

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://mobyli.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
<i>Arabidopsis thaliana</i>	NP_849732	Colmenero-Flores et al., 2007
<i>Oryza sativa</i>	ADB03187.1	Kong et al., 2011
<i>Nicotiana tabacum</i>	AAC49874.1	Harling et al., 1997
<i>Vitis vinifera</i>	VIT10s0003g04530	This study
<i>Lotus tenuis</i>	ACE78321.1	Teakle et al., unpublished
<i>Citrus clementina</i>	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)
<i>VviCCC</i>	Forward	ATGGACAACGGAGACATTGAA	XM_002274763	Cloning	2949
	Reverse	CTATGTGAAAAGGGTGACAACAT			
	Reverse no stop	TGTGAAAAGGGTGACAACATCT			
<i>VviCCC::YFP</i>	Reverse	CTAGATAGATCTCTGTACAGCTCGT	XM_002274763	Cloning	3714
<i>VviACT1^a</i>	Forward	CTTGCATCCCTCAGCACCTT	XM_002282480	qRT-PCR	82
	Reverse	TCCTGTGGACAATGGATGGA			
<i>VviEF1-α^b</i>	Forward	GAACTGGGTGCTTGATAGGC	XM_002284888	qRT-PCR	150
	Reverse	AACCAAAATATCCGGAGTAAAGA			
<i>VviUBQ-L40^a</i>	Forward	CATAACATTTGCGGCAGATCA	XM_002273532	qRT-PCR	80
	Reverse	TGGTGGTATTATTGAGCCATCCTT			
<i>VviCCC</i>	Forward	CCACCTCTCAACCACCCAG	XM_002274763	qRT-PCR RT-PCR	104
	Reverse	ACAACATCTCTACGGTATCCCCT			
<i>AtActin2</i>	Forward	TGAGCAAAGAAATCACAGCACT	At3g18780	qRT-PCR RT-PCR	166
	Reverse	CCTGGACCTGCCTCATCATA			
<i>AtCCC</i>	Forward	ATAGCGGCGACATTGAAGAAG	At1g30450	RT-PCR	325
	Reverse	GATGGTGCCTGAATTTGCTC			
<i>AtCCC</i>	LP	CCATTGACTCAAATCAGACGG	At1g30450	Screening Salk line for homozygous insertion	983 or 450-570
	RP	TTGTTTTCCGTTAATTCGTCG			
	LBa1	TGGTTCACGTAGTGGCCATCG			

^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

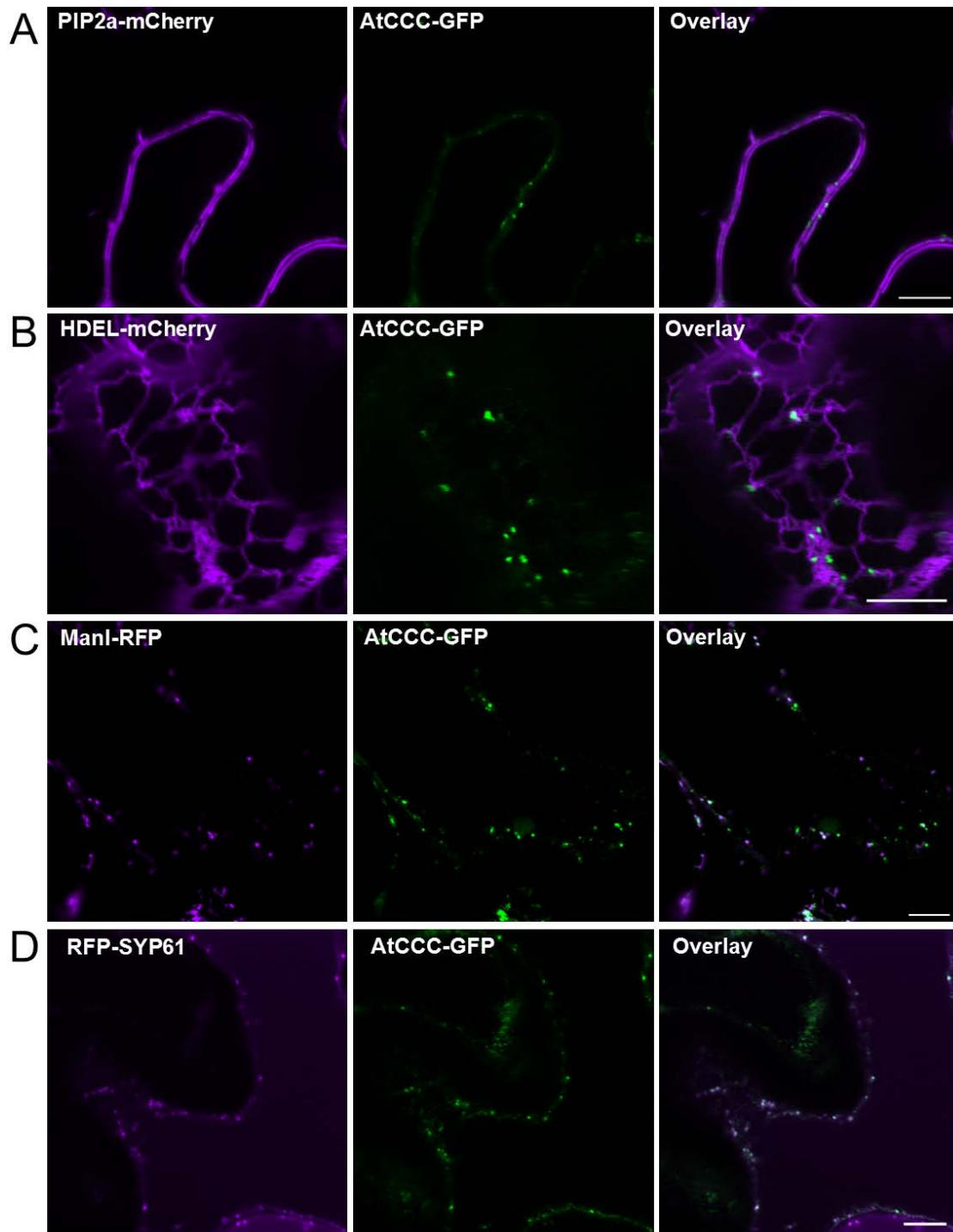


Figure S1

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 Man1-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 μ m.

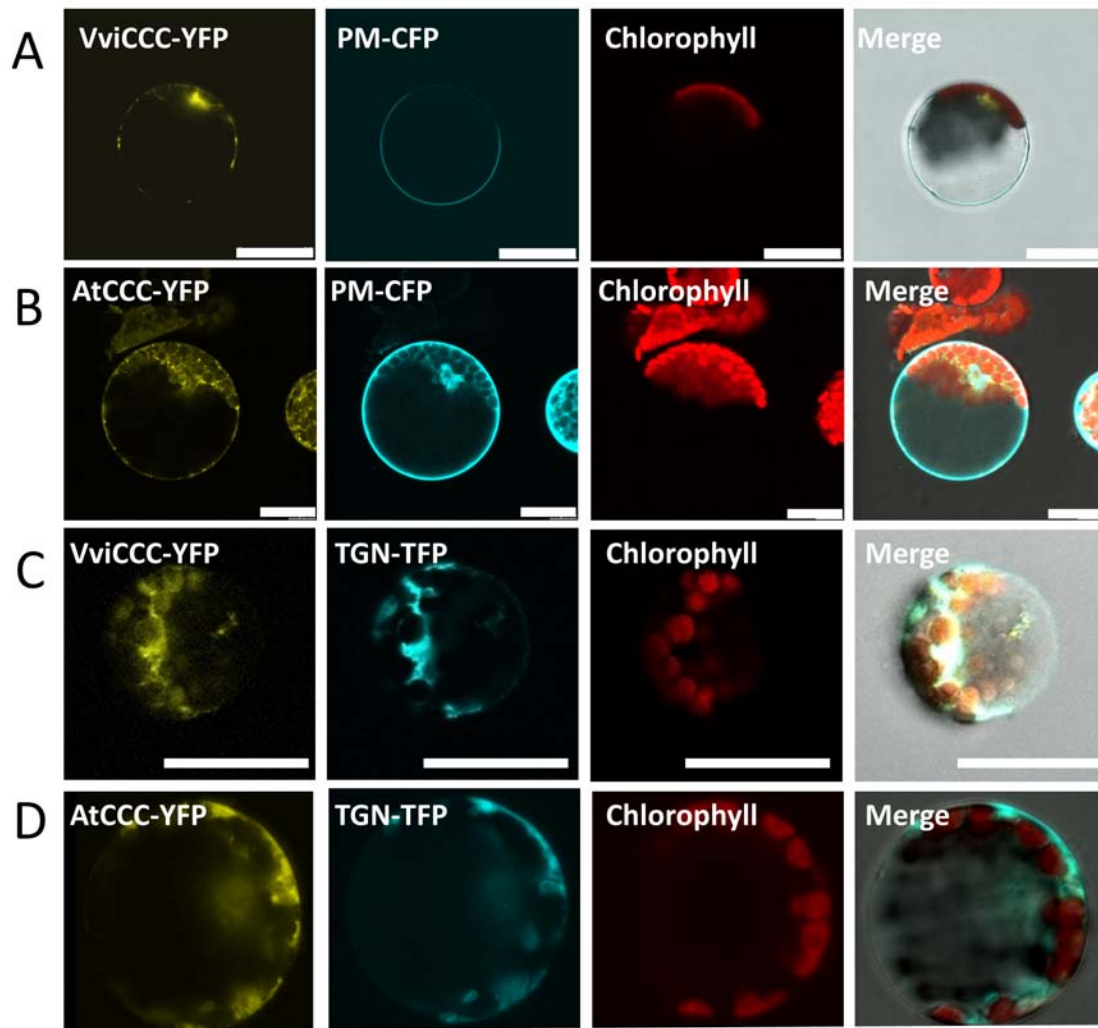


Figure S2

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
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AGAACTCAATGGTTCTGAAAGAGAATCCAAATTGGAATTGTTCCGGTTTTGACTCTTTGTTAATATTCTAGGTCTTA
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GCGTTCCAAGGCTAATGATCTCAAATTAGGGACATTGATGGGAGTATTTGTGCCGTGCTTGCAAAAACATTTTAGGA
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ACCTAGGCTTTTGATGGTAAGGGGATACCGTAGAGATGTTGTCACCCTTTTCACATAG

Coding sequence of *Vv*CCC from 140 Ruggeri

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TTTACGAACCTTAAAATAAGCATGCAGGGAAACATGAGTTCTGATGCAAGAGAAGAGTCATCTACTAATCATGA
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CGTTCCAAGGCTAATGATCTCAAATTAGGGACATTGATGGGAGTATTTGTGCCGTGCTTGCAAAACATTTTAGGAA
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Figure S4

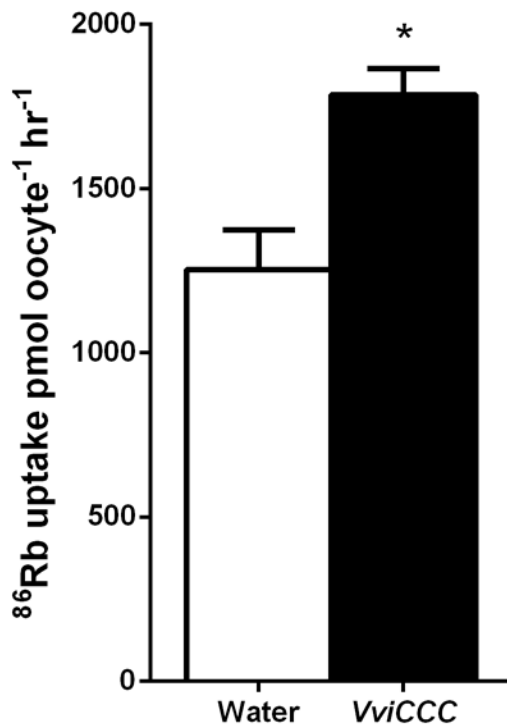


Figure S4

^{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 \pm 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 $\mu\text{Ci/ml}$ ^{86}Rb .

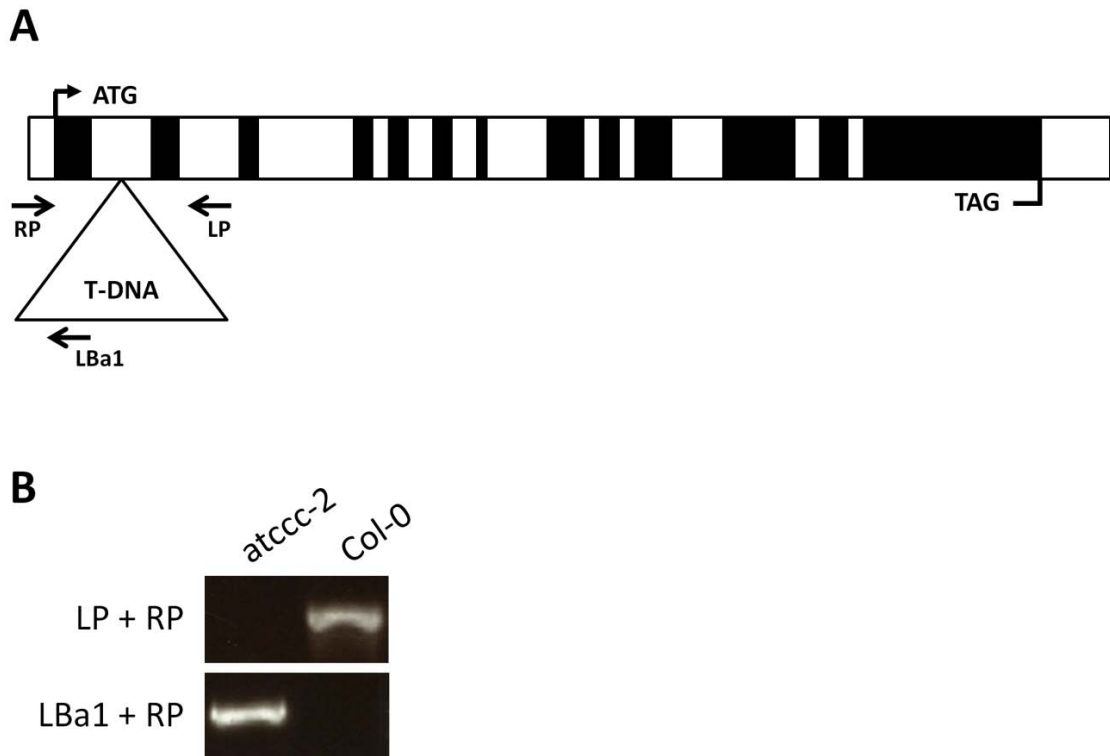


Figure S5

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.

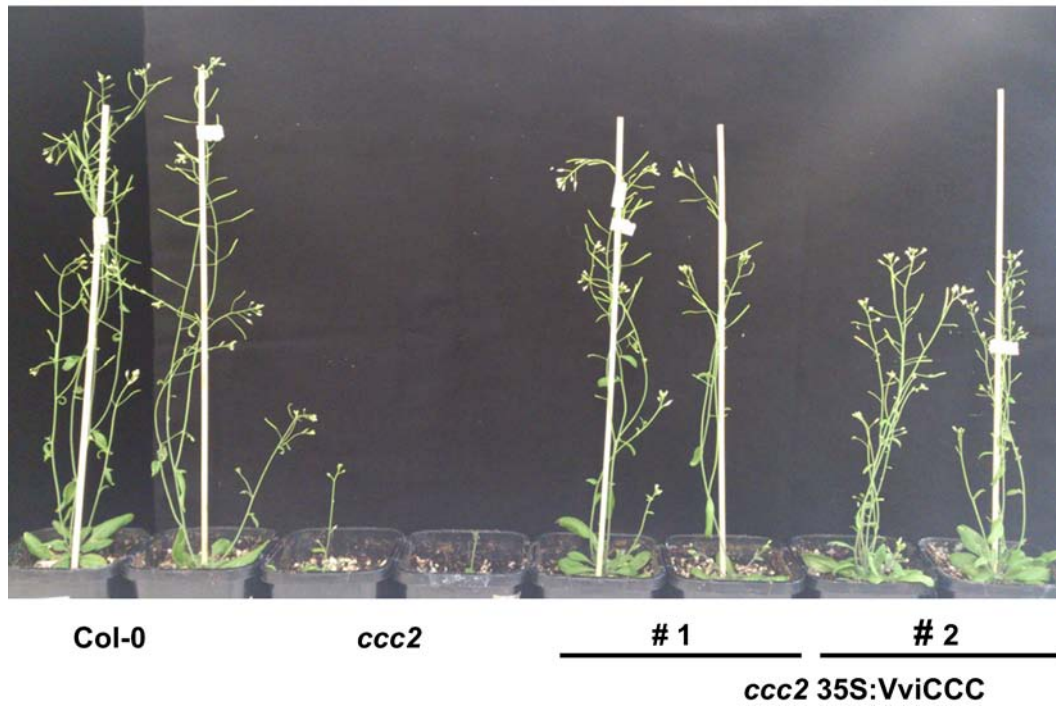


Figure S6

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.

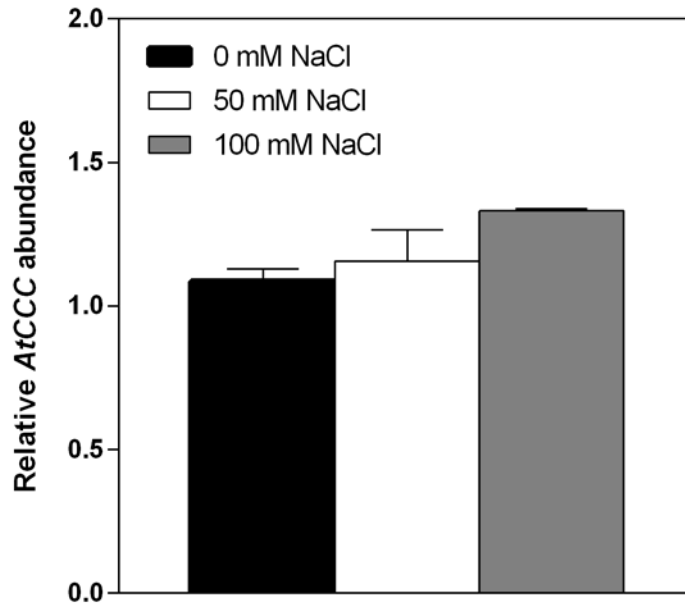


Figure S7

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 \pm 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).

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VviCCC MDCGDIENAEDE.....FCGQSCRKYPVYSRDL...AVLQWSSLDGSSSSLPV...PQSPFTNLISMQGNMSSDAREESSTNHEELGSERPESKLELFGFDSLVLNIGLRSMTCEA 108
CcCCC MDCNEDLGGEE.....FRAQLCRKYPVYAHDE...AVLQWSSMDGSSS.....DSSPKNVYIDGKENMSSDAREGAPDNLRVNGSERD SKLELFGFDSLVLNIGLRSMTCEG 103
NtAXI4 MHSDDNKEAIDGEDIETADDINQPTGVCHKYSPPVYAHVNDSSAVVEVSHHPCSSSSFF.....KHELKVVYGVQPNMASERREESAANHVINCPDRSKLELFGFDSLVLNIGLRSMTCEG 120
AtCCC MDCGDIENAEDE.....EFRSGPRLGSKYRPVYAHDE...AVVESSIDGSSSS.....LNKLVYVAPGDVAGV...GPEDC.VNCHKESKLELFGFDSLVLNIGLRSMTCEG 105
OsCCC1 MEGADLCAADDGVP.....VPAPNCRVYRVPYSSDR...AVIQWSSMDGSSS.....AVAAVSGITPQPPRNLTVPDMSQEDHTVSGQSKLELFGFDSLVLNIGLRSMTCEG 105
LtCCC WAGADLVEAGADG.....GRSSPICAKYRPVYAHDE...AVLEWSSIDGSSSSSSVIPDPPNLRKINVGSSSSASDAXGKSSHPQPQCPQDQSKLELFGFDSLVLNIGLRSMTCEG 115

VviCCC LAAPSSPR...DGEVSNTPRRSKANDLRLGTMGVFVPCQLQNLGIIYYIRF...VIVGMAGIGDQLLVVFCGCTFLTSLSLSAIATNGAMKGGGPPYILIGRALGPEVGVSTGLCFFLGNAAVAGSL 232
CcCCC IVAPSSPREGRDGEDAPITYPKPKSDVKLGTMGVFPCLQNLGIIYYIRF...VIVGMAGIGDQLLVVFCGCTFLTSLSLSAIATNGAMKGGGPPYILIGRALGPEVGVSTGLCFFLGNAAVAGM 230
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AtCCC IQAPSSPR...DGEISITDGHKPKPALKGTMGVFPCLQNLGIIYYIRF...VIVGMAGIGDQLLVVFCGCTFLTSLSLSAIATNGAMKGGGPPYILIGRALGPEVGVSTGLCFFLGNAAVAGL 229
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VviCCC YVVGAVETFLDALPAGLFGVVTIKVN.....GTEAAVAVPSPNHDQLQVYGVVITLCLFVFGGVKMINRVAPFLIPVLSLFCIFVGVAVARKDHPAVGVTLGSLKSLKNWSSVYQNTNN 352
CcCCC YVVGAVETFLKAVPAGMFRITIKVN.....GTATPEPQSPSLHDLQIYGVITLCLFVFGGVKMINRVAPFLIPVLSLFCIFVGVILLASKDDPAPGTTGLKKTCKDNWFSYQKNTN 350
NtAXI4 YVVGAVETFLNAPVAGLFRITIKVN.....GTDIAEPTSPSLHDLQIYGVITLCLFVFGGVKMINRVAPFLIPVLSLFCIFVGFSAHRHRPAPVGTGLNHESEKKNWSSVYQNTNN 364
AtCCC YVVGAVETFLKAVPAGLFRITIKVN.....GTAVSESTQSPNSHDLQVYGVITLCLFVFGGVKMINRVAPFLIPVLSLFCIFVGFIAKTDDEPDKGALQSLKSEKKNWSSVYQNTND 349
OsCCC1 YVVGAVETFLDAPVAGLFRITIKVNTLVNQTATSTATITSTPSSHDLQVYGVITLCLFVFGGVKMINRVAPFLIPVLSLFCIFVGFVAPRNAPKGTGLSLTTEKKNWSSVYQNTND 356
LtCCC YVVGAVETFLKAVPAGLFRITIKVN.....GTTIAQPLSPSSHDLQIYGVITLCLFVFGGVKMINRVAPFLIPVLSLFCIFVGFILAREHHPAEGITGLSEETLKNWSSVYQNTND 359

VviCCC AGIPDPDQAVSNFNALVGLFFPAVVTGIMAGSNRSASLKDQRSIPVGLTAAITSTAMVYFVSVLLFGSLATREKLLTDRLLTATIAWPLPAIHYGIILSTGAALQSLTGAPRLAAIANDDILP 479
CcCCC AGIPDPNKAQVSNFNALVGLFFPAVVTGIMAGSNRSASLKDQRSIPVGLTAAITTTTAYVIVSVLLFGSLAATREKLLTDRLLTATIAWPPAVHYGIILSTGAALQSLTGAPRLAAIANDDILP 477
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AtCCC AGIPDPGCTIYDFNALVGLFFPAVVTGIMAGSNRSASLKDQRSIPVGLTAAITTTISLYIVSVLFGAVATREKLLTDRLLTATIAWPPPAIVHVYGIILSTGAALQSLTGAPRLAAIANDDILP 476
OsCCC1 AGIPDPNNSIYDFNALVGLFFPAVVTGIMAGSNRSASLKDQRSIPVGLTAAITTTTAMYFVSVLLFGSLAATREKLLTDRLLTATVWPPAVHYGIILSTGAALQSLTGAPRLAAIANDDILP 483
LtCCC AGIPDPDQAVSNFNALVGLFFPAVVTGIMAGSNRSASLKDQRSIPVGLTAAITVTFMYIVSVLFGSLAATREKLLTDRLLTATVWPPPSLIRHYGIILSTGAALQSLTGAPRLAAIANDDILP 486

VviCCC VLYYFVAVGSEPHIATFTALICIGCVIIGNLDDLTPITFMFLCYAGVNLSCFLDLDLAPSWRPRWKEHHWSLSLVGLICVIMFLISWSFTVSLALASLIYYVYCKKAGDWGDDGKSA 606
CcCCC VLYYFVAVGSEPHIATFTAFICIGCVIIGNLDDLTPITFMFLCYAGVNLSCFLDLDLAPSWRPRWKEHHWSLSLVGLICVIMFLISWSFTVSLALASLIYYVYCKKAGDWGDDGKSA 604
NtAXI4 VLYYFVAVGSEPHIATFTAFICIGCVIIGNLDDLTPITFMFLCYAGVNLSCFLDLDLAPSWRPRWKEHHWSLSLVGLICVIMFLISWSFTVSLALASLIYYVYCKKAGDWGDDGKSA 618
AtCCC VLYYFVAVGSEPHIATFTAFICIGCVIIGNLDDLTPITFMFLCYAGVNLSCFLDLDLAPSWRPRWKEHHWSLSLVGLICVIMFLISWSFTVSLALASLIYYVYCKKAGDWGDDGKSA 603
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VviCCC YFQLALRSLRSLGASQVHPKNWYPIPLFCRPPWGLPENVPCHPKLADFANCMKKKGRGMSIFVSIIDGDYHECAEADAKACRQLSTYIDYKRCGVAEIVVAPNMSGFRGIVQTMGLGNLKNPIV 733
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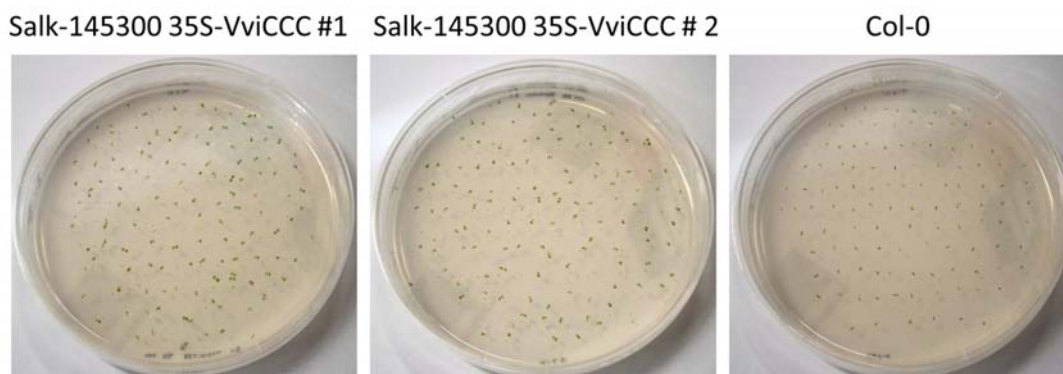
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Figure S8

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 ($15 \mu\text{g}\cdot\text{ml}^{-1}$). (Lower panel) number of T-DNA inserts was estimated by measuring Mendelian segregation ratios and performing chi square analysis.