The quest for selective Cs⁺ transport in plants

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Nuclear accidents have led to the release to the environment of radioactive isotopes, mainly radioactive cesium (Cs⁺), contaminating large areas of soil for very long periods of time (Burger and Lichtscheidl, 2018). These contaminated soils contain ¹³⁴Cs⁺ and ¹³⁷Cs⁺ radioisotopes that are readily accumulated into plants and that, through the food chain, are transferred to animals and humans, causing important health problems (Burger and Lichtscheidl, 2018).

Solutions to this problem range from removal of radiocesium from the soil by physical or chemical technologies to extensive fertilizer application (Burger and Lichtscheidl, 2018). These strategies are costly, hazardous, time-requiring or detrimental for agricultural soils. Besides the use of soil microorganisms (Burger and Lichtscheidl, 2018), plant biotechnology could be considered as a promising alternative. Improved plants could be used in two ways: (i) as radiocesium hyperaccumulators to remove this contaminant from the soil and (ii) as radiocesium hypoaccumulators to produce food in a contaminated environment. For such approaches, the identification of the molecular pathways for Cs⁺ uptake at the root is crucial. The transport systems to be considered should mediate high-affinity transport because radiocesium is present at micromolar-nanomolar concentrations in the soil.

Because Cs⁺ and K⁺ share chemical properties, some K⁺ transport systems also allow Cs⁺ permeation (Burger and Lichtscheidl, 2018). Thus, many studies on Cs⁺ transport have focused on K⁺ transporters, such as HAK/KUP/KT transporters. HAK5-like transporters from different plant species, including the *Arabidopsis thaliana* AtHAK5, the *Oryza sativa* OsHAK1 and the *Solanum lycopersicum* SlHAK5, have been directly associated to root Cs⁺ uptake. These high-affinity transporters poorly discriminate between K⁺ and Cs⁺ and are able to transport Cs⁺ into the root from very diluted solutions (Qi et al., 2008; Nieves-Cordones et al., 2017; Nieves-Cordones et al., 2020). It is worth to take into account that the relative contribution of these transport systems to Cs⁺ and K⁺ accumulation in the plant varies among species. For example, rice *oshak1* and tomato *slhak5* CRISPR-generated KO-mutants accumulate much less Cs⁺ than wild-type plants but the former mutant is more affected in K⁺ nutrition (Nieves-Cordones et al., 2017; Nieves-Cordones et al., 2020).

Despite Cs^+ is not an essential nutrient for plants, there are evidences indicating that K^+ -independent Cs^+ transport systems do exist. For example, Cs^+ transport to vacuoles is selectively compromised after mutating the SEC22 protein in Arabidopsis (Draxl et al., 2013) and, in poplar, Cs^+ and K^+ transport are differentially regulated (Noda et al., 2016). Identification of the transport systems underlying K^+ -independent Cs^+ uptake pathways is of great interest because they could circumvent penalties on K^+ nutrition caused by mutating K^+ uptake systems. It is at this point where the study by Ashraf et al., 2021 makes a highly relevant and very interesting contribution. The authors selected transporters of the ABC family for their study due to their metal transport activity and broader substrate specificity. They focused on subfamilies ABCB and ABCG because they predominantly localize to the plasma membrane. A screening for Cs^+ tolerant plants among 37 Arabidopsis available mutants in ABCB and ABCG transporters allowed the identification of two members, ABCG33 and ABCG37, as involved in root Cs^+ uptake. They showed that the supply of Cs^+ affected the amount of protein of these two transporters at the plasma membrane. After a short-time exposure to Cs^+ , the protein abundance increased but with longer ones, its amount was reduced. This is an interesting posttranslational regulatory mechanism, which has been observed in transporters of heavy metals (Vallières and Avery, 2017) and that deserves further investigation because of its biotechnological potential. ABCG33 and ABCG37 exhibited functional redundancy and only the double *abcg33 abcg37* KO mutant showed lower Cs^+ uptake and reduced Cs^+ accumulation. Importantly, this double mutant was not affected in K^+ nutrition. Moreover, when ABCG33 and ABCG37 were expressed in yeast, they mediated high-affinity Cs^+ uptake but not $\text{K}^+(\text{Rb}^+)$ uptake. Nevertheless, ABCG33 and ABCG37 seemed to have other substrates besides Cs^+ (auxinic or phenolic compounds, for instance) (Hwang et al., 2016) and this aspect should be kept in mind for future biotechnological approaches based on these transport systems. It is worth to mention that, as a result of the screening carried out, a gain of function mutant of ABCG37 (*abcg37-1*, previously described as *pdr9-1*) was also selected. This mutant showed increased abundance of ABCG37 and higher Cs^+ accumulation, which may be of interest for developing Cs^+ -hyperaccumulating varieties to be used in phytoremediation. Importantly, this hyperaccumulating plants should also be endowed with enhanced cellular Cs^+ detoxification mechanisms. For example, glutathione accumulation (Adams et al., 2020) or sequestration of Cs^+ into vacuoles (Draxl et al., 2013) have been suggested as possible detoxification mechanisms. In conclusion, this study describes the existence of K^+ -independent Cs^+ root uptake systems, and expands our knowledge on the mechanisms for Cs^+ accumulation in plants. However, a successful strategy for a given plant species may not only rely on mutating a certain transport system but on acting on several transporters at the same time. A recent study identified in rice a member of the ABC family,

OsABCG45 as well as the non-specific metal transporter OsNRAMP4, the ammonium transporter OsAMT1;1 or the amino acid transporter OsANT4 as involved in Cs⁺ uptake (Yamaki et al., 2017). Therefore, the set of transport systems mediating root Cs⁺ uptake can be much larger than expected and further research is needed to identify them and to establish their relative contribution to root Cs⁺ uptake and to Cs⁺ toxicity, and the possible interactions with other nutrients. Higher order mutants should be generated to determine the degree of redundancy and to identify compensatory or additive effects. Once the relevant transporters have been identified, increase or decrease of their selectivity for Cs⁺ over other substrates can be achieved either by random mutagenesis or by site-directed mutagenesis driven by a structural model. An example of the first approach is a single point mutation (F130S) in the high-affinity K⁺ transporter AtHAK5. This mutant, isolated in a screening of random mutagenized AtHAK5 transporters, showed a higher K⁺/Cs⁺ selectivity than WT AtHAK5, leading to Cs⁺ tolerance in yeast (Aleman et al., 2014). Regarding the second one, structure-function studies in relevant root Cs⁺ uptake systems can offer new possibilities to modify their Cs⁺ transport capacity. For example, residues directly involved in K⁺ permeation or involved in shaping the selective filter were recently identified in AtHAK5 (Ródenas et al., 2021) and their mutation could be used to alter Cs⁺ transport. This kind of studies on ABCG33 and ABCG37 will also provide us with information on the structural basis for their K⁺-independent Cs⁺ transport capacity, which will be of great help in this matter. This type of approaches may also be applied to transporters involved in the accumulation of other toxic metals. Nevertheless, further research is needed to understand regulatory mechanisms such as those shown by Ashraf et al., 2021 that could impact overall transport of Cs⁺ by the plant. Besides, natural variation could be an additional source for hyper- or hypoaccumulator genotypes that together with gene edition approaches will assist researchers and breeders to develop biotechnological approaches for phytoremediation or to maintain food production in radiocesium contaminated environments.

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Figure Legend

Figure 1. Cs⁺ transport systems in plant roots and workflow for obtaining plants suited for radiocesium-contaminated environments. Root Cs⁺ uptake is a key step to achieve hyper- or hypoaccumulation of Cs⁺ in plants. Three main groups of transport systems seem to mediate Cs⁺ uptake in plant roots. ABCG33 and ABCG37 operate as Cs⁺-selective K⁺-independent transport systems in Arabidopsis. HAK K⁺ transporters poorly discriminate K⁺ over Cs⁺ and thus transport substantial amounts of Cs⁺. Its involvement in root Cs⁺ uptake has been demonstrated in several plant species. Besides these two transporter types, there is a group of additional unidentified transport systems that can mediate Cs⁺ uptake as well. These transport systems may belong to different gene families according to expression studies in yeasts and pharmacological features *in planta*. Once inside root cells, Cs⁺ can be stored in vacuoles where the SEC22 protein seems to play a specific role. Glutathione (GSH) and its intermediates have been suggested as a possible mechanism for Cs⁺ excess alleviation. Cs⁺ can also move to other cells and it can reach the stele where it is transported to aerial parts through xylem vessels. In order to use plants in Cs⁺-contaminated environments, a workflow is proposed. (1) Transport systems involved in Cs⁺ uptake and variants of interest may be identified in high-throughput screenings or in natural variation studies. (2) If the transport systems identified have additional substrates besides Cs⁺, random or structure-driven mutagenesis can be performed to increase or decrease their Cs⁺ transport capacity, depending on the expected use. For Cs⁺ hyperaccumulation in plants, selectivity of Cs⁺ over other substrates should be high whereas for Cs⁺ hypoaccumulation, the contrary is desired. (3) Once the transport systems have been improved, they should be tested *in planta* for validation (either by using gene-editing or overexpression strategies). Additionally, engineered plants could be combined with non-plant strategies such as microorganisms to further decrease Cs⁺ entry into the food chain.

Figure 1