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Characterisation of proteins involved in plant defense responses by an LC-MS/MS approach for phytohormone quantification

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Plants are sensitive to different bioagressors and have to adapt their defense mechanisms accordingly. We use the model plant *Arabidopsis thaliana* to study the interaction with the downy mildew oomycete pathogen, *Hyaloperonospora arabidopsidis (Hpa*). Defense responses against this pathogen are governed by cellular signaling pathways that are coordinated by the phytohormone salicylic acid (SA). In its unconjugated form, SA is the active defense molecule, but it becomes cytotoxic when it accumulates to high levels. UDP-glucosyltransferases (UGTs) catalyze the transfer of a glucose residue to SA thus forming SA-glucoside (SAG) or SA-glucose ester (SGE), which both are inactive forms destined for vacuolar storage. Arabidopsis mutants for the glucosytranferase UGT76B1 are more resistant to *Hpa* infection, whereas plants that overexpress the enzyme are more susceptible. *Ugt76b1* mutants accelerate and enhance the onset of SA-dependant defenses, when compared to wild-type plants. A comparative transcriptome analysis between the *ugt76b1* mutant and wild-type plants revealed EXTRACELLULAR LIPASE 4 (EXL4) as a potential regulatory protein in UGT76B1-mediated defense responses.

In order to validate and explain the functions of both UGT76B1 and EXL4 in the SA-dependent defense pathway, the Analytical Biochemistry Platform (Sophia Agrobiotech Institute) set up a metabolomics approach to quantify free and storage forms of SA in mutant and wild-type Arabidopsis by LC-MS (microTOFQII, Bruker – PlantBIOs facilities *https://www6.paca.inra.fr/institut-sophia-agrobiotech/Infrastructure-PlantBIOs*). Our results partially validate a hypothetical model and lead us to propose other potential hypotheses about the function of both enzymes.