

Table 1 Suppl. PCR primer sequences for cloning, reverse transcription (RT)-PCR, and real time quantitative (q)-PCR assays.

	Primers	Sequences
35S: <i>CIPK2</i>	<i>AtCIPK2</i> -SacI- F	AGGAGCTCATGGAGAACAAACCAAGTGTATTA
	<i>AtCIPK2</i> - BamHI-R	AGGGATCCCTATGATGGTTCTTGCTCTCCTTG
	<i>HsCIPK2</i> -SacI-F	AGGAGCTCATGAAGGGTGGGCTCACAGATCAG
	<i>HsCIPK2</i> -BamHI-R	GTGGATCCCTTAACATGGTTGCTGCTGCTGCGGCTG
<i>ProAtCIPK2::GUS</i>	<i>ProAtCIPK2</i> -HindIII-F	TGGAAGCTTCGCAAAAGCAGCATCACCATAAACTCC
	<i>ProAtCIPK2</i> -XbaI-R	AGGTCTAGAAAAAAGCACAATAGGATCAGGACTCAGAAG
35S: <i>HsCIPK2-GFP</i>	<i>HsCIPK2</i> -KpnI-F	ACCGGTACCATGGGGGAGCAGAAGGGGAATATTC
	<i>HsCIPK2</i> -BamHI-R	TTGGGATCCACAAGATTGCTGCTGCTGCGGCTGCG
RT-PCR	<i>AtCIPK2</i> -527-F	CTTATGTTGCGCCCGAGGTTATTA
	<i>AtCIPK2</i> -R	CTATGATGGTTCTTGCTCTCCTTG
	<i>HsCIPK2</i> -420-F	TGACGGCGCCAAGGCTGACATCT
	<i>HsCIPK2</i> -R	TTAACATGGTTGCTGCTGCTGCGGCTG
	<i>AtActin11</i> -F	GGCTGGATTTGCTGGAGATGATGC
	<i>AtActin11</i> -R	CAATTTCTCGCTCTGCTGAGGTGG
real time qPCR	<i>qAtCIPK2</i> -F	GAAGTTGGGAGGTTATTGGG
	<i>qAtCIPK2</i> -R	AACGACATTCGGGTGTTAG
	<i>qHsCIPK2-GFP</i> -F	GAAGACAAACGGCGATACT
	<i>qHsCIPK2-GFP</i> -R	TCCAGCTCGACCAGGATG
	<i>qAtActin2</i> -F	TTGACTACGAGCAGGAGATGG
	<i>qAtActin2</i> -R	ACACGAGGGCTGGAACAAG

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HsCIPK2 : MGEQKGNILMHKYEMGKMLGQGTFAKVYHARNIETSQSVAIKVTDKEKVL : 50
OsCIPK2 : MAEQRGNMLMKKYEMGKLLGQGTFAKVYHARNITETSESVAIKMIDKEKVL : 50
AtCIPK2 : -MENKPSVLTERYEVGRLLGQGTFAKVYFGRSNHTNESVAIKMIDKDKVM : 49

HsCIPK2 : KGGLTDQIKREISVMKLVKHPNIVQMYEVMATKTKIYFVLEHVKGGELEFN : 100
OsCIPK2 : KGGLMDQIKREISVMKLVKHPNIVQLYEVMATKTKIYFVLEHVKGGELEFN : 100
AtCIPK2 : RVGLSQQIKREISVMRIAKHPNVVELYEVMATKSRIYFVIEYCKGGELEFN : 99

HsCIPK2 : KVQGRGLKEDAARKYFQQLICAVDFCHSRGVYHRDLKPENLLLDNSNLK : 150
OsCIPK2 : KVQGRGLKEDAARKYFQQLICAVDFCHSRGVYHRDLKPENLLLDNSNLK : 150
AtCIPK2 : KVAKGKGLKEDVARKYFYQLISAVDFCHSRGVYHRDIKPENLLLDNDNLK : 149

Activation loop
HsCIPK2 : VSDFGSLSTIIECCRRLDGLLHTSCGTPAYVAPEVINRKGYDGAKADIWSCG : 200
OsCIPK2 : VSDFGLSALADCKRODGLLHTTCGTPAYVAPEVINRRGYDGAKADIWSCG : 200
AtCIPK2 : VSDFGLSALADCKRODGLLHTTCGTPAYVAPEVINRKGYEGETKADIWSCG : 199
      *           *           *

HsCIPK2 : VILFVLMAGYLPFDKNLNMYYKKIGKAEFKCPSWFSSDIRRLLLRILDP : 250
OsCIPK2 : VILFVLLAGYLPFHDKNLMDYYKKIGKAEFKCPSWFNTDVRLLLRILDP : 250
AtCIPK2 : VVLFVLLAGYLPFHDNLNMEMYRKIGKADFKCPSWFAPEVKRLLCKMLDP : 249

HsCIPK2 : NPSTRISIEKIMEHPWFRKGLDAKLRLYNLQAKDAVPAS--DMTATSDSL : 298
OsCIPK2 : NPSTRISMDKIMENPWFRKGLDAKLRLYNLQPKDAIPV---DMSTDFDSF : 297
AtCIPK2 : NHETRITIAKIKESSWFRKGLHLKQKKMEKMEKQQVREATNPMEAGGSGQ : 299

NAF motif
HsCIPK2 : SSSNSAIEGKEQETKKLSNMNAFDIISLSTGLDLSGMFEDNDKKRESKFT : 348
OsCIPK2 : N-SAPTL-----KKPSNLNAFDIISLSTGLDLSGMFEESDKK-ESKFT : 339
AtCIPK2 : NENGENHE-----EPRLATLNAFDIIALSTGFGLAGLFGDVYDKRESRFA : 344

HsCIPK2 : STNSASTIVSKIEDIAKGMQLKLVKKDGGMLKMEGSKPGRKGVMSSIDAEI : 398
OsCIPK2 : STSTASTIISKIEDIAKGLRLKLTKKDGGMLKMEGSKPGRKGVMGIDAEI : 389
AtCIPK2 : SQKPASEIISKLVEVAKCLKLRKQAGLFLKLERVKEGKNGILTMDAEI : 394

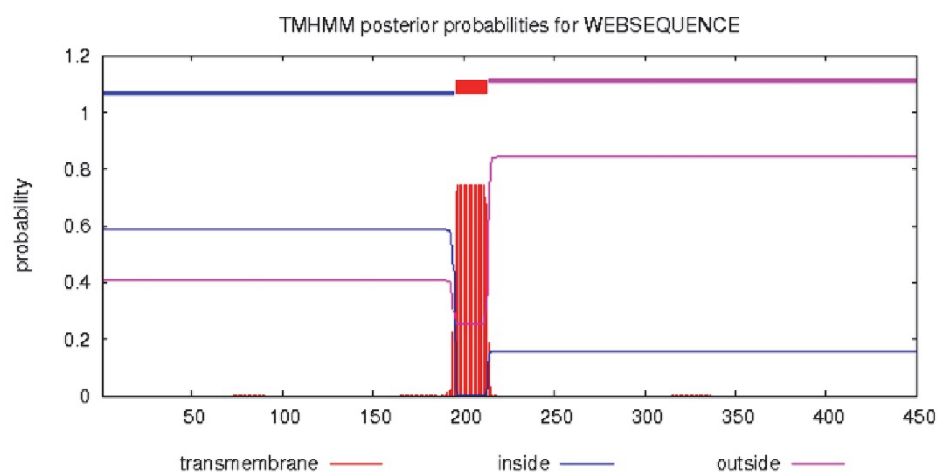
HsCIPK2 : FEVTPDFHLVELKKTNGDTLEYQRVENQEMRPALKDIVVAWQGE-PQPQQ : 447
OsCIPK2 : FEVTPNFHLVELKKTNGDTLEYRKVLNQEMRPALKDIVVAWQGEQPKQQQ : 439
AtCIPK2 : FQVTPTFHLVEVKKCNNGDTMEYQKLVEEDLRPALADIVVWVQGEKEKEEQ : 444

HsCIPK2 : Q-SC----- : 450
OsCIPK2 : QPTC----- : 443
AtCIPK2 : LLQDEQGEQEPS- : 456

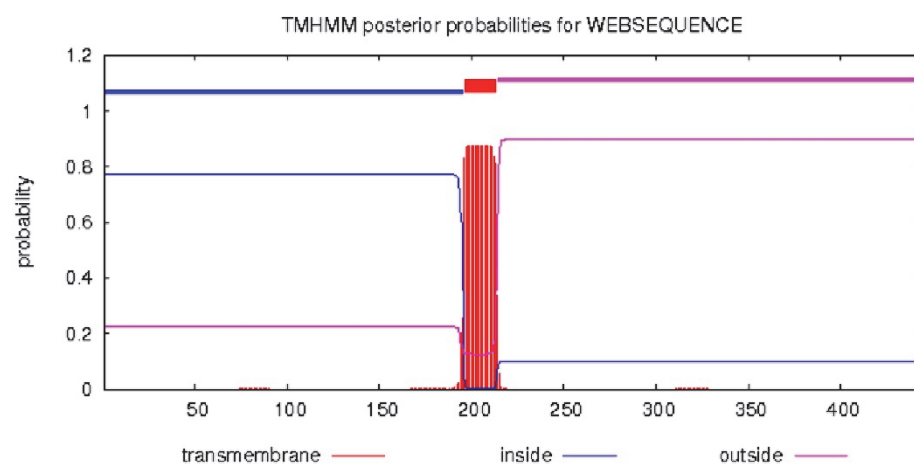
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Fig. 1 Suppl. Amino acid sequence alignment of HsCIPK2 with OsCIPK2 and AtCIPK2. Accession numbers of CIPK2 from three different plant species are wild barley HsCIPK2 (KP638475), *Arabidopsis* AtCIPK2 (AAF86506), and rice OsCIPK2 (ACD76974). Blue and red boxes denote two conserved functional domains, activation loop and NAF motif, respectively. Asterisks indicate three highly conserved residues (Ser, Thr, and Tyr) for putative phosphorylation sites in the activation loop.

A HsCIPK2



B OsCIPK2



C AtCIPK2

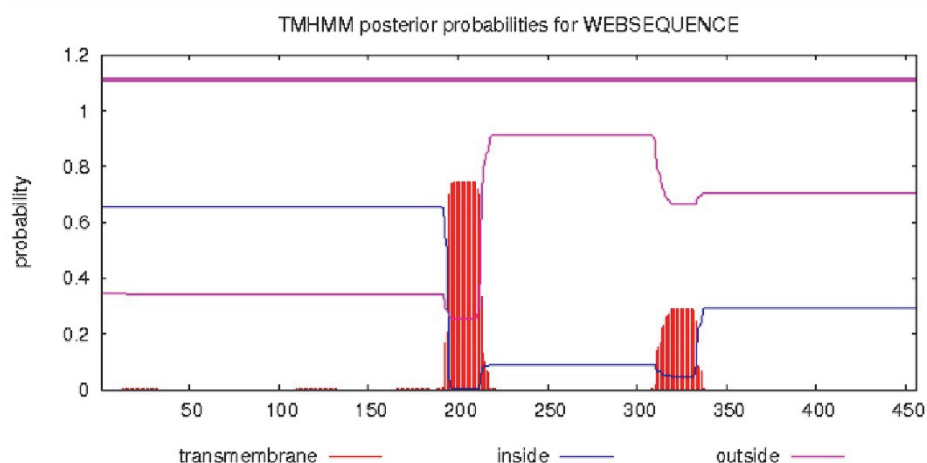


Fig. 2 Suppl. Prediction of transmembrane helix domain in HsCIPK2. *A* to *C* - a potential transmembrane helix domain of CIPK2 homologues, predicted by *TMHMM2.0* program, is located between amino acid residues 196 and 213 in HsCIPK2 (*A*) and OsCIPK2 (*B*) but not in AtCIPK2 (*C*).

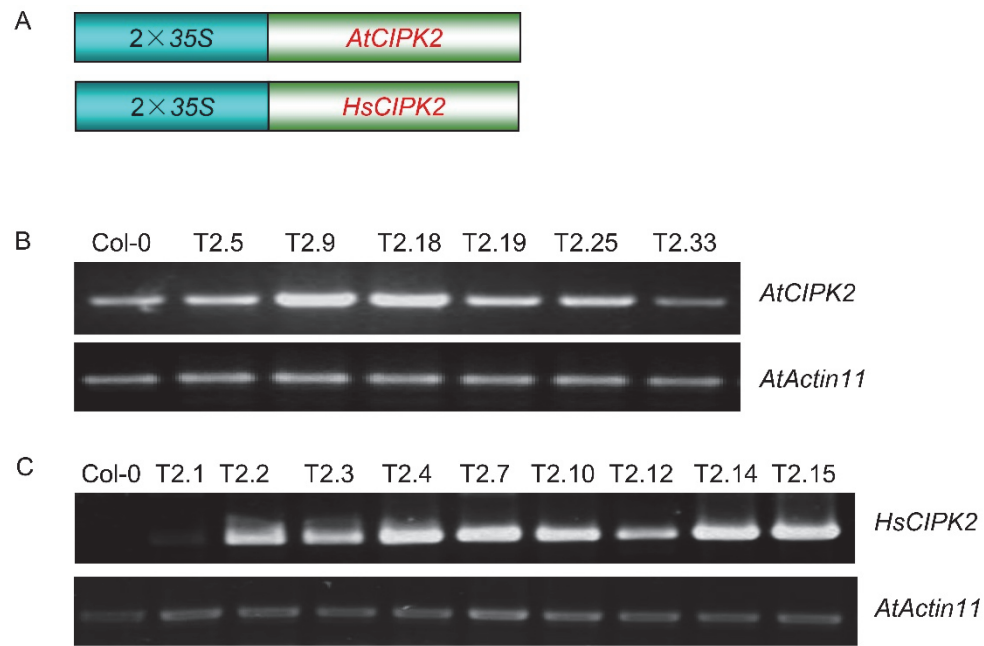


Fig. 3 Suppl. Molecular identification of *CIPK2*-overexpressing transgenic lines. *A* - Schematic diagrams of *AtCIPK2* and *HsCIPK2* constructs under control of double CaMV 35S promoters. *B* and *C* - RT-PCR analysis of kanamycin-resistant transgenic lines in T2 generation.

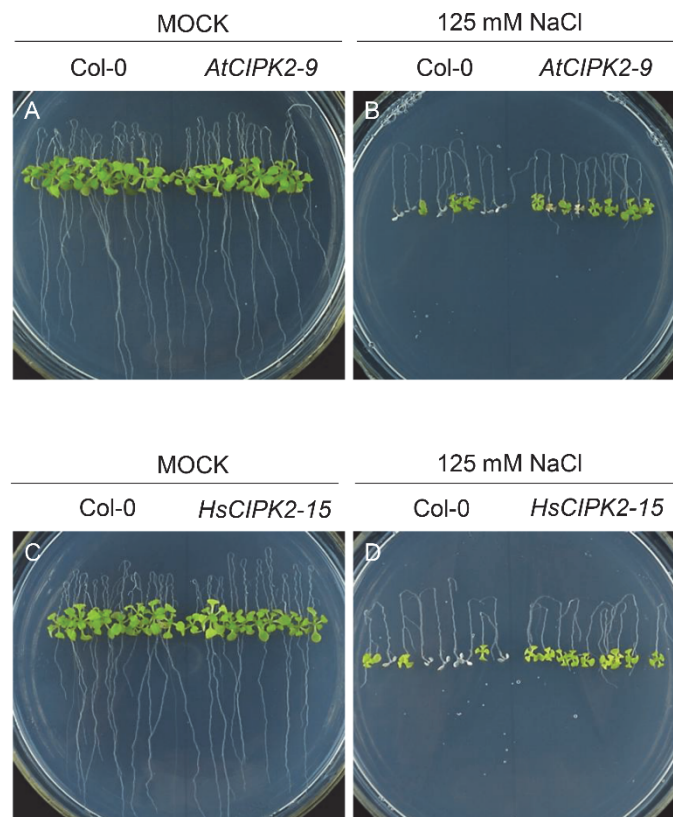


Fig. 4 Suppl. Effects of salt stress on seedling growth. *A - D* Representative images for growth of the wild-type, *AtCIPK2-9*, and *HsCIPK2-15* seedlings after 2-week exposure to 125 mM NaCl.

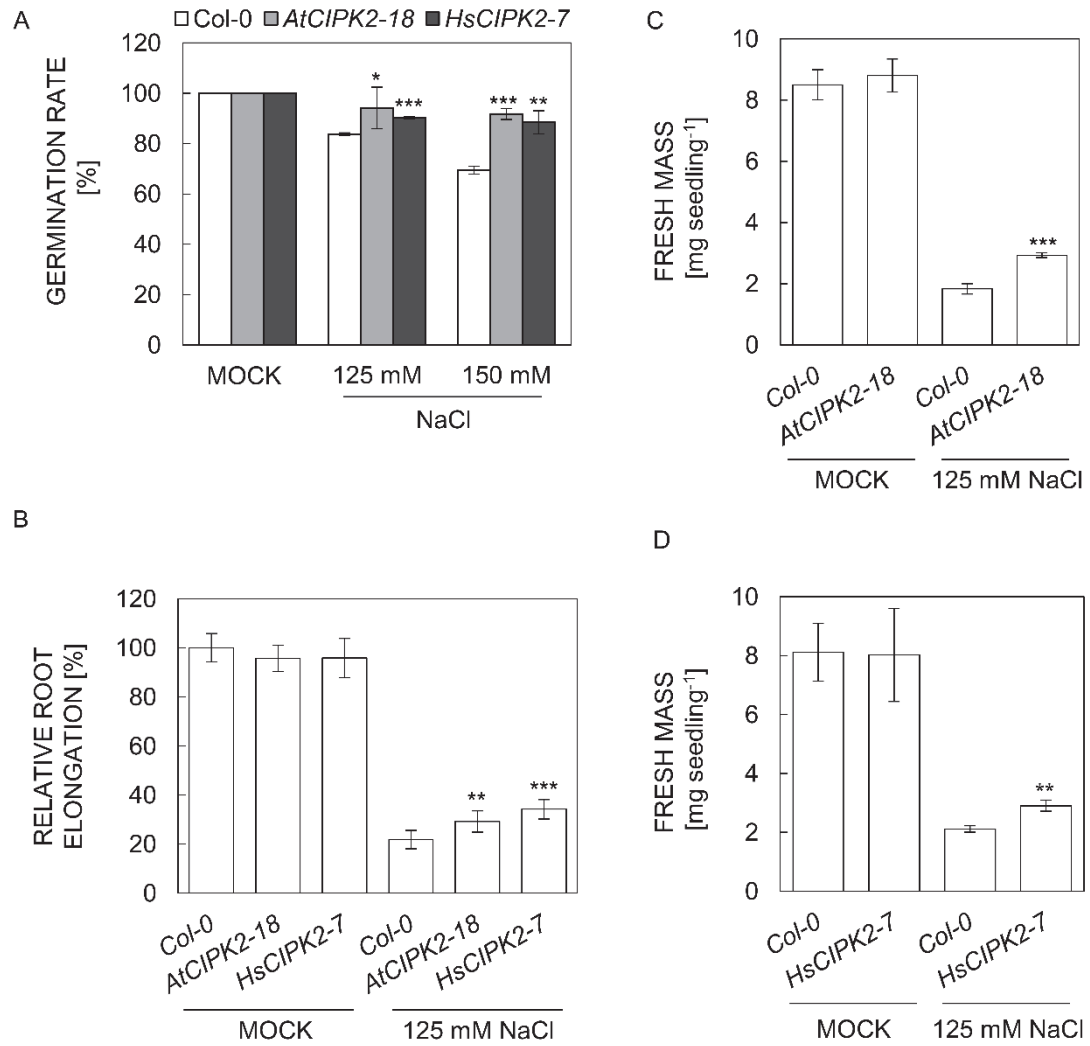


Fig. 5 Suppl. Effects of salt stress on seed germination and seedling growth in another independent lines. *A* - Seed germination rate of the wild-type, *AtCIPK2-18*, and *HsCIPK2-7* after 7-d exposure to 125 mM NaCl ($n = 112$ to 123 seeds). Root elongation (*B*) and fresh mass (*C* and *D*) of the wild-type, *AtCIPK2-18*, and *HsCIPK2-7* seedlings after 3-d (*B*) and 2-week (*C* and *D*) exposure to 125 mM NaCl ($n = 30$ to 32 seedlings). Means $\pm$ SD. Single, double, and triple asterisks indicate $P < 0.05$, 0.01, and 0.001, respectively (Student's t test; compared to the corresponding wild-type control).

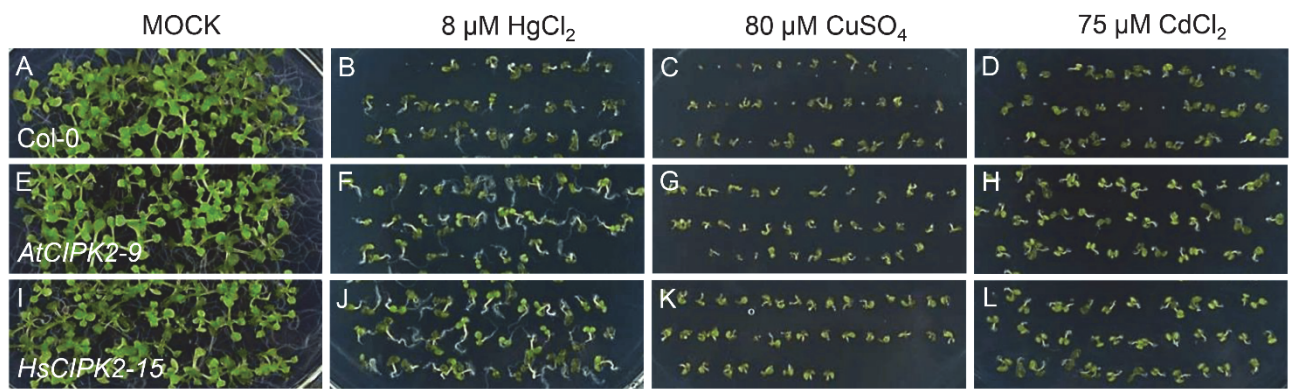


Fig. 6 Suppl. Effects of heavy metals on seed germination. *A* to *L* - Representative images of germination of the wild-type, *AtCIPK2-9*, and *HsCIPK2-15* seeds after 7-d exposure to HgCl_2 , CuSO_4 , and CdCl_2 .