 ml of a scintillation cocktail and counted in a Beckman LS 6000SC (Fullerton, CA, U.S.A.) scintillation counter.

Results

Cs^+, Rb^+ and K^+ influxes in maize root segments—Subapical segments from maize roots took up Cs^+; Figure

1 shows the influxes of Cs^+ as function of the external concentrations in the range 0.01–5 mM. The influxes of Cs^+ increased markedly up to an external concentration of 0.1 mM; at higher concentrations, the influxes increased slowly without reaching saturation. Figure 1 also shows that the influxes of Rb^+, in the same range of concentrations, and K^+, up to 0.5 mM, had a similar behaviour to that of the influxes of Cs^+.

Because the changes in E_m were small for each cation (≤ 15 mV) at external concentrations of 0.01–0.5 mM Cs^+, Rb^+ or K^+ (Fig. 2), the electrochemical activity [i.e. chemical activity $\times$ exp(FEm/RT)] (Rodriguez-Navarro et al. 1986) depends mainly on the external cation concentration. Thus, the influx can be fitted conveniently to an Eadie-Hofstee plot to calculate the apparent kinetic constants. For external concentrations of Cs^+, Rb^+ or K^+ ≤ 0.25 mM the Eadie-Hofstee plot was linear with an r^2 of 0.97, 0.97, 0.99, respectively (data not shown). Table 1 shows that at external cation concentrations of 0.01–0.25 mM, the values of the apparent K_m and V_{max} for the influx of Cs^+, Rb^+ or K^+, calculated by the Eadie-Hofstee plot, were not significantly different ($P > 0.05$).

At concentrations higher than 0.5 mM (Fig. 1, segmented abscissa), the influxes of Cs^+ and Rb^+ (at high concentrations it is not possible to measure K^+ influx by depletion) increased slowly but with different patterns. The influx of Cs^+ increased sharply between 0.5 and 1 mM, reaching values higher than those of Rb^+, then increased little up to 5 mM; the influx of Rb^+ increased slightly from 0.5 to 5 mM.

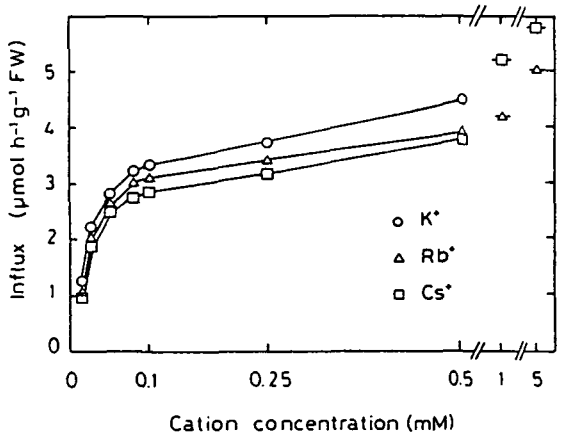

Fig. 1 Cs^+, Rb^+ and K^+ influx in subapical segments of maize roots. The root segments (15) were incubated in 15 ml of 0.5 mM $CaSO_4$, 0.1 mM MES-Ca (pH 6.0) and Cs^+, Rb^+, and K^+ (as SO_4^{2-}) at the indicated concentrations. Influxes were assayed by measuring the amount of the cation taken up after 15- and 30-min incubation at 26°C in an agitated, thermoregulated water bath; the K^+ influx values were evaluated as the sum of the net K^+ influx, assayed by the depletion of the cation in the incubation media, and the K^+ efflux. The values are the means of at least five experiments run in triplicate. SE did not exceed $\pm 5\%$ for Cs^+, $\pm 4\%$ for Rb^+ and $\pm 3\%$ for K^+.

Table 1 Kinetic constants calculated using the Eadie-Hofstee plot for the mean values of Cs^+, Rb^+ and K^+ influx in subapical maize root segments reported in Fig. 1

	K_m (μM)	V_{max} (μmol h^{-1} (g FW)$^{-1}$)
Cs^+	26±2.3	3.62±0.13
Rb^+	26±2.3	3.94±0.14
K^+	22±0.5	4.09±0.04

The kinetic constants were calculated for external cation concentrations of 0.01–0.25 mM. The values are means±SE (n=6).

Effects of the presence of Cs^+, Rb^+ or K^+ in the incubation medium on E_m and H^+ release—The effects of increasing concentrations of Cs^+ in the incubation medium on the value of E_m in cortical cells of maize root segments were examined in relation to the well-known depolarising action of K^+ and Rb^+ (Cocucci et al. 1976). Figure 2 shows that 0.01 mM Cs^+ in the incubation medium depolarised the E_m, which reached values of about -130 mV. This depolarising effect was lower than that induced by the same concentrations of K^+ or Rb^+. Increasing the external concentration of the cations to 0.05 mM and 0.5 mM induced a further depolarisations of the E_m, but in the presence of Cs^+ the value of E_m was always more negative (about 8 mV) than the values recorded in the presence of K^+ or Rb^+; at 5 mM, Cs^+ induced no significant effect ($P > 0.05$).

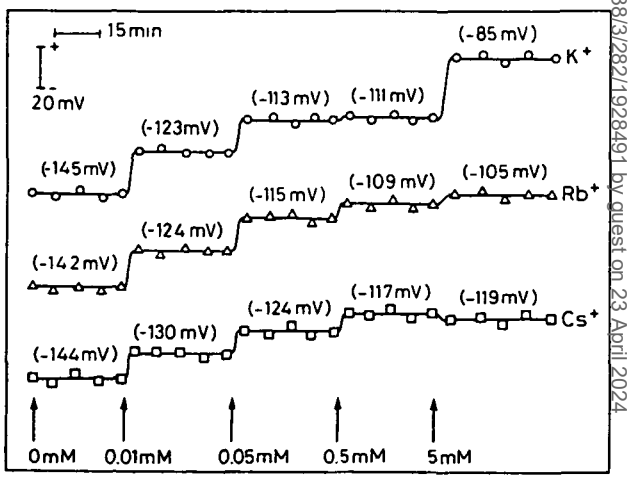

Fig. 2 Effects of the presence of Cs^+, Rb^+ or K^+ on the transmembrane electric potential (E_m) of cortical cells of maize root segments. The incubation medium, aerated, thermoregulated at 26°C and maintained in continuous flow (200 ml h^{-1}) contained 0.5 mM $CaSO_4$, 0.1 mM MES-Ca (pH 6.0) and Cs^+, Rb^+ or K^+ (as SO_4^{2-}) at the indicated concentrations. The values are the means of four experiments run in quadruplicate. SE did not exceed 1.5%.

In the presence of 5 mM Rb^+, there was a slight depolarisation (about 5 mV) of E_m and in the presence of 5 mM K^+ E_m became more positive (up to about −85 mV) with a depolarisation of about 25 mV.

The effects of Cs^+ on the release of H^+ into the incubation medium (which depends mainly on the H^+ efflux caused by the activity of the plasmalemma H^+-ATPase and on the H^+ influx from co-transport activity) were examined and compared with the effect of Rb^+ and the well-known stimulatory effect of K^+ on H^+ release (Marrè 1991). Table 2 shows that the presence of Cs^+ or Rb^+ promoted, as does K^+, the release of H^+ into the medium; the effects of Cs^+ and Rb^+ were not significantly different from that of K^+ at external concentrations up to 0.5 mM and at 5 mM the effect of Cs^+ on the release of H^+ was only slightly lower than that of Rb^+, but much lower (about −30%) than that induced by K^+.

Competition between Cs^+, Rb^+ and K^+ for the influx mechanisms in maize root segments—The effects of the presence of Rb^+ or K^+ in the incubation medium on the influx of Cs^+ were examined and the reciprocal effects of Cs^+ or K^+ on the influx of Rb^+ were analysed; however, the influx of K^+ in the presence of Rb^+ or Cs^+ cannot be studied, as K^+ influx cannot be measured in these conditions (Cocucci and Sacchi 1993). Figures 3A and 3B show the effects of the presence of 0.05 or 0.1 mM K^+ or Rb^+ on the influx of Cs^+ at 0.01–0.25 mM in the incubation medium, presented in Eadie-Hofstee plots. Both K^+ and Rb^+ competitively inhibited Cs^+ influx; the K_m for Cs^+ was about 4- and 9-fold higher in the presence of 0.05 or 0.1 mM, respectively, of both K^+ and Rb^+, whereas the value of V_{max} was not significantly affected. Figure 3C shows that, reciprocally, Cs^+ competitively inhibited the influx of Rb^+ at external concentrations of 0.01–0.25 mM, but to a lesser extent; the K_m for Rb^+ was only ca. 1.5- and 2-fold higher in the presence of 0.05 and 0.1 mM Cs^+, respectively. Figure 3D shows that the influx of Rb^+ was also competitively inhibited by K^+, and that this inhibition was higher than that induced by Cs^+.

The competition between the cations was also determined by measuring the influxes in root segments exposed to Cs^+ and Rb^+ supplied together at the same concentration; in these conditions, the electrochemical potential of the two cations in the medium was the same. Table 3 shows that the influxes of Cs^+ and Rb^+, when present alone, were very similar to those shown in Figure 1. The overall value of the influxes of the two cations ($Cs^+ + Rb^+$) when present together (each at half of the total concentration) fell between the influx values of Cs^+ and Rb^+, but the contribution of the influx of Cs^+ was always lower than that of Rb^+, and decreased with the increase in external concentration; the Cs^+/Rb^+ ratio decreased from a value of about 0.6 at 0.05 mM to <0.3 at 5 mM (Table 3, last column).

Effect of TEA^+ on E_m in the presence of Cs^+, Rb^+ or K^+—TEA^+ is a well-known specific K^+-channel blocker that has been used to inhibit K^+ transport in nerve fibres

Table 2 Effect of the presence of Cs^+, Rb^+ or K^+ in the incubation medium on H^+ release from subapical segments of maize roots

Concentration of monovalent cation (mM)	ΔH^+ (μmol h^{-1} (g FW)$^{-1}$) in the presence of		
	Cs^+	Rb^+	K^+
nil		0.83±0.07	
0.05	1.16±0.06	1.15±0.05	1.24±0.06
0.5	1.21±0.03	1.24±0.04	1.26±0.04
5	1.64±0.04	1.84±0.04	2.27±0.05

Root segments (15) were incubated in 10 ml of 0.5 mM $CaSO_4$, 0.1 mM MES-Ca (pH 6.0) containing Cs^+, Rb^+ or K^+ (as SO_4^{2-}) at the concentrations indicated. H^+ release was measured by titrating the media after 30-min incubations. The values are the mean±SE of three experiments run in triplicate.

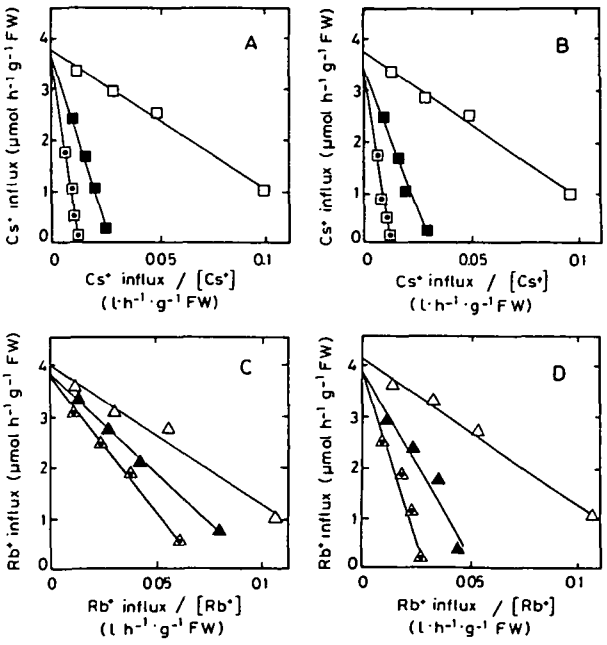

Fig. 3 Eadie-Hofstee plots of K^+ or Rb^+ inhibition on Cs^+ influx and of K^+ or Cs^+ inhibition on Rb^+ influx in subapical segments of maize roots. The influx of Cs^+ were determined in the range of concentrations 0.01–0.25 mM in the absence (□ in A and B) or in the presence (A) of 0.05 mM (■) and 0.1 mM K^+ (⊡) or in the presence (B) of 0.05 mM (■) and 0.1 mM Rb^+ (⊡). The influx of Rb^+ were determined in the range of concentrations 0.01–0.25 mM in the absence (△ in C and D) or in the presence (C) of 0.05 mM (▲) and 0.1 mM Cs^+ (△) or in the presence (D) of 0.05 mM (▲) and 0.1 mM K^+ (△). The values are the means of at least five experiments run in triplicate. SE of the calculated kinetic constants did not exceed 3% for V_{max} and 7% for K_m.

Table 3 Influxes of Cs^+, Rb^+ and Cs^+ Rb^+, present together in the incubation medium at equimolar concentration, in subapical segments of maize roots

Concentration of monovalent cation (mM)	Influx (μmol h^{-1} (g FW)$^{-1}$)			Flux ratio Cs^+/Rb^+
	Cs^+	Rb^+	$Cs^+ + Rb^+$	
0.05 Cs^+	2.45±0.08			
0.05 Rb^+		2.77±0.09		
0.025 Cs^+ + 0.025 Rb^+	1.01±0.04	1.71±0.06	2.72	0.59
0.1 Cs^+	2.75±0.07			
0.1 Rb^+		3.10±0.10		
0.05 Cs^+ + 0.05 Rb^+	1.06±0.02	2.02±0.07	3.08	0.52
0.2 Cs^+	3.06±0.08			
0.2 Rb^+		3.46±0.11		
0.1 Cs^+ + 0.1 Rb^+	1.02±0.06	2.42±0.08	3.44	0.42
2 Cs^+	5.31±0.21			
2 Rb^+		4.42±0.15		
1 Cs^+ + 1 Rb^+	1.15±0.07	4.15±0.06	5.30	0.28
5 Cs^+	5.71±0.19			
5 Rb^+		4.90±0.10		
2.5 Cs^+ + 2.5 Rb^+	1.30±0.09	4.61±0.11	5.91	0.28

Root segments (15) were incubated in 10 ml of 0.5 mM $CaSO_4$, 0.1 mM MES-Ca (pH 6.0) and Cs^+ or Rb^+ or Cs^+ plus Rb^+ (as SO_4^{2-}) at the concentrations indicated.The values are the mean±SE of four experiments run in triplicate.

(Tasaki and Hagiara 1957) and plant systems (Kochian et al. 1985, Lew 1991). Its effect on the E_m of the cells of root segments was studied in the presence of Cs^+, Rb^+ or K^+ (Fig. 4). As already shown in Figure 2, the depolarisation of E_m induced by 5 mM Cs^+ was less than that induced by the same concentration of Rb^+ or K^+, which caused marked depolarisation, and thus when TEA^+ was supplied in the presence of one of the three cations, the E_m value differed greatly. Figure 4 shows that in the presence of Cs^+, TEA^+ had no effect on E_m; the inhibitor strongly hyperpolarised (by about 35 mV) the E_m previously depolarised by the presence of K^+ and in the presence of Rb^+, the hyperpolarising effect of TEA^+ was lower (about 9 mV) but, as E_m in Rb^+ was higher than in the presence of K^+, the final value was similar.

Effect on cell pH of incubation in Cs^+, Rb^+ or K^+— The influxes of Cs^+, Rb^+ or K^+ into the root segments were accompanied by differences in H^+ release into the incubation medium (Table 2) and by differences in E_m (Fig. 2). These effects might depend on the differing involvement of metabolism in cation transport (Marrè 1991), or on the toxicity of Cs^+ on cell metabolism (Sheahan et al. 1993). The effects of Cs^+, Rb^+ or K^+ on energy metabolism were studied by in vivo ^{31}P-NMR of the subapical maize root segments. Figure 5 shows well-defined peaks of resonance for some phosphorylated metabolites; in particular, there were clear peaks of G6P, cytoplasmic Pi, vacuolar Pi, γ-phosphate of nucleosides triphosphates and β-phosphate of nucleoside diphosphates, α-phosphates of nucleosides di- and tri-phosphate, UDP-Glc, NAD(P)(H) and UDP-Glc. After 1 h of incubation in the presence of 5 mM K^+, there was only a slight change in the chemical shift (δ) of cytoplasmic Pi, suggesting an insignificant increase in cytoplasmic pH (about 0.03 pH units); no other change was detected (traces A and B in Fig. 5). The ^{31}P-NMR spectra obtained after 1 h incubation in 5 mM Rb^+ or Cs^+ were very similar to those obtained in the presence of K^+; there were no differences in the chemical shifts of either vacuolar and cytoplasmic Pi; indicating that the cytoplasmic and vacuolar pH were not affected differently by 1 h of incubation with the three cations. The resonance intensities of the γ-phosphate of the nucleoside triphosphate and β-phosphate of nucleoside diphosphate in the control and in the segments incubated in the presence of Cs^+, Rb^+ or K^+ were similar, suggesting that there was no direct toxicity on energy metabolism. These results were confirmed by the ATP levels, which were very similar after 1 h incubation in Cs^+, Rb^+ or K^+ (data not shown). The stability of the cytoplasmic and vacuolar pH could be due to the activation of the metabolic pH-stat mechanism which depends mainly on the activity of the phospoenolpyruvic- carboxylase (Davies 1973, Bown 1985, Guern et al. 1991, Marrè 1991). The activity of this system has been measured by assaying the dark fixation of CO_2; Table 4 shows that Cs^+, Rb^+ or K^+ in the incubation medium enhanced the dark fixation of CO_2, but this stimulation was very low in the presence of

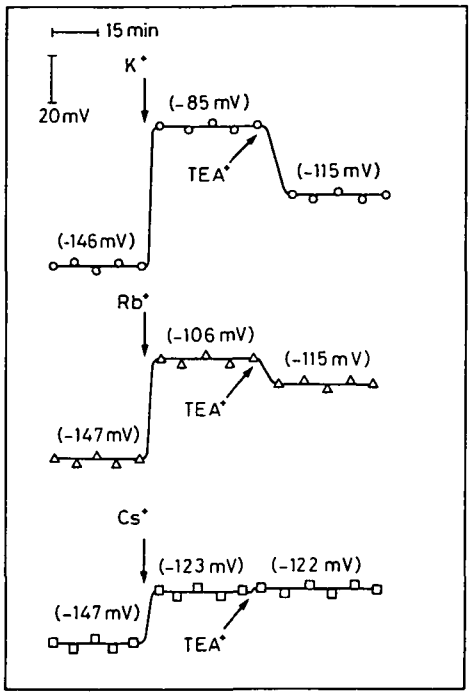

Fig. 4 Effects of tetraethylammonium-chloride on the transmembrane electric potential differences of cortical cells of maize root segments incubated in the presence of Cs^+, Rb^+ or K^+. The incubation medium, aerated, thermoregulated at 26°C and maintained in continuous flow (200 ml h^{-1}) contained 0.5 mM $CaSO_4$, 0.1 mM MES-Ca (pH 6.0), 5 mM K^+ or Rb^+ or Cs^+ (as SO_4^{2-}). 10 mM TEA^+ (as Cl^-) was added where indicated by the arrows. The values are the means of three experiments run in quadruplicate (n=12) in which SE did not exceed 2%.

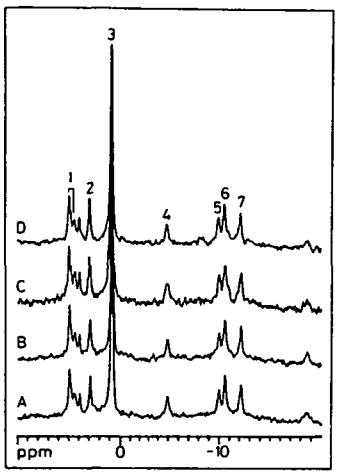

Fig. 5 ^{31}P-NMR spectra of maize root subapical segments in basal incubation medium and after 1 h incubation in K^+, Rb^+ or Cs^+. The segments (150), packed in an NMR tube (10 mm in diameter), were maintained for 30 min in continuous flow (8 ml min^{-1}) of the incubation medium, aerated, thermoregulated at 26°C containing (A) 0.5 mM $CaSO_4$, 0.1 MES-Ca (pH 6.0) added with (B) 5 mM K^+, or (C) 5 mM Rb^+ or (D) 5 mM Cs^+ (as SO_4^{2-}). After this period spectra were acquired for 21 min; chemical shifts were quoted relative to MDP (external reference) at 18.5 ppm (see Material and Methods section). The resonance assignments are as follows: 1, Glc-6-P and other phosphomonoesters; 2, cytoplasmic Pi; 3, vacuolar Pi; 4, γ-phosphate of nucleosides triphosphates and β-phosphate of nucleoside diphosphate; 5, α-phosphate of nucleoside triphosphates and nucleoside diphosphates; 6, UDP-Glc and NAD(P)(H); 7, UDP-Glc.

Cs^+ and much higher in the presence of Rb^+ (+46%) or K^+ (+53%).

Discussion

Subapical maize root segments, like other plant materials (Avery et al. 1993, Sheahan et al. 1993, Bellando et al. 1995), absorb Cs^+, as well as Rb^+ and K^+, starting from very low external concentrations ($<10^{-5}$ M). Kinetic analysis showed that at external concentrations up to 0.25 mM, the influxes of Cs^+, Rb^+ and K^+ were linear with concentration on an Eadie-Hofstee plot. The calculated kinetic constants of Cs^+ were very similar to those of Rb^+ and K^+; with values close to those reported previously in maize root segments for K^+ and Rb^+ for the high-affinity system (Kochian et al. 1985, Cocucci and Sacchi 1993). At external concentrations of up to 0.25 mM, the competition of the three cations (Fig. 3) suggested that they use the same transport system, as already reported in *Chlorella salina* (Avery et al. 1993) and *Arabidopsis thaliana* (Sheahan et al. 1993). This system, operating at low external concentrations, might be the K^+-uptake mechanism which it has been suggested to be energised by plasmamembrane proton gradient via a K^+-H^+ symport (Rodriguez-Navarro et al. 1986, Maathuis and Sander 1994).

At concentration >0.25 mM the competition between Cs^+ and Rb^+ (Table 3, concentration ≥2 mM) and the fact

Table 4 Effects of Cs^+, Rb^+ or K^+ on CO_2 dark fixation in subapical segments of maize roots

Concentration of monovalent cation (mM)	Radioactivity (Bq h^{-1} (g FW)$^{-1}$)
nil	68±5
5 Cs^+	72±6
5 Rb^+	99±7
5 K^+	104±7

The root segments (15) were incubated in 10 ml of 0.5 mM $CaSO_4$, 0.1 mM MES-Ca (pH 6.0) and 5 mM Cs^+, Rb^+ or K^+ (as SO_4^{2-}). 37 kBq $NaH^{14}CO_3$ (2.2 GBq $mmol^{-1}$) were injected into sealed flasks; after 1 h the root segments were homogenised in 1 ml 0.1 M HNO_3 and, after bubbling with N_2 the radioactivity was determined. The values are the means±SE of one typical experiment, run in quadruplicate.

that the presence of K^+ in the medium reduced the influx of Cs^+ or Rb^+ (data not shown; the influx of K^+ cannot be measured, see methods) might suggest the involvement of a same transport system or an effect of E_m that was very different at these concentrations when K^+, Rb^+ and Cs^+ were present in the incubation medium. That the influx of Cs^+ decreased with respect to that of Rb^+ at increasing concentrations of external cations, when they were present together in the incubation medium and driven by the same electrochemical potential (Table 3, compare the values of the last column at 0.2 and 2 mM), indicates that at higher concentrations there was discrimination between Rb^+ and Cs^+. This suggests the involvement of transport mechanisms with different discrimination at low and high concentrations.

Cation influx, at an external concentration where E_m is sufficient to drive K^+ uptake, can be mediated by K^+-channels (Kourie and Goldsmith 1992, Gassmann et al. 1993, Kochian and Lucas 1993, Gassmann and Schroeder 1994) that are highly selective for K^+ with respect to Rb^+ and, to a greater extent, to Cs^+ (Kourie and Goldsmith 1992, Gassmann and Schroeder 1994); the reported in vivo results are in agreement with this high discrimination. The involvement of K^+ channels in the transport has been studied using TEA^+, a K^+ channel blocker, used to indicate the presence of an inward K^+ current through ion channels (Lew 1991). The large hyperpolarisation induced by TEA^+ in the presence of K^+, the slight effect in the presence of Rb^+ and the lack of effect in the presence of Cs^+ (Fig. 4), suggests that, at high external concentration, K^+ and to a lesser extent Rb^+ uses channel-mediated transport; little Cs^+ should be transported through channels. An effect of Cs^+, as K^+ channels blocker, on the influx of Rb^+ through K^+ channels should be precluded because K^+ channels at an $E_m < -110$ mV and a Cs^+ concentration of <2.5 mM, are only partially blocked by Cs^+ (Kourie and Goldsmith 1992).

Even if the involvement of a non-specific permeation of Cs^+ cannot be excluded, the fact that the influx of Cs^+ reached values higher than the V_{max} (calculated up to 0.25 mM, Table 1), might depend on the electrochemical potential driving the transport of Cs^+ that, in absence of channel-permeating cations, was high. This higher electrochemical potential might induced a change in the kinetic characteristics of the high-affinity system (Blatt et al. 1987, Maathuis and Sanders 1994) explaining the stepwise increase of the influx of Cs^+ between 0.5 and 1 mM (Fig. 1).

It has been suggested that in fungi and plants the mechanism for K^+ high-affinity system is a K^+-H^+ co-uptake (Rodriguez-Navarro et al. 1986, Sacchi and Cocucci 1992, Maathuis and Sanders 1994, Schachtman and Schroeder 1994). Cation uptake is energised by the activity of the proton pump; the cation-H^+ co-uptake mechanism uses ΔH^+ together with E_m, whereas the channel-mediated mechanism uses E_m; in this case, the electrophoretic cation influx tends to reach the electrochemical equilibrium. In the channel-mediated influx, the lack of H^+ re-influx through the cation-H^+ co-uptake mechanism should be accompanied by a higher H^+ release in the medium; indeed, even if stoichiometric charge equilibrium cannot be determined in this material (i.e. tissues, walled cells), in the presence of a higher external K^+ concentration, when channel-mediated transport was used, there was a greater release of H^+ with respect to Cs^+ (Table 2). This suggestion was also supported as the release of H^+ was intermediate between the cations in the presence of Rb^+, for which the channel permeability is lower than that of K^+ but much greater with respect to Cs^+.

The activity of channel-mediated cation uptake should produce a greater release of H^+ in the medium and the cellular pH should increase. Neither cytoplasmic nor vacuolar pH changes were detected in the presence of 5 mM K^+ compared with Rb^+ and Cs^+ (^{31}P-NMR experiments, Fig. 5), but the higher dark fixation of CO_2 in the presence of K^+ than in the presence of Cs^+ indicated that the activation of the metabolic pH-stat mechanism counteracted the tendency for the pH to increase.

In conclusion, these results indicate that in maize root segments, Cs^+ is taken up by the K^+-high affinity system that discriminates little between Cs^+, Rb^+ and K^+ and not by the K^+ low-affinity system characterized by a higher selectivity for the three cations. The activities of these two transport mechanisms, both contributing to total uptake, might be controlled by the external cation concentration, probably by E_m and metabolic variables (i.e. cytosolic pH).

That Cs^+ is transported essentially by putative cation-H^+ co-uptake suggests that this mechanism might be examined using Cs^+, thus contributing to the clarification of the mechanism(s) of Cs^+ toxicity.

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