

THESEUS1 and RALF34 monitor cell wall integrity

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1 The cell wall is a rigid network being the first barrier between a plant cell and its environment, but at the same time has to be a dynamic network whose cell growth and shape is given by deposition and remodeling of the cell wall. Maintaining cell wall integrity (CWI) is essential for correct plant development and stress response. Members of the family of *Catharanthus roseus* receptor-like kinase 1-like (*CrRLK1L*) proteins have been shown to play a role in cell wall homeostasis, mechanoperception, CWI maintenance and growth control. One of the 17 members in Arabidopsis, THESEUS1 (THE1), was identified in a suppressor screen of a cellulose deficient mutant, revealing that the reduction in growth is part of a THE1-mediated compensatory response to cell wall perturbation (Hématy et al., 2007). Interestingly, several *CrRLK1L* members have been shown to be receptors for Rapid Alkalinisation Factor (RALF) peptides. RALFs are on average 50 amino acids highly basic, cysteine-rich peptides, most of which are predicted to be cleaved from a highly acidic prodomain by a subtilisin protease. Recently, THE1 has been identified as a receptor for RALF34 (Gonneau et al., 2018). However, this peptide might not be the only THE1 ligand, since *ralf34* loss-of-function mutant do not phenocopy the aspects of *the1* mutant.

2 *ralf34* loss-of-function mutant presents a wild type response under cell wall damage.

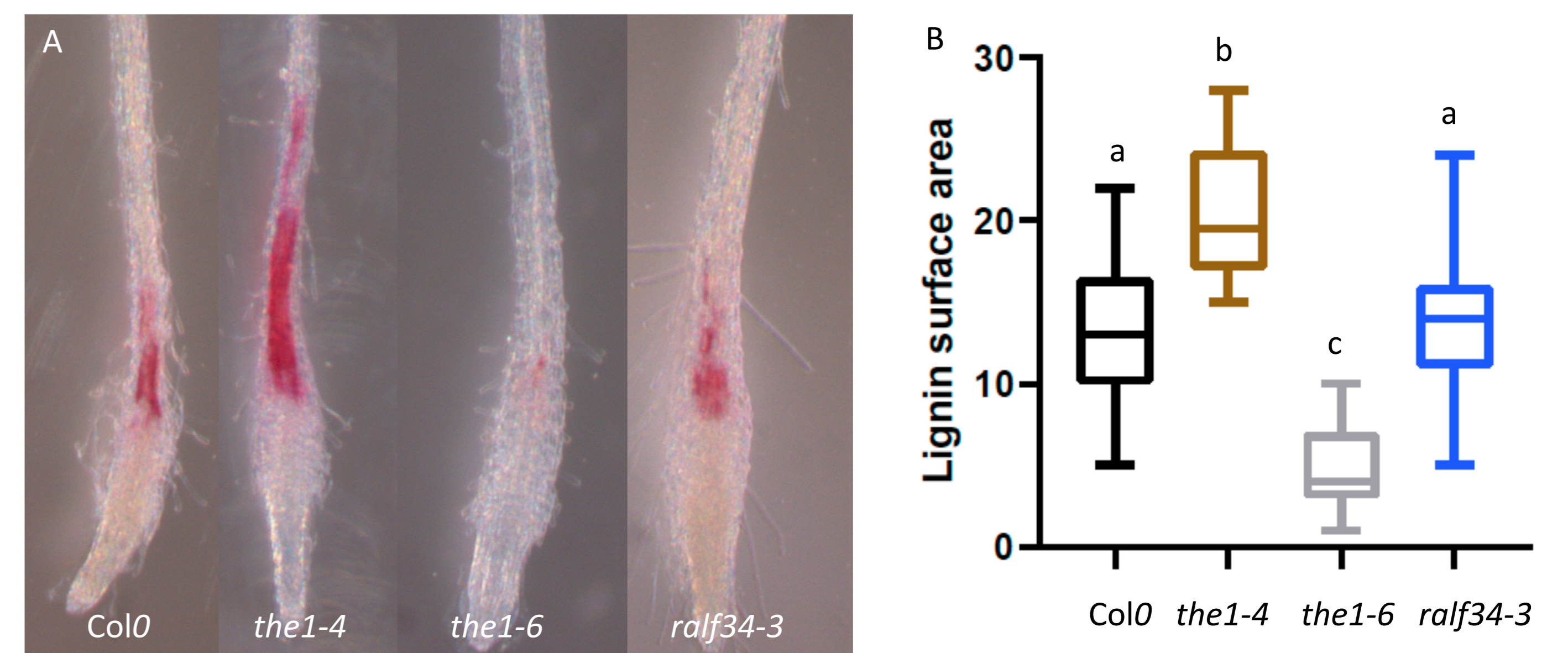


Figure 1: Response of the1 and ralf34 mutants under isoxaben treatment. (A) Representative images of phloroglucinol-stained root tips. **(B)** Quantification of the stained area.

A wild type plant induces an ectopic lignification in the root tip growing area as compensatory response in presence of the cellulose biosynthesis inhibitor Isoxaben. This response is THE1-dependent, since *the1-6* loss-of-function mutant allele presents very low amount of lignin accumulation, and *the1-4* gain-of-function mutant allele shows higher lignin accumulation than wild type (Merz et al., 2017). However, *ralf34-3* loss-of-function mutant allele does not phenocopy this aspect of *the1* mutant.

3 *ralf34* reduces adult plant growth size in combination with *eli1-1* mutation.

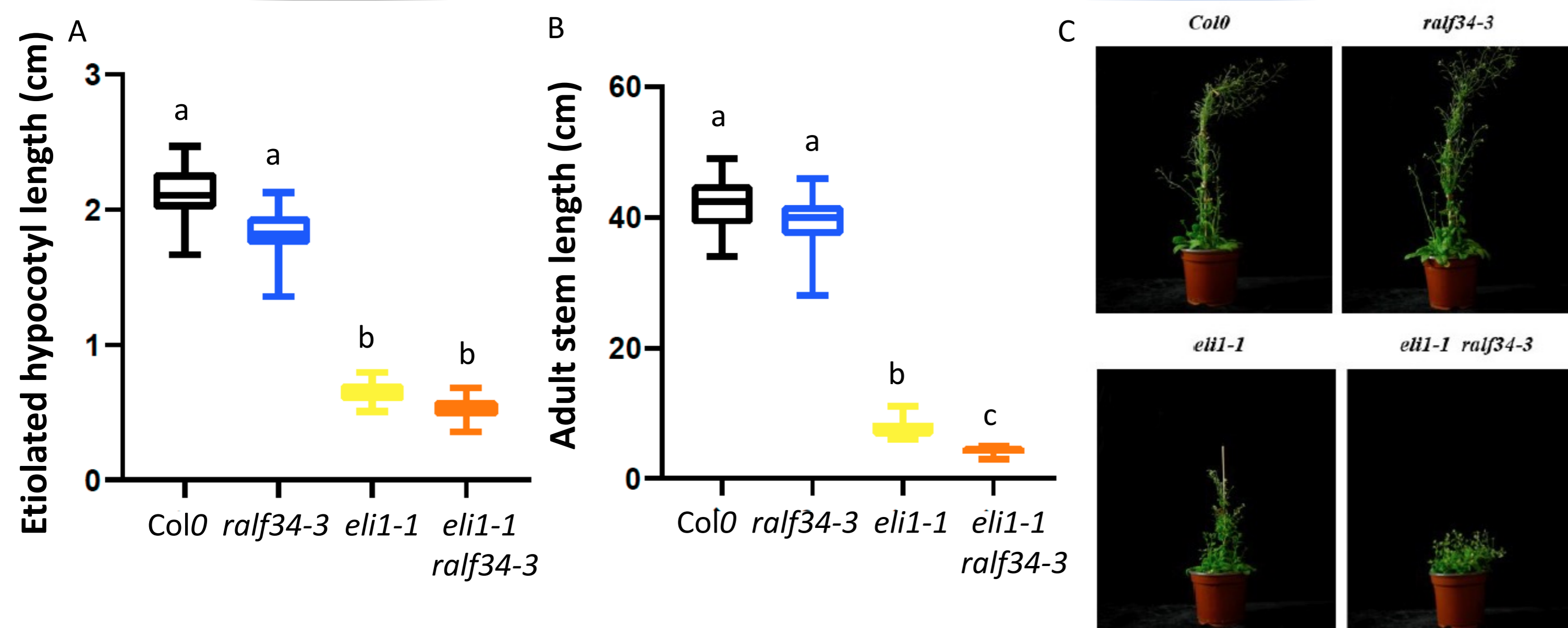


Figure 2: Phenotype of eli1-1 ralf34-3 combination. (A) Quantification of hypocotyl length grown in dark condition. **(B)** Quantification of stem length of adult plants. **(C)** Representative images of adult plants.

It is already reported that a combination of *the1* loss-of-function alleles with cellulose-deficient mutants, such as *eli1-1*, produces an improvement of the growth defects of these mutants (Merz et al., 2017). However, a combination with *ralf34-3* does not phenocopy this aspect. On the contrary, adult plants *eli1-1 ralf34-3* double mutants present shorter stem than single *eli1-1* mutant. This phenotype is similar to *the1-4* gain-of-function combination.

4 Multiple *ralf* mutants present an increased response to cell wall damage.

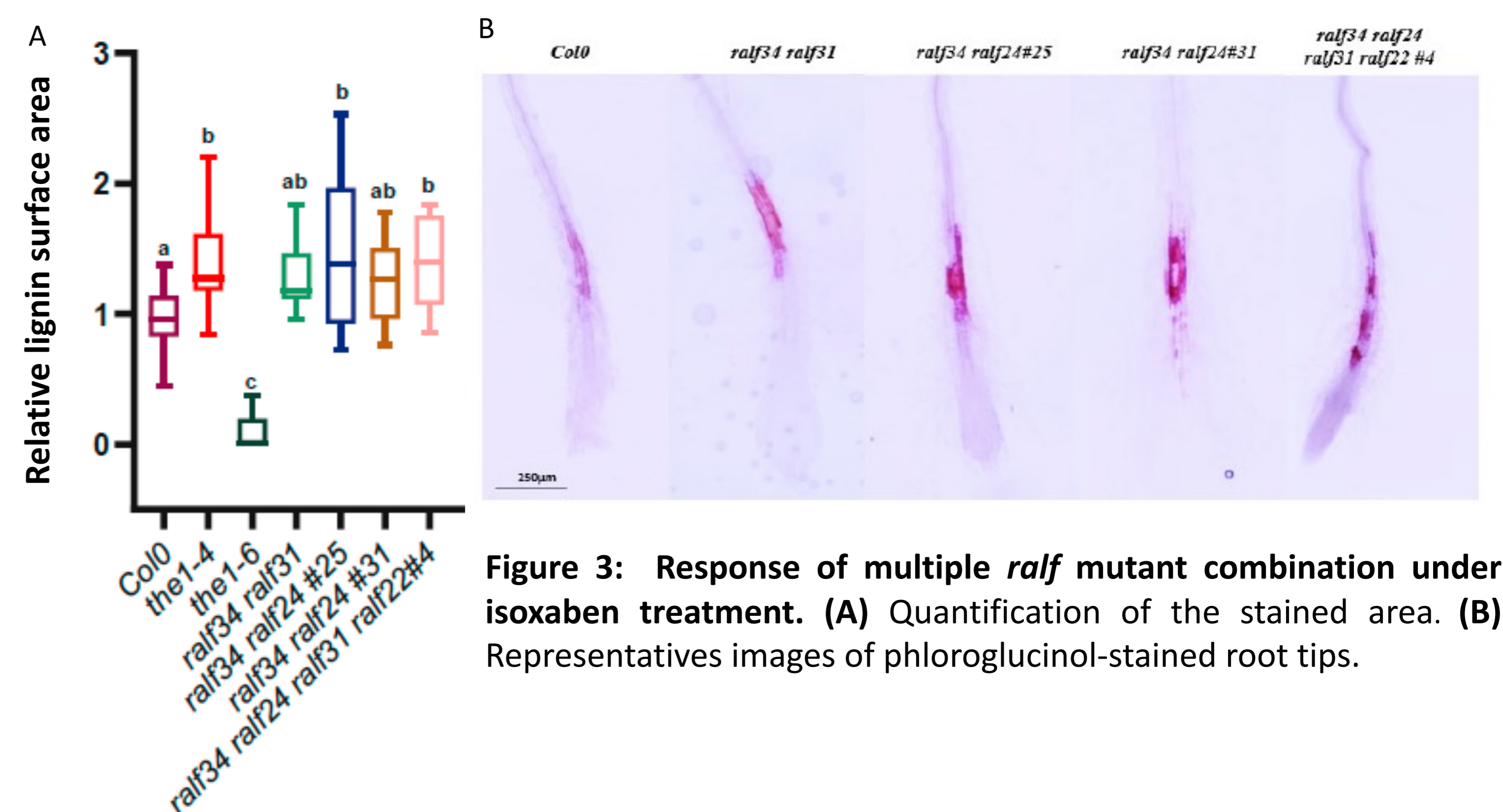


Figure 3: Response of multiple ralf mutant combination under isoxaben treatment. (A) Quantification of the stained area. **(B)** Representative images of phloroglucinol-stained root tips.

For the majority of small peptides described, there are several members of the family working on the same process. Based on expression values in Genevestigator database across different tissues, RALF24 and RALF31 clustered together with RALF34. We generated CRISPR/Cas9 mutant lines with the aim to study if they are involved in THE1 responses to cell wall damage. Combination of double mutant *ralf34 ralf31* and *ralf34 ralf24* slightly increased the lignin accumulation after isoxaben treatment.

5 Exogenous RALF34 peptide addition inhibits lignin deposition.

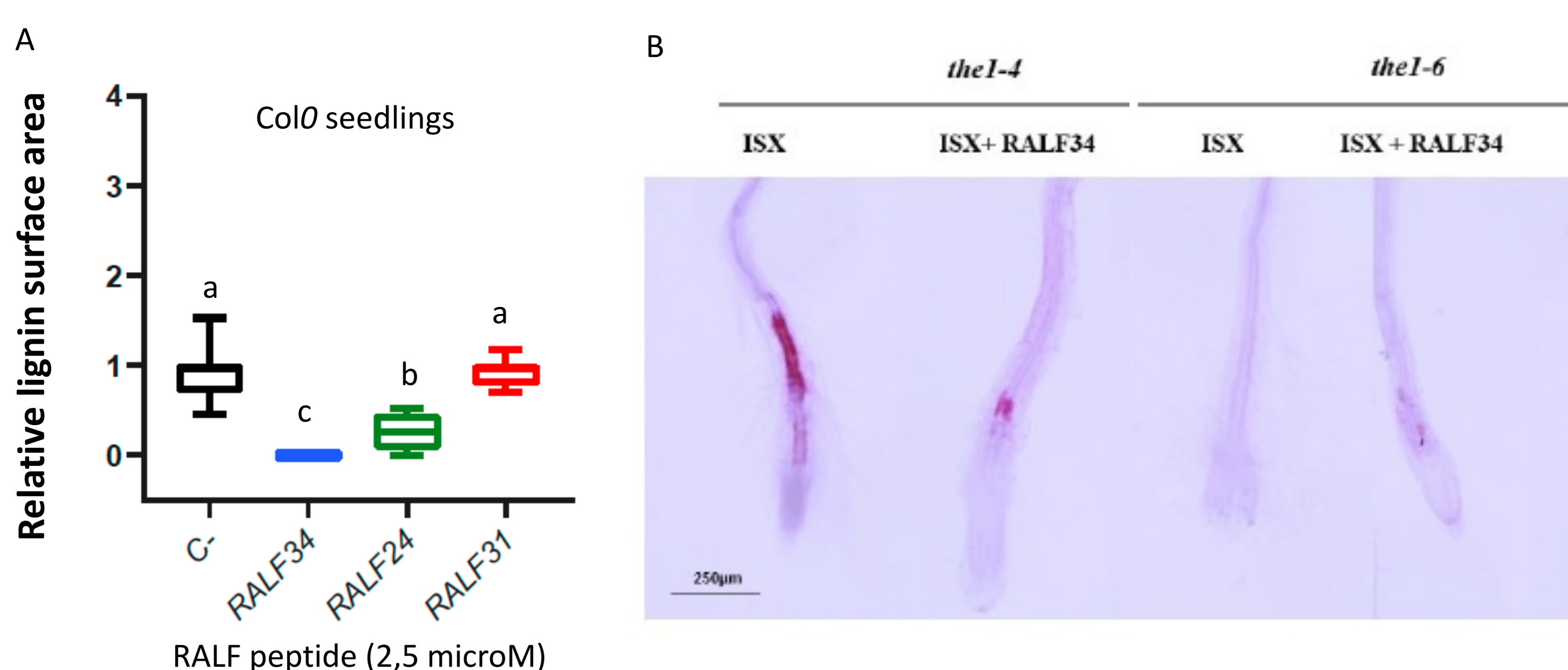


Figure 4: Response to isoxaben and RALF peptides co-treatment. (A) Quantification of the stained area. **(B)** Representative images of phloroglucinol-stained root tips.

Exogenous addition of RALF34 together with isoxaben, abolished completely the lignin accumulation in wild type plants. Interestingly, in the gain-of-function *the1-4* mutant, addition of RALF34 decreased the over-accumulation of lignin. RALF24 also affected lignin accumulation, causing a reduced stained area. However, exogenous RALF31 didn't affect the response of wild type plants.

6 Conclusion.

Our data suggest that RALF34 is not the unique THE1 ligand for CWI response. RALF24 seems to have an implication together with RALF34. However, the most interesting result are coming from exogenous addition of RALF34. When RALF34 is present in high amount, is acting negatively through THE1, inhibiting its response to cell wall damage. Future experiments are needed in order to confirm this hypothesis.

7 References:

Gonneau et al., *Current Biology* (2018), 28(15), 2452-2458; Hématy et al., *Current Biology* (2007), 17(11), 922-931; Merz et al., *Journal of Experimental Botany* (2017), 68(16), 4583-4593.

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