Modulation of K^+ translocation by AKT1 and AtHAK5 in Arabidopsis plants

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Keywords: K^+ uptake, K^+ translocation, K^+ retrieval from xylem, AtHAK5, AKT1, SKOR

Abstract

Root cells take up K^+ from the soil solution and a fraction of the absorbed K^+ is translocated to the shoot after being loaded into xylem vessels. K^+ uptake and translocation are spatially separated processes. K^+ uptake occurs in the cortex and epidermis whereas K^+ translocation starts at the stele. Both uptake and translocation processes are expected to be linked but the connection between them is not well characterized. Here, we studied K^+ uptake and translocation using Rb^+ as a tracer in wild type *Arabidopsis thaliana* and in T-DNA insertion mutants in the K^+ uptake or translocation systems. The relative amount of translocated Rb^+ to the shoot was positively correlated with net Rb^+ uptake rates and the *akt1 athak5* T-DNA mutant plants were more efficient in their allocation of Rb^+ to shoots. Moreover, a mutation of *SKOR* and a reduced plant transpiration prevented the full upregulation of *AtHAK5* gene expression and Rb^+ uptake in K^+-starved plants. Lastly, Rb^+ was found to be retrieved from root xylem vessels, with AKT1 playing a significant role in K^+-sufficient plants. Overall, our results suggest that K^+ uptake and translocation are tightly coordinated via signals that regulate the expression of K^+ transport systems.

1. Introduction

Potassium (K^+) is an essential nutrient for plants, and is required at adequate levels throughout the plant's life cycle. K^+ concentrations in the soil can be highly dynamic depending on the season or year (Marschner & Rengel, 2012) and plants must adapt to these fluctuations. K^+ can be taken up from the soil solution into the epidermis and cortex cells or can freely diffuse in the apoplasm up to the endodermis (White, 2012a). K^+ has to enter the cytosol of a root cell to cross the endodermis and to reach the vascular tissues at the stele. Once in the stele, K^+ can be loaded into xylem vessels and be transported towards the shoot (Amtmann, Armengaud, Volkov & Michael, 2004; White & Karley, 2010). Interestingly, aside from being an essential nutrient, K^+ is recognized as a signaling agent at the cell and at the whole-plant level. For instance, plasma membrane K^+ efflux may trigger reallocation of cellular energy into other metabolic processes under stress conditions (Shabala, 2017). At a larger scale, K^+ concentration in the phloem can act as a signal for root cells, providing information about the shoot's K^+ demand, so that K^+ uptake can be modulated accordingly (Ahmad & Maathuis, 2014; Dreyer, Lucia Gomez-Porras & Riedelsberger, 2017). Root cells are equipped with a set of plasma membrane proteins that are specialized in taking up K^+ from the external soil solution. The current model for root K^+ uptake postulates that the activity of AKT1-type voltage-gated K^+ channels (Shaker-like channels) and high-affinity K^+ transporters from the HAK/KUP/KT family is critical for plant K^+ nutrition at external K^+ concentrations below 5 mM (Nieves-Cordones *et al.*, 2016b; Santa-María, Oliferuk & Moriconi, 2018). According to the Arabidopsis T-DNA insertion mutants phenotypes, AtHAK5 is the main K^+ uptake system at concentrations below 20-30 µM (Pyo, Gierth, Schroeder & Cho, 2010; Qi *et al.*, 2008; Rubio, Nieves-Cordones, Alemán & Martinez, 2008), although at higher K^+ concentrations, both AtHAK5 and AKT1 can contribute to K^+ uptake. AtHAK5 contribution is residual at K^+ concentrations higher than 0.5 mM and AKT1 becomes the main pathway for K^+ acquisition (Nieves-Cordones, Martínez, Benito & Rubio, 2016a). At external K^+ concentrations higher than 1 mM, non-selective cation channels become important for root K^+ uptake (Rubio, Alemán, Nieves-Cordones & Martínez, 2010). The range of concentrations at which each system is significant may vary among plant species. For example, in rice OsHAK1 is the major K^+ uptake system in a wider range of K^+ concentrations (Chen *et al.*, 2015; Nieves-Cordones *et al.*, 2017). With this regard, at 1 mM K^+, OsHAK1 accounts for ~70% of the membrane depolarization induced by K^+ (Nieves-Cordones *et al.*, 2017).

K^+ translocation from root to shoot is mediated by specialized transport systems such as the K^+ channel SKOR (Stelar K^+ Outward-Rectifying channel) (Drechsler *et al.*,

2015; Gaymard *et al.*, 1998), the Nitrate Transporter1 (NRT1)/Peptide Transporter (PTR) NRT1.5 (Drechsler *et al.*, 2015; Li *et al.*, 2017) and the K^+ transporter KUP7 (Han, Wu, Wu & Wang, 2016). These systems are prominently expressed in vascular tissues and their contribution to K^+ translocation seems to vary depending on culture conditions. In particular, NRT1.5 and KUP7 have been shown to be especially relevant for K^+ translocation in low-K^+ plants (Drechsler *et al.*, 2015; Han *et al.*, 2016; Li *et al.*, 2017). Also important, aside from being involved in K^+ uptake, HAKs and AKTs have also been proposed to take part in K^+ translocation in Arabidopsis and rice, as they have been shown to be expressed in vascular tissues as well (Chen *et al.*, 2015; Gierth, Maser & Schroeder, 2005; Li *et al.*, 2014; Yang *et al.*, 2014).

The processes of K^+ uptake and translocation appear to be linked. For example, in barley, it has been shown that external K^+ supply, and thus uptake rates, has a strong effect on K^+ translocation rates (Hooymans, 1976; Kochian & Lucas, 1988). Moreover, K^+ uptake and translocation are well–known to be influenced by plant transpiration rates due to the interaction between water flow and ion transport (Kochian & Lucas, 1988; White, 2012b). Again in barley, it has also been shown that reducing transpiration by incubating plants in conditions of high humidity or darkness reduces both uptake and translocation of K^+ (Hooymans, 1969; Jeschke, 1984; Russell & Shorrocks, 1959). Interestingly, K^+ (Rb^+) translocation was reduced to a higher extent than its uptake by low transpiration conditions (Jeschke, 1984; Russell & Shorrocks, 1959). This effect was best observed when the external K^+ (Rb^+) concentration was in the micromolar range.

The identification of transport systems involved in K^+ uptake and translocation allows for a better characterization of the relationships between these two processes under different transpiration and K^+ regimes. With this in mind, experiments on uptake/translocation relationships in Arabidopsis using Rb^+ as an analog for K^+ were carried out in the present work. In particular, the research sought to clarify the specific contribution of the K^+ uptake systems AKT1 and AtHAK5 to K^+ translocation. Interestingly, the results showed that the double mutant *akt1 athak5* had a higher Rb^+ translocation efficiency than WT plants. And as related to this, it was shown that Rb^+ transport to shoots was dependent on Rb^+ resorption from xylem vessels in Arabidopsis roots and that AKT1 played a significant role in this process in $+K^+$ plants.

2. Materials and Methods

2.1 Plant growth and material

Plants of *Arabidopsis thaliana* ecotype Col-0 wild-type (WT) and the following mutants (in Col-0 background): the *athak5-3 akt1-2* single and double mutants (Rubio *et al.*, 2010), *nrt1.5-5* (Drechsler *et al.*, 2015), *skor-2* (Drechsler *et al.*, 2015) and *kup7-1* mutants (Han *et al.*, 2016) were used in this study.

Arabidopsis seeds were germinated in microtubes filled with Rockwool on 2-L containers with nutrient solution and grown in a controlled-environment chamber with a 8/16 h day/night cycle at 150 µmol m^{-2} s^{-1} light, 22 ◦C and 65 % relative humidity (Gibeaut, Hulett, Cramer & Seemann, 1997).

Plants were grown for 30 days in a modified 1/5-strength Hoagland solution with the following macronutrients (mM):10 KCl, 1.4 $Ca(NO_3)_2$, 0.35 $MgSO_4$ and 0.1 $Ca(H_2PO_4)_2$, and the following micronutrients (µM): 50 $CaCl_2$, 12.5 H_3BO_3, 2 $MnSO_4$, 1 $ZnSO_4$, 0.5 $CuSO_4$, 0.1 H_2MoO_4, 0.1 $NiSO_4$ and 10 Fe-EDDHA. Afterwards, the $+K^+$ plants were grown with 1.4 mM K^+, whereas $–K^+$ plants were grown in 1/5-strength Hoagland solution without the addition of KCl for 14 days. The pH was adjusted daily to 5.5 and the nutrient solutions were renewed weekly.

pAKT1::GUS and *pAtHAK5::GUS* plants were obtained in the Col-0 background by transforming WT plants with the *pAKT1::GUS* in pMOG502 plasmid (Lagarde *et al.*, 1996) and *pAtHAK5::GUS* in pMDC162, respectively, with the floral dip method (Clough & Bent, 1998). The *AtHAK5* promoter (2Kb upstream from the start codon) was amplified by PCR with the primers listed on Table S1 and cloned into pCR8 plasmid (Thermo Fisher Scientific, Waltham, MA). Then, it was transferred to pMDC162 by LR cloning (Curtis & Grossniklaus, 2003).

2.2 Rb^+ uptake and translocation

For determining Rb^+ uptake and translocation in Arabidopsis, plants were rinsed with a cold K^+-free solution at the end of the growth period and transferred to 2-L containers with a nutrient solution supplemented with 0.02, 0.2 or 1 mM RbCl as indicated. To reduce plant transpiration, plants were kept under high humidity (>95%) 24h before and during the Rb^+ uptake experiment. The high humidity treatment was applied by keeping the plants in a 27-L seed propagator whose atmosphere was saturated with

water vapor. Rb^+ uptake and translocation rates were calculated as described in (Ródenas *et al.*, 2017).

In experiments that aimed at measuring Rb^+ retrieval from the xylem sap, $+K^+$ or $–K^+$ plants were rinsed with a cold K^+-free solution and transferred to two 50 mL plastic tubes with the upper/proximal half of the root in one tube (R-Rb^+ compartment) and the lower/distal part in the other one (R+Rb^+ compartment) (Figure S1). The R-Rb^+ compartment was filled with K^+-free solution. A Rb^+-containing solution was added to the R+Rb^+ compartment and plants were maintained under these conditions for 6 h. Then, shoots and the two root halves were harvested and their Rb^+ content was analyzed separately, as described above. External Rb^+ concentration was adjusted for each genotype to show a net Rb^+ uptake rate of ~4 µmol gDW_{root} h^{-1} based on the results shown in Figure S1c,d. Further details about this adjustment are given in the legend of Figure S1. Thus, the external Rb^+ concentration in the R+Rb^+ compartment was: (mM Rb^+, concentration in parentheses) WT (0.2), *athak5* (0.2) *akt1* (0.5) and *athak5 akt1* (0.75) for $+K^+$ plants, and WT (0.03), *athak5* (0.075) *akt1* (0.03) and *athak5 akt1* (0.75) for $–K^+$ plants. In these experiments 0.2-0.4 (in $+K^+$ plants) and 0.1-0.2 (in $-K^+$ plants) µmoles of Rb^+ were absorbed by the plants (total Rb^+ content), thus representing ~1-5% of the total µmoles of Rb^+ in the R+Rb^+ tube. Therefore, the external Rb^+ concentration in the R+Rb^+ tube did not notably change during the experiment.

To measure shoot-to-root Rb^+ transport, drops of 1, 10 and 30 mM RbCl plus 0.02% Silwet L77 solutions (total volume 0.1 mL $plant^{-1}$) were deposited on Arabidopsis rosette leaves at the beginning of the light period. Plants were harvested 30 h later and the Rb^+ content was determined as described above.

2.3 GUS staining

GUS staining of roots from 6-week old transgenic plants expressing the ß glucuronidase (GUS) reporter gene under the control of *AtHAK5* or *AKT1* promoters was performed as described elsewhere (Jeanguenin *et al.*, 2011). Similar expression patterns were obtained in two independent transgenic lines for each promoter.

2.4 Real-time PCR in Arabidopsis plants

Total RNA isolation, cDNA synthesis and real-time polymerase chain reaction (PCR) were performed as described elsewhere (Nieves-Cordones, Aleman, Martinez & Rubio, 2010) except for the reference gene which was *AtPP2A* (*At1g13320*) (Czechowski, Stitt, Altmann, Udvardi & Scheible, 2005). The expression level of genes studied in this work were calculated by using the relative quantification method (Livak & Schmittgen, 2001). The calibrator sample was the control full nutrient solution treatment in WT Col-0 plants. The primers employed are described in Table S1.

2.5 Mineral composition determination in plants

Plant material was harvested after the nutritional treatments as indicated, separated into roots and shoots, dried at 65 °C for 4 days and the dry weights determined. Then, ion content analyses of plant organs were performed after ion extraction with 0.1 N hydrochloric acid for 2 days. Diluted samples were analyzed by ICP mass spectrometry by using an Iris Intrepid II ICP spectrometer (Thermo Electron Corporation) to determine their Rb^+ and K^+ contents.

2.6 Statistical analyses

Analysis of variance was performed with the Statistix v.8 software for Windows (Analytical software, Tallahassee, FL). The differences in means were compared by using a Tukey's multiple range test ($P<0.05$). Sigma Plot 9.0 was used for data fitting.

3. Results

3.1 Plant transpiration enhances the rates of Rb^+ uptake and translocation in Arabidopsis

With the aim of gaining insights into the K^+ uptake vs translocation relationship, a series of transport experiments using Rb^+ as an analog for K^+ were carried out. Plants were subjected to treatments that were expected to affect Rb^+ uptake and translocation rates: growth in the presence/absence of K^+ ($+K^+$ and $-K^+$ plants, respectively) (Santa-María, Danna & Czibener, 2000) and normal / high relative humidity (60% and >95% RH, respectively) which changes transpiration rate of plants (Hooymans, 1969).

To our knowledge, the effect of reduced plant transpiration on K^+ (Rb^+) uptake has not been studied thus far in Arabidopsis; therefore, a brief analysis of the effect of

different rates of transpiration on Rb^+ uptake in wild-type (WT) Col-0 plants was performed (Figure 1). When the experiment was carried out at >95% RH, where plant transpiration rate is very low, a reduction of Rb^+ uptake and translocation rates in both $+K^+$ and $–K^+$ plants was observed (Figures 1a and 1b). To assess the relative impact of the high relative humidity (>95%), Rb^+ uptake and translocation rates at >95% RH were expressed as a percentage with respect to the 60% RH condition. A high RH reduced K^+ uptake and translocation to a similar extent in $+K^+$ and $–K^+$ plants (Figure 1c). With respect to the effect of external Rb^+ concentration, Rb^+ uptake and translocation rates in the presence of high RH were higher at 1 mM Rb^+ than at 0.2 mM Rb^+ (Figure 1c), and Rb^+ translocation rates were higher at 1 mM Rb^+ than at 0.02 mM Rb^+ (Figure 1c). Interestingly, Rb^+ translocation was particularly low at 0.02 mM Rb^+ at high RH in comparison to Rb^+ uptake at the same Rb^+ concentration and, such an effect was not observed at higher Rb^+ concentrations (Figure 1c).

3.2. The relative shoot Rb^+ content is strongly dependent on uptake rates in Arabidopsis

Since the Rb^+ uptake and translocation curves in Figures 1a and 1b had a similar shape, a strong correlation seemed to exist between these two processes. To study this possible correlation, the net Rb^+ uptake rates were plotted against the relative shoot Rb^+ content (expressed as % of total Rb^+ in the plant). The plotted data included data from WT plants and those from knock-out mutants lacking the transport systems involved in K^+ uptake (*akt1*, *athak5* and *akt1 hak5* plants) and translocation (*skor, nrt1*.5 and *kup7* plants) (Figure 2). It is worth highlighting that a large range of net Rb^+ uptake rates was achieved by changing external Rb^+ concentration, relative humidity and K^+ regime as shown in Figure 1 (Figures 2a and 2b). The WT data for both $+K^+$ (Figure 2a) and $–K^+$ (Figure 2b) plants could be fitted to a hyperbolic equation. This indicates that the relative Rb^+ content in shoots increased as Rb^+ uptake rates increased, but the trend leaned towards saturation at high net Rb^+ uptake rates. With respect to $+K^+$ plants, knock-out mutants followed the WT trend, except for the *akt1 athak5* plants which exhibited high relative Rb^+ shoot contents at low net Rb^+ uptake rates (Figure 2a, red symbols). Moreover, the relative Rb^+ content of *akt1* shoots was similar to that of *akt1 athak5* shoots at the highest net Rb^+ uptake rate data point ($[Rb^+]_{ext}$ = 1 mM, 60%RH) (Figure 2a). Regarding $–K^+$ plants, knock-out mutants were similar to the WT trend except for *akt1 athak5* and *skor* plants (Figure 2b). *akt1 athak5* data were located above and *skor* data was found slightly below the WT trend

respectively (Figure 2b). The *skor* phenotype was particularly evident at the lowest net Rb^+ uptake rate measured ($[Rb^+]_{ext}$ = 0.02 mM, 60%RH).

In order to compare WT and *akt1 athak5* data in terms of Rb^+ translocation efficiency, a "Translocation Efficiency Factor" (TEF) was utilized. This factor was defined as the ratio between the relative shoot Rb^+ content and the net Rb^+ uptake rate, with a high TEF value indicating a high efficiency of Rb^+ translocation. Figure 2c shows TEF values calculated from regression curves in Figure 2a and b. TEF values are plotted against net Rb^+ uptake rates for WT and *akt1 athak5* plants grown in $+K^+$ and $–K^+$ conditions. WT $+K^+$ plants have higher TEF values than WT $–K^+$ plants at net Rb^+ uptake rates below 20 µmol Rb^+ gDW_{root}^{-1} h^{-1} which denotes a higher Rb^+ translocation efficiency of $+K^+$ plants (Figure 2c). On the other hand, *akt1 athak5* plants show higher TEF values than WT plants in both $+K^+$ and $–K^+$ conditions, especially below 10 µmol Rb^+ gDW_{root}^{-1} h^{-1} (Figure 2c). Thus, Rb^+ translocation is particularly efficient in *akt1 athak5* plants at low net Rb^+ uptake rates.

Further analyses were also carried out on the relative impact of the >95% RH condition on *akt1*, *athak5*, *akt1 hak5* and *skor* $–K^+$ plants to check if a K^+ transport system was particularly affected by low plant transpiration rates (Figure S2). Although the results obtained were rather complex, a few conclusions could be drawn. In *akt1*, *athak5* and *akt1 hak5* $–K^+$ plants, Rb^+ uptake and translocation rates were lower at >95% RH than at 60% RH. This general inhibition by a high RH indicates that plant transpiration enhances Rb^+ uptake and translocation in these mutant lines. As a high RH exerted a similar effect on WT and *akt1*, *athak5* and *akt1 hak5* $–K^+$ plants (Figure S2), it could be deduced that plant transpiration did not produce a specific effect on AKT1- and AtHAK5-mediated Rb^+ uptake. However, the results obtained in *skor* $-K^+$ plants were different. Neither Rb^+ uptake nor translocation rates were affected by high RH at 0.02 mM and 1mM Rb^+. Further analysis of the K^+-starved *skor* data are carried out in the next section.

3.3 The skor *mutation and a high-humidity reduce* Rb^+ *uptake and* AtHAK5 *expression in* $–K^+$ *plants*

skor $–K^+$ plants, aside from having an impaired Rb^+ translocation (Figure 2b), also showed lower net Rb^+ uptake rates under 60% RH than WT plants (Figure 3a). Since this occurred only in $–K^+$ plants, it was hypothesized that the expression of the K^+ transport systems involved in K^+ uptake could be affected in the *skor* background. Thus, the *AtHAK5* and *AKT1* expression levels in WT and *skor* roots under different

conditions were checked, as shown in Figure 3a. As expected, *AtHAK5* was upregulated in WT plants by K^+ starvation (Figure 3b). Interestingly, incubation of plants at >95% RH decreased *AtHAK5* expression (Figure 3b). Moreover, *skor* plants showed lower *AtHAK5* mRNA levels than WT plants in the $–K^+$ 60% RH condition (Figure 3b). In contrast, neither K^+ starvation nor incubation under high RH had a remarkable effect on WT *AKT1* expression (Figure 3c). With respect to *skor* plants, *AKT1* mRNA levels were lower in the $+K^+$ condition, but not in the $–K^+$ one, in comparison to WT plants (Figure 3c). It is worth highlighting that plants with a lower *AtHAK5* expression (WT grown in $–K^+$, >95%RH and *skor* grown in $–K^+$, 60%RH and >95%RH) had lower net Rb^+ uptake rates than plants with high *AtHAK5* expression (WT grown in $–K^+$, 60%RH). Thus, lower net Rb^+ uptake rates in the aforementioned plants could be ascribed, in part, to a lower induction of *AtHAK5*.

3.4. akt1 athak5 *plants exhibit a reduced capacity to accumulate Rb^+ in roots*

Since *akt1 athak5* plants are impaired in Rb^+ uptake (Rubio *et al.*, 2010), further WT vs *akt1 athak5* comparisons at similar net Rb^+ uptake and translocation rates were made to better understand Rb^+ translocation in *akt1 athak5* plants. Under conditions of similar uptake rates (~14 µmol Rb^+ gDW_{root}^{-1} h^{-1}), the Rb^+ content of roots and shoots of *akt1 athak5* plants were reduced (approximately 50%) and increased (approximately 30% and 60% in $+K^+$ and $–K^+$ plants, respectively), with respect to those of WT plants (Figure 4a). These data confirmed the greater capacity of *akt1 athak5* plants to translocate Rb^+ to the shoot. On the other hand, if plants with similar Rb^+ translocation rates (~10 µmol Rb^+ gDW_{root}^{-1} h^{-1}) were compared, it was observed that *akt1 athak5* plants had a much lower Rb^+ content in roots than WT plants (Figure 4b). Thus, *akt1 athak5* plants were able to reach WT-like Rb^+ translocation rates with 60% less Rb^+ in the roots. Therefore, *akt1 athak5* showed higher TEFs than WT plants (Figure 2c) because the mutation of *akt1* and of *athak5* had a larger impact on Rb^+ accumulation in roots than in shoots.

One explanation of the observed reduction in Rb^+ accumulation in *akt1 athak5* roots is that Rb^+ translocation is more efficient due to the upregulation of K^+ translocation systems. To explore this possibility, *SKOR* and *NRT1.5* transcript levels were analyzed by qRT-PCR in *akt1 athak5* roots (Figure 5c and 5d). Regarding *SKOR*, the $–K^+$ treatment repressed its expression in WT roots with respect to the $+K^+$ condition but this repression disappeared under high RH ($–K^+$, >95%RH condition) (Figure 4c). Moreover, a higher repression of the *SKOR* gene was observed in the $–K^+$

60% RH condition in *akt1 athak5* roots as compared with the WT roots. In contrast to the WT roots, at the highest RH treatment, the *akt1 athak5* roots showed a much lower *SKOR* expression level (Figure 4c). The $-K^+$ treatment induced the expression of the *NRT1.5* gene in roots of WT plants (Figure 4d). With respect to *NRT1.5* expression, a slight increase was observed in *akt1 athak5* roots as compared to the WT ones in the $+K^+$ condition (Figure 4d). In contrast, the $-K^+$ treatment either repressed (at 60% RH) or had no effect (at >95% RH) on *NRT1.5* expression in roots of *akt1 athak5* plants (Figure 4d). In summary, a more efficient Rb^+ translocation of *akt1 athak5* plants did not seem to originate from an increased expression of *SKOR* and *NRT1.5* genes. However, an increase in SKOR and NRT1.5 protein activity could take place in *akt1 athak5* plants and this cannot be discarded at the present.

3.5. AKT1 *and* AtHAK5 *are expressed in root vascular tissues*

Thus far, the role of AKT1 and AtHAK5 in Rb^+ translocation within vascular cells cannot be discarded. Expression of AKT1-like and HAK5-like genes in vascular tissues has been established before (Costa *et al.*, 2017; Chen *et al.*, 2015; Li *et al.*, 2014; Yang *et al.*, 2014) . Thus, *AKT1* and *AtHAK5* expression in root tissues was verified with *promoter::GUS* fusions (Figure 5). *AtHAK5* showed a different expression pattern depending on the K^+ status of the plant, being is expressed in the stele K^+ sufficient plants, while in K^+-starved plants it is expressed in cortex, epidermis and stele (Figure 5a and c). By contrast, *AKT1* was expressed in all root tissues irrespective of the K^+ supply (Figure 5b and d). It is worth mentioning that *AKT1* transcripts have been detected in vascular tissues in transcriptomic and RNA-seq analysis (eFP browser and Genevestigator) (Figure S3). Thus, *AtHAK5* and *AKT1* are expressed in vascular tissues and may play a role in these cells.

3.6. AKT1 and AtHAK5 do not have a dominant role in shoot-to-root Rb^+ transport

Since *AKT1* and *AtHAK5* are expressed in root vascular tissues, they could take part in K^+ movements associated to xylem or phloem transport. Our experiments with Rb^+ are almost unidirectional (from the root to the shoot) and a putative role of these transport systems in Rb^+ recirculation through phloem cells cannot be studied with this experimental design. To assess whether AKT1 and AtHAK5 contributed to K^+ transfer from the shoot to the root via phloem cells, different amounts of Rb^+ (0.1, 1 and 3 µmoles Rb^+) were placed on Arabidopsis leaves and the quantity of Rb^+ that was

transferred to the roots after 30 h was measured (Figure 6). Shoot-to-root Rb^+ transport rates were comparable between $+K^+$ and $–K^+$ plants. Regarding the mutant lines, no significant differences were found among WT, *athak5*, *akt1* and *akt1 athak5* genotypes, except in *akt1 athak5* single and double mutant plants, where the shoot-to-root Rb^+ transport rate was lower as compared to WT at 0.1 µmoles Rb^+ (Figure 6b). It is worth mentioning that Rb^+ shoot-to-root transport experiments (Figure 6) may have provided an underestimation of the real K^+ transport through the phloem since part of the Rb^+ transported to roots may have been sent back to the shoot via the xylem and Rb^+ was used as an analog for K^+. Nevertheless, AKT1 and AtHAK5 did not have a dominant role in shoot-to-root transfer of Rb^+ in $+K^+$ plants, whereas in $–K^+$ plants they could be involved in the transport of Rb^+ at very low concentrations.

3.7 Rb^+ is reabsorbed from xylem vessels in roots and AKT1 contributes to this process in $+K^+$ plants

Reduced Rb^+ accumulation in roots of *akt1 athak5* mutants (Figure 4) could be influenced by an altered Rb^+ exchange between vascular cells and xylem vessels. For instance, reduced Rb^+ unloading from xylem vessels could enhance Rb^+ translocation to the shoot. Therefore, further analysis of AKT1 and AtHAK5 contribution to Rb^+ translocation required a new experimental setup that allowed distinguishing between Rb^+ release to or reabsorption from the xylem. WT, *akt1*, *athak5* and *akt1 athak5* plants grown under the $+K^+$ and the $–K^+$ treatments were used in these experiments. The roots from each plant were maintained in two isolated compartments, one proximal and one distal (Figure S1), with the root biomass in each compartment being comparable (Table S2). Rb^+ was provided only to the distal compartment, which was named $R+Rb^+$. The proximal compartment was not provided with Rb^+ and was named $R\text{-}Rb^+$. External Rb^+ concentration in the compartment $R+Rb^+$ was adjusted so each genotype exhibited comparable net Rb^+ uptake rates (~4 µmol Rb^+ gDW_{root}^{-1} h^{-1}). After 6 h of incubation with Rb^+, plant material of the two root compartments as well as that of shoots was collected separately to determined their internal Rb^+ concentrations. In $+K^+$ WT plants, the % of Rb^+ accumulated in the shoot was higher than that in the $R+Rb^+$ compartment (55.2% vs 35.6%, respectively), while 9.2% of total Rb^+ was found in the $R\text{-}Rb^+$, indicating that part of the Rb^+ loaded into the xylem sap at the $R+Rb^+$ compartment was reabsorbed in the $R\text{-}Rb^+$ compartment (Figure 7a). *athak5* plants had a lower Rb^+ content in shoots and a higher one in the $R+Rb^+$ root, respectively, in comparison with WT plants. In addition, there were no significant differences with

respect to the Rb^+ content in the R-Rb^+ between *athak5* and WT plants. These results indicate that AtHAK5 contributes to Rb^+ translocation from the distal root tissue (compartment R+Rb^+). With respect to the rest of the genotypes, both *akt1 and akt1 athak5* mutants exhibited a higher Rb^+ content in shoots and a lower content in the R+Rb^+ compartment, respectively, as compared to WT plants. More importantly, the Rb^+ content in the R-Rb^+ compartment was ~50% lower in the *akt1* and *akt1 athak5* plants when compared to WT (5.3% and 4.3% in the *akt1* and *akt1 athak5* lines, respectively, vs 9.2% in WT plants). This result points to a reduced retrieval of Rb^+ from the xylem in both *akt1* and *akt1 athak5* plants, suggesting that AKT1 is involved in that process.

Also important, a reduced retrieval of Rb^+ at the root is expected to increase the Rb^+ content in the shoots. To quantify the contribution of AKT1-mediated Rb^+ reabsorption at the root to the shoot Rb^+ content, the following calculations were performed. The differences between mutant (*akt1* and *akt1 athak5*) and WT Rb^+ contents in shoots and in the R-Rb^+ compartment were calculated (Figure 7b). These data showed that the amount of Rb^+ that was not recovered by the R-Rb^+ root of *akt1* and *akt1 athak5* plants (Figure 7b, black bars) accounted for ~1/4 of the Rb^+ excess measured in *akt1* and *akt1 athak5* shoots (Figure 7b, grey bars). Therefore, AKT1, by mediating Rb^+ retrieval from xylem vessels, negatively contributed with a remarkable amount of Rb^+ that was transported to the shoot in $+K^+$ plants.

Concerning $–K^+$ plants, WT plants transported less Rb^+ to the shoots in comparison to the WT $+K^+$ ones (13% vs 55% of total Rb^+, respectively) (Figure 7b). Similarly, there was less Rb^+ in the R-Rb^+ root of WT $–K^+$ plants (Figure 7b) when compared to the $+K^+$ condition (Figure 7a) (5.0% vs 9.2% of total Rb^+, respectively). Therefore, Rb^+ translocation and Rb^+ resorption from xylem vessels were reduced when growing plants without K^+. With respect to the mutant genotypes, *athak5* and *akt1* plants displayed a similar Rb^+ pattern to the WT ones, whereas *akt1 athak5* displayed a more efficient Rb^+ translocation to the shoot (Figure 7b). This suggests that AtHAK5 and AKT1 have a redundant role in Rb^+ translocation in $–K^+$ plants since a more efficient Rb^+ translocation was only observed in the double mutant (Figure 7b). Unlike $+K^+$ plants, there were no significant differences with respect to the Rb^+ content in the R-Rb^+ root among all the genotypes. Thus, AtHAK5 and AKT1 did not contribute to Rb^+ retrieval from xylem vessels in $–K^+$ plants. To sum up, the experiments with the $–K^+$ plants led us to propose that AKT1 and AtHAK5 did not play a significant role in Rb^+ retrieval from xylem vessels in $–K^+$ plants.

4. Discussion

4.1. Translocation efficiency factor as a concept to study mutants affected in nutrient translocation in Arabidopsis

Our experiments on Rb^+ uptake and translocation clearly showed that Rb^+ translocation (expressed as % of total Rb^+ present in the shoot) was highly dependent on the rate at which Rb^+ was taken up by the roots. Thus, we proposed that the relevance of K^+ transport systems and environmental conditions on K^+ uptake and translocation should be examined using Translocation vs Uptake plots and calculation of factors that illustrate the relationship between these two processes, such as the TEF (Translocation Efficiency Factor) defined in this study (Figure 2). Consequently, comparisons between group of plants should be made under similar uptake rates. The relationship between uptake and translocation can be fitted to a hyperbolic function similar to that used in enzyme kinetics (Figure 2). TEF values showed that Rb^+ translocation was less efficient in $–K^+$ than in $+K^+$ WT plants (Figure 2c). This result indicates a higher capacity of $–K^+$ WT roots to retain K^+ and may be related to an adaptive response of the root to the $–K^+$ treatment. In line with this idea, K^+ starvation triggers the deposition of suberin lamellae in the endodermis, which is expected to slow down the movement of nutrients up to the xylem stream (Barberon *et al.*, 2016), and suberin-deficient mutants develop K-deficiency symptoms in shoots (Pfister *et al.*, 2014). Another aspect to take into account in TEF calculations is the amount of Rb^+ transported from the shoot to the root through the phloem at the end of the experiment. It has been documented that a significant amount of K^+ is recycled back to the root and such amount seems to vary among species (for example, 20% in tomato (Armstrong & Kirkby, 1979) and 85% in castor bean (White, 2012b)). Thus far, no data is available on the percentage of K^+ recycled in Arabidopsis plants. A preliminary experiment (1 µmol of Rb^+ added to the leaf; roots harvested after 6h) showed that WT plants exhibited a root Rb^+ content of 0.009±0.003 µmoles. Although this value could be an underestimation, it is ~50 times lower than the shoot Rb^+ content in an opposite experiment (6h incubation of WT roots with Rb^+; shoot Rb^+ content (0.48±0.07 µmoles); total Rb^+ content (0.93±0.11 µmoles)). Thus, we expect that the amount of Rb^+ recycled back to the root was markedly lower than the amount of Rb^+ moving towards the shoot during a 6h experiment such as the one carried out to calculate TEFs (Figure 2). However, the quantification of these shoot-to-root Rb^+ fluxes deserves further experiments.

All mutant genotypes, except for the *akt1 athak5* plants, aligned with the WT trend for shoot Rb^+ content versus net Rb^+ uptake (Figure 2), which indicated that the single mutants and WT plants had comparable TEFs and that the mutant lines did not show a dramatic effect on Rb^+ translocation. Thus, higher or lower shoot Rb^+ % in mutant lines in comparison to WT originated from variations in net Rb^+ uptake rate. For example, lower shoot Rb^+ content in the *skor* mutant in comparison to the WT in the $-K^+$ condition (56±3% vs 71±1 % Shoot Rb^+, respectively, at 1mM Rb^+ in the external solution and 70% RH) (Figure 2b) was not fully explained by impaired Rb^+ translocation but also by a lower net Rb^+ uptake rate (for example, 48±4 vs 73±4 µmol Rb^+ gDW_{root}^{-1} h^{-1}, respectively) (Figure 3a). This is a key point in the discussion of mutant genotypes with low Rb^+ content in shoots. In line with this idea, it is likely that lower translocation rates observed in *oshak1* and *oshak5* rice mutants for K^+ and Cs^+ could be explained in part because of the lower uptake rates shown by these plants, aside from a direct involvement of the protein in K^+ translocation (Chen *et al.*, 2015; Nieves-Cordones *et al.*, 2017; Yang *et al.*, 2014). Similarly, N, P or S deprivation have been recently shown to reduce both K^+ uptake and translocation in tomato and Arabidopsis (Ródenas *et al.*, 2017). Again, the reduced K^+ translocation rates of nutrient-starved plants could be partially explained by the effect of nutrient deprivation on K^+ uptake.

We propose that this sort of analysis could be useful for evaluating the effects of gene disruption or growth conditions on root-to-shoot transport of other nutrients.

4.2 Reciprocal regulation of K^+ uptake and K^+ translocation

Root K^+ uptake and xylem K^+ load are interrelated processes. However, little information is available on the molecular events that take part in the cross-regulation of these two processes. In our experiments with Rb^+, new insights into this matter have been gained (Figure 8). We observed a clear reduction in the net Rb^+ uptake rates in K-starved *skor* plants in comparison to WT plants (Figure 3a). Such a reduction coincided with a lower expression level of *AtHAK5* in the roots of the *skor* mutant in comparison to those of WT plants (Figure 3b). On the other hand, *skor* $+K^+$ plants exhibited lower *AKT1* expression levels than WT plants (Figure 3c). Therefore, the *skor* mutation has a strong influence on K^+ uptake systems, affecting AtHAK5 or AKT1 depending on the K^+ status of the plant. Other mutants affected in K^+ translocation such as *nrt1.5* (Drechsler *et al.*, 2015) and *cpr5* (Borghi, Rus & Salt, 2011) also displayed reduced *AtHAK5* expression levels. Thus, it is tempting to speculate that xylem K^+ load may regulate K^+ uptake by a retrograde signal from the root stele to the

cortex and epidermis. Additionally, mutation of *AKT1 and AtHAK5* led to a lower *SKOR* expression in $-K^+$ plants (Figure 4c) which suggests that there was also an anterograde signal from the epidermis and cortex to the stele. Changes in the expression levels of genes encoding the transport system (involving either *AtHAK5*, *AKT1*, *NRT1*.5 or *SKOR*) did not provide a conclusive evidence for feedback mechanisms and further research in this line is required.

Another factor involved in the regulation of K^+ uptake is plant transpiration. Incubating plants at high RH gave rise to lower net Rb^+ uptake and translocation rates, evidencing the influence of transpiration on Rb^+ transport. Quantitatively, ~20-70% of Rb^+ uptake and translocation is dependent on plant transpiration (Figure 1c). High RH reduced Rb^+ translocation to a higher extent than Rb^+ uptake at 0.02 mM external Rb^+ (Figure 1c). Russell & Shorrocks (1959) obtained similar results in barley. This indicates that Rb^+ translocation is particularly dependent on transpiration when the external Rb^+ concentration is very low, probably because transpiration compensates for the low Rb^+ concentration gradient that exists under such conditions in xylem loading sites. Analysis of the high RH impact on net Rb^+ uptake and translocation rates in mutant lines did not reveal substantial differences in *akt1*, *athak5* and *akt1 athak5* plants (Figure S2) with respect to WT plants (Figure 1c) indicating that AKT1 and AtHAK5 activity was not dramatically affected by low plant transpiration conditions. Thus, plant transpiration may enhance Rb^+ uptake and translocation in WT and *akt1*, *athak5* and *akt1 hak5* $-K^+$ plants indirectly by favoring water and solute movement (White, 2012b). However, high RH did not inhibit Rb^+ uptake and translocation in skor -K^+ plants (Figure S2). This result seemed to be related with a lower *AtHAK5* expression in *skor* -K^+ plants at 60% RH which prevented full upregulation of Rb^+ uptake under these conditions (Figures 3a,b). Also, reducing plant transpiration diminished Rb^+ uptake and also *AtHAK5* expression in WT $-K^+$ plants (Figures 3a and b). One attractive hypothesis related to these observations is that a lower transfer of K^+ to the shoot due to high RH or mutation of *SKOR* downregulated *AtHAK5* and reduced root Rb^+ uptake capacity. One consequence of the model proposed is that enhancing plant transpiration in low K^+-plants could, to some extent, improve K^+ acquisition. In agreement with this idea, in olive trees and sunflower plants, K^+ starvation prevents stomatal closure induced by water-stress (Benlloch-González, Arquero, Fournier, Barranco & Benlloch, 2008) and such a regulation is mediated by ethylene production during K^+-starvation (Benlloch-Gonzalez *et al.*, 2010). Moreover, in pea and wheat, leaf K^+ content is inversely correlated with plant transpiration and stomatal aperture (Brag, 1972). Nevertheless, this issue requires further research.

4.3.A role for AKT1 and AtHAK5 in K^+ translocation

Experiments presented in Figure 2 showed that the shoot Rb^+ contents of *akt1 athak5* plants at different net Rb^+ uptake rates were found above the WT trend, which was indicative of a more efficient Rb^+ translocation. *akt1 athak5* mutants displayed a higher TEF value than WT plants irrespective of the K^+ supply (Figure 2c). Given that *SKOR* and *NRT1.5* were not upregulated in *akt1 athak5* roots (Figures 4c and d) and that AtHAK5 and AKT1 were expressed in vascular tissues as well (Figure 5), a direct involvement of AKT1 and AtHAK5 on Rb^+ translocation could not be ruled out. Two-compartment experiments revealed that AtHAK5 positively contributed to Rb^+ translocation from distal root tissues in $+K^+$ plants (Figure 7a). Interestingly, in $+K^+$ plants *AtHAK5* expression was mainly observed in the root stele (Figure 5a) which is in agreement with Rb^+ translocation data. These results showed that the role of AtHAK5 on K^+ nutrition was dependent on the K^+-status of the plant: in K^+-sufficient plants, AtHAK5 contributed to K^+ translocation (Figure 7) whereas in K^+-starved ones it mediates root K^+ uptake (Nieves-Cordones *et al.*, 2016a). However, it remains unclear how a well-characterized uptake system such as AtHAK5 contributes to K^+ translocation from root vascular cells, as other transport systems involved in this process, for example, SKOR and NRT1.5, mediate K^+ efflux at the plasma membrane. Given the range of pH values reported for root xylem sap (pH=6-7) (Wilkinson, Corlett, Oger & Davies, 1998), it seems unlikely that AtHAK5 mediates H^+/K^+ symport into xylem vessels. Nevertheless, AtHAK5 may help, for instance, in establishing K^+ gradients in the root stele apoplast necessary for the release of K^+ into xylem vessels from xylem parenchyma cells. By contrast, AKT1 plays a different role regarding Rb^+ translocation: AKT1 seems to contribute to Rb^+ resorption from xylem vessels in $+K^+$ plants (Figure 7a). Thus, in the absence of AKT1, more Rb^+ is translocated to the shoot, and this is due, in part, to a lower unloading rate of Rb^+ in the R-Rb^+ compartment. Such contribution of AKT1 to Rb^+ unloading from the xylem was not observed in $–K^+$ plants. It is worth noting that the % of Rb^+ in the R-Rb^+ compartment was approximately one half in $–K^+$ plants than that observed in $+K^+$ plants, indicating that this pathway was less relevant under low K^+ conditions. The activity of inward-rectifying K^+ channels in xylem parenchyma cells from barley was reported two decades ago (Amtmann, Jelitto & Sanders, 1999; Wegner & De Boer, 1997; Wegner, De Boer & Raschke, 1994; Wegner & Raschke, 1994). However, it was unclear what the role of these channels was in K^+ translocation. Interestingly, a significant expression of AKT1 was detected in stellar cells (Figure S3) which would support a

direct role of AKT1 in K^+ unloading in these cells. Thus, K^+ resorption from xylem vessels in concert with K^+ loading may contribute to the fine-tuning of K^+ supply to the root and shoot. Also, the importance of this K^+ resorption may vary among plant species. Unlike Arabidopsis, rice retained radioactive K^+ in distal root parts rather than translocating it to the shoot, which may be indicative of a strong K^+ unload from the xylem sap (Kobayashi, Sugita, Nobori, Tanoi & Nakanishi, 2016). Localized K^+ unloading from xylem vessels may be of relevance in soils with heterogeneous K^+ availability, as the reabsorption of K^+ from xylem vessels would guarantee K^+ provision to some root parts that are surrounded by low external K^+ concentrations. Another possibility is that K^+ retention in the root due to K^+ unloading from xylem vessels may be of relevance under abiotic stress conditions as well. In line with this idea, it has been recently shown that a K^+-permeable HKT transporter, ZmHKT2, negatively contributed to maize salt tolerance by favoring K^+ resorption from xylem vessels and reducing K^+ transport rates to shoots (Cao, Liang, Yin, Zhang & Jiang, 2018). Therefore, an increase in root K^+ retention by ZmHKT2 activity seemed to be detrimental for salt stress tolerance. Besides K^+, unloading of other ions from xylem vessels has been reported, for example for Na^+, SO_4^{2-}, PO_4^{3-} and NO_3^- (Li *et al.*, 2010; White, 2012b) . The case of Na^+ has been studied in depth in Arabidopsis, with AtHKT1 being the main protein involved (Davenport *et al.*, 2007). Na^+ unloading from xylem vessels mediated by AtHKT1 prevented massive Na^+ transport to the shoot. Interestingly, Davenport and colleagues (2007) also concluded that mutation of AtHKT1 also had a negative and independent effect on root Na^+ vacuolar accumulation aside from reducing Na^+ unloading from xylem vessels. In our experiments, it seemed that reduced root Rb^+ accumulation in *akt1 athak5* plants underlined the increase in their TEF values in comparison to WT plants (Figure 2c). Indeed, a decrease in root Rb^+ content will affect calculation of net Rb^+ uptake rates but not of Rb^+ translocation rates. Both AKT1 and AtHAK5 mediate plasma membrane $Rb^+(K^+)$ influx into root cortex and epidermal cells. Thus, it is clear that less Rb^+ will be stored in the root symplast of *akt1 athak5* plants in particular when the external Rb^+ concentration is low (<1mM) (Rubio *et al.*, 2010) (Figure 2). However, under other circumstances, high Rb^+ accumulation in root cells is not necessary for showing proper Rb^+ translocation rates. For example, at 1 mM Rb^+ WT and *akt1 athak5* $+K^+$ plants exhibited comparable Rb^+ translocation rates despite root Rb^+ concentration being ~60% lower in *akt1 athak5* as compared with WT plants (Figure 4b). It is worth noting that part of the reduced Rb^+ accumulation in *akt1 athak5* roots originated from decreased retrieval of Rb^+ from xylem vessels by AKT1 in the $+K^+$ condition (Figures 7a and b).

5. Conclusions

Long-distance K^+ transport is of critical importance for plant nutrition. In the present research a detailed study on Arabidopsis plants was conducted to gain insights into the connections that may exist between K^+ uptake and K^+ translocation (Figure 8). The first major outcome of this study was the observation of a clear relationship between Rb^+ uptake rates and the amount of Rb^+ transported to the shoot. Many mutations have an impact on Rb^+ translocation, which is indirectly originates from an effect on Rb^+ uptake. This aspect has been systematically overlooked and we propose that it should be taken into account in future studies. Secondly, the mutation of SKOR or a reduction of plant transpiration by high RH has a negative impact on Rb^+ uptake and *AtHAK5* expression in $-K^+$ plants. This reveals the presence of additional layers of regulation of K^+ uptake that should be further studied. It also suggests that K^+ translocation may act on K^+ uptake via a feedback mechanism to adapt K^+ uptake rates to the K^+ translocation ones. Lastly, an AKT1-dependent pathway for K^+ unloading from xylem vessels was described, which is likely to contribute to the fine regulation of K^+ transport to the shoots. Available data suggests that K^+ resorption from xylem vessels is widespread in land plants. Further study of K^+ uptake-translocation relationships will be of great help in improving crop performance within a context of limited resources and climate change.

Acknowledgements

This work was supported by grant numbers AGL2015-66434-R (to F.R.) and AGL2015-74011-JIN (to M.N.-C.) from the Spanish Ministry of Economy and Competitiveness. R.R. and A.L. are recipients of a F.P.U. Fellowship from the Spanish Ministry of Education, Culture and Sports. This work was also supported by the Network of Excellence BIO2016-81957-REDT. We thank Cristina López and Maria del Carmen García for her skillful technical assistance. We thank Dr. Navina Drechsler and Prof. Reinhard Kunze for providing *skor* and *nrt1.5* seeds, Dr. Yi Wang for providing *kup7* seeds and Dr. Hervé Sentenac for providing *pAKT1::GUS* construct. We also thank Mario Fon for English editing assistance.

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Supporting information

Table S1. Primer list.

Table S2. Biomass and net Rb^+ uptake rates of plants used in two-compartment experiments shown in Figure 7.

Figure S1. Overview of the two-compartment experiments shown in Figure 7.

Figure S2. Effect of high-humidity on net Rb^+ uptake and translocation rates of *akt1*,*athak5*, *akt1 athak5* and *skor* $-K^+$ plants.

Figure S3. *AKT1* expression levels among different root cell types.

Figure legends

Figure 1. Net Rb^+ rates for the uptake and translocation of Arabidopsis WT plants at two relative humidity levels and at two K^+ supplies. (a) Rb^+ uptake and translocation rates in WT Col-0 plants grown in the $+K^+$ solution. (b) Rb^+ uptake and translocation rates in WT Col-0 plants grown in the $-K^+$ solution. These experiments were performed either at 60%RH or at >95% RH. Rb^+ uptake and translocation rates were calculated based on total or shoot Rb^+ contents, respectively, as described in Ródenas *et al.* 2017. (c) net Rb^+ uptake and translocation rates at >95% RH expressed as a % of the corresponding rate observed at 60% RH. Shown are mean values ± SE and bars with different letters are significantly different at $P < 0.05$ according to Tukey's test. ns depicts not significant.

Figure 2. Relationship between shoot Rb^+ content and net Rb^+ uptake rates in Arabidopsis plants. WT Col-0 and T-DNA mutants affected in K^+ uptake and translocation systems were used in Rb^+ transport experiments. (a) Data obtained from $+K^+$ plants. (b) Data obtained from $–K^+$ plants. WT and *akt1 athak5* data were fitted to a hyperbolic equation (black line for WT and red for *akt1 athak5* plants in (a) and (b)) and yielded the following R^2 values 0.95 (WT $+K^+$), 0.87 (*akt1 athak5* $+K^+$), 0.93 (WT $–K^+$) and 0.95 (*akt1 athak5* $–K^+$). (c) Translocation efficiency factor (TEF) trends obtained for each fitted curve shown in (a) and (b). Shown are mean values ± SE .

Figure 3. Effect of high RH and *skor* mutation on net Rb^+ uptake rates and on *AtHAK5* and *AKT1* expression in roots. (a) Net Rb^+ uptake rates of WT and *skor* plants. (b) and (c) *AtHAK5* (b) and *AKT1* (c) expression in WT and *skor* roots determined by qRT-PCR. Expression levels are shown as $\log_2$(Fold-Change) of *AtHAK5* and *AKT1* with respect to the calibrator sample ($+K^+$ 60%RH) according to the $\Delta\Delta C_t$ method. Shown are mean values ± SE. ** denotes $P<0.01$ in Student's test. Letters indicates homogeneous group of data at $P < 0.05$ according to Tukey's test.

Figure 4. Rb^+ tissue distribution and expression of genes encoding transport systems involved in K^+ translocation in WT and *akt1 athak5* plants. (a) Rb^+ tissue distribution of

WT and *akt1 athak5* plants with comparable net Rb^+ uptake rates. (b) Rb^+ tissue distribution of WT and *akt1 athak5* plants with comparable Rb^+ translocation rates. Comparable net Rb^+ uptake and translocation rates were obtained at different K^+ supplies, external Rb^+ concentrations and relative humidity levels. In (a), external conditions were 1 mM Rb^+/>95%RH (WT $+K^+$), 1 mM Rb^+/60%RH (*akt1 athak5* $+K^+$), 0.02 mM Rb^+/>95%RH (WT $-K^+$) and 1 mM Rb^+/60%RH (*akt1 athak5* $-K^+$). In (b), external conditions were 1 mM Rb^+/60%RH (WT $+K^+$), 1 mM Rb^+/60%RH (*akt1 athak5* $+K^+$), 0.02 mM Rb^+/60%RH (WT $-K^+$) and 1 mM Rb^+/60%RH (*akt1 athak5* $-K^+$). (c) and (d) expression levels of *SKOR* (c) and *NRT1.5* (d) in WT and *akt1 athak5* roots determined by real-time PCR. Expression levels are shown as $\log_2$(Fold-Change) of *SKOR* and *NRT1.5* with respect to the calibrator sample ($+K^+$ 60%RH) according to the ΔΔCt method. Shown are mean values ± SE. ** denotes $P<0.01$ in Student's t-test. *ns* denotes not significant. Letters indicates homogeneous group of data at $P<0.05$ according to Tukey's test.

Figure 5. GUS staining of Arabidopsis WT roots expressing *pAtHAK5::GUS* ((a) and (c)) or *pAKT1::GUS* ((b) and (d)) constructs. Plants were grown in the presence ((a) and (b)) or absence ((c) and (d)) of K^+. Scale bar = 250µm

Figure 6. Shoot-to-root Rb^+ transport rates of WT, *athak5*, *akt1* and *akt1 athak5* plants. Rb^+ was added to the shoot and transport rates were calculated taking into account the Rb^+ content present in roots. (a) Data obtained from $+K^+$ WT and mutant plants. (b) Data obtained from $-K^+$ WT and mutant plants. Shown are mean values ± SE and bars with different letters are significantly different at $P<0.05$ according to Tukey's test. *ns* denotes not significant.

Figure 7. Rb^+ distribution in WT, *athak5*, *akt1* and *akt1 athak5* plants in two-compartment experiments. Roots were separated in two compartments (R-Rb^+ and R+Rb^+) and Rb^+ was added to the distal compartment (R+Rb^+). After 6h, each root compartment and the shoots were harvested separately. External Rb^+ concentration was adjusted to produce comparable net Rb^+ uptake rates among genotypes. (a) Data obtained from $+K^+$ WT and mutant plants. (b) Difference in the Rb^+ content in shoot (grey bar) and in the R-Rb^+ root (black bar) between mutant genotypes (*akt1* and *akt1 athak5*) and WT plants. This graph represents the relative weight of the Rb^+ that is not

reabsorbed in *akt1* and *akt1 athak5* R-Rb^+ roots (black bars) with respect to the excess of Rb^+ that is transported to the shoot in such mutant genotypes (grey bars). (c) Rb^+ distribution obtained in two-compartment experiments carried out with $–K^+$ WT and mutant plants. Shown are mean values ± SE and bars with different letters are significantly different at $P < 0.05$ according to Tukey's test. *ns* denotes not significant.

Figure 8. Hypothetic model for the regulation of K^+ uptake and translocation depending on the K^+-status of the plant. (a) in $+K^+$ plants, AKT1 constitutes the main K^+ uptake pathway in epidermal and cortex root cells. Within the stele, AKT1 contributes to K^+ retrieval from the xylem sap whereas AtHAK5 and SKOR contribute to K^+ translocation to the shoot. It remains unclear how an uptake system such as AtHAK5 takes part in K^+ translocation (indicated by a question mark), since a K^+ efflux system would fit better in the direct release of K^+ into xylem vessels. However, an indirect contribution of AtHAK5 to K^+ translocation by aiding in K^+ uptake in vascular cells before K^+ release into xylem vessels cannot be ruled out. Mutation of SKOR leads to lower *AKT1* expression levels which suggests the presence of an intercellular signal associated to SKOR activity (Green arrow) in the stele that modulates K^+ uptake in outer root cell layers. (b) in $–K^+$ plants, both AKT1 and AtHAK5 mediate K^+ uptake from root outer cell layers. Within vascular tissues, AKT1 does not play a significant role in K^+ resorption from xylem vessels whereas SKOR is involved in K^+ translocation to the shoot. Mutation of *SKOR* and reduced transpiration (due to high RH) give rise to lower *AtHAK5* mRNA levels in $–K^+$ plants. Thus, transpiration (blue arrow) and SKOR activity (Green arrow) seem to regulate K^+ uptake by controlling expression of *AtHAK5* in $–K^+$ plants. On the other hand, the mutation of *AKT1* and *AtHAK5* produced a downregulation of the *SKOR* gene, suggesting that K^+ uptake also has an influence on K^+ translocation regulation. It is tempting to speculate that K^+ uptake and translocation are linked in such a way that if one slows down the other follows suit. This regulation mechanism is particularly evident at the gene expression levels.