

1 **Uptake and translocation of cesium by *Arabidopsis thaliana* in hydroponics conditions: links**
2 **between kinetics and molecular mechanisms**

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12

13 **ABSTRACT**

14 Early studies have shown that cesium (Cs⁺) competes with the macronutrient potassium (K⁺) for
15 uptake by plants. The present study investigates the effect of K⁺ supply on Cs⁺ uptake and
16 translocation in *Arabidopsis thaliana*. Taking advantage of the frequent use of this model plant in
17 previous molecular studies, we discuss the link between functional features described for transporters
18 involved in K⁺ (and sometimes in Cs⁺) uptake and results obtained here in both Cs⁺ influx and
19 accumulation experiments under different K⁺-treatments. In low K⁺ condition (10 μM), we observed
20 that roots affinity for Cs⁺ increased significantly and Cs⁺ concentration in the external medium clearly
21 affected the efficiency of Cs⁺ uptake. Our results are consistent with previous molecular studies
22 indicating the role of the high-affinity K⁺ carrier AtHAK5 in Cs⁺ uptake under K⁺-deprivation. Further
23 experiments show that the lack of *AtHAK5* has no more effect on Cs⁺ uptake for external Cs⁺
24 concentration above 100 μM. We propose that non-selective cation channels, likely involved in Cs⁺
25 uptake under K⁺-sufficient conditions according to previous studies, could also mediate Cs⁺ uptake
26 under K⁺-starvation and high Cs⁺ concentrations. Finally, evidences for Cs⁺ translocation mediated by
27 K⁺ channels are discussed.

28 **Keywords:** Cesium uptake, Potassium uptake, kinetics parameters, transporters, *Arabidopsis*
29 *thaliana*.

30

31 **1. Introduction**

32 Cesium (Cs^+) has no known physiological role in plant but, because of its chemical similarity with the
33 essential macronutrient potassium (K^+), the monovalent cation Cs^+ can be taken up from the soil
34 solution by plant roots through the K^+ uptake pathway (White & Broadley, 2000). Some biological
35 processes involving K^+ can be altered by Cs^+ but, at the natural concentrations occurring in soil
36 solutions, stable isotope ^{133}Cs rarely causes environmental toxicity (Hampton et al., 2004). However,
37 radiocesium (^{134}Cs and ^{137}Cs), which may occur in the environment after accidental release from
38 nuclear plant facilities or resulting of nuclear weapon tests, is a major concern. These radioisotopes
39 emit harmful β and γ radiations during its decay and its uptake by plant is the predominant first step for
40 its entry in the terrestrial food chain (Avery, 1996).

41 In order to minimize the entry of radiocesium in the food chain, contaminated soils are usually
42 removed from agricultural uses or managed through the elimination of contaminated surface or by
43 using countermeasures such as fertilization with competitive cations to minimize Cs^+ uptake by plants
44 (Zhu & Shaw, 2000). Alternatively, with new biotechnologies emerging, phytoremediation is studied for
45 contaminated site rehabilitation using plants as extractor or developing “safe” crops which do not
46 accumulate radiocesium (Lasat & Kochian, 1997; Kobayashi et al., 2014).

47 The transport of monovalent cations from the soil solution to plant roots and shoots is mediated
48 symplastically by transporters. Therefore, identification and characterization of genes encoding
49 transporters involved in Cs^+ fluxes across the membranes of plant cells are thought to be helpful to
50 understand its uptake and accumulation. Phytoextraction and development of safe crops could be
51 optimized using plant selection based on this Cs^+ accumulation related knowledge (White et al., 2003).

52 Several recent studies on the model plant *Arabidopsis thaliana* have deciphered part of the molecular
53 mechanisms involved in Cs^+ uptake and accumulation, found among the plant K^+ transporters system.

54 Since the level of K^+ (both in the external and intracellular medium) modifies the relative contribution of
55 each transporters in K^+ fluxes (see Alemán et al., 2011 and references therein), Cs^+ uptake pathway
56 also depends on the K^+ -status. Up to now, non-selective cation channels (NSCC) also called Voltage-
57 Independent Cation Channels (VICC) encoded by members of the cyclic-nucleotide gated channel
58 (*AtCNGC*) and glutamate like-receptor (*AtGLR*) gene families are suspected to mediate the largest
59 part of Cs^+ uptake in *Arabidopsis* roots under K^+ -sufficient conditions (White & Broadley, 2000;
60 Hampton et al., 2005). Under K^+ -starvation, the high-affinity transporters encoded by the

61 *AtKUP/HAK/KT* gene families and in particular the HAK5 transporter mediate a significant part of Cs⁺
62 uptake in *A. thaliana* (Qi et al., 2008).

63 The competitive effect of K⁺ on kinetics of Cs⁺ uptake have been described for several crops species:
64 barley (Middleton et al., 1960), wheat (Shaw & Bell, 1989; Smolders et al., 1996; Zhu, 2001), maize
65 (Sacchi et al., 1997), rice (Kondo et al., 2015), spinach (Buysse et al., 1995), radish (Prorok et al.,
66 2016). However, only few studies report the kinetics aspect of Cs⁺ uptake in *A. thaliana* (Broadley et
67 al., 2001; Kanter et al., 2010) due to the fact that it is nor a commercial crop nor a potential plant for
68 phytoremediation uses. Conversely, *A. thaliana* is the preferred organism for molecular studies (see
69 above). Consequently, links between kinetics data and molecular characterization of transporters
70 involved in Cs⁺ uptake is reported only in few studies.

71 In this study, we report the effect of K⁺-supply on Cs⁺ uptake, distribution and accumulation by the
72 model plant *A. thaliana* in hydroponics condition. Links between transporters involved in K⁺ and Cs⁺
73 uptake related-knowledge and changes observed in Cs⁺ transport under K⁺-starvation are discussed.

74

75 **2. Materials and methods**

76 **2.1. Plant material and growing before exposure to cesium**

77 *Arabidopsis thaliana* seeds of Columbia-0 (Col-0) and *athak5-3* mutant line (SALK_074868) were used
78 in this study. As described before (Qi et al., 2008; Rubio et al., 2008), the T-DNA insertion is located in
79 exon 4 in *athak5-3*. Plants homozygous for the T-DNA insertion were identified by polymerase chain
80 reaction (PCR) using primers annealing upstream (HAK5A-F1:
81 CGCAGGAGGAACATTTGCATTGTACTC) and downstream (HAK5B-R1:
82 AGTGCCTTTAAGACGGTAATGTCATGCTTG) of the insertion site and a T-DNA left-border primer
83 (Lbb1.3: ATTTTGCCGATTTTCGGAAC) as advised by the Salk Institute Genomic Analysis Laboratory
84 (SIGNAL, <http://signal.salk.edu/tdnaprimers.2.html>).

85 Seeds were surface-sterilized using a mix of 70% ethanol (v/v)/ 0.05% SDS (v/v) and rinsed in ethanol
86 96% before sowing in Petri dishes (120 mm * 120 mm) on a half-strength Murashige and Skoog
87 medium (MS½, Murashige & Skoog, 1962) containing 1% (w/v) agar and 1% (w/v) sucrose. To
88 synchronize germination and to break the dormancy, the sowing boxes were placed at 4°C during 48 h
89 before transfer in a growth chamber set to 23°C, 50% HR with 8 h/16 h day/night cycle. After 7 days
90 on MS½ agar medium, seedlings were transferred on sand (Zolux) and watered with nutrient solution

91 to allow roots and shoots growing for a further 14 days. Finally, plants were transferred in a hydroponic
92 system over 1L of aerated nutrient solution.

93 The nutrient solution (pH 5.8) contained 1.1 mM MgSO₄, 805 μM Ca(NO₃)₂, 2 mM KNO₃, 60 μM
94 K₂HPO₄, 695 μM KH₂PO₄ and micronutrients (3.6 μM MnSO₄, 74 nM (NH₄)₆Mo₇O₂₄, 3 μM ZnSO₄, 9.25
95 μM H₃BO₃, 785 nM CuSO₄, 20 μM Na₂EDTA and 20 μM FeSO₄).

96

97 **2.2. Potassium treatments**

98 These experiments were designed to estimate the effect of K⁺-supply on uptake and accumulation of
99 Cs⁺ by *A. thaliana*. Three distinct concentrations of K⁺ (10 μM, 100 μM or 3000 μM) were supplied
100 during five days before addition of Cs⁺ in the medium.

101 K⁺ treatments were performed during the hydroponic step. After 3-5 days of acclimatization to
102 **hydroponics conditions** with the nutrient solution described in section 2.1, plants were transferred
103 over 1L of a K⁺-treatment solution (pH 5.8) containing 0.75 mM MgSO₄, 2 mM Ca(NO₃)₂, 0,5 mM
104 H₃PO₄, and micronutrients. Three different treatments were tested through adding different amounts of
105 KCl in the K⁺-treatment solution: 10 μM (starved level), 100 μM (intermediate level) or 3000 μM
106 (replete level).

107 In the same hydroponic box, 5-6 plants were allowed to grow over 1 L of K⁺-treatment solution during
108 5 days.

109

110 **2.3. Exposure to cesium in short-term influx experiments**

111 After 30 days (+/- 3 days) of growing and K⁺ treatment in the conditions described in sections 2.1 and
112 2.2 (summarized in Table 1), seedlings were transferred into individual wells containing 8 mL of
113 exposure solution taking care to not contaminate shoots. The exposure solution contained the K⁺-
114 treatment solution plus a range of ¹³³Cs from 0.1 μM to 3000 μM traced by ¹³⁷Cs (approximately 140
115 Bq.mL⁻¹ representing 3.3.10⁻⁴ μM Cs⁺). Plants were exposed during 15 min in order to determine
116 kinetics parameters of net influx or during 6 h to evaluate the evolution of Cs⁺ distribution between
117 roots and shoots. Activities of ¹³⁷Cs in the exposure solution were followed during the course of assays
118 and reveal no significant depletion (data not shown).

119 After exposure to Cs⁺, plants were transferred in 8 mL of a fresh solution corresponding to the
120 exposure solution without Cs⁺ (*i.e.* the K⁺-treatment solution) for 1 min to remove Cs⁺ bound to the cell

121 wall. Roots and shoots of plants tested were separated and blotted on Benchkote paper before
122 recording of fresh weights.

123 For each parameters (time of exposure, concentration of Cs⁺ in the exposure solution, K⁺-treatment),
124 experiments were repeated at least two times with a minimum of three plants per repetition.

125

126 ***2.4. Exposure to cesium in long-term accumulation experiments***

127 Long-term accumulation experiments were performed into 1 L exposure solution containing the three
128 different K⁺-treatment solutions described in section 2.2 (10, 100 or 3000 μM K) plus 1 μM stable Cs⁺
129 (no tracer was used). After 30 days (+/- 3 days) of growing in the conditions described in sections 2.1
130 and 2.2 (summarized in Table 1), plants were exposed for 7 days with renewing of the exposure
131 solution every 2-3 days to avoid significant decrease of Cs⁺ concentration in the medium due to uptake
132 by plants.

133 After 7 days exposure to Cs⁺, roots and shoots were harvested as described in section 2.3 for the
134 short-term influx experiments. For each K⁺-treatments condition, three tests were performed with a
135 minimum of five plants per test.

136

137 ***2.5. Measure of cesium and potassium***

138 Fresh roots and shoots of plants were mineralized in 5 mL HNO₃ 65% and 1.5 mL H₂O₂ 30% at 100-
139 150°C on a sand bath. Mineralisates were evaporated to dryness and redissolved in HNO₃ 2% v/v
140 before measuring the different elements.

141 Activity of ¹³⁷Cs accumulated in plants in the short-term experiments was measured by β liquid
142 scintillation counting. Liquid scintillation cocktail (Instagel-Plus, Perkin Elmer) was added to the
143 mineralized samples. The photon emissions following interaction between the liquid scintillator and the
144 β particles emitted in the radioactive decay of ¹³⁷Cs accumulated in plants were counted during 30
145 min. In parallel, concentration of ¹³⁷Cs and ¹³³Cs into the exposure solution were measured by β liquid
146 scintillation counting and ICP-MS respectively. The amount of Cs⁺ accumulated in plant sample was
147 deduced from the content of ¹³⁷Cs in plant and the ratio ¹³⁷Cs/¹³³Cs into the exposure solution.

148 ¹³³Cs concentrations in roots and shoots of plants and in the exposure solutions used for the long-term
149 accumulation experiments were measured by ICP-MS.

150 K⁺ content in plants after the K⁺-treatments was measured by ICP-AES on a minimum of 3 non-
151 exposed samples per experiment.

152

153 **2.6. Data analysis**

154 Using data from the 15 min influx assays, we calculated a solution to plant transfer factor ($TF_{ext \rightarrow plant}$)
155 according to **Eq.(1)**, defined here as the ratio between Cs⁺ uptake by plant roots and the Cs⁺
156 concentration in the solution (named $[Cs]_{ext}$ in the following). Cs⁺ uptake was calculated by dividing its
157 amount in the whole plant by the roots fresh-weight (FW). This $TF_{ext \rightarrow plant}$ is a modified version of the
158 usual TF defined as the ratio between concentration in shoots and concentration in the medium.
159 $TF_{ext \rightarrow plant}$ represents influx of Cs⁺ by the plant roots depending on the external Cs⁺ concentration.
160 Thus $TF_{ext \rightarrow plant}$ estimates the efficiency of Cs⁺ net uptake by roots.

$$TF_{ext \rightarrow plant}(Cs) = \frac{(Cs \text{ uptake})_{plant \ roots}}{[Cs]_{ext}} \quad \text{Eq.(1)}$$

161

162 As described in Zhu et al. (2000), Eadie-Hofstee plot was used to calculate the kinetics parameters
163 (K_m and V_{max}) of Cs⁺ influx from the 15 min experiment data. Linear regression on the range of $[Cs]_{ext}$
164 comprised between 0.1 and 200 μM was performed on this plot, with $V = Cs^+$ uptake rate (μmol.g⁻¹ FW
165 roots.h⁻¹) expressed as a function of the ratio $V/[Cs]_{ext}$ according to the **Eq.(2)**:

$$V = -K_m \frac{V}{[Cs]_{ext}} + V_{max} \quad \text{Eq.(2)}$$

166

167 Using data from the long-term accumulation experiment, we calculated a discrimination factor (DF) in
168 order to estimate the selectivity for uptake between K⁺ and Cs⁺, as described in Smolders et al. (1996)
169 and in Kanter et al. (2010):

$$DF(Cs) = \frac{([Cs] / [K])_{plant}}{([Cs] / [K])_{solution}} \quad \text{Eq.(3)}$$

170

171 ANOVA analysis were performed to evaluate the effect of K⁺-treatment on plant K⁺ content, fresh
172 weight and Cs⁺ content separately (NS, Non-Significant and *, **, *** Significant at the $\alpha = 0.05, 0.01$
173 and 0.001 level respectively). In tables, different letters in bold indicate significant differences between
174 means (Tuckey post-hoc test, p-value < 0.05).

175

176 **3. Results and discussion**

177 Effects of K⁺-supply on Cs⁺ influx, accumulation and distribution was estimated using both short-term
178 (from 15 min to 6 h exposure) and 7-days exposure assays performed on *A. thaliana* (Col-0 ecotype).

179 The experiments were designed to compare the effects of three different K⁺-treatments on:

180 (i) Kinetics parameters K_m and V_{max} for Cs⁺ influx,

181 (ii) Cs⁺ uptake efficiency estimated by transfer factor,

182 (iii) Distribution of Cs⁺ between roots and shoots.

183 Role of the high-affinity HAK5 K⁺ carrier in Cs⁺ uptake under both low and high K⁺-supply is also
184 described for a range of Cs⁺ external concentrations ($[Cs]_{ext}$).

185

186 **3.1. Effect of K⁺-treatment on plants potassium content and on plant growth**

187 Early studies have shown that K⁺ content of plant affects uptake of monovalent cations (Kochian &
188 Lucas, 1982). In order to evaluate the effect of K⁺-supply on Cs⁺ uptake, 25 days-old plants were first
189 acclimated during five days to three distinct levels of K⁺. K⁺ content of plants after acclimation is
190 significantly different between the three distinct K⁺-treatments (Table 2): plant K⁺ content is 1.5 times
191 higher for K⁺-replete seedlings (3000 μM K⁺ condition) compared to the K⁺-starved conditions (10 μM
192 K⁺).

193 However, we did not observe visible K⁺ starvation symptoms like chlorosis of older leaves nor plant
194 growth effects (Table 2). Plants were growing in standard K⁺ condition (2 mM) before K⁺-treatments
195 were applied. As stated by Kanter et al. (2010), this pre-culture in sufficient K⁺ conditions can prevent
196 the further effects of turning to low K⁺ supply.

197 Plants were exposed to Cs⁺ after acclimation period with the three different K⁺-supplies. The level of
198 K⁺ during the pre-treatment was maintained in the exposure solution containing Cs⁺. Thus, our
199 experiments describe the effect of global level of K⁺-supply *i.e.* in the solution outside the plants during
200 the exposure to Cs⁺ but also inside the plants due to the K⁺-treatment in pre-culture.

201

202 **3.2. Effect of potassium supply and cesium concentration in solution on cesium uptake** 203 **efficiency**

204 Effects of $[Cs]_{ext}$ on transfer factor for the three K^+ -conditions are shown in Figure 1. As expected,
205 $TF_{ext \rightarrow plant}$ values (calculated as described in **Eq.(1)**) are higher for plants in the 10 μM K^+ -condition
206 compare to the K^+ -replete plants (between 17 and 1.6 times higher, depending on the $[Cs]_{ext}$). As
207 stated before (Waegeneers et al., 2001), there are different reasons to record a higher Cs^+ transfer
208 factor in low K^+ condition:

- 209 i) the high depletion of K^+ at the root surface due to high demand of plant to sustain growth
210 whereas K^+ is weakly available. This depletion of K^+ in the medium favors Cs^+ uptake.
- 211 ii) the higher uptake rate potential of Cs^+ at low K^+ due to prevalence of high-affinity transport
212 (HAT) system. In *A. thaliana*, this HAT system is mainly mediated by HAK5 which is known to
213 be involved in Cs^+ uptake (Rubio et al., 2000, Qi et al., 2008).

214 When cultured in low K^+ condition (10 μM K^+), the plasma membrane potential of *A. thaliana* roots
215 cells (in the resting state) can become as negative as -215mV (Hirsch et al., 1998). In these
216 conditions, K^+ uptake is mainly mediated by high-affinity carriers that move K^+ against the
217 electrochemical gradient. In maize, it has been shown that these carriers display low selectivity
218 between K^+ and Cs^+ (Sacchi et al., 1997). Activity of low selective carriers, by contrast with highly
219 selective transport system, should display strong sensitivity to K^+/Cs^+ competition in the external
220 medium. This could explain why increase in K^+/Cs^+ competition with increasing $[K]_{ext}$ in the low
221 concentration range (between 10 μM and 100 μM K^+ -conditions in this study) results in significant
222 decrease of Cs^+ transfer factor (Figure 1).

223 Efficiency of Cs^+ uptake also depends on the $[Cs]_{ext}$. Thus, $TF_{ext \rightarrow plant}$ decreases with increasing Cs^+ in
224 the external solution (Figure 1). Linear regression indicates that this effect is significant ($p < 0.01$) for
225 the 10 μM and 100 μM K^+ condition with adjusted- R^2 between 0.91 and 0.83 respectively. This could
226 be due to the behavior of carriers which became saturated because conformational changes are
227 needed for each transport event. As a consequence of transporters saturation, efficiency of Cs^+
228 uptake (and thus $TF_{ext \rightarrow plant}$) could be reduced with the $[Cs]_{ext}$ increasing. Membrane depolarization
229 due to the cation Cs^+ accumulation in root cells and due to the fact that Cs^+ blocks some of the K^+
230 channels should also be addressed as a hypothesis to explain this observation. Indeed, lower
231 membrane potential reduces the driving force for positively charged element and subsequently
232 reduces Cs^+ uptake as described previously for an *Arabidopsis* mutant disrupted in a plasma
233 membrane proton pump (Haruta & Sussman, 2012).

234 When $[K]_{ext}$ increases ($>100 \mu\text{M}$), the electrochemical gradient is reduced (Hirsch et al., 1998) and the
235 membrane potential follows the Nernst potential of K^+ (Hedrich, 2012). In these conditions, K^+ uptake
236 is mainly mediated by channels. For higher K^+ levels in this study ($100 \mu\text{M}$ and $3000 \mu\text{M}$), we
237 observed only a slight decrease of $TF_{ext \rightarrow plant}$ value with the increase of $[Cs]_{ext}$ which is consistent with
238 a channel-type transport system: when the pore is open, no more conformational changes are needed
239 to transport ions. Therefore, saturation pattern are not observed conversely to carrier-mediated
240 pathway.

241 Eadie-Hofstee plots (**Eq.(2)**) derived from data of the 15 min influx experiments are presented in
242 Figure 2. The non-linear pattern of these plots let us to distinguish at least two systems for Cs^+ uptake
243 depending on the $[Cs]_{ext}$. In the range of $[Cs]_{ext}$ comprised between 0.1 and $200 \mu\text{M}$, kinetics
244 parameters (Table 3) were estimated by linear regression on the Eadie-Hofstee plots for the three K^+ -
245 conditions. In K^+ -starved plants ($10 \mu\text{M}$ K^+ -supply), we observed a drastic reduction of K_m that signs a
246 higher affinity of the roots transport system for Cs^+ . The slight increase of V_{max} , which represent
247 maximal rate of Cs^+ influx when the uptake system is saturated, can be interpreted as a higher number
248 of transporters with ability to transport Cs^+ in the low- K^+ condition. The well-known positive regulation
249 of high-affinity transporters encoding gene expression, in particular *AtHAK5*, by low K^+ supply likely
250 contributes to this effect of K^+ on both K_m and V_{max} . Additionally, early study in excised roots of winter
251 wheat (Shaw & Bell, 1989) described a dual uptake mechanism for Cs^+ uptake in the global high-
252 affinity range ($[Cs]_{ext}$ below $200 \mu\text{M}$). The high-affinity HAK5 carrier contributing in this dual uptake
253 system specifically for low K^+ condition, this could explain the lower R^2 obtained for K^+ -starved plants
254 in our experiments (Table 3).

255

256 **3.3. Distribution of cesium in roots and shoots**

257 *3.3.1. Short-term cesium uptake experiment: evolution of cesium distribution with time and with* 258 *$[Cs]_{ext}$*

259 Distribution of Cs^+ between roots and shoots (related to fresh weight) after 15 min and 6 h exposure
260 for the three K^+ -conditions and a range of $[Cs]_{ext}$ are shown in Figure 3. Cs^+ root:shoot concentration
261 ratio decreased rapidly over time. Thus ratios after 6 h exposure are in average 5 to 10 times lower
262 than after 15 min exposure, depending on the K^+ -treatment. We also found that, on average, 30 % of
263 the total quantity of Cs^+ was found in shoots after only 15 min of exposure in the highest K^+ -conditions

264 (data not shown) suggesting that Cs^+ is highly mobile in these conditions. In contrast, Cs^+ root:shoot
265 concentration ratio is up to ten times higher for K^+ -starved plant compared to the highest K^+ -conditions
266 and between 3 and 20 % of Cs^+ were found in shoots in this condition depending on $[\text{Cs}]_{\text{ext}}$.
267 Interestingly, $[\text{Cs}]_{\text{ext}}$ seems to affect Cs^+ root:shoot concentration ratio only for the 10 μM K^+ -condition.
268 In this condition, translocation of Cs^+ from the root to the shoot is higher when $[\text{Cs}]_{\text{ext}}$ increases.
269 These data suggest that Cs^+ translocation is mediated by very efficient systems which could be
270 inhibited by decrease of K^+ and improved when Cs^+ concentrations increase in low K^+ condition.

271

272 3.3.2. Long-term cesium accumulation experiment

273 Cs^+ accumulation in roots and shoot tissues after 7 days exposure to 1 μM Cs^+ is given in Table 4 for
274 the three K^+ -treatments. The Cs^+ root:shoot concentration ratio is 8.9 (SD=0.8), 1.6 (SD=0.4) and 0.9
275 (SD=0.2) in the 10 μM , 100 μM and 3000 μM K^+ -condition respectively. For the highest K^+ -conditions,
276 these values are comparable with the root:shoot concentration ratio obtained after 6 h exposure to 1
277 μM Cs suggesting that equilibrium between roots and shoot Cs^+ content occurs quickly during the first
278 hours. This equilibrium between Cs^+ concentrations in roots and shoots likely involves both
279 translocation and recirculation mechanisms. Indeed, previous study on Cs^+ circulation in spinach
280 indicates that about 85 % of Cs^+ accumulated in shoots is recirculating to the roots under certain
281 conditions (Buysse et al., 1995).

282 As in short-term influx experiments, fresh weight-based Cs^+ concentration remains globally higher in
283 roots than in shoots of Col-0. This result is consistent with previous studies on different plant species
284 (see the review by Zhu & Smolders (2000)) but reasons for higher Cs^+ accumulation in roots than in
285 shoots remain unclear. As far as we know, limitation of Cs^+ storage in the vacuole which could
286 enhance recirculation from the shoots to the roots has not been proven yet. Another explanation is
287 that Cs^+ adsorbed on the root surface could be a factor of discrepancies between roots and shoot Cs^+
288 concentration. Roots are exposed to external Cs^+ whereas Cs^+ in shoots comes from roots only.
289 Furthermore, some authors suggest that larger amount of Cs^+ distributes in cell wall (and free space)
290 at low external K^+ concentration due to lower competition for adsorption site on the root surface (Zhu
291 et al., 1999). This could explain why differences between Cs^+ roots and shoot concentrations are
292 reduced when external K^+ concentration increases.

293 K^+ and Cs^+ do not display the same distribution pattern: shoots contain around 75% of the total
294 amount of K^+ whereas shoots contain between 25 and 80% of the total amount of Cs^+ depending on
295 the K^+ -treatment (Figure 4). When plants are K^+ -starved, Cs^+ distribution highly differs from K^+
296 distribution.

297 In accordance with others studies on different plant species (Buysse et al., 1996), we observed that
298 the Cs^+ root:shoot concentration ratio in *A. thaliana* is higher when plants are K^+ -starved. By analogy
299 with adjustment of K^+ distribution under low- K^+ supply condition in *A. thaliana*, the decrease of the part
300 of total Cs^+ allocated to the shoots could be the result of two distinct mechanisms:

301 (i) Limitation of Cs^+ loading in xylem for translocation from roots to the shoots. The K^+ -outward
302 rectifying channel SKOR, expressed in root stellar tissues (pericycle and xylem parenchyma)
303 and mediating K^+ loading into the xylem sap (Gaymard et al., 1998), is down-regulated by low
304 external K^+ concentration (Pilot et al., 2003). Subsequently, loading of K^+ in the xylem is
305 reduced in low- K^+ condition. Expressed in *Xenopus* oocytes, AtSKOR displays a permeability
306 to Cs^+ (Gaymard et al., 1998) but its role in Cs^+ transport remains unknown Taking these
307 previous findings with distribution pattern of Cs^+ described here, it is tempting to speculate that
308 inhibition of SKOR at low- K^+ could also limit Cs^+ transport from roots to shoots. It is worth
309 pointing out that modifications of internal K^+ fluxes with external K^+ concentrations are not
310 significant in our experiments maybe due to the high K^+ content of the plant before treatment
311 with low- K^+ supply.

312 (ii) Higher redistribution of Cs^+ from the shoots to the roots via the phloem sap.

313 The decrease of Cs^+ allocated to the shoots in low- K^+ condition could also be linked to the global
314 increase of Cs^+ content in plant. Indeed, preferential distribution in roots when plants accumulate high
315 amount of Cs^+ might reflect the limit of Cs^+ storage and/or translocation in shoots together with the
316 part of Cs^+ adsorbed in roots mentioned above which is not available for translocation and which is
317 taking account in roots Cs^+ concentration calculation.

318

319 **3.4. Discrimination between potassium and cesium**

320 Discrimination factor (DF , Eq.(3)) values calculated for plants from the long-term exposure
321 experiments are less than unity whatever the external K^+ level (Table 4), which means that in any case
322 K^+ is more efficiently absorbed than Cs^+ , as shown in most previous studies (Zhu & Smolders, 2000

323 and references therein). $DF(Cs)$ is significantly lower for the 10 μM K^+ -condition indicating a stronger
324 discrimination against Cs^+ for K^+ -starved plant. Interestingly, if only one system involved in K/Cs
325 uptake operated for all K^+ concentrations with a constant selectivity, we would have a reduction of
326 discrimination against Cs^+ at low K^+ level due to the lowest competition between the two in the
327 external medium. Decrease of discrimination against Cs^+ for lower K^+ level was observed for spring
328 wheat (Smolders et al., 1996) but previous study on *A. thaliana* indicates the same result that we
329 obtained here. Thereby, Kanter et al. (2010) recorded higher discrimination against Cs^+ for the lowest
330 K^+ concentration.

331 A part of the effects of K^+ -supply on plant $DF(Cs)$ can be imputed on the plural Cs^+ uptake system
332 involving transporters with different selectivity and with activity regulated by K^+ concentrations and
333 membrane potential. For example, K^+ deprivation affects positively expression of *AtHAK5* gene which
334 is a major contributor to K^+ uptake under very low and low- K^+ conditions and a major pathway for Cs^+
335 uptake in certain conditions.

336 It has been proposed that, conversely to channels functioning at high level of K^+ , high-affinity carriers
337 should display low discrimination against Cs^+ (Zhu, 2001). It is worth pointing out that, in our
338 experiments, the $DF(Cs)$ value is lower for K^+ -starved plants indicating a higher discrimination against
339 Cs^+ at low level of K^+ . From a molecular point of view, this result remains unclear. Otherwise, we think
340 that the calculation of $DF(Cs)$ could be affected by bias. K^+ -content of plants tested results from 25
341 days of culture in sufficient K^+ supply prior to K^+ -treatments. This pre-culture in sufficient- K^+ conditions
342 could lead to a K^+ -content higher than expected for plants supplied with 10 μM K^+ and therefore a ratio
343 $([Cs]/[K])_{plant}$ "underestimated". Potential differences in Cs^+/K^+ ratio at the root surface compared to
344 bulk solution as a resultant of uptake kinetics of both species should also be addressed as factor of
345 discrepancy as stated in Smolders et al. (1996).

346

347 **3.5. Comparison between *athak5-3* mutant line and *Col-0* wildtype**

348 We used a T-DNA insertion line in *AtHAK5* to study the effect of $[Cs]_{ext}$ and $[K]_{ext}$ on HAK5-mediated
349 Cs^+ influx. PCR amplification as described in the Material and Method section confirmed the
350 homozygosity of the T-DNA insertion in the mutant line used.

351 No significant differences on Cs^+ influx between *athak5-3* mutant line and *Col-0* wildtype were
352 detected for K^+ -replete plants (3000 μM K^+ -condition, Figure 5-B). This result can be explained by the

353 expression pattern of *HAK5* which is up-regulated by K⁺-starvation and down-regulated after K⁺-
354 resupply (Armengaud et al., 2004).

355 As expected for K⁺-starved plant (10 μM K⁺-condition, Figure 5-A), Cs⁺ uptake is lower in the *athak5-3*
356 mutant due to the lack of HAK5 transporter. However, significant differences between *athak5-3* mutant
357 and Col-0 disappear for high concentration of Cs⁺. According to the data, between 76% and 69% of
358 the total Cs⁺ uptake is mediated by HAK5 pathway for 1 μM and 10 μM $[Cs]_{ext}$ while contribution of
359 HAK5 to Cs⁺ uptake is negligible for high external Cs⁺ concentration (above 200 μM). In short-term
360 uptake experiments on 14 days-old seedlings, Qi et al. (2008) measure only a 20% decrease of Cs⁺
361 influx in *hak5* mutants compare to wildtype. Based on result obtained here, this may be partly due to
362 the high $[Cs]_{ext}$ (50 μM) they applied during the influx assay (with 500 μM of K⁺ supply). In long-term
363 exposure experiments (7 days) under K⁺-sufficient conditions, it has been shown that Cs⁺ induces
364 *AtHAK5* expression maybe due to K⁺-deficiency caused by Cs⁺ (Adams et al., 2013) and, up to now,
365 Cs⁺ has not been shown to inhibit HAK5 transporter. Therefore, specific blockade of HAK5-mediated
366 pathway for Cs⁺ uptake when $[Cs]_{ext}$ is high remains unclear. Conversely, we think that other systems
367 involved in Cs⁺ uptake, which can be efficient under low-K⁺ and high-Cs⁺ conditions, may exist. These
368 systems operating at high $[Cs]_{ext}$ could mediate Cs⁺ uptake in *athak5-3* mutant and explain why we do
369 not observe differences between plants lacking HAK5 and wildtype under this condition.

370 Several studies indicate that Non-Selective Cation Channels (NSCC) (also named Voltage-
371 Independent Cation Channels, VICC, and encoding by *CNGC* and *GLR* genes) are very promising
372 candidates for channel-mediated Cs⁺ transport pathway (White & Broadley, 2000 and references
373 therein; Hampton et al., 2005). According to the model described by White and Davenport (2002) for
374 permeation of monovalent cations through the VIC channels, at most two cations can bind
375 simultaneously and interact within the pore. Applied to Cs⁺ and K⁺, these interactions can result in the
376 inhibition of Cs⁺ influx by K⁺ and therefore, this model predicts the greatest Cs⁺ influx through VIC
377 channels when $[K]_{ext}$ is low and $[Cs]_{ext}$ is high (White & Broadley, 2000). This pattern is consistent with
378 the system described above to explain the *athak5-3* mutant and wildtype convergence with increasing
379 external Cs⁺ concentration.

380 In *Arabidopsis*, *HAK5* gene belongs to the *HAK/KUP/KT* family with 12 other genes (Mäser et al.,
381 2001). The knowledge about their role in Cs⁺ and K⁺ transport is still fragmentary but there are some
382 very promising candidates for Cs⁺ pathway. Besides *AtHK5*, *AtKUP9* expressed in *E. coli* mediates

383 Cs⁺ transport (Kobayashi et al., 2010). Further investigations are thus needed to understand the role
384 of each HAK/KUP/KT transporter but up to now this family appears as the dominant Cs⁺ transport
385 pathway for *A. thaliana* under low-K⁺ supply.

386

387 **4. Conclusions**

388 As for K⁺ uptake, Cs⁺ transport is mediated by both carriers and channels. Under low K⁺ condition (10
389 μM), we measured an increase of the affinity (calculated using Michaelis-Menten equation) of
390 *Arabidopsis thaliana* roots for Cs⁺. Additionally we observed a decrease of the transfer factor
391 calculated for Cs⁺ with Cs⁺ increasing in the external medium. This result about the effect of Cs⁺
392 concentration on Cs⁺ uptake efficiency suggests that different mechanisms operate depending on Cs⁺
393 concentration in the medium or/and mechanisms operating at low-Cs⁺ external concentration are
394 saturated at high Cs⁺ concentration.

395 Taking together, results on affinity and transfer factor are consistent with the known Cs⁺ uptake
396 mediated by the high-affinity K⁺ carrier AtHAK5 under K⁺-starvation. Testing a mutant lacking this
397 carrier, we found that discrepancies between wildtype and *athak5-3* disappear when Cs⁺ in the
398 external medium is high (above 100 μM). Based on predicted functioning of this type of channels, we
399 suggest that non-selective cation channels could mediate Cs⁺ influx in low K⁺ condition and high
400 external Cs⁺ concentrations.

401 Affinity of *A. thaliana* roots for Cs⁺ did not change substantially between intermediate and high level of
402 K⁺ (100 and 3000 μM). Varying the Cs⁺ concentration in the external medium, we found that Cs⁺ has
403 no significant effect on transfer factor calculated for Cs⁺ when K⁺ is high. These results are consistent
404 with the supposed channel-mediated pathway for Cs⁺ uptake under K⁺-sufficient conditions.

405 Studying the effect of K⁺-supply on Cs⁺ distribution between roots and shoots, we found that the part
406 of Cs⁺ allocated to the shoots was lower under K⁺-deprivation. As the total Cs⁺ accumulated into the
407 plant is higher in this condition, a part of the impairment of Cs⁺ translocation could be explained by a
408 limitation of Cs⁺ storage into shoots. Additionally, we suggest that inhibition of SKOR-mediated K⁺
409 translocation at low-K⁺ supply could also limit Cs⁺ translocation. However, further experiments on *skor*
410 *Arabidopsis* mutant for example are needed to better understand the role of K⁺ channels in Cs⁺
411 translocation.

412 Finally, recent identification of transporters involved in Cs⁺ uptake and functional studies on K⁺
413 transporters let us to discuss kinetics data in a molecular and mechanistic way. The understanding of
414 mechanisms leading to Cs⁺ uptake by plants is essential for modelling approaches to predict the
415 success of phytoremediation strategies for example. However, beside the plant part, integration of the
416 soil part to predict K⁺ and Cs⁺ concentration around the roots is also needed for a higher accuracy of
417 these modelling approaches.

418

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423

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506
507

508 **Table 1: Experimental scheme.**

Step	Composition of the medium	Duration
1- Growing on agar plates	MS½ , 1% agar, 1% sucrose	7 days
2- Growing on sand	Watered with nutrient solution	14 days
3- Growing in hydroponics	Nutrient solution	3-5 days
4- K ⁺ -treatment in hydroponics	K ⁺ -treatment solution	5 days
5a- Exposure to Cs ⁺ (short-term experiments)	K ⁺ -treatment solution + ¹³³ Cs (0.1-3000 µM) + ¹³⁷ Cs	15 min to 6 h
5b- Exposure to Cs ⁺ (long-term experiments)	K ⁺ -treatment solution + ¹³³ Cs (1 µM)	7 days

509

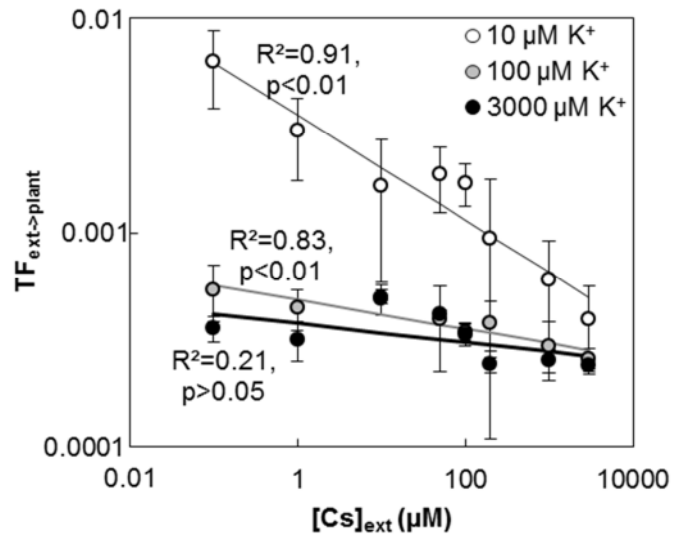
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511 **Table 2: Fresh-weight (FW) and K⁺ content of roots and shoots of Col-0 depending on the K⁺-**
 512 **treatment.** Plants were grown 7 days on MS½, 14 days on sand then 8 days in hydroponic system.
 513 K⁺-treatments were applied during the last 5 days of the hydroponic step. Values are means of at least
 514 ten plants with standard deviation in brackets. To evaluate the effect of K⁺-treatment, ANOVA analysis
 515 was performed on fresh-weight then on K⁺ content separately and results are indicated in the last line
 516 (NS, Non-Significant and **, *** Significant at the $\alpha = 0.01$ and 0.001 level respectively). Different
 517 letters in bold indicate significant differences between means (Tuckey post-hoc test, p-value < 0.05).
 518

K ⁺ -treatment (μM)	Shoots (g)	Roots (g)	K shoots (μmol.g ⁻¹ FW)	K roots (μmol.g ⁻¹ FW)
10	0.331 (0.166) NS	0.107 (0.060) NS	50.64 (9.55) a	60.48 (15.15) a
100	0.280 (0.176) NS	0.129 (0.094) NS	65.70 (18.58) b	73.13 (29.69) a
3000	0.363 (0.176) NS	0.135 (0.056) NS	69.18 (17.89) b	109.03 (10.56) b
K ⁺ -treatment effect	NS	NS	**	***

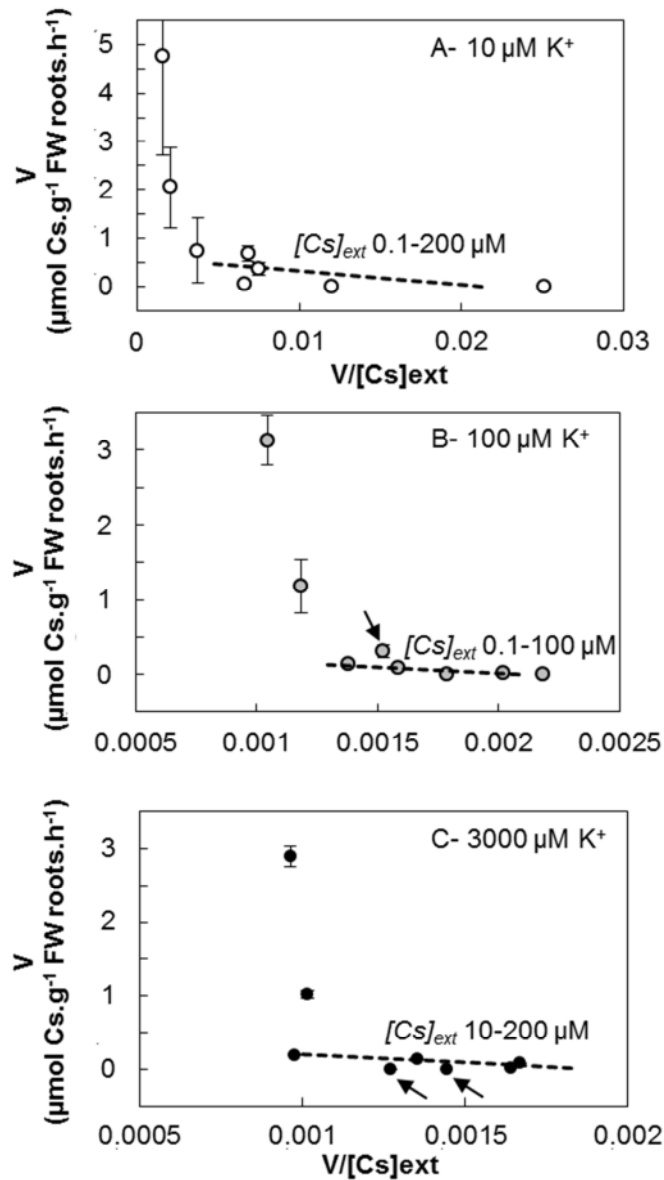
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Figure 1: Log-log plot of transfer factor ($TF_{ext \rightarrow plant}$) of Cs^+ for Col-0 grown with three different K^+ -treatments (10, 100 or 3000 μM) and exposed during 15 min to a range of Cs^+ concentrations. Transfer factor was calculated as described in Eq.(1). Values are means of at least three different plants and error bars indicate standard deviation.



527

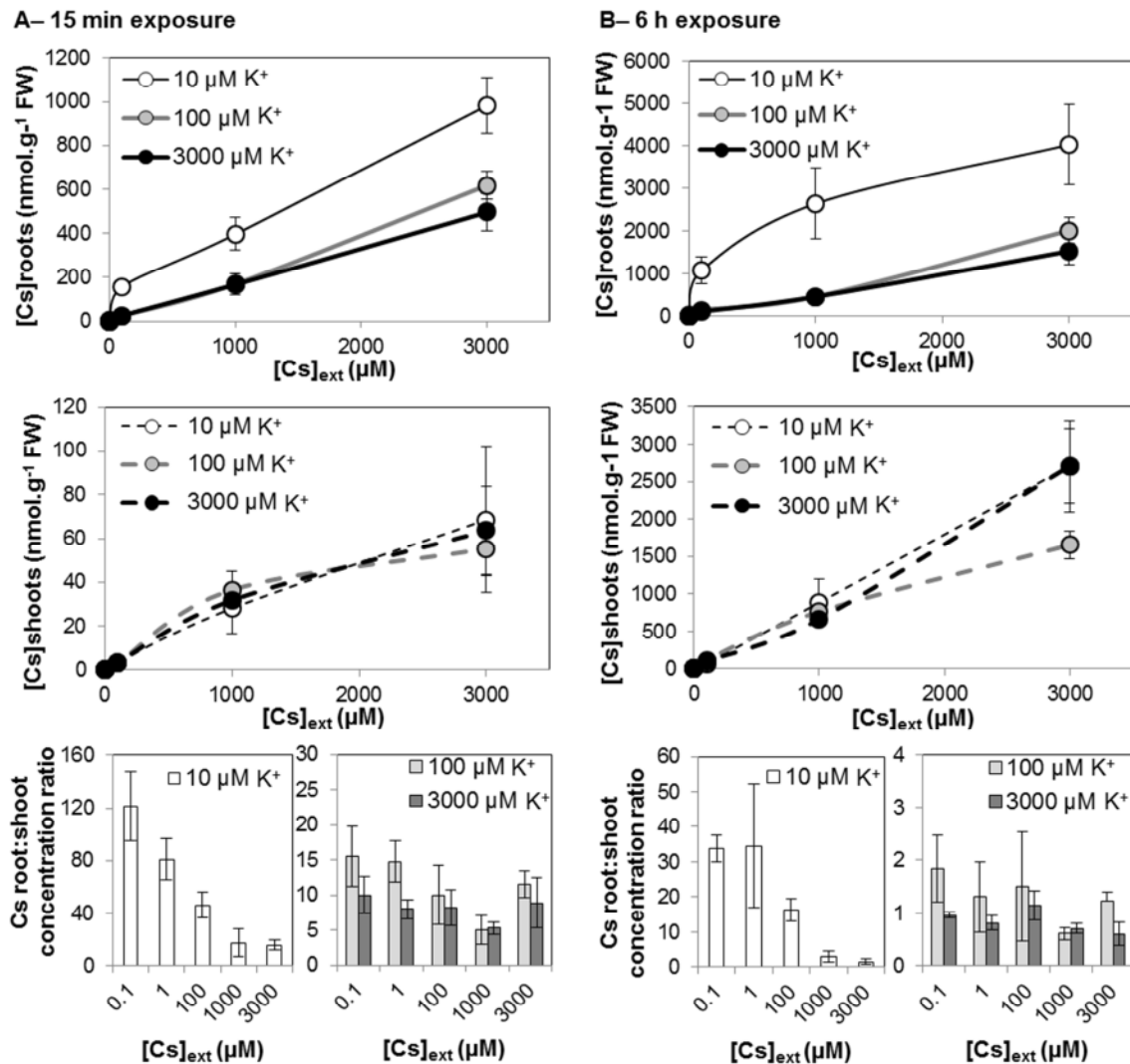
528 **Figure 2: Eadie-Hofstee plot for Cs⁺ uptake data from 15 min influx assays.** Plants were supplied
 529 with three distinct K⁺-treatments (A-10 μM, B-100 μM, C-3000 μM). Concentrations of Cs⁺ in the
 530 exposure solution range from 0.1 to 3000 μM. Values are means of at least three different plants and
 531 error bars indicate standard deviation. Broken lines represent the Michaelis-Menten function with
 532 kinetics parameters calculated in Table 3. Linear regression is calculated over the range of external
 533 Cs⁺ concentrations ($[Cs]_{ext}$) indicated on each figure. Arrows indicate extreme values which had to be
 534 removed from the calculation because they lead to absurd results.

535

536 **Table 3: Estimation of Cs⁺ influx kinetic parameters using Eadie-Hofstee plot for data from 15**
 537 **min influx assays.** Uncertainties on parameters estimation by linear regression are indicated in
 538 brackets. In order to avoid absurd results for kinetics parameters, some extreme external Cs⁺
 539 concentrations ($[Cs]_{ext}$ in the table) had to be removed of the linear regression. The R² values are
 540 associated with the linear regression represented in Figure 2 (broken lines).
 541

K ⁺ -treatment (μM)	$[Cs]_{ext}$ (μM)	K_m (μM)	V_{max} (μmol.g ⁻¹ FW roots.h ⁻¹)	R ²
10	0.1-200	28.68 (16.85)	0.61 (0.21)	0.42
100	0.1-100	163.48 (49.68)	0.34 (0.09)	0.78
3000	10-200	214.94 (70.03)	0.41 (0.10)	0.99

542



543

544 **Figure 3: Evolution of the distribution of Cs^+ between roots and shoots across a range of**

545 **$[Cs]_{\text{ext}}$ for plants from the short-term influx assays. Three distinct K^+ -treatments (10 μM, 100 μM,**

546 **3000 μM K^+) were applied during 5 days before exposure to Cs^+ . Levels of K^+ in the pre-culture were**

547 **maintained during the exposure to Cs^+ . Plants were exposed during A- 15min or during B- 6 h. Means**

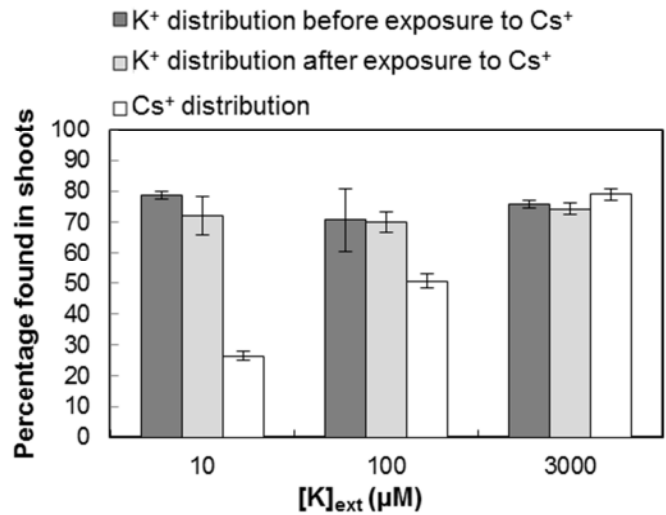
548 **of at least three different plants are represented with standard deviation.**

549

550 **Table 4: Cs⁺ accumulation in Col-0 exposed during 7 days to nutrient solution containing 1 μM**
 551 **Cs⁺ and three distinct K⁺ concentrations.** *DF(Cs)* is the discrimination factor calculated for Cs⁺ with
 552 **Eq.(3).** Values are mean of at least six different plants with standard deviation in brackets. Result of
 553 the ANOVA analysis is indicated in the last line. Different letters in bold indicate significant differences
 554 between means (Tuckey post-hoc test, p-value < 0.05).
 555

K ⁺ -treatment (μM)	Cs ⁺ shoot (nmol.g ⁻¹ FW)	Cs ⁺ roots (nmol.g ⁻¹ FW)	Cs ⁺ whole plant (nmol.g ⁻¹ FW)	Plant <i>DF(Cs)</i>
10	0.34 (0.04) a	3.08 (0.60) a	0.98 (0.10) a	0.37 (0.06) a
100	0.17 (0.02) b	0.28 (0.08) b	0.21 (0.03) b	0.54 (0.08) b
3000	0.01 (0.0007) c	0.01 (0.002) c	0.01 (0.0006) c	0.54 (0.03) b
K ⁺ -treatment effect	***	***	***	***

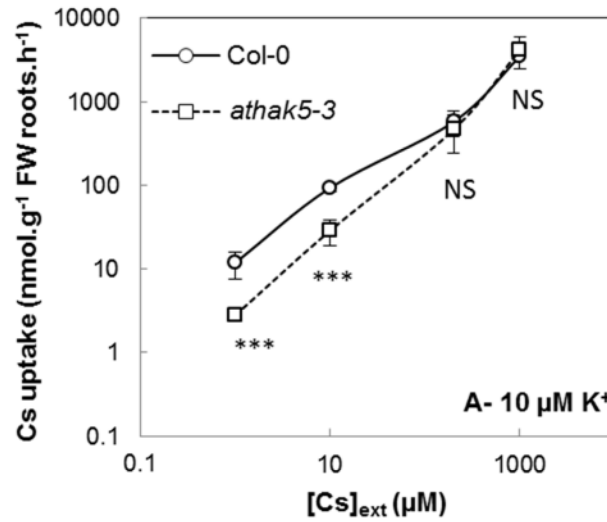
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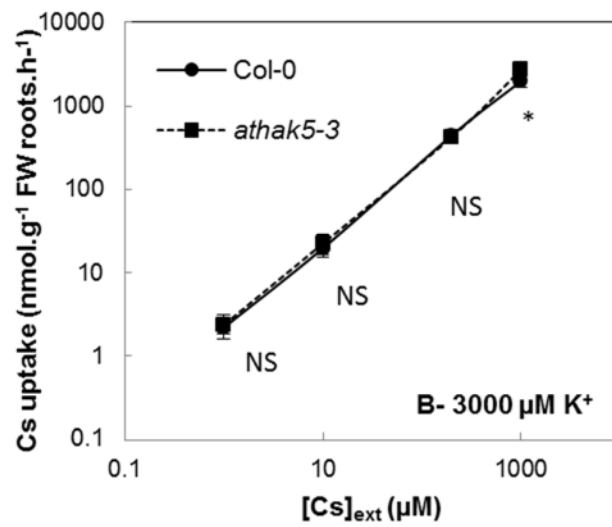
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558 **Figure 4: Comparison between K⁺ and Cs⁺ distribution in plants exposed during 7 days to a**
 559 **nutrient solution containing 1 μM Cs⁺ and three distinct K⁺ concentrations.** Percentage was
 560 calculated by dividing the quantity of element found in shoots by the quantity found in the whole plant.
 561 K⁺ distribution before exposure to Cs⁺ was measured on non-exposed plants from the same bulk than
 562 plants used for the Cs⁺ exposure assay. Means of at least four different plants are represented with
 563 standard deviation.

564



565



566

567 **Figure 5: Cs⁺ uptake measured over 15 min for the wild-type Col-0 and the mutant line *athak5-3*.**

568 Two distinct levels of K⁺ were used during pre-culture and during exposure to Cs⁺: A-10 μM and B-

569 3000 μM. Means of at least five different plants are represented with standard deviation. Student t-

570 tests were performed to compare Cs⁺ uptake between *hak5-3* and Col-0 for each [Cs]_{ext} (NS, Non-

571 significant and *, *** P < 0.05 and P < 0.001 respectively).

572