

The roles of the cation transporters CHX21 and CHX23 in development of *Arabidopsis thaliana*

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Supplementary Material

Figure S1

The relative positions of nine members of the CHX gene family. The arrows show the closest homologues between pairs of genes; the numbers indicate the percentage identity at the amino acid level.

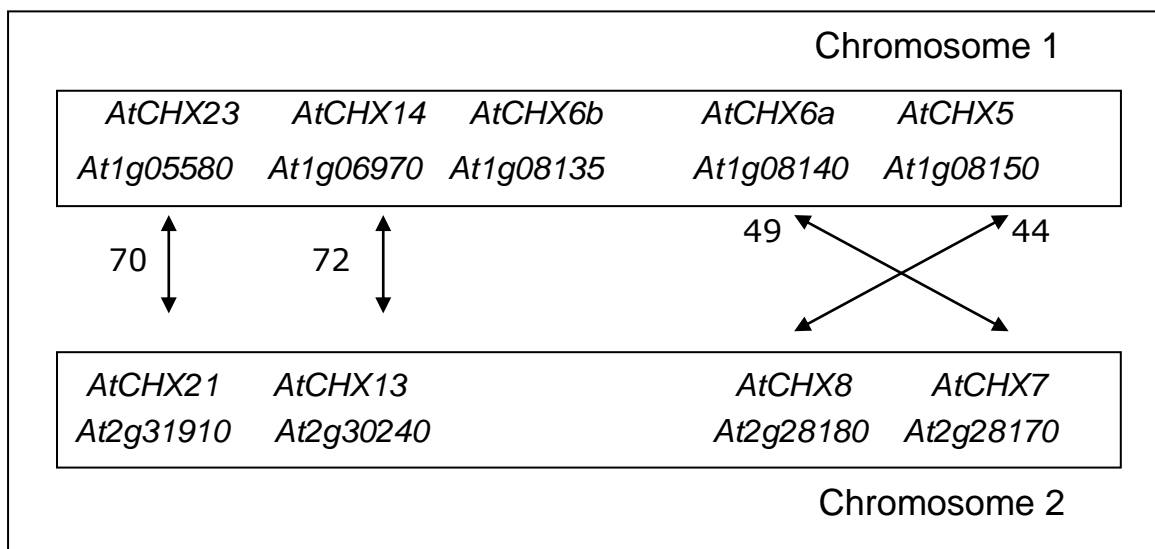


Figure S2

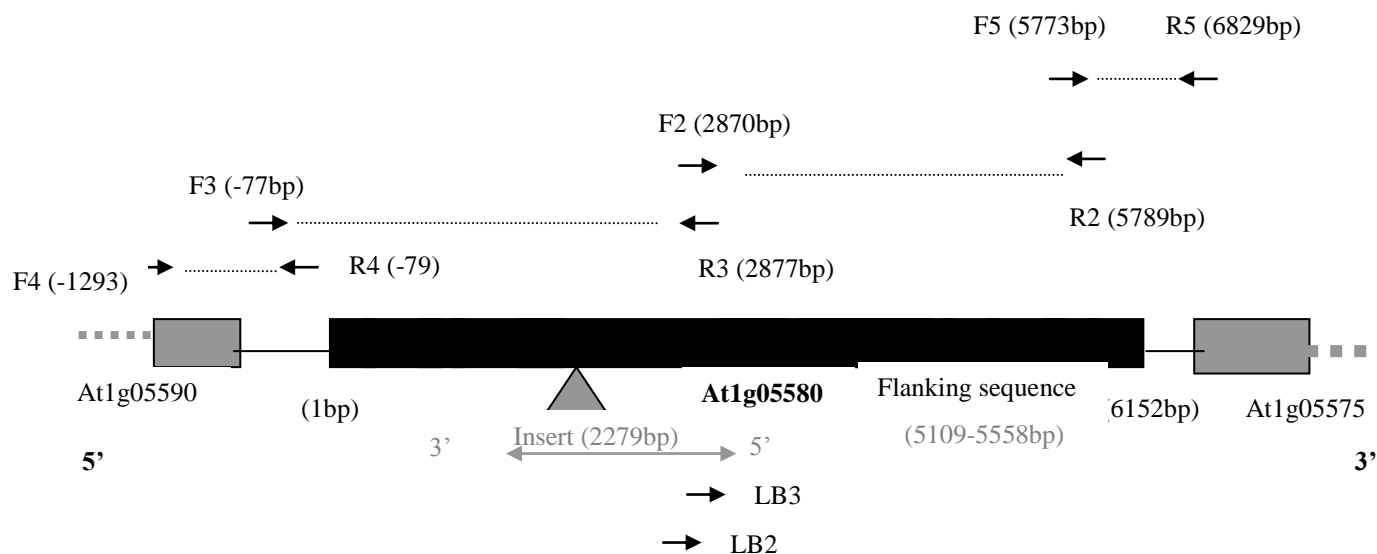
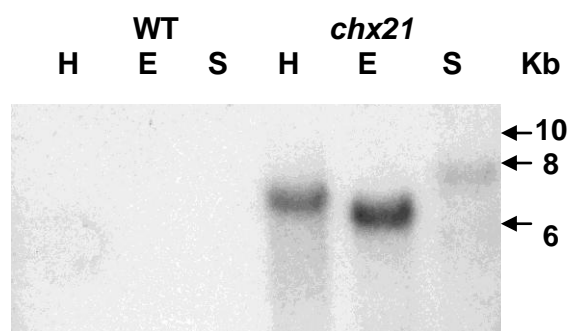


Diagram representing the knockout mutant of gene *At1g05580*, showing primer positions relative to base 1 of *At1g05580* coding region (arrows) and the regions they amplify. The sequence flanking the insertion (available on the SAIL database and used for selection of this mutant genotype) is shown at positions 5109-558). Introns are shown as stippled boxes in the following position: bases 256-339, 797-1016, 1134-1169, 1559-1851, 3198-3638, 3671-4725, 4796-4872, 4989-5060, 5560-5776, 5882-5956. The position of the T-DNA insertion is shown by a triangle at position 2279. The sizes of amplification products with different primers complementary to the wild type allele are as follows: F4 and R4, 1214bp; F3 and R3, 2954bp; F2 and R2, 2919bp; F5 and R5, 1056bp. The position of the insertion was determined by using combinations of these primers along with primers complementary to parts of the insertion (e.g. LB2 and LB3) and the sequencing of the amplification products.

Primers used:

05580F2	5'-AGA TTC TCC GGT GAC TGC CGG-3'
05580R2	5'-CAT CTT TAT GCC ACT CCA TGC TCC-3'
05580F3	5'-TCT GAA CCT AAC GTT GGG TTC AGG-3'
05580R3	5'-ATC GTT TAA ACC GGC AGT CAC CGG-3'
05580F4	5'-TGT GAT ACC GTG TTA CAG AGG CCG-3'
05580R4	5'-TGA ACC CAA CGT TAG GTT CAG ACG-3'
05580F5	5'-TTC GAA GGA GCA TGG AGT GGC-3'
05580R5	5'-CTG ATG GAT CTC GCT GAC CGG-3'
LB2	5'-GCT TCC TAT TAT ATC TTC CCA AAT TAC-3'
LB3	5'-TCC TGC CCG TCA CCG AGA TCT GA-3'
LB3Rev	5'-GTG TAT CGA GAT TGG TTA TGA AAT TCA G-3'

Figure S3



Southern analysis

DNA was extracted from leaf tissue of wild type and *chx21*^{-/-} plants, purified by two phenol/chloroform extractions and ethanol precipitated. DNA was digested with restriction enzymes (E, H and S = *EcoRV*, *HindIII* or *SpeI* respectively) run on a 0.8% agarose gel and blotted onto nitrocellulose by capillary transfer.

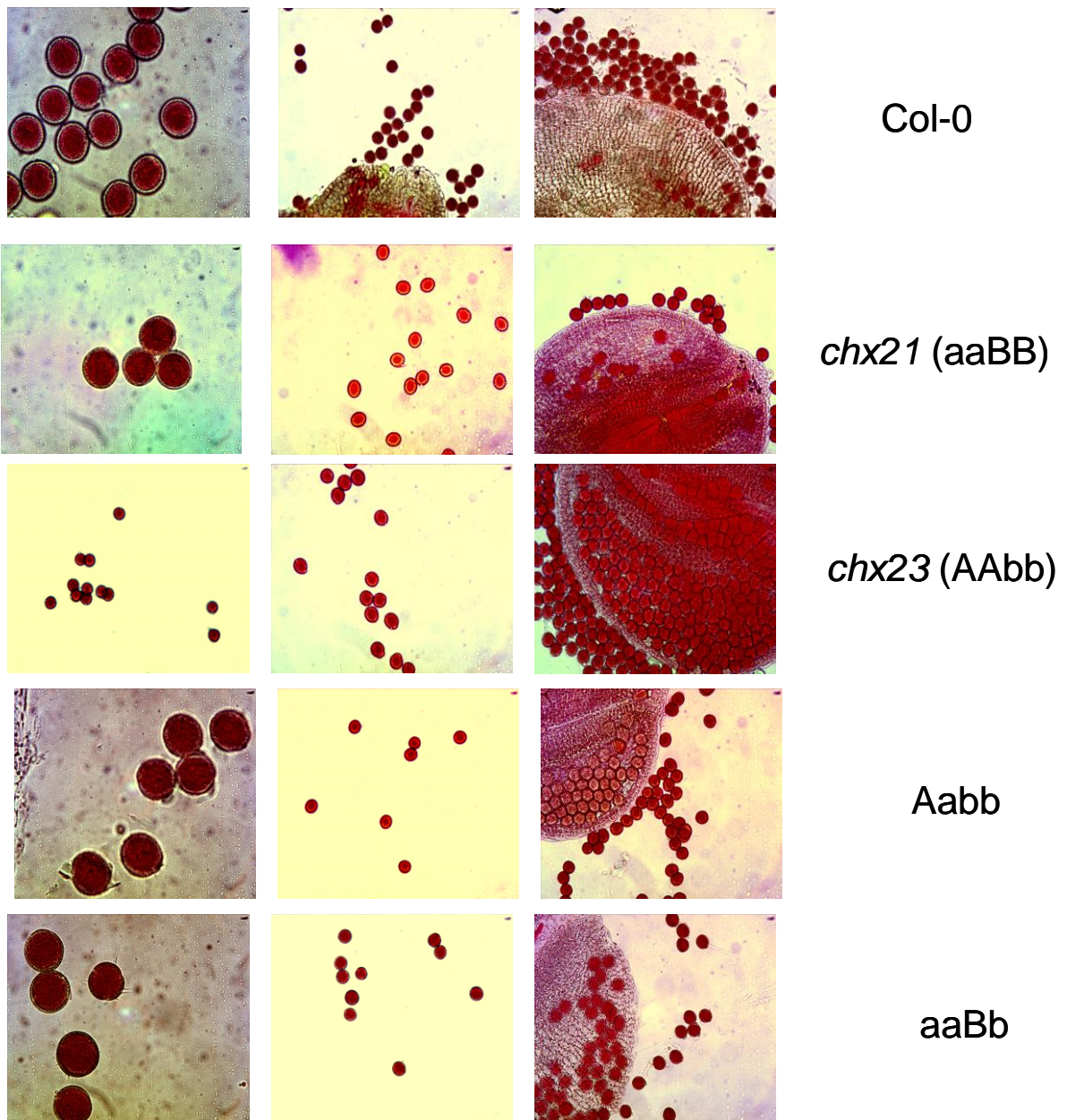
A probe was designed against a ~350bp region of the BASTA resistance gene, which is present in the the *dSpm* transposon construct used to insertionally inactivate genes in the SAIL lines. This was amplified with primers BastaF (CATGAGCCCAGA ACGACGCC) and BastaR (TCTTGAAGCCCTGTGCCTCC), ligated into pGEM-T, AND transformed into *E. coli*. 100ng of this recombinant plasmid was used to make a ³²P probe using the method of Feinberg and Vogelstein, 1983). After hybridisation and autoradiography the presence of a single band in all three patterns of restriction fragments demonstrates that only a single transposon construct copy has inserted into the CHX21 locus.

Feinberg, A. and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Annals of Botany* **132**, 6-13.

Figure S4

Pollen viability testing.

Pollen was collected from a series of genotypes, mounted with Alexander stain and viewed under a bright field microscope. Col-0 = wild type; *chx21* (aaBB) = *chx21*^{-/-} *chx23*^{+/+}; *chx23* (AAbb) = *chx21*^{+/+} *chx23*^{-/-}; Aabb = *chx21*^{+/+} *chx23*^{-/-}; aaBb = *chx21*^{-/-} *chx23*^{+/+}. Viable pollen absorbs the stain and thus turns red, whereas non-viable pollen remains green. 50% of the pollen grains from the latter two genotypes would be *chx21*⁻ *chx23*⁻ possessing no active copy of either gene. However, both these and the other genotypes produced pollen with almost 100% viability.



Production of CHX23-specific antiserum

A region of hydrophilic, extra-membrane loop of CHX23 was identified: GTW NKMMPFVIITT. Oligonucleotide sequences representing this peptide were produced and cloned into pGEX-6P-1 to allow the production of the target protein regions as GST fusion products in the protease-deficient *E. coli* strain Bc21. Following induction of the expression of the constructs using IPTG, protein was extracted from 10ml cell cultures using Bugbuster, purified using a GST resin and concentrated using a protein concentrator column (10kDa cut off, Millipore). Approximately 100µg of protein was sent to Scottish Blood transfusion for antiserum to be raised in rats.