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Effect of calcium, sodium and pH on uptake and accumulation of radiocesium by *Riccia fluitans*

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Abstract

The effect of external Ca^{2+}, Na^+ and H^+ concentrations on radiocesium uptake and concentration factor (CF) was analysed in *Riccia fluitans* plants grown in K^+-deficient and K^+-sufficient conditions. The kinetics of the high-affinity K^+/Cs^+ transporter were also analysed in K^+-deficient plants. The K^+ regime determined Cs^+ uptake rates and CF: both variables were higher in K^+-deficient plants than in K^+-sufficient plants irrespective of the Ca^{2+}, Na^+ or H^+ concentrations. The effect of Ca^{2+} depended on the K^+ regime. Cesium uptake rates and CF increased in K^+-sufficient plants, 6- and 12-fold, respectively, at decreasing Ca^{2+} concentrations, whereas the Cs^+ uptake rate decreased by 50% in K^+-deficient plants and CF showed a maximum at intermediate Ca^{2+} concentrations (0.1 mM). The observed effect of Cs^+ uptake rates in K^+-deficient plants can be explained by a decrease in the maximum velocity and the affinity of the transporter at low Ca^{2+} concentrations. Cesium uptake rates decreased by 75% at alkaline pH in K^+-deficient plants, as did (by 40%) the maximum velocity of the transporter, while CF reached almost zero values. CF declined by 90% at acid pH but Cs^+ uptake rates only decreased by 20% and the maximum velocity of the transporter was relatively constant. Both Cs^+ uptake rates and CF showed a maximum at pH 7.5. No significant pH effect was found in K^+-sufficient plants. Cesium uptake rates increased by almost three-fold at low Na^+ concentrations in both K^+ regimes. The increase in uptake rates in K^+-deficient plants paralleled the increment in both the maximum velocity of the transporter and its affinity for K^+ and Cs^+. CF also increased with low Na^+ concentrations at both K^+ regimes but less so in K^+-deficient (three-fold) than in K^+-sufficient plants (30-fold). In conclusion, both Cs^+

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uptake and accumulation would show a maximum in K^+-deficient *R. fluitans* plants in media with neutral pH, very low Na^+ and intermediate (0.1 mM) Ca^{2+} concentrations.

Keywords: Calcium: pH: Sodium: Radiocesium uptake: Cesium accumulation: Membrane potential: *Riccia fluitans*

1. Introduction

After the Chernobyl accident, great attention has been paid to the accumulation of radiocesium in terrestrial plants (Dahlberg et al., 1997; Rühm et al., 1997; Sacchi et al., 1997; Willey and Martin, 1997), as this ion was liberated in large quantities (Warner and Harrison, 1993). However, little is known about Cs^+ uptake and accumulation in freshwater plants despite the fact that, in ecosystem models that assess risk to the population, it is necessary to determine the uptake and accumulation of radioactive ions as well as the influence of major environmental variables on these processes, especially in relevant points of the aquatic food chain (Håkanson, 1997; Håkanson and Fernández, 2001). Nevertheless, there are few reports on the effect of environmental variables on Cs^+ uptake and accumulation in freshwater plants, only in some microalgae and cyanobacteria (Avery et al., 1991, 1993a,b).

It is known that Cs^+ enters into plant cells through K^+ transporters (Avery et al., 1993a; Sheahan et al., 1993; Sacchi et al., 1997), so any factor that influences K^+ uptake could affect Cs^+ uptake. It is known that the K^+ regime and pH affect the activity of K^+ transporters (Maathuis and Sanders, 1996), and the interaction between K^+ and Na^+ uptake is also well known (Schachtman and Liu, 1999). Apart from that, it is an old observation that Ca^{2+} stimulates K^+ uptake in terrestrial plants (Epstein et al., 1963). In fact, it has been proved that Ca^{2+} stimulates Cs^+ uptake in terrestrial plants (Bange and Overstreet, 1960), and that the K^+ regime affects Cs^+ uptake rates in some grasses (Smolders et al., 1997). However, little is known about the effect of these variables (K^+ regime, pH, Na^+ and Ca^{2+}) on Cs^+ uptake and accumulation in freshwater plants, except the previously mentioned reports in cyanobacteria and halophytic microalgae (Avery et al., 1991, 1993b).

Potassium enters into the cells through channels and high-affinity transporters, and not all of them show the same permeability for Cs^+ and Na^+, or the same pH or Ca^{2+} regulation. Inward and outward rectifying K^+ channels show a restricted permeability to Cs^+ (Maathuis and Sanders, 1995) and also are highly selective for K^+ over Na^+ (Amtmann and Sanders, 1999). However, voltage-insensitive monovalent-cation channels have been described recently in some plant cells (Tyerman et al., 1997; Amtmann and Sanders, 1999) which are permeable to a range of monovalent cations, including K^+, Cs^+ and Na^+ and which are inhibited by extracellular Ca^{2+} (White, 1999).

High-affinity K^+ transporters are considered the main way by which Cs^+ enters into plants (Avery et al., 1993a; Maathuis and Sanders, 1996; Sacchi et al., 1997). Molecular studies have revealed that some transporters show poor discrimination between K^+ and Cs^+ (Bañuelos et al., 1995; Rubio et al., 2000) and Na^+ has been shown to reduce K^+ uptake through them (Santa-María et al., 1997; Rubio et al., 2000). It seems also that these transporters could carry K^+ coupled with the entrance of protons (Rodríguez-Navarro, 2000),

which has been generally considered the mechanism for the high-affinity K^+ transport in plants (Maathuis and Sanders, 1996). However, apart from the requirements of external Ca^{2+} for the unimpaired functioning of the high-affinity K^+ transporter (Epstein et al., 1963), there appears to be little molecular or electrophysiological information about Ca^{2+} influence on high-affinity transporter kinetics.

Riccia fluitans is a liverwort that shows two distinct Cs^+ uptake kinetics depending on the K^+ regime. In K^+-deficiency, it shows saturating kinetics, while in K^+-sufficiency it remains linear. This and other evidence suggests the operation of different K^+ transporters systems at each K^+ regime, with high-affinity transporters or channels (Fernández et al., 1997) that could be differentially affected by environmental variables. In this paper, we report on the effect of Ca^{2+}, pH and Na^+ on Cs^+ uptake and accumulation in *R. fluitans* plants grown at two different K^+ regimes to analyse the influence that these variables may have on Cs^+ fluxes for plants in freshwater ecosystems. We have also characterised by classical electrophysiology the high-affinity K^+ transporter of this plant at different pH, Na^+ and Ca^{2+} concentrations, in order to explain the observed Cs^+ uptake rates.

2. Material and methods

2.1. Plant material

Riccia fluitans was grown as described by Felle (1981). Plants were cultivated in a solution which contained micronutrients (1% Murashige and Skoog plant salts, Serva, Germany), 0.1 mM KCl, 0.1 mM $CaCl_2$, 0.1 mM NaCl and 2 mM NaH_2PO_4/Na_2HPO_4, pH 6.7. Plants were maintained at constant temperature (25 °C) with a photoperiod of 16 h. The photon fluence rate was 30 $\mu mol\, m^{-2}\, s^{-1}$.

The plants used for experiments were preincubated for 3 days in a medium containing 0.1 mM $CaCl_2$, 0.1 mM KCl, 0.1 mM NaCl and 10 mM HEPES-CAPSO, pH 7.3. To induce K^+-deficiency, plants were preincubated in the same medium without KCl.

2.2. Electrophysiology

Membrane potentials were measured using the standard glass microelectrode technique as described in Felle (1981). Capillary glass containing internal filament (Hilgenberg, Germany) was pulled using a horizontal puller (PD-5, Narishige, Japan). Microelectrodes were backfilled with 0.5 M KCl. Micropipettes were fixed to electrode holders containing an Ag/AgCl pellet, connected to a high impedance voltmeter FD-223 (WPI, Sarasota, FL, USA).

Plants preincubated for 3 days in K^+-deficiency were used for impalements. Green thallus pieces were fixed with wax in a Plexiglas chamber (volume 1.1 ml) and a single cell was impaled each time in constant perfusion at a flux rate of 10 $ml\, min^{-1}$. Control membrane potentials values were around −245 mV (Ballesteros et al., 1998).

The medium used for impalement was the preincubation medium described above, without KCl and increasing K^+ (or Cs^+) concentrations were added sequentially. In the experiments with Na^+, the ion was added as NaCl. In the assays with Ca^{2+}, the cation was added buffered with EDTA. In the experiments at different pH, the medium was buffered at

pH 5.3, 6.3 (with MES-HEPES), 7.3, 8.3 and 9.3 (with HEPES-Bis Tris propane). Buffer concentrations were 10 mM.

2.3. Radiocesium uptake experiments

The assay medium was the same as used for preincubation (with or without K^+). However, in the experiments with Na^+, NaCl was added in the range 1 μM–10 mM final concentration. Ca^{2+} was added, buffered with 0.1 mM EDTA, in the range 0.1 μM–10 mM. Free Ca^{2+} ionic activity was calculated with the program MINTEQA2 version 3.11 (Jerry D. Allison, USEPA Environmental Research Lab, College Station Road, Athens, GA 30613, USA). Ion concentrations, [I] (in M), have been expressed as $pI = -\log [I]$. In the experiments at different pH, the assay medium was buffered at pH 6.5 (with MES-HEPES), 7, 7.5, 8, 8.5 or 9 (with HEPES-Bis Tris propane). Buffer concentrations were 10 mM.

Uptake experiments were performed with 0.5 g of thallus in 250 ml Erlenmeyer flasks containing 100 ml of assay medium. The flasks were maintained under continuous fluorescent light (photon fluence rate: 80 $\mu mol\, m^{-2}\, s^{-1}$) and gentle shaking. After 1 day of preincubation in the new media, the flasks were labelled with $^{137}CsCl$ up to a final activity of 0.5 $Bq\, ml^{-1}$. Cesium concentration and activity of the $^{137}Cs^+$ source was 7.4 10^{-10} g $Cs^+\, ml^{-1}$ and 0.2 10^{-6} $Ci\, ml^{-1}$, respectively. Every experiment was performed in triplicate.

Samples from the medium were taken at 5 min and at 1, 3, 7, 24, 48 and 72 h after labelling. Radioactivity was measured by means of a gamma-ray (NaI detector) 3MW3/3 (Bicron, OH, USA). Data acquisition was performed through an interface board AccuSpecNaI plus and software version 7.3 from AccuSpec (Canberra, IL, USA). Uptake rates were calculated as the slope of the line obtained by fitting of the initial $^{137}Cs^+$ concentration values as a function of time within the first 3 h of incubation.

Concentration factors (CF) were computed as the ratio between the activity inside the plants, expressed in terms of plant water volume and the activity per volume of assay medium at the end of the 72-h incubation, when no net flux of radiocesium could be detected. Plant water volume was determined as the difference between plant fresh weight and dry weight. Dry weight was obtained after incubation of the plant material at 110 °C for 24 h.

2.4. Data analysis

Data are given as the mean ± S.E. When indicated, data were fitted to the equation of Michaelis–Menten using a non-linear regression computer program (KaleidaGraph, Sinergy Software, PA, USA).

3. Results

3.1. Effect of Ca^{2+} concentration on $^{137}Cs^+$ uptake rate and accumulation (CF)

The effect of Ca^{2+} concentration on Cs^+ uptake rates depended on the K^+ regime (Fig. 1A). In K^+-deficient plants, Cs^+ uptake rates decreased at lower Ca^{2+} concentrations whilst they increased in K^+-sufficient plants. The decrease in uptake rates of K^+-deficient

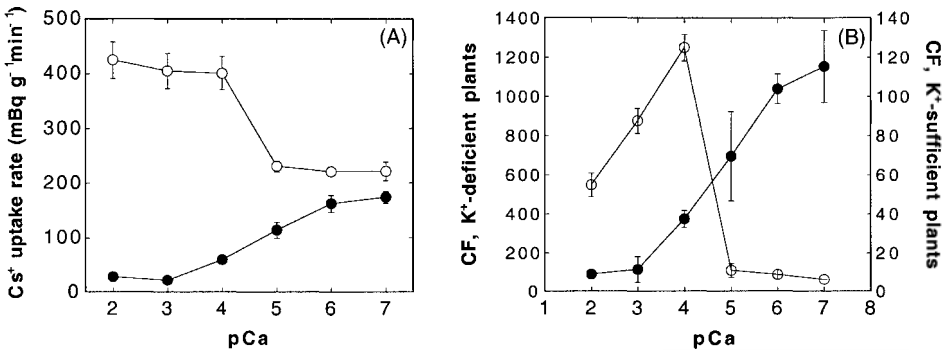

Fig. 1. Cesium uptake rates (A) and CF(B) at decreasing Ca^{2+} concentrations, expressed as pCa, in K^+-sufficient (closed symbols, right scale in B) or K^+-deficient (open symbols, left scale in B) plants. The values are the mean ± S.E. ($n = 3$).

plants was steeper in the range pCa 4–5 and the rates were reduced by half in the range pCa 2–7. In K^+-sufficient plants the uptake rates increased six-fold in the same range of Ca^{2+} activities, but were never higher than those observed in K^+-deficient plants.

The Ca^{2+} effect on CF also depended on the K^+ regime (Fig. 1B). In K^+-sufficient plants, a decrease in Ca^{2+} concentration induced an increase of CF, which was 12-fold higher at pCa 7 than at pCa 2. On the contrary, CF values declined at lower Ca^{2+} concentrations in K^+-deficient plants. However, the effect of Ca^{2+} was more complex in these plants. CF values increased from pCa 2 to 4, then decreased steeply at pCa 5 and showed a slight reduction at lower Ca^{2+} concentrations.

3.2. *Effect of pH on $^{137}Cs^+$ uptake rates and accumulation (CF)*

Proton concentration had a clear effect in Cs^+ uptake rates of K^+-deficient plants, whereas in K^+-sufficient plants the differences between Cs^+ uptake rates measured at different pHs were not significant (ANOVA, $P < 0.1$, Fig. 2A).

Cesium uptake rates in K^+-deficiency showed a maximum at pH 7.5, declining at more acid and more alkaline conditions. However, the decrease was higher at alkaline than at acid pHs: rates were reduced by approximately 75% from pH 7.5 to 9 and only decreased by 20% from pH 7.5 to 6.5.

As observed with Cs^+ uptake rates, there were no differences between CF values measured at different proton concentrations in K^+-sufficient plants (ANOVA, $P < 0.1$, Fig. 2B). However, in K^+-deficient plants, CF showed a maximum at pH 7.5 and then the values were reduced markedly both at acid and alkaline pH. The decrease in CF was steeper at alkaline pH, reaching almost zero values at pH 8.5 and 9.

3.3. *Na^+ effect on $^{137}Cs^+$ uptake rates and accumulation (CF)*

Cesium uptake rates increased at low external Na^+ concentrations both in K^+-sufficient and K^+-deficient plants (Fig. 3A). The differences in uptake values were significant with

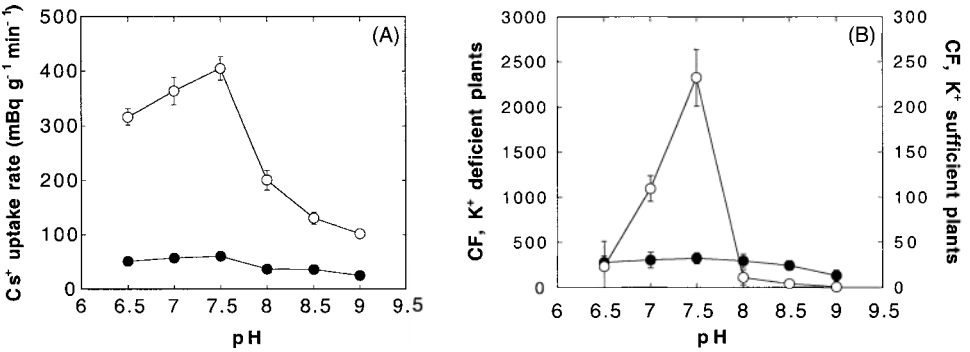

Fig. 2. Cesium uptake rates (A) and CF (B) at decreasing proton concentrations in K^+-sufficient (closed symbols, right scale in B) or K^+-deficient (open symbols, left scale in B) plants. The values are the mean ± S.E. ($n = 3$).

respect to external Na^+ concentration at both K^+ regimes (ANOVA, $P < 0.1$). The decrease in Cs^+ uptake rates from pNa 6 to 2 was also similar in percentage (by around 65%) in the two treatments.

Sodium external concentration had a significant effect on Cs^+ accumulation at both K^+ regimes (Fig. 3B, ANOVA, $P < 0.1$). CF values declined at high Na^+ concentrations, but they decreased more in K^+-deficient plants (by 97% from pNa 6 to 2) than in K^+-sufficient plants (by 60%). However, in K^+-deficient plants there were no significant differences in CF values in the range pNa 6–4, decreasing steeply from pNa 4 to 2.

3.4. *Effect of Ca^{2+}, pH and Na^+ on the high-affinity K^+ transporter*

Membrane depolarisations can be considered an estimate of the activity of the high-affinity K^+ transporters, as it is known that K^+ uptake in plants is coupled with the entrance of more than one proton and that it is not electrically silent (Maathuis and Sanders, 1994). As

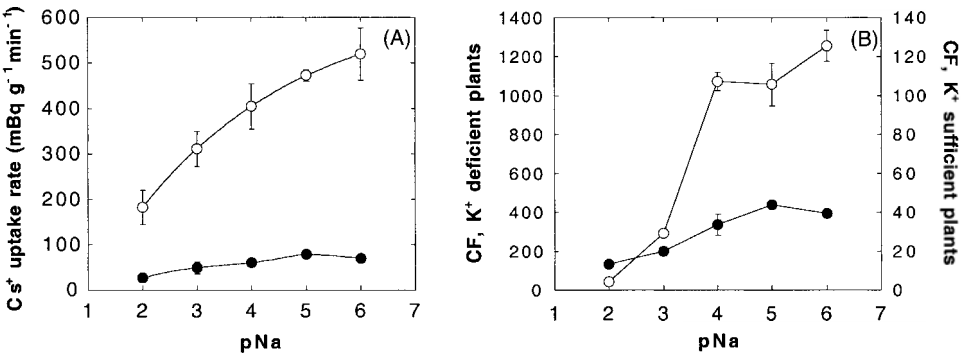

Fig. 3. Cesium uptake rates (A) and CF (B) at decreasing Na^+ concentrations (expressed as pNa) in K^+-sufficient (closed symbols, right scale in B) or K^+-deficient (open symbols, left scale in B) plants. The values are the mean ± S.E. ($n = 3$).

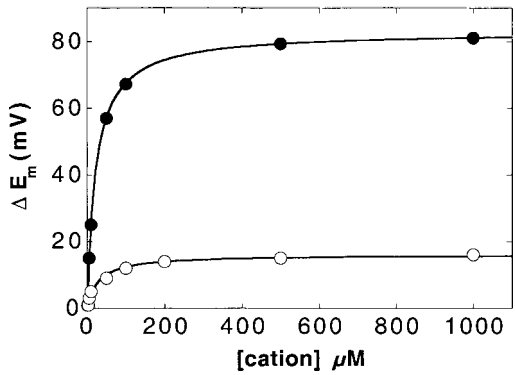

Fig. 4. Membrane depolarisations (ΔE_m, in mV) induced by increasing external concentrations of K^+ (closed symbols) or Cs^+ (open symbols) in K^+-deficient *R. fluitans* plants. Values are the mean ± S.E. ($n = 3$). In all cases, S.E. values were smaller than symbols.

Cs^+ seems to enter mainly through a high-affinity K^+ transporter in K^+-deficient plants (Avery et al., 1993a; Fernández et al., 1997; Sacchi et al., 1997), the effect of external Ca^{2+}, pH, Na^+ and on this transporter was further analysed in these plants by eletrophysiology.

Cesium, as well as K^+, induced a depolarisation of the membrane in K^+-deficient plants, and these depolarisations increased with higher concentrations, showing saturating kinetics (Fig. 4). However, the depolarisations induced by Cs^+ were always lower than the depolarisations produced by K^+. Curve-fitting of the depolarisation values to the Michaelis–Menten equation showed that the semisaturation constant, K_m, was slightly higher for Cs^+ (30.1 ± 4.4 μM) than for K^+ (22.9 ± 0.1 μM) and that the calculated maximum depolarisation (D_{max}, an estimate of the maximum velocity of the transporter) for K^+, was more than five-fold higher (82.9 ± 0.1 mV) than that obtained for Cs^+ (15.9 ± 0.5 mV). As the depolarisations induced by Cs^+ were not higher than 15 mV, K^+-induced depolarisations were used to further analyse the effect of pH, Na^+ and Ca^{2+} on the transporter.

K^+-induced membrane depolarisations were monitored at three different Ca^{2+} concentrations (Table 1). At high Ca^{2+} concentrations, the D_{max} obtained was higher than that observed at low concentrations, increasing by 30% at pCa 3 with respect to pCa 5. Furthermore, the K_m decreased by approximately 90% in the same range of concentrations.

Table 1
Kinetics parameters of the high-affinity K^+ transporter at different Ca^{2+} and Na^+ concentrations

pCa	D_{max} (mV)	K_m (μM)	pNa	D_{max} (mV)	K_m (μM)
5	63.0 ± 7.6	72.7 ± 29	5	48.5 ± 2.3	18.6 ± 0.4
4	82.9 ± 0.1	22.9 ± 0.1	4	47.4 ± 0.5	23.2 ± 0.2
3	89.3 ± 8.0	9.2 ± 5.4	2	37.0 ± 1.1	40.4 ± 2.1

Data were obtained by curve-fitting of the membrane depolarisations values induced by the addition of increasing K^+ concentrations. Each range of K^+ concentrations (up to 200 μM) was assayed at three different external Ca^{2+} or Na^+ concentrations, expressed as pI (−log [I], in M). Calculated maximum depolarisation (D_{max}, in mV) and semisaturation constant (K_m, in μM K^+) are shown in the table.

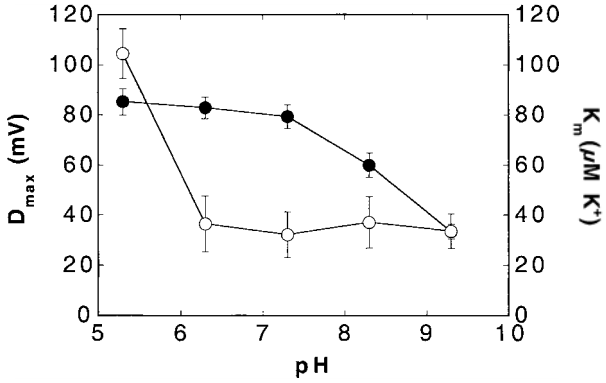

Fig. 5. Maximum depolarisation values (D_{max}, in mV, closed symbols) and semisaturation constant for K^+, K_m (in μM, open symbols), obtained from the curve-fitting of the membrane depolarisations values observed at increasing K^+ concentrations at different pH. Data were fitted at each pH to the Michaelis–Menten model using a non-linear regression computer program.

The effect of increasing Na^+ concentrations on the kinetics of the high-affinity K^+ transporter is also shown in Table 1. Maximum depolarisations (D_{max}) decreased by 24% when the external Na^+ concentration changed from pNa 5 to 2. The affinity of the transporter for K^+ also decreased with higher Na^+ concentrations, as K_m values were doubled in the same range of concentrations. The effect of Na^+ on Cs^+-induced membrane depolarisations was quite similar to that observed with K^+ (data not shown).

Maximum depolarisations (D_{max}) were similar from pH 5.3 to 7.3, but decreased by half from pH 7.3 to 9.3 (Fig. 5). The semisaturation constant for K^+ did not show significant differences from pH 6.3 to 9.3, but it was more than doubled at pH 5.3 (Fig. 5).

4. Discussion

4.1. The effect of Ca^{2+}

Changing of external Ca^{2+} affects both Cs^+ uptake rates and accumulation in *R. fluitans*. This contrasts with the result observed in the unicellular algae *Chlorella salina* in which no effect could be detected (Avery et al., 1993b) but agrees with the classical observation of Ca^{2+} stimulation of Cs^+ uptake in terrestrial plants (Bange and Overstreet, 1960). However, although Cs^+ uptake rates increase in K^+-deficient plants at high Ca^{2+} concentrations, they decrease in K^+-sufficient plants. This differential effect of Ca^{2+} on Cs^+ uptake rates does not appear to have been reported before and could be explained as different transporters seem to be involved in Cs^+ uptake in *R. fluitans* depending on the K^+ regime (Fernández et al., 1997).

Previous results suggest that Cs^+ uptake in K^+-sufficient *R. fluitans* plants, which shows linear kinetics, could be produced through channels (Fernández et al., 1997). Non-selective

cation channels, that show a significant permeability for Cs^+, are inhibited by high external Ca^{2+} concentrations in some plants (Tyerman et al., 1997; White, 1999), as observed with Cs^+ uptake rates in K^+-sufficient *R. fluitans* plants. However, external Ca^{2+} is known to produce several other effects at the membrane level that could also explain the decrease in Cs^+ uptake rates in K^+-sufficient plants.

On the other hand, *R. fluitans* plants subjected to K^+-deficiency exhibit Michaelis–Menten kinetics for Cs^+ uptake, suggesting that Cs^+ is taken up via a high-affinity K^+ transporter (Fernández et al., 1997). However, the electrophysiology results show that the transporter exhibits a lower affinity for Cs^+ compared to K^+, and also a lower maximum velocity. Calcium induced a change on the kinetic parameters of the transporter which could explain its effect on Cs^+ uptake rates. The increase in the estimated maximum velocity of the high-affinity K^+ transporter paralleled the increase in Cs^+ uptake rates. The effect of Ca^{2+} was also observed on the K_m value, as the affinity for K^+ (and therefore for Cs^+) increased at high Ca^{2+} concentrations. The addition of Ca^{2+} is known to promote the Cs^+ uptake capacity in barley roots at low external Cs^+ concentrations (Bange and Overstreet, 1960); however, there was no observed effect of Ca^{2+} on Cs^+ affinity in this species.

The Cs^+ CF is the result of both influx (uptake) and efflux processes. In K^+-sufficient plants, the effect of Ca^{2+} on CF was similar to that observed in Cs^+ uptake rates. However, in K^+-deficient plants, CF varied with Ca^{2+} concentration in a different way than uptake rates did. When Ca^{2+} concentrations changed from pCa 4 to 5, CF values decreased one order of magnitude, whereas Cs^+ uptake rates were reduced by less than 50%. This means that although Cs^+ could have been taken up initially, low Ca^{2+} conditions could be stimulating the efflux of Cs^+.

4.2. *Effects of pH and Na^+*

The effect of pH depended also on the K^+ regime. In K^+-sufficient plants there was no significant pH effect, but in K^+-deficient plants both Cs^+ uptake and accumulation showed a clear pH-dependence. High-affinity K^+ transport, which would be operating in K^+-deficiency, is proton-coupled in most plants analysed (Maathuis and Sanders, 1996). Therefore, a decrease in transport rates could be anticipated at alkaline pHs, when the proton motive force for the transport would be reduced. In fact, Cs^+ uptake rates in *R. fluitans* decreased by 75% at increasing pH values and so did (by 40%) the maximum velocity of the transporter. However, the affinity of the transporter for K^+ did not change significantly at higher pH values. The results observed in *R. fluitans* contrast with those found in the unicellular algae *C. salina*, in which Cs^+ uptake did not change over a range of external pH values (Avery et al., 1993b), and also with the results reported in *Synechocystis*, in which Cs^+ accumulation increased at alkaline pH (Avery et al., 1991).

On the other hand, we could anticipate an increment in the capacity of the K^+ transporter at higher external proton concentrations, unless there was an effect of pH per se on the transporter (Bush, 1993). However, Cs^+ uptake rates decreased by 20% at low pH. The increment in proton concentration did not modify significantly the maximum velocity of the K^+ transporter, and the affinity decreased significantly only at pH 5.3. Therefore, the decline in Cs^+ uptake rates at acid pH is difficult to explain by the variations observed in the kinetics of the K^+ transporter.

Changes on Cs^+ accumulation with pH in K^+-deficient plants followed the same pattern as Cs^+ uptake, but the effect of acid pH was more potent, whereas the decrease on Cs^+ uptake was slight (about 20%), Cs^+ accumulation decreased by 90%. In the same way, Cs^+ uptake rates decreased by 75% at alkaline pH in K^+-deficient plants; however Cs^+ accumulation decreased even more, to almost zero. This suggests that Cs^+ efflux could be being promoted at acid and alkaline pH.

Sodium inhibition of high-affinity K^+ (Epstein et al., 1963) and Cs^+ (Bange and Overstreet, 1960) uptake is a classical observation in terrestrial plants, which has been confirmed also in *R. fluitans*, and previously in the unicellular algae *Chlorella emersonii* (Avery et al., 1992). With respect to high-affinity K^+ transporter kinetics, an increase in Na^+ concentration produced both a decrease in the maximum velocity of the transporter and in its affinity for the substrate. These effects could explain the lower Cs^+ uptake rates observed at high Na^+ concentrations in *R. fluitans*.

The inhibition of Cs^+ transport by Na^+ in K^+-deficient plants was reflected in the lower rates of Cs^+ accumulation, which decreased at the same levels of Cs^+ accumulation of K^+-sufficient plants when the external Na^+ concentrations were in the range 1–10 mM. However, Cs^+ uptake rates were always higher in K^+-deficient than in K^+-sufficient plants. This suggests that Cs^+ efflux could be promoted in K^+-deficient plants at external Na^+ concentrations higher than 0.1 mM Na^+.

External proton concentration did not have a significant effect both in Cs^+ uptake and accumulation in K^+-sufficient plants, suggesting that pH could not influence the transporters used by Cs^+ in K^+-sufficiency, probably non-selective cation channels. However, the activity of these transporters could be affected by Ca^{2+} and Na^+, as an increase in their concentrations induced a decrease both in Cs^+ uptake and accumulation in K^+-sufficient plants.

In conclusion, external Ca^{2+} concentration is an important control variable as Cs^+ accumulation in plants can be dramatically affected. The use of K^+-deficient plants in media with intermediate Ca^{2+} concentrations (around 0.1 mM), low external Na^+ (up to 0.1 mM) concentrations and neutral pH (around 7.5) could be an efficient way for cesium removal. The results also suggest that low K^+, low Na^+ and high Ca^{2+} concentrations in freshwater environments would induce maximum Cs^+ accumulation in plants, while in high K^+, Na^+ and Ca^{2+} conditions, Cs^+ accumulation would be minimal.

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