RALF/LRX monitor cell wall integrity during tomato fruit formation

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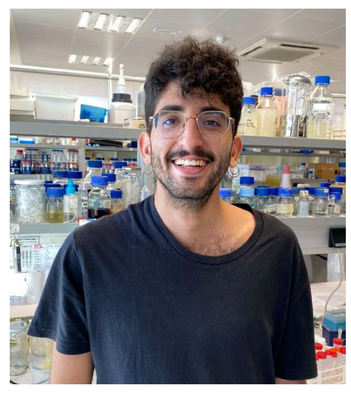


In the past decade, Small Signaling Peptides (SSPs) have been identified as key regulators coordinating an extensive range of developmental and stress processes. Plant cells perceive SSPs at the cell wall by Receptor-Like Kinases (RLKs), activating a huge range of biochemical and physiological processes. SSPs from Rapid Alkalinization Factor (RALF) family are ubiquitous in dicot plants and they have been associated to cell wall integrity during cell wall remodeling. It has already been reported the implication of some members of *Cr*RLK1L regulating fruit ripening in few species like tomato, strawberry or apple, remarking the importance of these receptors and their ligands sensing changes produced in the cell wall during the ripening process (Zhu et al; 2021). Here, we are interested in a deeper biochemical and phenotypical characterization of RALF/LRX proteins during tomato ripening process.

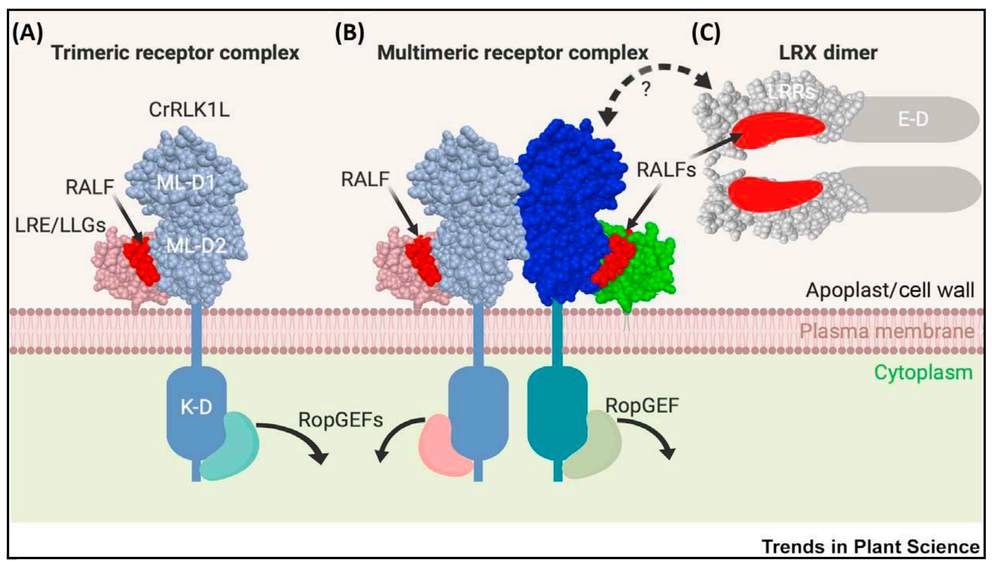


Fig.1 Scheme of RALF perception by CrRLK1L and LRX. Ge et al., 2019.

The RALF family has been classified as cysteine-rich peptides (less than 160 amino acids), containing four or more conserved cysteine residues responsible for the mature peptide conformation and their biological activity. There are some conserved motifs within the RALF family: an arginine-arginine (RR) motif has been identified as a cleavage site, separating the propeptide from the mature active RALF peptide and the **YISY** motif in the mature peptide is required for productive binding of the peptide to its target receptor. RALFs peptides can bind two types of receptors: **Leucine-Rich Repeat Extensin proteins (LRXs)**, and ***Catharanthus roseus* RLK1-Like (*Cr*RLK1L).** All recent discoveries remark the importance of RALF/LRX/CrRLK1L module regulating cell wall status (Zhang X. et al; 2020).

They have been associated to a several physiological and developmental processes. The application of these small molecules to the medium increase the pH and inhibit the elongation of the roots and hypocotyls, but not all members of the family induce the same phenotype.

Looking for candidates

To select the candidates for our study, we made a search in databases in order to find the members of RALF, CrRLK1L and LRX families that are expressed in tomato fruits.

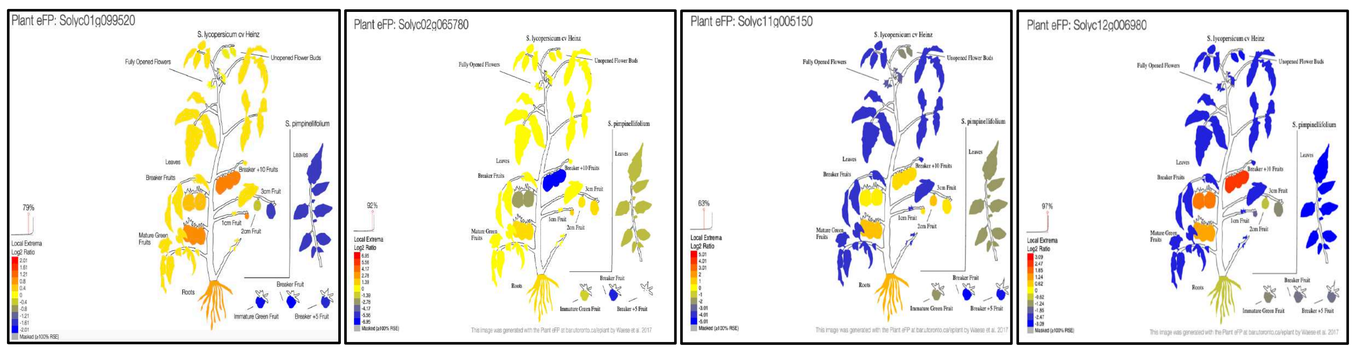


Fig. 2 From left to right, relative expression of **RALF33, RALF27, LRX2 and LRX5** in tomato tissues. Notice that the expression of LRXs is very restricted to fruits and roots. Data obtained from The Bio-Analytic Resource for Plant Biology.

Physiological charaterization

After three days in liquid MS medium with 5µM of the peptides we measured the weight of *Arabidopsis* and the root lenght of tomato seedlings.

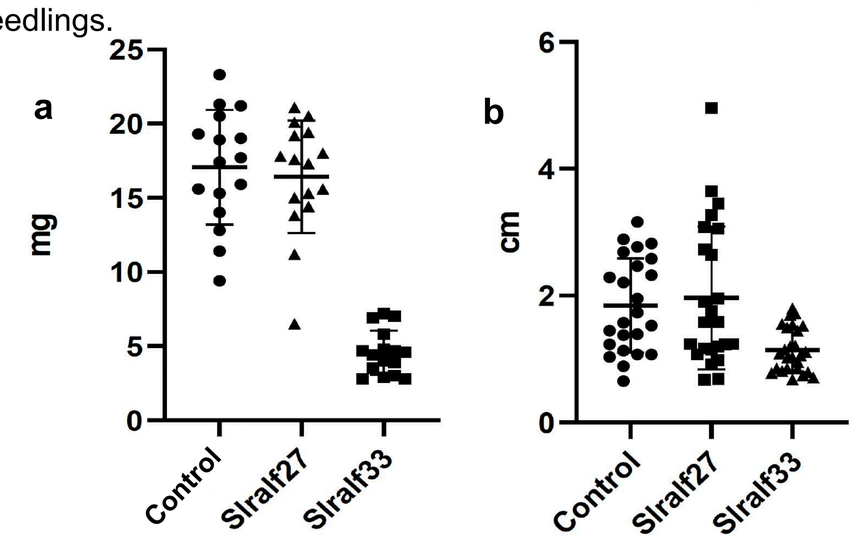
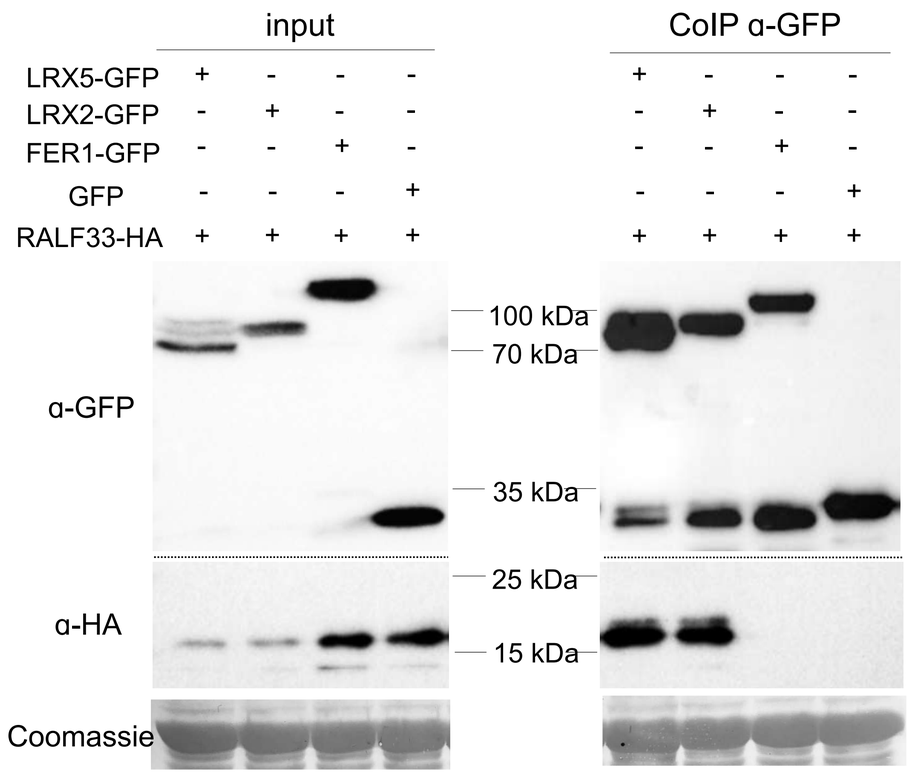


Fig 3. Measurements of weight in Arabidopsis (a) and root lenght in tomato (b) after treatment with SlRALF27 and SlRALF33

Looking for RALF receptors: RALF27 and RALF33 interact with LRX2 and LRX5



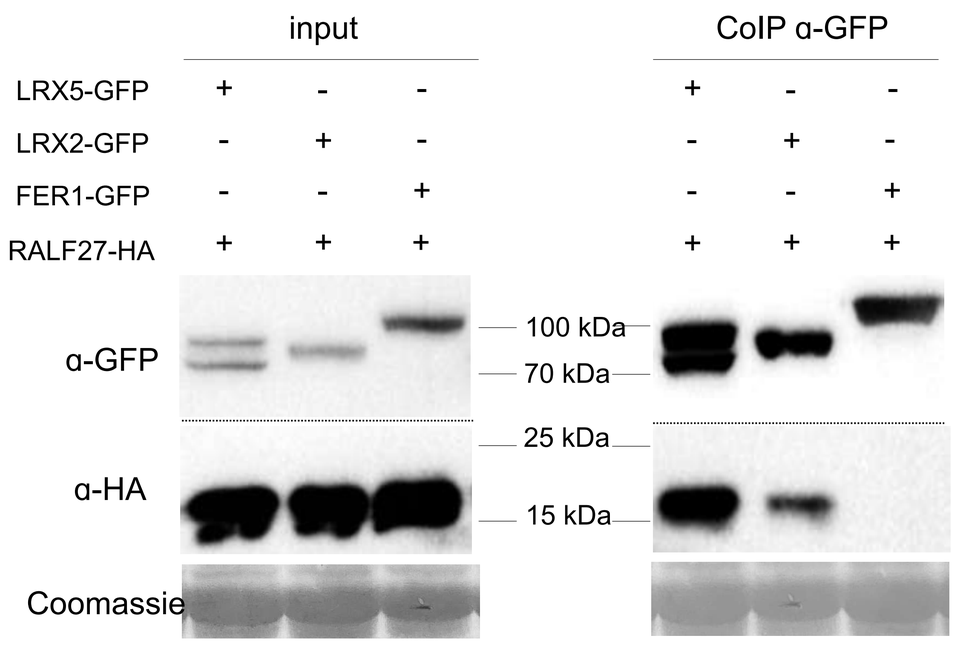


Fig. 4 Western blot analysis of coimmunoprecipitated RALF27-HA and RALF33-HA after immunoprecipitated LRX2-GFP and LRX5-GFP using antibody against GFP.

Future perspectives

According to their expression among the different tomato tissues they are good candidates to control cell wall softening during the ripening process. To confirm these data we will perform an ITC (Isothermal Titration Calorimetry).

To elucidate the function of these pairs (ligands and receptors) in the ripening process we will induce gene silencing by VIGS and generate CRISPR lines with knockout in each one of them so we can observe the phenotype obtained in fruits.

References

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