Kinetics of caesium and potassium absorption by roots of three grass pastures and competitive effects of potassium on caesium uptake in Cynodon sp.

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Abstract. Caesium uptake by plant roots has been normally associated with the uptake of potassium as the potassium transport systems present in plants have also the capacity to transport caesium. Three grass species (Eragrostis curvula, Cynodon sp and Distichlis spicata) growing in seminatural grassland of central Argentina were selected to study their capability to incorporate Cs+ (and K+) using electrophysiological techniques. Although the 137Cs soil inventory ranged between 328- 730 Bq m⁻² in this region, no 137Cs activity was detected in these plants. However, all the species, submitted previously to K+ starvation, showed the uptake of both Cs+ and K+ when micromolar concentrations of these cations were present in the medium. The uptake showed saturation kinetics for both cations that could be fitted to the Michelis-Menten model. KM values were smaller for K+ than for Cs+, indicating a higher affinity for the first cation. The presence of increasing K+ concentrations in the assay medium inhibited Cs+ uptake in Cynodon sp., as expected if both cations are transported by the same transport systems. This effect is due to the competition of both ions for the union sites of the high affinity potassium transporters. In field situation, where soil concentration of Cs+ is smaller than K+ concentration, is then expectable that caesium activity in plants is not detectable. Nevertheless, the studied plants would have the capacity to incorporate caesium if its availability in soil solution increases. In addition, studies of Cs/K interaction can help us to understand the variability in transfer factors.

Keywords: Michaelis-Menten Kinetics, Caesium, Potassium, Plant Uptake, Grasses.
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INTRODUCTION

Nuclear fission reactions release 134Cs and 137Cs into environment. Following soil contamination, these radioisotopes can be transferred from soil to plants and then incorporated into the food chain. It becomes necessary then to know and to understand
the mechanisms for the uptake of these elements into plants [1]. Caesium absorption by plant roots has been normally associated with the uptake of K\(^+\), as the influx of caesium into plant cells mainly occurs through potassium transporters [2]. Classically two discrete uptake systems for potassium have been defined: system 1 and 2. These systems operate at low and high substrate concentration, respectively. System 1 obeys Michaelis-Menten kinetics and it has been related to the operation of high affinity carriers that are expressed in radical cells of plants growing in K\(^+\) deficiency. This system is a saturable absorption mechanism. The second mechanism is not saturable and is normally associated to ionic channels [2]. There are reports in the literature on the kinetics of Cs\(^+\) root uptake in different plant species, but there are few studies on the influence of the K\(^+\) regime on Cs uptake or about Cs/K interactions [3, 4]. To gain insight about the capacity of some grass species to incorporate Cs\(^+\) from the soil, we have analyzed both Cs\(^+\) and K\(^+\) uptake kinetics by the roots of three grass species submitted to K starvation, as well as the relative permeability of the membrane of root cells to both cations.

**MATERIALS AND METHODS**

For this study, we selected three grass species from seminatural grassland of central Argentina: *Eragrostis curvula*, *Cynodon* sp and *Distichlis spicata*. These plants grow in soils in which \(^{137}\)Cs fallout inventory range from 328 to 730 Bq m\(^{-2}\), but no \(^{137}\)Cs activity was detected in these plants. Individuals of *Cynodon* sp and *D. spicata* were extracted from the field and cultivated in hydroponic culture (1 mM KCl, 0.1 mM NaCl, 1mM CaCl\(_2\), pH 7.3). Commercial seeds of *E. curvula* were germinated in distilled water and later transferred to hydroponic culture. To induce K\(^+\) deficiency, plants were preincubated in the same medium without KCl, for at least 4 days. Membrane potentials (\(E_m\)) were determined by electrophysiology techniques [2] and variations of this parameter were recorded at different external potassium or caesium concentrations. Changes of the membrane potential (\(\Delta E_m\)) can be considered an estimate of ion uptake and these data could be fitted to the Michaelis-Menten equation:

\[
V = \frac{V_{\text{MAX}} S}{K_m + S}
\]

where \(S\) (\(\mu\)M) is the substrate concentration in assay medium; \(V\) (estimated by \(\Delta E_{m}\), in mV), is the uptake by cell root, \(V_{\text{MAX}}\) (estimated by \(D_{\text{MAX}}\), maximum depolarization, in mV) is the maximal rate of uptake by cell root, and \(K_m\) (\(\mu\)M) is the Michaelis-Menten constant.

In uptake experiments, the assay medium was the same as used for preincubations, and K\(^+\) (KCl) or Cs\(^+\) (CsCl) were added in the range 1 \(\mu\)M-1mM. To estimate the relative permeability of the membrane for potassium with respect to caesium, respiration mechanisms were blocked with sodium cyanide (1mM NaCN) and salicylhydroxamic acid (1mM SHAM).
RESULTS AND DISCUSSION

Kinetic experiments indicated the presence of high affinity transport systems (System 1) for the uptake of potassium and caesium in the roots of the three analyzed species. Both potassium and caesium uptake data were fitted to Michaelis-Menten model, showing r² values higher than 0.8 (Table 1). In all plants, the affinity constant, $K_M$, was smaller for K than for Cs, indicating the higher affinity of the carriers for K than for Cs. Both ions compete for the union sites of the same carriers, but do not behave as strict analogues, as indicated also by the values of $D_{MAX}$ that were always lower for Cs⁺ than for K.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>D. spicata</th>
<th>Cynodon sp</th>
<th>E. curvula</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>18.4±0.9</td>
<td>13.7±1.5</td>
<td>21±3</td>
</tr>
<tr>
<td>Cs⁺</td>
<td>97.4±16.2</td>
<td>163.3±51.9</td>
<td>120.2±52.7</td>
</tr>
<tr>
<td>$K_M$ (µM)</td>
<td>97.4±16.2</td>
<td>163.3±51.9</td>
<td>120.2±52.7</td>
</tr>
<tr>
<td>$D_{MAX}$ (mV)</td>
<td>18.4±0.9</td>
<td>13.7±1.5</td>
<td>21±3</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.98</td>
<td>0.95</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Direct effect of increasing K⁺ concentrations on Cs⁺ uptake was evaluated in Cynodon sp, submitted to K⁺ deficiency (fig. 1). In culture medium without K⁺, the addition of a saturating concentration of Cs⁺ (0.5 mM) induced a membrane depolarization of 14 mV (fig. 1, left side). This depolarization value was similar to maximum depolarization obtained in Cs uptake kinetic experiments (Table 1). However, in the presence of 0.5 mM and 1 mM K⁺ in the assay medium, the addition of Cs⁺ (0.5 mM), after membrane potential stabilization, caused a depolarization 3- and 7-fold lower, respectively, than in absence of K⁺ (fig. 1, middle). The addition of 0.5 mM Cs at 5m M K⁺ did not induce a clear depolarization. An increase in external K⁺ concentration causes then a decrease in the absorption of Cs⁺. This effect is due to the competition of both ions for union sites of high affinity potassium carriers, as it has been shown for these transporters [2].

The effect of the external potassium or caesium concentrations on the diffusion potential that is the membrane potential measured in the presence of inhibitors of the respiration, can be used to estimate the relative permeability of the cellular membrane.
to these two ions. For *D. spicata* and *Cynodon* sp. the relative permeability was estimated to be 0.74 and 0.71, respectively. These results indicate that the membranes of the root cells of the species analyzed are highly permeable to Cs⁺, being around 70% of K⁺ permeability.

**CONCLUSION**

The membranes of plant cells are highly permeable to K⁺, an important macronutrient for growth and development. The results obtained show that, in the species analyzed, the permeability of the membrane of the root cells to Cs is also very high (being 70% of K⁺ permeability), indicating the potential capacity of these plants to absorb caesium from the soil.

When the roots are submitted to potassium starvation, a high affinity uptake of potassium and cesium can be detected. The transport of both ions are carried out by the same potassium carriers, but showing a lower affinity for Cs and also a lower maximum uptake rate, as it has been shown before [3]. We have detected inter-specific differences in *V*<sub>MAX</sub> and *K*<sub>M</sub> for K⁺ and Cs⁺ uptake, as it has been described for Cs uptake kinetics in another grass species [4].

In addition, we have shown that addition of K⁺ to assay culture decreases strongly Cs⁺ uptake in *Cynodon* sp. The external concentration of K⁺ has then a direct influence on Cs incorporation by plants.

In field situation, where Cs⁺ concentration in the soil is smaller than K⁺ concentration, is then expectable that caesium activity in plants will be not detectable. Nevertheless, the studied plants would have the capacity to incorporate Cs if its availability in soil solution increases. In addition, kinetic studies of Cs/K interaction can help us to understand the wide variability detected in transfer factors, and could also provide a screening procedure to select plants with different caesium affinity, for example with low caesium uptake capacity.

**ACKNOWLEDGMENTS**

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**REFERENCES**