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Laure GENIES[a, b], Daniel ORJOLLET[a], Loïc CARASCO[a], Virginie CAMILLERI[c], Sandrine FRELON[c], Alain VAVASSEUR[b], Nathalie LEONHARDT[b], Pascale HENNER[a]

[a] *Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PRP-ENV, SERIS, Laboratoire de Biogéochimie, Biodisponibilité et Transferts des radionucléides (L2BT), Cadarache, France*

[b] *Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), IBEB-SBVME, Laboratoire de Biologie du Développement des Plantes (LBDP), Cadarache, France*

[c] *Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PRP-ENV, SERIS, Laboratoire d'ECOtoxicologie des radionucléides (LECO), Cadarache, France*

Corresponding author: laure.genies@gmail.com

ABSTRACT

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

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methods

2.1. Plant material and growing before exposure to cesium

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

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

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

The nutrient solution (pH 5.8) contained 1.1 mM $MgSO_4$, 805 µM $Ca(NO_3)_2$, 2 mM KNO_3, 60 µM K_2HPO_4, 695 µM KH_2PO_4 and micronutrients (3.6 µM $MnSO_4$, 74 nM $(NH_4)_6Mo_7O_{24}$, 3 µM $ZnSO_4$, 9.25 µM H_3BO_3, 785 nM $CuSO_4$, 20 µM Na_2EDTA and 20 µM $FeSO_4$).

2.2. Potassium treatments

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

K^+ treatments were performed during the hydroponic step. After 3-5 days of acclimatization to **hydroponics conditions** with the nutrient solution described in section 2.1, plants were transferred over 1L of a K^+-treatment solution (pH 5.8) containing 0.75 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 0,5 mM H_3PO_4, and micronutrients. Three different treatments were tested through adding different amounts of KCl in the K^+-treatment solution: 10 µM (starved level), 100 µM (intermediate level) or 3000 µM (replete level).

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

2.3. Exposure to cesium in short-term influx experiments

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

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

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

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

2.4. Exposure to cesium in long-term accumulation experiments

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

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

2.5. Measure of cesium and potassium

Fresh roots and shoots of plants were mineralized in 5 mL HNO_3 65% and 1.5 mL H_2O_2 30% at 100-150°C on a sand bath. Mineralisates were evaporated to dryness and redissolved in HNO_3 2% v/v before measuring the different elements.

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

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

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

2.6. Data analysis

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

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

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

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

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

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

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

3. Results and discussion

Effects of K^+-supply on Cs^+ influx, accumulation and distribution was estimated using both short-term (from 15 min to 6 h exposure) and 7-days exposure assays performed on *A. thaliana* (Col-0 ecotype). The experiments were designed to compare the effects of three different K^+-treatments on:

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

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

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

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

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

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

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

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

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

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

i) the high depletion of K^+ at the root surface due to high demand of plant to sustain growth whereas K^+ is weakly available. This depletion of K^+ in the medium favors Cs^+ uptake.

ii) the higher uptake rate potential of Cs^+ at low K^+ due to prevalence of high-affinity transport (HAT) system. In *A. thaliana*, this HAT system is mainly mediated by HAK5 which is known to be involved in Cs^+ uptake (Rubio et al., 2000, Qi et al., 2008).

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

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

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

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

3.3. Distribution of cesium in roots and shoots

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

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

(data not shown) suggesting that Cs^+ is highly mobile in these conditions. In contrast, Cs^+ root:shoot concentration ratio is up to ten times higher for K^+-starved plant compared to the highest K^+-conditions and between 3 and 20 % of Cs^+ were found in shoots in this condition depending on $[Cs]_{ext}$. Interestingly, $[Cs]_{ext}$ seems to affect Cs^+ root:shoot concentration ratio only for the 10 µM K^+-condition. In this condition, translocation of Cs^+ from the root to the shoot is higher when $[Cs]_{ext}$ increases.

These data suggest that Cs^+ translocation is mediated by very efficient systems which could be inhibited by decrease of K^+ and improved when Cs^+ concentrations increase in low K^+ condition.

3.3.2. Long-term cesium accumulation experiment

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

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

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

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

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

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

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

No significant differences on Cs^+ influx between *athak5-3* mutant line and Col-0 wildtype were 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

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

4. Conclusions

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

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

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

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

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

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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

510

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 α = 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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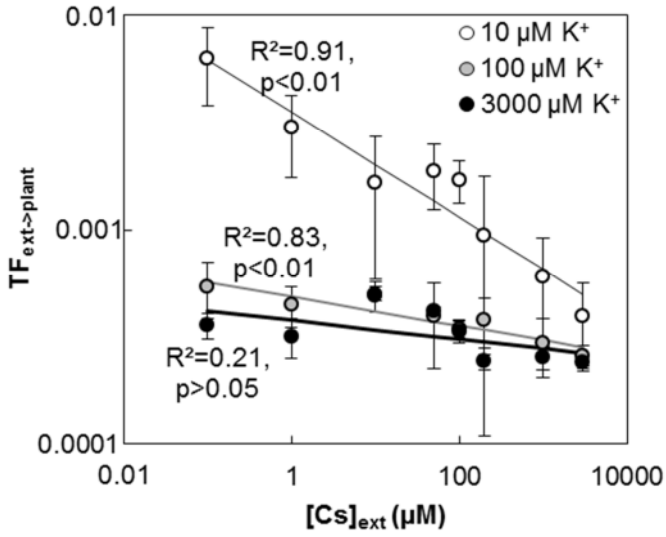

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522 **Figure 1: Log-log plot of transfer factor ($TF_{ext\to plant}$) of Cs^+ for Col-0 grown with three different**
523 **K^+-treatments (10, 100 or 3000 µM) and exposed during 15 min to a range of Cs^+**
524 **concentrations.** Transfer factor was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

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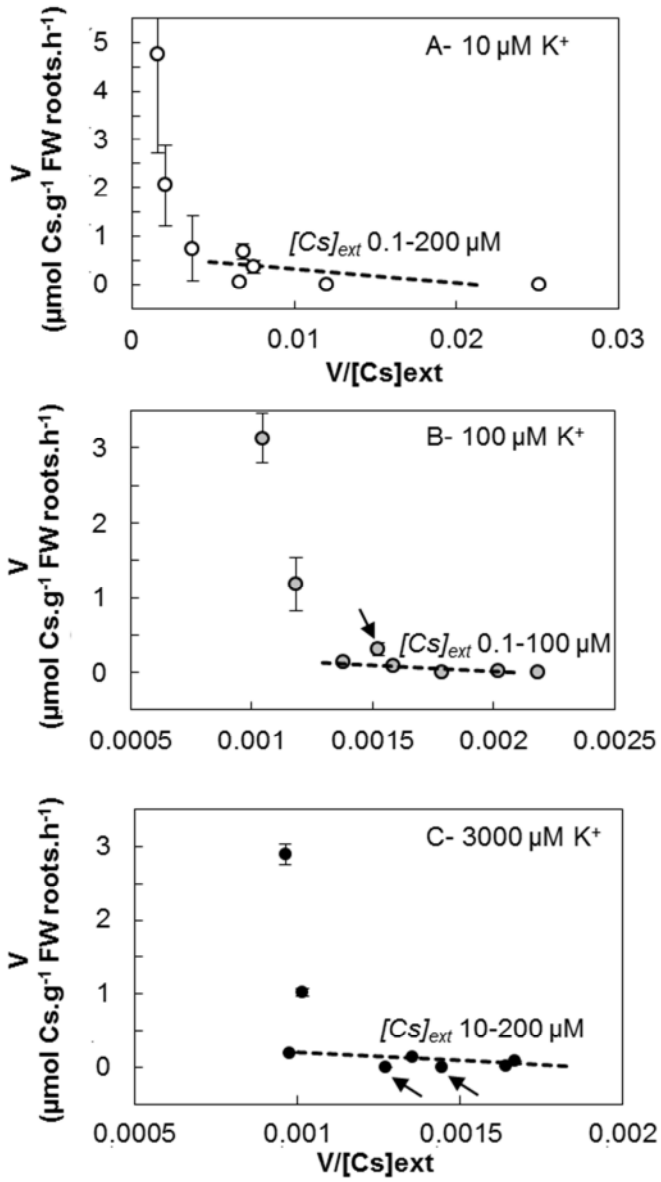

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528 **Figure 2: Eadie-Hofstee plot for Cs^+ uptake data from 15 min influx assays**. Plants were supplied
529 with three distinct K^+-treatments (A-10 µM, B-100 µM, C-3000 µM). Concentrations of Cs^+ in the
530 exposure solution range from 0.1 to 3000 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

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

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

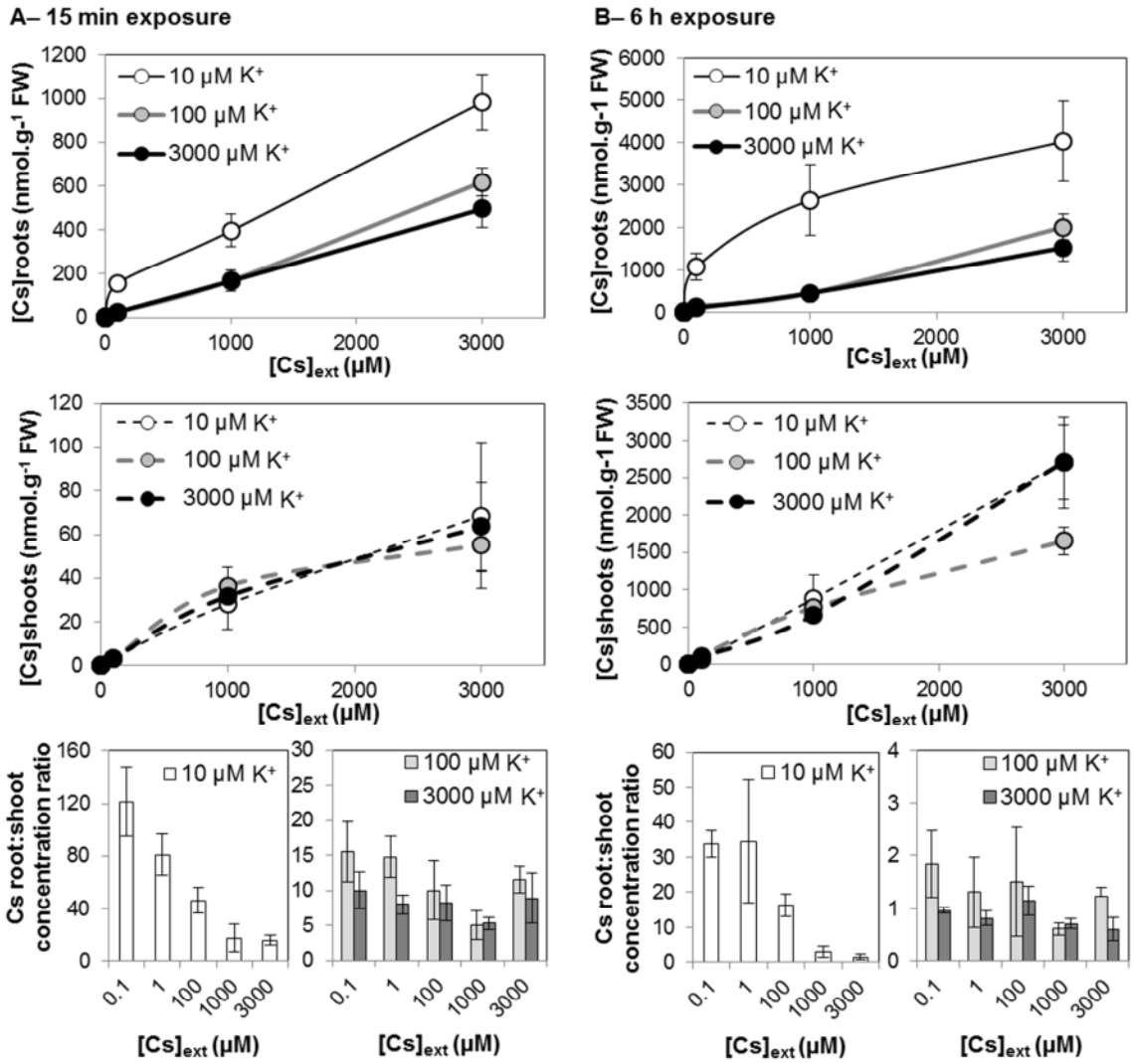

Figure 3: Evolution of the distribution of Cs^+ between roots and shoots across a range of *$[Cs]_{ext}$* for plants from the short-term influx assays. Three distinct K^+-treatments (10 µM, 100 µM, 3000 µM K^+) were applied during 5 days before exposure to Cs^+. Levels of K^+ in the pre-culture were maintained during the exposure to Cs^+. Plants were exposed during A- 15min or during B- 6 h. Means of at least three different plants are represented with standard deviation.

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

K^+-treatment (µM)	Cs^+ shoot (nmol.g^{-1} FW)	Cs^+ roots (nmol.g^{-1} FW)	Cs^+ whole plant (nmol.g^{-1} FW)	Plant *DF(Cs)*
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	***	***	***	***

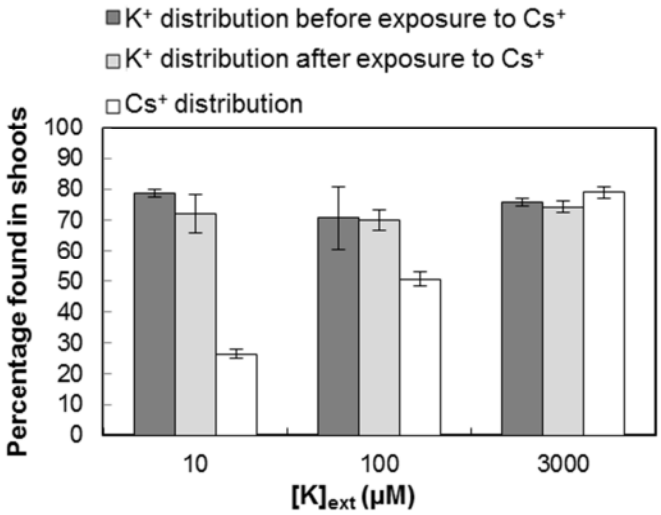

Figure 4: Comparison between K^+ and Cs^+ distribution in plants exposed during 7 days to a nutrient solution containing 1 µM Cs^+ and three distinct K^+ concentrations. Percentage was calculated by dividing the quantity of element found in shoots by the quantity found in the whole plant. K^+ distribution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

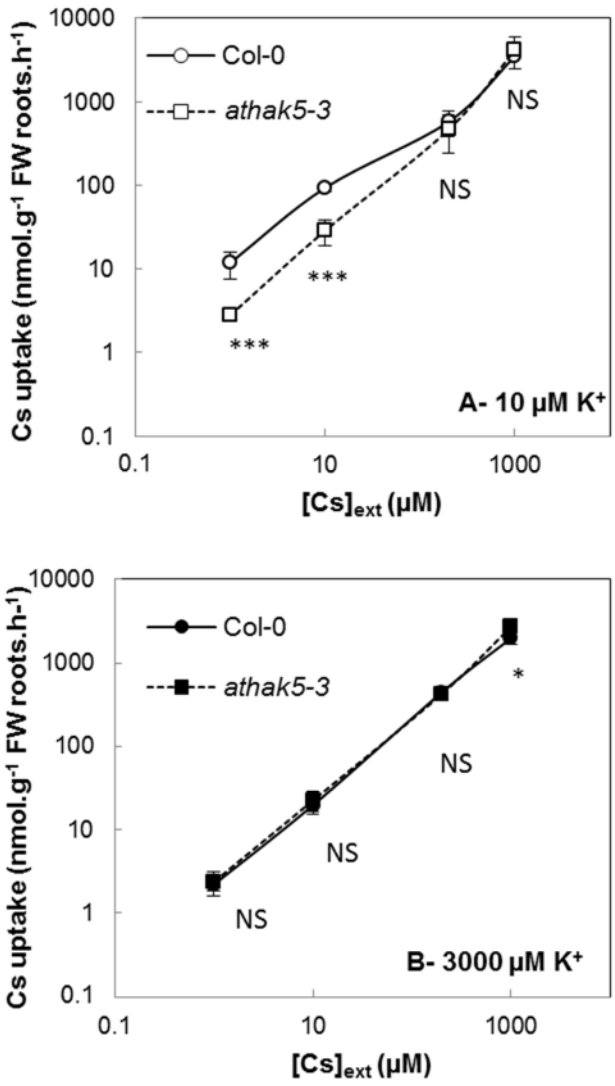

Figure 5: Cs^+ uptake measured over 15 min for the wild-type Col-0 and the mutant line *athak5-3*. Two distinct levels of K^+ were used during pre-culture and during exposure to Cs^+: A-10 µM and B-3000 µM. Means of at least five different plants are represented with standard deviation. Student t-tests were performed to compare Cs^+ uptake between *hak5-3* and Col-0 for each $[Cs]_{ext}$ (NS, Non-significant and *, *** $P < 0.05$ and $P < 0.001$ respectively).