**Table S1**

Summary of outputs generated from different trans-membrane prediction algorithms used to analyse the *VviCCC* full-length amino acid sequence.

| Server | Number of TM Helices | Website |
|---|---|---|
| SOSUI | 11 | http://bp.nuap.nagoya-u.ac.jp/sosui/ |
| TMHMM V2.0 | 11 | www.cbs.dtu.dk/services/TMHMM/ |
| HMMTOP | 11 | http://www.enzim.hu/hmmtop/ |
| TopPred | 11 (12th putative) | http://mobyle.pasteur.fr/cgi-bin/portal.py?#forms::toppred |
| PSIPRED (MEMSTAT 3) | 11 | http://bioinf.cs.ucl.ac.uk/psipred/ |

**Table S2**
Accession numbers of plant *CCC* genes used for multiple sequence alignment.

| Species | Accession | References |
|---|---|---|
| *Arabidopsis thaliana* | NP_849732 | Colmenero-Flores et al., 2007 |
| *Oryza sativa* | ADB03187.1 | Kong et al., 2011 |
| *Nicotiana tabacum* | AAC49874.1 | Harling et al., 1997 |
| *Vitis vinifera* | VIT10s0003g04530 | This study |
| *Lotus tenuis* | ACE78321.1 | Teakle et al., unpublished |
| *Citrus clementina* | CBJ19439.1 | Brumos et al., 2010 |

**Table S3**

Primers used in this study.

| Gene | Primer | Primer Sequence 5'-3' | NCBI accession number | Application | Product size (bp) |
|---|---|---|---|---|---|
| | Forward | ATGGACAACGGAGACATTGAA | | | |
| | Reverse | CTATGTGAAAAGGGTGACAACAT | | | |
| *VviCCC* | Reverse no stop | TGTGAAAAGGGTGACAACATCT | XM_002274763 | Cloning | 2949 |
| *VviCCC::YFP* | Reverse | CTAGATAGATCTCTTGTACAGCTCGT | XM_002274763 | Cloning | 3714 |
| | Forward | CTTGCATCCCTCAGCACCTT | | | |
| *VviACT1*[a] | Reverse | TCCTGTGGACAATGGATGGA | XM_002282480 | qRT-PCR | 82 |
| | Forward | GAACTGGGTGCTTGATAGGC | | | |
| *VviEF1-a*[b] | Reverse | AACCAAAATATCCGGAGTAAAAGA | XM_002284888 | qRT-PCR | 150 |
| *VviUBQ-L40*[a] | Forward | CATAACATTTGCGGCAGATCA | | | |
| | Reverse | TGGTGGTATTATTGAGCCATCCTT | XM_002273532 | qRT-PCR | 80 |
| | Forward | CCACCTCTCAACCACCCAG | | qRT-PCR | |
| *VviCCC* | Reverse | ACAACATCTCTACGGTATCCCCT | XM_002274763 | RT-PCR | 104 |
| | Forward | TGAGCAAAGAAATCACAGCACT | | qRT-PCR | |
| *AtActin2* | Reverse | CCTGGACCTGCCTCATCATAC | At3g18780 | RT-PCR | 166 |
| | Forward | ATAGCGGCGACATTGAAGAAG | | | |
| *AtCCC* | Reverse | GATGGTGCCTGAATTTGCTC | At1g30450 | RT-PCR | 325 |
| | LP | CCATTGACTCAAATCAGACGG | | Screening Salk line for homozygous insertion | |
| | RP | TTGTTTTCCGTTAATTCGTCG | | | 983 or 450-570 |
| *AtCCC* | LBa1 | TGGTTCACGTAGTGGGCCATCG | At1g30450 | | |

[a] Designed by Reid et al., (2006)

[b] Designed by Terrier et al., (2005)

**Reid K, Olsson N, Schlosser J, Peng F, Lund S** (2006) An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biology **6:** 27

**Terrier N, Glissant D, Grimplet J, Barrieu F, Abbal P, Couture C, Ageorges A, Atanassova R, Léon C, Renaudin J-P, Dédaldéchamp F, Romieu C, Delrot S, Hamdi S** (2005) Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. Planta **222:** 832-847

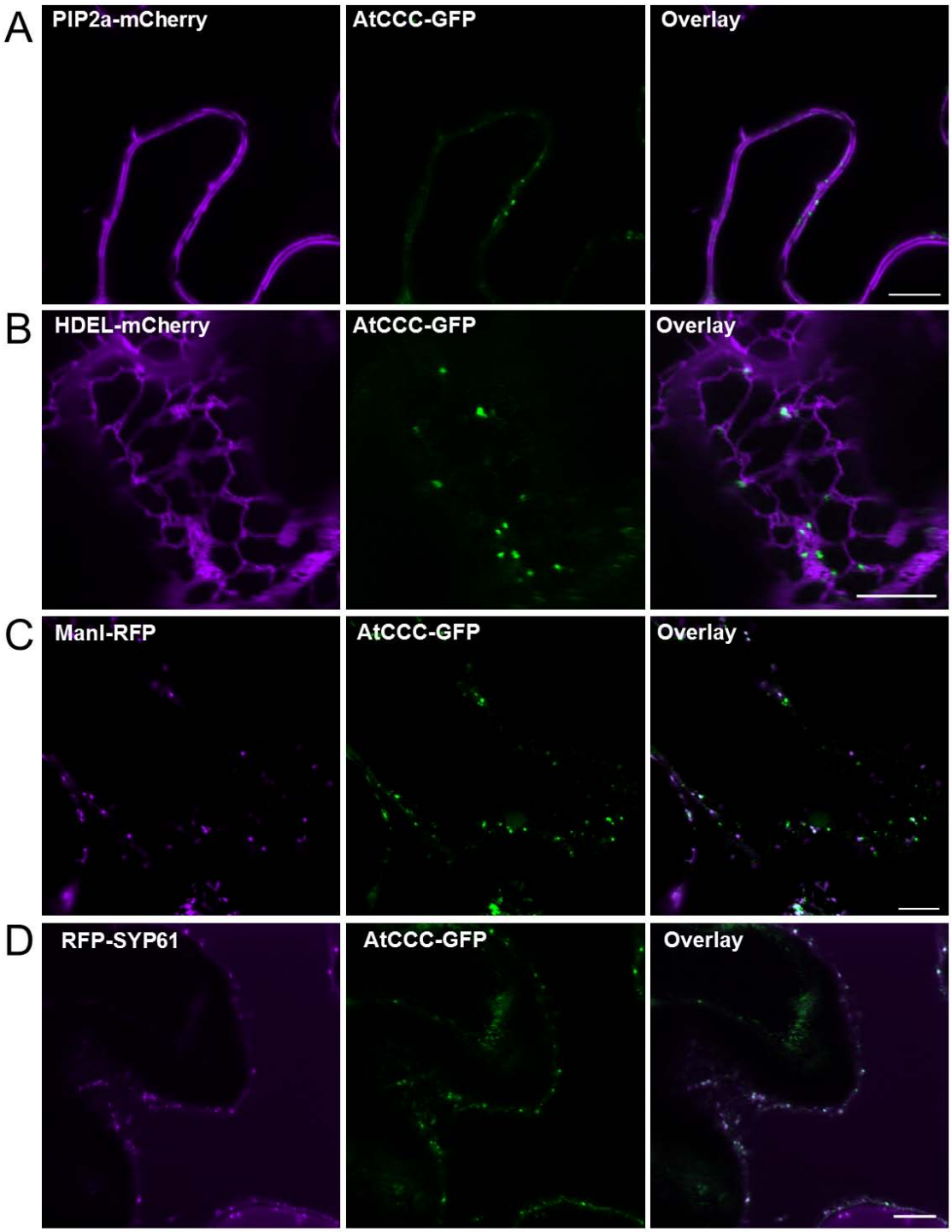


**Figure S1**
Transient co-expression of AtCCC-GFP with various subcellular markers in epidermal cells of *Nicotiana benthamiana*. *N. benthamiana* leaves were co-infiltrated with *A. tumefaciens* strains harbouring AtCCC-GFP and either the plasma membrane-marker AtPIP2a-mCherry (A), ER marker HDEL-mCherry (B), Golgi marker ManI-RFP (C) or TGN-marker RFP-SYP61 (D). Leaf sections were imaged by confocal laser scanning microscopy. mCherry- and RFP-signals are shown in magenta in the left panel. GFP-signals are shown in green in the middle panel. Co-localization of green and magenta signals appears in white in the right panel. Scale bar = 10 µm.

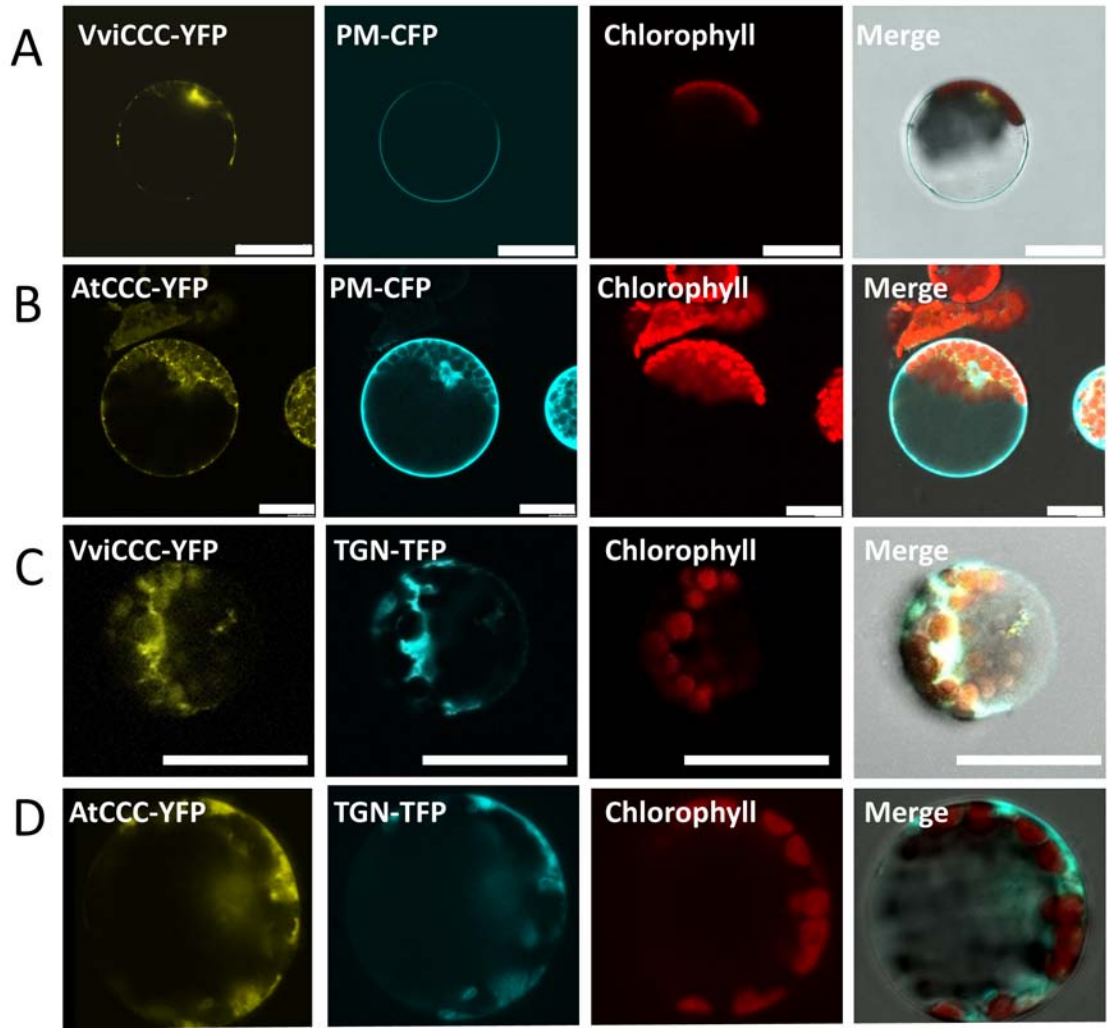


**Figure S2**

Subcellular localisation of VviCCC and AtCCC in Arabidopsis mesophyll protoplasts. Protoplasts were transiently co-transfected with the PM marker Rop11-CFP and (A) VviCCC-YFP (Scale = 25 µm) or (B) AtCCC-YFP (Scale = 20 µm), or the TGN marker Vti12-TFP and (C) VviCCC-YFP (Scale = 20 µm) or (D) AtCCC-YFP. Protoplasts were imaged by confocal laser scanning microscopy. Fluorescent signals are indicated within each panel. Merged images include the brightfield channel.

**Figure S3**

cDNA coding sequences of *VviCCC* from K51-40 and 140-Ruggeri. Heterozygous regions within the cDNAs that do not affect the amino acid sequence of their respective proteins are shown in red. The only nucleotide polymorphism that could result in an amino acid change is conserved between rootstocks, and this is shown in blue.

Coding sequence of *VviCCC* from K51-40

ATGGACAACGGAGACATTGAAAACGCGGAAGACGAGTTCGGCGGGCAAAGCGGACGCAAATACAGACCAGTCGT
CTCGCATGACCGCGCTGTTCTCCAAATGTCCTCTTTGGATTCCGGATCTTCCTCTTCTCTCCCCGTCCCTCAATCACC
TTTCACGAACCTTAAAATAAGCATGCAGGGAAACATGAGTTCTGATGCAAGAGAAGAGTCATCTACTAATCATGA
AGAACTCAATGGTTCTGAAAGAGAATCCAAATTGGAATTGTTCGGTTTTGACTCTCTTGTTAATATTCTAGGTCTTA
AGAGTATGACAGGGGAGGCAATTGCAGCTCCT**T/C**TCGAGTCCTAGAGATGGCGAGGATGTTTCTA**T/A**TACACCTGG
GCGTTCCAAGGCTAATGATCTCAAATTAGGGACATTGATGGGAGTATTTGTGCCGTGCTTGCAAAACATTTTAGGA
ATTATCTACTATATACGATTTTCTTGGATCGTTGGTATGGCAGGCATTGGTCAGTCGCTTTTGCTGGTGTCCTTTTGT
GGTTTGTGTACTTTCCTGACTTCGATATCATTGAGTGCTATCGCAACTAATGGTGCAATGAAGGGTGGGGGGCCAT
ATTATCTCATTGGTCGTGCCCTTGGTCCAGAAGTTGGAGTTAGCATTGGACTATGTTTCTTCCTTGGGAATGCAGTA
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ACAAGGCAGTTGTGAT**T/C**GTCAAGGGCCTTGATGAATGGCCCAATGAGTATCAAAGGCAGTATGGTACTATTGAT
CTGTATTGGATTGTGAGAGATGGGGGCCTTATGCTTCTCCTCTCCCA**A/G**CTCCTCCTTACCAAGGAGAGCTTTGAG
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TGTCCCAGCAAGATGAATCAATAGAGGCATTCACGGGTGCTCAGCGGCGGATTGCTGGTTACTTGAGTGAGATGA
AGGAGGCAGCTAAGAGGGAAGGAACTCCACTGATGGCTGATGGGAAATCTGTGGTTGTGAATGAGCAACAGGTG
GAGAAATTTCTTTACACAACACTGAAGCTTAATTCGACAATACTTAGATACTCAAGAATGGCTGCAGTCGTGCTTG

TGAGCTTACCACCACCACCTCTCAACCACCCAGCGTATTTCTACATGGAGTACATGGATCTTTTGGTAGAGAATGT
ACCTAGGCTTTTGATGGTAAGGGGATACCGTAGAGATGTTGTCACCCTTTTCACATAG

## Coding sequence of *VviCCC* from 140 Ruggeri

ATGGACAACGGAGACATTGAAAACGCGGAAGACGAGTTCGGCGGGCAAAGCGGACGCAAATACAGACCAGTCGT
CTCGCATGACCGCGCTGTTCTCCAAATGTCCTCTTTGGATTCCGGATCTTCCTCTTCTCTCCCCGTCCCTCAATCACC
TTTCACGAACCTTAAAATAAGCATGCAGGGAAACATGAGTTCTGATGCAAGAGAAGAGTCATCTACTAATCATGA
AGAACTCAATGGTTCTGAAAGAGAATCCAAATTGGAATTGTTCGGTTTTGACTCTCTTGTTAATATTCTAGGTCTTA
AGAGTATGACAGGGGAGGCAATTGCAGCTCCTTCGAGTCCTAGAGATGGCGAGGATGTTTCTAT/ATACACCTGGG
CGTTCCAAGGCTAATGATCTCAAATTAGGGACATTGATGGGAGTATTTGTGCCGTGCTTGCAAAACATTTTAGGAA
TTATCTACTATATACGATTTTCTTGGATCGTTGGTATGGCAGGCATTGGTCAGTCGCTTTTGCTGGTGTCCTTTTGTG
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CTGGATCTCTTTATGTGCTGGGAGCTGTAGAAACCTTCTTAGATGCCTTGCCAGGTGCTGGGATTTTTGGAGAGGTT
GTCACAAAAGTTAATGGCACAGAAGCTGCAGTGGCCGTTCCAAGTCCAAATTTGCATGACCTGCAAGTCTATGGG
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CTACTTCTGCAATGTACCTATTTTCC/TGTGCTTTTATTTGGATCTCTAGCAACAAGGGAGAAACTTTTGACAGACA
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**Figure S4**

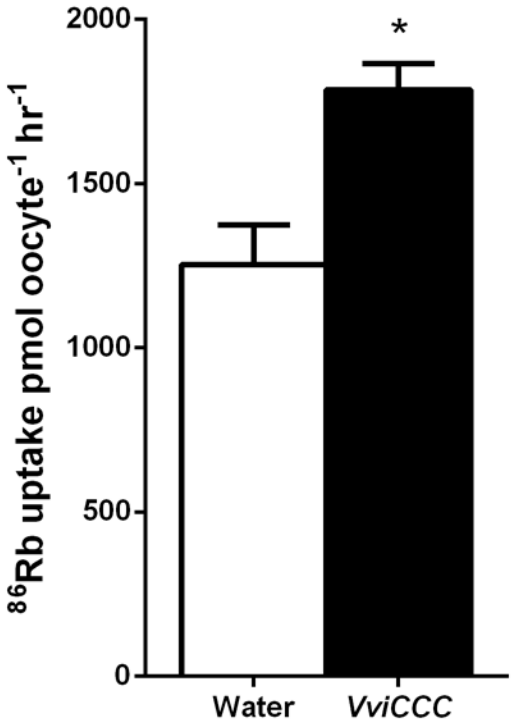



**Figure S4**

$^{86}$Rb uptake by *Xenopus* oocytes in high K buffer. Oocytes injected with water (white bars) or cRNA encoding *VviCCC* (black bars) were bathed in radioactive uptake buffer containing $^{86}$Rb for 1 hour. Each data point is the mean ± SEM of 10 oocytes. Asterisk indicates significant difference between water and *VviCCC* injected oocytes (t-test, $P < 0.01$).

**Method**

Oocytes were preincubated in $Cl^-$-free ND96 for 2 hours before the experiment ($Cl^-$ replaced with gluconate). Oocytes were then transferred to $K^+$ and $Cl^-$- free ND96 plus ouabain for 10 minutes to block the $Na^+/K^+$ ATPase. Oocytes were then transferred to flux buffer for 60 minutes. Oocytes were washed 3 times in ice cold flux buffer without isotope, and dissolved overnight in 10 % SDS. Uptake was then measured by scintillation counting. The flux buffer contained: 96 mM KCl; 2 mM NaCl; 1.8 mM $CaCl_2$; 1 mM $MgCl_2$, 5 mM Hepes pH 7.4 Tris, 1 mM Ouabain, 2 uCi/ml $^{86}$Rb.

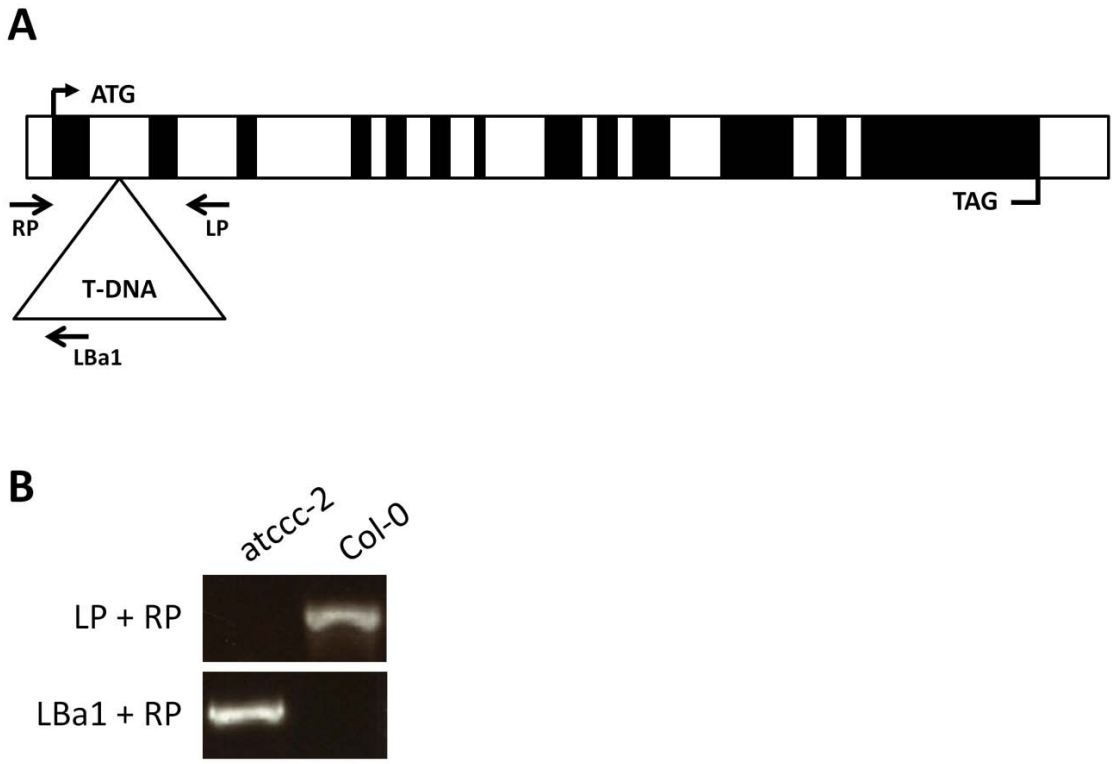



**Figure S5**

Confirmation of a homozygous T-DNA insertion in the Arabidopsis mutant line Salk_145300. (A) Schematic representation of *AtCCC* gene structure with black boxes representing exons and position of the T-DNA insertion (triangle) and primers (arrows) shown. (B) PCR of genomic DNA to confirm homozygosity.

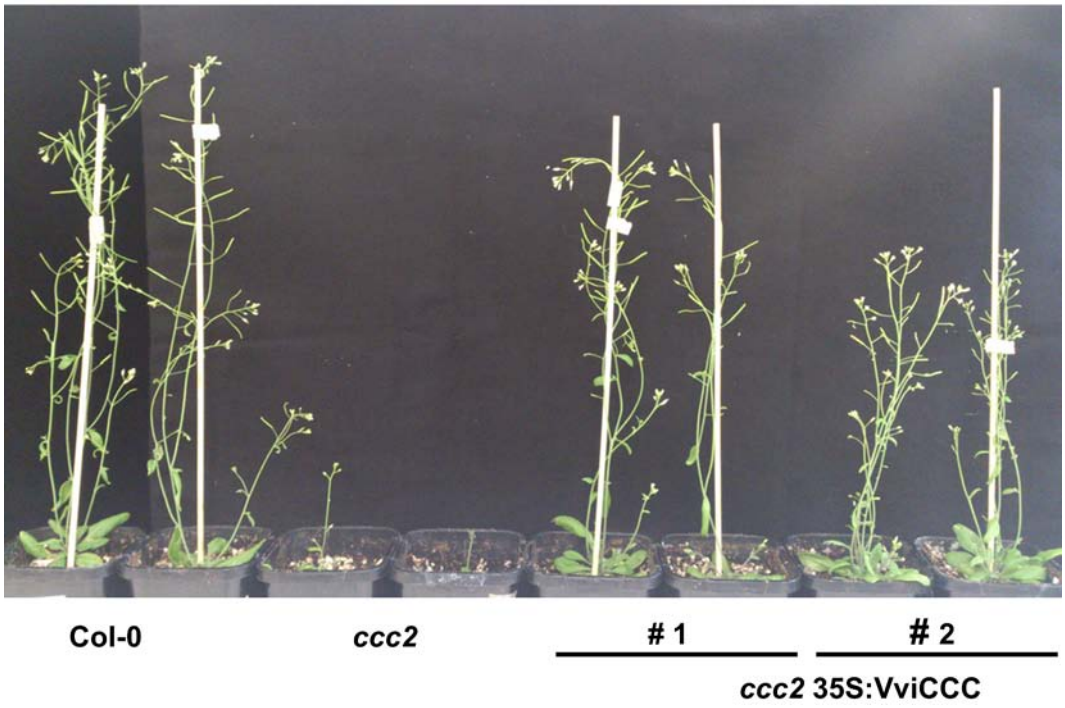



**Figure S6**

Complementation of the *ccc* phenotype with *VviCCC*. Two plants of each Arabidopsis line are shown for further confirmation of the ability of *VviCCC* to complement the *atccc* phenotype.

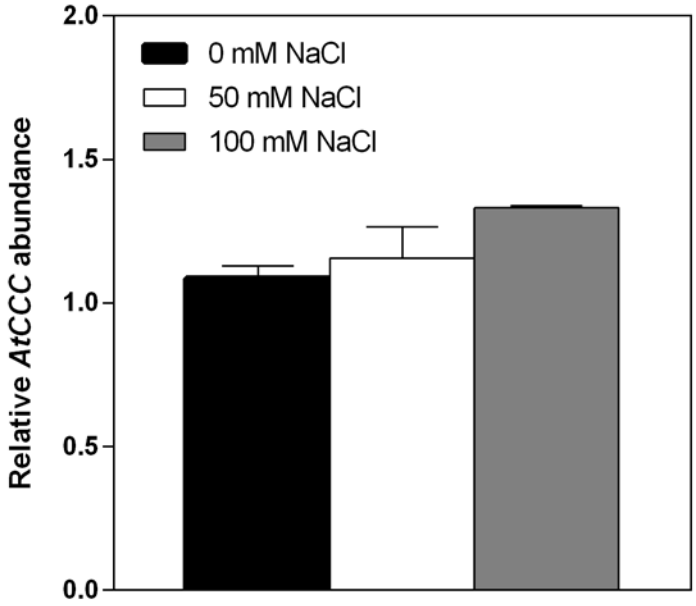



**Figure S7**

*AtCCC* is not regulated by salt stress in Arabidopsis roots. Quantitative real time PCR of whole roots of hydroponically grown, 5-week old wild-type Columbia-0 exposed to 0 mM (black bars), 50 mM (white bars) or 100 mM (grey bars) NaCl for 7 days. Bars represent the mean of 3 biological replicates ± SEM. Data are relative to the untreated (0 mM NaCl) replicated with lowest transcript abundance (set to 1). No significant differences were observed between treatments (ANOVA with Tukey's post hoc test).

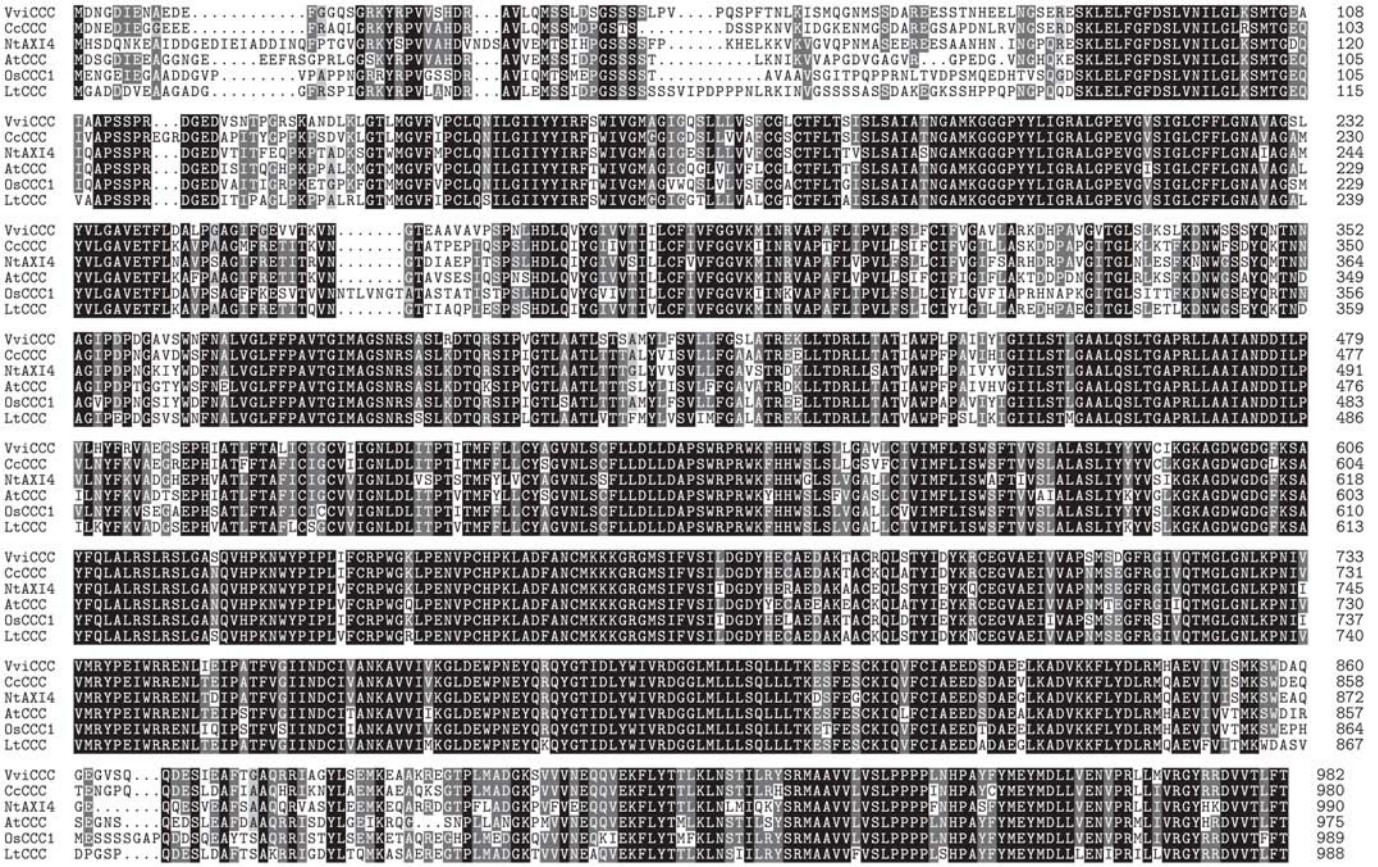
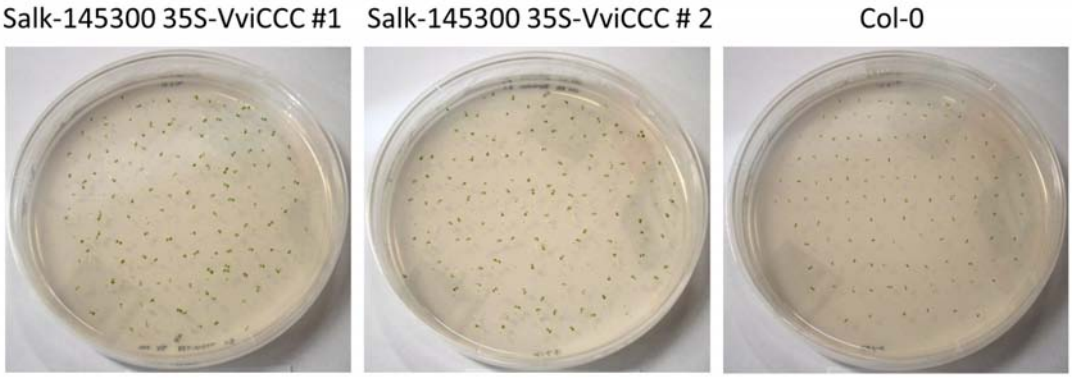


**Figure S8**

Multiple sequence alignment of six plant *CCC* family members. This alignment was used to generate the alignment and neighbour joining tree of Figure 1A. Shading is white (non-similar), light grey (similar), dark grey (> 50% conserved) and black (all match).




**Salk-145300 35S:VviCCC # 1**

For 1 insertion segregating at 3:1 ratio

| | Observed | Expected | $(Obs-Exp)^2/Exp$ |
|---|---|---|---|
| Resistant | 110.00 | 101.25 | 0.76 |
| Sensitive | 25.00 | 33.75 | 2.27 |
| **Total** | 135.00 | 135.00 | **3.02** |

$\chi^2$= 3.02 for 1 d.f.
0.05 < P < 0.1. Not significant deviation from expected.

**Salk-145300 35S:VviCCC # 2**

For 2 insertions segregating at 15:1 ratio

| | Observed | Expected | $(Obs-Exp)^2/Exp$ |
|---|---|---|---|
| Resistant | 119.00 | 120.94 | 0.03 |
| Sensitive | 10.00 | 8.06 | 0.47 |
| **Total** | 129.00 | 129.00 | **0.50** |

$\chi^2$= 0.5 for 1 d.f.
0.3 < P < 0.5. Not significant deviation from expected.

**Figure S9**

Identification of T-DNA copy number in complemented Arabidopsis mutants. (Upper panel) Two independent transformants from the T2 generation were grown on MS Agar plates containing hygromycin (15 µg.ml$^{-1}$). (Lower panel) number of T-DNA inserts was estimated by measuring Mendelian segregation ratios and performing chi square analysis.