



**HAL**  
open science

## **Uptake and translocation of cesium by *Arabidopsis thaliana* in hydroponics conditions: Links between kinetics and molecular mechanisms**

Laure Genies, Daniel Orjollet, Loïc Carasco, Virginie Camilleri, Sandrine Frelon, Alain Vavasseur, Nathalie Leonhardt, Pascale Henner

### ► **To cite this version:**

Laure Genies, Daniel Orjollet, Loïc Carasco, Virginie Camilleri, Sandrine Frelon, et al.. Uptake and translocation of cesium by *Arabidopsis thaliana* in hydroponics conditions: Links between kinetics and molecular mechanisms. *Environmental and Experimental Botany*, 2017, 138, pp.164 - 172. 10.1016/j.envexpbot.2017.03.013 . hal-01682673

**HAL Id: hal-01682673**

**<https://amu.hal.science/hal-01682673>**

Submitted on 12 Jan 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3 Laure GENIES<sup>a, b</sup>, Daniel ORJOLLET<sup>a</sup>, Loïc CARASCO<sup>a</sup>, Virginie CAMILLERI<sup>c</sup>, Sandrine FRELON<sup>c</sup>,  
4 Alain VAVASSEUR<sup>b</sup>, Nathalie LEONHARDT<sup>b</sup>, Pascale HENNER<sup>a</sup>

5 <sup>a</sup> *Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PRP-ENV, SERIS, Laboratoire de*  
6 *Biogéochimie, Biodisponibilité et Transferts des radionucléides (L2BT), Cadarache, France*

7 <sup>b</sup> *Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), IBEB-SBVME, Laboratoire*  
8 *de Biologie du Développement des Plantes (LBDP), Cadarache, France*

9 <sup>c</sup> *Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PRP-ENV, SERIS, Laboratoire*  
10 *d'ECOTOxicologie des radionucléides (LECO), Cadarache, France*

11 Corresponding author: [laure.genies@gmail.com](mailto:laure.genies@gmail.com)

12

13 **ABSTRACT**

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

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

30

31           **1. Introduction**

32   Cesium (Cs<sup>+</sup>) has no known physiological role in plant but, because of its chemical similarity with the  
33   essential macronutrient potassium (K<sup>+</sup>), the monovalent cation Cs<sup>+</sup> can be taken up from the soil  
34   solution by plant roots through the K<sup>+</sup> uptake pathway (White & Broadley, 2000). Some biological  
35   processes involving K<sup>+</sup> can be altered by Cs<sup>+</sup> but, at the natural concentrations occurring in soil  
36   solutions, stable isotope <sup>133</sup>Cs rarely causes environmental toxicity (Hampton et al., 2004). However,  
37   radiocesium (<sup>134</sup>Cs and <sup>137</sup>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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> uptake and accumulation, found among the plant K<sup>+</sup> transporters system.

54   Since the level of K<sup>+</sup> (both in the external and intracellular medium) modifies the relative contribution of  
55   each transporters in K<sup>+</sup> fluxes (see Alemán et al., 2011 and references therein), Cs<sup>+</sup> uptake pathway  
56   also depends on the K<sup>+</sup>-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<sup>+</sup> uptake in *Arabidopsis* roots under K<sup>+</sup>-sufficient conditions (White & Broadley, 2000;  
60   Hampton et al., 2005). Under K<sup>+</sup>-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<sup>+</sup>  
62 uptake in *A. thaliana* (Qi et al., 2008).

63 The competitive effect of K<sup>+</sup> on kinetics of Cs<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> uptake is reported only in few studies.

71 In this study, we report the effect of K<sup>+</sup>-supply on Cs<sup>+</sup> uptake, distribution and accumulation by the  
72 model plant *A. thaliana* in hydroponics condition. Links between transporters involved in K<sup>+</sup> and Cs<sup>+</sup>  
73 uptake related-knowledge and changes observed in Cs<sup>+</sup> transport under K<sup>+</sup>-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 MgSO<sub>4</sub>, 805 μM Ca(NO<sub>3</sub>)<sub>2</sub>, 2 mM KNO<sub>3</sub>, 60 μM  
94 K<sub>2</sub>HPO<sub>4</sub>, 695 μM KH<sub>2</sub>PO<sub>4</sub> and micronutrients (3.6 μM MnSO<sub>4</sub>, 74 nM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 3 μM ZnSO<sub>4</sub>, 9.25  
95 μM H<sub>3</sub>BO<sub>3</sub>, 785 nM CuSO<sub>4</sub>, 20 μM Na<sub>2</sub>EDTA and 20 μM FeSO<sub>4</sub>).

96

## 97 **2.2. Potassium treatments**

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

101 K<sup>+</sup> 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<sup>+</sup>-treatment solution (pH 5.8) containing 0.75 mM MgSO<sub>4</sub>, 2 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0,5 mM  
104 H<sub>3</sub>PO<sub>4</sub>, and micronutrients. Three different treatments were tested through adding different amounts of  
105 KCl in the K<sup>+</sup>-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<sup>+</sup>-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<sup>+</sup> 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<sup>+</sup>-  
114 treatment solution plus a range of <sup>133</sup>Cs from 0.1 μM to 3000 μM traced by <sup>137</sup>Cs (approximately 140  
115 Bq.mL<sup>-1</sup> representing 3.3.10<sup>-4</sup> μM Cs<sup>+</sup>). 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<sup>+</sup> distribution between  
117 roots and shoots. Activities of <sup>137</sup>Cs 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<sup>+</sup>, plants were transferred in 8 mL of a fresh solution corresponding to the  
120 exposure solution without Cs<sup>+</sup> (*i.e.* the K<sup>+</sup>-treatment solution) for 1 min to remove Cs<sup>+</sup> 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<sup>+</sup> in the exposure solution, K<sup>+</sup>-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<sup>+</sup>-treatment solutions described in section 2.2 (10, 100 or 3000 μM K) plus 1 μM stable Cs<sup>+</sup>  
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<sup>+</sup> concentration in the medium due to uptake  
132 by plants.

133 After 7 days exposure to Cs<sup>+</sup>, roots and shoots were harvested as described in section 2.3 for the  
134 short-term influx experiments. For each K<sup>+</sup>-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 HNO<sub>3</sub> 65% and 1.5 mL H<sub>2</sub>O<sub>2</sub> 30% at 100-  
139 150°C on a sand bath. Mineralisates were evaporated to dryness and redissolved in HNO<sub>3</sub> 2% v/v  
140 before measuring the different elements.

141 Activity of <sup>137</sup>Cs 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 <sup>137</sup>Cs accumulated in plants were counted during 30  
145 min. In parallel, concentration of <sup>137</sup>Cs and <sup>133</sup>Cs into the exposure solution were measured by β liquid  
146 scintillation counting and ICP-MS respectively. The amount of Cs<sup>+</sup> accumulated in plant sample was  
147 deduced from the content of <sup>137</sup>Cs in plant and the ratio <sup>137</sup>Cs/<sup>133</sup>Cs into the exposure solution.

148 <sup>133</sup>Cs 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<sup>+</sup> content in plants after the K<sup>+</sup>-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<sup>+</sup> uptake by plant roots and the Cs<sup>+</sup>  
156 concentration in the solution (named  $[Cs]_{ext}$  in the following). Cs<sup>+</sup> 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<sup>+</sup> by the plant roots depending on the external Cs<sup>+</sup> concentration.  
160 Thus  $TF_{ext \rightarrow plant}$  estimates the efficiency of Cs<sup>+</sup> 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<sup>+</sup> influx from the 15 min experiment data. Linear regression on the range of  $[Cs]_{ext}$   
164 comprised between 0.1 and 200  $\mu\text{M}$  was performed on this plot, with  $V = \text{Cs}^+$  uptake rate ( $\mu\text{mol.g}^{-1}$  FW  
165 roots.h<sup>-1</sup>) 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<sup>+</sup> and Cs<sup>+</sup>, 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<sup>+</sup>-treatment on plant K<sup>+</sup> content, fresh  
172 weight and Cs<sup>+</sup> 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<sup>+</sup>-supply on Cs<sup>+</sup> 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<sup>+</sup>-treatments on:

180 (i) Kinetics parameters  $K_m$  and  $V_{max}$  for Cs<sup>+</sup> influx,

181 (ii) Cs<sup>+</sup> uptake efficiency estimated by transfer factor,

182 (iii) Distribution of Cs<sup>+</sup> between roots and shoots.

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

185

#### 186 **3.1. Effect of K<sup>+</sup>-treatment on plants potassium content and on plant growth**

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

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

197 Plants were exposed to Cs<sup>+</sup> after acclimation period with the three different K<sup>+</sup>-supplies. The level of  
198 K<sup>+</sup> during the pre-treatment was maintained in the exposure solution containing Cs<sup>+</sup>. Thus, our  
199 experiments describe the effect of global level of K<sup>+</sup>-supply *i.e.* in the solution outside the plants during  
200 the exposure to Cs<sup>+</sup> but also inside the plants due to the K<sup>+</sup>-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  $\mu M$   $K^+$ -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  $\mu M$   $K^+$ ), 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  $\mu M$  and 100  $\mu M$   $K^+$ -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  $\mu M$  and 100  $\mu M$   $K^+$  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  $\text{Cs}^+$  is highly mobile in these conditions. In contrast,  $\text{Cs}^+$  root:shoot  
265 concentration ratio is up to ten times higher for  $\text{K}^+$ -starved plant compared to the highest  $\text{K}^+$ -conditions  
266 and between 3 and 20 % of  $\text{Cs}^+$  were found in shoots in this condition depending on  $[\text{Cs}]_{\text{ext}}$ .  
267 Interestingly,  $[\text{Cs}]_{\text{ext}}$  seems to affect  $\text{Cs}^+$  root:shoot concentration ratio only for the 10  $\mu\text{M}$   $\text{K}^+$ -condition.  
268 In this condition, translocation of  $\text{Cs}^+$  from the root to the shoot is higher when  $[\text{Cs}]_{\text{ext}}$  increases.  
269 These data suggest that  $\text{Cs}^+$  translocation is mediated by very efficient systems which could be  
270 inhibited by decrease of  $\text{K}^+$  and improved when  $\text{Cs}^+$  concentrations increase in low  $\text{K}^+$  condition.

271

### 272 3.3.2. Long-term cesium accumulation experiment

273  $\text{Cs}^+$  accumulation in roots and shoot tissues after 7 days exposure to 1  $\mu\text{M}$   $\text{Cs}^+$  is given in Table 4 for  
274 the three  $\text{K}^+$ -treatments. The  $\text{Cs}^+$  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  $\mu\text{M}$ , 100  $\mu\text{M}$  and 3000  $\mu\text{M}$   $\text{K}^+$ -condition respectively. For the highest  $\text{K}^+$ -conditions,  
276 these values are comparable with the root:shoot concentration ratio obtained after 6 h exposure to 1  
277  $\mu\text{M}$   $\text{Cs}$  suggesting that equilibrium between roots and shoot  $\text{Cs}^+$  content occurs quickly during the first  
278 hours. This equilibrium between  $\text{Cs}^+$  concentrations in roots and shoots likely involves both  
279 translocation and recirculation mechanisms. Indeed, previous study on  $\text{Cs}^+$  circulation in spinach  
280 indicates that about 85 % of  $\text{Cs}^+$  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  $\text{Cs}^+$  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  $\text{Cs}^+$  accumulation in roots than in  
285 shoots remain unclear. As far as we know, limitation of  $\text{Cs}^+$  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  $\text{Cs}^+$  adsorbed on the root surface could be a factor of discrepancies between roots and shoot  $\text{Cs}^+$   
288 concentration. Roots are exposed to external  $\text{Cs}^+$  whereas  $\text{Cs}^+$  in shoots comes from roots only.  
289 Furthermore, some authors suggest that larger amount of  $\text{Cs}^+$  distributes in cell wall (and free space)  
290 at low external  $\text{K}^+$  concentration due to lower competition for adsorption site on the root surface (Zhu  
291 et al., 1999). This could explain why differences between  $\text{Cs}^+$  roots and shoot concentrations are  
292 reduced when external  $\text{K}^+$  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  $\mu M$   $K^+$ -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  $\mu M$   $K^+$  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  $\mu M$   $K^+$ -condition, Figure 5-B). This result can be explained by the

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

355 As expected for K<sup>+</sup>-starved plant (10 μM K<sup>+</sup>-condition, Figure 5-A), Cs<sup>+</sup> 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<sup>+</sup>. According to the data, between 76% and 69% of  
358 the total Cs<sup>+</sup> uptake is mediated by HAK5 pathway for 1 μM and 10 μM  $[Cs]_{ext}$  while contribution of  
359 HAK5 to Cs<sup>+</sup> uptake is negligible for high external Cs<sup>+</sup> 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<sup>+</sup>  
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<sup>+</sup> supply). In long-term  
363 exposure experiments (7 days) under K<sup>+</sup>-sufficient conditions, it has been shown that Cs<sup>+</sup> induces  
364 *AtHAK5* expression maybe due to K<sup>+</sup>-deficiency caused by Cs<sup>+</sup> (Adams et al., 2013) and, up to now,  
365 Cs<sup>+</sup> has not been shown to inhibit HAK5 transporter. Therefore, specific blockade of HAK5-mediated  
366 pathway for Cs<sup>+</sup> uptake when  $[Cs]_{ext}$  is high remains unclear. Conversely, we think that other systems  
367 involved in Cs<sup>+</sup> uptake, which can be efficient under low-K<sup>+</sup> and high-Cs<sup>+</sup> conditions, may exist. These  
368 systems operating at high  $[Cs]_{ext}$  could mediate Cs<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> and K<sup>+</sup>, these interactions can result in the  
376 inhibition of Cs<sup>+</sup> influx by K<sup>+</sup> and therefore, this model predicts the greatest Cs<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> and K<sup>+</sup> transport is still fragmentary but there are some  
382 very promising candidates for Cs<sup>+</sup> pathway. Besides *AtHK5*, *AtKUP9* expressed in *E. coli* mediates

383 Cs<sup>+</sup> 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<sup>+</sup> transport  
385 pathway for *A. thaliana* under low-K<sup>+</sup> supply.

386

#### 387 **4. Conclusions**

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

395 Taking together, results on affinity and transfer factor are consistent with the known Cs<sup>+</sup> uptake  
396 mediated by the high-affinity K<sup>+</sup> carrier AtHAK5 under K<sup>+</sup>-starvation. Testing a mutant lacking this  
397 carrier, we found that discrepancies between wildtype and *athak5-3* disappear when Cs<sup>+</sup> 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<sup>+</sup> influx in low K<sup>+</sup> condition and high  
400 external Cs<sup>+</sup> concentrations.

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

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

412 Finally, recent identification of transporters involved in Cs<sup>+</sup> uptake and functional studies on K<sup>+</sup>  
413 transporters let us to discuss kinetics data in a molecular and mechanistic way. The understanding of  
414 mechanisms leading to Cs<sup>+</sup> 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<sup>+</sup> and Cs<sup>+</sup> concentration around the roots is also needed for a higher accuracy of  
417 these modelling approaches.

418

#### 419 **Acknowledgments**

420 This work was conducted within a PhD cofunded by IRSN and CEA and has benefited from funds of  
421 the French government, managed by the Agence Nationale de la Recherche, originating from the  
422 funding program "Investissement d'Avenir" under the reference ANR-11-RSNR-0005.

423

#### 424 **References**

- 425 Adams, E., Abdollahi, P., Shin, R., 2013. Cesium inhibits plant growth through jasmonate signaling in  
426 *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **14**, 4545-59.
- 427 Alemán, F., Nieves-Cordones, M., Martínez, V., Rubio, F., 2011. Root K<sup>+</sup> acquisition in plants: the  
428 *Arabidopsis thaliana* model. *Plant Cell Physiol.* **52**, 1603-12.
- 429 Armengaud, P., Breitling, R., Amtmann, A., 2004. The potassium-dependent transcriptome of  
430 *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signaling. *Plant Physiol.* **136**,  
431 2556-76.
- 432 Avery, S.V., 1996. Fate of caesium in the environment: Distribution between the abiotic and biotic  
433 components of aquatic and terrestrial ecosystems. *J. Environ. Radioact.* **30**, 139-71.
- 434 Broadley, M.R., Escobar-Gutiérrez, A.J., Bowen, H.C., Willey, N.J., White, P.J., 2001. Influx and  
435 accumulation of Cs<sup>+</sup> by the *akt1* mutant of *Arabidopsis thaliana* (L.) Heynh. lacking a dominant K<sup>+</sup>  
436 transport system. *J. Exp. Bot.* **52**, 839-44.
- 437 Buysse, J., Van den Brande, K., Merckx, R., 1995. The distribution of radiocesium and potassium in  
438 spinach plants grown at different shoot temperatures. *J. Plant Physiol.* **146**, 263-267.
- 439 Buysse, J., Van Den Brande, K., Merckx, R., 1996. Genotypic differences in the uptake and  
440 distribution of radiocaesium in plants. *Plant Soil* **178**, 265-71.



441 Deeken, R., Geiger, D., Fromm, J., Koroleva, O., Ache, P., Langenfeld-Heyser, R., Sauer, N., May,  
442 S.T., Hedrich, R., 2002. Loss of the AKT2/3 potassium channel affects sugar loading into the  
443 phloem of Arabidopsis. *Planta* **216**, 334-44.

444 Gaymard, F., Pilot, G., Lacombe B, Bouchez, D., Bruneau, D., Boucherez, J., Michaux-Ferrière, N.,  
445 Thibaud, J.-B., Sentenac, H., 1998. Identification and disruption of a plant shaker-like outward  
446 channel involved in K<sup>+</sup> release into the xylem sap. *Cell* **94**, 647-55.

447 Hampton, C., Broadley, M., White, P., 2005. Short review: the mechanisms of radiocaesium uptake by  
448 Arabidopsis roots. *Nukleonika* **50**, 3-8.

449 Haruta, M., & Sussman, M.R., 2012. The effect of a genetically reduced plasma membrane  
450 protonmotive force on vegetative growth of Arabidopsis. *Plant Physiol.* **158**, 1158-71.

451 Hedrich, R., 2012. Ion channels in plants. *Physiol. Rev.* **92**, 1777-811.

452 Hirsch, R.E., Lewis, B.D., Spalding, E.P., Sussman, M.R., 1998. A role for the AKT1 potassium  
453 channel in plant nutrition. *Science* **280**, 918-21.

454 Kanter, U., Hauser, A., Michalke, B., Dräxl, S., Schäffner, A.R., 2010. Caesium and strontium  
455 accumulation in shoots of Arabidopsis thaliana: genetic and physiological aspects. *J. Exp. Bot.*,  
456 erq213.

457 Kobayashi, D., Uozumi, N., Hisamatsu, S.I., Yamagami, M., 2010. AtKUP/HAK/KT9, a K<sup>+</sup> transporter  
458 from Arabidopsis thaliana, mediates Cs<sup>+</sup> uptake in Escherichia coli. *Biosci. Biotech. Biochem.* **74**,  
459 203-5.

460 Kobayashi, D., Okouchi, T., Yamagami, M., Shinano, T., 2014. Verification of radiocesium  
461 decontamination from farmlands by plants in Fukushima. *J. Plant Res.* **127**, 51-6.

462 Kochian, L.V., & Lucas, W.J., 1982. Potassium transport in corn roots I. Resolution of kinetics into a  
463 saturable and linear component. *Plant Physiol.* **70**, 1723-31.

464 Lacombe, B., Pilot, G., Michard, E., Gaymard, F., Sentenac, H., Thibaud, J.-B., 2000. A shaker-like K<sup>+</sup>  
465 channel with weak rectification is expressed in both source and sink phloem tissues of Arabidopsis.  
466 *Plant Cell* **12**, 837-51.

467 Mäser, P., Thomine, S., Schroeder, J.I., Ward, J.M., Hirschi, K., Sze, H., Talke, I. N., Amtmann, A.,  
468 Maathuis, F.J.M., Sanders, D., Harper, J.F., Tchieu, J., Gribskov, M., Persans, M.W., Salt, D.E.,  
469 Kim, S.A., Guerinot, M.L., 2001. Phylogenetic relationships within cation transporter families of  
470 Arabidopsis. *Plant Physiol.* **126**, 1646-67.

471 Murashige, T., & Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco  
472 tissue cultures. *Physiol. Plant.* **15**, 473-97.

473 Pilot, G., Gaymard, F., Mouline, K., Chérel, I., Sentenac, H., 2003. Regulated expression of  
474 *Arabidopsis* Shaker K<sup>+</sup> channel genes involved in K<sup>+</sup> uptake and distribution in the plant. *Plant*  
475 *Mol. Bio.* **51**, 773-87.

476 Qi, Z., Hampton, C.R., Shin, R., Barkla, B.J., White, P.J., Schachtman D.P., 2008. The high affinity K<sup>+</sup>  
477 transporter AtHAK5 plays a physiological role in planta at very low K<sup>+</sup> concentrations and provides  
478 a caesium uptake pathway in *Arabidopsis*. *J. Exp. Bot.* **59**, 595-607.

479 Rubio, F., Guillermo, E., Rodríguez-Navarro, A., 2000. Cloning of *Arabidopsis* and barley cDNAs  
480 encoding HAK potassium transporters in root and shoot cells. *Physiol. Plant.* **109**, 34-43.

481 Rubio, F., Nieves-Cordones, M., Alemán, F., Martínez, V., 2008. Relative contribution of AtHAK5 and  
482 AtAKT1 to K<sup>+</sup> uptake in the high-affinity range of concentrations. *Physiol. Plant.* **134**, 598-608.

483 Sacchi, G.A., Espen, L., Nocito, F., Cocucci, M., 1997. Cs<sup>+</sup> uptake in subapical maize root segments:  
484 Mechanism and effects on H<sup>+</sup> release, transmembrane electric potential and cell pH. *Plant Cell*  
485 *Physiol.* **38**, 282-9.

486 Shaw, G., & Bell, J., 1989. The kinetics of caesium absorption by roots of winter wheat and the  
487 possible consequences for the derivation of soil-to-plant transfer factors for radiocaesium. *J.*  
488 *Environ. Radioact.* **10**, 213-31.

489 Smolders, E., Kiebooms, L., Buysse, J., Merckx, R., 1996. <sup>137</sup>Cs uptake in spring wheat (*Triticum*  
490 *aestivum* L. cv. Tonic) at varying K supply. *Plant Soil* **181**, 211-20.

491 Waegeneers, N., Camps, M., Smolders, E., Merckx, R., 2001. Genotypic effects in phytoavailability of  
492 radiocaesium are pronounced at low K intensities in soil. *Plant Soil* **235**, 11-20.

493 White, P.J., & Broadley, M.R., 2000. Tansley Review No. 113. *New Phytol.* **147**, 241-56.

494 White, P.J., & Davenport, R.J., 2002. The voltage-independent cation channel in the plasma  
495 membrane of wheat roots is permeable to divalent cations and may be involved in cytosolic Ca<sup>2+</sup>  
496 homeostasis. *Plant Physiol.* **130**, 1386-95.

497 Zhu, Y.-G., Shaw, G., Nisbet, A., Wilkins, B., 1999. Effects of external potassium supply on  
498 compartmentation and flux characteristics of radiocaesium in intact spring wheat roots. *Ann. Bot.*  
499 **84**, 639-44.

500 Zhu, Y.-G., & Smolders, E., 2000. Plant uptake of radiocaesium: a review of mechanisms, regulation  
501 and application. *J. Exp. Bot* **51**, 1635-45.

502 Zhu, Y.-G., Shaw, G., Nisbet, A., Wilkins, B., 2000. Effect of potassium starvation on the uptake of  
503 radiocaesium by spring wheat (*Triticum aestivum* cv. Tonic). *Plant Soil* **220**, 27-34.

504 Zhu, Y.-G., 2001. Effect of external potassium (K) supply on the uptake of <sup>137</sup> Cs by spring wheat  
505 (*Triticum aestivum* cv. Tonic): a large-scale hydroponic study. *J. Environ. Radioact.* **55**, 303-14.

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 <sup>+</sup> -treatment in hydroponics	K <sup>+</sup> -treatment solution	5 days
5a- Exposure to Cs <sup>+</sup> (short-term experiments)	K <sup>+</sup> -treatment solution + <sup>133</sup> Cs (0.1-3000 µM) + <sup>137</sup> Cs	15 min to 6 h
5b- Exposure to Cs <sup>+</sup> (long-term experiments)	K <sup>+</sup> -treatment solution + <sup>133</sup> Cs (1 µM)	7 days

509

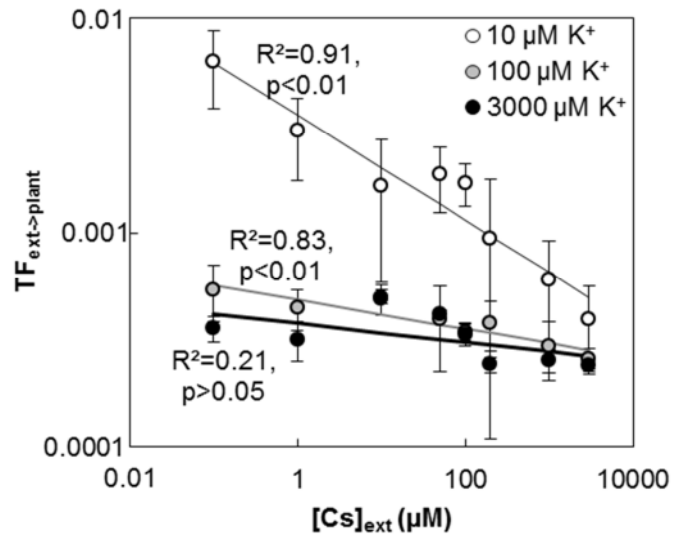
510

511 **Table 2: Fresh-weight (FW) and K<sup>+</sup> content of roots and shoots of Col-0 depending on the K<sup>+</sup>-**  
 512 **treatment.** Plants were grown 7 days on MS½, 14 days on sand then 8 days in hydroponic system.  
 513 K<sup>+</sup>-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<sup>+</sup>-treatment, ANOVA analysis  
 515 was performed on fresh-weight then on K<sup>+</sup> 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 <sup>+</sup> -treatment (μM)	Shoots (g)	Roots (g)	K shoots (μmol.g <sup>-1</sup> FW)	K roots (μmol.g <sup>-1</sup> FW)
10	0.331 (0.166) <b>NS</b>	0.107 (0.060) <b>NS</b>	50.64 (9.55) <b>a</b>	60.48 (15.15) <b>a</b>
100	0.280 (0.176) <b>NS</b>	0.129 (0.094) <b>NS</b>	65.70 (18.58) <b>b</b>	73.13 (29.69) <b>a</b>
3000	0.363 (0.176) <b>NS</b>	0.135 (0.056) <b>NS</b>	69.18 (17.89) <b>b</b>	109.03 (10.56) <b>b</b>
K <sup>+</sup> -treatment effect	<b>NS</b>	<b>NS</b>	<b>**</b>	<b>***</b>

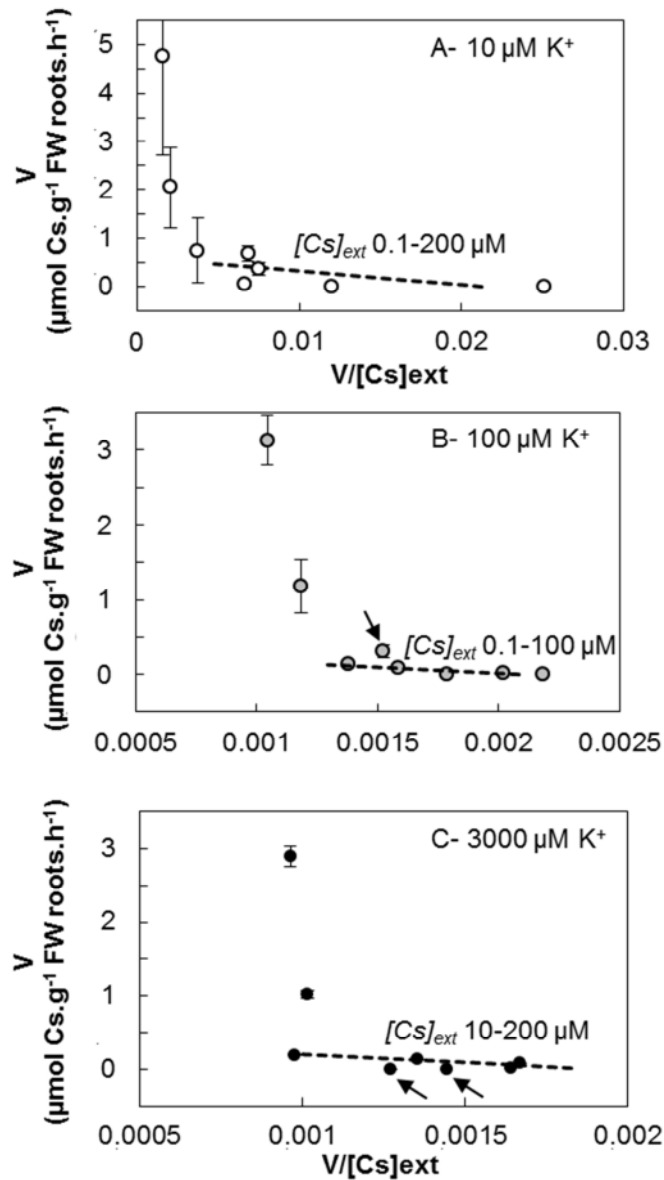
519

520



521  
 522  
 523  
 524  
 525  
 526

**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  $\mu M$ ) 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<sup>+</sup> uptake data from 15 min influx assays.** Plants were supplied  
 529 with three distinct K<sup>+</sup>-treatments (A-10 μM, B-100 μM, C-3000 μM). Concentrations of Cs<sup>+</sup> 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<sup>+</sup> concentrations ([Cs]<sub>ext</sub>) indicated on each figure. Arrows indicate extreme values which had to be  
 534 removed from the calculation because they lead to absurd results.

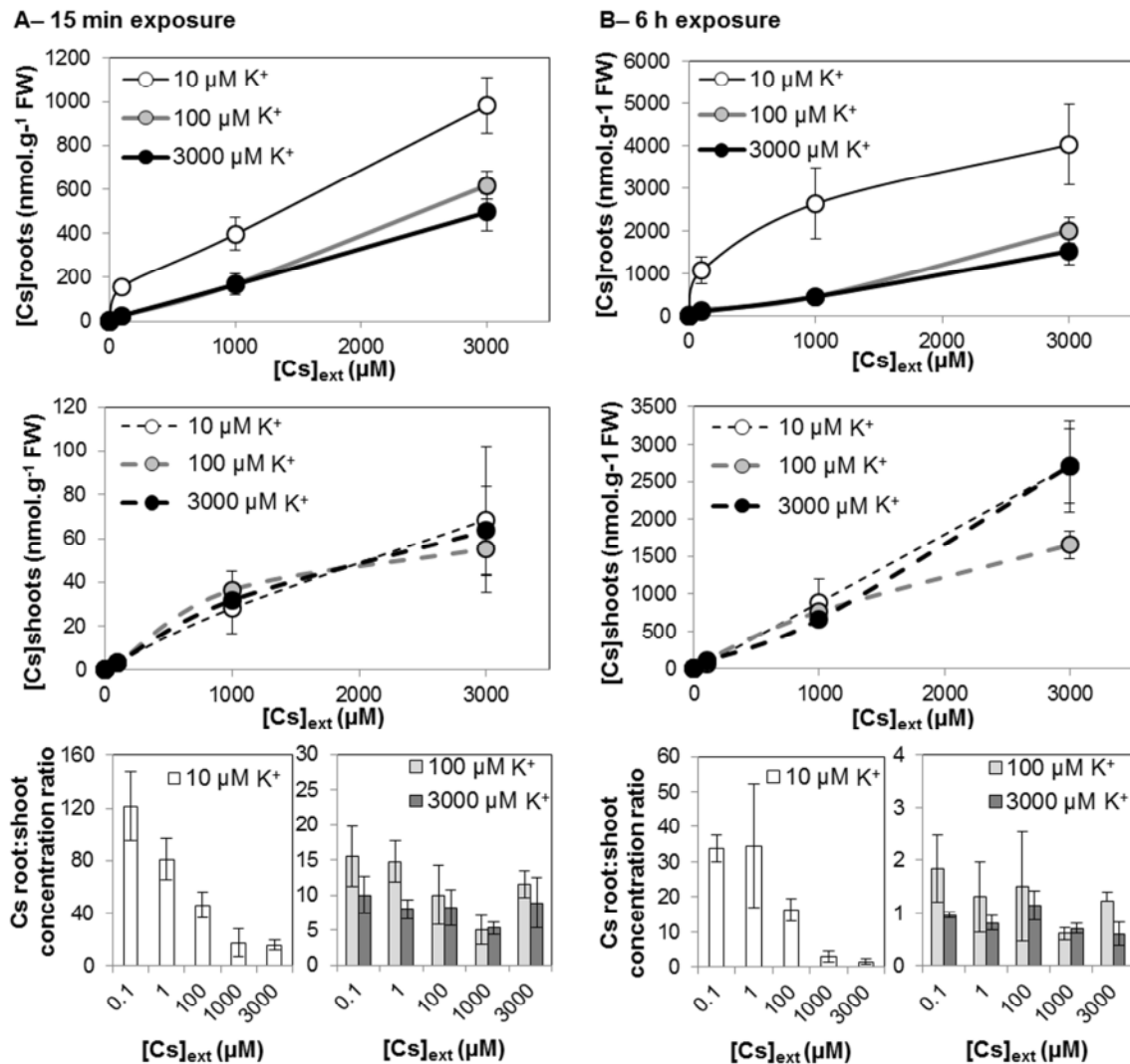
535

536 **Table 3: Estimation of Cs<sup>+</sup> 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<sup>+</sup>  
 539 concentrations ( $[Cs]_{ext}$  in the table) had to be removed of the linear regression. The R<sup>2</sup> values are  
 540 associated with the linear regression represented in Figure 2 (broken lines).  
 541

K <sup>+</sup> -treatment (μM)	$[Cs]_{ext}$ (μM)	$K_m$ (μM)	$V_{max}$ (μmol.g <sup>-1</sup> FW roots.h <sup>-1</sup> )	R <sup>2</sup>
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<sup>+</sup> between roots and shoots across a range of**

545 **[Cs]<sub>ext</sub> for plants from the short-term influx assays. Three distinct K<sup>+</sup>-treatments (10 µM, 100 µM,**

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

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

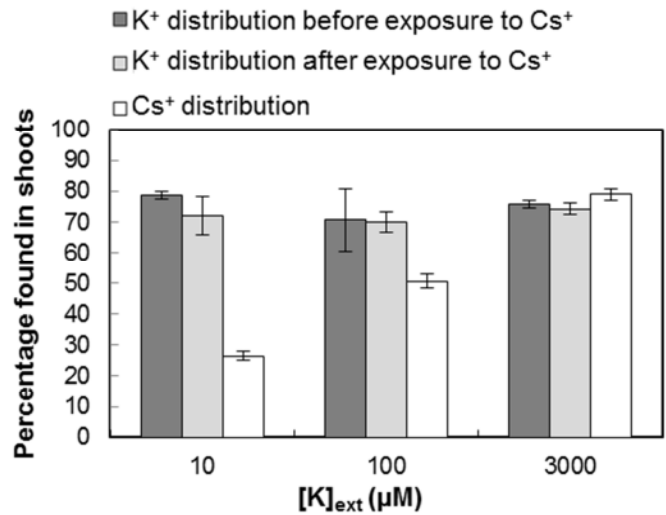
548 **of at least three different plants are represented with standard deviation.**

549

550 **Table 4: Cs<sup>+</sup> accumulation in Col-0 exposed during 7 days to nutrient solution containing 1 μM**  
 551 **Cs<sup>+</sup> and three distinct K<sup>+</sup> concentrations.** *DF(Cs)* is the discrimination factor calculated for Cs<sup>+</sup> 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 <sup>+</sup> -treatment (μM)	Cs <sup>+</sup> shoot (nmol.g <sup>-1</sup> FW)	Cs <sup>+</sup> roots (nmol.g <sup>-1</sup> FW)	Cs <sup>+</sup> whole plant (nmol.g <sup>-1</sup> FW)	Plant <i>DF(Cs)</i>
10	0.34 (0.04) <b>a</b>	3.08 (0.60) <b>a</b>	0.98 (0.10) <b>a</b>	0.37 (0.06) <b>a</b>
100	0.17 (0.02) <b>b</b>	0.28 (0.08) <b>b</b>	0.21 (0.03) <b>b</b>	0.54 (0.08) <b>b</b>
3000	0.01 (0.0007) <b>c</b>	0.01 (0.002) <b>c</b>	0.01 (0.0006) <b>c</b>	0.54 (0.03) <b>b</b>
K <sup>+</sup> -treatment effect	***	***	***	***

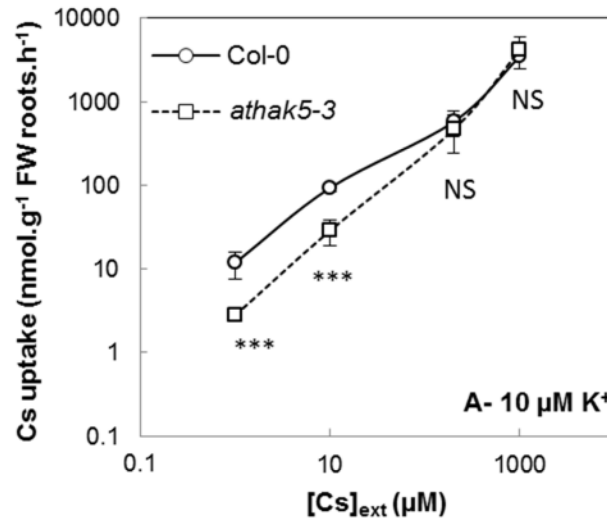
556



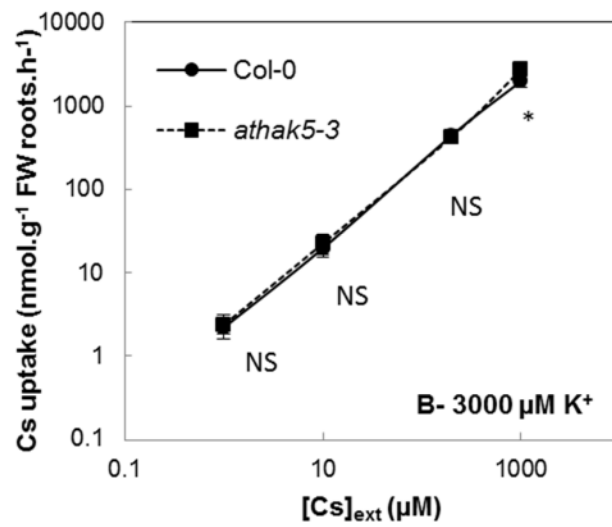
557

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

564



565



566

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

568 Two distinct levels of K<sup>+</sup> were used during pre-culture and during exposure to Cs<sup>+</sup>: 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<sup>+</sup> uptake between *hak5-3* and Col-0 for each [Cs]<sub>ext</sub> (NS, Non-

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

572