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RESEARCH PAPER

Caesium and strontium accumulation in shoots of *Arabidopsis thaliana*: genetic and physiological aspects

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Abstract

Due to the physico-chemical similarities of caesium (Cs^+) to potassium (K^+) on the one hand and strontium (Sr^{2+}) to calcium (Ca^{2+}) on the other hand, both elements can easily be taken up by plants and thus enter the food chain. This could be detrimental when radionuclides such as ^{137}Cs and ^{90}Sr are involved. In this study, both genetic and physiological aspects of Cs^+ and Sr^{2+} accumulation in *Arabidopsis thaliana* were investigated using 86 *Arabidopsis* accessions and a segregating F_2 population of the low Cs^+ accumulating Sq-1 (Ascot, UK) crossed with the high uptaking Sorbo (Khurmatov, Tajikistan). Hydroponically grown plants were exposed to subtoxic levels of Cs^+ and Sr^{2+} using radioactive isotopes as tracers. In the natural accessions shoot concentration of Cs^+ as well as Sr^{2+} varied about 2-fold, whereas its heritability ranged for both ions between 0.60 and 0.73. Shoot accumulation of Cs^+ and Sr^{2+} could be compromised by increasing concentrations of their essential analogues K^+ and Ca^{2+}, respectively, causing a reduction of up to 80%. In the case of the segregating F_2/F_3 population Sq-1×Sorbo, this study identified several QTL for the trait Cs^+ and Sr^{2+} accumulation, with main QTL on chromosomes 1 and 5. According to the correlation and discrimination surveys combined with QTL-analysis Cs^+ and Sr^{2+} uptake seemed to be mediated mostly via non-selective cation channels. A polymorphism, affecting amino acids close to the K^+-pore of one candidate, CYCLIC-NUCLEOTIDE-GATED CHANNEL 1 (CNGC1), was identified in Sorbo and associated with high Cs^+ concentrating accessions.

Key words: Accumulation, *Arabidopsis*, caesium (Cs^+), discrimination, quantitative trait loci (QTL), strontium (Sr^{2+}).

Introduction

Radionuclide contamination of the environment can be caused by intentional releases of radionuclides by nuclear power testing, nuclear waste disposal, nuclear weapons production on the one hand, and nuclear accidents on the other hand. There is particular concern about radiocaesium (^{137}Cs) and radiostrontium (^{90}Sr) due to the long physical half-lives of these two major contaminants (^{137}Cs, $t_{1/2}$= 30.17 years and ^{90}Sr, $t_{1/2}$=28.64 years) and their similarity to the essential minerals, potassium (K^+) and calcium (Ca^{2+}), respectively, with which they share many physico-chemical properties. Therefore, they have a high biological availability. Both radionuclides can be taken up by plants and by animals, and it is postulated that potassium-related pathways are involved in the accumulation of Cs^+ (White and Broadley, 2000; Zhu and Smolders, 2000; Hampton *et al.*, 2005). In addition to the apoplastic pathway into the xylem, different Ca^{2+} channels are associated with Sr^{2+} accumulation (White, 2001; White *et al.*, 2002). After ingestion into the human body, the internal radiation exposure can be a major health hazard (UNSCEAR, 2000;

IAEA, 2006). Recent studies of different plant species as well as plant varieties have shown a huge variation in the uptake of ^{137}Cs and ^{90}Sr. Among distinct varieties within Poaceae species (rye, maize, wheat, and barley), for example, a variation of 1.5–4.3-fold for Cs^+ accumulation and 1.6–4.6-fold for Sr^{2+} accumulation was described (Rasmusson *et al.*, 1963; Prister *et al.*, 1992; Schimmack *et al.*, 2004; Gerstmann and Schimmack, 2006; Putyatin *et al.*, 2006; Schneider *et al.*, 2008). Among different plant families, the reported variation in Cs^+ accumulation can be up to 100-fold higher than the variation within different varieties (Broadley *et al.*, 1999). It is assumed that the traits for either Cs^+ or Sr^{2+} are controlled by a number of genes, which act together as a complex quantitative trait, since there are considerable variations for both Cs^+ and Sr^{2+} accumulation via the roots within different plant species (Schneider *et al.*, 2008). Following this assumption, it is hypothesized that it might be possible to select and/or breed crop plants with less radionuclide accumulation in their shoots, taking advantage of evolutionary developed properties. This might be a suitable countermeasure to reduce radiation exposure via the food chain.

To identify the genetic factors underlying differences in ion accumulation within the closely related individuals of one species, QTL analyses are useful. By relating polymorphic markers in a segregating population to the phenotypic properties of the individuals; genetic loci and genes can be identified which exert an impact on the trait of interest (Alonso-Blanco and Koornneef, 2000). By correlating the genetic data against the phenotypic data, information on both genetic and environmental effects of the trait and genetic loci (QTL) can be detected simultaneously (Payne *et al.*, 2004). For such studies either a population of immortalized progeny of recombinant inbred lines (RILs) in *Arabidopsis thaliana*, or genotyped F_2 individuals in combination with phenotypic data of the derived F_3 families can be used (Hayashi and Ukai, 1999). Until now, two linkage mapping approaches were carried out for Cs^+ accumulation in different RIL populations from *Arabidopsis thaliana* (White *et al.*, 2003; Payne *et al.*, 2004), but none for Sr^{2+} accumulation. The results of these mapping studies concerning Cs^+ accumulation had shown that genomic regions associated with Cs^+ enrichment depended on the genetic background of the RILs, but the significance of QTL peaks and their explained phenotypic variance was also influenced by the experimental set-up.

Arabidopsis accessions from different geographic regions all over the world reveal considerable genetic variation resulting from adaptation to various habitats (Alonso-Blanco and Koornneef, 2000). They represent a natural source, in contrast to laboratory-induced mutants, for the investigation of the genetic variation of specific and, in particular, multifactorial traits. The aim of this work was to gain more insights into the natural variation of Cs^+ and Sr^{2+} uptake and the influence of K^+ and Ca^{2+} in these processes, respectively. Furthermore, the focus of this study was the detection of additional quantitative loci with an impact on Cs^+ and Sr^{2+} uptake, using F_2/F_3 plants crossbred from two antithetically accumulating parents to detect additional genes with an impact on Cs^+ or Sr^{2+} accumulation besides the K^+ transporters and Ca^{2+} channels already identified by other studies (White *et al.*, 2003; Payne *et al.*, 2004).

Materials and methods

Plant material, cultivation, and analysis of Cs^+- and Sr^{2+}-concentrations

All experiments were carried out two to three times, (i) to determine either adequate growth and experimental conditions, (ii) to detect the natural variation within the accessions related to radionuclide uptake and the influence of K^+ and Ca^{2+}, respectively, and (iii) to identify QTLs with an impact on Cs^+ and Sr^{2+} accumulation. Eighty-six *Arabidopsis thaliana* accessions (N22564–N22571, N22581–N22585, N22587–N22659) according to Nordborg *et al.* (2005) were obtained from the European *Arabidopsis* Stock Centre (Nottingham, UK). These accessions were genotyped in an association mapping study (Aranzana *et al.*, 2005; Nordborg *et al.*, 2005). For uptake experiments, plants were grown hydroponically in a growth chamber (16/8 h light/dark regime; 23–25 °C; 65% relative humidity). Firstly, seeds were surface-sterilized using NaOCl (0.8% active hypochlorite), rinsed, and stratified in deionized water for 4–6 d at 5 °C to break dormancy. After swelling, seeds were sown on 1.5 ml Eppendorf tubes with a sawn tip containing 0.65% bacto agar (Becton, USA) (Tocquin *et al.*, 2003) and placed on racks in plastic beakers filled with a modified Hoagland's liquid medium, including 1.5 mM $Ca(NO_3)_2.4H_2O$, 1.25 mM KNO_3, 0.75 mM $MgSO_4.7H_2O$, 0.5 mM KH_2PO_4, 0.1 mM $Na_2O_3Si.9H_2O$, 0.072 mM Fe-EDTA, 0.05 mM KCl, 0.05 mM H_3BO_3, 0.01 mM $MnSO_4.H_2O$, 0.002 mM $ZnSO_4.7H_2O$, 0.0015 mM $CuSO_4.5H_2O$, 0.075 µM $(NH_4)_6Mo_{24}.4H_2O$, and 0.5 g l^{-1} MES at pH 5.7 (Gibeaut *et al.*, 1997). 10 d after sowing (DAS), the tubes were transferred to a modified Hoagland's medium supplemented with stable (chemo-toxicity and competition assay) or stable and radioactive isotopes (kinetics, accession screen, and phenotyping of F_3 families) of Cs^+ and Sr^{2+}, respectively, at the indicated concentrations. For chemo-toxicity tests, six different concentrations of CsCl (0, 0.01, 0.1, 0.3, 0.7, and 1.0 mM) and $SrCl_2$ (0, 0.01, 1.0, 10, 20, and 40 mM) were tested. Screening of accessions was conducted with modified Hoagland's liquid medium supplemented with Cs^+ [0.003 mM stable CsCl, radiolabelled with 14.2 kBq l^{-1} ^{134}Cs (AEA Technology, Germany) and Sr^{2+} (0.01 mM stable $SrCl_2$, spiked with 5.8 kBq l^{-1} ^{85}Sr (Perkin Elmer, USA)]. Cs^+ and Sr^{2+} were added together, since it was observed in several experiments, that they do not influence each other in their uptake (Eapen *et al.*, 2006; Singh *et al.*, 2008). For competition analyses, the medium was supplemented with 0.003 mM CsCl or 0.01 mM $SrCl_2$ and the indicated concentrations of K^+ or Ca^{2+}. To reduce $[K]_{medium}$ in Cs^+/K^+ competition experiments below 1.8 mM, KNO_3, KH_2PO_4, and KCl were successively replaced by $Ca(NO_3)_2$, NaH_2PO_4, and NaCl, to increase $[K^+]_{medium}$ KCl was added. To decrease $[Ca^{2+}]_{medium}$ in Sr^{2+}/Ca^{2+} competition experiments below 1.5 mM, $Ca(NO_3)_2$ was successively substituted by KNO_3, to increase $[Ca^{2+}]_{medium}$ $CaCl_2$ was used.

Finally, except for the kinetic study where shoots were harvested at 13–20 DAS, shoots were harvested at 20 DAS and dried in a lyophylizer Beta A (Christ, Osterode, Germany). When ^{134}Cs and ^{85}Sr were added as tracers to the medium, their tissue concentrations were determined in a well type gamma spectrometer WIZARD 1480 (Perkin Elmer, USA) by counting the γ-emissions of both radionuclides. To reduce the statistical counting error below 1%, each sample was measured with a count limit of 25 000 recorded counts, and because no discrimination

between different isotopes of Cs^+ and Sr^{2+} was detected in biological systems (White and Broadley, 2000; Soudek *et al.*, 2004, 2006), their tissue concentrations were directly estimated from the measured activity. Concentrations of stable isotopes in tissues were quantified after pressurized wet digestion of the sample material. To this end, samples were properly weighed into quartz vessels. Subsequently, 1 ml HNO_3, suprapure, subboiling distilled and 100 µl HCl, 33% (both Merck, Darmstadt), were added. The vessels were closed and introduced into a pressure digestion system (Seif, Unterschleissheim, Germany) for 10 h at 170 °C. The resulting clear solution was filled up to exactly 5 ml with double-distilled H_2O and was then ready for element determination (Schramel *et al.*, 1980; Schramel and Wendler, 1998). An ICP-AES 'Spectro Ciros Vision' system (SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, Germany) was used for Ca^{2+}, K^+, and Sr^{2+} determination in samples. Sample introduction was carried out using a peristaltic pump equipped with an 'anti-pulse-head' (SPETEC, Erding, Germany), connected to a Meinhard nebulizer with a cyclon spray chamber. The measured spectral element lines were: Ca^{2+}, 182.801 nm; K^+, 766.490 nm; Sr^{2+}, 407,771 nm. The RF power was set to 1000 W (Ca, 1400 W), the plasma gas was 15 l Ar min^{-1}, whereas the nebulizer gas was 0.6 l Ar min^{-1}. An ELEMENT 1, Thermo-Finnigan (Bremen, Germany) ICP-sf-MS instrument was employed for ^{133}Cs determination in the low resolution mode. Sample introduction was carried out using a peristaltic pump equipped with an 'anti-pulse-head' (SPETEC, Erding, Germany), connected to a Meinhard nebulizer with a cyclon spray chamber. The RF power was set to 1200 W, the plasma gas was 15 l Ar min^{-1}, whereas the nebulizer gas was 0.9 l Ar min^{-1}.

Every ten measurements three blank determinations and a control determination of a certified standard for all the elements mentioned were performed for quality control of ICP-AES and ICP-sf-MS. Calculation of results was carried out on a computerized lab-data management system, relating the sample measurements to calibration curves, blank determinations, control standards, and the weight of the digested sample.

All statistical data analyses were performed with the software package STATISTICA 6 (StatSoft, Tulsa, USA, 2004). Mann-Whitney *U* tests were used to check whether the data were statistically different at $P=0.05$ significance threshold. Normal distribution was confirmed by using Kolmogorov–Smirnov tests. Correlations were tested with Pearson correlation test and the effect of multiple predictors on a depending variable was studied with analyses of variance (ANOVA).

*Calculation of discrimination factor (*DF*) and transfer factor (*TF*)*

The transfer factor provides information about the extent of accumulation in plants in comparison to the medium. Transfer factors (*TF*) for Cs^+ and Sr^{2+} were related to fresh weight (FW).

$$TF(Cs) = \frac{\text{Caesium concentration (FW)}}{\text{Caesium concentration (medium)}}$$

$$TF(Sr) = \frac{\text{Strontium concentration (FW)}}{\text{Strontium concentration (medium)}}$$

To estimate the discrimination or selectivity of absorption and uptake of two ions in roots, a discrimination factor (*DF*) was calculated according to (Smolders *et al.*, 1996). Discrimination factors for Cs^+ and Sr^{2+} were related to dry weight (DW). *DF* value of unity indicates that no discrimination occurs. *DF* values below unity indicate a more selective K^+ or Ca^{2+}, respectively. The smaller the *DF* values are the larger is the discrimination against Cs^+ or Sr^{2+}, respectively. *DF* does not necessarily reflect the absolute amount of accumulated ions, for example, Sr^{2+} versus Ca^{2+}, since it is dependent on both Sr^{2+} and Ca^{2+}.

$$DF(Cs) = \frac{\text{Caesium concentration (DW)}/\text{Potassium concentration (DW)}}{\text{Caesium concentration (medium)}/\text{Potassium concentration (medium)}}$$

$$DF(Sr) = \frac{\text{Strontium concentration (DW)}/\text{Calcium concentration (DW)}}{\text{Strontium concentration (medium)}/\text{Calcium concentration (medium)}}$$

Dissection of the complex genetic traits 'Cs^+ and Sr^{2+} accumulation' by performing QTL-analyses

As an approach for detecting the underlying genetic factors of the traits 'Cs^+ and Sr^{2+} accumulation' a QTL-analysis of a segregating F_2 population was conducted. To construct such an F_2 population, the two accessions Sorbo (N22653) and Sq-1 (N22600), which significantly differ in their Cs^+ accumulation in shoots, were selected as parents. The offspring included 108 F_2 individuals that were used for genotyping and 108 related F_3 families that were used for phenotyping. 75 CAPS (cleavage amplified polymorphic sequence) and three DFLP (DNA fragment length polymorphism) markers for genotyping were designed according to the DNA fragments sequenced by Nordborg (Nordborg *et al.*, 2005). The genetic linkage map obtained, covering in total 366.3 cM (see Supplementary Fig. S1 and Supplementary Tables S1 and S2 at *JXB* online), was calculated with the Kosambi mapping function of the software JoinMap 4 (Van Ooijen, 2006). Distances between markers were (mean) 4.69 cM and (maximum) 13.2 cM.

For phenotyping, five to seven plants of each F_3 family were hydroponically grown and harvested at 20 DAS. Three independent replications in a randomized design were carried out. Finally, a QTL analysis was carried out with MapQTL 5 software (Van Ooijen, 2004), performing a parametric approximate multiple QTL mapping (MQM) with all genotypic data (78 markers) and phenotypic data (mean of three replications of the accumulations). The genome-wide 5% significance threshold of the LOD scores was calculated on 2000 permutations to avoid the probability of a type I error.

Results

Due to their chemical and physical similarities to essential elements like K^+ (for Cs^+) and Ca^{2+} (for Sr^{2+}), Cs^+ and Sr^{2+} are taken up by plants although they are not relevant for any cellular function as yet known. Radionuclide accumulation in the shoots of plants is governed by diverse processes encompassing uptake and efflux by the root, intracellular storage or transport within the plant. Several different transporters and channels with variable specificity for the ions Cs^+ and Sr^{2+} have been discussed and might be involved in these processes. Therefore, a large number of genes are possibly involved in the natural variation of Cs^+ and Sr^{2+} accumulation in *Arabidopsis*, and to narrow down on these genes, a QTL analysis is useful (White, 2001; White *et al.*, 2004).

Establishment of assay conditions to measure Cs^+ and Sr^{2+} accumulation in Arabidopsis

Since it is well known that the non-essential ions Sr^{2+} and Cs^+ can be toxic, a growth assay was conducted with increasing concentrations of either Cs^+ or Sr^{2+} to determine the toxic levels for *Arabidopsis thaliana* under the

hydroponic growth conditions used in this study. Ten days after transferring the plants to Cs^+ or Sr^{2+} containing modified Hoagland medium (20 DAS), the *Arabidopsis thaliana* accession L*er*-1 (N22618) showed visible symptoms like chlorosis and necrotic leaves as well as growth depression at Cs^+ concentrations higher than 0.3 mM in accordance with a previous study (Hampton *et al.*, 2004). By contrast, Sr^{2+} concentrations higher than 10 mM only led to a depression of growth but not to chlorotic effects (data not shown). The toxicity analysis for Cs^+ and Sr^{2+} showed that the widely used accession L*er*-1 tolerated Sr^{2+} concentrations up to 1 mM without any visible symptoms. To avoid toxic effects all following experiments were performed using 0.003 mM Cs^+ and 0.01 mM Sr^{2+}, which is in the range of naturally occurring concentrations in the soil (Kabata-Pendias and Pendias, 1984).

Similar to Payne *et al.* (2004) the shoot fresh weight of accession L*er*-1 (N22618) almost increased exponentially for 22 DAS and shoot Cs^+ and Sr^{2+} concentration rose with plant age until saturation occurred at 20 DAS (see Supplementary Fig. S2 at *JXB* online). This approximately constant Cs^+ concentration based on FW might be explained by a balance of the relative accumulation rate for absolute Cs^+ content and the relative growth rate at this stage. Furthermore, when the relative growth rate was at maximum and Cs^+ concentration remained approximately the same, the contribution of non-genetic variance components to the phenotypic variation in shoot Cs^+ concentration was likely to be greatest. Thus, 20 DAS was selected as the time point to quantify the constant Cs^+ and Sr^{2+} concentration of 86 different accessions.

Quantifying the natural genetic variation in Cs^+ and Sr^{2+} concentration within Arabidopsis thaliana

To dissect the natural variation in Cs^+ and Sr^{2+} concentration, the shoots of 86 *Arabidopsis thaliana* accessions (according to Nordborg *et al.*, 2005), representing the worldwide distribution of *Arabidopsis thaliana* were analysed. The 86 accessions varied 1.9-fold in Cs^+ concentration (Fig. 1A), quantified by the maximum–minimum ratio of accumulation, with accessions Tamm-2 (N22604) and Sorbo (N22653) showing the lowest (35.3 nmol g^{-1} FW) and the highest (66.6 nmol g^{-1} FW) Cs^+ concentration, respectively (see Supplementary Table S3 at *JXB* online). Shoots of the accessions Wa-1 (N22644) and Omo2-3 (N22585) contained the lowest (273.3 nmol g^{-1} FW) and the highest (586.8 nmol g^{-1} FW) Sr^{2+} content, respectively (see Supplementary Table S3 at *JXB* online), representing the observed variation by a factor 2.1 (Fig. 1B). Due to a normal distribution of the Cs^+ and Sr^{2+} concentrations within the accessions, a Pearson correlation analysis was conducted revealing almost no correlation (r=0.482, P=0.002) between the enrichment of Cs^+ and Sr^{2+}. Some accessions showed a clear distinction in Cs^+ and Sr^{2+} accumulation, for example, accession Ga-0 (N22634), indicated by a relatively low Cs^+ concentration along with a relatively high Sr^{2+} content (Fig. 1C).

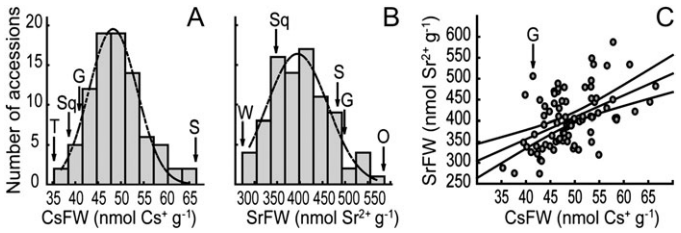

Fig. 1. Frequency distributions of Cs^+ (CsFW) and Sr^{2+} (SrFW) concentration g^{-1} fresh weight (FW) of shoots of *Arabidopsis thaliana* accessions (n=86) grown on nutrient replete medium supplemented with 3 μM Cs^+ and 10 μM Sr^{2+}, respectively. (A) Arrows indicate the values of Cs^+ concentration in the accessions Tamm-2 (N22604)=T; Ga-0 (N22634)=G; Sq-1 (N22600) =Sq, and Sorbo (N22653)=S. (B) Arrows indicate the values of Sr^{2+} concentration in the accession Wa-1 (N22644)=W; Ga-0 (N22634)=G, Omo2-3 (N22585)=O, Sq-1 (N22600)=Sq, and Sorbo (N22653)=S. Dashed curves show Gaussian distribution. According to Kolmogorov Smirnov tests both Cs^+ and Sr^{2+} concentration were normally distributed. (C) Pearson correlation analysis.

Transfer factors (*TF*) obtained in the screening, which are parameters for the accumulation of a special element in relation to the medium or soil, ranged between 11.8 and 22.2 for Cs^+ and between 27.3 and 58.7 for Sr^{2+} (see Supplementary Table S3 at *JXB* online), demonstrating a strong accumulation of both ions in *Arabidopsis* shoots from plants grown under hydroponic conditions.

Heritability, which is estimated as the fraction of the genotypic variance of the total phenotypic variance, was calculated according to the method introduced by Schneider *et al.* (2008). Therefore, an assessment of a two-factorial ANOVA with the variables accession/genotype and replication was used. The calculated heritability ranged between 0.69 for Cs^+ concentration (FW) and 0.73 for Sr^{2+} concentration according to FW, indicating that in the variety of 86 different accessions 69% and 73% of the phenotypic variance was contributed by the genetic variance, respectively. The heritability estimated in this study was in a similar range to that reported for K^+ concentration in *Arabidopsis* grown under connatural conditions (Harada and Leigh, 2006).

Potassium competition with Cs^+ uptake

After rescreening the extreme Cs^+ accumulating accessions, three accessions were selected for further investigation. Firstly, the very highly accumulating Sorbo (N22653), secondly, Sq-1 (N22600) exhibiting very low Cs^+ concentrations, and thirdly, L*er*-1 (N22618), a well-characterized accession used for normalizing the initial ecotype screen, were chosen to elucidate whether the Cs^+ accumulation was dependent on $[K^+]_{medium}$. In these competition analyses, Cs^+-containing growth media (0.003 mM) were supplemented with different K^+ concentrations, which caused a significant reduction in Cs^+ accumulation in shoots by up to 76% relative to the data of $[K^+=0]_{medium}$ (Fig. 2A). In all three accessions, the reduction was largest between 0 mM

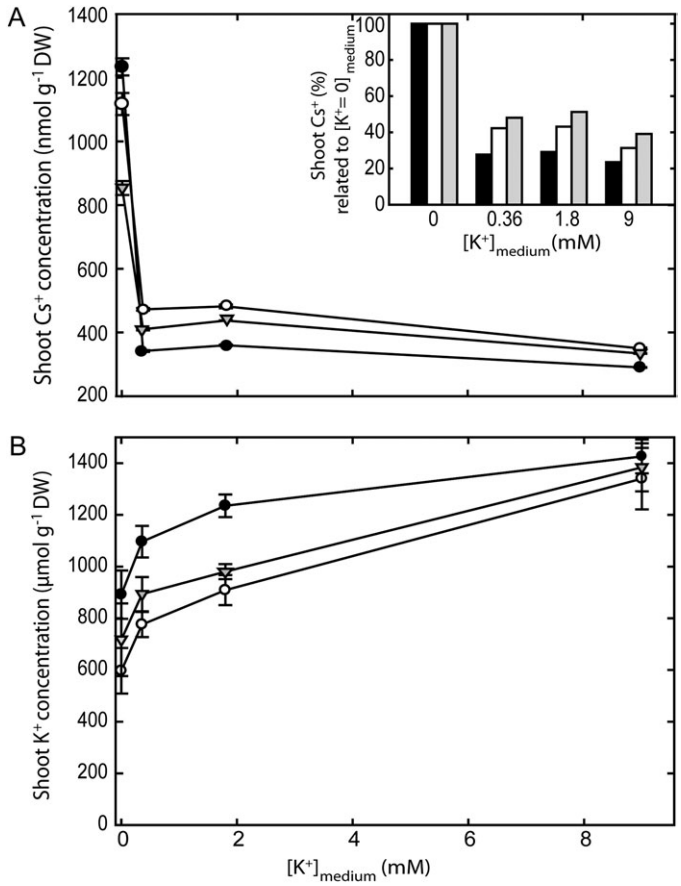

Fig. 2. K^+ competition analysis and K^+ concentration in shoot with different $[K^+]_{medium}$. (A) Shoot Cs^+ concentration of *Arabidopsis thaliana* grown for 10 d in normal nutrient replete medium and another 10 d in nutrient replete medium with different potassium concentrations (0 mM, 0.36 mM, 1.8 mM, and 9 mM) supplemented with 3 µM ^{133}Cs (stable isotope). (B) Shoot potassium concentration of different *Arabidopsis thaliana* accessions grown for 10 d on normal nutrient replete medium and further 10 d on nutrient replete medium supplemented with increasing concentrations of $[K^+]_{medium}$ (0 mM, 0.36 mM, 1.8 mM, and 9 mM). Closed circles and black column, low Cs^+ accumulating accession Sq-1 (N22600); open circles and white column, high Cs^+ accumulating accession Sorbo (N22653); triangles and grey column, accession L*er*-1 (N22618). Mean values ±SD (n=3, ten plants each) are shown.

and 0.36 mM $[K^+]_{medium}$. At $[K^+]_{medium}$ greater than 0.36 mM only a slight, continuing reduction was observed. The high Cs^+ concentration of Sq-1 at 0 mM $[K^+]_{medium}$ might be due to the fact that Sq-1 is able to accumulate Cs^+ in large amounts when no K^+ is available, but when K^+ is accessible it is much more favoured over Cs^+, reflected by the low DF. Therefore Sq-1 might have a more functional discrimination system. Shoot K^+ concentration of all three accessions analysed increased significantly with rising $[K^+]_{medium}$. In contrast to Hampton *et al.* (2004), no significant differences were detected in this study for shoot dry weight (DW) in relation to increasing $[K^+]_{medium}$ according to the analysis of variance (ANOVA; data not shown). This might be due to the fact that plants were transferred to different $[K^+]_{medium}$ after growth in standard medium (1.8 mM K^+) for 10 d resulting in a particular amount of K^+ in the shoot before the treatment. This might prevent growth delays or differences in growth. Accordingly, the value at 0 mM $[K^+]_{medium}$ reflected the K^+ accumulation before the treatment and the loss of K^+ from the plant during the treatment (Karley and White, 2009). Between 0 and 9 mM $[K^+]_{medium}$ a 1.7-fold increase for Sq-1 and a 2.5-fold increase for Sorbo in shoot K^+ concentration was observed, respectively. The low Cs^+ accumulating accession Sq-1 accumulated significantly more K^+ than Sorbo at 0 mM, 0.36 mM, and 1.8 mM $[K^+]_{medium,}$ in contrast to 9 mM $[K^+]_{medium}$, where no significant differences could be detected (Fig. 2B). In addition, the accessions analysed exhibited altered ion levels under normal growth conditions. Sq-1 showed a significantly elevated K^+ content (1.37-fold, P=0.02; Sq-1, average 60 225 mg kg^{-1}; Sorbo, average 43 925 mg kg^{-1}), but had a notable lower Ca^{2+} content (0.86-fold, P=0.02; Sq-1, average 25 300 mg kg^{-1}; Sorbo, average 29 100 mg kg^{-1}) as well as a lowered Mg^{2+} content (0.89-fold, P=0.04; Sq-1, average 5480 mg kg^{-1}; Sorbo, average 6095 mg kg^{-1}) compared with Sorbo.

With increasing $[K^+]_{medium}$, the discrimination factors for $[Cs^+/K^+]$ increased by a factor of 16.5 (Sq-1), 10.7 (Sorbo), and 13.2 (L*er*-1), respectively, implying that at 9 mM $[K^+]_{medium}$ ($DF_{Cs/K}$: Sq-1=0.610; Sorbo=0.783; L*er*-1=0.724) all accessions were 10.7–16.5-fold more selective for Cs^+ than at 0.36 mM ($DF_{Cs/K}$: Sq-1=0.037; Sorbo=0.073; L*er*-1=0.055). On the other hand, when comparing different accessions, Sq-1 was about 2-fold less selective for Cs^+ at $[K^+]_{medium}$ of 0.36 and 1.8 mM than Sorbo, and even in the presence of 9 mM external $[K^+]_{medium}$ Sq-1 showed a 1.3-fold higher discrimination against Cs^+ than Sorbo (Table 1).

Calcium competition with strontium uptake

Likewise, the dependency of Sr^{2+} uptake on the Ca^{2+} system was examined. The competition effect of Ca^{2+} on the Sr^{2+} accumulation was investigated in detail for three accessions, which showed opposite behaviour in the initial accession screening: Wa-1 (N22644) with low Sr^{2+} accumulation and Omo2-3 (N22585), which showed high Sr^{2+} concentration, as well as L*er*-1 (N22618) were used for normalization in the accession screen. By increasing $[Ca^{2+}]_{medium}$ from 0 mM to 7.5 mM, the shoot Sr^{2+} concentration of all three accessions decreased by about 80%, with a significant reduction in all three accessions between 0 mM and 1.5 mM and between 1.5 mM and 7.5 mM (Fig. 3A). As expected, shoot Ca^{2+} concentrations were significantly enhanced with increasing Ca^{2+} concentrations in the medium; merely the low rise of Omo2-3 between 1.5 mM and 7.5 mM $[Ca^{2+}]_{medium}$ was not significant. From 0–7.5 mM $[Ca^{2+}]_{medium}$, the Ca^{2+} concentration was elevated 3.2-fold for both accessions, Wa-1 and Omo2-3. For the accession L*er*-1, a 4.5-fold boost was detected. The high Sr^{2+} accumulating accession Omo2-3 enriched significantly more Ca^{2+} in shoot tissue than Wa-1

Table 1. Discrimination factors (*DF*) for [Cs^+/K^+] discrimination of the low Cs^+ accumulating accession Sq-1 (N22600), the high Cs^+ concentrating accession Sorbo (N22653), and L*er*-1 (N22618) for different total $[K^+]_{medium}$ and constant $[Cs^+]_{medium}$ (3 μM), as well as *DF* for [Sr^{2+}/Ca^{2+}] discrimination of the low Sr^{2+} acquiring accession Wa-1 (N22644), the high Sr^{2+} uptake accession Omo2-3 (N22585), and L*er*-1, with varying total $[Ca^{2+}]_{medium}$ and constant $[Sr^{2+}]_{medium}$ (10 μM)

$[K^+]_{medium}$	Accession	*DF* [Cs^+/K^+]	$[Ca^{2+}]_{medium}$	Accession	*DF* [Sr^{2+}/Ca^{2+}]
0.36 mM	Sq-1	0.037	0.3 mM	Omo2-3	0.338
	Sorbo	0.073		Wa-1	0.412
	L*er*-1	0.055		L*er*-1	0.442
1.8 mM	Sq-1	0.175	1.5 mM	Omo2-3	0.799
	Sorbo	0.318		Wa-1	0.867
	L*er*-1	0.268		L*er*-1	0.816
9 mM	Sq-1	0.610	7.5 mM	Omo2-3	0.993
	Sorbo	0.783		Wa-1	1.037
	L*er*-1	0.724		L*er*-1	1.043

at 0 mM, 0.3 mM, and 7.5 mM $[Ca^{2+}]_{medium}$ (Fig. 3B), whereas no significant distinction was detected at 1.5 mM $[Ca^{2+}]_{medium}$ between these two accessions. According to ANOVA, increasing $[Ca^{2+}]_{medium}$ did not significantly influence shoot dry weight (data not shown). There was still a detectable discrimination between Sr^{2+} and Ca^{2+} at $[Ca^{2+}]_{medium}$ of 0.3 and 1.5 mM (Table 1). Between $[Ca^{2+}]_{medium}$ of 0.3 mM and 7.5 mM, *DF*s increased 2.4–2.9-times for all three accessions. At 7.5 mM $[Ca^{2+}]_{medium}$ no discrimination between Sr^{2+} and Ca^{2+} was detectable. Interestingly, while there was no discrimination at $[Ca^{2+}]_{medium}$ of 7.5 mM, Sr^{2+} concentration was reduced to about 20% related to the 0 mM $[Ca^{2+}]_{medium}$ (Fig. 3A).

Detecting underlying genetic factors of Cs^+- and Sr^{2+}-concentration by QTL-analyses

Several traits for Cs^+ and Sr^{2+} concentration, according to fresh weight (FW), dry weight (DW), and tissue water (TW) were analysed to identify the genetic loci involved in the variation of Cs^+ and Sr^{2+} content in shoots. A segregating F_2 popluation with corresponding F_3 families was produced by cross-breeding the low Cs^+ accumulator Sq-1 (N22600) and the high Cs^+ accumulating accession Sorbo (N22653). These parental accessions were not significantly distinct in their Sr^{2+} accumulation. Cs^+ and Sr^{2+} concentration data were collected from the resulting F_3 families and a QTL analysis was conducted. Traits were named using the ion analysed, together with the abbreviation of the reference weights (FW, DW, and TW). Among Sq-1×Sorbo F_3-families a 1.3–1.7-fold variation in Cs^+ concentration according to FW, TW, and DW was detected. For the trait Sr^{2+} enrichment, a phenotypic segregation and variation by a factor of 1.7 and 1.8 were detected in relation to FW and DW, respectively (Table 2). Following Kolmogorov–Smirnov tests, the traits Cs^+ and Sr^{2+} concentration approximated a normal distribution. Transgressive segregation occurred for the traits CsFW, SrFW, SrDW, plant FW, and to a lesser extent for CsTW (Fig. 4).

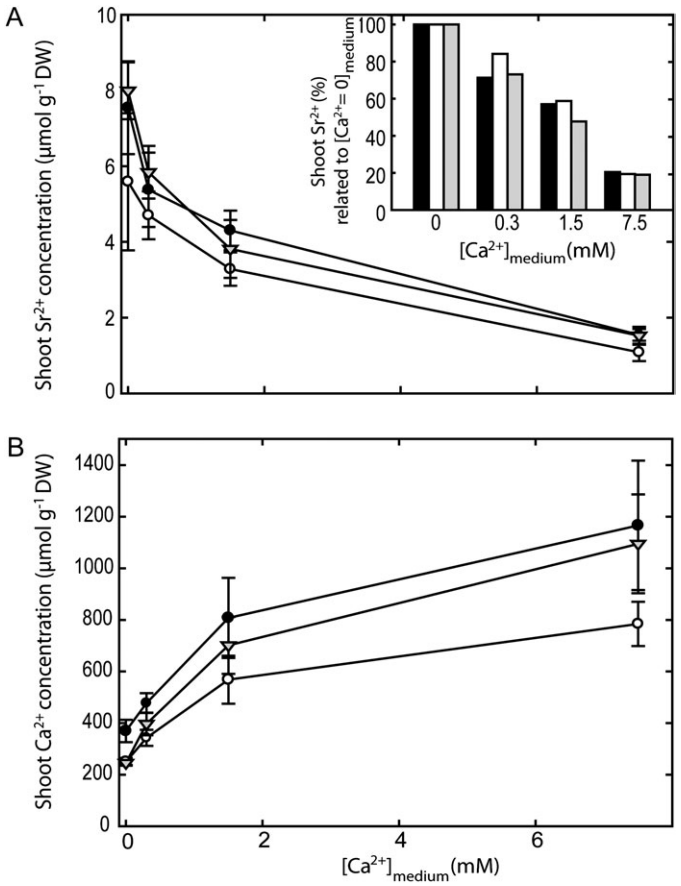

Fig. 3. Ca^{2+} competition analysis and Ca^{2+} concentration in shoot with different $[Ca^{2+}]_{medium}$. (A) Shoot Sr^{2+} concentration of *Arabidopsis thaliana* grown for 10 d in normal nutrient replete medium and for further 10 d in nutrient replete medium with different calcium concentrations (0 mM, 0.3 mM, 1.5 mM, and 7.5 mM) supplemented with 10 μM ^{88}Sr (stable isotope). (B) Plants are grown for 10 d in normal nutrient replete medium and for another 10 d in nutrient replete medium with increasing Ca^{2+} concentration (0 mM, 0.3 mM, 1.5 mM, and 7.5 mM). Closed circles and black bars, high Sr^{2+} concentrating accession Omo2-3 (N22585); open circles and white bars, low Sr^{2+} concentrating accession Wa-1 (N22644); triangles and grey bars, accession L*er*-1 (N22618). Mean values ±SD (n=3, ten plants each) are shown.

Out of the 108 F_3 families, 45 F_3 families representing the whole Cs^+ enrichment spectrum were investigated further. Cs^+ and K^+ contents were almost not correlated (correlation factors between –0.347 and 0.211, depending on the trait FW, TW, or DW). In contrast to this, however, positive correlations between Cs^+ and Ca^{2+} concentrations were observed (0.495, P=0.01 to 0.754, P=0.001), as well as for Cs^+ and Sr^{2+} (0.474, P=0.01 to 0.784, P=0.001) (see Supplementary Table S4 at *JXB* online). Furthermore, a strong and significant correlation of Sr^{2+} and Ca^{2+} accumulation was detected either related to FW or DW (0.782–0.932, P=0.001). No or only a very weak negative correlation between K^+ and Sr^{2+} (–0.350, P=0.05 to 0.139 not significant) and K^+ and Ca^{2+} (–0.498, P=0.001 to 0.062 not significant) was observed.

Table 2. Statistical parameters for Cs^+ concentration and Sr^{2+} concentration of F3-families and the corresponding parental accessions

Trait	Sorbo ±% SD[a]	Sq-1 ±% SD[a]	F_3-family ±% SD[a]	Least value[a,e]	Maximum value[a,e]	V_G [b,e]	V_{GY}[c,e]	H^2 [d,e]
CsFW	68.1±18.4	43.2±7.2	57.6±9.2	47.1	79.1	25	85	0.71
CsTW	75.6±13.8	51.9±9.0	63.5±9.6	51.8	89.7	30	112	0.73
CsDW	677.5±18.8	507.7±17.2	595.2±6.6	520.11	692.2	1821	4578	0.60
SrFW	470.3±9.6	353.2±2.6	413.0±10.2	322.0	534.4	199745	643932	0.69
SrDW	4451.1±12.1	4101.8±14.5	4286.4±10.9	3214.1	5605.4	0.50	1.31	0.62

[a] Mean concentrations are shown in nmol g^{-1}.
[b] Variance associated with the genotype effect.
[c] Variance associated with genotype and replication.
[d] H^2, heritability.
[e] According to F_3-families.

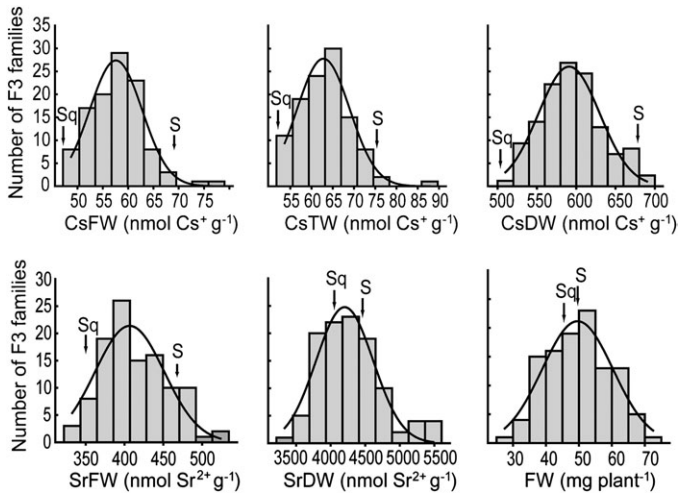

Fig. 4. Frequency distributions of Cs^+ (CsFW, CSTW, and CsDW)) and Sr^{2+} (SrFW and SrDW) concentration g^{-1} fresh weight (FW), tissue water (TW), and dry weight (DW) of shoots of *Arabidopsis thaliana* F_3 families as well as their shoot fresh weight, grown on nutrient replete medium supplemented with 3 μM Cs^+ and 10 μM Sr^{2+}, respectively. Arrows indicate the values of Sq-1 (N22600)=Sq and Sorbo (N22653)=S. Black curves show Gaussian distribution. According to Kolmogorov–Smirnov tests all traits were normally distributed.

QTL analysis

Test statistics and thresholds for QTL evidence as well as the genetic effect and percentage of variance explained by the QTLs were calculated by MapQTL5 for all traits using a genetic map of Sq-1×Sorbo based on 78 markers with JoinMap4 covering 366.3 cM (Van Ooijen, 2004, 2006) (see Supplementary Fig. S1, Supplementary Tables S1 and S2 at *JXB* online). In the case of the Cs^+ traits, several putative QTLs exceeding a LOD value ⩾3.1 were allocated to three chromosomes. QTLs were named using the trait abbreviation annexed by the chromosome number and by an ordering number from top to bottom (Table 3). Putative QTLs on chromosome 1 CsFW1.1 and CsTW1.1, which co-localized, were very close to CsDW1.1 (Fig. 5) and explained 9%, 10.2%, and 9.8% of the total variance of their traits, respectively (Table 3). These QTLs represent the major QTLs for Cs^+ concentration in *Arabidopsis* shoots. CsFW1.3 likewise coincided with CsTW1.2, and CsTW3.1 was concordant to a non-significant QTL peak of the trait CsFW. CsTW5.1 was overlapping to the non-significant QTL of CsFW. The QTLs CsFW5.1 and CsTW5.2 were concordant. There was just one positive genetic effect of the Sq-1 allele on CsTW5.1. For the Sr^{2+} traits some putative QTLs were detected as well (Fig. 6). SrFW1.2 overlapping to SrDW1.1 on chromosome 1 explained 14.8% and 14.5% of the total variance, respectively and the QTLs SrFW5.2 and SrDW5.1 explained 14.6% and 12.3% of variance, respectively. In addition, the QTL SrFW2.1 displayed a positive genetic effect of Sq-1. The QTLs CsFW1.2, CsDW1.2, (CsTW), and SrFW1.2 as well as SrDW1.2 showed the same peak maxima and CsFW5.1, CsTW5.2, and SrFW5.2 coincided.

Based on theoretical models as well as physiological studies, candidate genes with an impact on Cs^+ concentration are thought to express monovalent cation transporters localized in the tonoplast or the plasma membrane of root cells (White and Broadley, 2000; Broadley *et al.*, 2001). In the case of Sr^{2+} enrichment, depolarization-activated and hyperpolarization-activated Ca^{2+} channels as well as depolarization-activated outward-rectifying K^+ channels (KORCs), cyclic-nucleotide gated channels (CNGCs), and ionotropic glutamate receptors (GLRs) homologues are proposed to be involved (White *et al.*, 2002). According to the performed QTL analysis, at least 16 QTLs with a possible impact on Cs^+ and/or Sr^{2+} accumulation were compiled (Table 3). *In silico* analysis detected several candidate genes, which are putatively involved in the uptake and accumulation of Cs^+ and Sr^{2+} in *Arabidopsis* shoots, when only ion permeation is taken into account. Within the QTL peak areas, K^+ transporters and channels, which may be involved in the Cs^+ accumulation, as well as putative Sr^{2+} permeable channels known to participate in Ca^{2+} uptake and were also expressed in root tissue, have been pointed out (Table 4).

A deeper view on two candidate genes

Three QTLs related to the traits CsFW and CsTW nicely overlapped with high LOD scores. Only CsFW1.3/CsTW1.2 and CsFW5.1/CsTW5.2 contained genes encoding membrane proteins putatively involved in K^+ permeation and, therefore, also possibly involved in Cs^+ permeation. These two candidate genes (*ATCHX16* and *CNGC1*), were chosen

Table 3. Quantitative trait loci (LOD >3.1) affecting the concentration of Caesium and strontium in 3-week-old F_3 plants of a crossbreeding of Sq 1 ×Sorbo

Positive (+) effects indicate that Sq-1 alleles at that marker increased levels of caesium and strontium ions, negative (–) effects indicate that Sorbo alleles increased the caesium and strontium ion content. The percentage of explained variance is shown for all identified QTL (PVE).

Trait	QTL	Nearest marker	Position (cM)	LOD	PVE (%)	Effect
CsFW	CsFW1.1	At1g17840	21.3	7.6	9.0	–
	CsFW1.2	At1g43780	56.7	3.2	3.4	–
	CsFW1.3	At1g66245	86.6	6.3	7.6	–
	CsFW5.1	At5g54240	70.5	5.4	5.8	–
CsTW	CsTW1.1	At1g17840	21.3	7.7	10.2	–
	CsTW1.2	At1g66245	86.6	6.0	8.4	–
	CsTW3.1	At3g15260	20.0	4.7	6.4	–
	CsTW5.1	At5g05350	0.0	3.5	4.0	+
	CsTW5.2	At5g54240	70.5	6.4	8.0	–
CsDW	CsDW1.1	At1g20730	25.3	6.0	9.8	–
	CsDW1.2	At1g43780	56.7	6.3	11.7	–
	CsDW3.1	At3g53190	79.3	5.2	10.8	–
SrFW	SrFW1.1	At1g08520	11.1	7.0	8.6	–
	SrFW1.2	At1g43780	56.7	11.1	14.8	–
	SrFW2.1	At2g45340	52.7	6.7	8.3	+
	SrFW3.1	At3g53190	79.3	4.0	5.2	–
	SrFW4.1	At4g26750	43.3	3.5	3.7	+
	SrFW5.1	At5g44310	53.8	3.2	3.6	+
	SrFW5.2	At5g54240	70.5	8.1	14.6	–
SrDW	SrDW1.1	At1g43780	55.6	5.1	14.5	–
	SrDW1.2	At1g71350	102.7	4.3	12	+
	SrDW5.1	At5g24400	26.8	4.4	12.3	–

to look for polymorphisms, which would result in a change of an amino acid (AA) or any other possibly discriminating mutation. For this purpose, the parental alleles were sequenced. For *ATCHX16* only one polymorphism was identified leading to the exchange of the encoded amino acids R^{692} (Sq-1 and EMBL ID: Q1HDT3-1) and M^{692} (Sorbo). This residue is located in a non-conserved part of the hydrophilic carboxyterminal domain (Sze *et al.*, 2004). By contrast, *CNGC1* on chromosome 5 (CsFW5.1; CsTW5.2, and SrFW5.2) displayed more interesting differences in the nucleotide sequence of Sorbo (N22653) compared with Sq-1 (N22600), resulting in a change of five amino acids (Fig. 7). The residues E^{178}, N^{297}, P^{298}, P^{299}, and S^{302} of Sq-1 were replaced by Q^{178}, S^{297}, S^{298}, T^{299}, and P^{302} in the accession Sorbo. Amino acids 297–302 are located in an external loop between transmembrane domains S5 and S6 close to the K^+-transporting pore (P). Analysis of the hydropathy of the proteins indicated a slight change from less hydrophilic in Sq-1 to more hydrophilic in Sorbo due to the change of NPP to SST.

Discussion

Genetic variability

Among distinct varieties of wheat and maize a genetic variation in Cs^+ enrichment was observed by a factor of 1.5–3.1 (Schimmack *et al.*, 2004; Putyatin *et al.*, 2006; Schneider *et al.*, 2008). Similar to this study, Payne *et al.* (2004) detected a natural genetic variation in Cs^+ concentration by a factor of 2 in shoots of *Arabidopsis*. For Sr^{2+}, the genetic variation for enrichment was monitored as well. Within different wheat varieties, a 1.6–2.6-fold variation in Sr^{2+} concentration was detected (Gerstmann and Schimmack, 2006; Putyatin *et al.*, 2006). To date, no comparable data about Sr^{2+} concentration have been available for *Arabidopsis thaliana* shoots, but the ascertained natural variation of a factor of 2.1 fitted to the data known for other species. Outdoor studies showed that the environmental

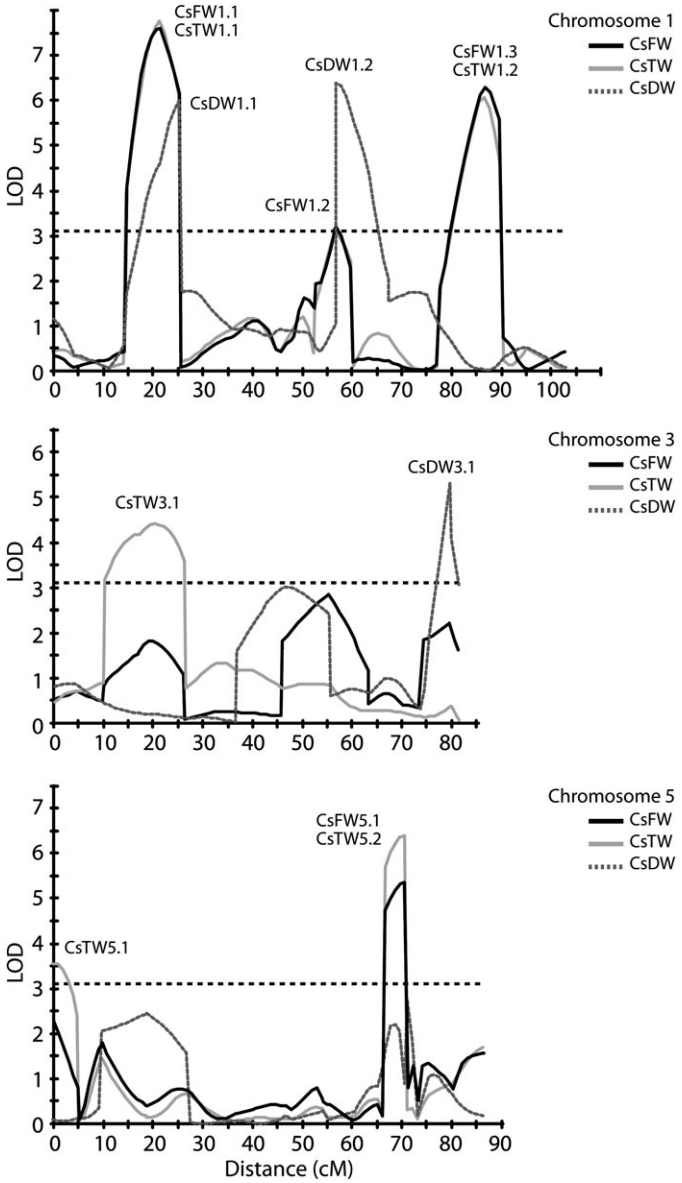

Fig. 5. Location of QTL for the trait Cs^+ concentration (CsFW, CsTW, CsDW) of the Sq-1×Sorbo F_2/F_3 families. Test statistics were calculated using the computer program MapQTL5 (Van Ooijen, 2004) and genome-wide threshold indicated by the horizontal dashed line was calculated by a permutation test with 2000 replications to keep the probability of type I error below 5%. The names of QTL are indicated.

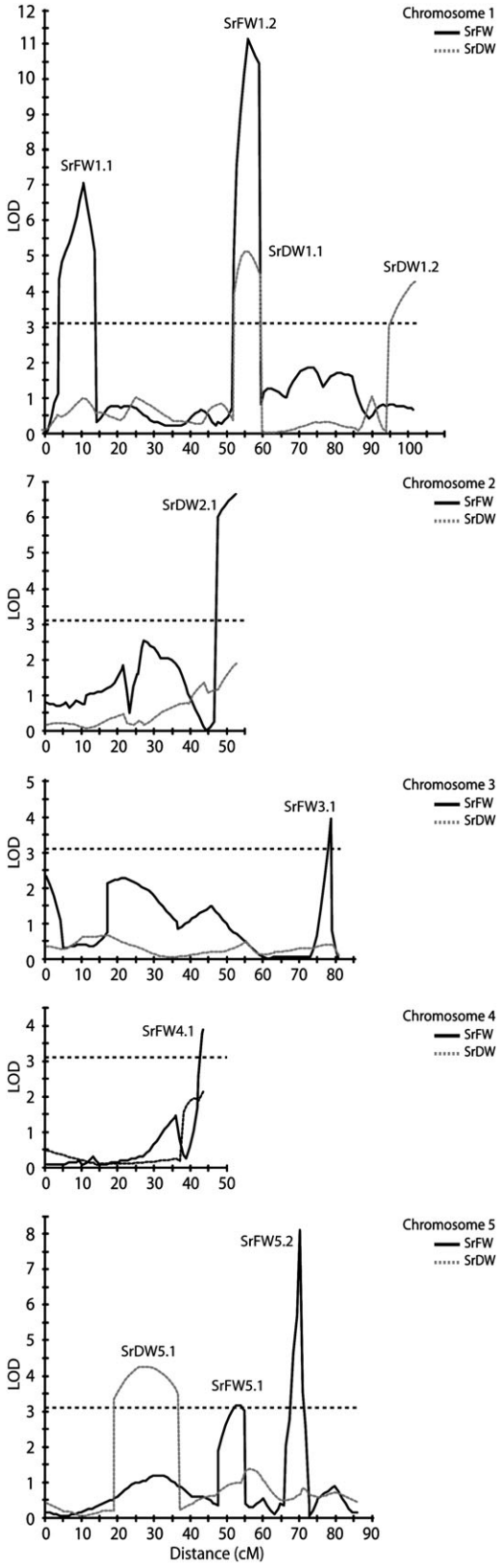

Fig. 6. Locations of QTLs involved in Sr^{2+} accumulation (SrFW, SrDW) of the Sq-1×Sorbo F_2/F_3 families. The computer program MapQTL5 (Van Ooijen, 2004) was used to calculate test statistics as well as the genome-wide threshold indicated by the horizontal dashed line. The threshold was calculated by a permutation test with 2000 replications.

Table 4. Candidate cation transporter/channel genes also expressed in root cells detected in this study within QTLs for Cs^+ and Sr^{2+} concentration

QTL (position in cM)	Candidate genes within the QTL peak
CsFW1.3; CsTW1.2 (86.6)	*AtCHX16*
CsTW3.1	*CNGC19; CNGC20*
CsDW3.1	*AtCHX20*
CsFW5.1; CsTW5.2 (70.5)	*CNGC1*
SrFW1.1 (11.1)	*CAX11*
SrFW1.2; SrDW1.1 (56.7)	*GLR3.3*
SrFW2.1 (52.7)	*CNGC3; CNGC11; CNGC12*
SrFW3.1 (79.3)	*CAX3*
SrFW4.1 (43.3)	*CNGC9; CNGC17; ACA10; GLR3.2*
SrDW5.1 (26.8)	*GLR2.1*
SrFW5.2 (70.5)	*CNGC1*

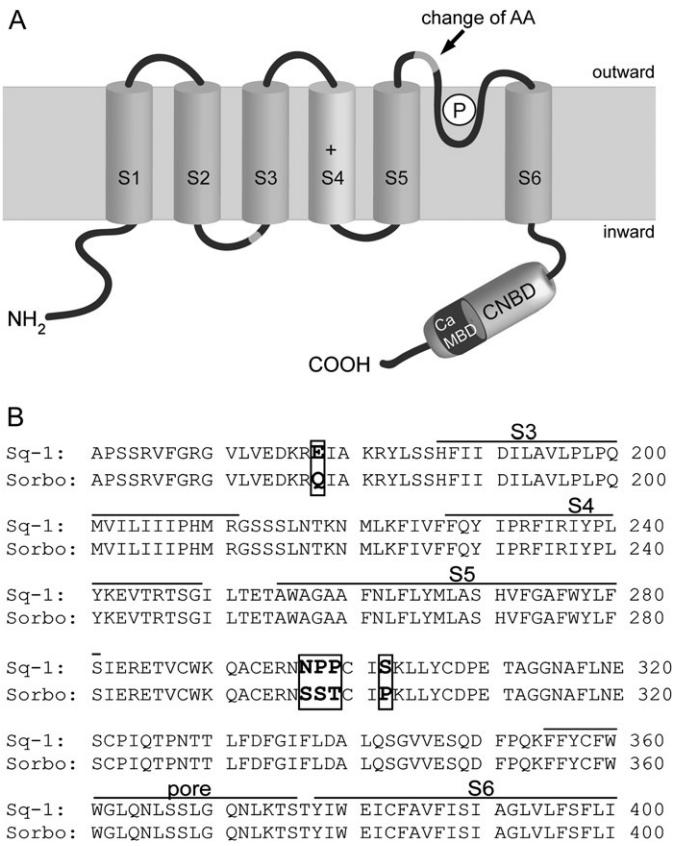

Fig. 7. CNGC1 sequence and structure. (A) The predicted membrane topology of CNGC1 with six transmembrane spanning helices (S1–S6), the pore region (P), the cyclic nucleotide binding (CNBD) domain with the overlapping CaM binding site (CaMBD). S4 is positively charged. (B) Sequence alignment of Sq-1 and Sorbo representations of CNGC1. Four transmembrane spanning regions (S3–S6) and the pore (P) are indicated. Differences of the amino acid sequence are marked with frames.

conditions such as site, soil or precipitation also had a strong impact on the extent of phenotypic variation (Schimmack *et al.*, 2004; Gerstmann and Schimmack, 2006; Schneider *et al.*, 2008).

In this study, transfer factors (*TF*) varied from 11.8 and 22.2 for Cs^+ and from 27.3 and 58.7 for Sr^{2+} in 86 *Arabidopsis* accessions (see Supplementary Table S3 at *JXB* online), indicating that the accumulation potential for Sr^{2+} in *Arabidopsis* shoots was, on average, about 2.2 times higher than that for Cs^+. Such elevated *TF*s for Sr^{2+} in comparison to Cs^+ were also reported for other plant species grown in soil culture (Melnitchouck and Hodson, 2004; Lu *et al.*, 2006).

Cs^+ accumulation is partly independent of external potassium

The accessions analysed (Sq-1, Sorbo, and L*er*-1) showed a significant decrease in Cs^+ concentration (by a maximum of 76%) at increasing $[K^+]_{medium}$ (Fig. 2A). This observation is in good agreement to Smolders *et al.* (1996) and Hampton *et al.* (2004), who demonstrated a reduction in Cs^+ accumulation upon the addition of K^+ in wheat and the *Arabidopsis* accession Ws-2. However, it has to be pointed out that the high Cs^+ concentrating accession Sorbo enriched significantly more Cs^+ than the low-accumulating accession Sq-1 at all inspected $[K^+]_{medium}$ (Fig. 2B). According to Zhu and Smolders (2000), almost no further reduction in Cs^+ concentration has been seen in this study at $[K^+]_{medium}$ >0.36 mM. Moreover, the accessions analysed exhibited altered ion levels. Sq-1 was characterized by a significantly elevated K^+ content (1.37-fold, P=0.02) and a notably lowered Ca^{2+} and Mg^{2+} content (0.86-fold and 0.89-fold, P=0.02 and P=0.04) compared with Sorbo. There are also ion content data available for soil-grown Sq-1 and Sorbo plants at the PIIMS database (Baxter *et al.*, 2007). A slight elevation of K^+ content in Sq-1 compared with Sorbo was also observed for soil-grown plants ($Sq\text{-}1_{soil}$, average 46 270 mg kg^{-1}; $Sorbo_{soil}$, average 39 490 mg kg^{-1}), whereas the database depicted that Mg^{2+} ($Sq\text{-}1_{soil}$, average 19 520 mg kg^{-1}; $Sorbo_{soil}$, average 17 150 mg kg^{-1}), and Ca^{2+} ($Sq\text{-}1_{soil}$, average 34 620 mg kg^{-1}; $Sorbo_{soil}$, average 32 940 mg kg^{-1}) concentrations of Sq-1 were also slightly higher or almost the same as Sorbo. Interestingly, the Mg^{2+} content of hydroponically grown plants was 2.8–3.5 times lower compared with soil-grown plants. In addition, hydroponically grown plants showed a 1.1–1.3-fold higher K^+ concentration and a 1.1–1.3-fold lower Ca^{2+} content than soil-grown plants. It is well known that the internal content of K^+ also has an influence on the expression or activity of different K^+ transporters/channels (Gierth and Mäser, 2007; Qi *et al.*, 2008; Karley and White, 2009). This was, for example, the case for the high affinity K^+ transporter AtHAK5, known to transport K^+ as well as Cs^+ under very low K^+ conditions (Qi *et al.*, 2008). Furthermore, internal Ca^{2+} could regulate K^+ transporter activity (Li *et al.*, 2006; Xu *et al.*, 2006; Cheong *et al.*, 2007). Importantly, Cs^+ concentration was almost independent of $[K^+]_{medium}$ for a range from 0.36–9 mM (Fig. 2A). Based on these data and on data from the literature, it can be concluded that, in addition to variable uptake mechanisms for K^+ and Cs^+ at different $[K^+]_{medium}$, the accumulation of Cs^+ within the plant might not be completely dependent on $[K^+]_{medium}$. Thus, the results presented here might be explained by a differentially regulated internal K^+ and Ca^{2+} content in the accessions analysed.

With increasing $[K^+]_{medium}$, less discrimination against $[Cs^+]$ occurred. This increase in Cs^+ selectivity suggested different uptake mechanisms with variable discrimination against Cs^+. Sq-1 was up to 2-fold less selective for Cs^+ at $[K^+]_{medium}$ compared with Sorbo (Table 1). This also supported the assumption that there had to be different controls of the uptake mechanisms provided, if the same, principal uptake mechanisms were expected in all three accessions.

Arabidopsis *accessions can discriminate against Sr^{2+}*

Competition experiments (Fig. 3A) as well as correlation studies indicated that the non-essential Sr^{2+} and the essential Ca^{2+} were closely correlated (0.78–0.93, P=0.001, depending on the FW or DW of the F_3-families). Nevertheless, discrimination was still detectable at 0.3 and 1.5 mM $[Ca^{2+}]_{medium}$ between Sr^{2+} and Ca^{2+} (Table 1), although the discrimination of Ca^{2+} versus Sr^{2+} was much less pronounced than the discrimination of K^+ versus Cs^+ (less sensitive by up to a factor of 9) (Table 1). Interestingly, whereas no discrimination occurred at $[Ca^{2+}]_{medium}$ of 7.5 mM, Sr^{2+} concentration was reduced to about 20% relative to the control at 0 mM $[Ca^{2+}]_{medium}$ (Fig. 3A). This phenomenon could not be due to a crowding-out effect, because a 25-fold decrease in the ratio Sr^{2+}/ Ca^{2+} from 1:30 at 0.3 mM $[Ca^{2+}]_{medium}$ to 1:750 at 7.5mM $[Ca^{2+}]_{medium}$, resulted only in a 3-fold increase in the *DF*. It might indicate that two parallel pathways for Ca^{2+}/Sr^{2+} movement to the xylem could imply a functional separation of a symplastic and an apoplastic Ca^{2+}/Sr^{2+} flux within the root. Accordingly, at low $[Ca^{2+}]_{medium}$ the more discriminating symplastic pathway and at high external Ca^{2+} the apoplastic pathway were more prominent (White, 2001). For several seaweeds, distinct *DF* $[Sr^{2+}/Ca^{2+}]$ have been observed, which showed either discrimination against Ca^{2+} or Sr^{2+} compared with seawater (Ophel and Fraser, 1970). Contrary to the results presented here, Bowen and Dymond (1956) could not detect any discrimination between Sr^{2+} and Ca^{2+} in tomato shoots grown in nutrient solution. However, in contrast to this study, they changed Ca^{2+} and Sr^{2+} in the medium simultaneously to maintain an overall concentration of 6 mM for both ions. Our results indicated that different uptake mechanisms for Sr^{2+} and Ca^{2+} might be used at variable $[Ca^{2+}]_{medium}$. Until now, several Ca^{2+} permeable channels have been identified and were electrophysiologically characterized (Very and Davies, 2000; White and Broadley, 2000; Demidchik *et al.*, 2002), and it is possible that these channels might also be regulated by external Ca^{2+} concentrations or other divalent ions. This would explain why no discrimination was detectable at high concentrations of external divalent ions (Bowen and Dymond, 1956), whereas at low concentrations of divalent ions discrimination took place (Ophel and Fraser, 1970).

In shoots of 108 F_3 families, the Cs^+ concentration varied in a range of 1.3 to 1.7 (Table 2). In contrast to this observation, Payne *et al.* (2004) calculated a variation of shoot Cs^+ concentration up to a factor of 3 in different RIL populations. An explanation for these differences might be different experimental set-ups and genetic backgrounds. Under the conditions chosen in this study, the F_3 families of (Sq-1×Sorbo) showed a relatively high heritability for Cs^+ and Sr^{2+} traits between 60% and 73% (Table 2). Continuative correlation analysis on Cs^+, K^+, Sr^{2+}, and Ca^{2+} of 45 F_3 families suggested that Cs^+ accumulation seemed to be more closely correlated to Sr^{2+} content and Ca^{2+} accumulation than to K^+ uptake (see Supplementary Table S4 at *JXB* online). Taken together these correlation data confirmed the results of other studies, which could not find a correlation between the accumulation of Cs^+ and K^+ in wheat either (Schimmack *et al.*, 2004; Putyatin *et al.*, 2006). In addition, a strong and significant correlation of Sr^{2+} and Ca^{2+} accumulation was observed either related to fresh weight or dry weight (0.782–0.932, P=0.001). Similar conclusions were found for several other plant species, for example, spring wheat Ca^{2+}/Sr^{2+} (Andersen, 1967; White, 2005; Putyatin *et al.*, 2006). The correlation studies combined with the discrimination data (Table 1) provided strong evidence that the mechanisms of Cs^+ and K^+ uptake were not absolutely identical.

QTL analysis

Candidate genes, which are expressed in root cells and are thought to participate in K^+ accumulation (White and Karley, 2010) were compiled within the QTLs involved in the trait Cs^+ concentration. The overlapping CsFW1.3 and CsTW1.2 enclosed the locus of *ATCHX16*, a cation/H^+ exchanger, which might have a role in K^+ homeostasis since it shows >50% similarity to *ATCHX17*, involved in K^+ homeostasis under K^+ starvation (Cellier *et al.*, 2004; Sze *et al.*, 2004). The CsTW3.1 peak comprised the loci of two cyclic-nucleotide-gated channels, *CNGC19* and *CNGC20*, known to assist the plant in coping with toxic effects caused by salt stress (Kugler *et al.*, 2009). Accordingly, they might also have an impact on Cs^+ accumulation. Within the QTL CsDW3.1, *CHX20*, a cation/H^+ exchanger encoding gene, was located. It could maintain K^+ homeostasis, influence pH under certain conditions, and was induced under Cs^+ stress (Hampton *et al.*, 2004; Padmanaban *et al.*, 2007), but its impact on Cs^+ concentration has still to be clarified. Finally, CsFW5.1 CsTW5.2 included the candidate gene *CNGC1*, a cyclic-nucleotide-gated channel, which is known to be involved in metal uptake and can conduct Ca^{2+}, K^+, and Na^+ (Köhler *et al.*, 1999; Hua *et al.*, 2003; Ali *et al.*, 2006; Ma *et al.*, 2006). The gene for *CNGC1* was up-regulated under Cs^+ stress, and a *cngc1* loss-of-function mutant displayed higher tolerance against Pb^{2+} (Sunkar *et al.*, 2000; Hampton *et al.*, 2004). *CNGC1* was also identified by Harada and Leigh (2006), who mapped the genetic variation of K^+ content in *Arabidopsis* shoots. The genes identified by Payne *et al.* (2004) to be involved in Cs^+ accumulation could not be confirmed in this study. However, one QTL detected by these authors was overlapping with the QTL KFM5.2 from Harada and Leigh (2006) using the same RIL population, which included *CNGC1*, therefore it is possible that CsFW5.1/CsTW5.2 also coincided with one QTL detected by Payne *et al.* (2004). Although the QTLs CsFW1.1, CsTW1.1, and CsDW1.1 (with the same flanking markers) had the highest LOD scores and included *CNGC7* and *CNGC8*, these genes were not considered, because they were not expressed in roots, but preferentially in pollen (Sherman and Fromm, 2009). Since no other K^+ transporters or channels were found within this region, other genes or pathways had to be responsible for the QTL.

In addition a QTL analysis was performed for potassium on the 45 F_3 families and the peaks for Cs^+ and K^+ concentration were compared (see Supplementary Table S5 and Supplementary Fig. S3 at *JXB* online). QTL peaks detected for K^+ concentration in shoots matched, in part, with QTLs identified for Cs^+ concentration (CsFW1.1=KFW1.2 and CsFW5.1/CsTW5.2=KFW5.3), other peaks overlapped either with QTLs from Payne *et al.* (2004) for Cs^+ accumulation (KFW1.1) or for K^+ concentration in shoots (KFW3.1, KFW5.3) (Harada and Leigh, 2006) or for K^+ content in *Arabidopsis* seeds (KFW1.2, KFW3.1, KFW5.2) (Vreugdenhil *et al.*, 2004). The QTLs detected for K^+ concentration included several loci for K^+ transporters (e.g. *KUP1*, *KUP6*, *KUP7*) (Fu and Luan, 1998; Kim *et al.*, 1998; Ahn *et al.*, 2004). KUPs are thought to belong to the high affinity K^+ transporters (Britto and Kronzucker, 2008; Szczerba *et al.*, 2009) and, until now, several KUPs have been experimentally analysed either in plants or in heterologous systems operating in different affinity ranges. Heterologous expression of *KUP1* in yeast (Fu and Luan, 1998) yielded different kinetic results compared with *in planta* studies (Kim *et al.*, 1998), which might be due, for example, to differing membrane potentials in yeast (Gierth and Mäser, 2007). Also eight putative K^+/H^+ antiporter (*KEA*) and the cyclic-nucleotide-gated Ca^{2+} channel *CNGC2*, known to conduct several ions such as K^+, Na^+, Cs^+, Li^+, and Rb^+ (Leng *et al.*, 1999, 2002; Chin *et al.*, 2009) as well as *CNGC1*, *CNGC13*, and *CNGC15* were detected within the QTL analysis. Further, *TPK4* and *TPK5*, two tandem-pore K^+ channels (Becker *et al.*, 2004; Voelker *et al.*, 2006; Dunkel *et al.*, 2008), two genes of the *CPA2* cation/proton exchanger family (Zhao *et al.*, 2008), one gene of the *CPA1* cation/proton exchanger family (An *et al.*, 2007), and *SKOR* (Lacombe *et al.*, 2000) have been detected due to the QTL analysis. Since not all peaks detected for Cs^+ accumulation overlapped with QTL peaks revealed for K^+, other uptake mechanisms might be involved in Cs^+ accumulation. Besides the two *CHX* cation/H^+ antiporters only non-specific cation channels have been detected as putative candidate genes involved in Cs^+ accumulation. *AtHAK5*, a high affinity transporter shown to conduct Cs^+ at very low K^+ conditions (Qi *et al.*, 2008) was not revealed within this study, which might be due to the experimentally high K^+ conditions.

In the case of Sr^{2+} accumulation, the following candidate genes were detected within QTLs (Table 4). SrFW1.1 included the locus for *CAX11*, a cation/H^+ antiporter. SrFW1.2 and SrDW1.2 harboured the locus of the putative plasma membrane glutamate receptor channel *GLR3.3* (Qi *et al.*, 2006), SrFW2.1 included three loci for *CNGC*s (*CNGC3*, *CNGC11*, *CNGC12*) (Gobert *et al.*, 2006). The locus for *CAX3*, a H^+/Ca^{2+} antiporter was found within the QTL SrFW3.1 (Cheng *et al.*, 2005). SrFW4.1 displayed the loci for two additional *CNGC*s (*CNGC9* and *CNGC17*) as well as for GLR3.2. Within SrDW5.1 the locus of another glutamate receptor channel *GLR2.1* was found, playing a role in Ca^{2+} homeostasis (White, 1998; White *et al.*, 2002). QTL SrFW5.2 included *CNGC1*, which is known to participate in Ca^{2+} transport (Ali *et al.*, 2006).

The highly accumulating accession Sorbo contains a mutated CNGC1 *allele*

Sequencing the Sorbo and Sq-1 alleles of *CNGC1* revealed a change of five amino acids between the two accessions, the replacement of amino acids N^{297}, P^{298}, P^{299}, and S^{302} of Sq-1 by S^{297}, S^{298}, T^{299}, and P^{302} in the accession Sorbo (Fig. 7). Analysis of the same genomic region in 87 accessions (http://www.1001genomes.org/) (Ossowski *et al.*, 2008; Schneeberger *et al.*, 2009) indicated two equally sized populations with 43 Sorbo-type and 41 Sq-1-type alleles; the latter included the reference accession Col-0. The regional distribution of both alleles was random. Since the accessions from the 1001-genome-project were considerably different from the accessions used in this study, the *CNGC1* polymorphic genomic region of the top ten accessions with high and low Cs^+ concentration were also sequenced. Six out of the ten high accumulating accessions [Sorbo, Lp2-6, Spr1-2, Omo2-3, Bor-4, Cvi-1, and Sha (S^{297}, P^{302})] displayed the high accumulator Sorbo-type *CNGC1* allele, whereas only one accession of the low accumulating *Arabidopsis* showed the Sorbo-type allele (Ga-4). These findings provided independent support for the involvement of CNGC1 in Cs^+ accumulation and the correlation with the Sorbo-type allele, even though this allele cannot be the only cause of the high accumulating phenotype, since Ga-4 also showed the Sorbo-type allele. Because the regions involved are closely located to the pore helix, the two alternatives might have an impact on the protein conformation and/or the substrate specificity. Proline residues have an influence on the protein structure and a strong bias in favour of β sheet regions (MacArthur and Thornton, 1991). Proline-pairs are most common in coiled regions, as also predicted for $P^{298}$and P^{299} of the Sq-1-type CNGC1 by PSIPRED (Jones, 1999). Due to the change of S^{302} in P^{302} of the Sorbo-type CNGC1, the structural prediction changed from coiled to beta strand. These changes might alter the protein conformation, which would be predicted to be more suitable for Cs^+ accumulation in the case of the Sorbo-type protein. Considering the observation by Hampton *et al.* (2005), who detected an increased uptake of Cs^+ in a *cngc1* mutant line together with a lowered K^+ content, as observed for Sorbo, a second assumption could be hypothesized. The amino acid alteration in the Sorbo CNGC1 may lead to a less functional CNGC1 and therefore to an altered ion content and possibly to an altered expression profile of K^+ transporters and channels.

In this study almost no correlations of Cs^+ and K^+ concentration were detected in shoots of *Arabidopsis thaliana* grown under controlled conditions, indicating different mechanisms of Cs^+ and K^+ uptake, whereas significant correlations of Sr^{2+} and Ca^{2+} (0.782–0.932, P=0.001) suggested very similar uptake mechanisms. The QTL analyses identified several candidate genes, which may have an impact on Cs^+ and Sr^{2+} accumulation. For the trait Cs^+ concentration non-specific cation channels, such as the CNGCs, were associated with Cs^+ accumulation. In the case of CNGC1 a polymorphism could be identified that was potentially associated with shoot Cs^+ concentration. Taking the results of Cs^+/K^+ discrimination studies into account, these non-specific cation channels (CNGCs) might have an even stronger impact on the Cs^+ concentration than the K^+ specific pathways under the given experimental set-up, since no high affinity K^+ transporters have been detected in this study.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Tables S1. Summary of the genetic linkage map.

Supplementary Table S2. CAPS and DFLP markers.

Supplementary Table S3. Cs^+ and Sr^{2+} concentration of 86 *Arabidopsis* accessions.

Supplementary Table S4. Pearson correlation coefficients.

Supplementary Table S5. Candidate genes detected in QTL analysis for the trait K^+ concentration.

Supplementary Fig. S1. Genetic linkage map of a segregating F_2 population from Sq-1 x Sorbo.

Supplementary Fig. S2. Time kinetic for Cs^+ and Sr^{2+} concentration and shoot fresh weight.

Supplementary Fig. S3. Location of QTL for trait K^+ concentration.

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