Metal sensing of the cell wall

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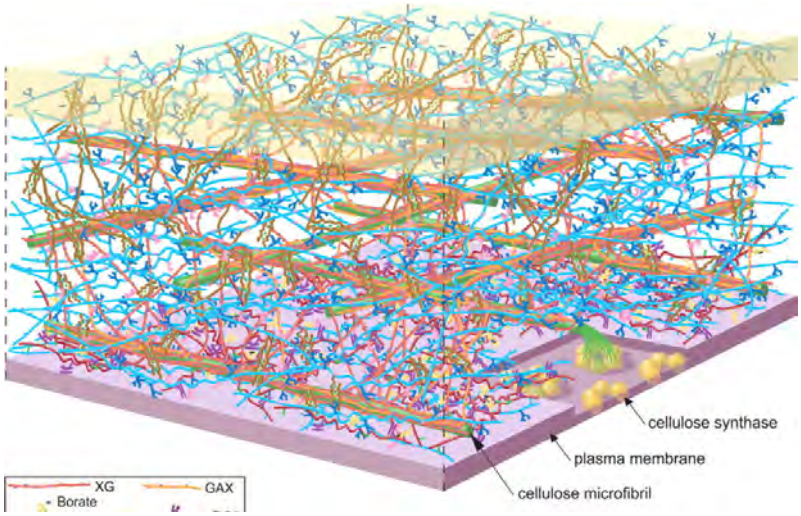
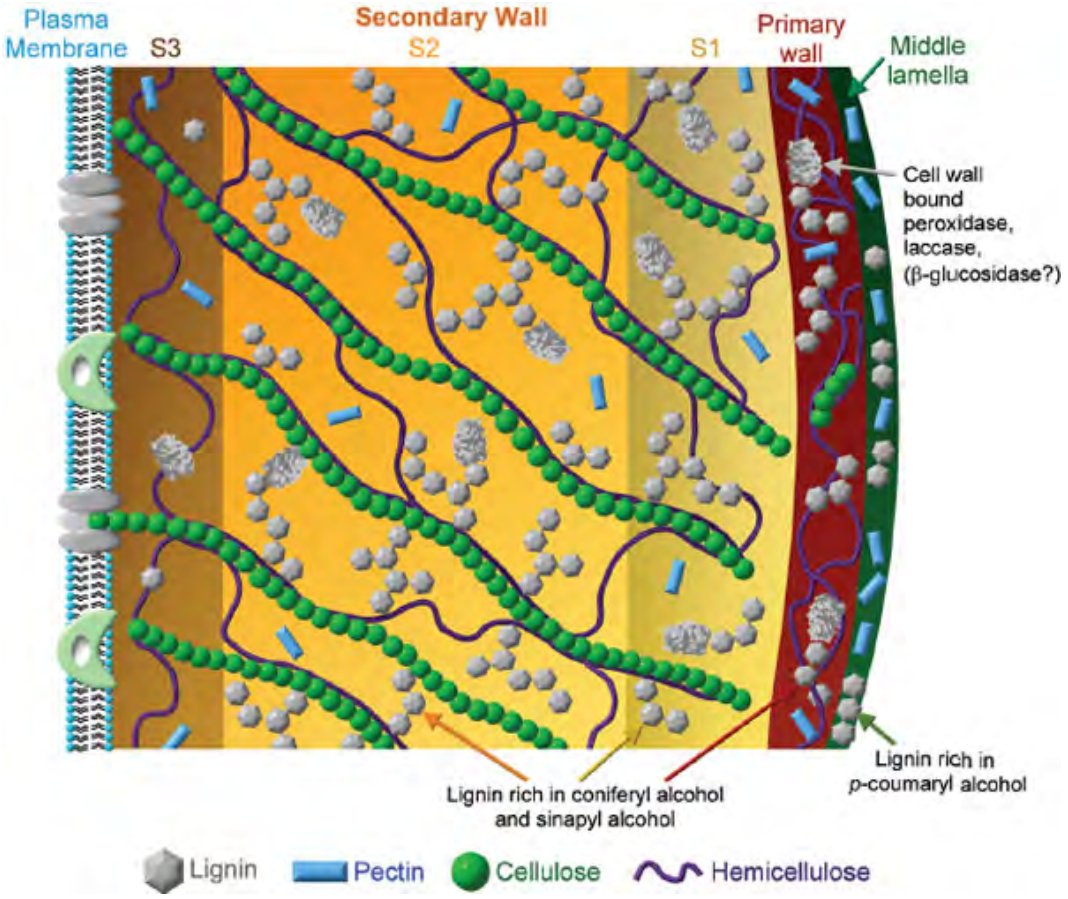
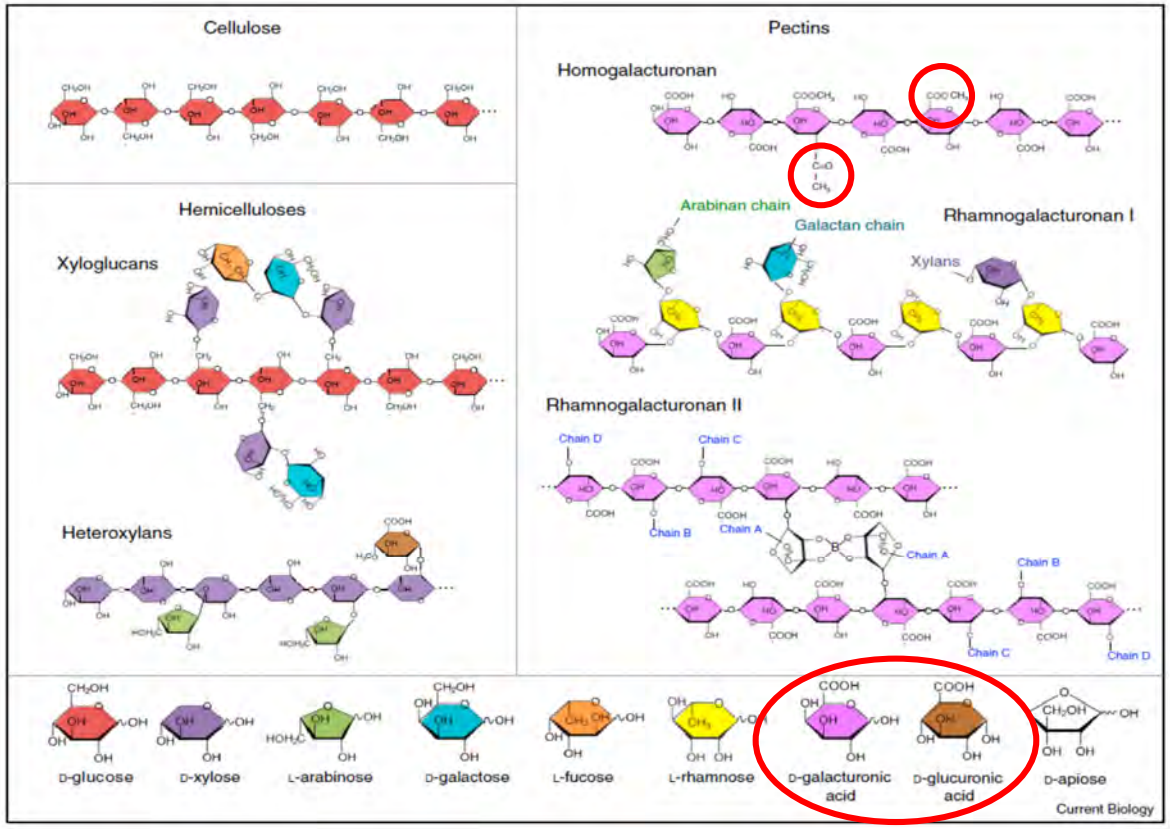


Major components of the plant cell wall

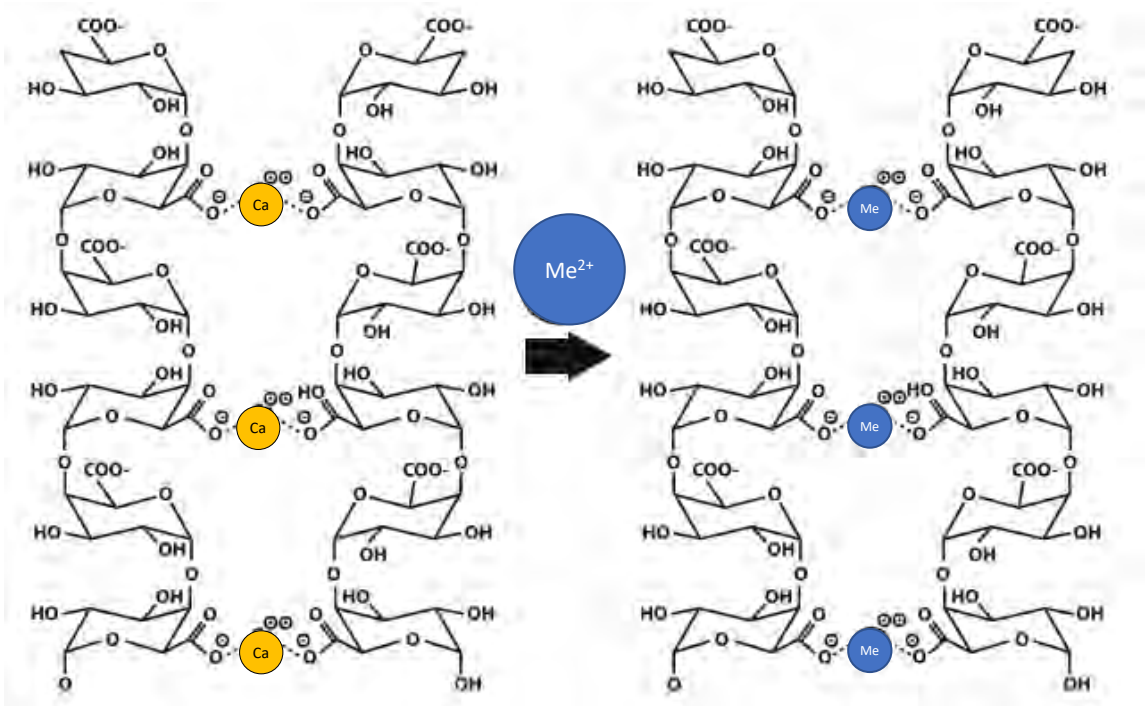


Capacity for binding divalent/trivalent metal cations depends mainly on the amount of polysaccharides abundant in carboxyl groups



Voxeur & Höfte, 2017

Demethylated Pectins Bind Cations/Metal Ions



modified from Krzesłowska, 2011

Demethylated Homogalacturonane (HGs) and Oligogalacturonides (OGs) bind heavy metals and trace elements

Sr> Pb> Ni> Cd> Mn> Cu> Ba> Co> Zn> Fe> Mg> Ca> Cr> Hg (Waldron-Edward et al., 1965)

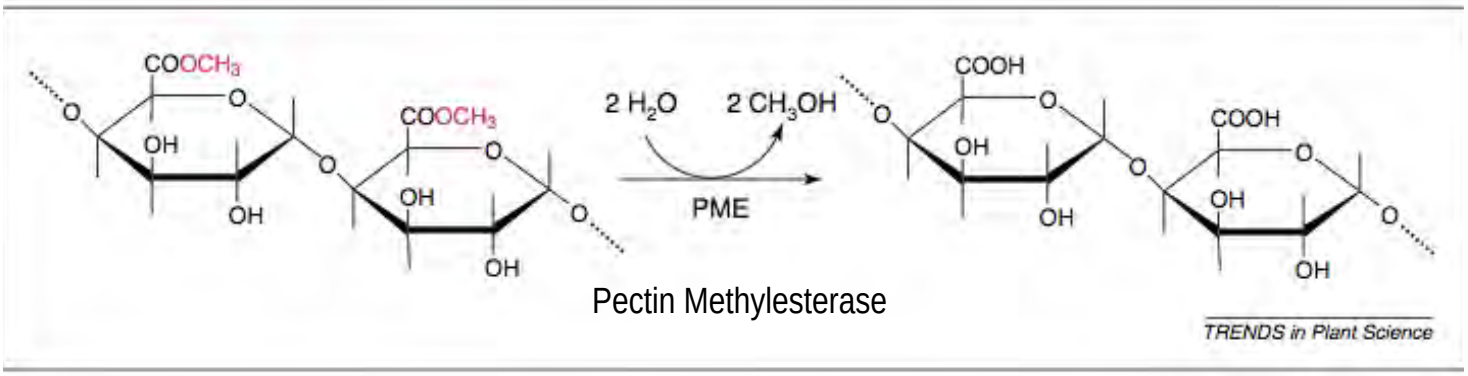
Pb^{2+}=Cu^{2+}>Cd^{2+}>Ni^{2+}>Ba^{2+}>Zn^{2+}>Ca^{2+}>Sr^{2+}>Co^{2+}>Mn^{2+}>Mg^{2+} (Haug and Smidsrod, 1970)
sunflower head, apple

Cu^{2+} ~ Pb^{2+} >> Zn^{2+} ≥ Cd^{2+} ~ Ni^{2+} > Ca^{2+} (Dronnet et al.1996) citrus, sugar beet

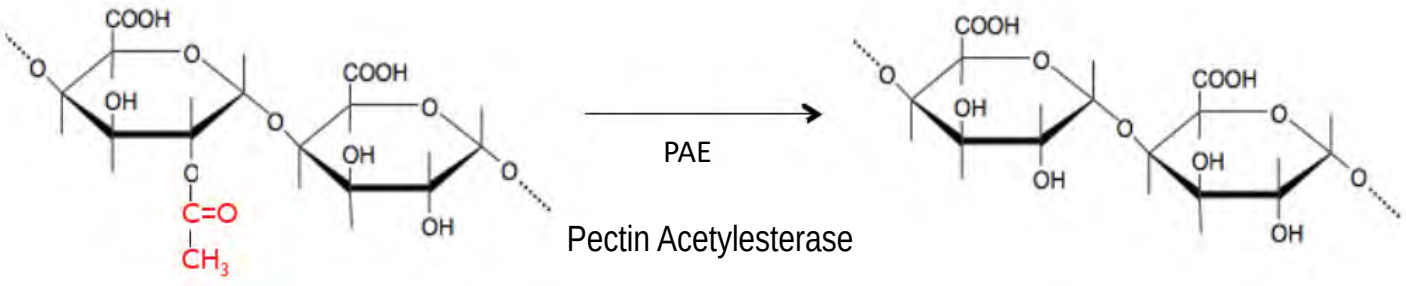
Pb> Ba> Cd> Sr> Zn> Cu> Co> Ni> Fe> Hg> Cr> Mn> Mg (Braudo et al., 1996, 1991)

$Pb^{2+(C>A>B)}$ >>$Cu^{2+(B>C>A)}$ >$Co^{2+(A>C>B)}$ >$Ni^{2+(C>A>B)}$ >> $Zn^{2+(C\sim A\sim B)}$ ~ $Cd^{2+\ (B>C\sim A)}$ (Kartel et al. 1999)
apple, beet, citrus

Pectin modifying enzymes



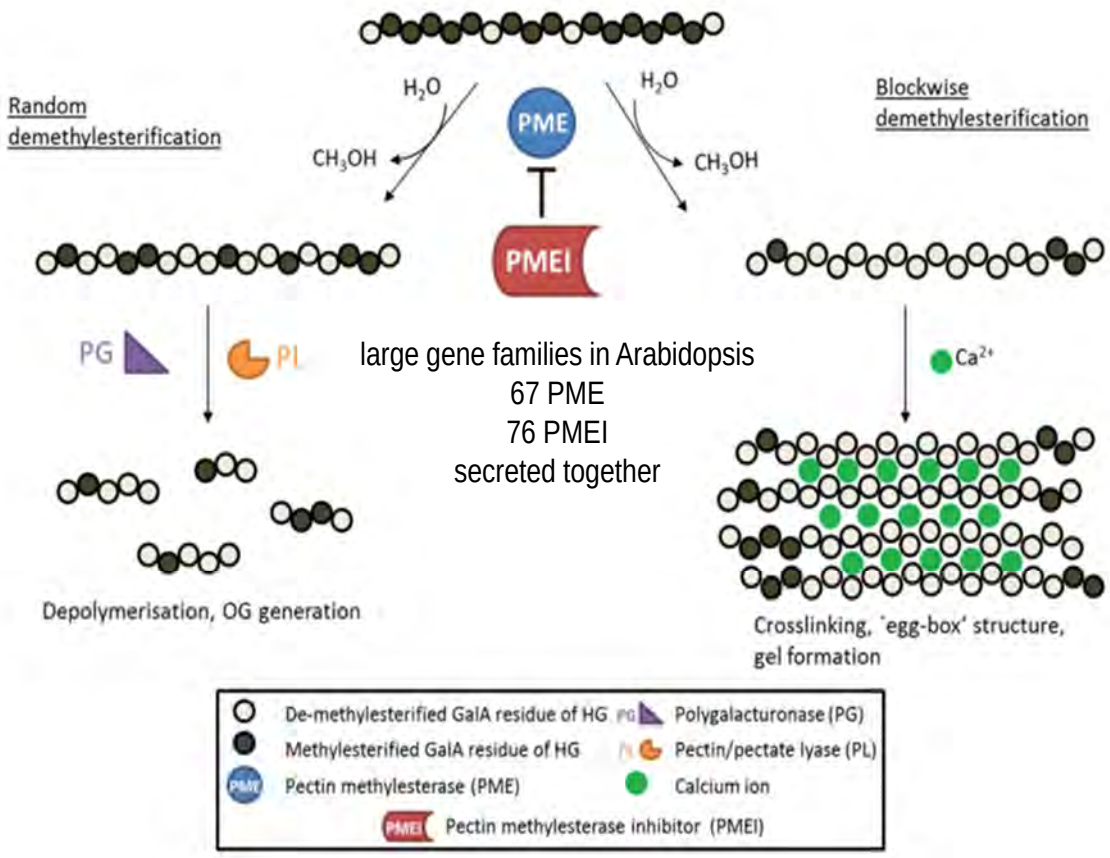
Pectin Methylesterase



Pectin Acetylesterase

Modified from Micheli, 2001

PME activity is tightly regulated



Wormit et al., 2018

PMEs and their inhibitors PMEIs

Large gene families

in Arabidopsis

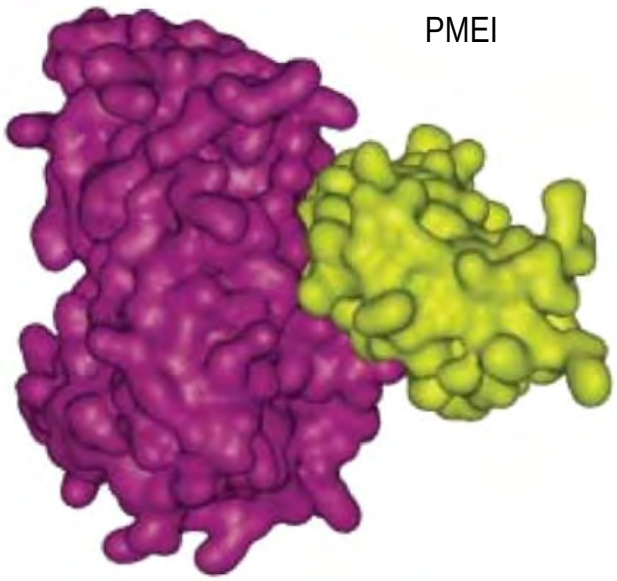
67 PME

76 PMEI

Secreted together

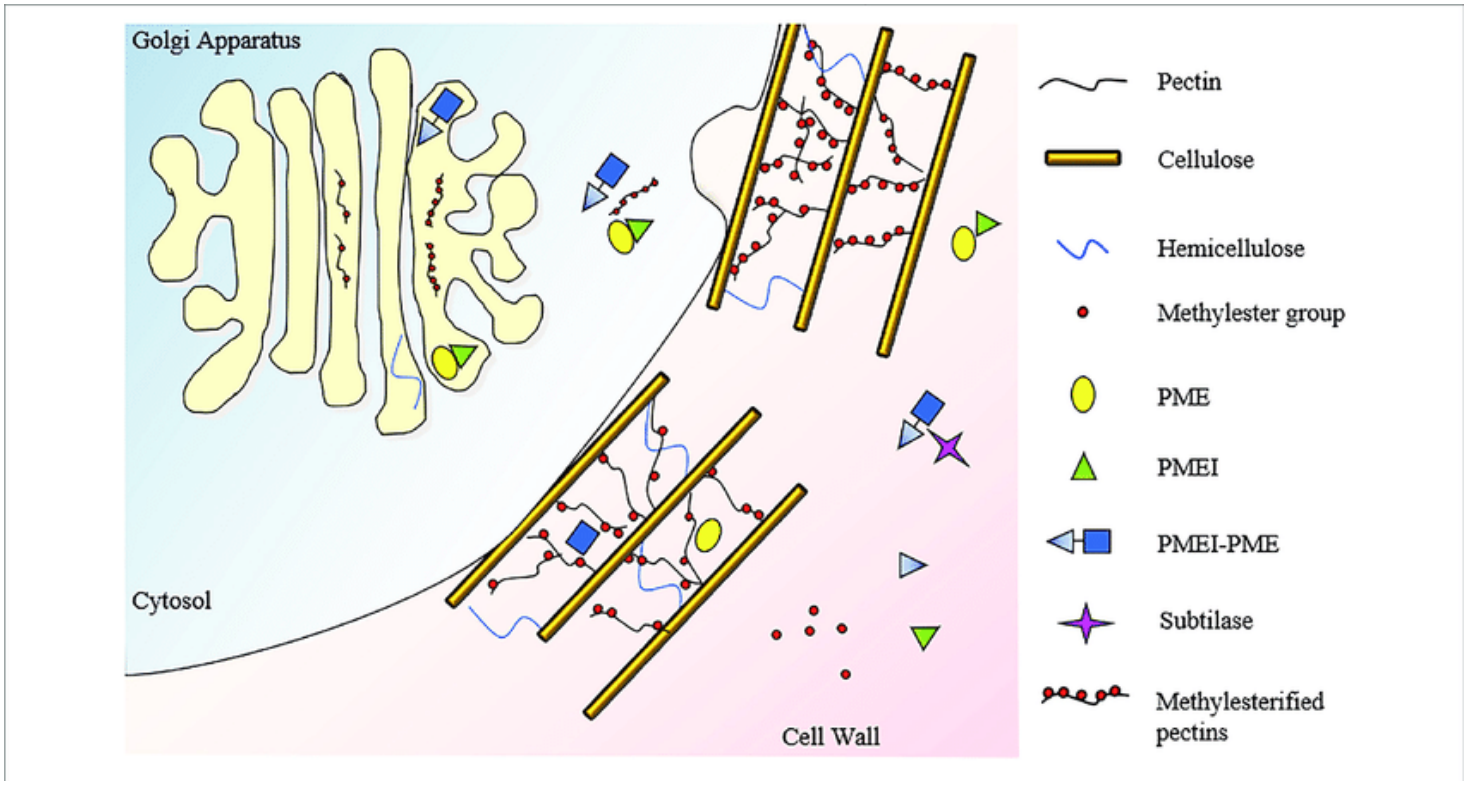
PME

PMEI



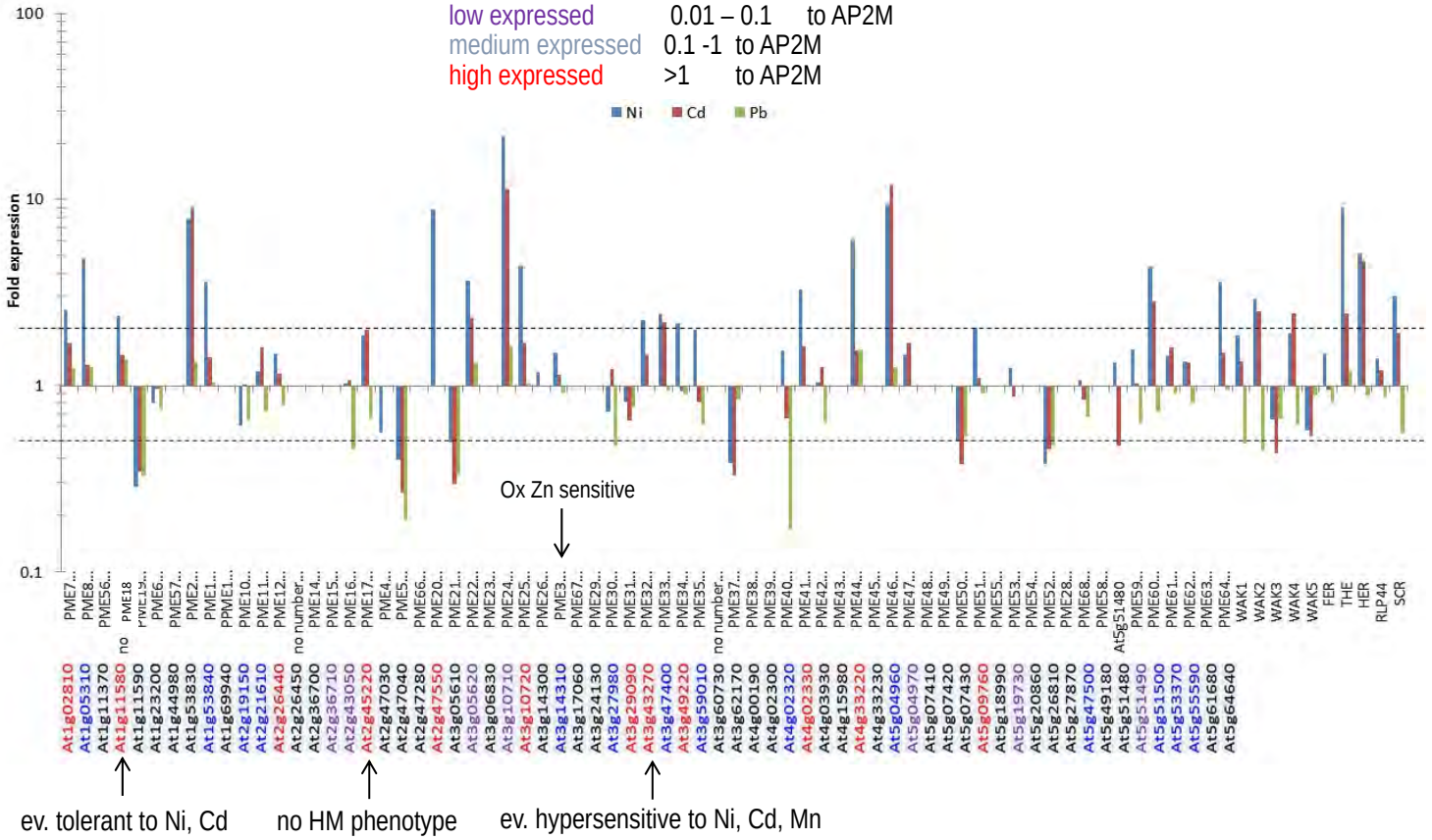
Jolie et al. 2010

Pectin is synthesized in the Golgi and secreted together with PME and PMEI into the apoplast/cell wall



PME Expression upon Cd^{2+}, Ni^{2+}, Pb^{2+} exposure

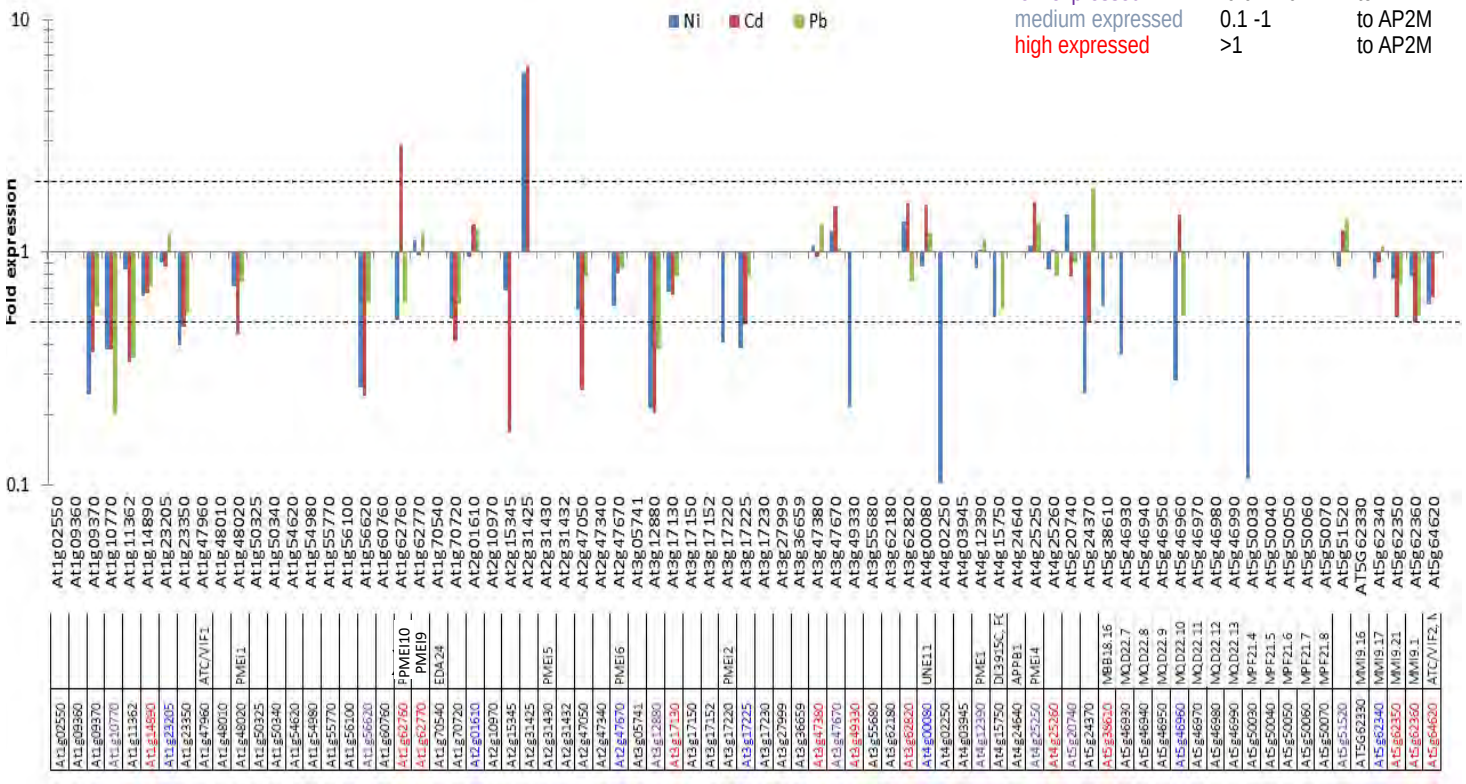
very low expressed <0.01 to AP2M
low expressed 0.01 – 0.1 to AP2M
medium expressed 0.1 -1 to AP2M
high expressed >1 to AP2M



Marie Ploderer + Amiens

PMEI Expression upon Cd^{2+}, Ni^{2+}, Pb^{2+} exposure

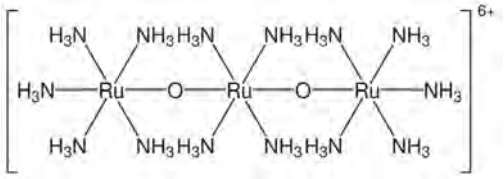
very low expressed	<0.01	to AP2M
low expressed	0.01 – 0.1	to AP2M
medium expressed	0.1 -1	to AP2M
high expressed	>1	to AP2M



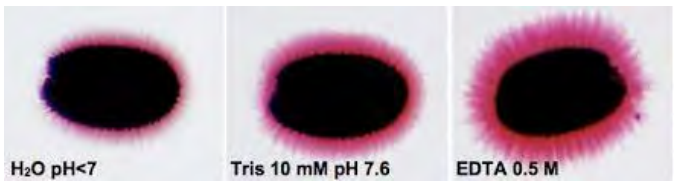
PMEI expression is generally repressed by Cd^{2+}, Ni^{2+}, Pb^{2+}

Marie Ploderer + Amiens

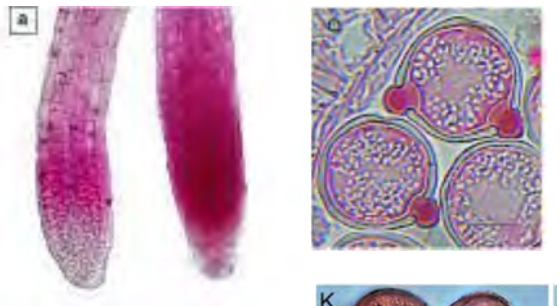
Visualization of PME Activity



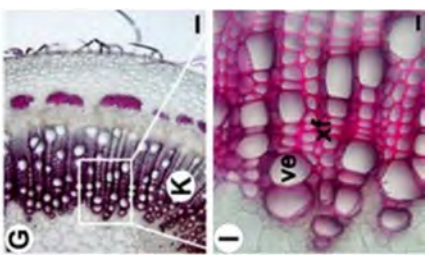
Ruthenium red binds to negative charges and stains acidic polysaccharides of pectin.



Seed mucilage



Root tips



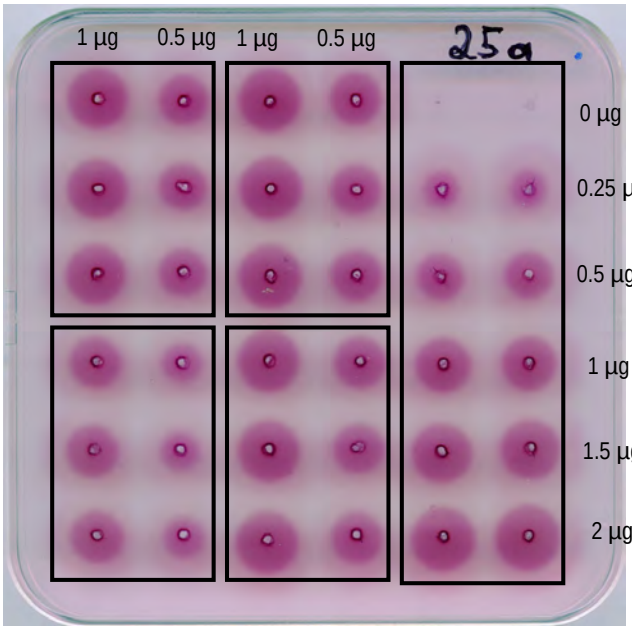
Vasculature

Pollen tubes

Quantification of PME Activity by a Diffusion Assay

Agarose gel with esterified pectin incubated with protein extracts and stained with Ruthenium red. Dark red indicates de-esterified pectins, diffusion area quantified with ImageJ.

Samples in triplicates and 2 concentrations



Standards in duplicates

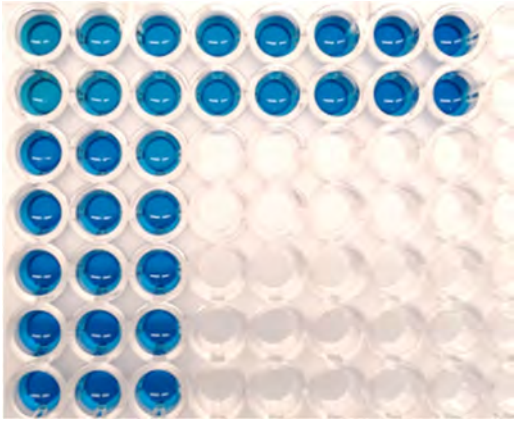
Quantification of PME Activity by measuring the released MeOH (MBTH – assay)

MeOH enzymatically oxidized to formaldehyde by alcohol oxidase.

Formaldehyde and 3-methyl-2-benzothiazolinon-hydrazon hydrochloride (MBTH) form a blue formazan dye in the presence of Fe^{3+} under acidic conditions.

Absorbance is measured at 620 nm

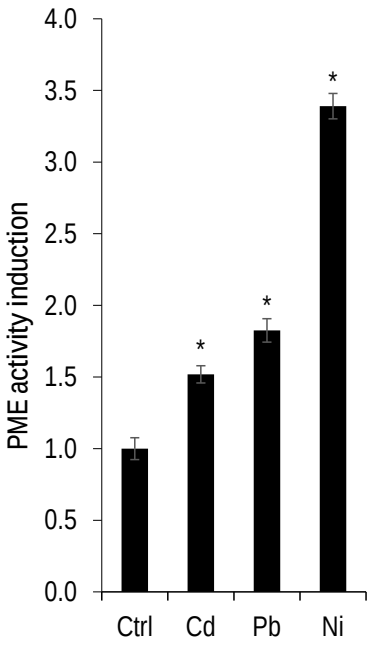
Samples in triplicates



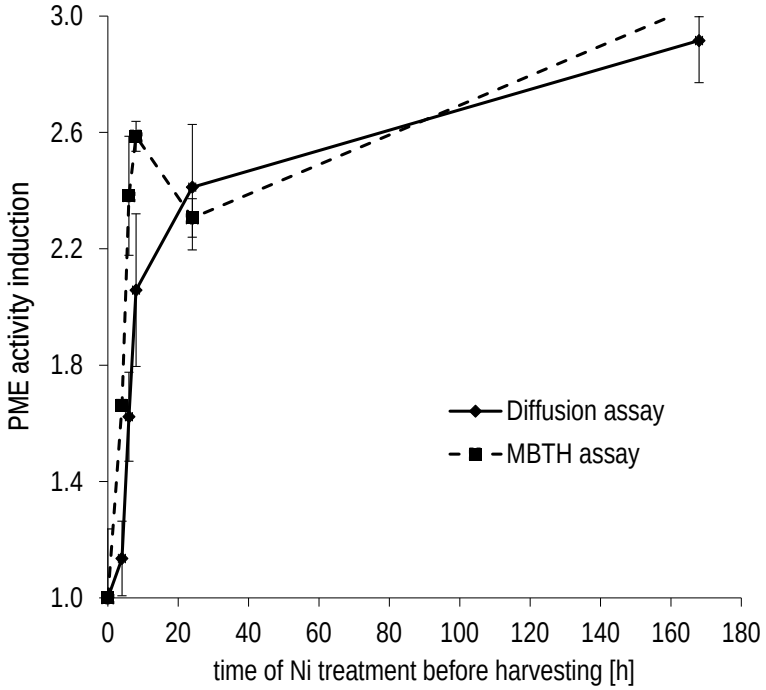
Standards in duplicates

Anthon and Barrett, 2004

PME Activity is induced in seedlings by Ni, Cd and Pb

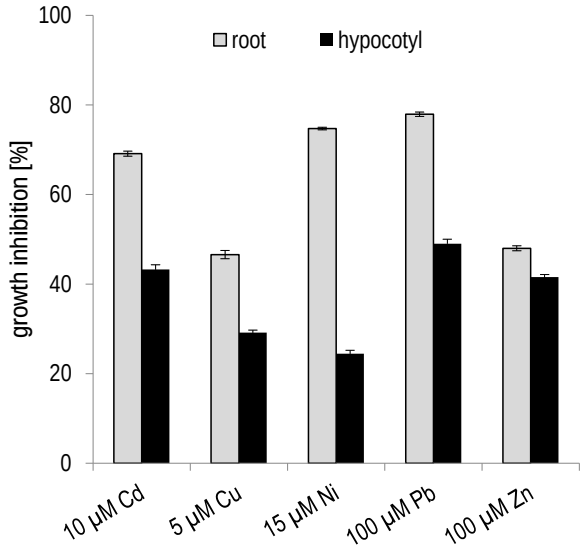


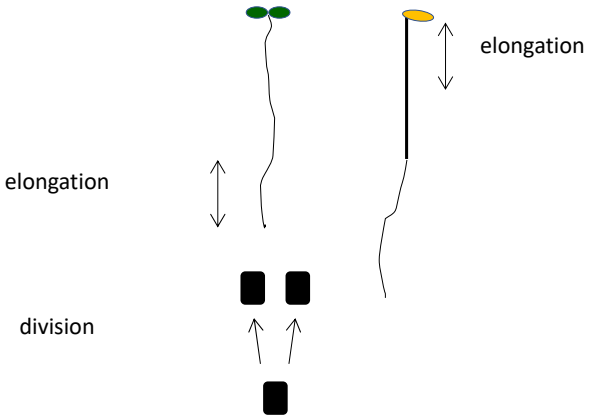
Strobl & Richter, unpublished



Strobl, Ünal & Richter, unpublished

Growth inhibition varies between light/dark grown seedlings, roots and hypoctoyls





Richter et al., 2017

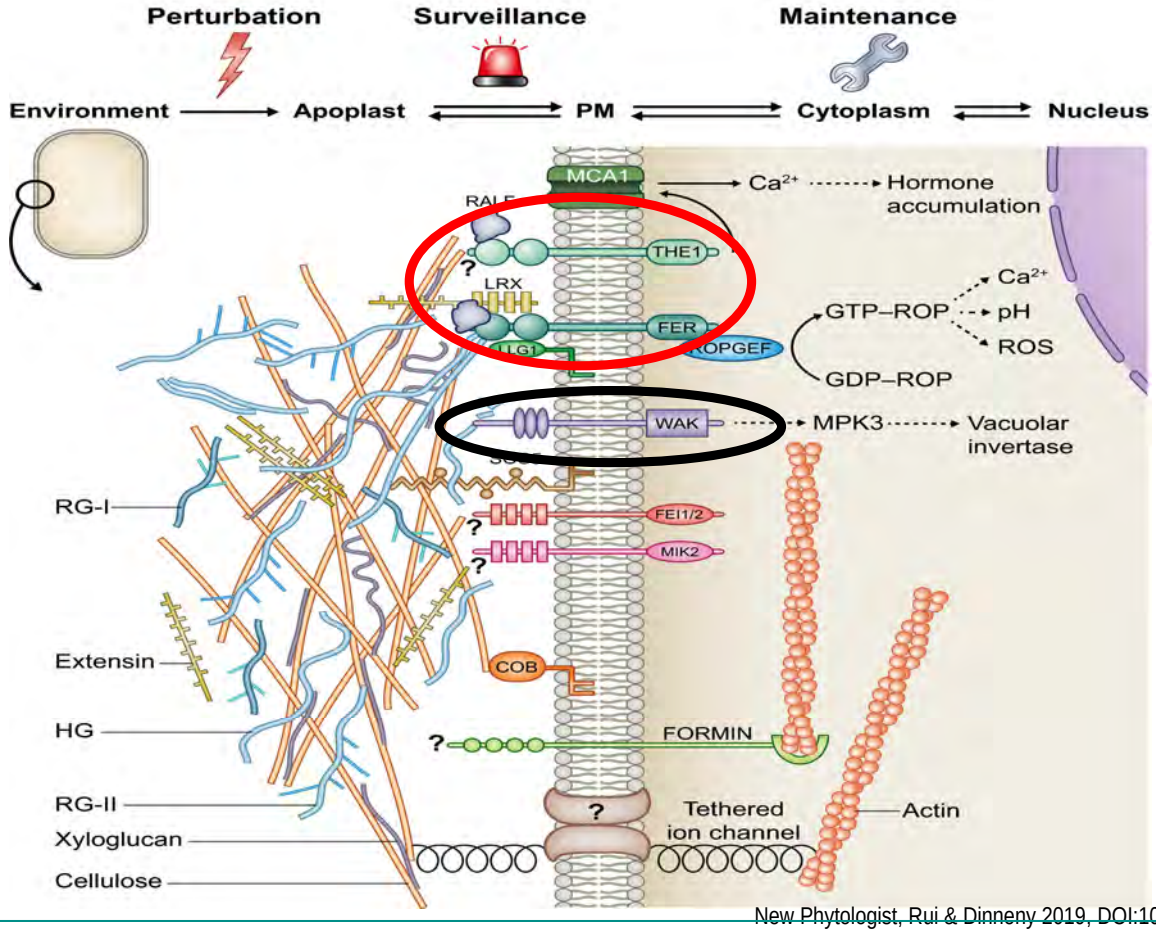
Metal ions change the balance between PME and PMEI

more PMEI
less PME activity
less demethylation
less cation binding
more flexible cell wall

less PMEI
more PME activity
more demethylation
more cation binding
stiffer cell wall
Ni, Cd, Pb

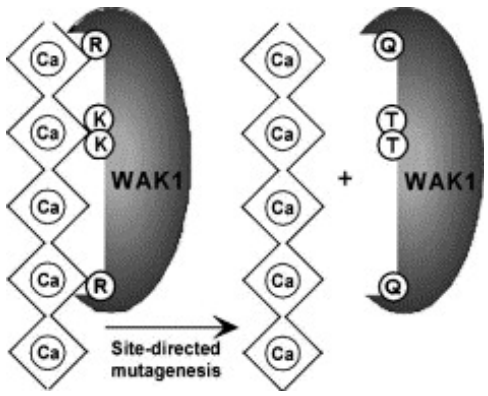
How are cell wall changes monitored?

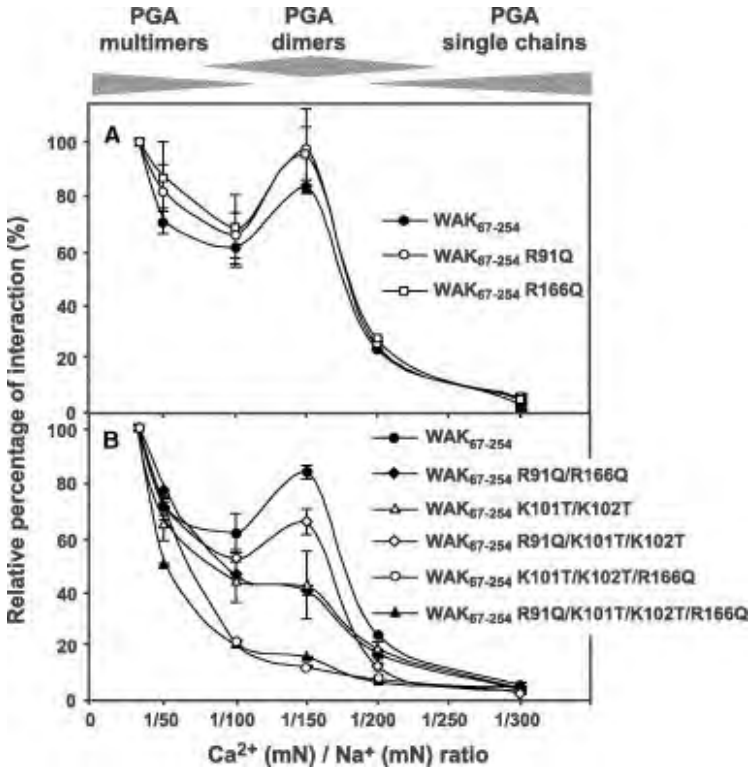
Components of Cell Wall Integrity Signaling



New Phytologist, Rui & Dinneny 2019, DOI:10.1111/nph.16166

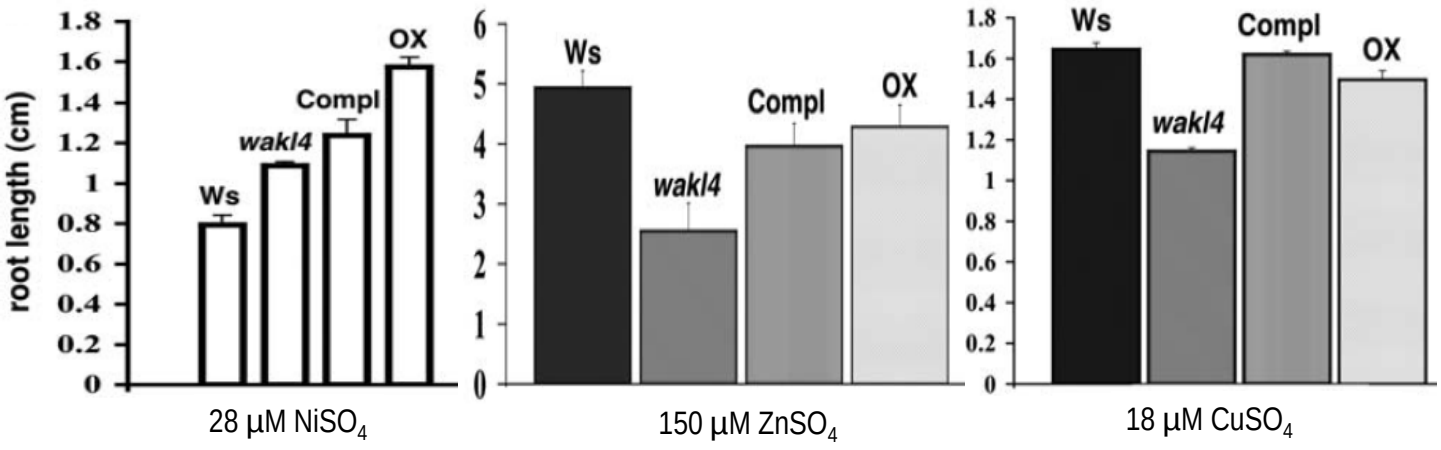
Recombinant extacellular domain of Arabidopsis WallAssociatedKinase (WAKs) bind PGA





Devreux et al., 2006

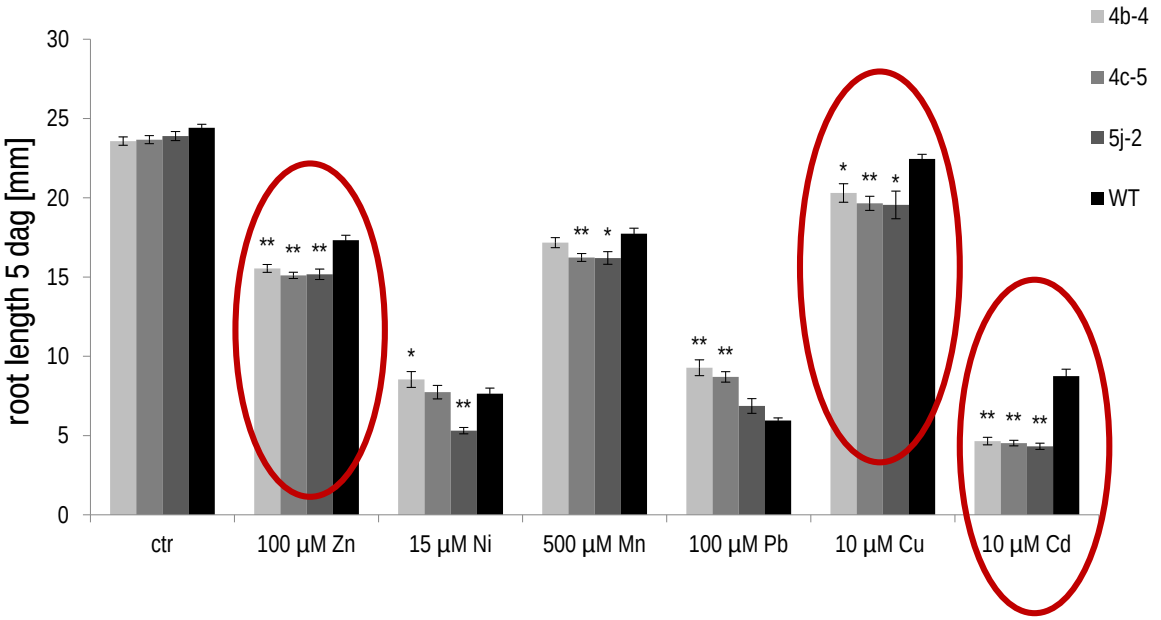
Wall associated kinase like 4 (WAKL4) involved in metalloid responses



wakl4 mutant hypersensitive to K^+, Na^+, Cu^{2+}, Zn^{2+} and tolerant to Ni^{2+}

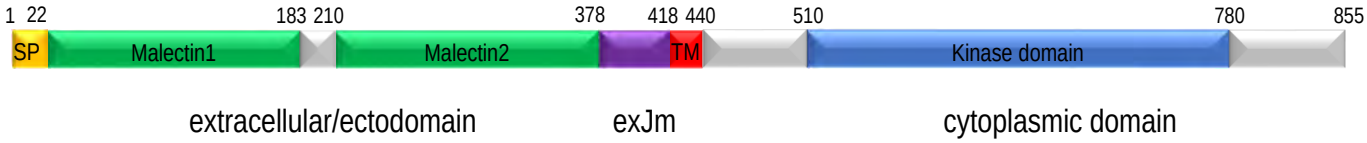
Hou et al. 2005

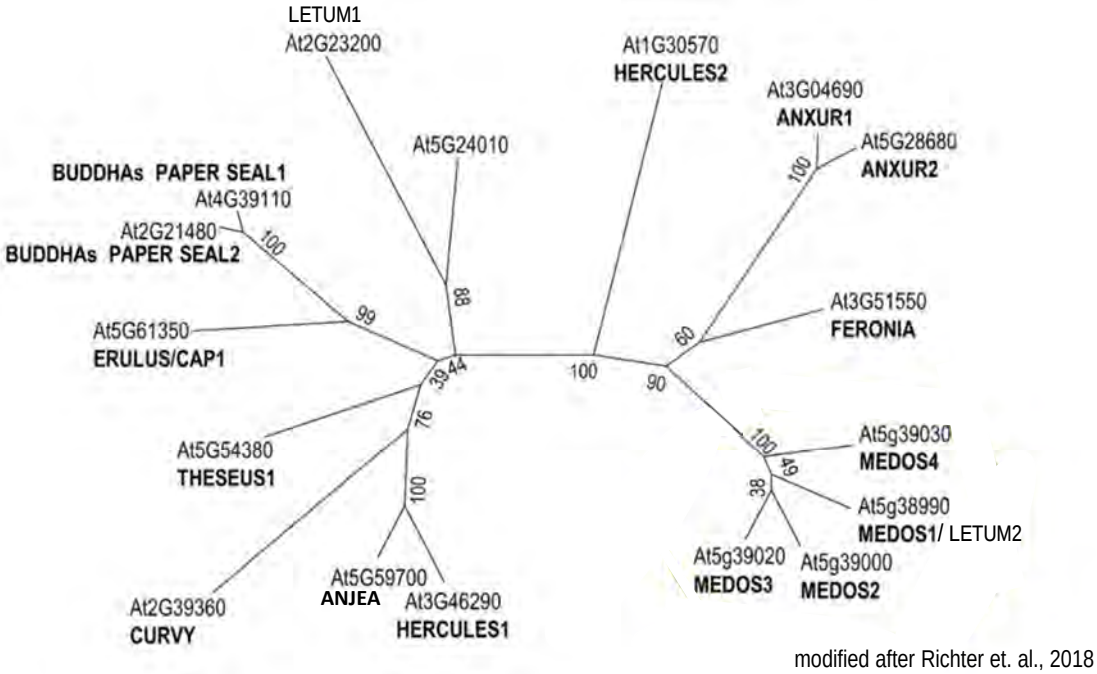
Overexpression of *Salix caprea* WallAssociatedKinaseLike (ScWAKL) in Arabidopsis causes ion specific root growth inhibition



Julia Richter & Shi unpublished

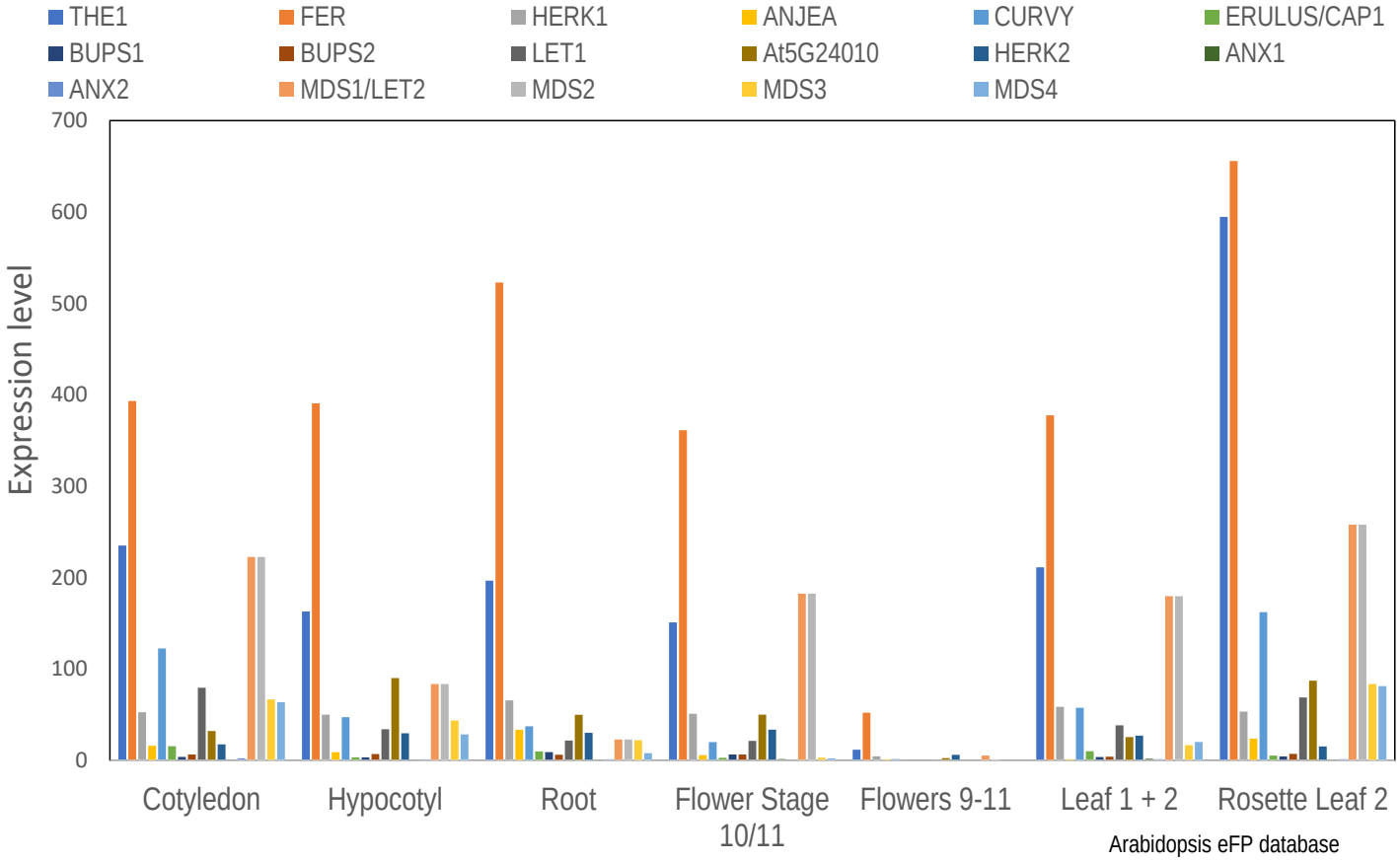
Catharanthus roseus Receptor Like Kinsase 1 Like Family (*Cr*RLK1L)





modified after Richter et. al., 2018

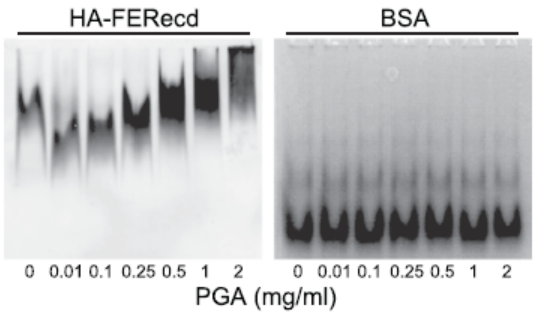
Organ specific Expression of *Cr*RLK1L



Arabidopsis eFP database

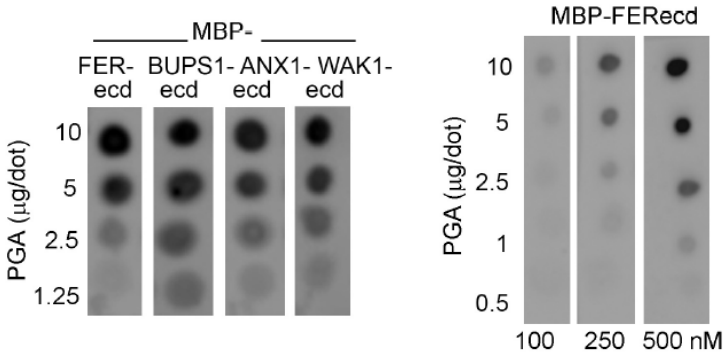
Pectates bind to the malectin A domain of *Cr*RLK1Ls

Electrophoretic mobility shift assay



HA-FERecd +PGA mixed before gel

Dot blot assay



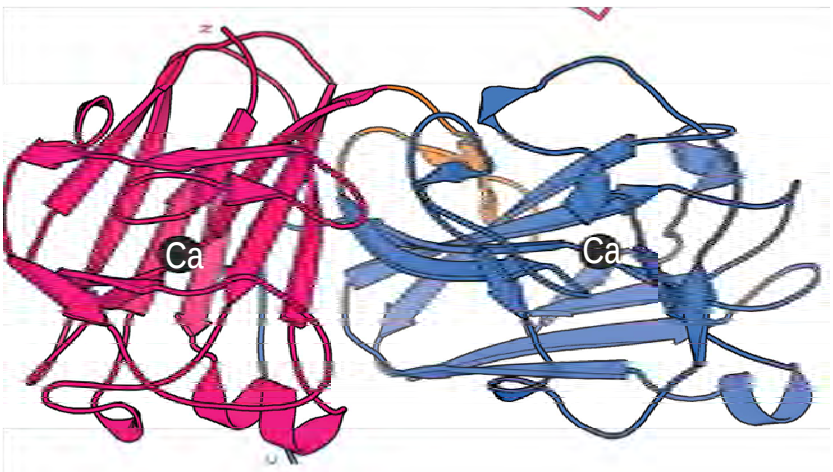
PGA immobilized on nitrocellulose membrane – rec. Protein – anti-MBP

Feng et al., 2018

Lin et al., 2018

Domain characteristics of *Cr*RLK1Ls

extracellular/ectodomain exJm cytoplasmic domain



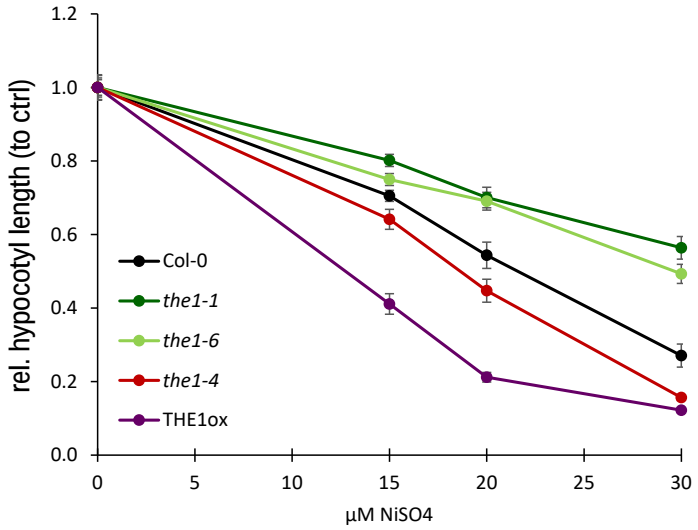
Malectin-like domains of ANX1 are structurally stabilized by Ca^{2+}

Moussu et al., 2018

Du et al., 2018

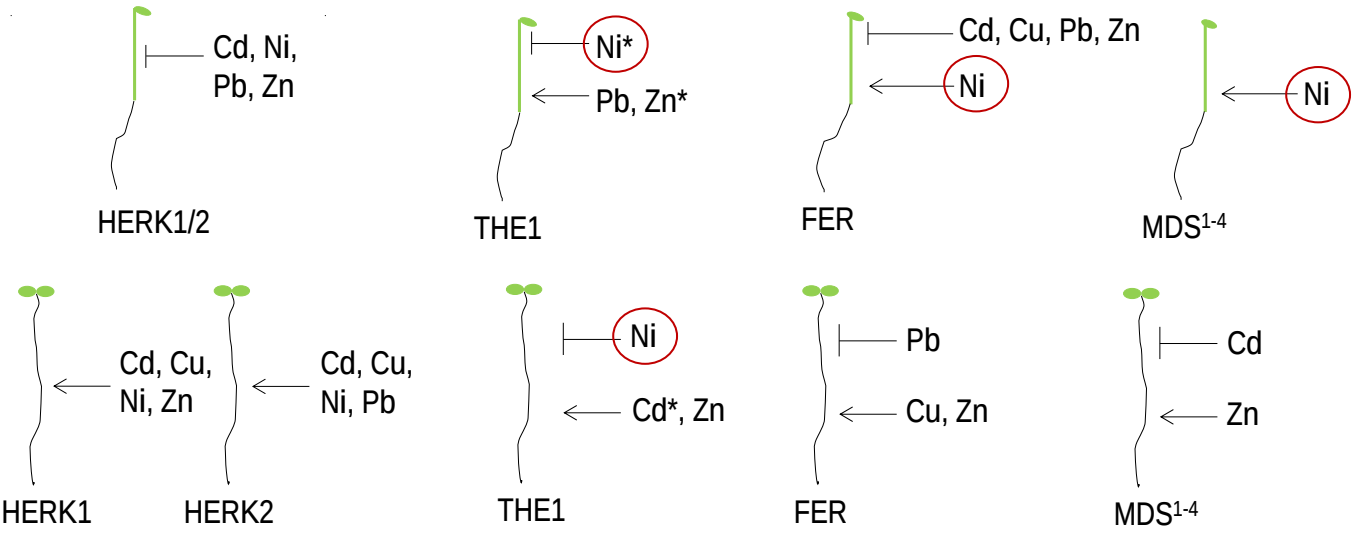
THESEUS1 mutants are tolerant and overexpressors sensitive to Nickel

Col-0 *the1-1* *the1-6* *the1-4* THE1ox



Richter et al., 2017

Organ specific network of positively and negatively acting *Cr*RLK1Ls



Complex pattern of gene specific, overlapping and antagonistic growth responses

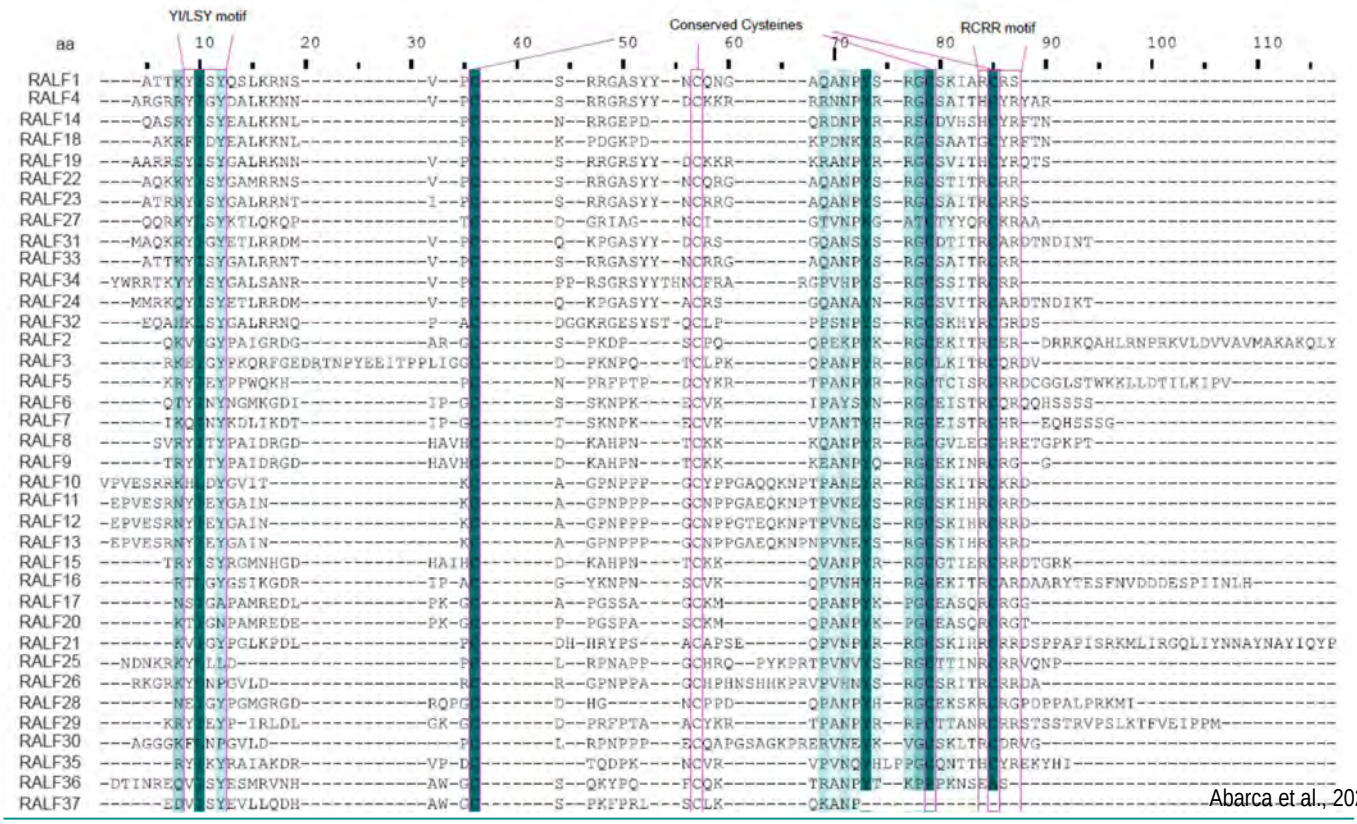
*Opposing between the1-6 and the1-4/THEox

Richter et al., 2017 and 2018

Pectates are not the only Ligand of *Cr*RLK1L

RAPID ALKALINIZATION FACTOR (RALFs) Peptides in Arabidopsis

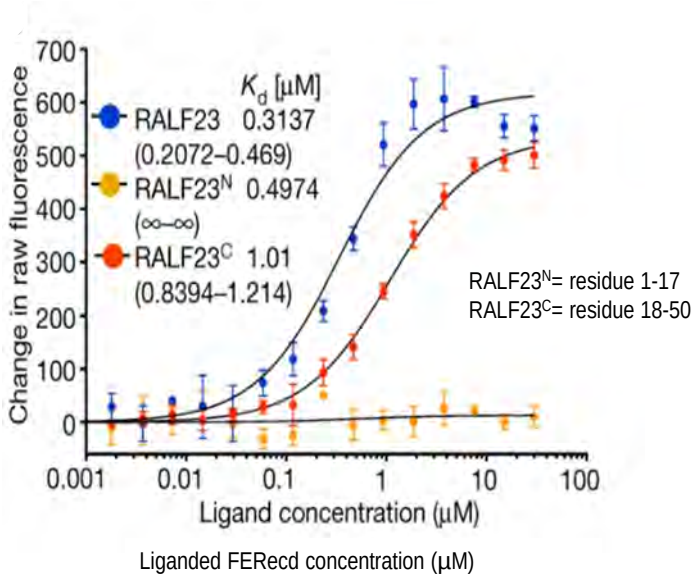
37 members of secreted cysteine-rich peptids derived from a precuror protein with signal peptide and dibasic processing site , YI/LSY and RCRR important for



Abarca et al., 2021

The extracellular domain of *Cr*RLK1Ls binds to Rapid Alkalinization Factors (RALFs)

Quantification of binding affinity by Microscale thermophoresis



RALF1 – FER, not ERU, not THE1

RALF4 – ANX1/2, BUPS1/2

RALF7 – FER

RALF16 - FER

RALF19 - ANX1/2, BUPS1/2

RALF22/23 – FER

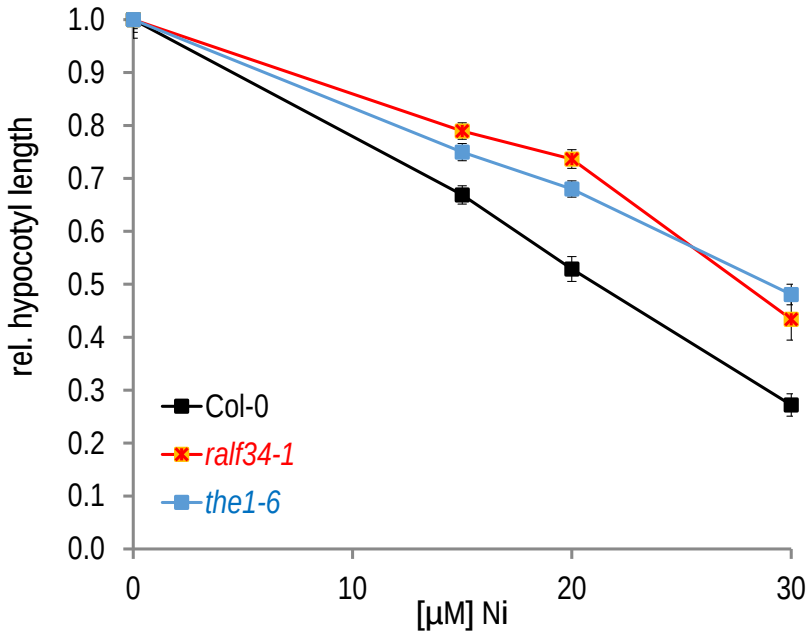
RALF32 -

RALF33 - FER

RALF34 – THE1, FER (weak), BUPS1/2

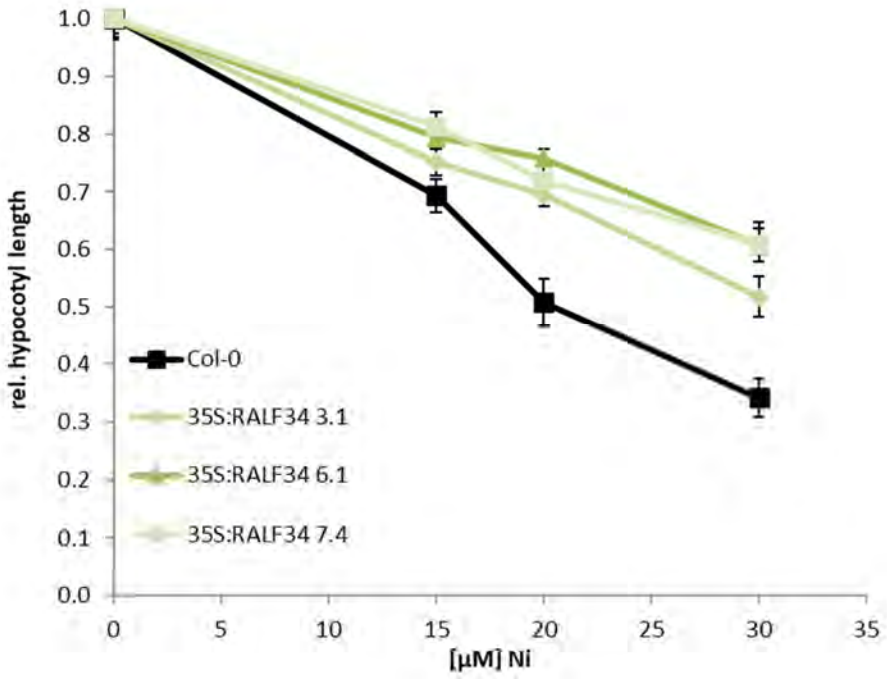
Xiao et. al., 2019
Gonneau et al,2018

Knock-down of RALF34 is Less Sensitive to Ni^{2+}



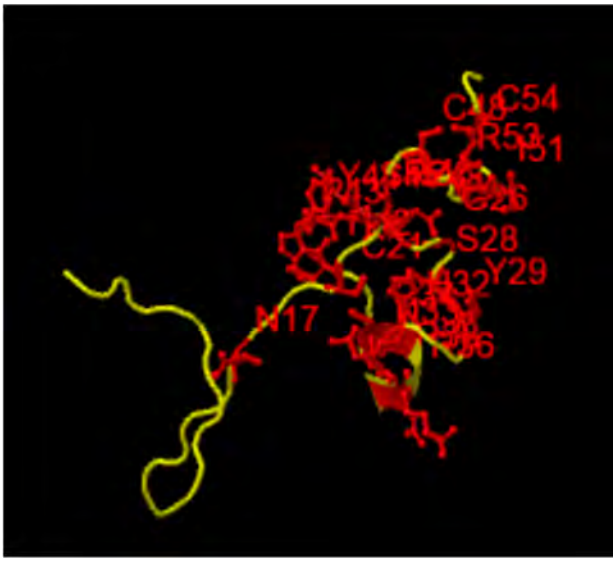
Richter unpublished

But also RALF34 Overexpressors are Less Sensitive to Ni^{2+}



Richter unpublished

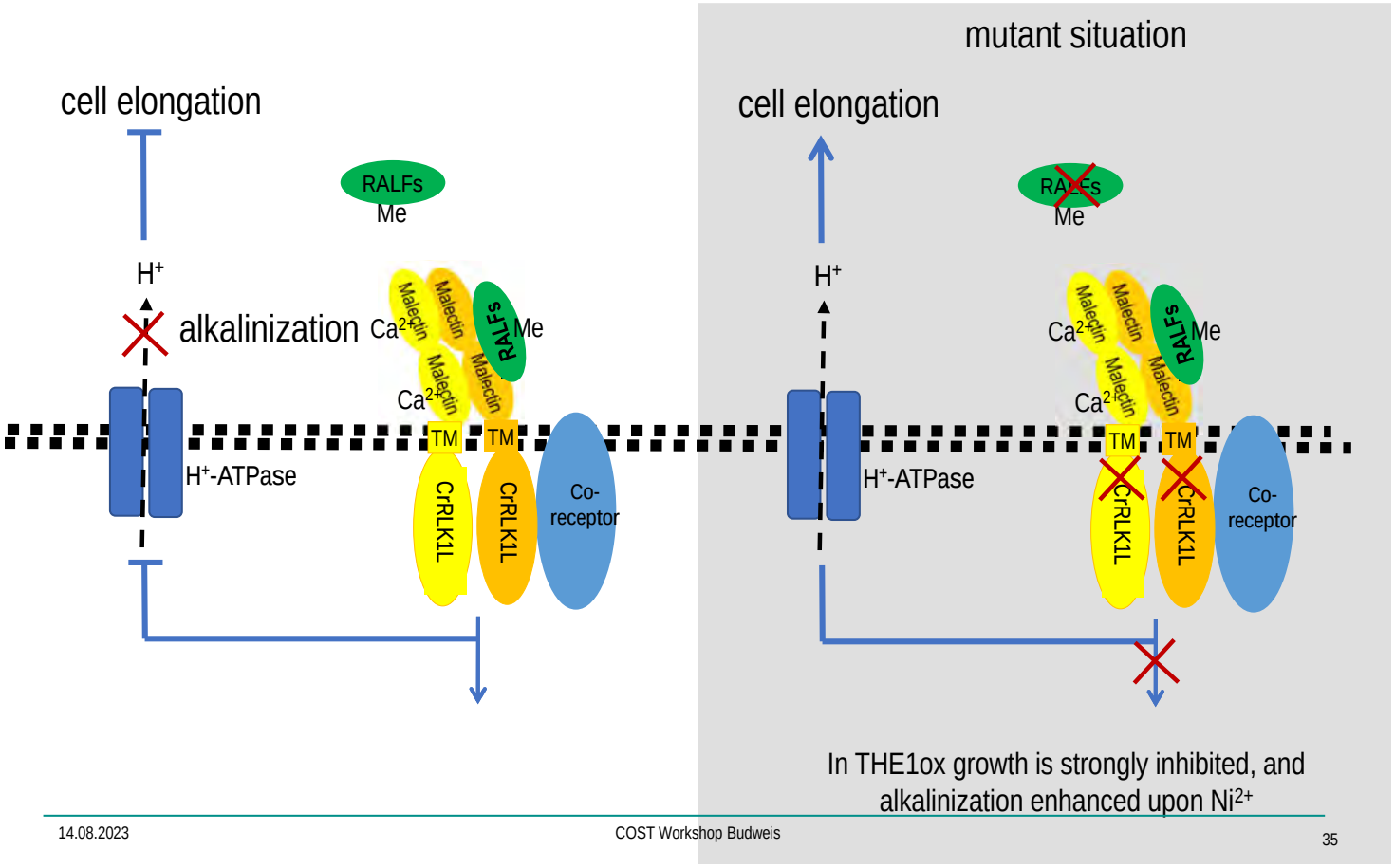
IonCom – Metal Ion Binding Prediction of the THE1 Ligand RALF34 for Zn^{2+}, Fe^{2+}, Ca^{2+}, Mn^{2+}, Na^{+}



YWRRTKYYISYGALSAN RVPC PPR SG RS Y YTH N C FR AR GPVH P Y S R GC SSI TR C RR

https://zhanggroup.org/IonCom/

Hypothetical Model of *Cr*RLK1Ls mediated cell wall signalling upon metal ions



In THE1ox growth is strongly inhibited, and alkalinization enhanced upon Ni^{2+}

Thanks to

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FWF L433-B17, L561-B17, I1725-B16