

co-ordinated with the Director of the Institute / Head of Department

Institute/ Independent Department / Clinical Co-operation Group / Junior Research Group:

Biochemical Plant Pathology – Molecular Plant Physiology

PSP-Element:

G-504900-002

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Title of the Highlight:

The Arabidopsis small-molecule glucosyltransferase UGT76B1 conjugates isoleucic acid and modulates plant defense

Keywords:

Systems biology, Arabidopsis thaliana, ab initio substrate identification, non-targeted metabolomics, plant defense

Central statement of the Highlight in one sentence:

The orphan small-molecule glycosyltransferase UGT76B1 is a novel player in plant pathogen response that conjugates 2-hydroxy-3-methyl-pentanoic and reciprocally affects salicylate- and jasmonate-dependent defense.

Text of the Highlight:

Plants coordinate and tightly regulate pathogen defense by the mostly antagonistic salicylate (SA)- and jasmonate (JA)-mediated signaling pathways. The previously uncharacterized glucosyltransferase UGT76B1 is a novel player in this SA-JA signaling crosstalk. UGT76B1 has been selected as the top-stress induced isoform among the UGT gene family. UGTs (UDP-carbohydrate-dependent glycosyltransferases) comprise a large gene family with 122 members in the model plant Arabidopsis thaliana, which transfer sugar moieties onto small organic molecules. Loss of UGT76B1 function leads to enhanced resistance to the biotrophic pathogen Pseudomonas syringae and accelerated senescence, but increased susceptibility towards necrotrophic Alternaria brassicicola. This is accompanied by constitutively elevated SA levels and SA-related marker gene expression, and reduced levels of JA-dependent markers. Conversely, UGT76B1 overexpression has the opposite effect. Thus, UGT76B1 attenuates SA-dependent plant defense in the absence of infection, promotes the JA response and delays senescence.

Non-targeted metabolomic analysis of UGT76B1 knockout and overexpression lines using ultra-high resolution mass spectrometry and activity assays with the recombinant enzyme led to the ab initio identification of isoleucic acid (2-hydroxy-3-methyl-pentanoic acid) as a substrate of UGT76B1. This is the first elucidation of the substrate of an enzyme based on non-targeted metabolomics without any prior knowledge on its chemical nature.

Exogenously applied isoleucic acid increased resistance against *Pseudomonas* infection. These findings indicate a novel link between amino acid-related molecules and plant defense that is mediated by small-molecule glucosylation. In addition, isoleucic acid offers a potential biotechnological application as a plant protective agent.

Publication:

von Saint Paul, V., Zhang, W., Kanawati, B., Geist, B., Faus-Keßler, T., Schmitt-Kopplin, P., **Schäffner, A.R.** (2011) The Arabidopsis glucosyltransferase UGT76B1 conjugates isoleucic acid and modulates plant defense and senescence. *Plant Cell* (in press)

Taking account of the HMGU mission:

The discovery offers novel insights into plant defense against pathogens. The identification of isoleucic acid represents an analytical breakthrough in fundamental research and offers a potential application for plant protection.

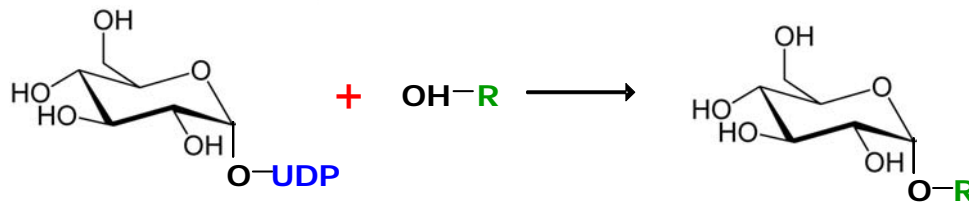
The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:

Philippe Schmitt-Kopplin	G-505190-001
Theresa Faus-Keßler	G-500500-001

The small-molecule glucosyltransferase UGT76B1 conjugates isoleucic acid and modulates plant defense

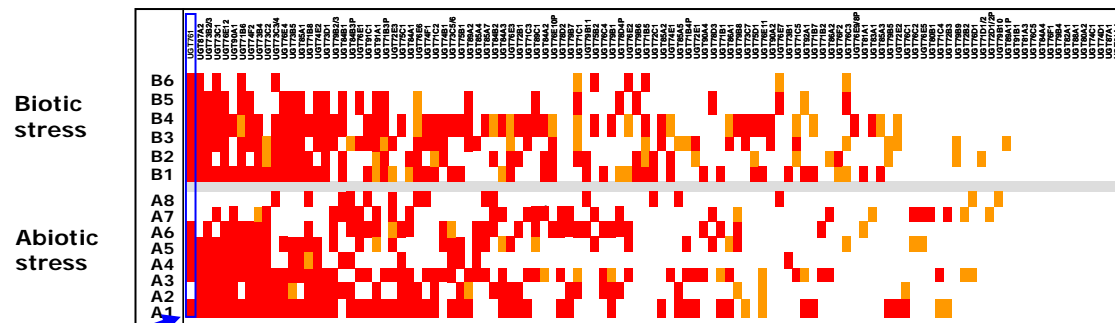
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The 'orphan' glycosyltransferase UGT76B1 is highly stress-responsive



■ UGTs glycosylate small organic aglycon molecules R-OH.
Glycosylation modulates the biological activity of aglycons.

- Stress-responsive induction of >100 *UGT* genes in *Arabidopsis thaliana*.

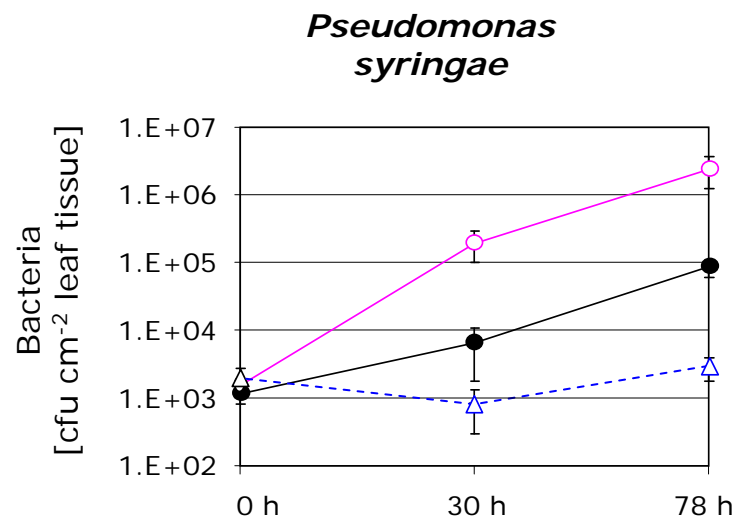


■ **UGT76B1** is the top **stress-induced** *UGT* gene, but its aglycon and relation to metabolic or signaling pathways were unknown.

UGT76B1 expression affects pathogen defense

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- *ugt76b1* knockouts: resistant to biotrophic pathogen (*Pseudomonas*), susceptible to necrotrophic pathogen (*Alternaria*).
- Constitutive *UGT76B1* overexpressors show the opposite behaviour.

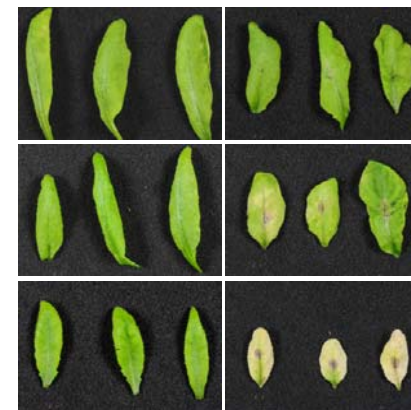


UGT76B1
overexpressor

Wild type

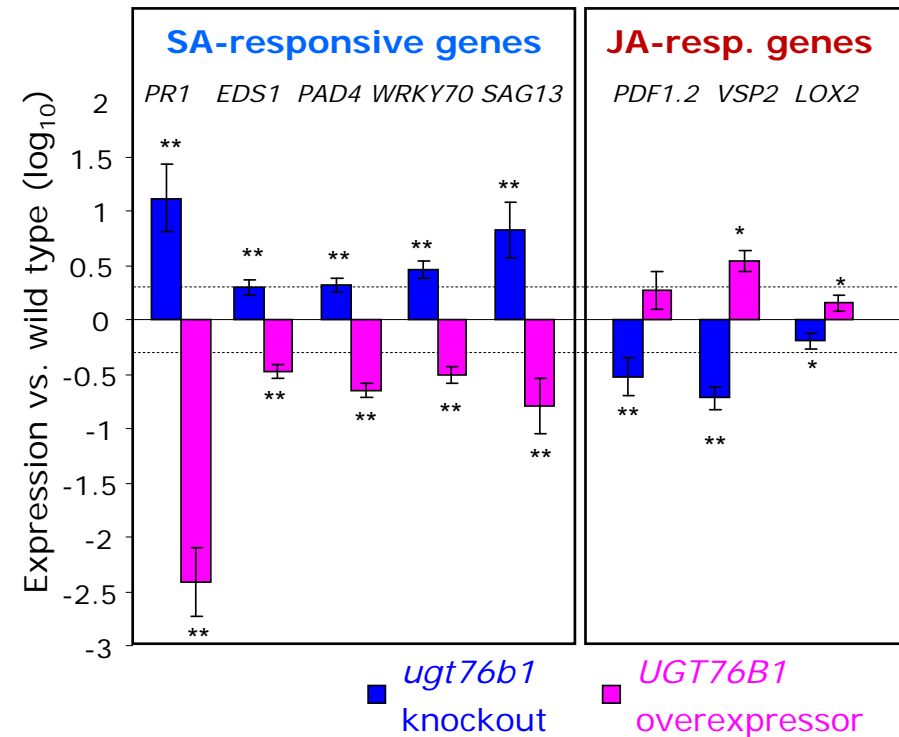
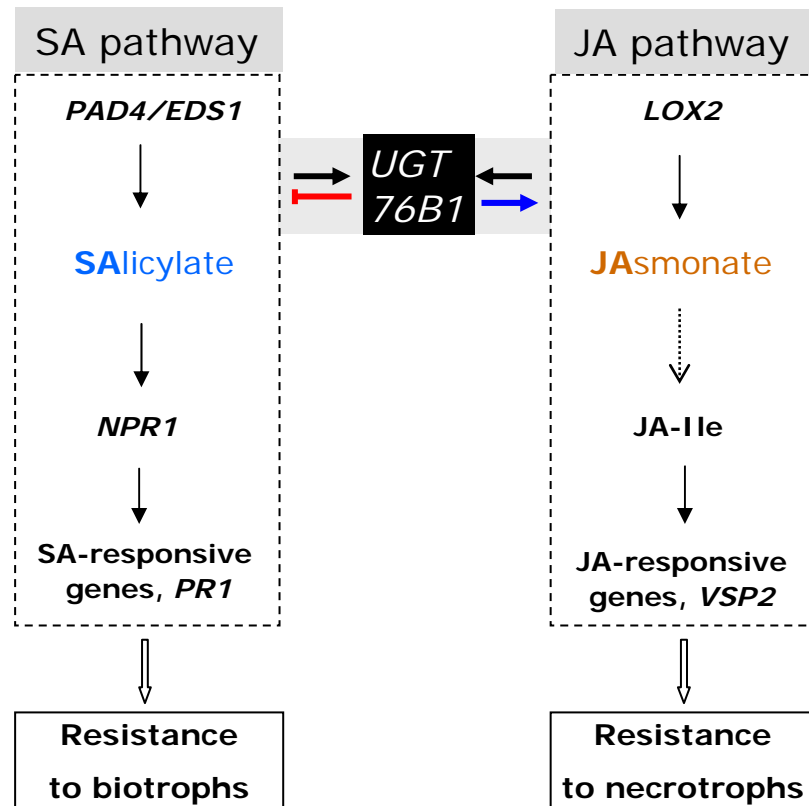
ugt76b1
knockout

mock *Alternaria brassicicola*



UGT76B1 affects antagonistic plant defense pathways

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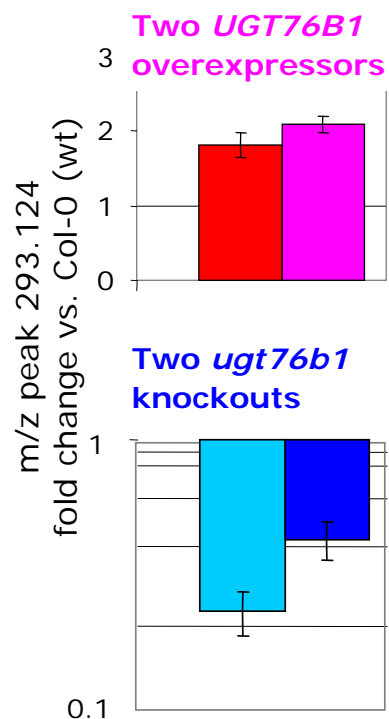


■ UGT76B1 suppresses SA and activates JA defense pathways.

Ab initio identification of the UGT76B1 substrate: isoleucic acid

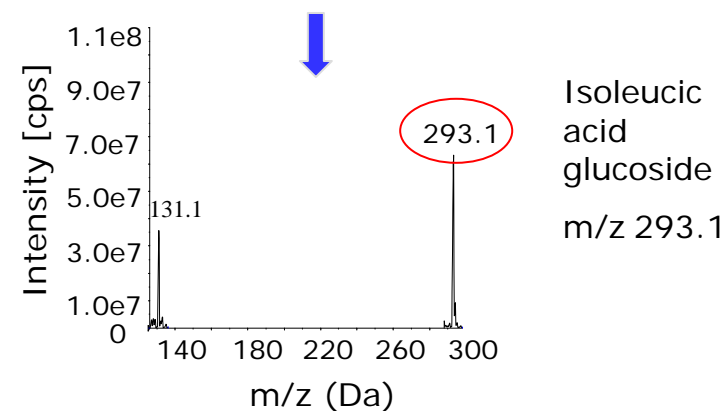
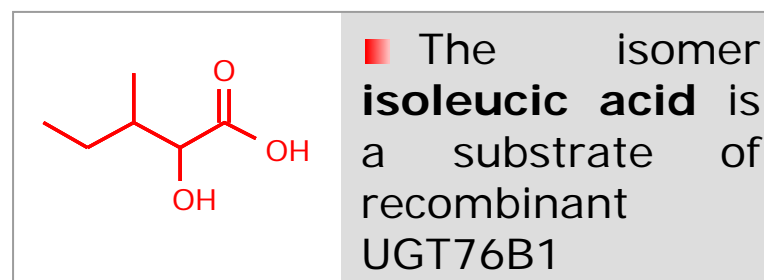
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- Non-targeted metabolomics using ultra-high resolution FT-ICR mass spectrometry led to the identification of the UGT product *isoleucic acid glucoside*



■ **m/z [M-H] 293.124**

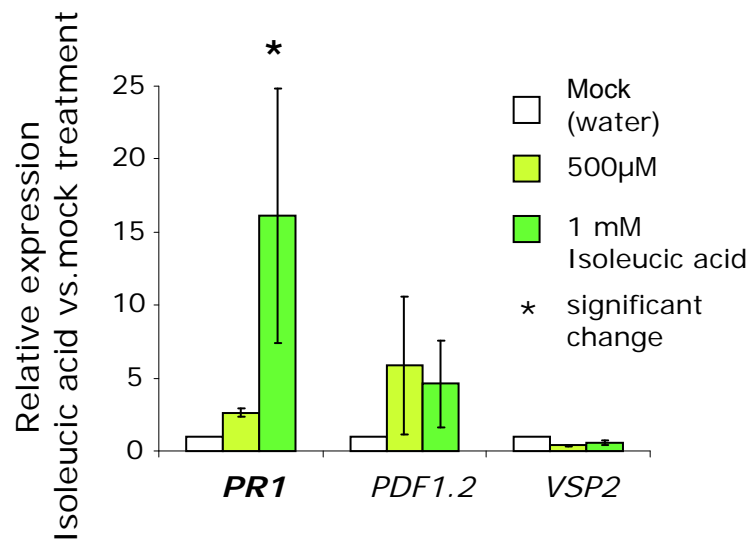
- is correlated with UGT76B1 expression
- is a glucoside
- of an α -hydroxy hexanoic acid (six possible isomers)



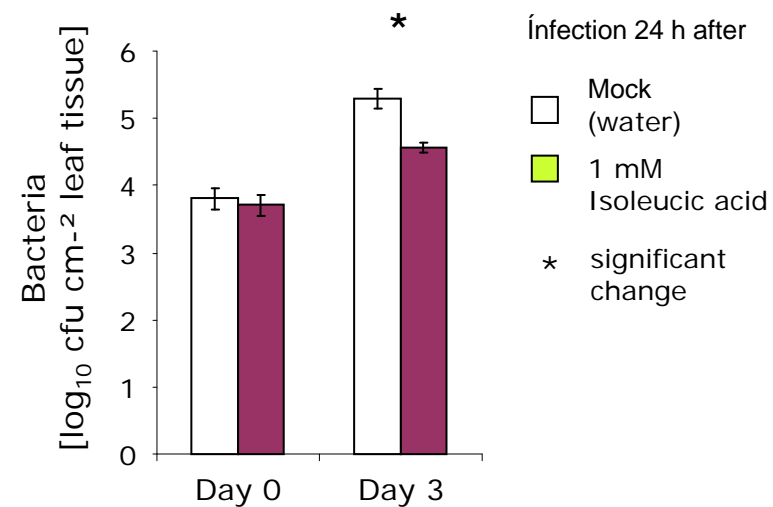
Isoleucic acid promotes plant defense

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- Isoleucic acid induces SA marker gene *PR1*



- Pretreatment with isoleucic acid induces resistance to *Pseudomonas*



- Isoleucic acid is explored as a novel plant protective agent and signaling molecule