Supplementary Material

CmRCC1 Gene from Pumpkin Confers Cold Tolerance in Tobacco by Modulating Root Architecture and Photosynthetic Activity

Running title: Molecular regulation of cold tolerance

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Table S1. List of primer sequences.

Gene name	Forward primer 5’-3’	Reverse primer 5’-3’
For subcellular localization		
CmRCC1-N-GFP	Acggcatggacgagctgtacagatct	Gccgggcggccgcttta
	GCAAATGCCGACCGTGATGTC	CTCACGGTTCTCTGACCACC
For transgenic plants		
CmRCC1-pHellgate8	Catttggagaggacacgctcgag	Tctcattaaagcaggactctaga
	TGCAAATGCCGACCGTG	GCTGGGAAGCTATTGAAACTGC
For yeast two-hybrid		
pGBKT7-CmRCC1	Aggccgaattcccggggatccgt	Ccgctgcaggtcgacggatccc
	GCAAATGCCGACCGTGATGTC	CTCACGGTTCTCTGACCACC
pGADT7-CmLAZY1	Gtgggcatcgatacgggatccgt	Cagctcgagctcgatggatcc
	ATGAAGTTATTAGGATGGATGCACA	CTAAAGCTCCAACACCAAGT
For luciferase complementation imaging assay		
pCAMBIA-CmRCC1-cLUC	Tcccggggcggtacc	Gctctgcaggtcgac
	GCAAATGCCGACCGTGATGTC	CTCACGGTTCTCTGACCACC
pCAMBIA-CmLAZY1-nLUC	Tcccggggcggtacc	Gctctgcaggtcgac
	ATGAAGTTATTAGGATGGATGCACA	CTAAAGCTCCAACACCAAGT
For qRT-PCR		
CmRCC1	GGTGGTCAGAGAACCGTGAG	CTGTGACGACAGTCCCGAAA
CmCAC	GGACAAACAGAACCAACCATGA	GGTTTCCTTTCCGTCACTGTAGA
NbPIN1	CCGATATACTGAGCACAGGGG	ACTGAGAAGTGAGAGCTTAGCA
NbPIN2	ATGCTAGTTGCCTTGCCCATT	TCTTTCTTTCATGAATCCAGTGGC
NbPIN3	AATAACGGTTTTTCTTCTCCCAC	CCACCAACGAACAGAACCATA
NbPIN6	TGTTTCGGCAGCAATCTCCA	TCAATAGCTCCTCCTTGCACT
NbACTIN	TGGTCGTACCACCGGTATTGTGTT	TCACTTGCCCATCAGGAAGCTCAT

Table S2. Segregation of kanamycin resistance in *CmRCC1* transgenic T_1 generation lines.

Line	R	S	Total	Segregation Ratio
OxCmRCC1-1	26	10	36	3:1
OxCmRCC1-3	23	8	31	3:1
OxCmRCC1-6	28	9	37	3:1

R, positive plants with kanamycin resistance; S, negative plants with kanamycin sensitivity.

Figure S1. CmRCC1 protein domain prediction and alignment with RLD family proteins in *Arabidopsis*. (A) Online Pfam software (http://pfam.xfam.org/search/sequence) was used to predict conserved domains of CmRCC1 protein. **(B)** Multiple sequence alignment of CmRCC1 with RLD family proteins in *Arabidopsis* using Geneious software. The sequences in the blue box indicate the RCC1 repeats domain.

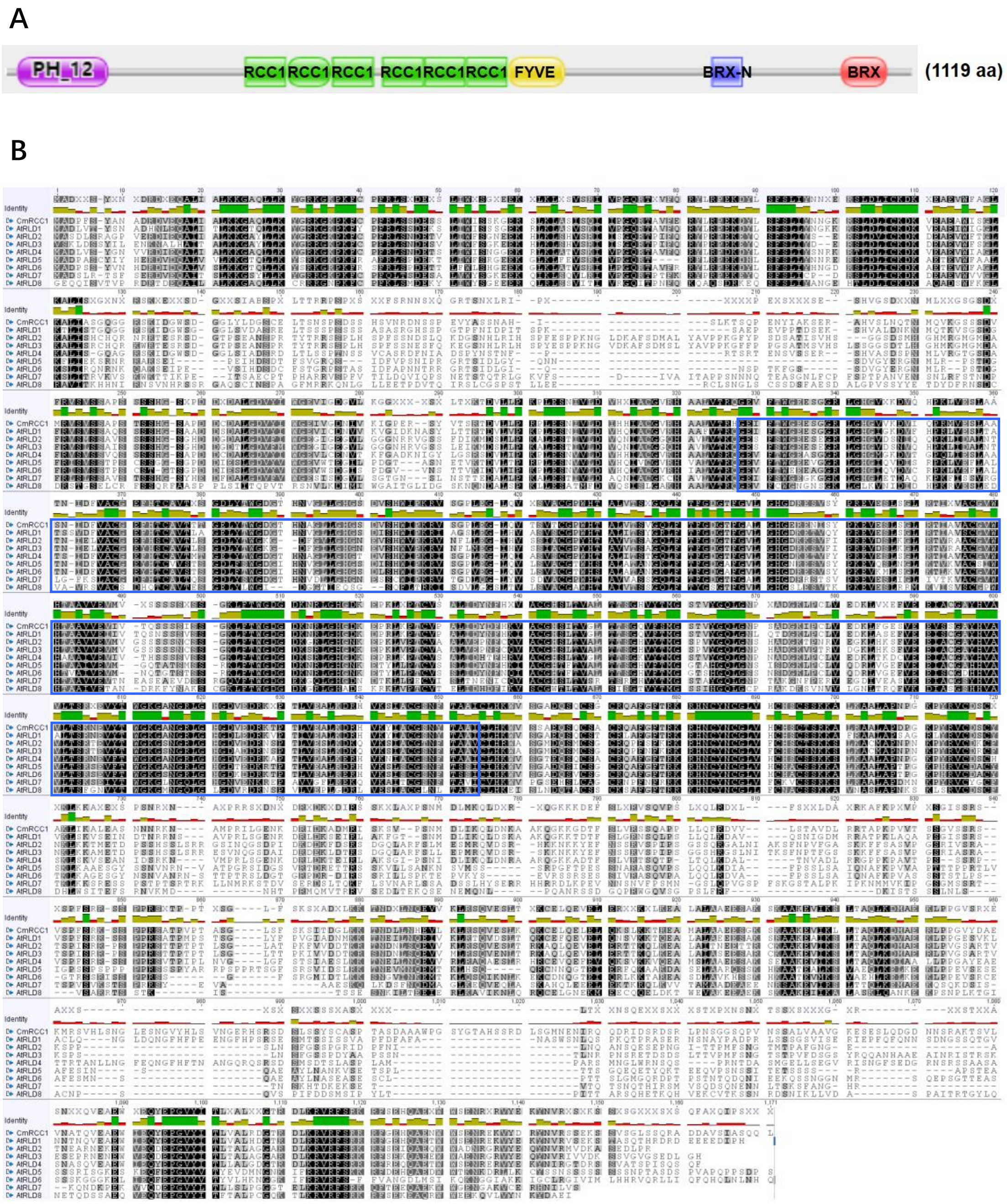

Figure S2. Identification of *CmRCC1* in transgenic tobacco lines. RT-PCR was performed to detect *CmRCC1* in transgenic T_0 generation lines with the primers from pHellgate8 vector and downstream of *CmRCC1* coding region, respectively.