

Supplementary materials

(a)

SaCLCa2	1 MEASMQSPLSENNSSNAIDESARLNEGANTQNTPEPEVAANGEPDLESNFLHQPLLLKRNRTLSASHLAMVGAKVSHIESLDYEINEN	86
SfCLCa2	1 MEASAESPLSENNSSNAIDESTRFNEGANTONMEAAANGEPDLESNFLQDPLLRNRTLSASHLAMVGAKVSHIESLDYEINEN	86
SaCLCa2	87 DLFKQDWRSRSKAQVLOYVFLKWKLAFLVGLLTGLIATAINLAENIAGYKLLLVSQYVLEKRYIMGLITLTGANFLTLSSSTLLV	172
SfCLCa2	87 DLFKQDWRSRSKAQVLOYVFLKWKLAFLVGLLTGLIATAINLAENIAGYKLLLVSQYVLEKRYMMGLITLTGANFLTLSSSTLLV	172
SaCLCa2	173 VFFAPTAAGPGIPEIKAYLNGIDTPNMYGAPTLLVKIFGSIGAVSAGLDLGKEGPLVHIGACIASLLGQQGTDKHRVKWRWYRIMN	258
SfCLCa2	173 VFFAPTAAGPGIPEIKAYLNGIDTPNMYGASTLLVKIFGSIGAVSAGLDLGKEGPLVHIGACVASLLGQQGTDKHRVRWRWYRIMN	258
SaCLCa2	259 NDRDRRDLLTCGSSAGVCAAFRSPVGGVLFALEEEVATWWRSAALLWRTFFFSTAVVVVVLRAFI EYCSGGKGCGFGNGGLIMFDVSNV	344
SfCLCa2	259 NDRDRRDLLTCGSSAGVCAAFRSPVGGVLFALEEEVATWWRSAALLWRTFFFSTAVVVVVLRAFI EYCSGGKGCGFGNGGLIMFDVSNV	344
SaCLCa2	345 TVSYRLMDIIPIALIGLIGGVLGSLYNYTLLHKVRLRLLSNERGKLAVALLLSLTVSIFTSVCLYGLPFLVGCTKCDPSLTECSTTG	430
SfCLCa2	345 TVSYRLMDIIPIALIGLIGGALGSLYNYTLLHKVRLRLLSNERGKLAVALLLSLTVSFTSVCLYGLPFLVGCTKCDPSLTECSTTG	430
SaCLCa2	431 RTGNYKRFNCPDGYYNDLASLFLSTNDAAVRNIFSTNTPGEFHPLSLVIYFLYLICILGLTFGIAVPSGLFLPIILMG SAYGRLLG	516
SfCLCa2	431 RTGNYKRFNCPDGYYNDLASLFLSTNDAAVRNIFSTNTPGEFHPLSLVIYFLYLICILGLTFGIAVPSGLFLPIILMG SAYGRLLG	516
SaCLCa2	517 IAMGSYTKIDRQLFAVLGAASLMASSMRMTVSLCVIFLELTNNLLLPVTMLVLLIAKSVGDCFNLISIYEIILELKGLPFLDANPE	602
SfCLCa2	517 IAMGSYTKIDRQLFAVLGAASLMASSMRMTVSLCVIFLELTNNLLLPVTMLVLLIAKSVGDCFNLISIYEIILELKGLPFLDANPE	602
SaCLCa2	603 PWMRNITVGEELADVKPPVVTLRGIEKVSIVDILKNTTHNAFPVIDDNVTVPEROQTKATELHGLIRAHLVAVLKRKWFLAQSRR	688
SfCLCa2	603 PWMRNITVGEELADVKPPVVTLRGIEKVSIVDILKNTTHNAFPVIDDNVTVPETOQTKATELHGLIRAHLVAVLKRKWFLAQSRR	688
SaCLCa2	689 TEEWEVREKFVWVELAERDLNFEQVAITKQEMEMYIDLHPFTNKTPTVVEDMSAAKAMVLFRQVALRHLIVPKYQGAGIYPVIG	774
SfCLCa2	689 TEEWEVREKFVWVELAERDLNFEQVAITKQEMEMYVDLHPFTNKTPTVVEDMSAAKAMVLFRQVALRHLIVPKYQGAGIYPVIG	774
SaCLCa2	775 VLTRQDLRAHNQSVFPHVAKSKKK	800
SfCLCa2	775 VLTRQDLRAHNQSVFPHVAKSKKK	800

(b)

SaCLCc2	1 MENELFKEDWRSRTKSEIFQYIFFLKWTLSLLIGFTGVVGFFNNLGVENISGFKFLQTDYVRHEKHFKGFTYFAGCNLLAVAAG	86
SfCLCc2	1 MSMVCLV-----TK-----FLYIMLCWSLMI-----E-----	26
SaCLCc2	87 ALCAFIAPAAAAGSGIPEVKAYLNGVDAHSILAPSTLFWKTIIGSILGVAAAGFVVGKEGPVMVHTGACLASLLGQQGSRKYHLTWKWL	172
SfCLCc2	27 -----QIIGSILGVAAAGFVVGKEGPVMVHTGACLASLLGQQGSRKYHLTWKWL	74
SaCLCc2	173 YFKNDRDRDILT CGSAAAGVAAA FRAPVGGVLFALEEVASWWRSALLWRTFTTAVVAVVLRSLIGFCRHGNCDLFGEGGLIMFDV	258
SfCLCc2	75 YFKNDRDRDILT CGSAAAGVAAA FRAPVGGVLFALEEVASWWRSALLWRTFTTAVVAVVLRSLIGCDRHGNCDLFGEGGLIMFDV	160
SaCLCc2	259 NHATSKWSVPDLILLILGI VGGVLGSFYNYLVDKVLRTYSIINEQGPVFVKKLLVMTISLLTSCCSYGLPWALPCPCPPELQDEC	344
SfCLCc2	161 NHATSKWSAPDEMILLILGI VGGVLGSFYNYLVDKVLRTYSIINEQGPVFVKKLLVMTISLLTSCCSYGLPWALPCPCPPELQDEC	246
SaCLCc2	345 PSIGRSGNYKFNCPAGHYNDLASLMFTNDAAIRNLNSNSNVKDFHVSTLYTYFGAMYCLGIITYGIAIPSGLFIPVILAGASYG	430
SfCLCc2	247 PSIGRSGNYKFNCPAGHYNDLASLMFTNDAAIRNLNSNSNVKDFHVSTLYTFGATYCLGIITYGIAIPSGLFIPVILAGASYG	332
SaCLCc2	431 RLVGTIMGPFCSDPGFLFAILGAASFGLGTMRRMVTSLCVILLELTNDLMLPLVMLVLLISKSVADEFNKGVYDQIVKMKGLPFME	516
SfCLCc2	333 RLVGTIMGPFCQDPLGFLFAILGAASFGLGTMRRMVTSLCVILLELTNDLMLPLVMLVLLISKSVADEFNKGVYDQIVKMKGLPFME	418
SaCLCc2	517 IHAEPYMRHLAASDVTGPLVVFSSVERVGTIMHALRATGHNGFPVIDDEPPFTDAPELCGIVLRSHLLVLLKAGKFTQHRELMRSN	602
SfCLCc2	419 IHAEPYMRHLAASDVTGPLVAFSSVERVGTIMHALRATGHNGFPVIDDEPPFTDAPELCGIVLRSHLLVLLKAGKFTQHRELMRSN	504
SaCLCc2	603 IKRSFQAFDFAKPGSGKGPKIDDLVITDEEMDMYVDLHPITNTCPYTVETMSLAKAAVLRELGLRHLCVVPKTPPEKPPIVGILT	688
SfCLCc2	505 IKKSFQAFDFAKPGSGKGPKIDDLVITDEEMDMYVDLHPITNTCPYTVETMSLAKAAVLRELGLRHLCVVPKTPPEKPPIVGILT	500
SaCLCc2	689 RHDFTPEHILGLYPHLLEVHK	708
SfCLCc2	591 RHDFTPEHILGLYPHLKVHK	610

Figure S1. Alignment of the proteins from *Suaeda altissima*, SaCLCa2 (a) and SaCLCc2 (b), with their homologs from closely related species *Suaeda fruticosa*, SfCLCa2 (a) and SfCLCc2 (b), respectively. SaCLCc2, unlike to SaCLCa2, is shortened from N-terminus.

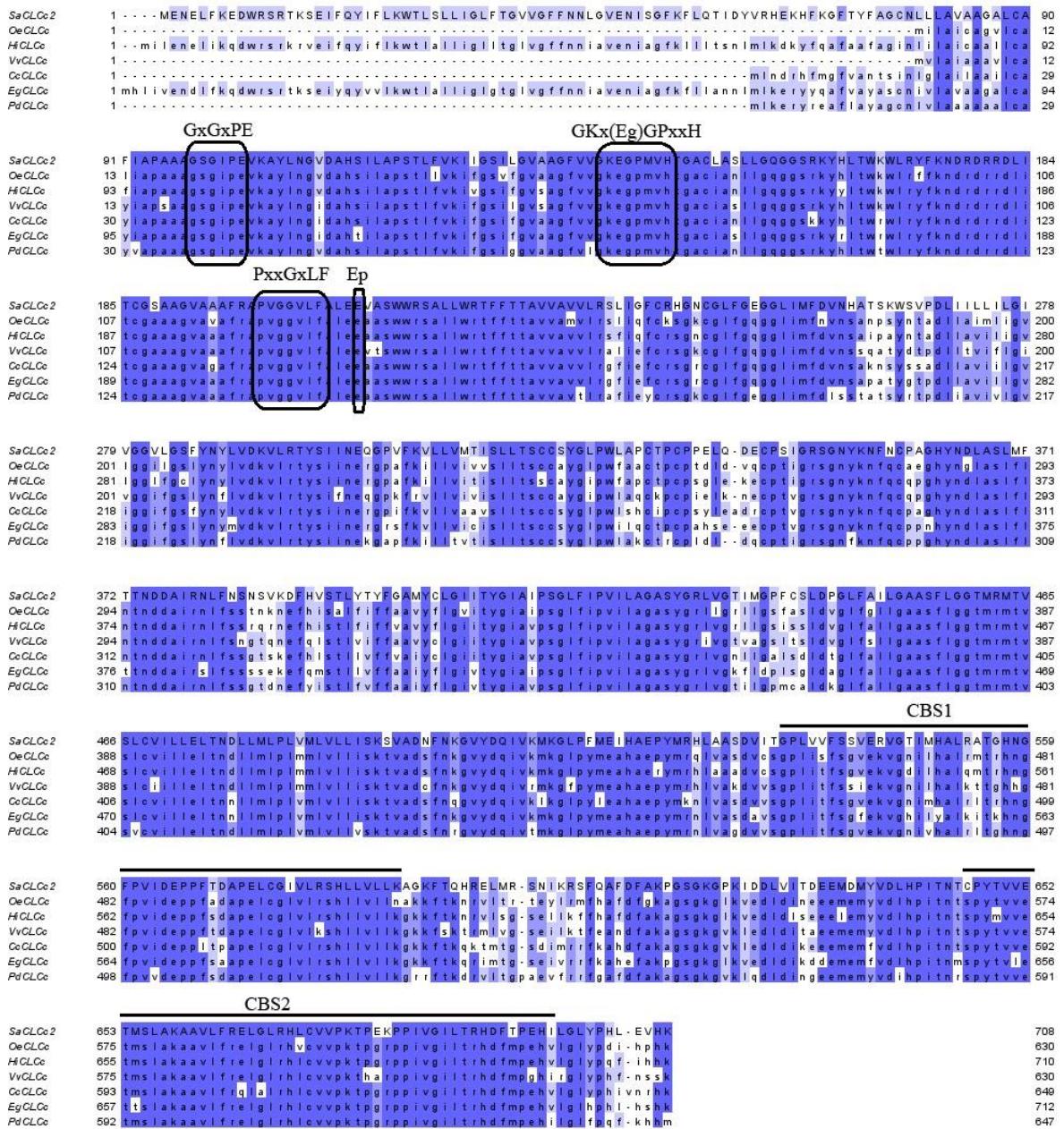
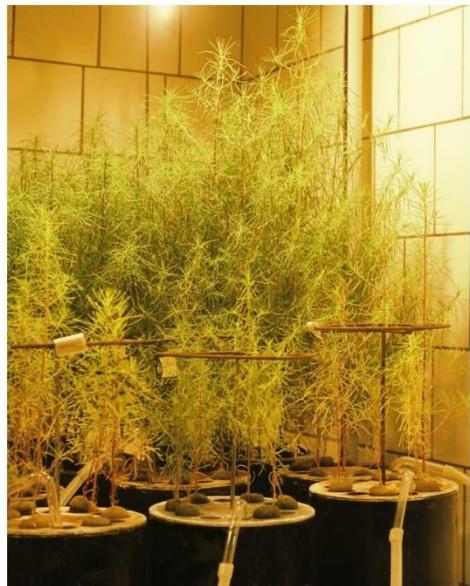


Figure S2. The CLCc proteins found in transcriptomes of different plants: *Suaeda altissima* (L.) Pall (GenBank, acc. no. OM994379), *Olea europaea* subsp. *europaea* (CAA 2963713.1), *Handroanthus impetiginosus* (PIN10391.1), *Vitis vinifera* (XP_010657886.1), *Olea europaea* var. *sylvestris* (XP_022861637.1), *Eucalyptus grandis* (XP_010030224.1), *Phoenix dactylifera* (XP_038975861.1).

(a)



(b)



Figure S3. *Suaeda altissima* plants grown under different concentrations of NaCl and nitrate in the aerated nutrient medium. **(a)** General view of the plants in the growth chamber. **(b)** *S. altissima* 45-day-old plants grown under different concentrations of NaCl (0, 250, 750 mM) and nitrate (15 mM or 0.5 mM) in the nutrient medium. Scale bar represents 15 cm.

Table S1. Conserved amino acids motifs and residues and their positions in SaCLCa2 and SaCLCc2 sequences.

Conserved amino acids motifs and residues	SaCLCa2	SaCLCc2
Gating Glu	E 225	E 142
Proton Glu	E 292	E 209
(1) residues in the selectivity filter, a.a.	GPGIPE 181–186	GSGIPE 98–103
(2) GKxGPxxH, a.a.	GKEGPLVH 223–230	GKEGPMVH 140–147
(3) PxxGxLF, a.a.	PVGGVLF 282–288	PVGGVLF 199–205
CBS1, a.a.	619–677	535–587
CBS2, a.a.	733–786	646–697

Table S2. The composition of the nutrient media used in the research.

Nº	15 mM NO ₃ ⁻		Nº	0.5 mM NO ₃ ⁻	
1.	0 mM NaCl	4 mM Ca(NO ₃) ₂ 7 mM KNO ₃ 1 mM KH ₂ PO ₄ 2 mM MgSO ₄	4.	0 mM NaCl	4 mM CaCl ₂ 6.5 mM KCl 0.5 mM KNO ₃ 1 mM KH ₂ PO ₄
2.	250 mM NaCl	50 µM FeSO ₄ 50 µM Na ₂ EDTA 50 µM H ₃ BO ₃ 10 µM MnCl ₂	5.	250 mM NaCl	2 mM MgSO ₄ 50 µM FeSO ₄ 50 µM Na ₂ EDTA 50 µM H ₃ BO ₃
3.	750 mM NaCl	1 µM ZnSO ₄ 0.5 µM CuSO ₄ 0.1 µM Na ₂ MoO ₄	6.	750 mM NaCl	10 µM MnCl ₂ 1 µM ZnSO ₄ 0.5 µM CuSO ₄ 0.1 µM Na ₂ MoO ₄

Table S3. List of the primers used in the research.

Primer name	Nucleotide sequences (5'-3')	Primer designation
SaCLCa2R	GCCCTCAAATCTTGCCTT	Amplification of 3'- and 5'-ends <i>SaCLCa2</i> transcript sequences
SaCLCa2_5'RACE_R1	GATCTAACAAAGCAATGTTGGTGCT	
SaCLCa2_5'RACE_R2	GCCCCTGTCAACGTAATCAGC	
SaCLCa2_3'RACE_F1	TCTTGAGCATTGGAGC	
SaCLCa2F	TCCCGGAGAATTCATCCAC	
pVR2_SaCLCa2_F	AAGGATCAAAACCATCATATGGAAGCAAGTGCAGAA TCACCA	Cloning of <i>SaCLCa2</i> into pVR2 vector
pVR2_SaCLCa2_R	ACTGACTCGACCCTATCATTCTGCTTTGGATTTGCT	
pMB1_SaCLCa2_F	ACACACATAAACAAACCATGGAAGCAAGTGCAGAAATCACCA	Cloning of <i>SaCLCa2</i> into pMB1 vector
pMB1_SaCLCa2_R	ATCGATACCGTCGACCTCGAGTCATTCTGCTTTGGATTTGCTACA	
SaCLCa2_P182S_F	CCTACTGCTGGTCTGGTATCCCTG	Site-directed mutagenesis of <i>SaCLCa1(C544T)</i>
SaCLCa2_528_R	AGCAAAGAAAACAACCAGCAGCGTAG	
SaCLCc2F	TGTCCTCCTGAAGTGCAGA	Amplification of 3'- and 5'-ends <i>SaCLCc2</i> transcript sequences
SaCLCc2R	GCCTAATTCCCGAAACAAACACT	
SaCLCc2_5'RACE_R1	GTTGTAAACATGAGTGAGGCAAGATC	
pVR2_SaCLCc2_F	CGAAGGATCAAAACCATCATAGAGACATTGAATAGCTGTGAGGTGA	Cloning of <i>SaCLCc2</i> into pVR2 vector
pVR2_SaCLCc2_R	CAGTACTGACTCGACCCTAAAGGGAAACTGCTCCTAACATTGTA	
pMB1_SaCLCc2_F	ACACACATAAACAAACCATGGAAAATGAACCTTTAAAGAGGGATTGGA	Cloning of <i>SaCLCc2</i> into pMB1 vector
pMB1_SaCLCc2_R	ATCGATACCGTCGACCTCGAGCTACTTGTGGACTTCGAGGTGC	
SaClCa1_F1	GAGACCTTGCTGAAGCCAAGC	RT-qPCR
SaClCa1_R1	TGTCCCTTCACTAGTAAGGATTG	

SaCLCa2_F1	GTCCCAGAAAGACAAGGCACG	
SaClCa2_R1	CCCATGTAAACTCTCTCGCAC	
SaClCc1_F1	GGCAAAGTATTCTCTGAGGAG	
SaClCc1_R1	AGATATATCCTCCAATGTGAGCC	
SaClCc2_F1	CGACCAAATTGTTAAGATGAAGG	
SaClCc2_R1	TGATCGTCCCAACTCTTCAAC	
SaClCd_F1	GGCTTCCCTGTGATAGATC	
SaClCd_R1	CACCTCTTGAATCACTAGGC	
SaClCg_F1	GGCTCCATGAGAATGACT	
SaClCg_R1	TGAAACCATCAGCCACG	
SaClCf_F1	GAAATGCTGCTGTTGCC	
SaClCf_R1	CAGAAGCAGAACAGATGTCA	
SaAct7_F1	AGATTCCGTTGCCAG	
SaAct7_R1	ATTTCCTTGCTCATACGGTCA	
SaeEF1alfa_F1	TGAGATGTGTGGCAATCC	
SaeEF1alfa_R1	GTTGCTTCTGACTCCAAGAAT	
pMB1_F	CATGGTTGTTATGTGTGTTATTG	Amplification of a linear form of pMB1 vector
pMB1_R	CTCGAGGTCGACGGTATCGATAAGC	