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Abstract

Pectin MethylEsterases (PME) catalyze the hydrolysis of the C6 Galacturonic Acid (GalA) methylester group present in newly synthesized HomoGalacturonans (HG) [1]. Analysis of recent literature suggests a potential role for pectin demethylesterification in the plant's response to nutritional stresses such as nitrogen and phosphorus [2, 3, 4] or metal stresses such as cadmium and aluminum [5, 6, 7]. This is evidenced by changes in PME activity under these conditions. These observations raise the question of whether pectin methylesterification rate is involved in the apoplastic transport and translocation of ions in both root and aerial plant tissues.

Objective

Assess how modifications to the cell wall pectic fraction impact the absorption and translocation of cadmium within *Arabidopsis thaliana*

Results

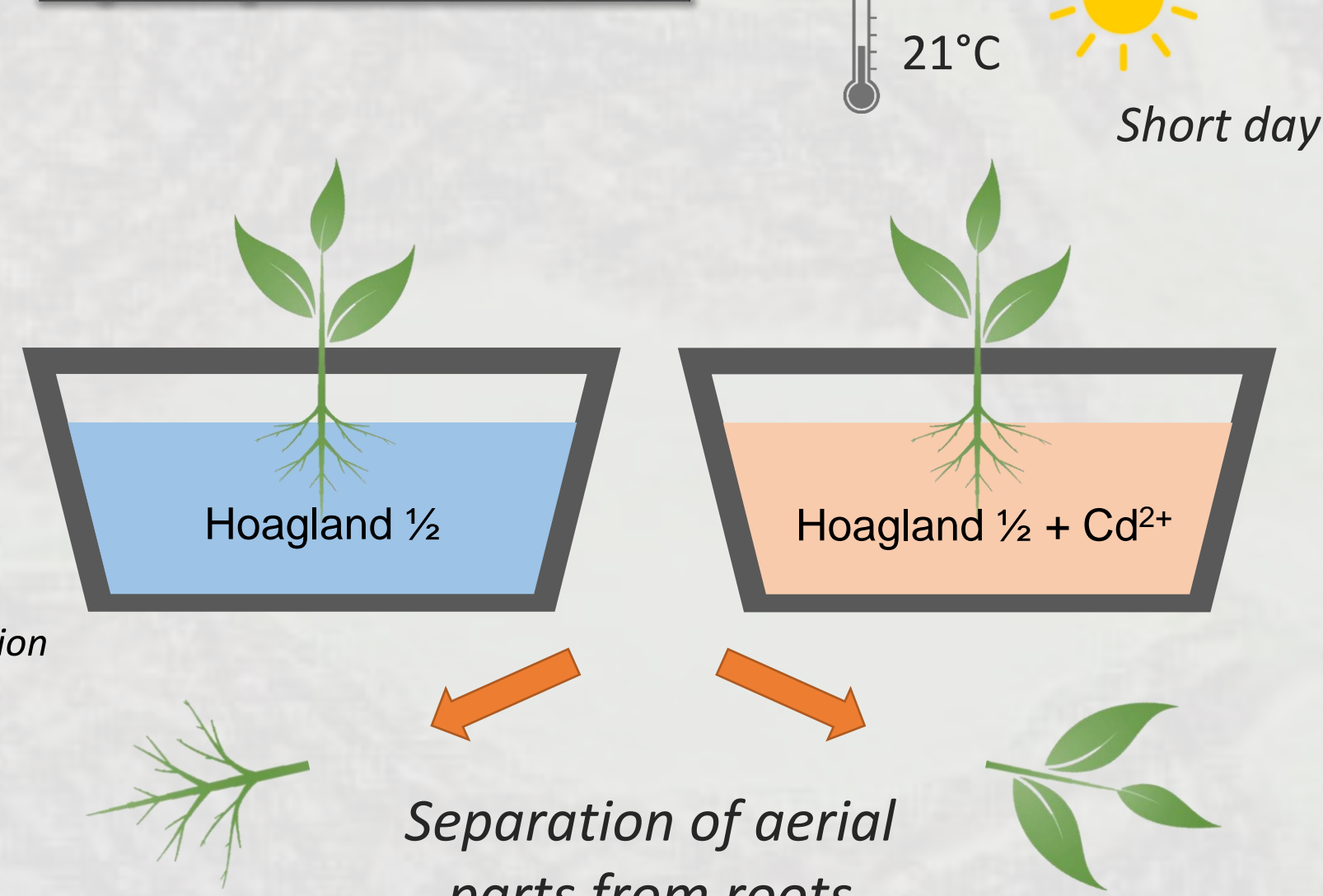
1 Impact of modifications on the absorption and transport of Cadmium

Pre-culture on MS ½ (WT + mutants):



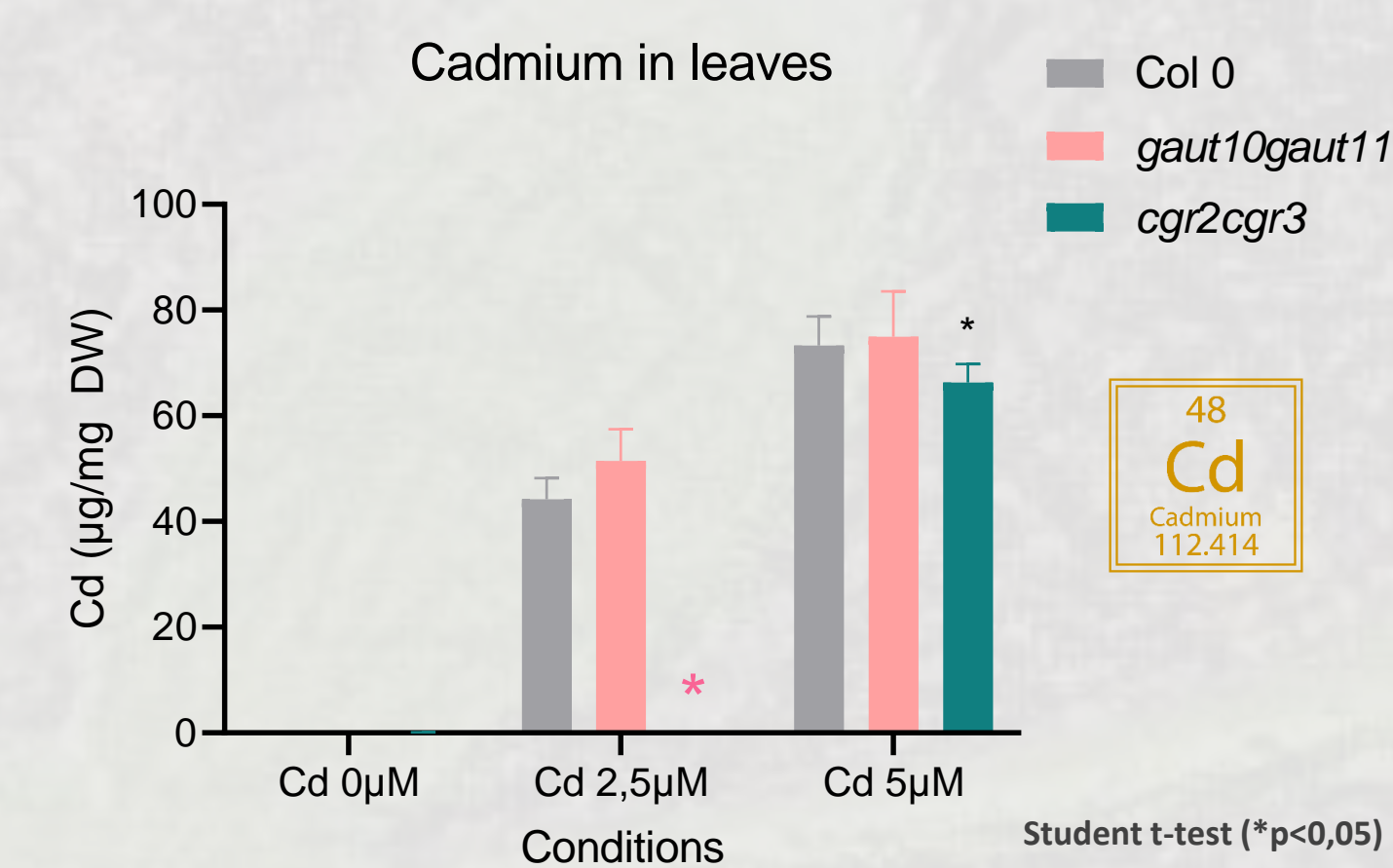
Transfer + 2 weeks of adaptation

Hydroponic culture:



→ Cell wall analysis + measurement of different ions quantity in plant tissues (sugar composition of cell wall, quantification of metal ions)

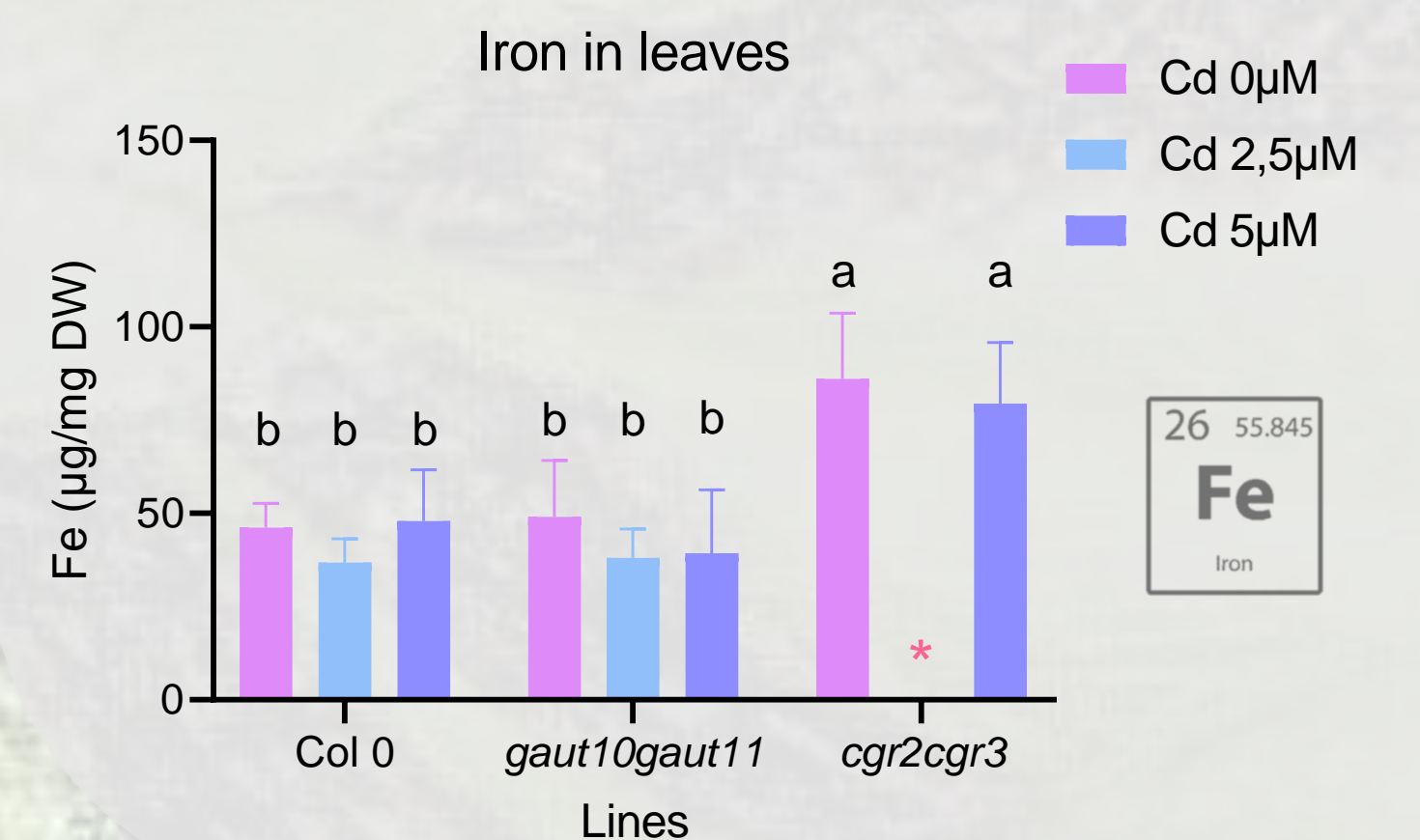
3 Accumulation of cadmium in leaves



➤ Significant ↓ of Cadmium in *cgr2cgr3* mutant compared to Col 0

➤ Significant ↑ of Copper and Iron in *cgr2cgr3* mutant compared to Col 0

➤ No significant difference between Col 0 and *gaut10gaut11*



Significant differences (letters) were determined according to one-way ANOVA followed by a multiple comparison with Tukey's test

2 Characterization of *A. thaliana* cell wall mutants

Double mutant *gaut10gaut11*

(GalactUronosyl Transferase → synthesis of HG)

- Ø visible phenotypic difference between *gaut10gaut11* and Col 0
- Lower amount of GalA in cell wall (obtained by F. Sénéchal and P. Trezel : BioEcoAgro)



Double mutant *cgr2cgr3*

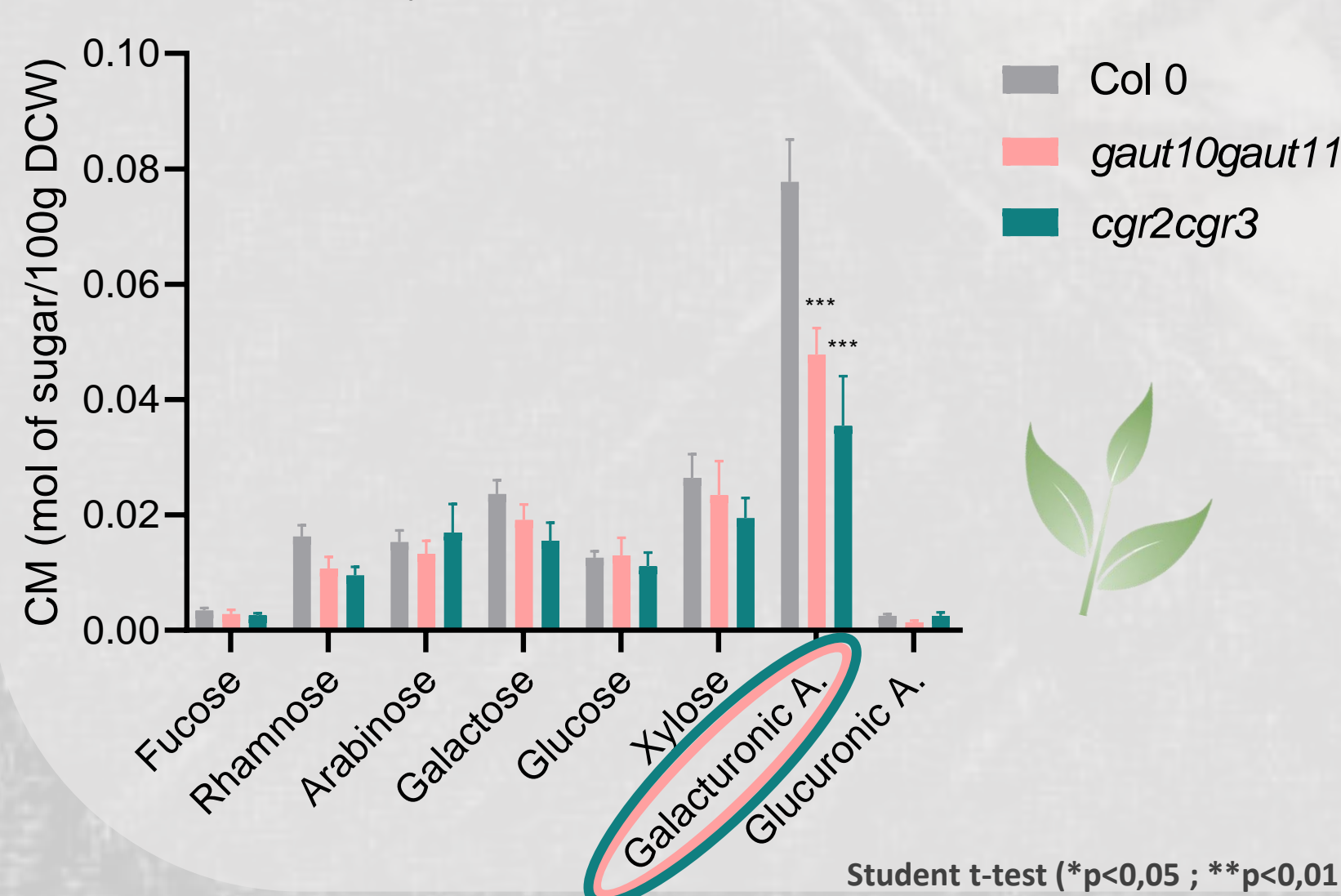
(HG MethylTransferase → HG methylesterification)

- Growth ↓ (Dwarf phenotype)
- Early appearance of inflorescence
- Defect in silique formation
- Lower methylesterification rate (Kim et al. 2015)

Obtained thanks to F. Brandizzi

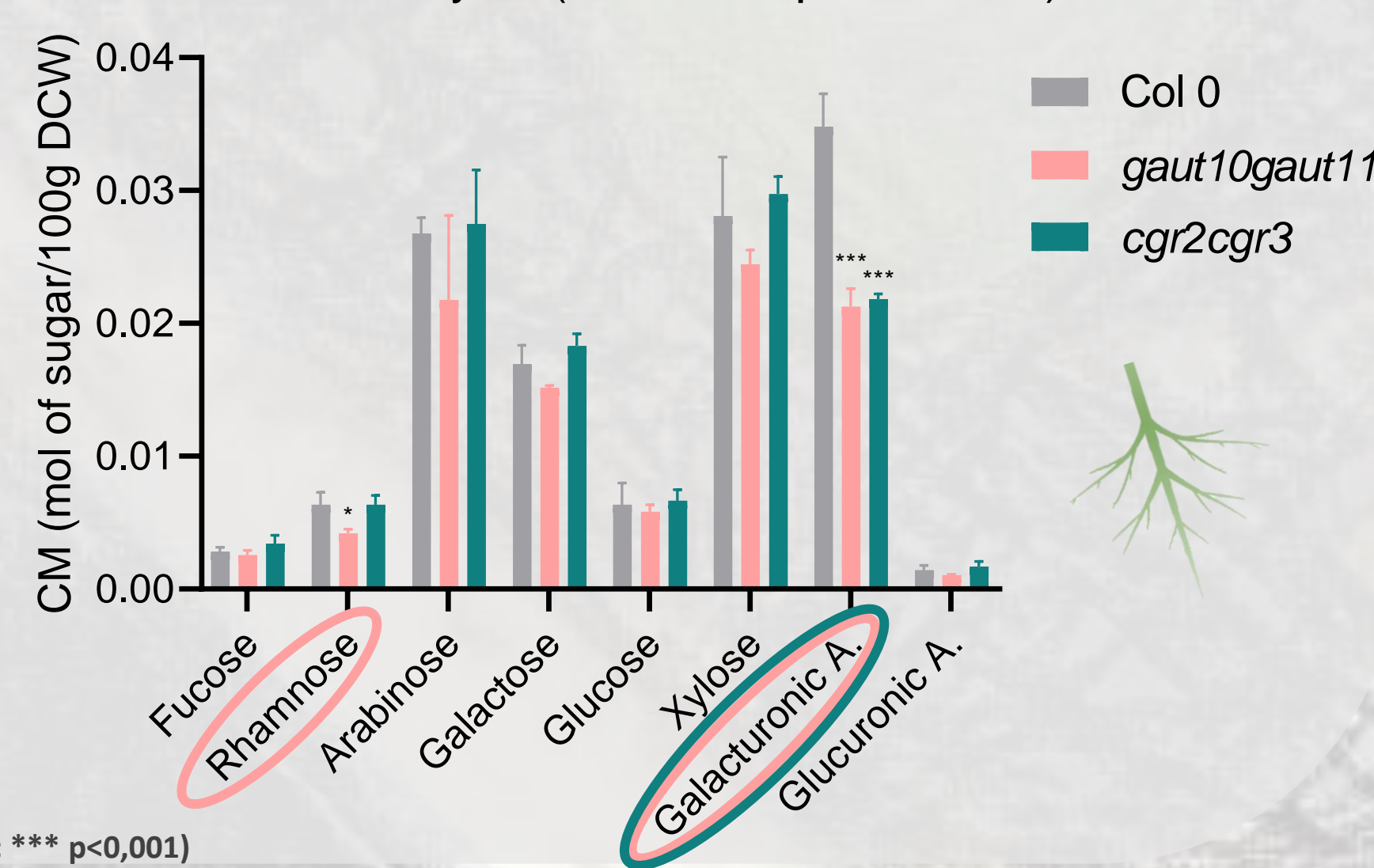


Cell wall analysis (Leaves at 0µM of Cd²⁺)



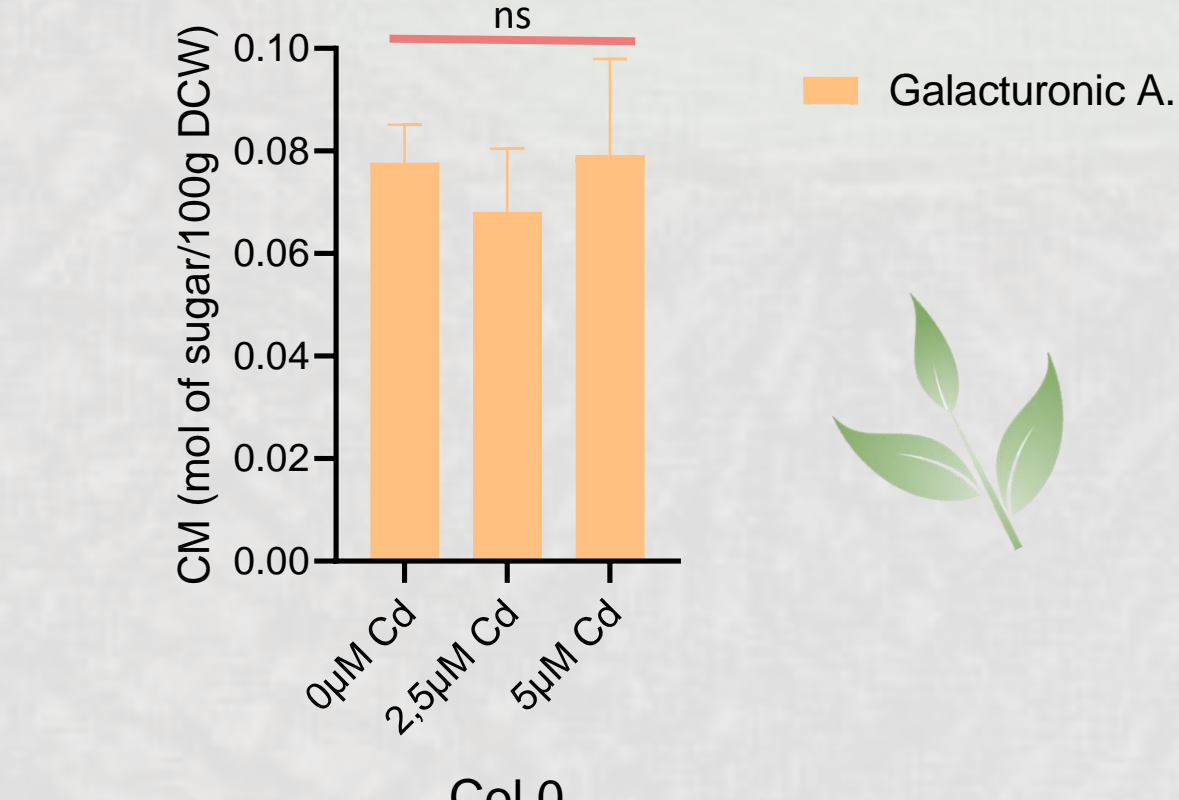
Student t-test (*p<0,05; **p<0,01; *** p<0,001)

Cell Wall analysis (Roots at 0µM of Cd²⁺)



4 Effect of Cadmium on cell wall composition

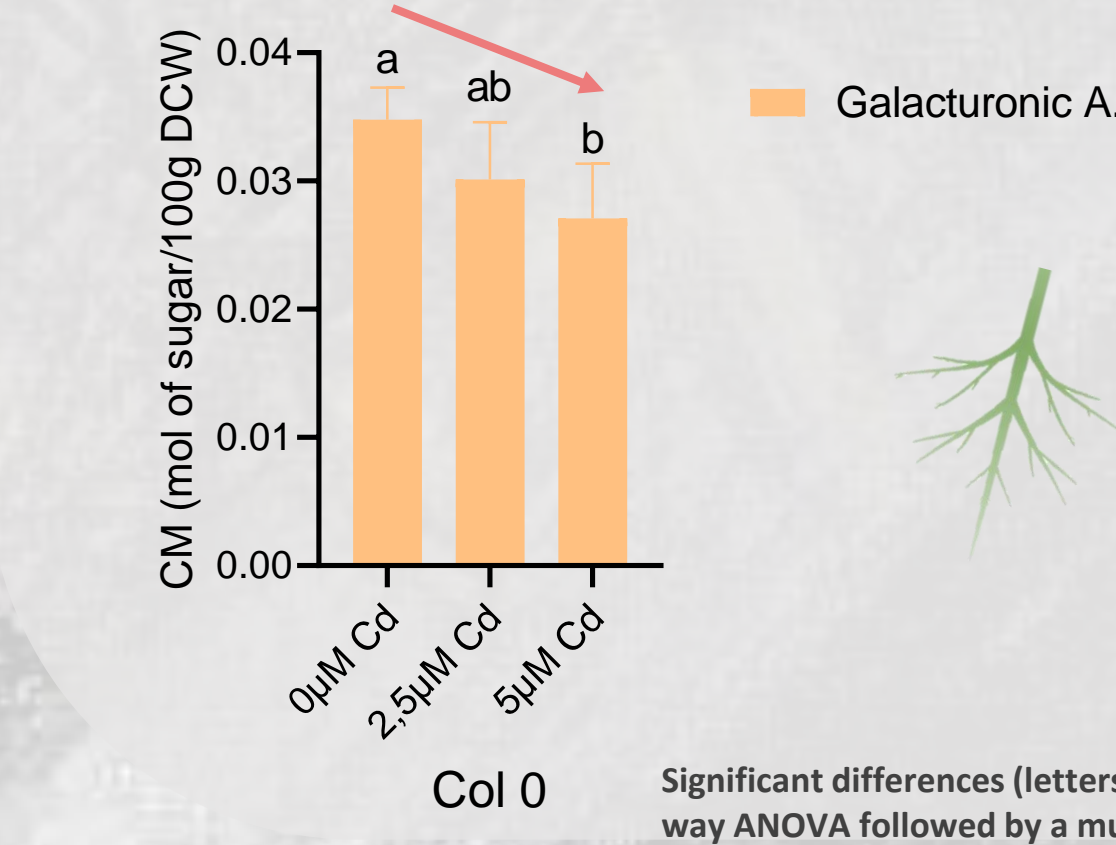
Cell wall analysis (Leaves: Galacturonic A.)



➤ Significant ↓ of Gal A on WT as Cd concentration ↑ : in roots only

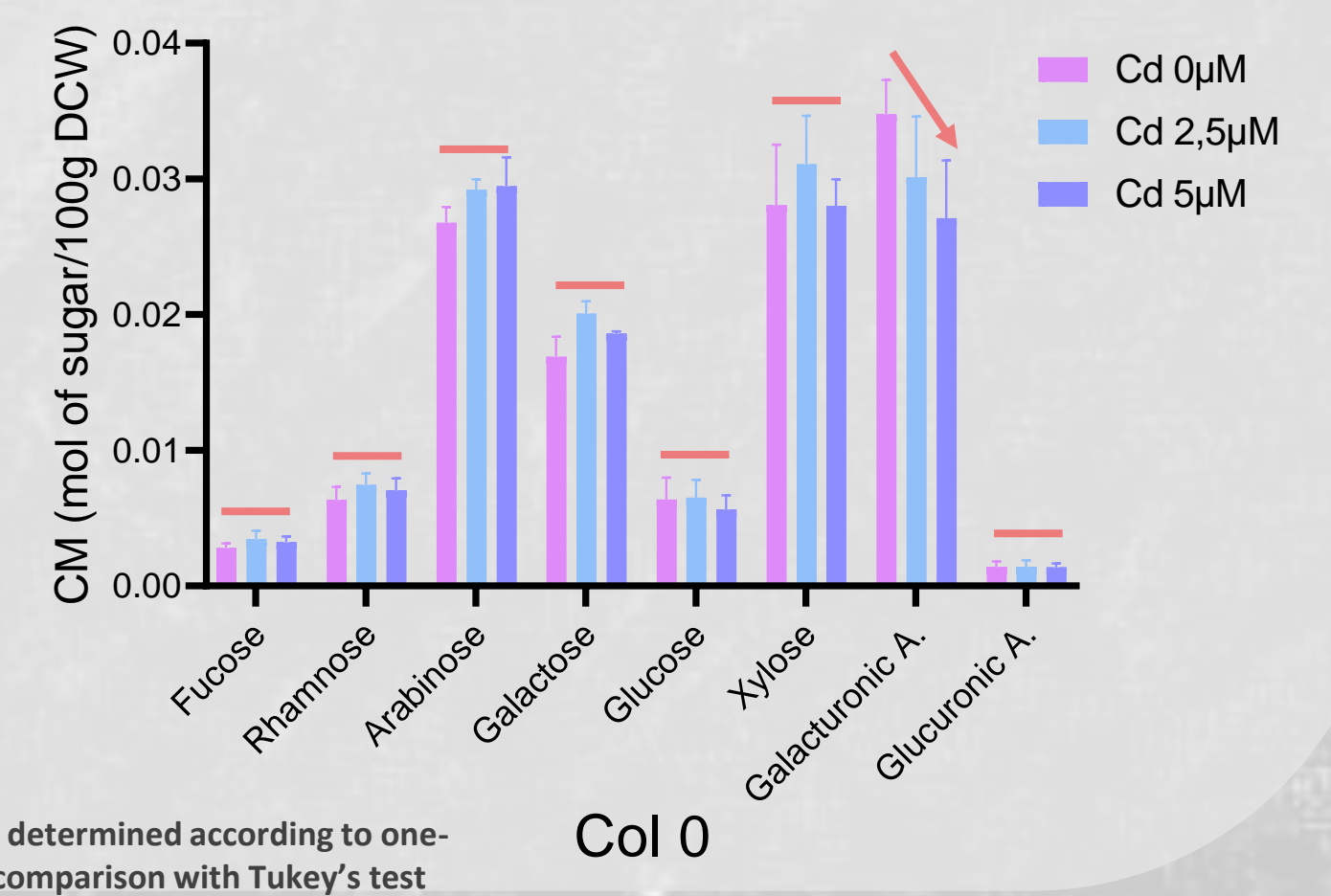
➤ Other cell wall components (roots or leaves) remain unchanged between cadmium conditions

Cell wall analysis (Roots: Galacturonic A.)



Significant differences (letters) were determined according to one-way ANOVA followed by a multiple comparison with Tukey's test

Cell wall analysis (Roots)



Conclusions

- *gaut10gaut11* mutant did show a significant decrease of GalA in addition to a decrease of Rhamnose
- Cadmium stress induces a decrease in GalA content in roots cell wall
- Metallic ions accumulation in *cgr2cgr3* leaves, whatever condition, suggests that lower methylesterification level results in cation accumulation
- Upon cadmium stress, accumulation of cadmium in *cgr2cgr3* mutants is slightly decreased. Which can be explained by an overload of cell wall by other ions as shown by copper and iron hyperaccumulation

Perspectives

- Plants overexpressing a fungal PME [8] in different tissues will be tested to confirm importance of methylesterification rate under cadmium stress conditions
- Cadmium accumulation kinetics and the effect of cadmium on cell wall modifications will be tested
- Influence of cadmium on root architecture will be tested on different mutants
- Capacity of cell wall to bind cadmium will be tested using extracted cell wall from different mutants
- PME and PMEi genes expression upon cadmium stress will be measured using RT-qPCR

References

- [1] Sénéchal et al. (2014) J Exp Bot 65:5125. ; [2] Landi S. and Esposito S. (2017) Front. Plant Sci. 8: 1376. ; [3] Podgorska et al. (2017) Front. Plant Sci. 8: 1344. ; [4] Hoehenwarter et al. (2015) BMC Plant Biol 16: 106. ; [5] Li et al. (2017) Plant Physiology and Biochemistry 119: 265. ; [6] Corso et al. (2021) New Phytol. 230: 669. ; [7] Wu et al. (2021) Environmental Pollution doi.org/10.1016/j.envpol.2021.117218 ; [8] Safran J et al. (2021) Int J Biol Macromol. 15:176:165-176. doi: 10.1016/j.ijbiomac.2021.02.035. ; [9] Schürholz AK et al. (2018) Plant Physiol. 2018 Sep;178(1):40-53. doi: 10.1104/pp.18.00463. Epub 2018 Jul 19.