Sensing osmotic stress: A quantitative analysis of the osmotic-induced calcium response in *Arabidopsis* seedlings

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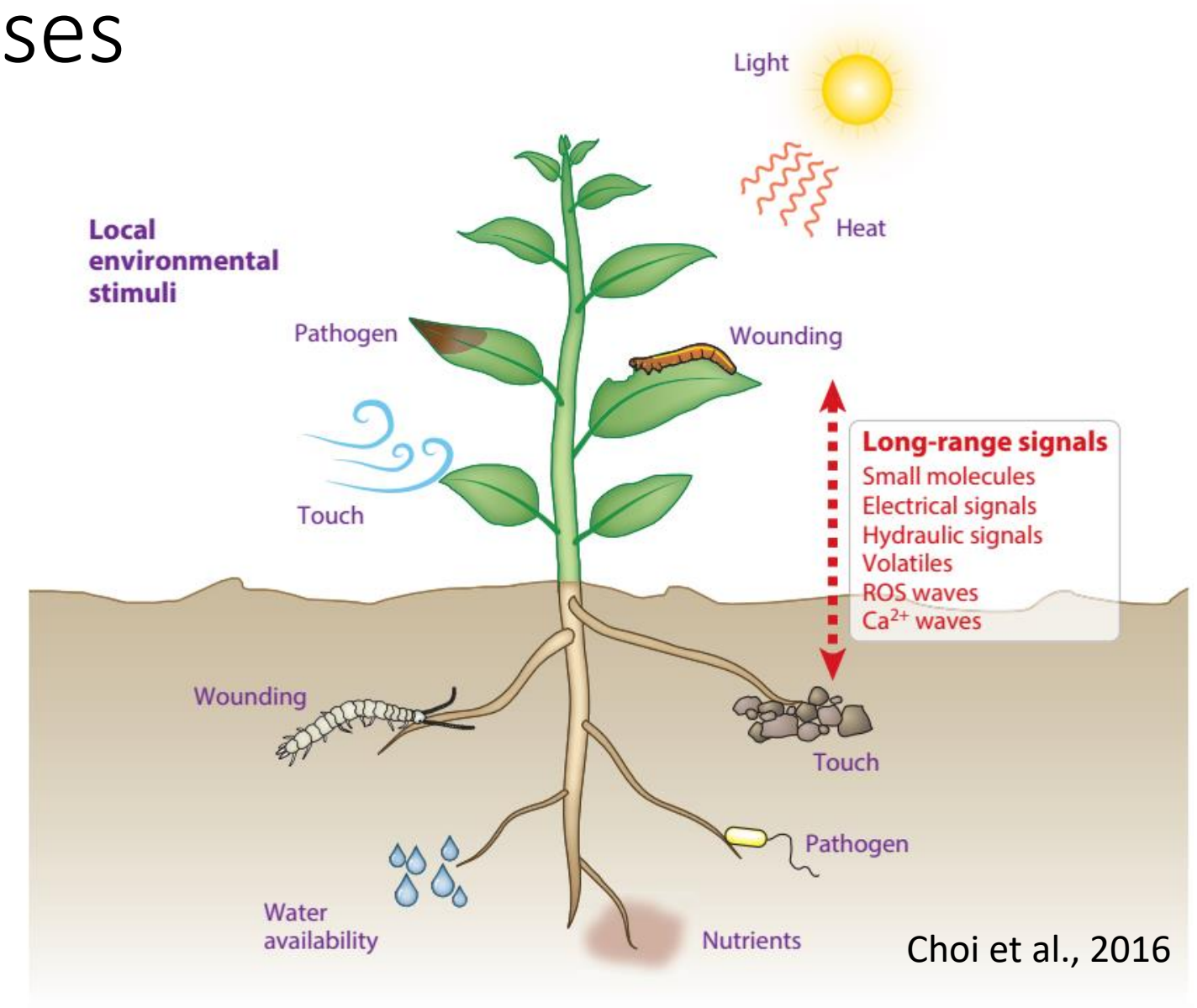
Projektmodul: University of California San Diego, Schroeder laboratory

17.05.2017 Berlin

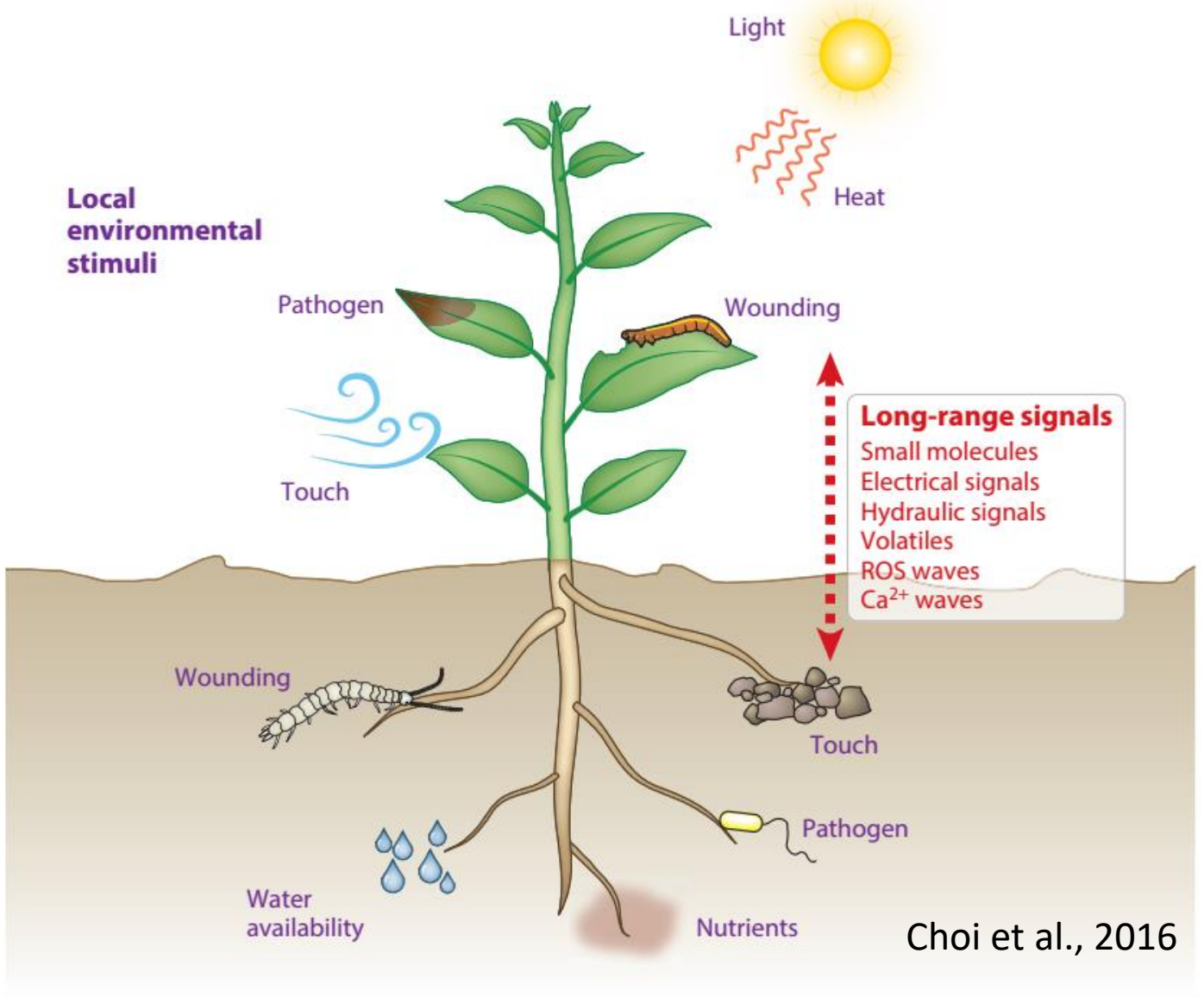
Introduction

Plants are exposed to biotic and abiotic stresses



Choi et al., 2016

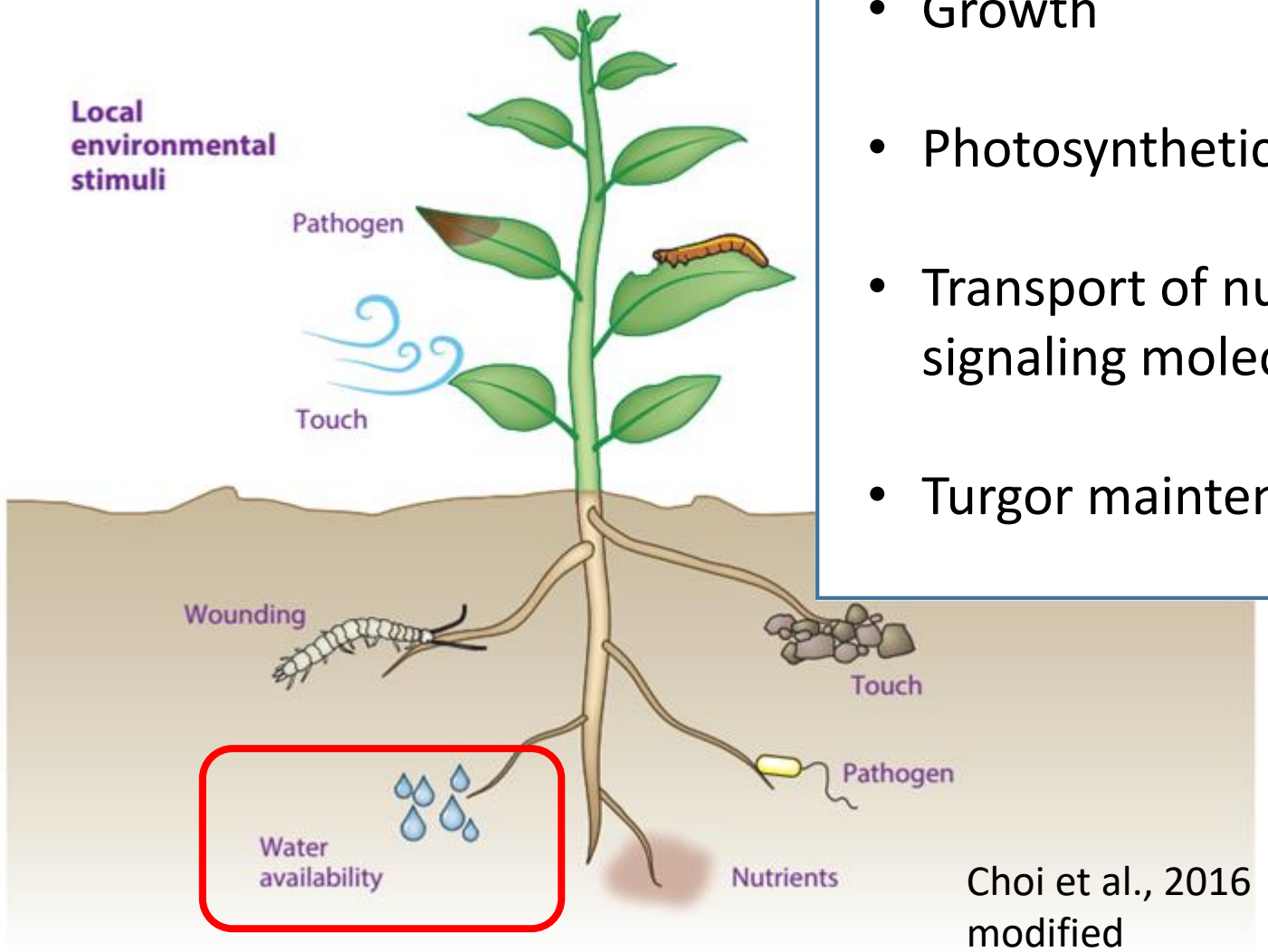
Stress: Primary receptor → Sensor → Response



Choi et al., 2016

Importance of Water

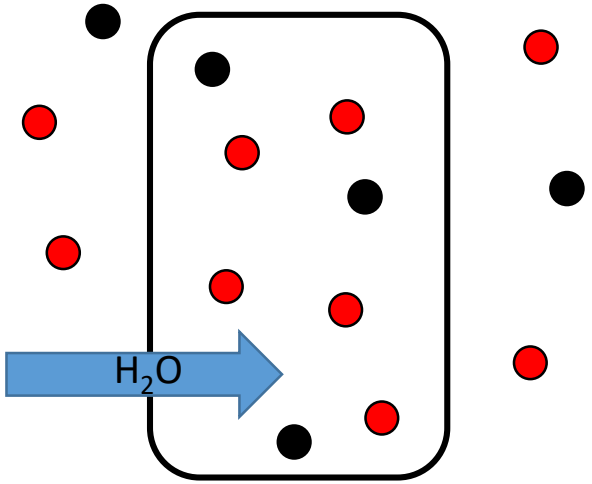
- Growth
- Photosynthetic activity
- Transport of nutrients and signaling molecules
- Turgor maintenance



Choi et al., 2016 modified

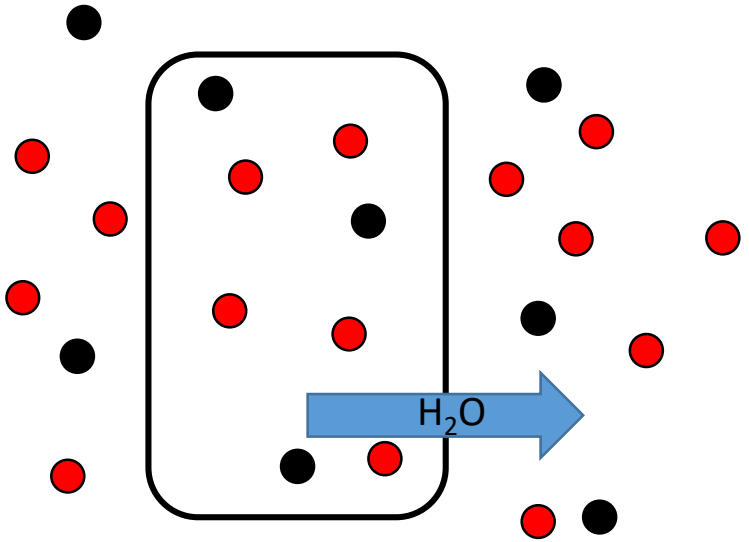
Salt stress reduces water availability

Normal salt conditions



$\Psi_{Soil} > \Psi_{Root\ Cell}$

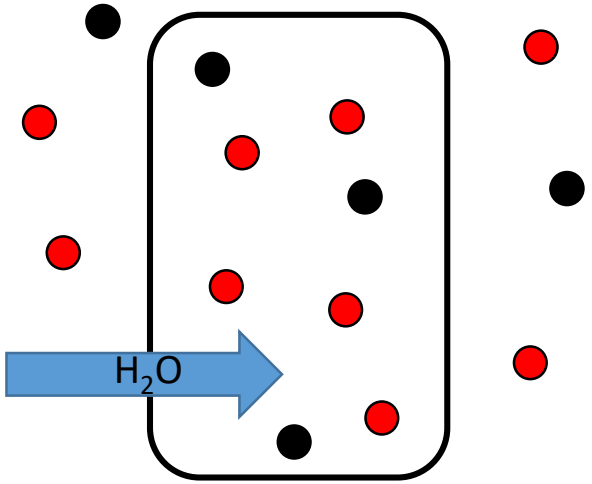
Elevated salt conditions



$\Psi_{Soil} < \Psi_{Root\ Cell}$

Salt stress has an osmotic and ionic component

Normal salt conditions



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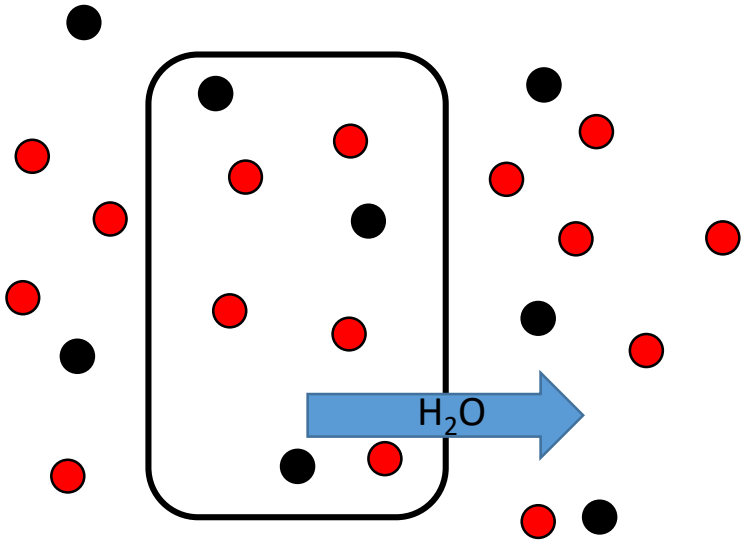
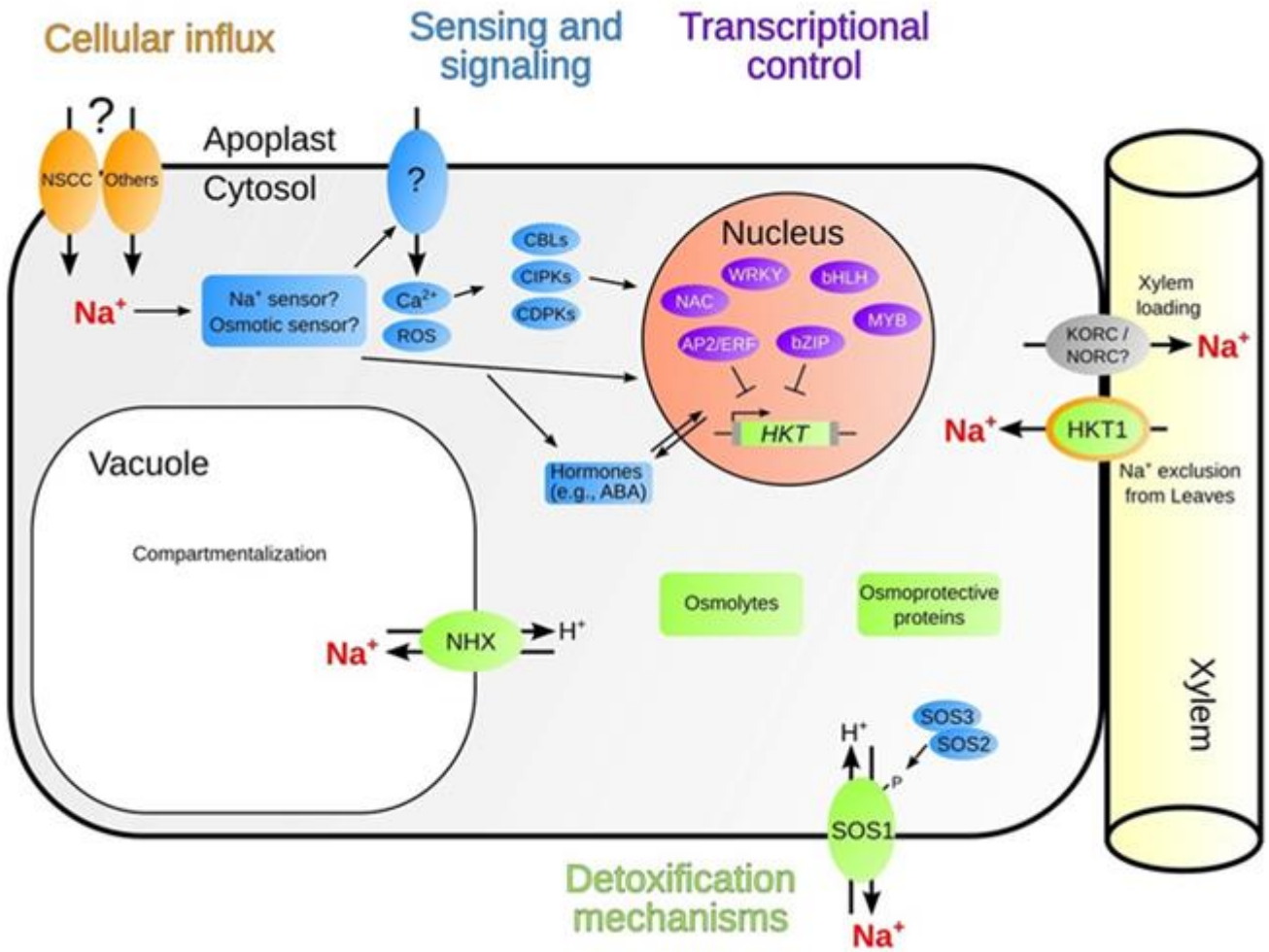
Elevated salt conditions



$\Psi_{Soil} < \Psi_{Root\ Cell}$

Perception of salt stress



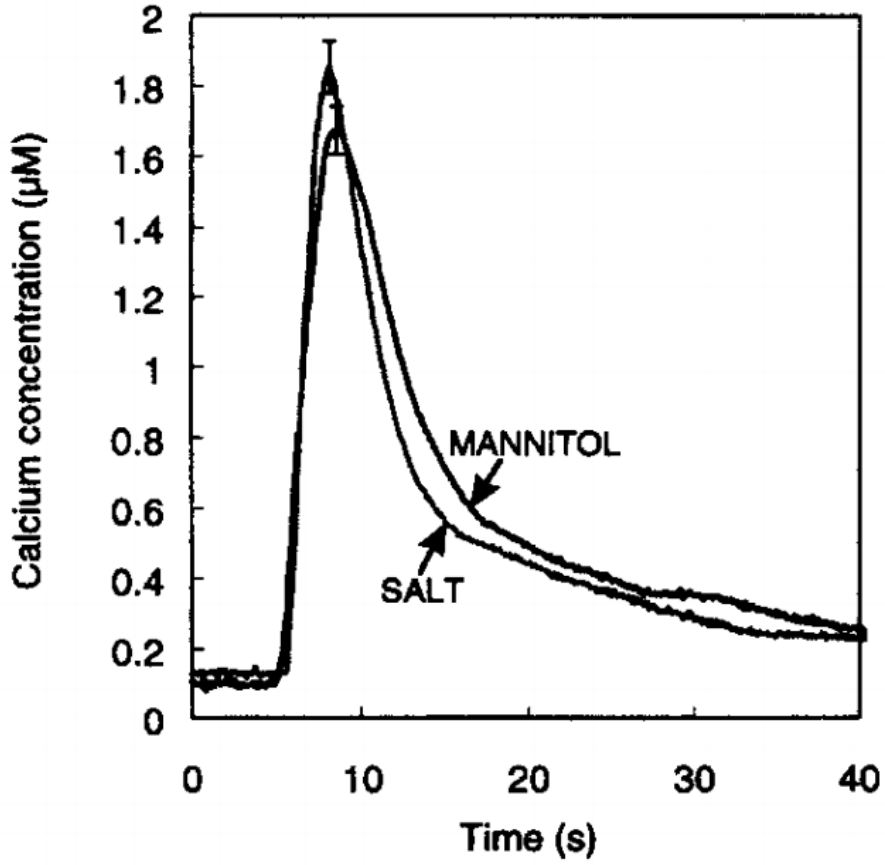
Deinlein et al., 2014

Osmotic stress and salt stress induce an increase in cytosolic Ca^{2+}

- Found in maize, corn, *Arabidopsis*

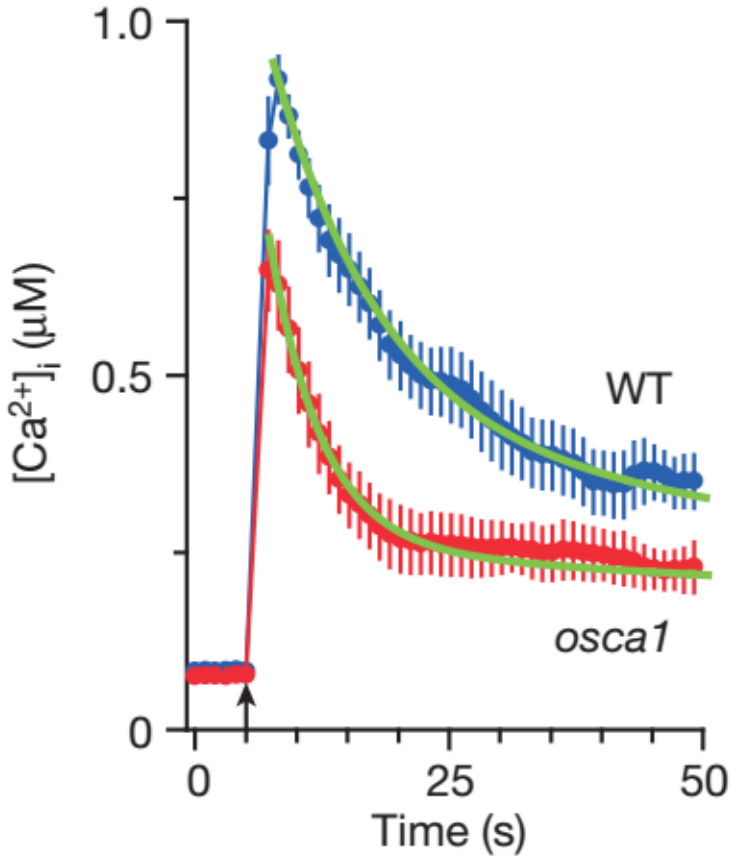
→ Probably highly redundant

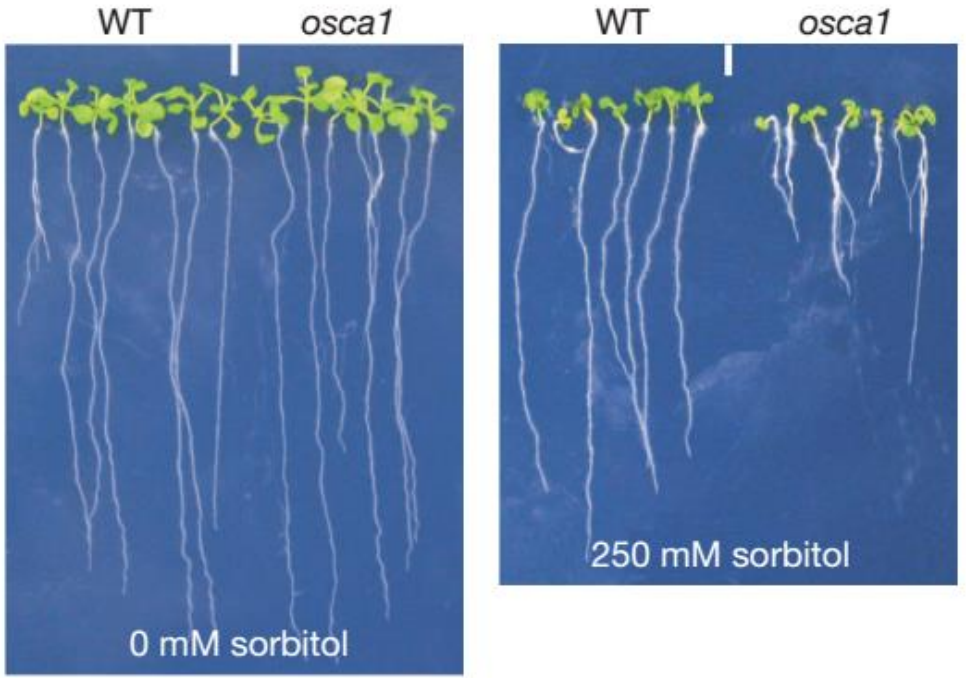
→ Analysis might help to detect the channels involved in osmoperception



Knight et al., 1997

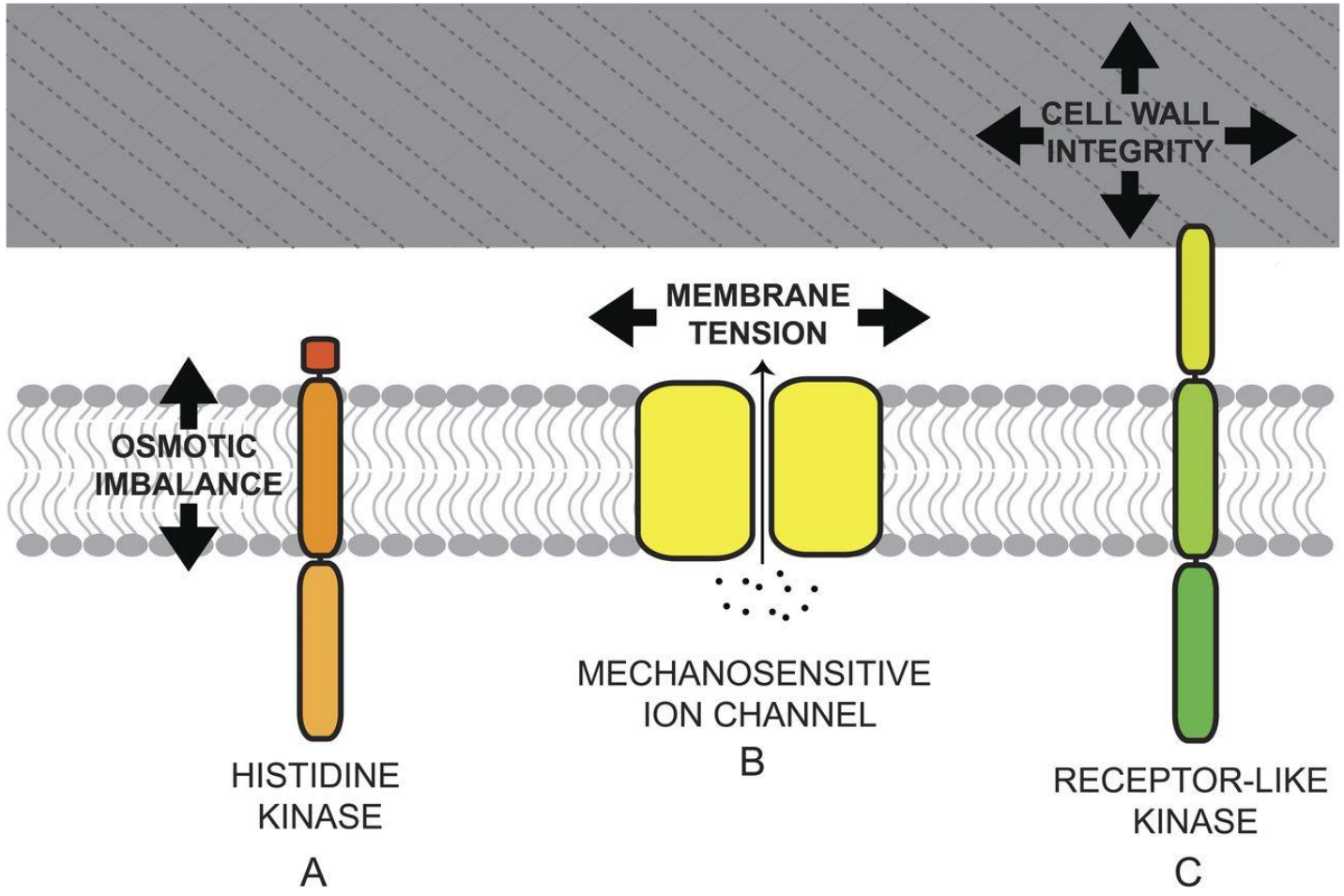
osca1 is the first mutant line with an impaired osmotic-induced Ca^{2+} response





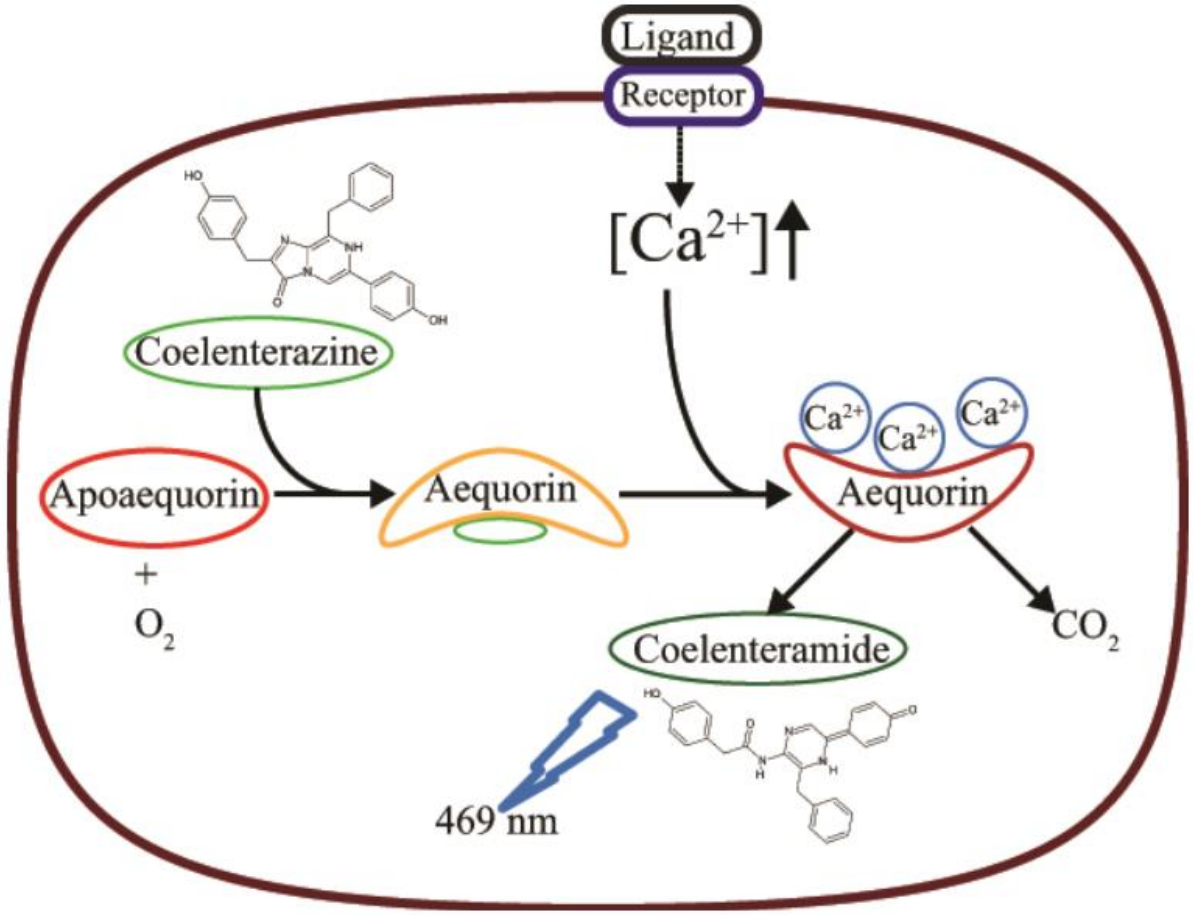
Yuan et al., 2014

The molecular basis for plant osmoperception is unknown



Haswell and Verslues, 2015

Aequorin-based Ca^{2+} measurements



Kanchiswamy et al., 2014

Aequorin luminescence experiment

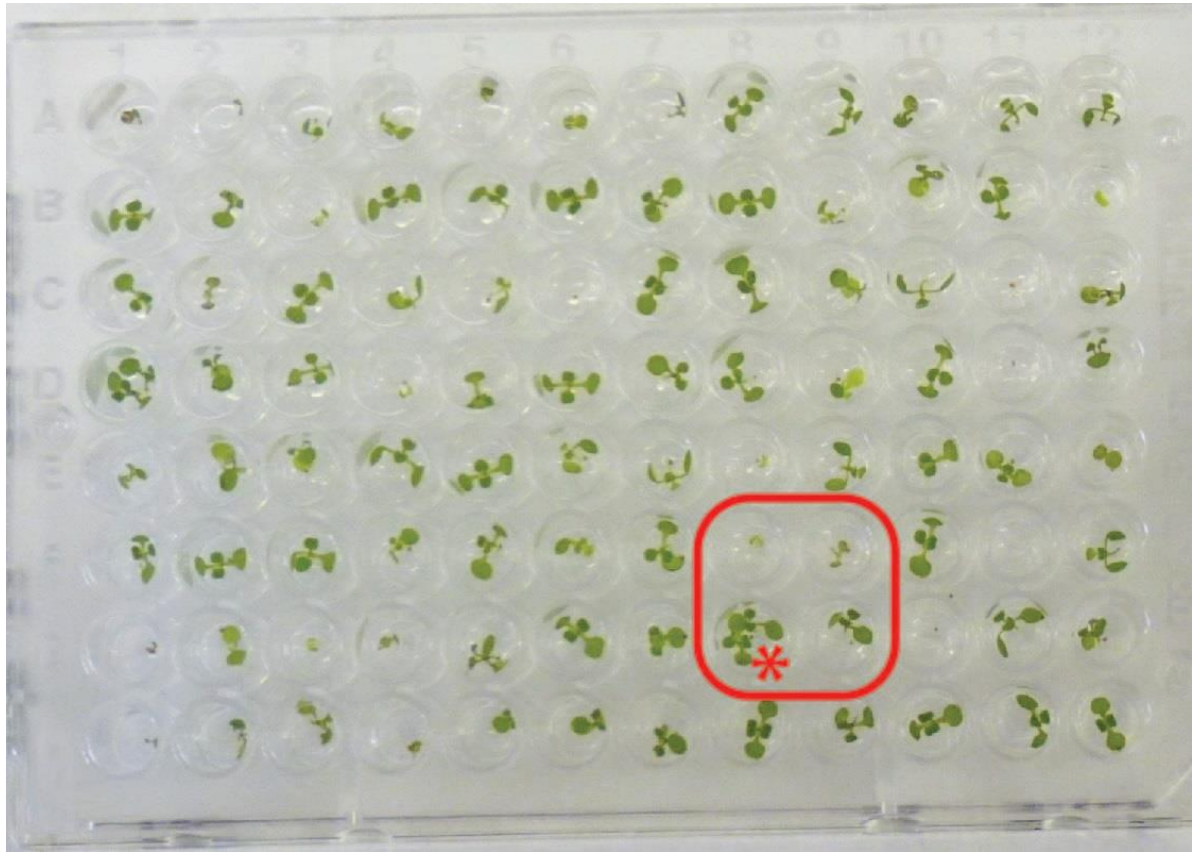
- Grow seedlings in 96-well plate for 7 days in ½ MS with 0.08 % phytoagar

- Incubate in coelenterazine for 6 hours

→ Inject 100 µl stimulus into each well

→ Measure luminescence

→ Normalize aequorin expression



http://www.bio-protocol.org/e1519

Key points

- Ion channels involved in osmosensing are unknown
- Osmosensing probably involves Ca^{2+} signaling
- Osmotic sensors could be mechano-sensitive channels or receptor-like kinases
- *osca1* first line with impaired osmotic-induced Ca^{2+} response
- Ca^{2+} can be measured using aequorin luminometry

What are the mechanisms involved in osmoperception?

- Approach: Forward genetic screen

→ Mutagenize Col-0 aequorin expression *Arabidopsis* line

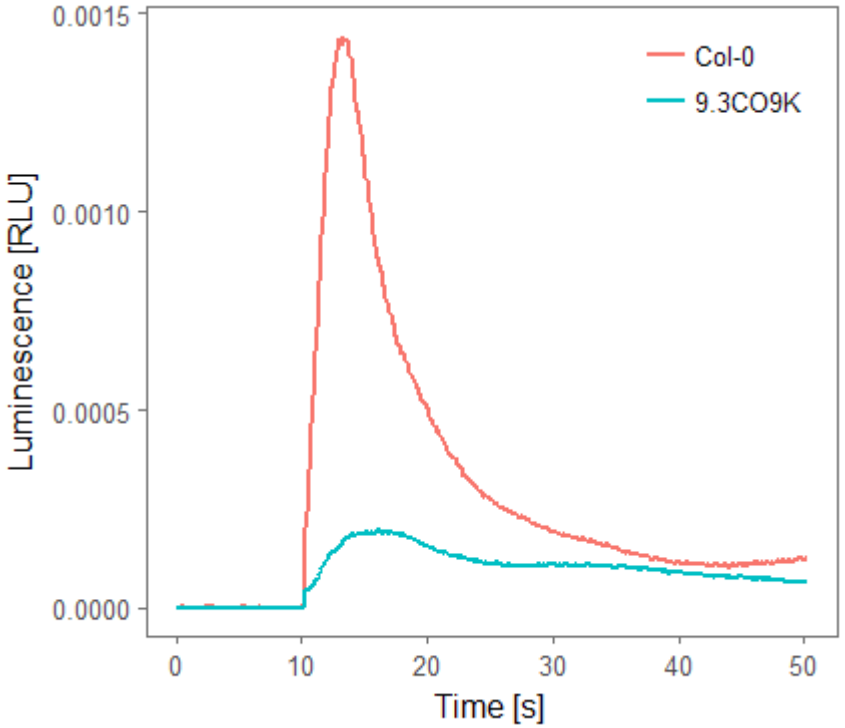
→ Luminescence screen for M1 seedlings with reduced Ca^{2+} response

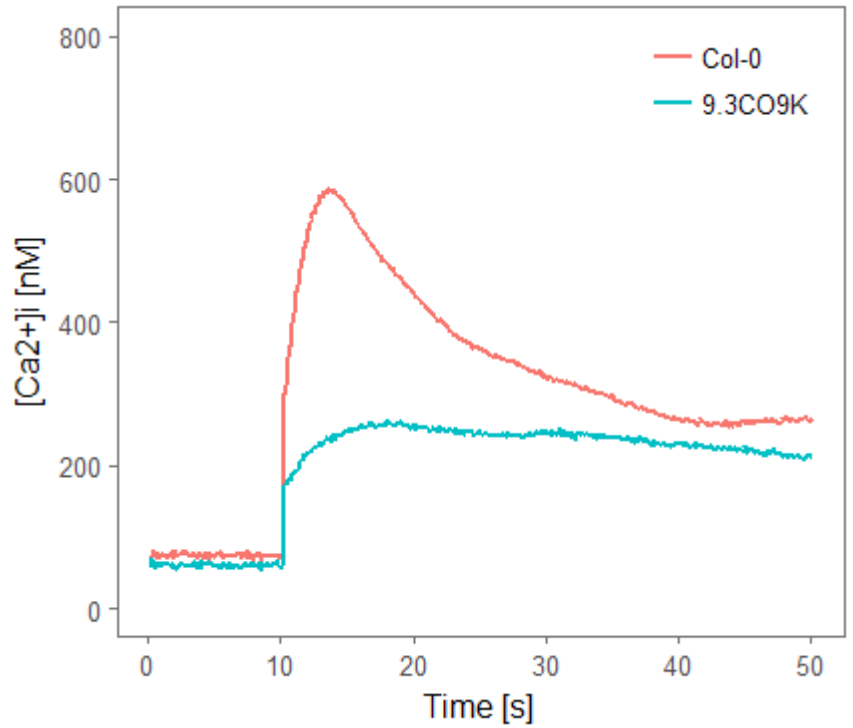
→ Select mutant with strongest phenotype

→ Repeat with aequorin normalization and high sample sizes

Results

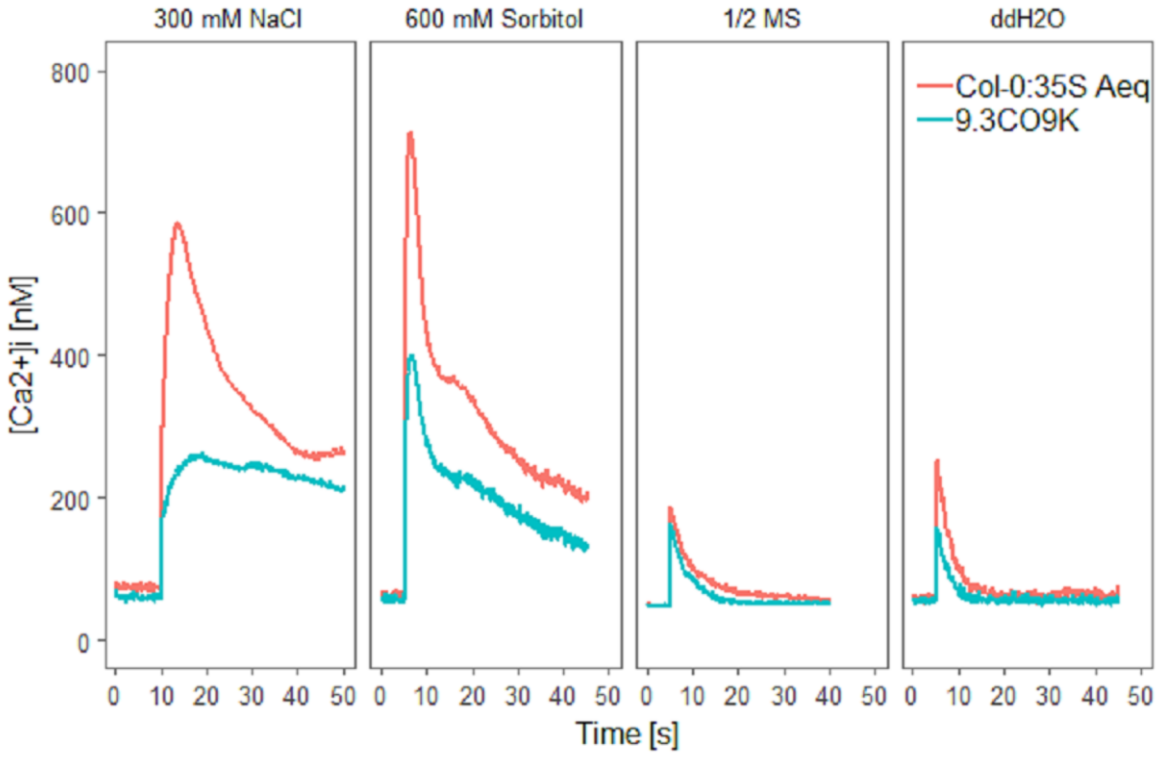
The 9.3CO9K line shows a significantly reduced Ca^{2+} response

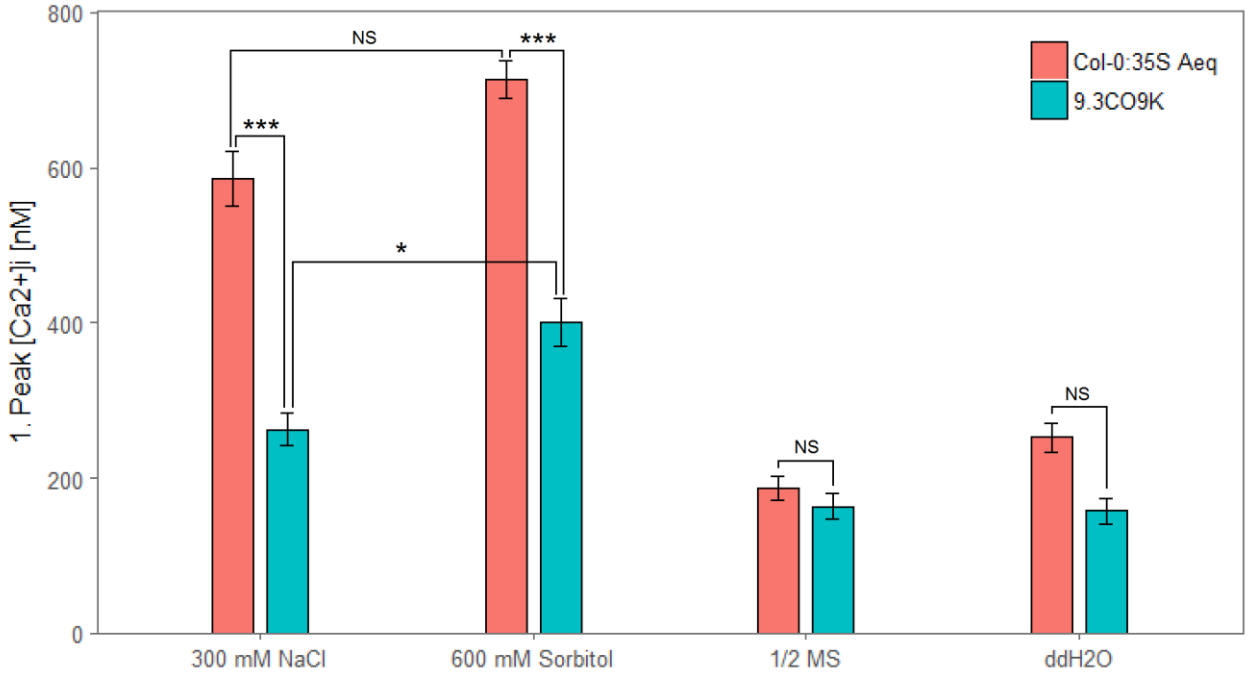




Average traces, n = 80 – 96 seedlings per line
Stimulus: 300 mM NaCl

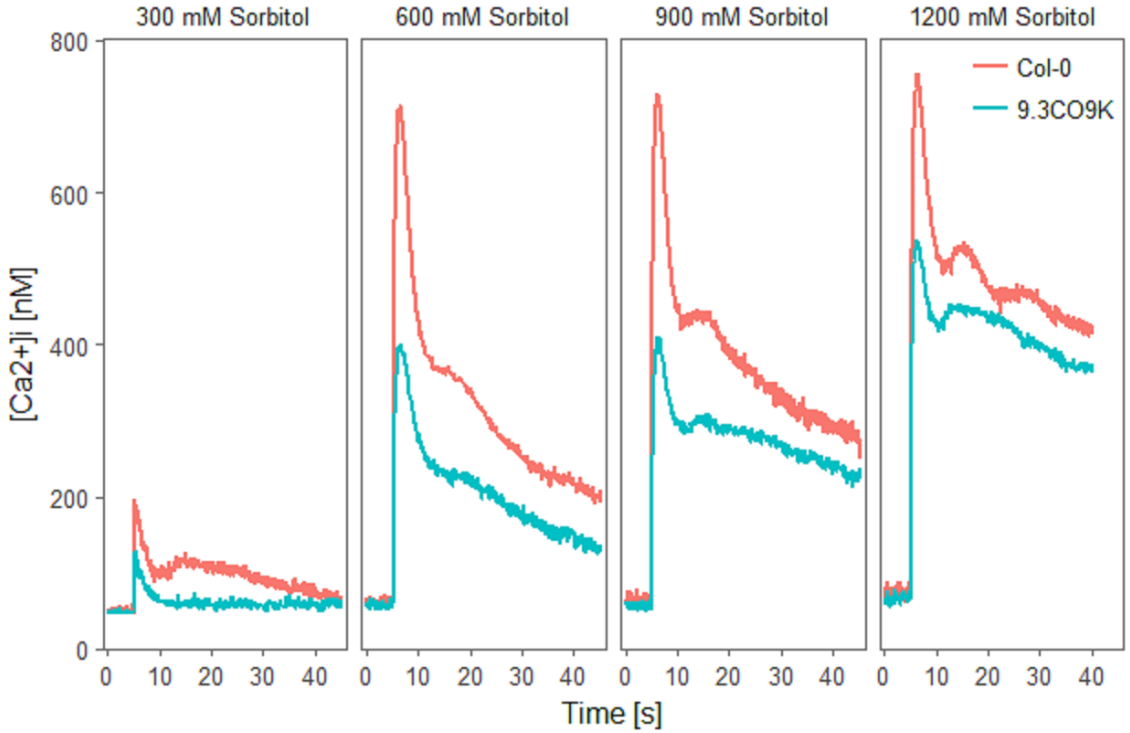
Sorbitol and NaCl induced Ca^{2+} responses are impaired in the 9.3CO9K line

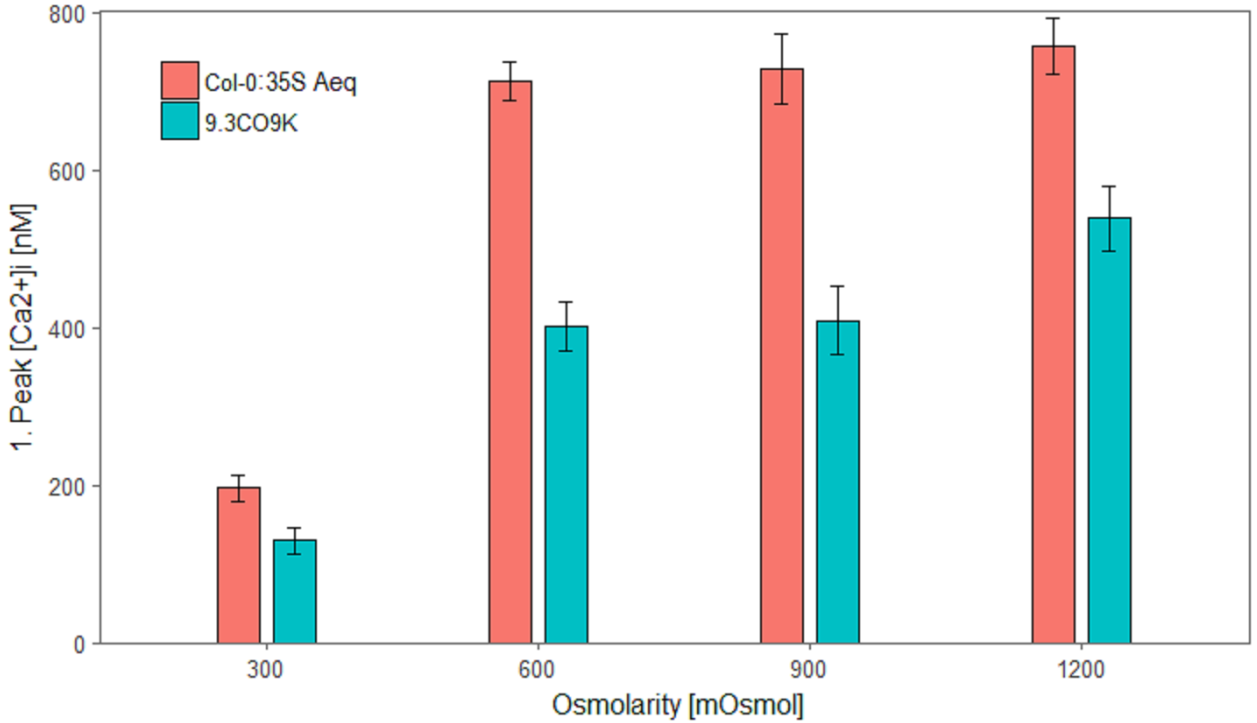




Average traces, n = 40 – 48 seedlings per line per condition

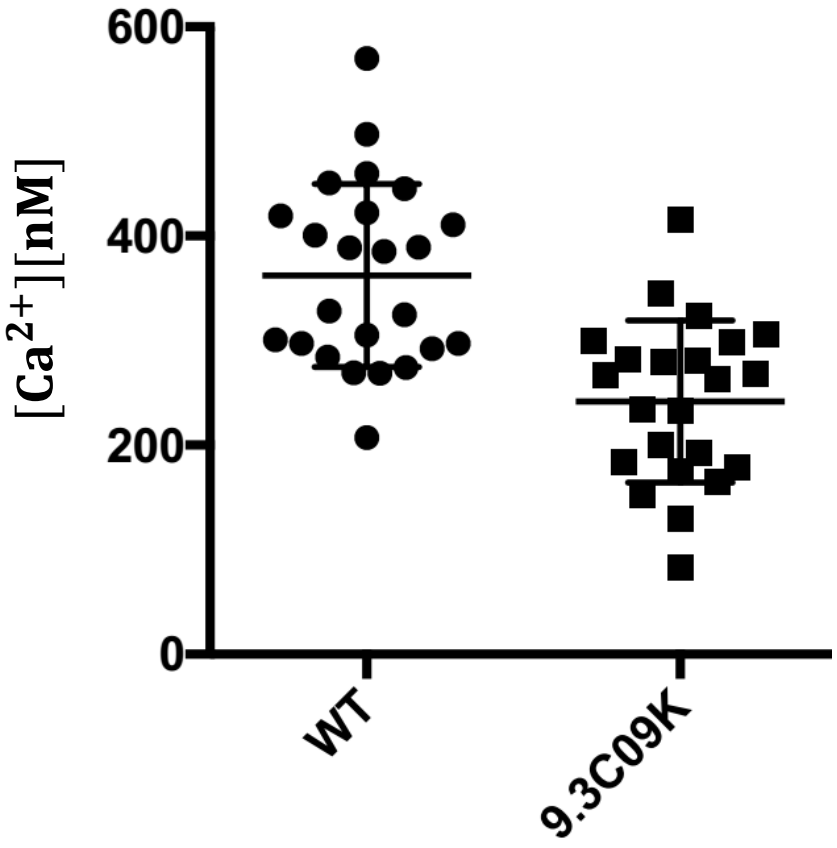
Optimization of stimulus osmolarity for luminescence assays





Average traces, n = 40 – 48 seedlings per line per condition

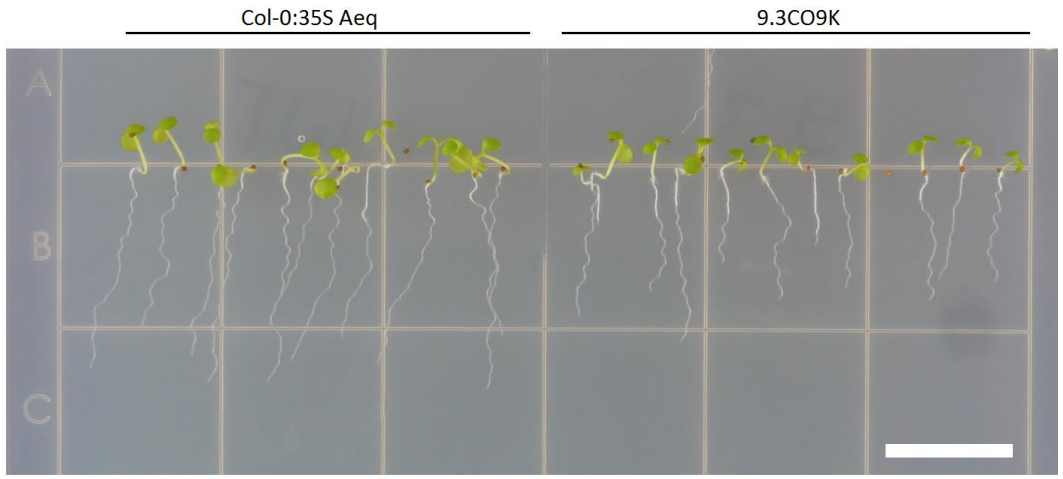
Ca^{2+} peak distribution of individual seedlings

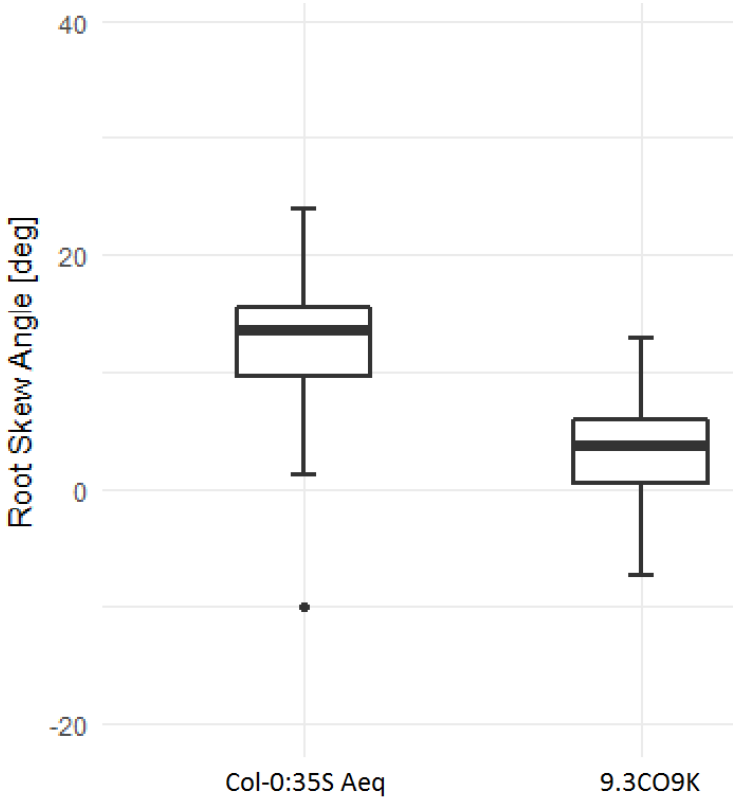


Data and figure: Po-Kai Hsu

→ Not easily possible to map the Ca^{2+} phenotype causing mutation based on Ca^{2+} Phenotype

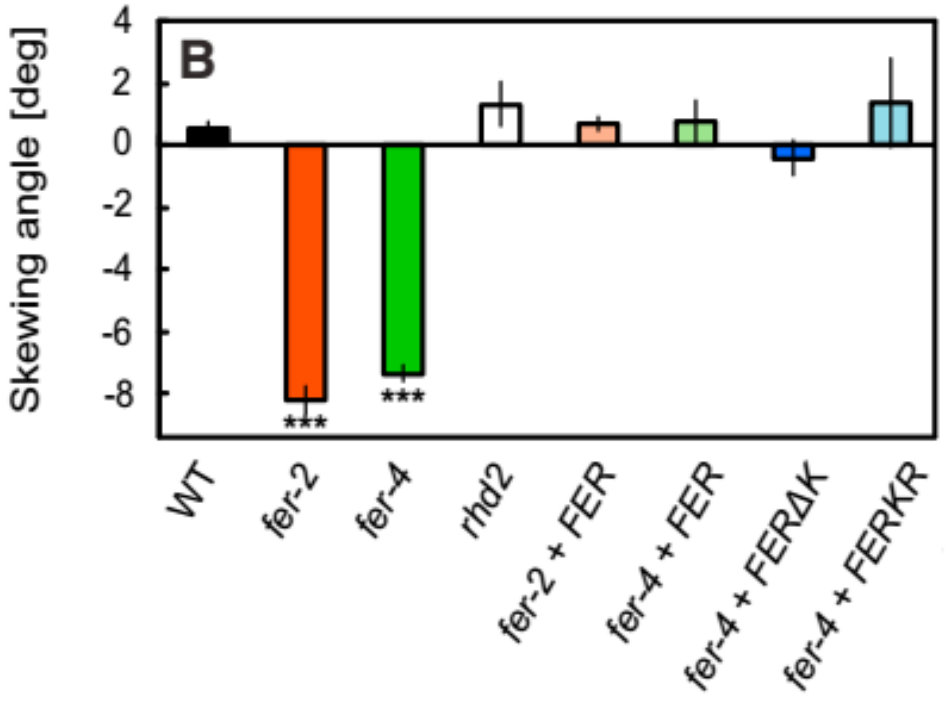
9.3CO9K seedlings have a root skew phenotype

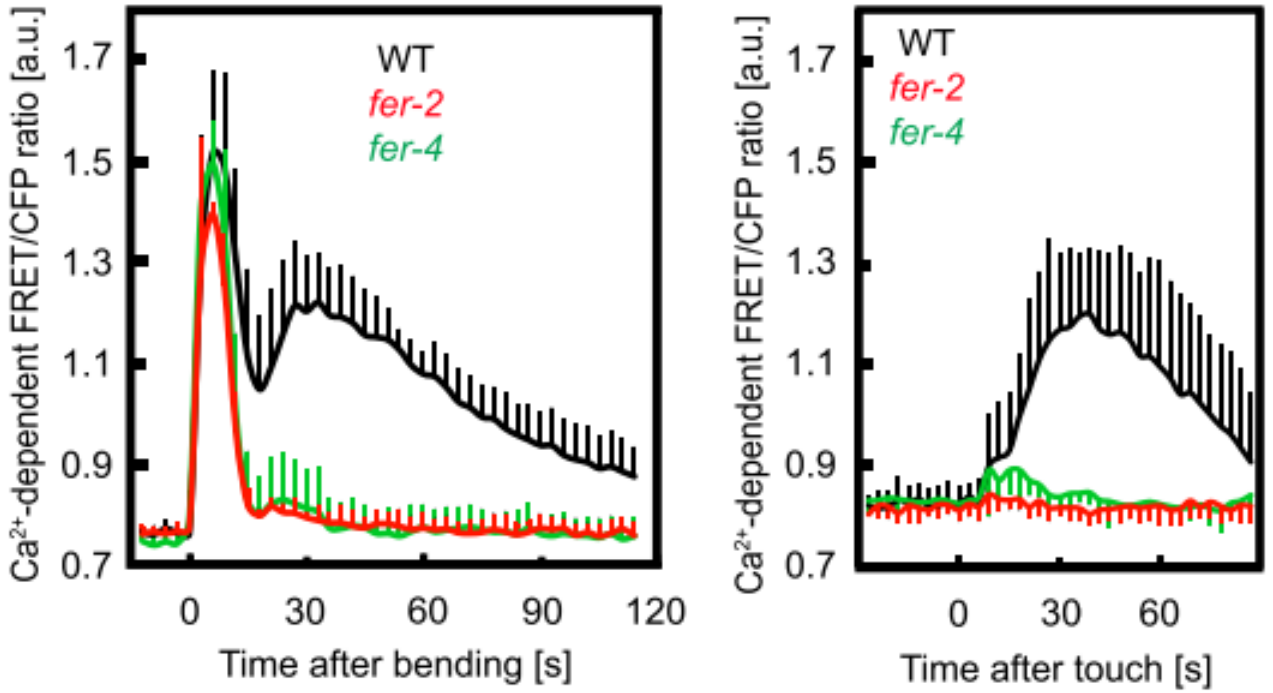




n = 20 – 30 seedlings per line

FERONIA defective mutants show different root growth patterns and Ca^{2+} signatures compared to WT





Shih et al., 2014

1. Hypothesis: Root skew and Ca^{2+} phenotype in the 9.3CO9K line are caused by the same mutation

→ Root skew phenotype was mapped to the bak1 gene (A. Stephan)

Approach: Reproduce root skew phenotype in *bak1* T-DNA lines

1. Hypothesis: Root skew and Ca^{2+} phenotype in the 9.3CO9K line are caused by the same mutation

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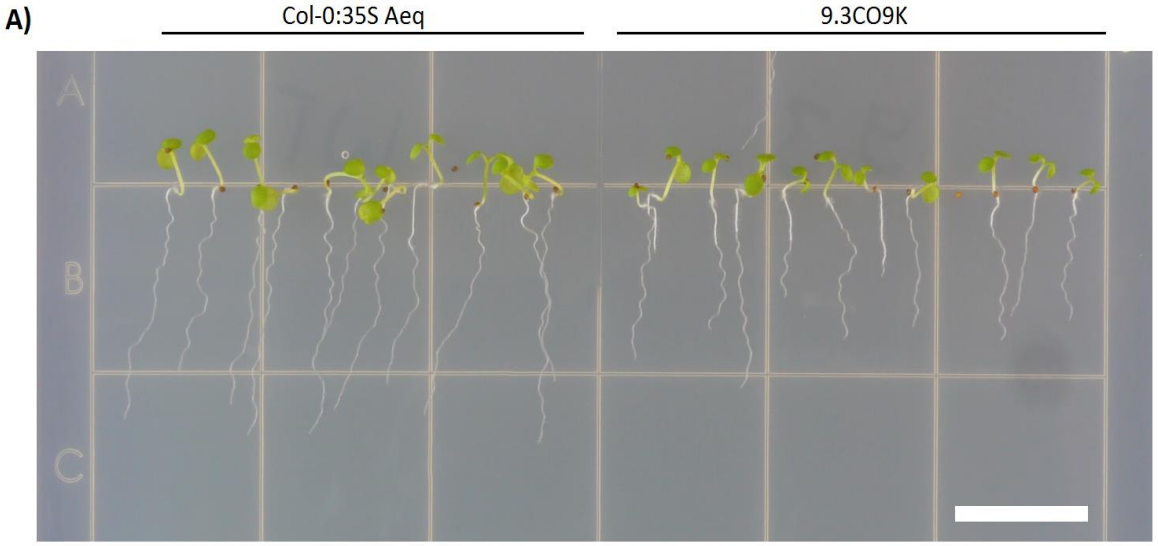
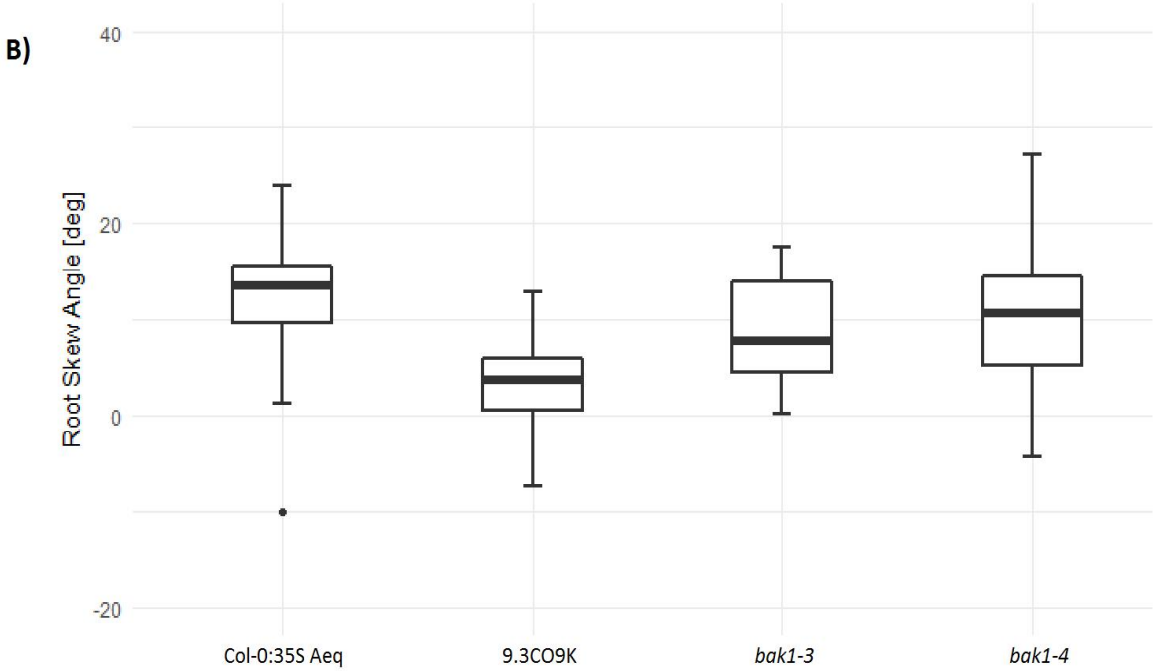
Approach: Reproduce root skew phenotype in *bak1* T-DNA lines

2. Hypothesis: Root skew phenotype and Ca^{2+} phenotype are caused by a defect in BAK1

Approach: Generate *bak1/Aeq* lines and measure Ca^{2+} response





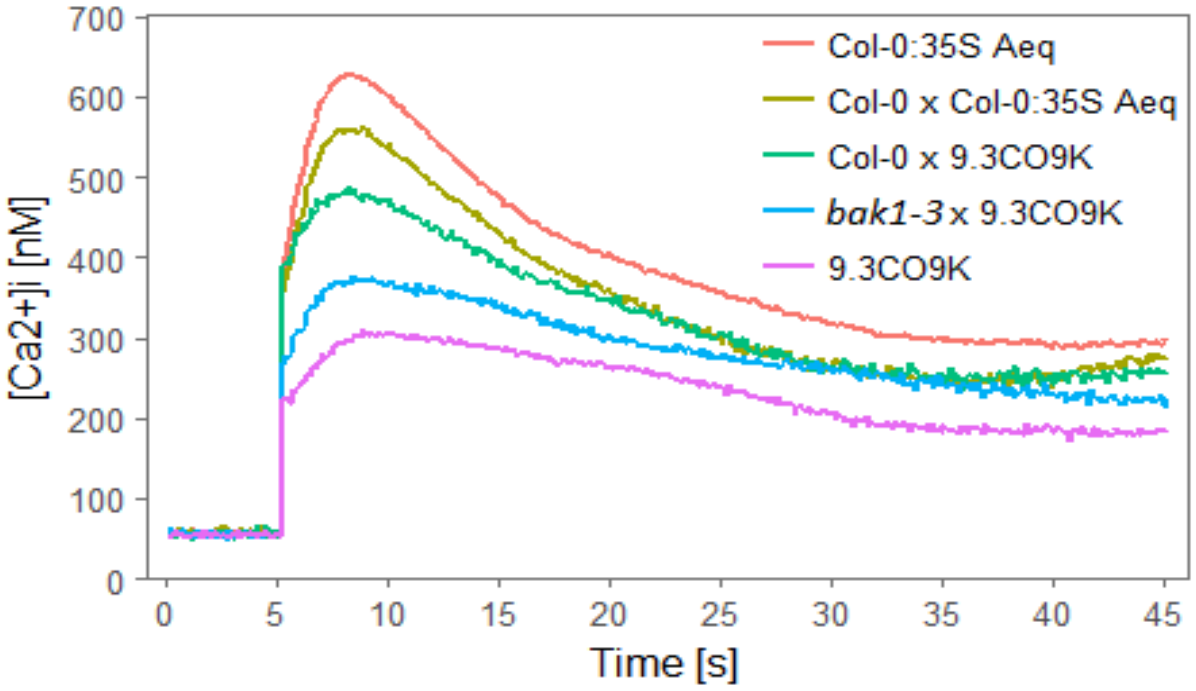
→ *bak1-3* and *bak1-4* root skew phenotype not reproducible with current setup

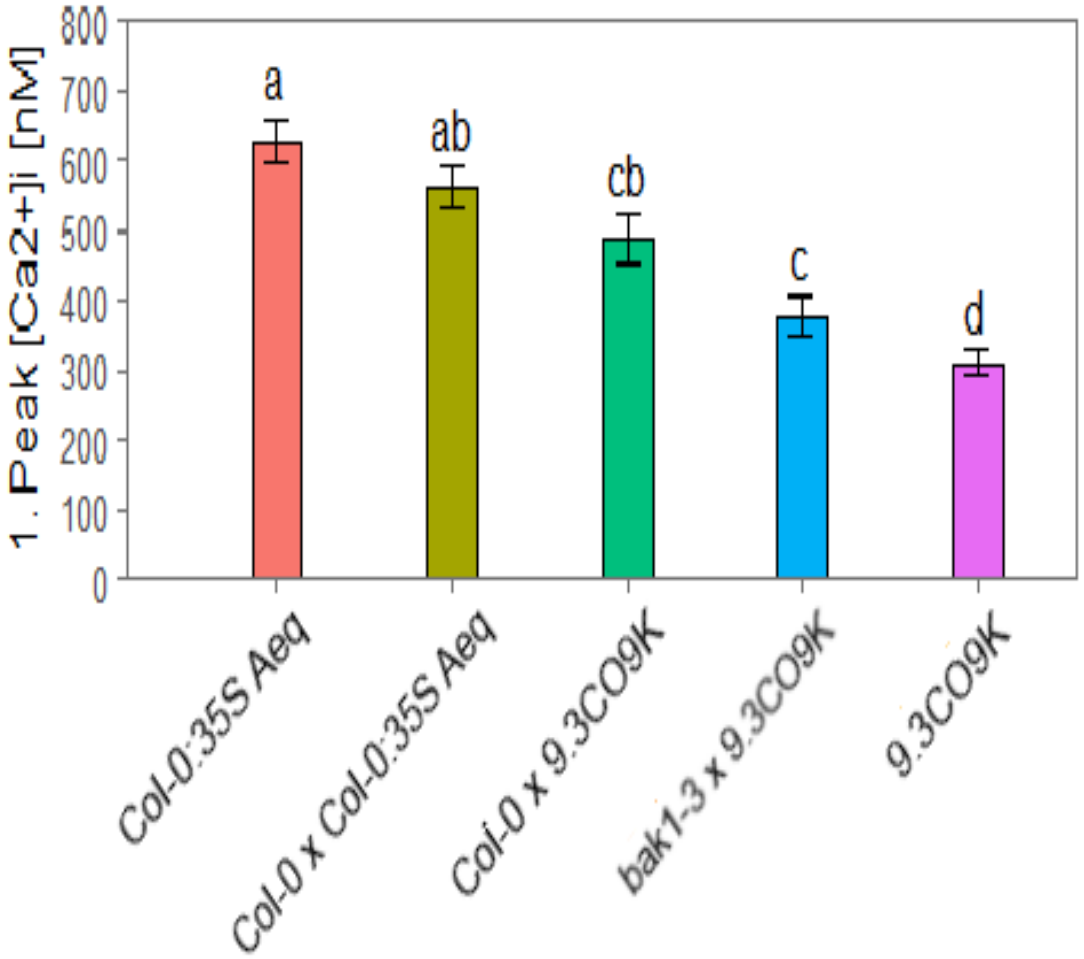
→ High sample sizes required

→ Phenotype might not be perfectly suited for gene mapping

n = 20 – 30 seedlings per line

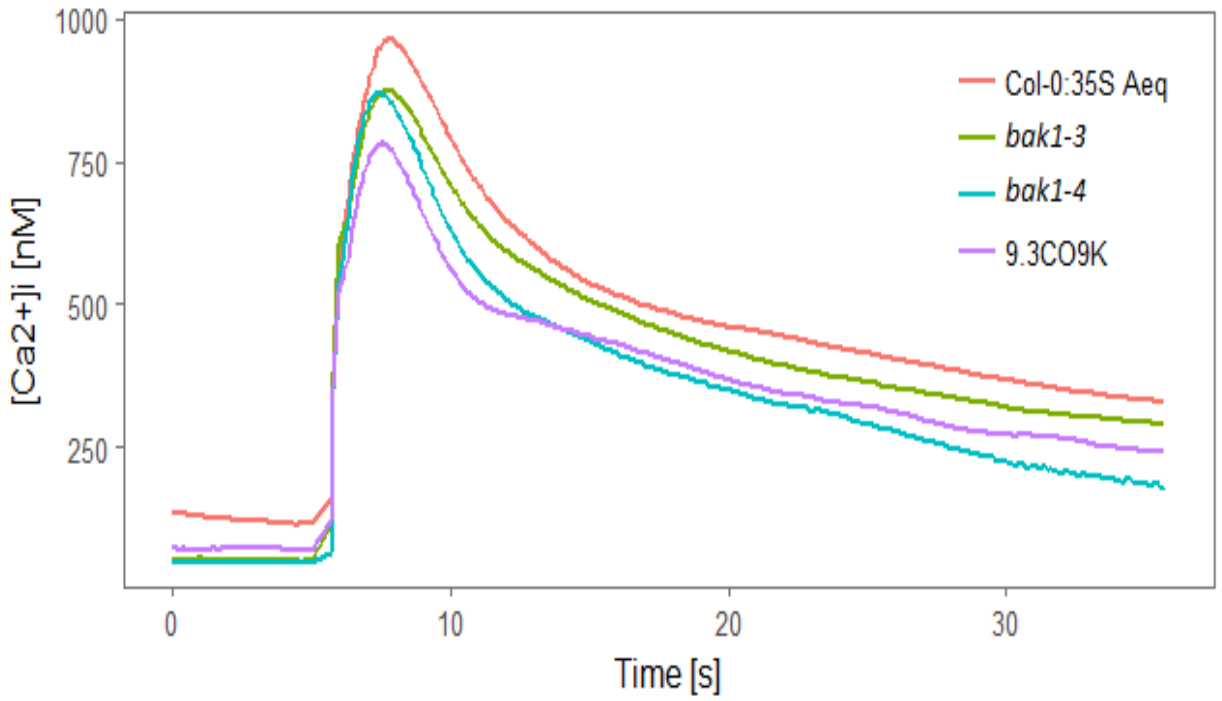
bak1-3 and 9.3CO9K complementation test

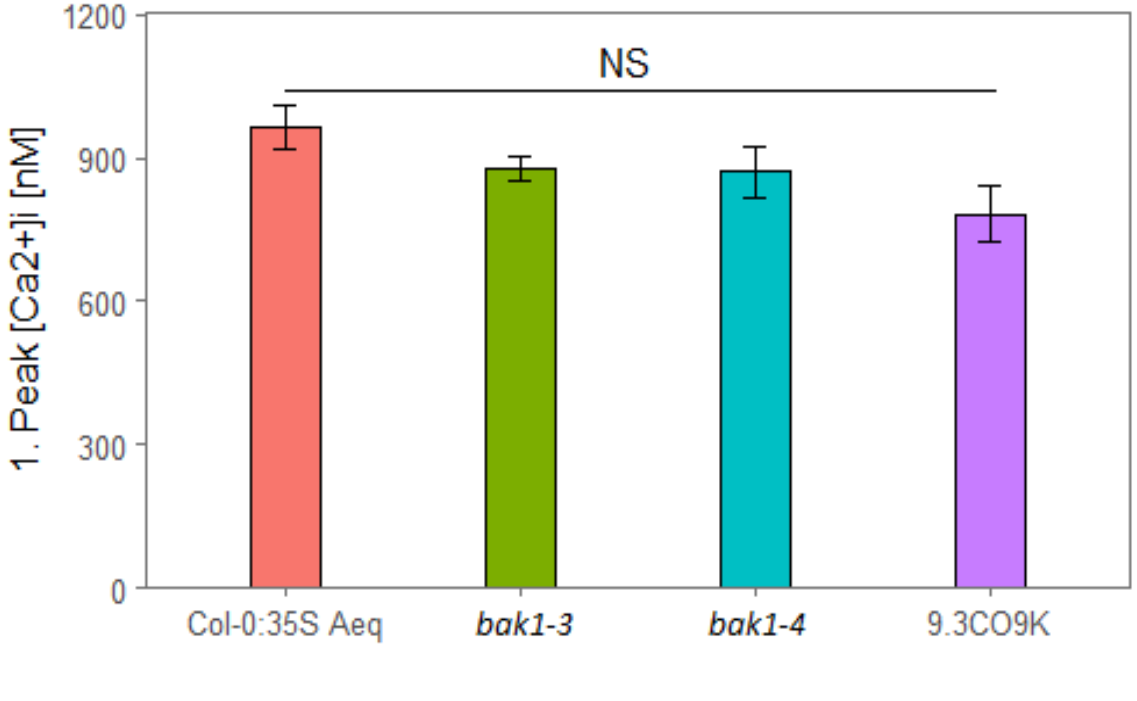




n = 20 – 48 seedlings per line
Stimulus: 300 mM NaCl

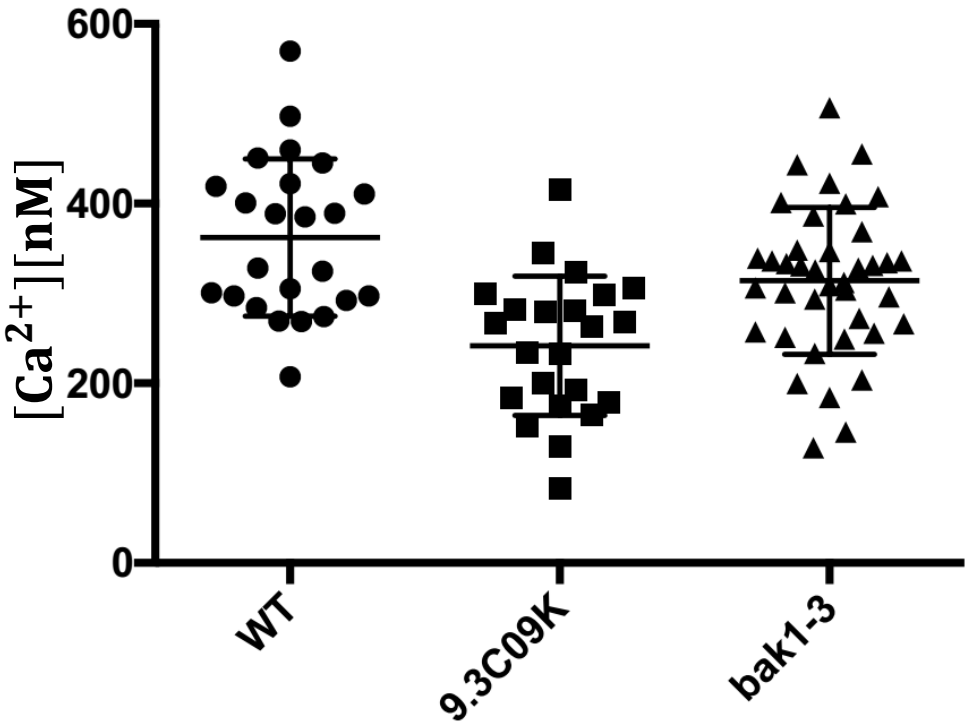
bak1-3 and *bak1-4* do not show an impaired osmotic-induced Ca^{2+} response

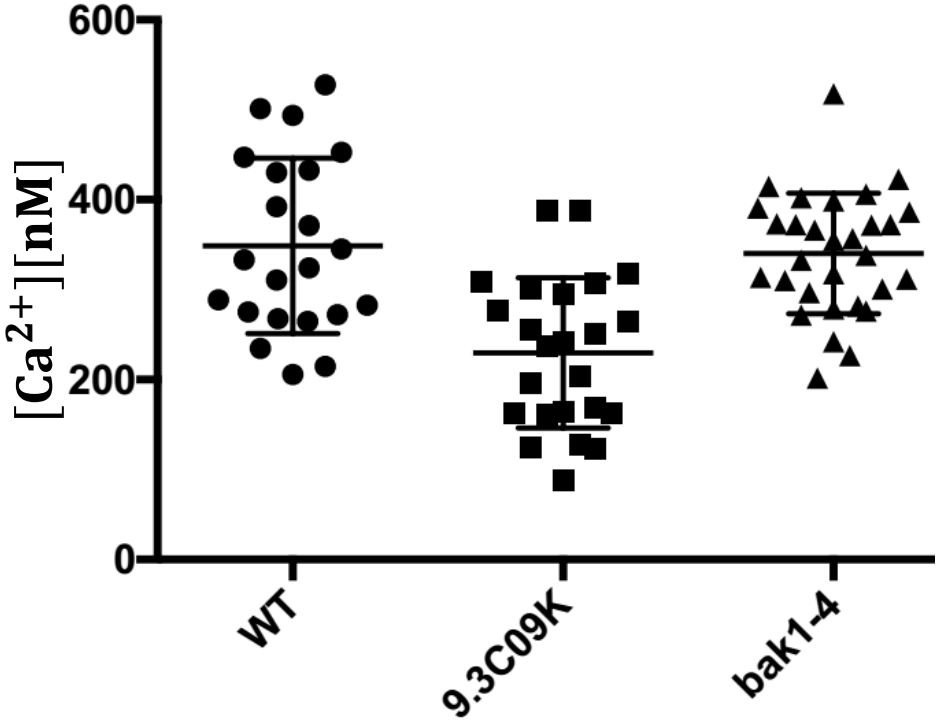




n = 20 – 24 seedlings per line
Stimulus: 600 mM sorbitol

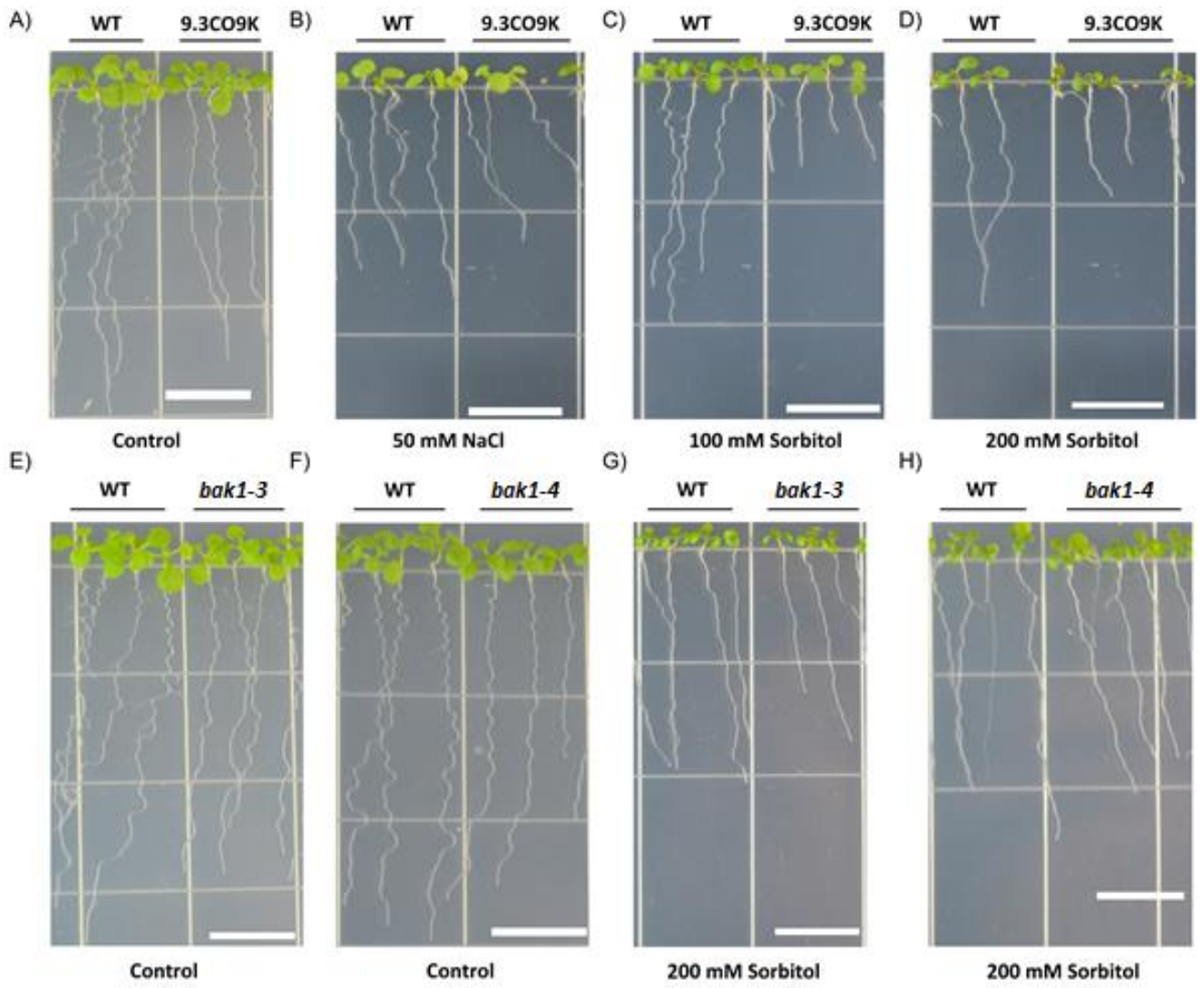
Ca^{2+} peak distribution of individual seedlings

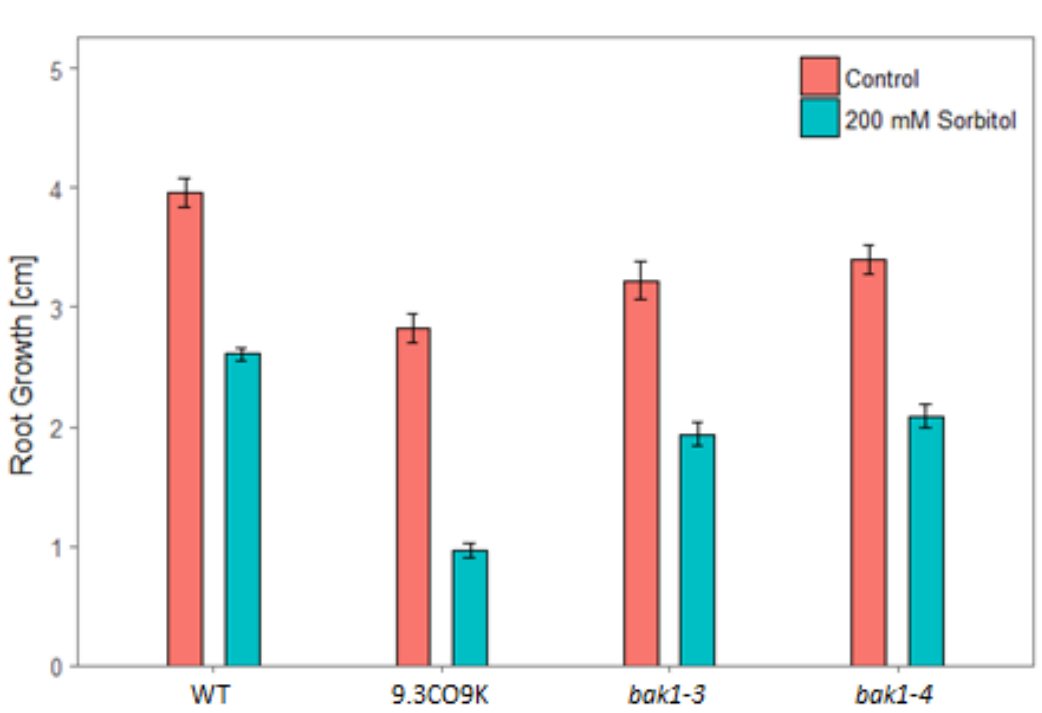




Data and figures: Po-Kai Hsu

9.3CO9K root growh is affected by osmotic stress





n = 16 – 49 seedlings per line per condition

Summary

- 9.3CO9K line has a significantly reduced Ca^{2+} response
- 9.3CO9K line has a root skew phenotype
- 9.3CO9K shows reduced root growth when exposed to osmotic stress

Summary

- 9.3CO9K line has a significantly reduced Ca^{2+} response
- 9.3CO9K line has a root skew phenotype
- 9.3CO9K shows reduced root growth when exposed to osmotic stress

- Root skew phenotype might not be suitable for genetic mapping
- Ca^{2+} measurements and root growth experiments implicate that BAK1 might not be responsible for the Ca^{2+} phenotype in the 9.3CO9K line

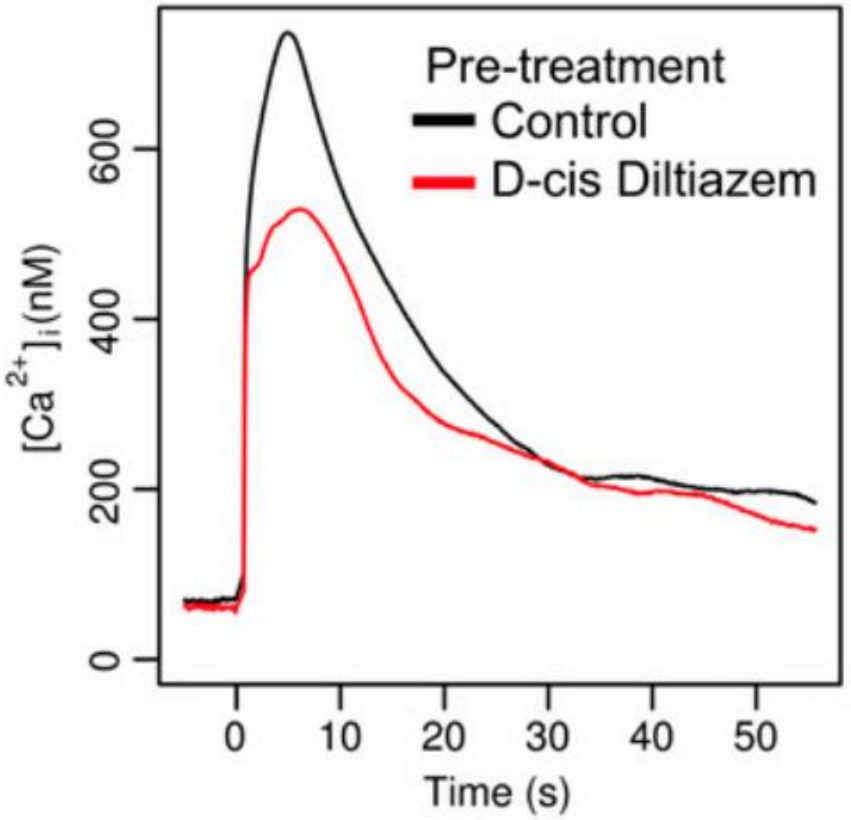
→ Root growth phenotype promising for genetic mapping

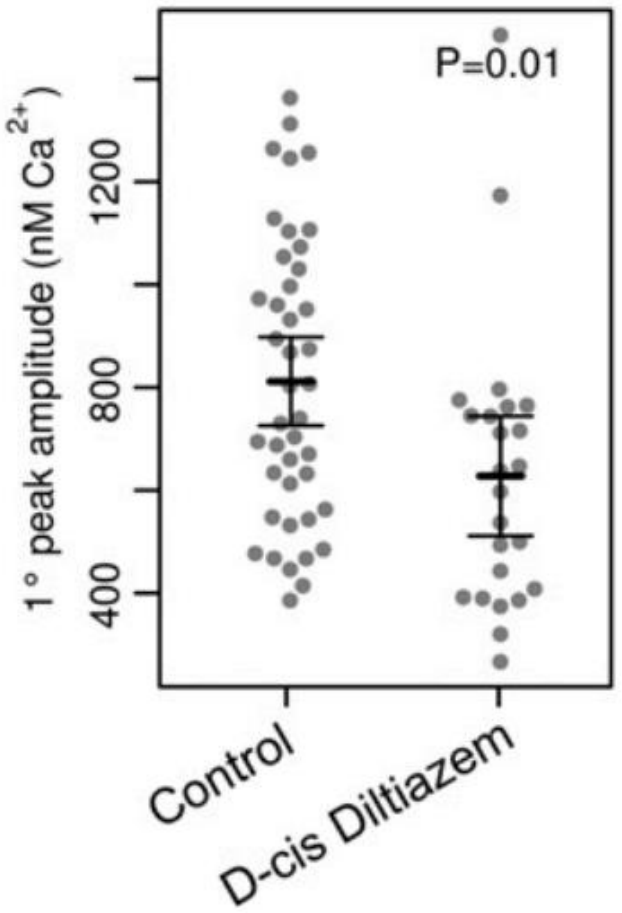
Outlook

- Genetic mapping of root growth phenotype in 9.3CO9K line
- Further phenotypical characterization of 9.3CO9K line
- Analysis of 9.3CO9K line with other Ca^{2+} reporters
- *osca1* and 9.3CO9K lines might help to reveal channels involved in osmosensing

Further work

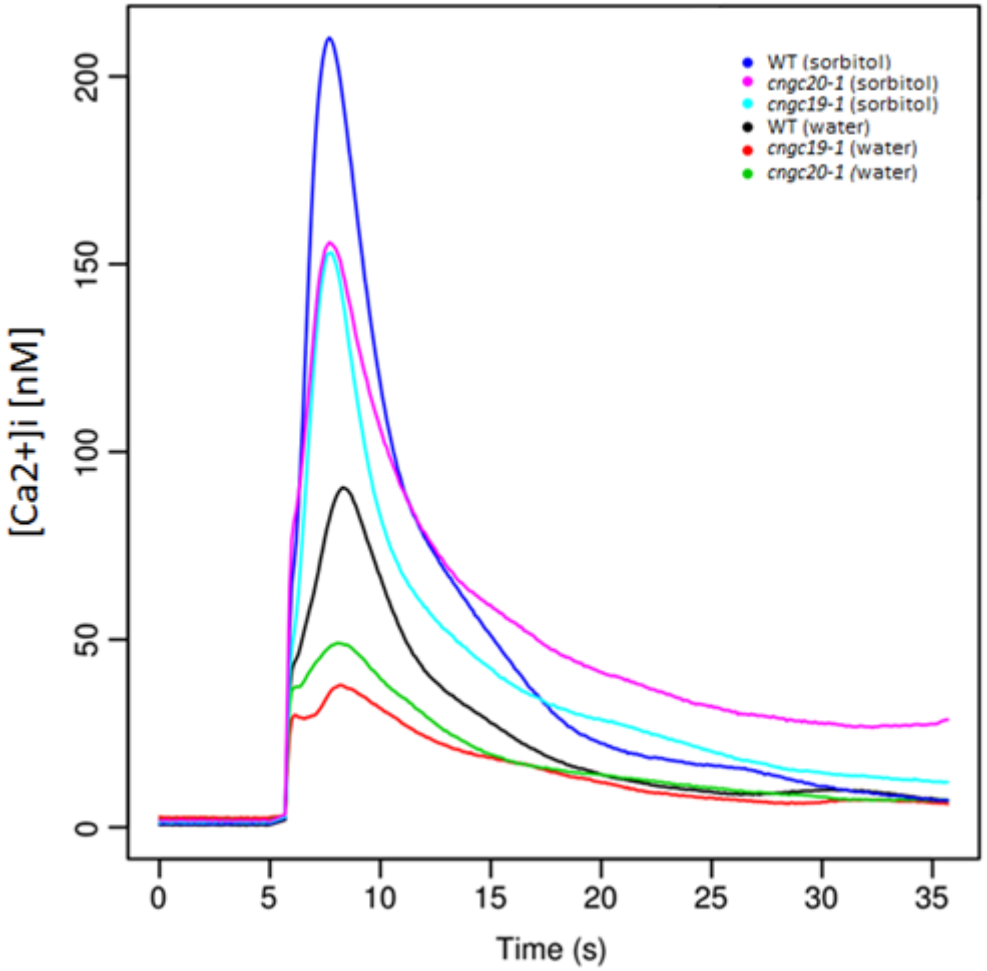
Cyclic nucleotide gated channel (CNGC) inhibitors impair the osmotic-induced Ca^{2+} response





Stephan et al., 2015

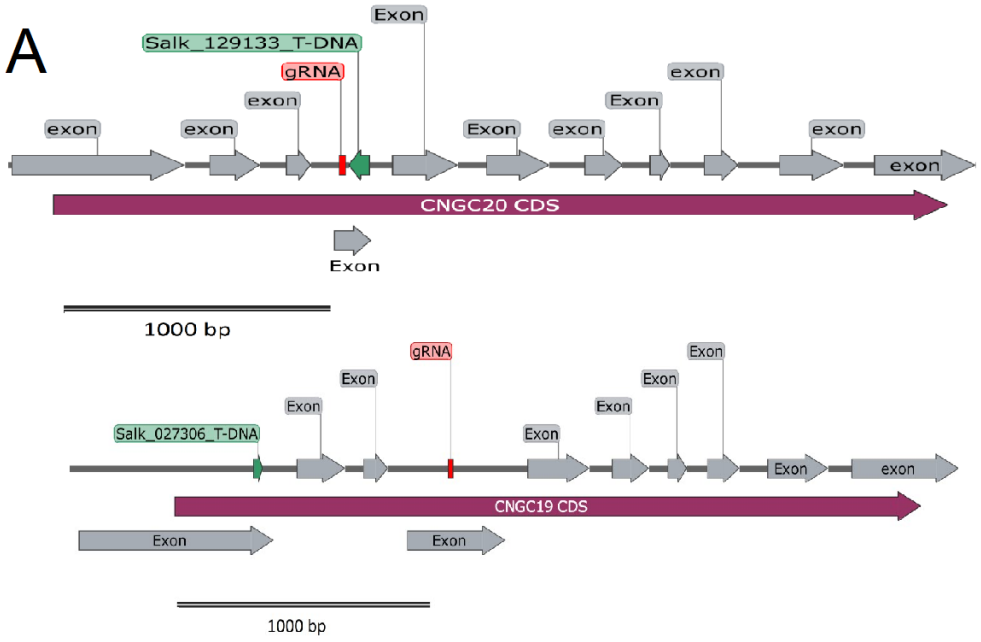
CNCG19 and CNGC20 might be involved in the osmotic-induced Ca^{2+} response

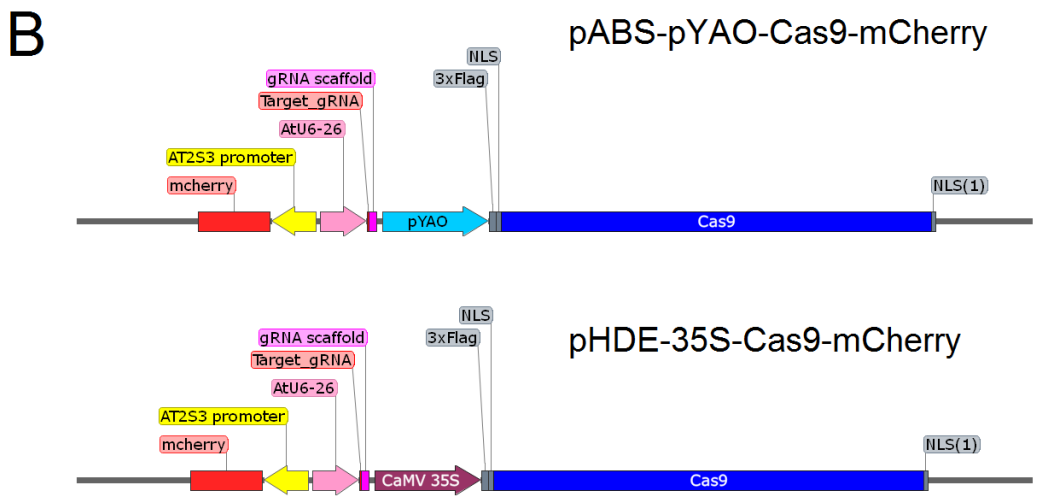


Data: A. Stephan

Hypothesis: CNGC19 and CNGC20 together act as osmosensors

→ Use CRISPR/Cas9 to create a CNGC19/CNGC20 double knockout line





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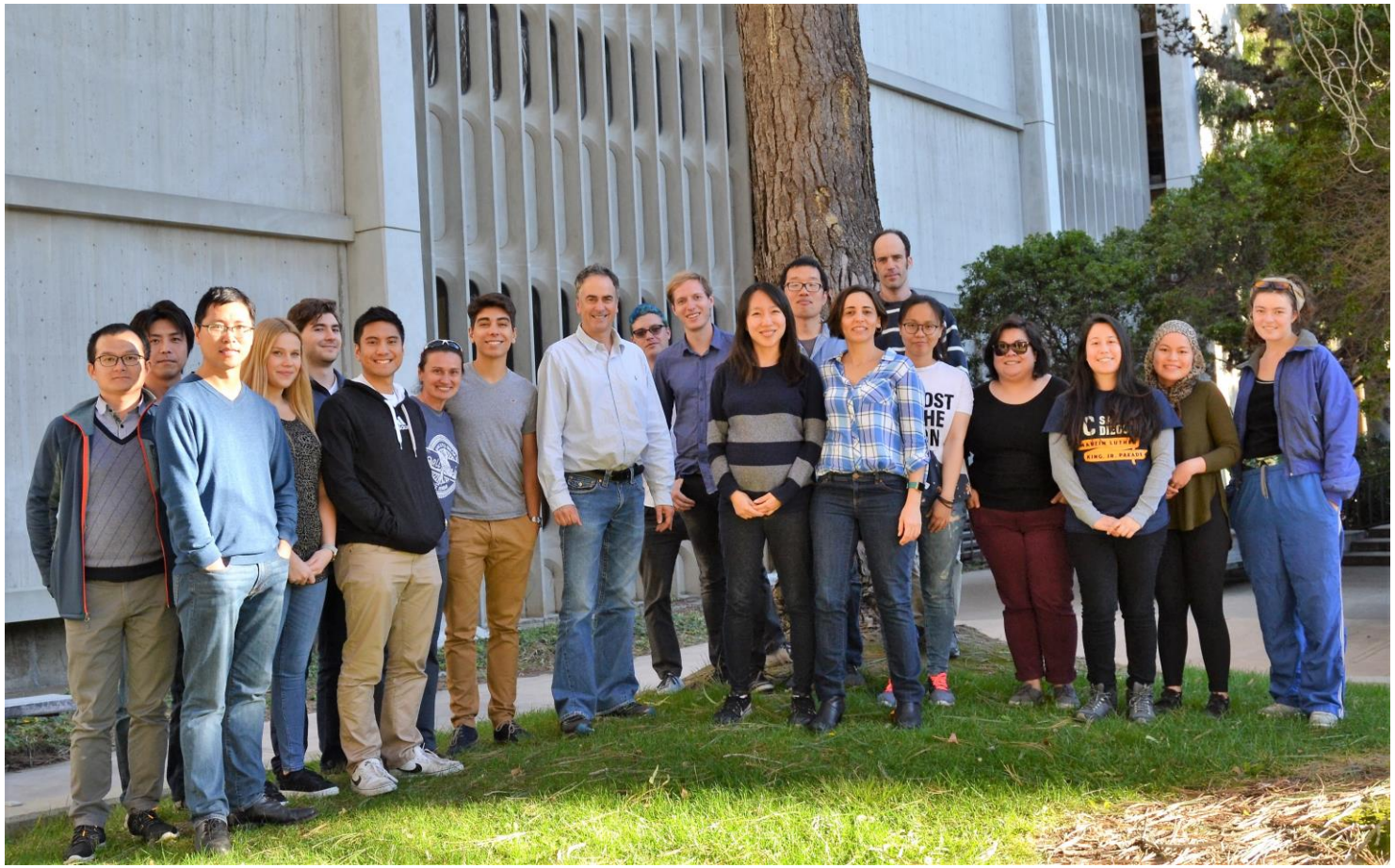
Acknowledgements



- PD. Dr. Christina Kühn
- Aleksandra Hackel
- Prof. Dr. Julian Schroeder and lab members
- Dr. Jiyoung Park
- Dr. Po-Kai Hsu

Thank You!

Slides and data:

https://github.com/burtphil/UCSD

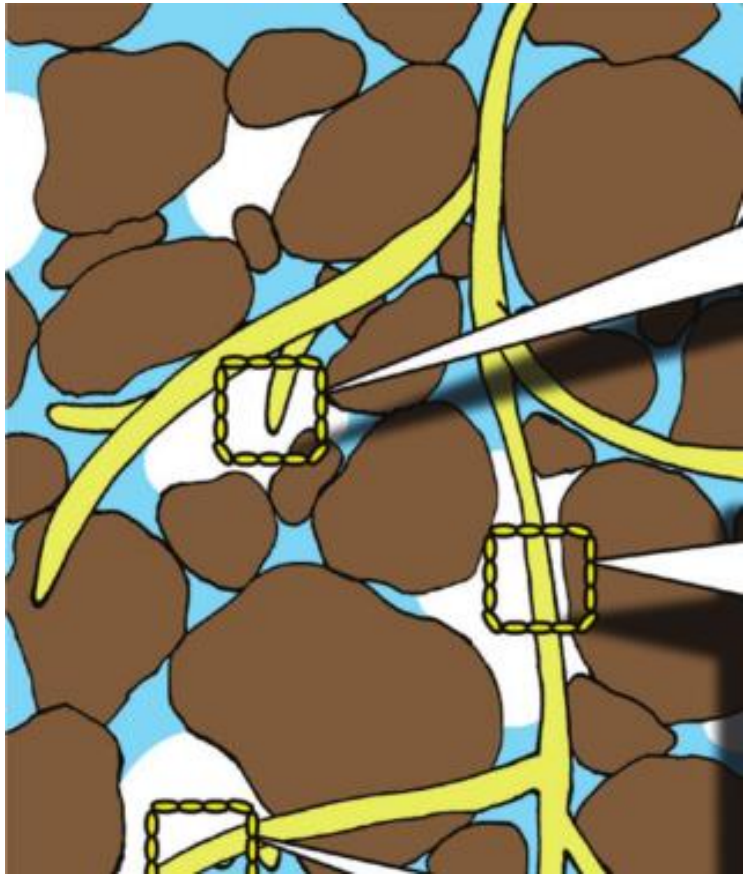
Online aequorin data analysis:

https://burtphil.shinyapps.io/aequorin_luminescence/

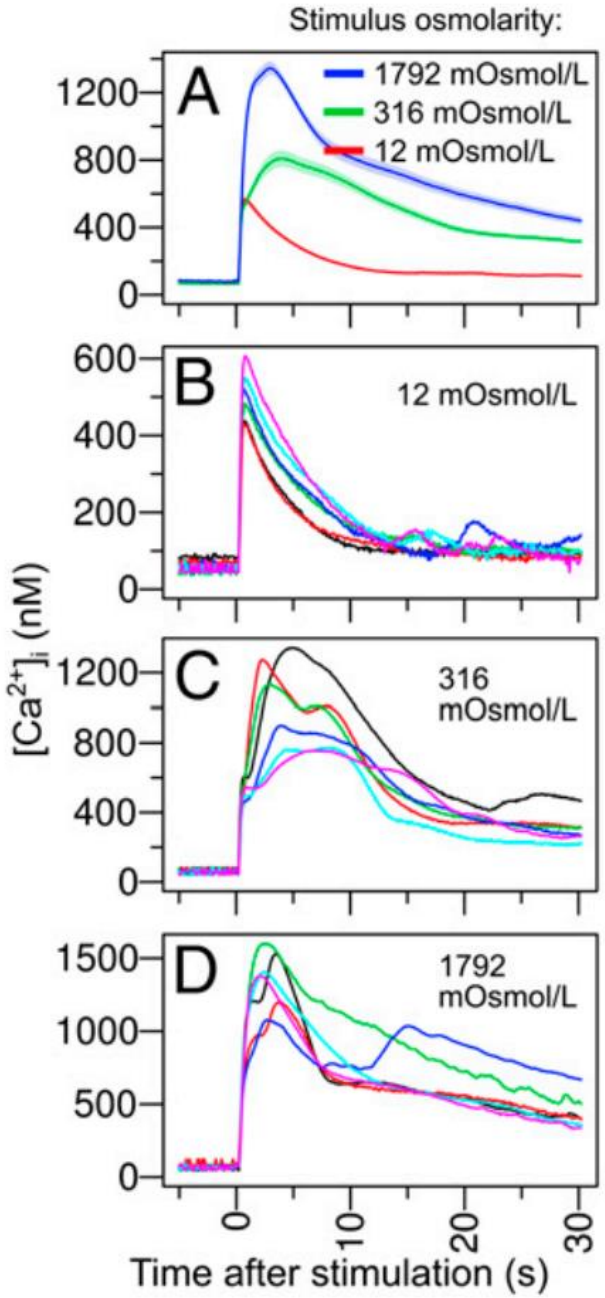
Water availability is dynamic

- Soil matrix - root hair microenvironment

→ Water availability is different throughout the soil



J. Dinneny, 2015



Stephan et al., 2015

Table 1. Some Physiological Stimuli That Elevate $[Ca^{2+}]_c$ in Plant Cells

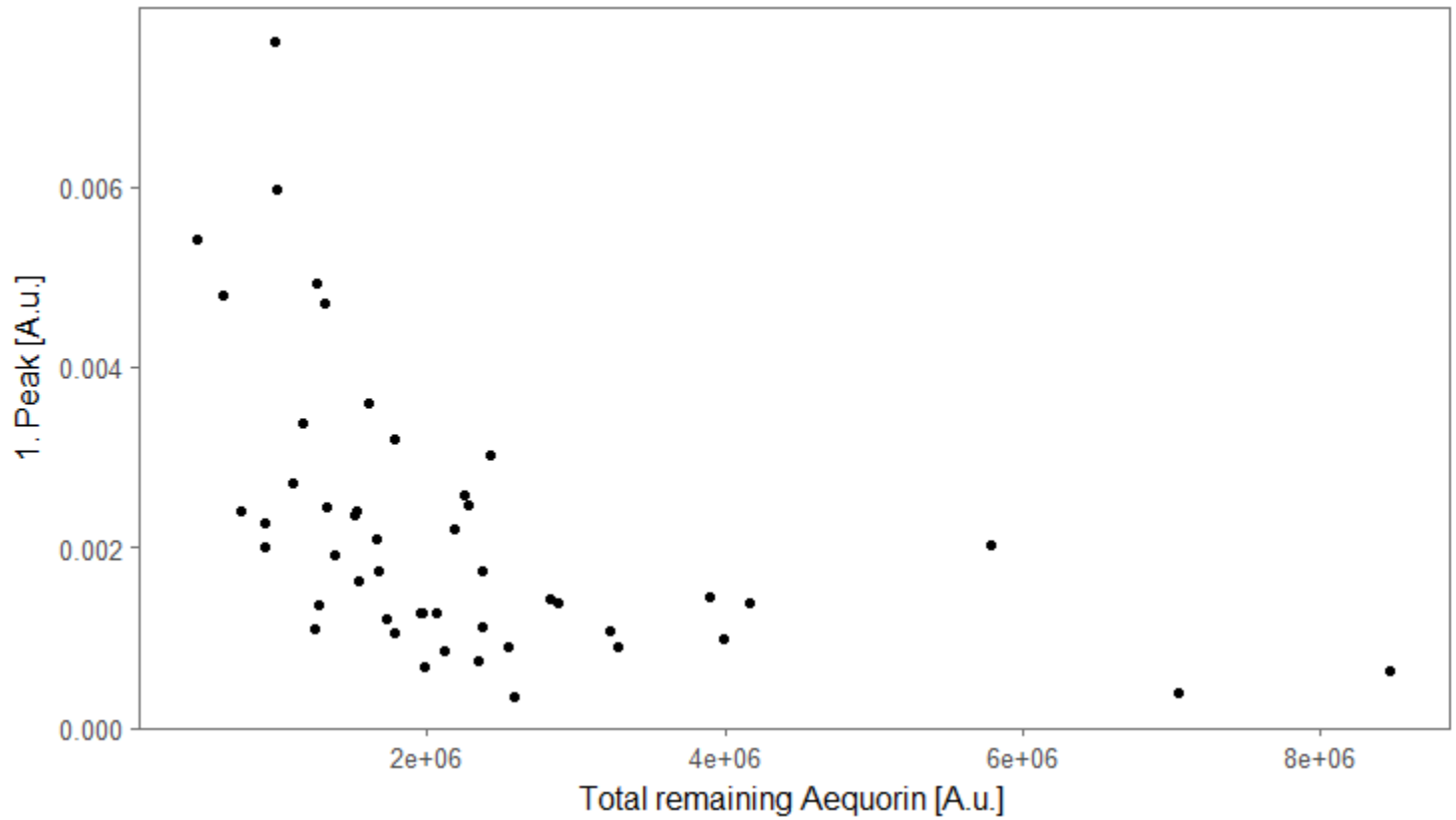
Stimulus	Example of Response	Reference
Red light	Photomorphogenesis	Shacklock et al. (1992)
Abscissic acid	Stomatal closure	McAinsh et al. (1990)
Gibberellin	α-Amylase secretion	Bush and Jones (1988)
Salinity/drought	Proline synthesis	Knight et al. (1997)
Hypoosmotic stress	Osmoadaptation	Taylor et al. (1996)
Touch	Growth retardation	Knight et al. (1991)
Fungal elicitors	Phytoalexin synthesis	Knight et al. (1991)
Cold	*KIN1* gene expression	Knight et al. (1996)
Heat shock	Thermotolerance	Gong et al. (1998)
Oxidative stress	Free radical scavenger induction	Price et al. (1994)
NOD factors	Root hair curling	Ehrhardt et al. (1996)

Normalization

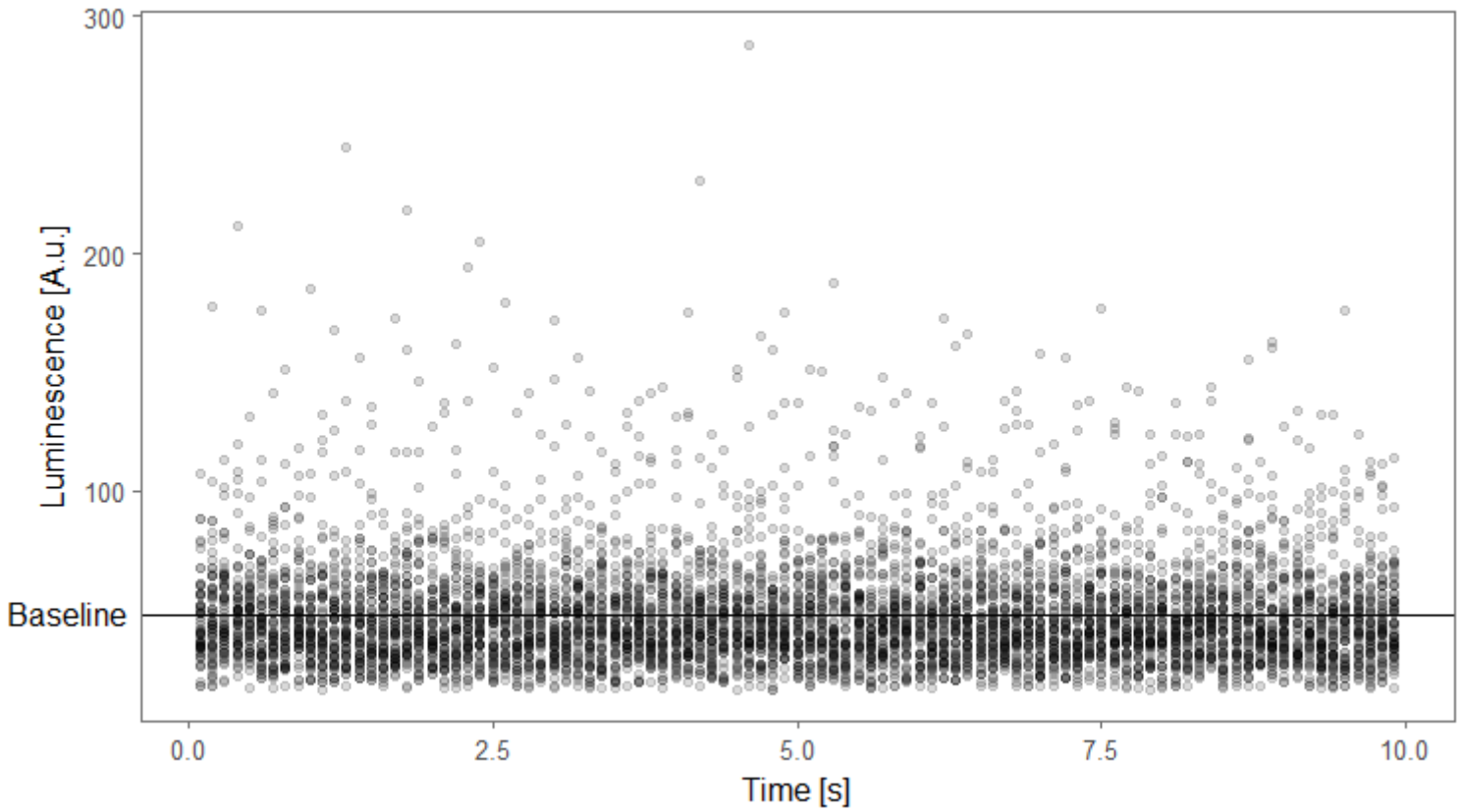
$$[Ca^{2+}] = 0.332588\,(-logk)\ +\ 5.5593$$

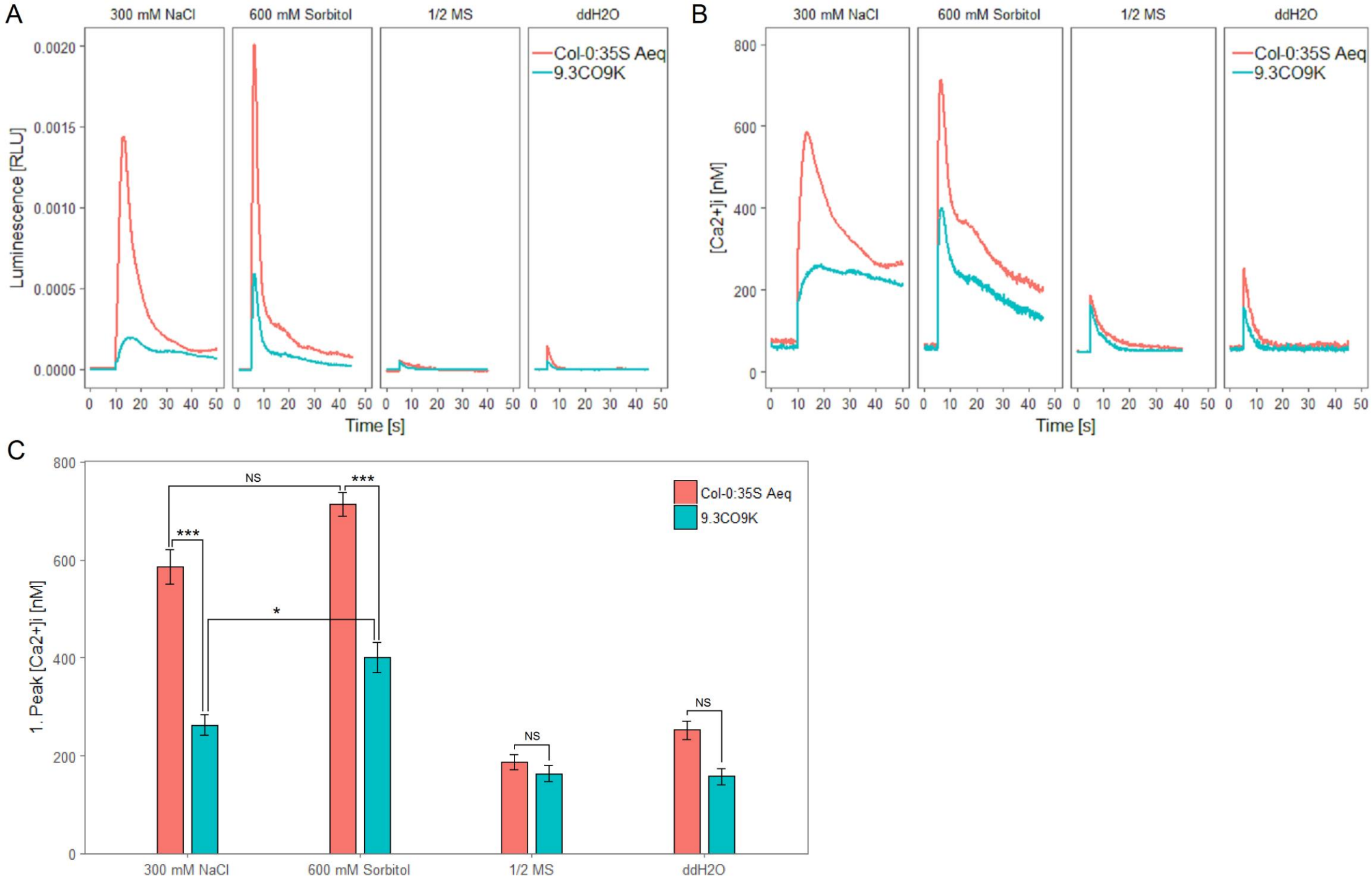
k: Stimulus luminescence counts / total luminescence counts

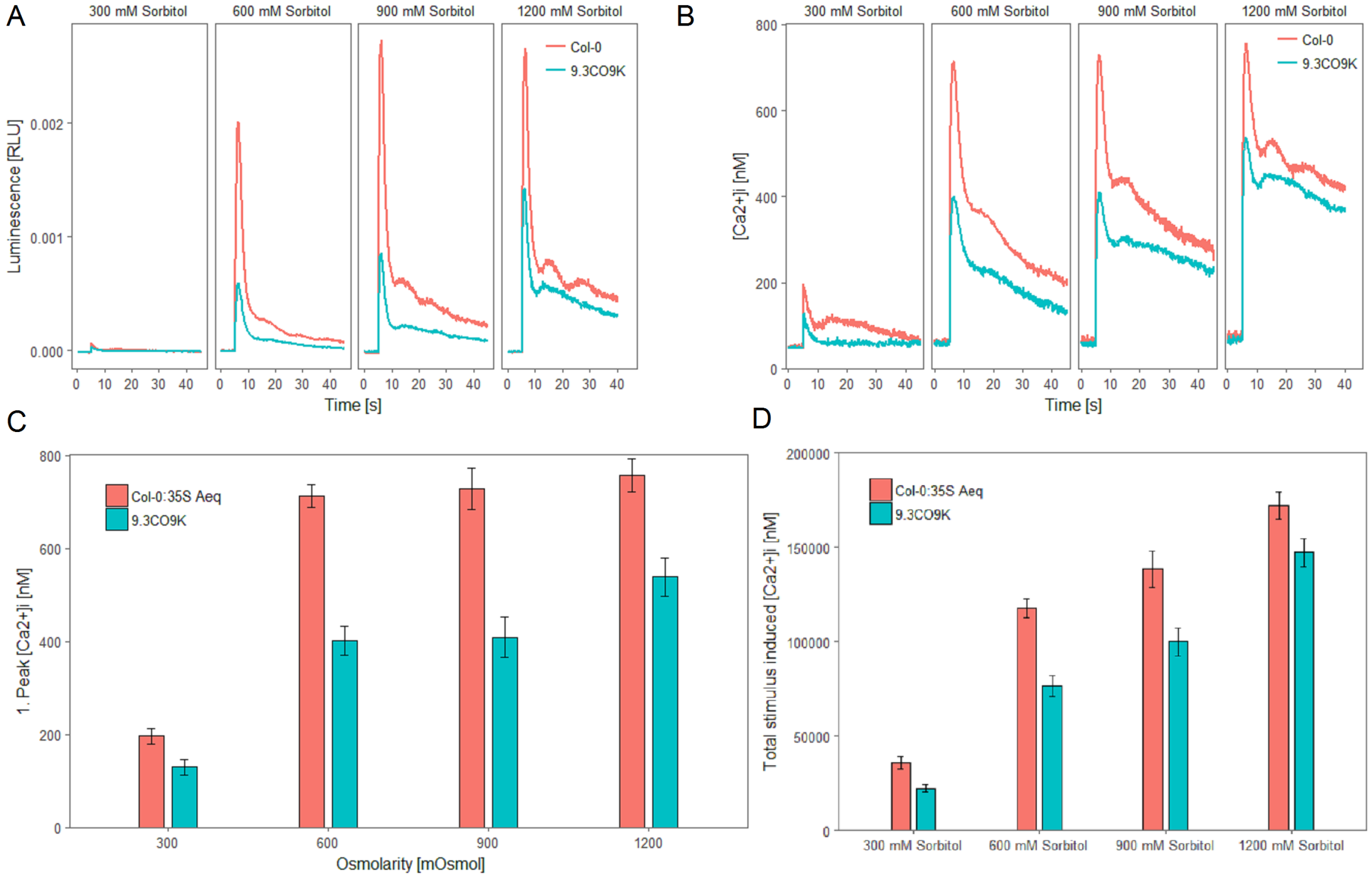
Ca2+ peak amplitude is independent of aequorin expression

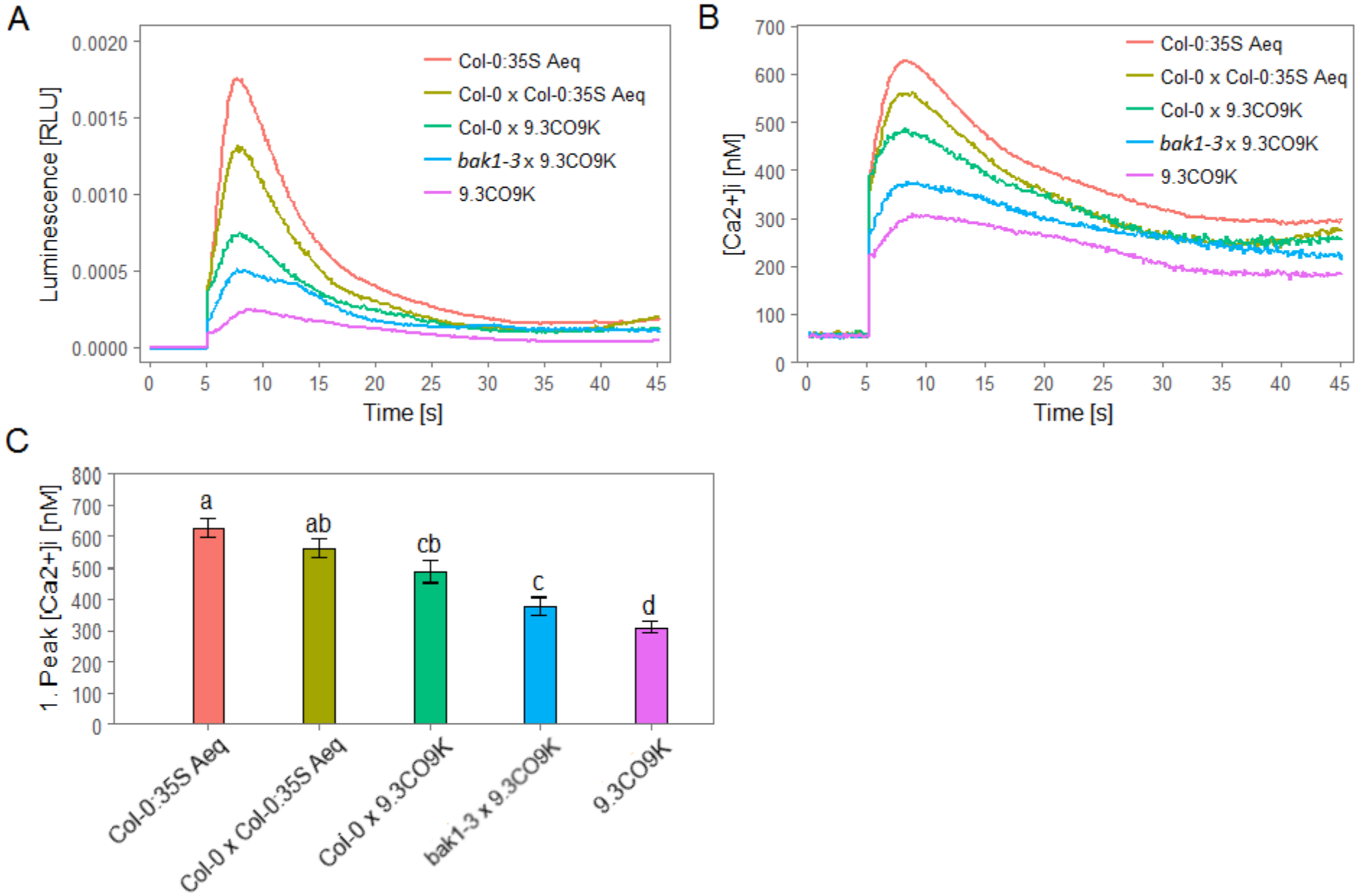


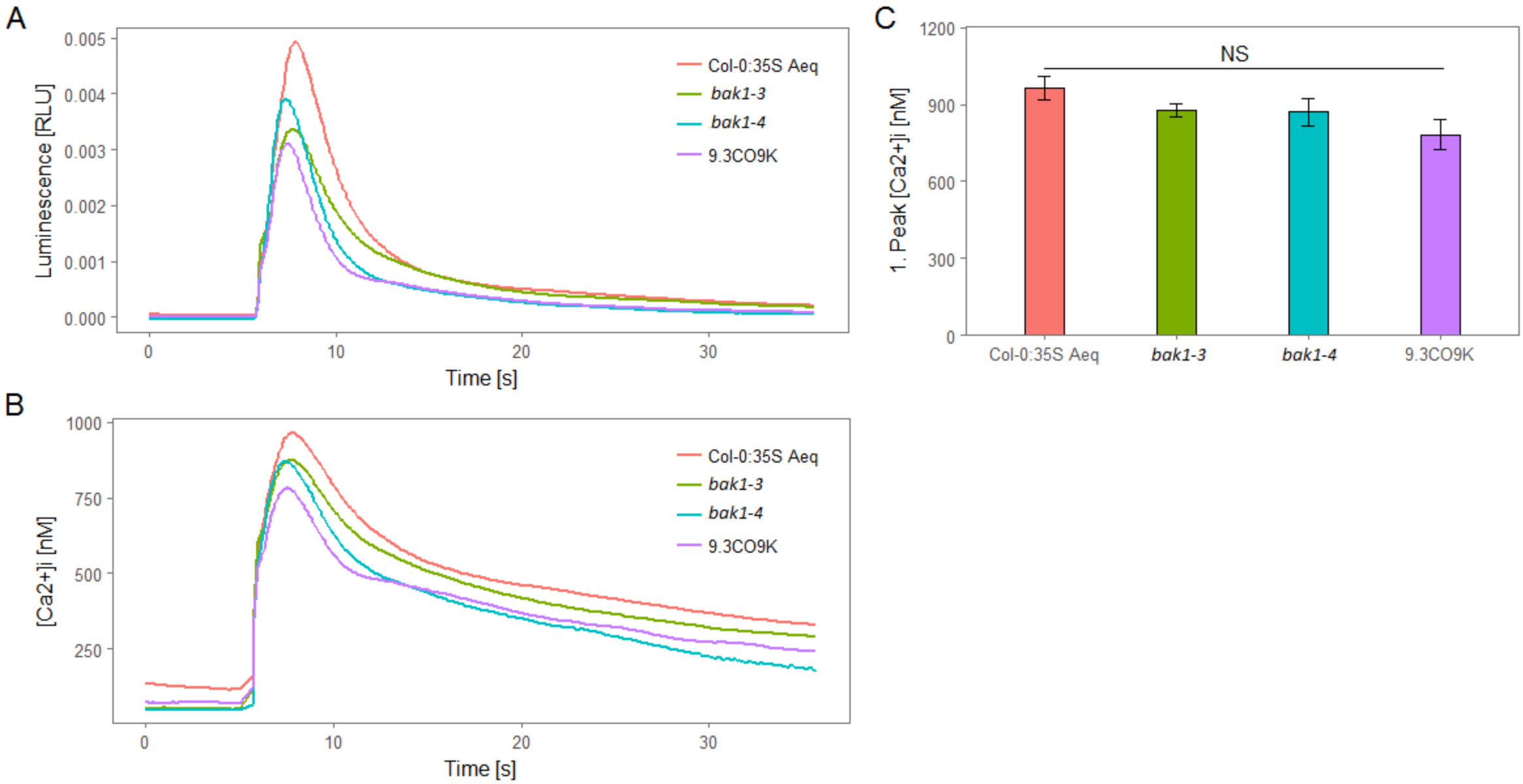
Luminescence baseline has a uniform distribution




A
300 mM NaCl
600 mM Sorbitol
1/2 MS
ddH2O
Col-0:35S Aeq
9.3CO9K
Luminescence [RLU]
0.0020
0.0015
0.0010
0.0005
0.0000
0 10 20 30 40 50
Time [s]
B
300 mM NaCl
600 mM Sorbitol
1/2 MS
ddH2O
Col-0:35S Aeq
9.3CO9K
[Ca2+]i [nM]
800
600
400
200
0
0 10 20 30 40 50
Time [s]
C
Col-0:35S Aeq
9.3CO9K
1. Peak [Ca2+]i [nM]
800
600
400
200
0
NS
***
***
*
NS
NS
300 mM NaCl
600 mM Sorbitol
1/2 MS
ddH2O


A
300 mM Sorbitol
600 mM Sorbitol
900 mM Sorbitol
1200 mM Sorbitol
Col-0
9.3CO9K
Luminescence [RLU]
0.000
0.001
0.002
0 10 20 30 40
Time [s]
B
300 mM Sorbitol
600 mM Sorbitol
900 mM Sorbitol
1200 mM Sorbitol
Col-0
9.3CO9K
[Ca2+]i [nM]
0
200
400
600
800
0 10 20 30 40
Time [s]
C
Col-0:35S Aeq
9.3CO9K
1. Peak [Ca2+]i [nM]
0
200
400
600
800
300
600
900
1200
Osmolarity [mOsmol]
D
Col-0:35S Aeq
9.3CO9K
Total stimulus induced [Ca2+]i [nM]
0
50000
100000
150000
200000
300 mM Sorbitol
600 mM Sorbitol
900 mM Sorbitol
1200 mM Sorbitol


A
Luminescence [RLU]
0.0020
0.0015
0.0010
0.0005
0.0000
0
5
10
15
20
25
30
35
40
45
Time [s]
Col-0:35S Aeq
Col-0 x Col-0:35S Aeq
Col-0 x 9.3CO9K
bak1-3 x 9.3CO9K
9.3CO9K
B
[Ca2+]j [nM]
700
600
500
400
300
200
100
0
Time [s]
Col-0:35S Aeq
Col-0 x Col-0:35S Aeq
Col-0 x 9.3CO9K
bak1-3 x 9.3CO9K
9.3CO9K
C
1. Peak [Ca2+]j [nM]
800
700
600
500
400
300
200
100
0
a
ab
cb
c
d
Col-0:35S Aeq
Col-0 x Col-0:35S Aeq
Col-0 x 9.3CO9K
bak1-3 x 9.3CO9K
9.3CO9K


A
Luminescence [RLU]
0.005
0.004
0.003
0.002
0.001
0.000
0
10
20
30
Time [s]
Col-0:35S Aeq
bak1-3
bak1-4
9.3CO9K
B
[Ca2+]i [nM]
1000
750
500
250
0
10
20
30
Time [s]
Col-0:35S Aeq
bak1-3
bak1-4
9.3CO9K
C
1. Peak [Ca2+]i [nM]
1200
900
600
300
0
NS
Col-0:35S Aeq
bak1-3
bak1-4
9.3CO9K