**Supplemental Data**

**Supplemental Figure 1.** Elevated expression of *CIPK21* gene in the WT (Col-0) plants under various conditions such as ABA (100 μM), cold (4˚C), drought, or PEG (30%) treatments. Semi-quantitative RT-PCR analysis (25 cycles) was performed with *CIPK21*-specific primers. *ACTIN2* was used as a loading control.

**Supplemental Figure S2.** Expression of the *CIPK21* gene in *Arabidopsis eFP Browser*.

**Supplemental Figure S3.** Subcellular localization of the the CIPK21 in the epidermal peel cells of *Nicotiana benthamiana*.

**Supplemental Figure S4.** CIPK21:GFP co-expressed either with CBL1n:OFP (upper panel, red) or TPC1:OFP (lower panel, red) in the epidermal cells of *Nicotiana benthamiana*. Scale bar = 40 μm.

**Supplemental Figure S5.** Investigation of interaction of CBLs with CIPK21 by bimolecular fluorescence complementation in the epidermal peel cells of *Nicotiana benthamiana*. Plasmid combinations are indicated on the left. Scale bars =40 μm.

**Supplemental Figure S6.** Determination of the relative fluorescence produced to monitor the effects of salt stress treatment on pGPTV-II-BAR-35S::mVENUS vector, which were incubated with 125mM NaCl or control (10 mM MES pH5.6, 10 mM $MgCl_2$) medium. Results are presented as average values along with standard errors from three experiments.

**Supplemental Table S1.** List of primers used for quantitative PCR/RT-PCR

| Name | Sequence 5' → 3' | quantitative PCR/RT-PCR |
|---|---|---|
| CIPK21F | GAAATCCGCAGGCGAGTTAA | quantitative PCR |
| CIPK21R | GCTTACTTCCGCGGTAAGTAAGC | quantitative PCR |
| Actin2F | CTTGCACCAAGCAGCATGAA | quantitative PCR |
| Actin2R | CCACCGATCCAGACACTGTACTT | quantitative PCR |
| CIPK21F | AACTTTGAGATTACCCTTTGAATCTAG | RT-PCR |
| CIPK21R | ATGGGGTTTGTTTGGAACGAAGAAGATC | RT-PCR |

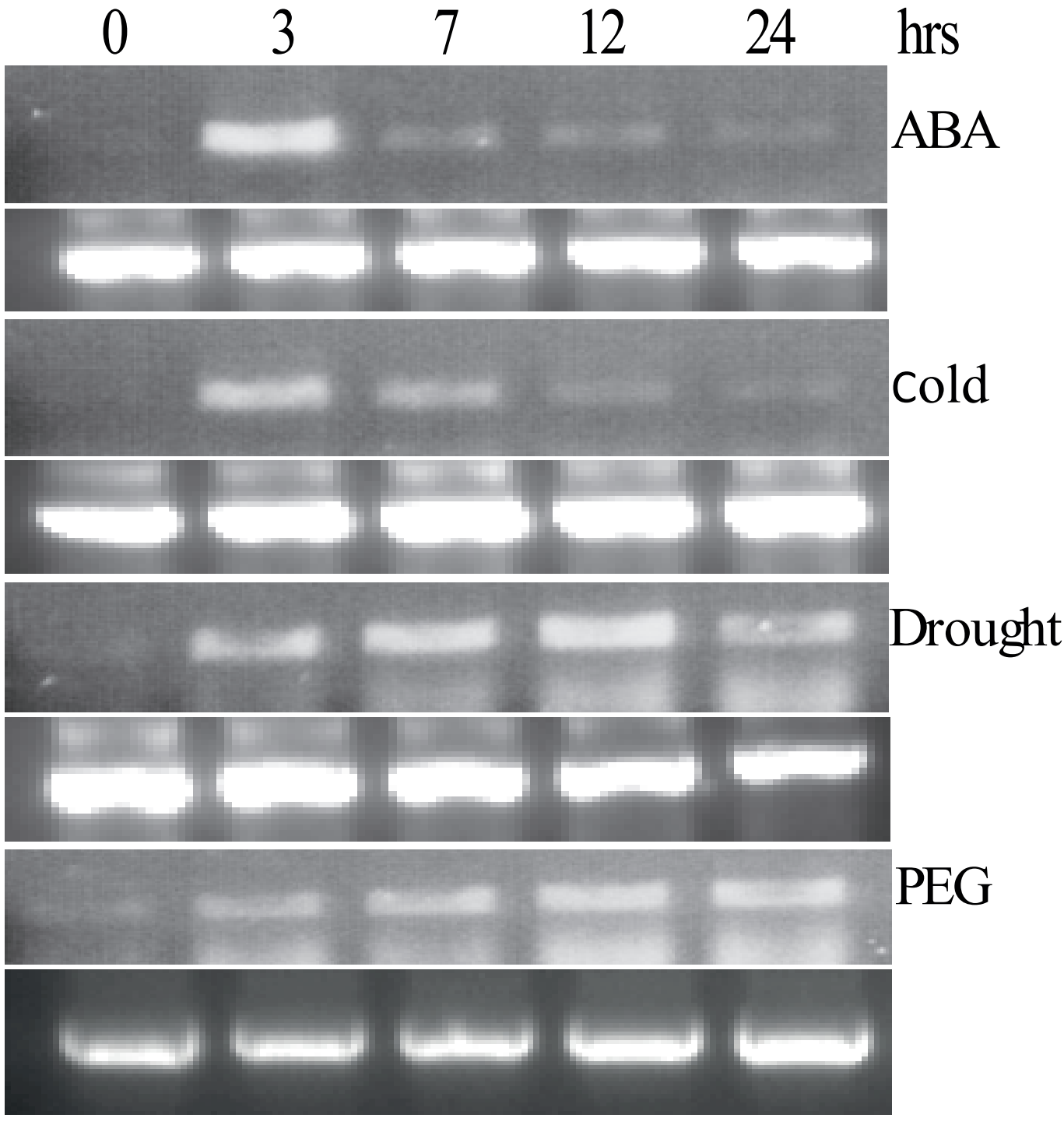
**Supplemental Table S2.** List of primers used for generation of constructs

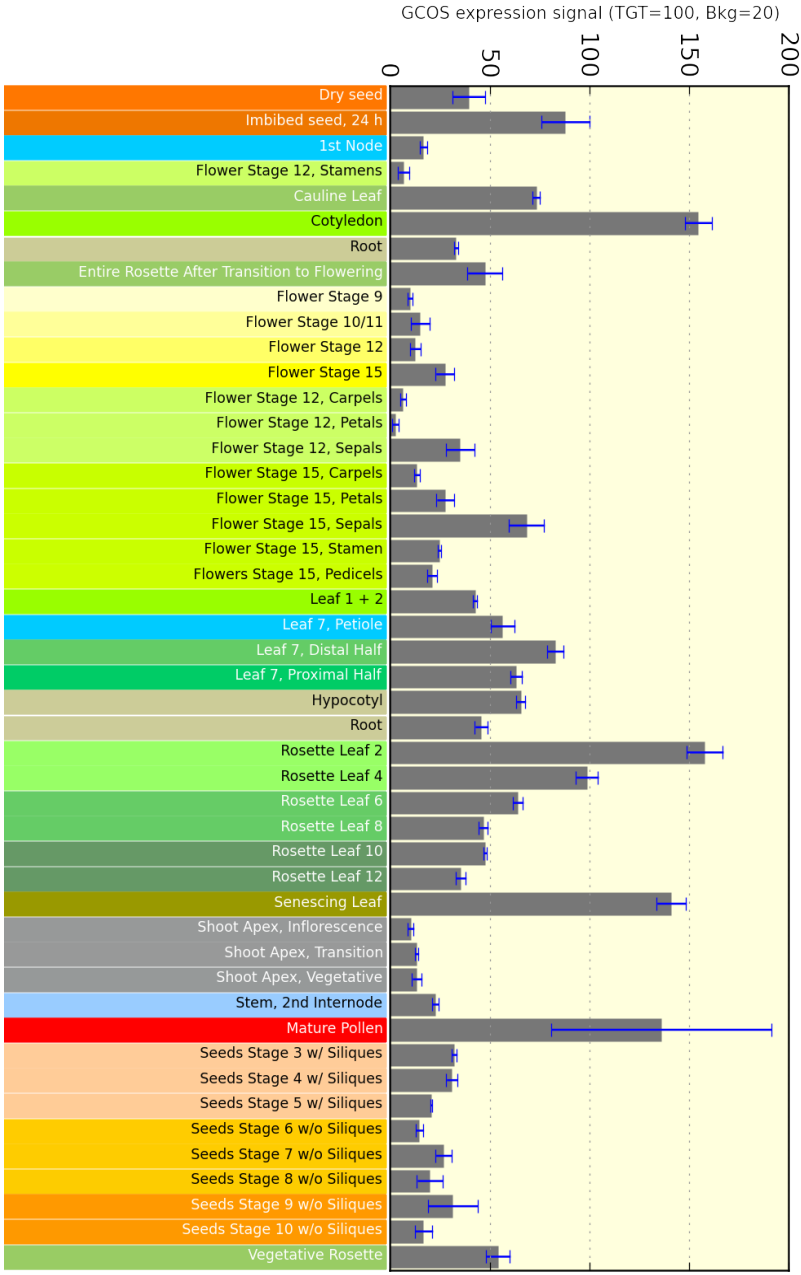
| Name | Sequence 5' → 3' | Constructs |
|---|---|---|
| cipk21_speforw | TTTACTAGTATGGGTTTGTTTGGAACGAAG | GFP::CIPK21, CIPK21:: GFP |
| | | CIPK21::SPYNE-173, SPYNE-173:: CIPK21 |
| cipk21_xhorev | TTTCTCGAGGCTTACTTCCGCGGTAAG | GFP::CIPK21, CIPK21:: GFP |
| | | CIPK21::SPYNE-173, SPYNE-173:: CIPK21 |
| CBL2GFPfor | TTTTCTAGAGGATCCTGAATGTCGCAGTGCGTTGA | CBL02::mCHERRY |
| CBL2GFPrev | TTTCCCGGGGGTATCTTCAACCTGAGAATGG | |
| CIPK21_promoterxbafor | AAATCTAGACAGGTTCTTTTGTCTCACAAAACG | CIPK21 promoter::GUS |
| CIPK21_GUSbamrev | AAAGGATCCCGTGATTGAATCGAAAGACTGGAAAG | |
| CIPK21_compecofor | AAAGAATTCCGGATTTAAGGTTTTTGCTACACAAGGT | CIPK21 promoter::CIPK21 |
| CIPK21_compbamrev | AAAGGATCCCCGTCGTTTGAAAATGTCCAATATAAACGA | CIPK21 promoter::CIPK21 |
| CIPK21_ADbamfor | AAAGGATCCCATGGGTTGTTTGGAACGAAGAAGATCGG | AD::CIPK21 |
| CIPK21_ADsalrev | AAAGTCGACTTAGCTTACTTCCGCGGTAAGTAAGCTTG | |

Restriction endonuclease sites are underlined

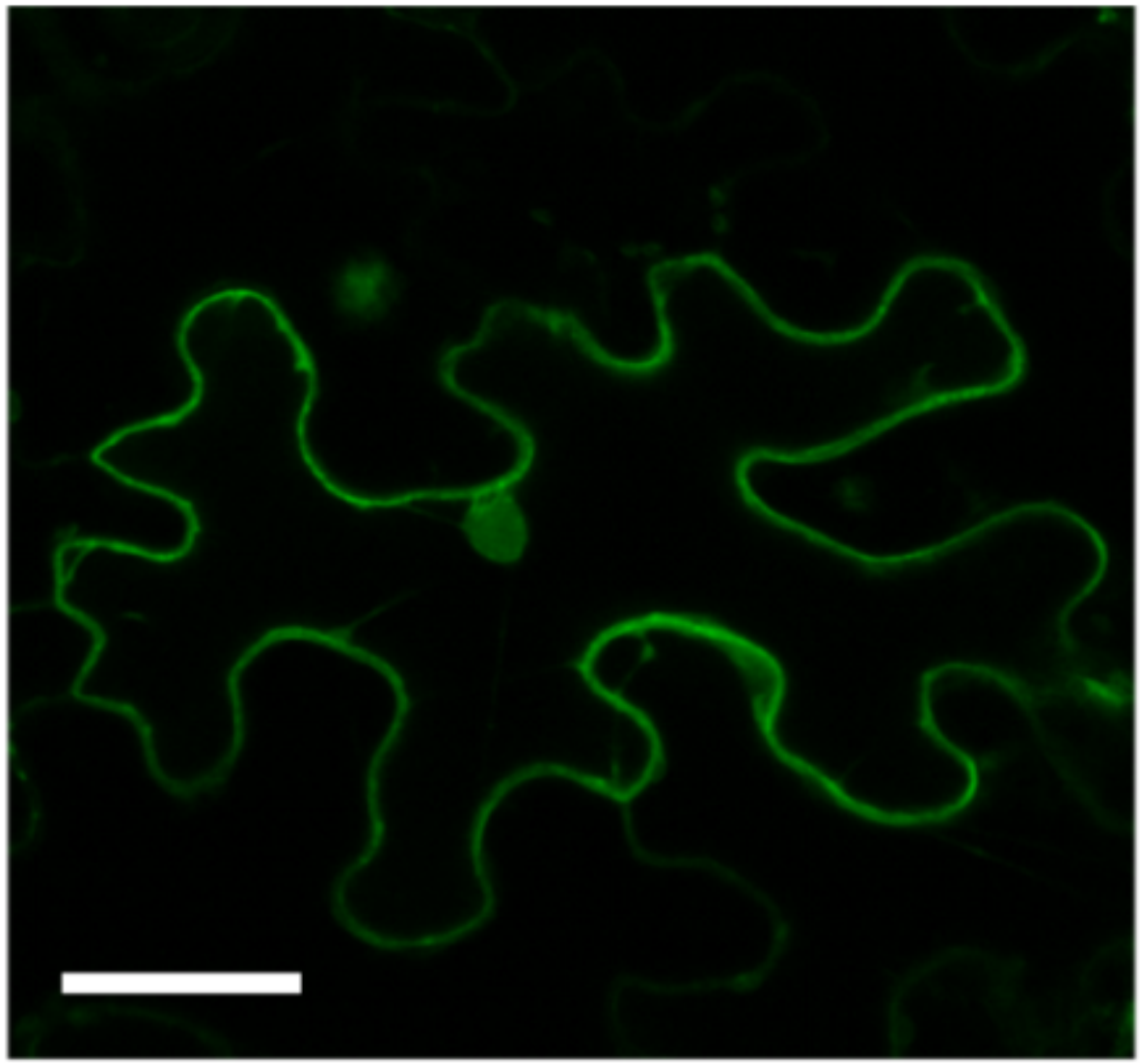
**Supplemental Table S3.** Published constructs used in this work.

| Constructs | References |
|---|---|
| pGPTV-II-BAR-pUBQ10-GFP::CIPK21<br>pGPTV-II-KAN-35S-CBL02::SPYCE-M<br>pGPTV-II-KAN-35S-CBL03::SPYCE-M<br>pGPTV-II-KAN-35S-CBL03::OFP | Batistič O, Waadt R, Steinhorst L, Held K, Kudla J. CBL-mediated targeting of CIPKs facilitates the decoding of calcium signals emanating from distinct cellular stores. Plant J; 2010 **61** (2):211-222. |
| BD.CBL1<br>BD.CBL2<br>BD.CBL3<br>BD.CBL4<br>BD.CBL5<br>BD.CBL6<br>BD.CBL7<br>BD.CBL8 | Kim KN, Cheong YH, Gupta R, Luan S. Interaction specificity of Arabidopsis calcineurin B-like calcium sensors and their target kinases. Plant physiology 2000; **124** (4):1844-1853.<br>Albrecht V, Ritz O, Linder S, Harter K, Kudla J. The NAF domain defines a novel protein-protein interaction module conserved in Ca2+-regulated kinases. The EMBO journal 2001; **20** (5):1051-1063. |
| BD.CBL9 | Kolukisaoglu Ü, Weinl S, Blazevic D, Batistič O, Kudla J. Calcium sensors and their interacting protein kinases: genomics of the Arabidopsis and rice CBL-CIPK signaling networks. Plant Physiol 2004; **134:** 43-58. |
| BD.CBL10 | Kim BG, Waadt R, Cheong YH*, et al.* The calcium sensor CBL10 mediates salt tolerance by regulating ion homeostasis in Arabidopsis. Plant J 2007; **52** (3):473-484. |


0
3
7
12
24
hrs
ABA
cold
Drought
PEG


GCOS expression signal (TGT=100, Bkg=20)
0
50
100
150
200
Dry seed
Imbibed seed, 24 h
1st Node
Flower Stage 12, Stamens
Cauline Leaf
Cotyledon
Root
Entire Rosette After Transition to Flowering
Flower Stage 9
Flower Stage 10/11
Flower Stage 12
Flower Stage 15
Flower Stage 12, Carpels
Flower Stage 12, Petals
Flower Stage 12, Sepals
Flower Stage 15, Carpels
Flower Stage 15, Petals
Flower Stage 15, Sepals
Flower Stage 15, Stamen
Flowers Stage 15, Pedicels
Leaf 1 + 2
Leaf 7, Petiole
Leaf 7, Distal Half
Leaf 7, Proximal Half
Hypocotyl
Root
Rosette Leaf 2
Rosette Leaf 4
Rosette Leaf 6
Rosette Leaf 8
Rosette Leaf 10
Rosette Leaf 12
Senescing Leaf
Shoot Apex, Inflorescence
Shoot Apex, Transition
Shoot Apex, Vegetative
Stem, 2nd Internode
Mature Pollen
Seeds Stage 3 w/ Siliques
Seeds Stage 4 w/ Siliques
Seeds Stage 5 w/ Siliques
Seeds Stage 6 w/o Siliques
Seeds Stage 7 w/o Siliques
Seeds Stage 8 w/o Siliques
Seeds Stage 9 w/o Siliques
Seeds Stage 10 w/o Siliques
Vegetative Rosette

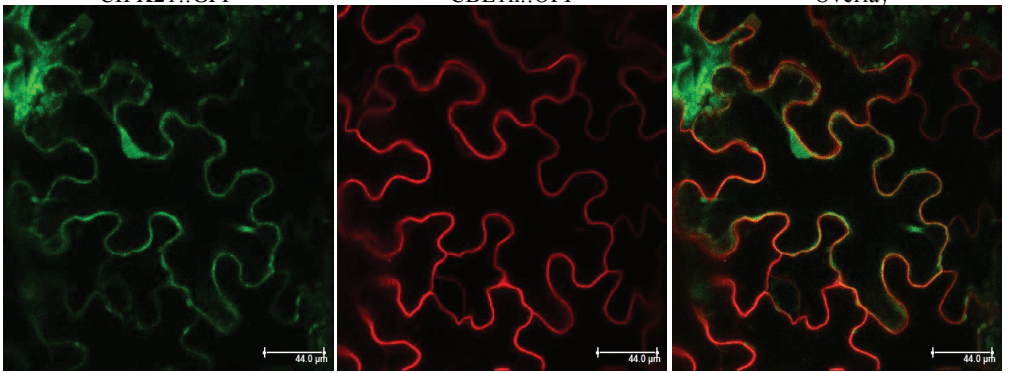
CIPK21::GFP



CIPK21::GFP

CBL1n::OFP

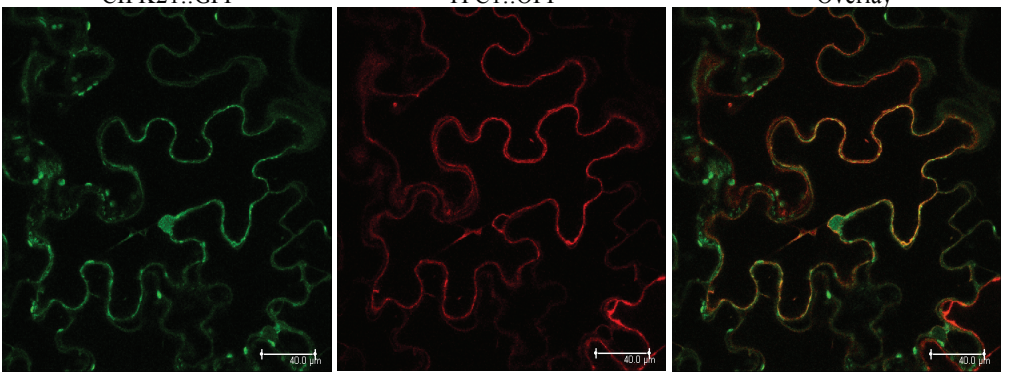
Overlay



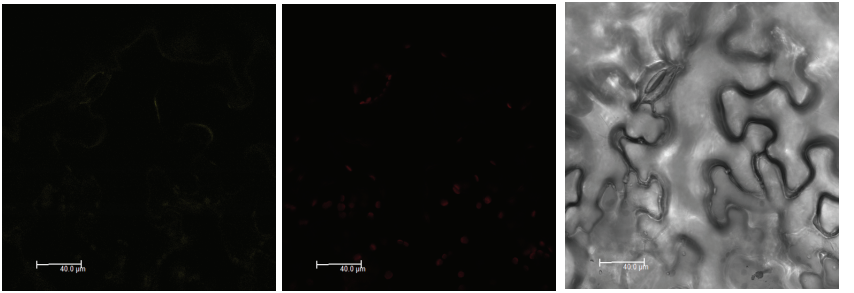

CIPK21::GFP

TPC1::OFP

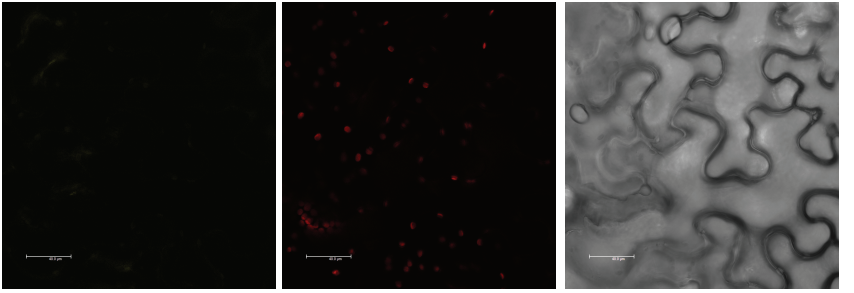
Overlay



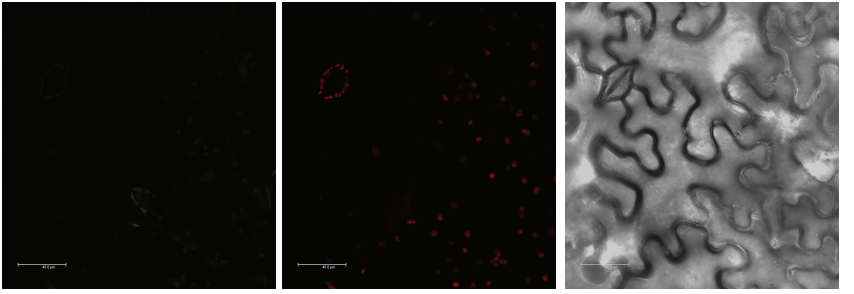
SPYNE(R)173::CIPK21
+CBL5::SPYCE(M)

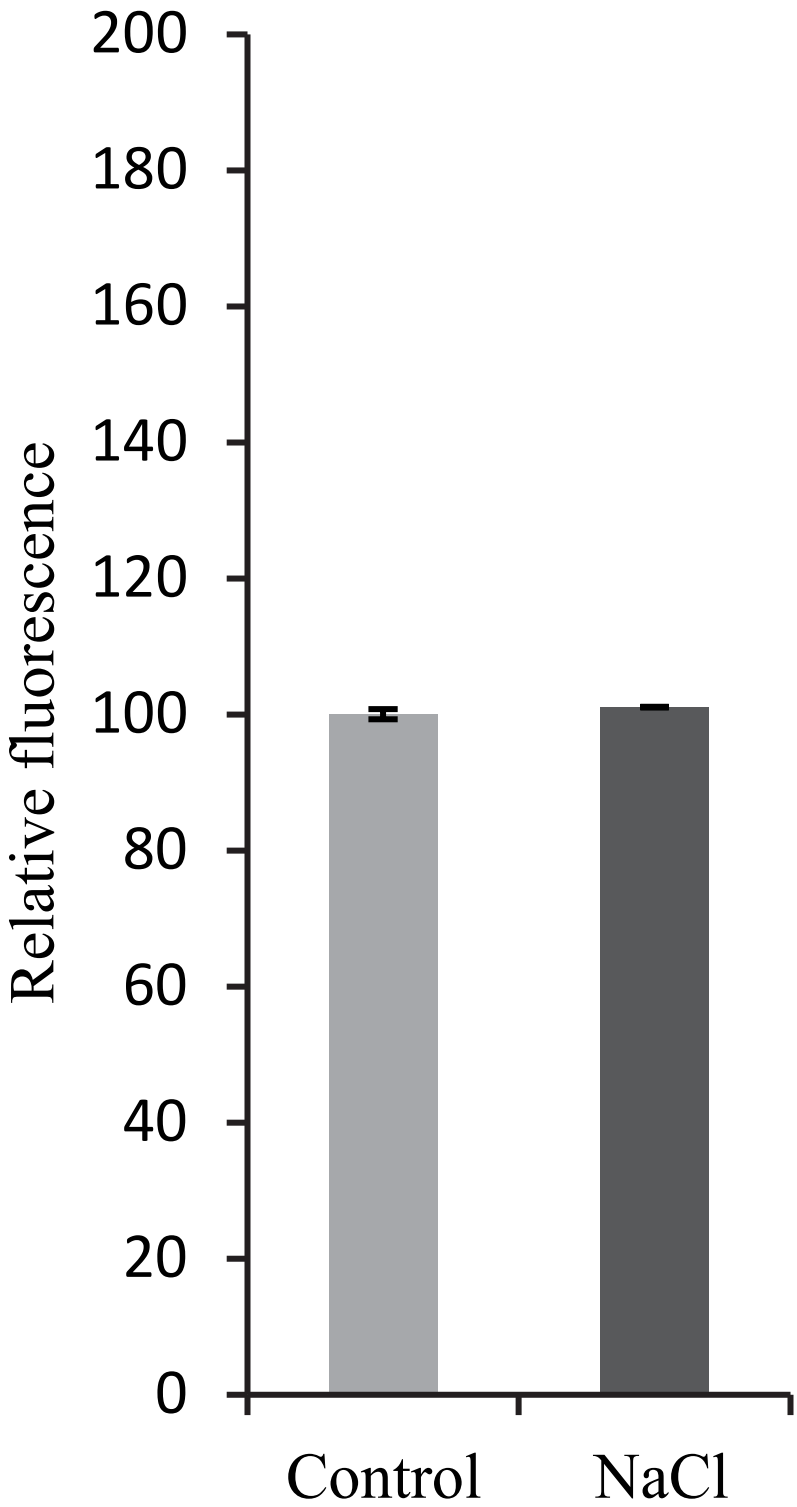


SPYNE(R)173::CIPK21
+CBL7::SPYCE(M)



SPYNE(R)173::CIPK21
+SPYCE(M)




Relative fluorescence
200
180
160
140
120
100
80
60
40
20
0
Control
NaCl