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Unraveling the biosynthetic pathway of triterpenoid saponins in *Barbarea vulgaris*

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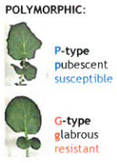
INTRODUCTION

The *Barbarea vulgaris* - *Phyllotreta nemorum* model system

The wild crucifer *Barbarea vulgaris* is polymorphic in respect to resistance towards the herbivorous pest *Phyllotreta nemorum*. Thereby, the G-**type** is resistant to *Phyllotreta* larvae and adults. In contrary, both can feed on the P-**type** without showing any decrease in their viability.

Barbarea vulgaris (winter cress)



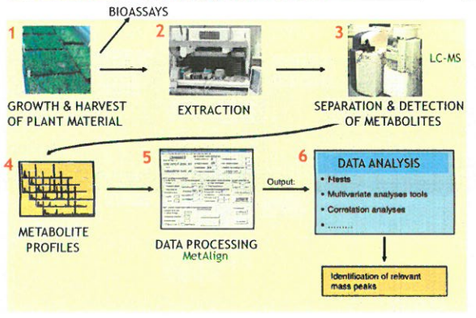
To identify metabolites responsible for the resistance, an untargeted metabolomic investigation was performed. The resistance of F_2-population plants, gained from a cross between G- and P-**type**, was correlated with their metabolite profile. Four triterpenoid saponins were found to show the most significant correlation with resistance.

Subsequently, to unravel the biosynthetic pathway of saponins in *Barbarea vulgaris*, a strategy based on 454 pyrosequencing is pursued. Accordingly, transcriptomic data was obtained from the resistant G-**type** and used for comparative genomics towards *Arabidopsis thaliana* as well as mapping of quantitative trait loci (QTL).

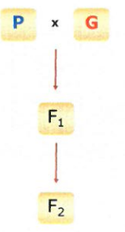
Phyllotreta nemorum (flea beetle)

METABOLOMICS

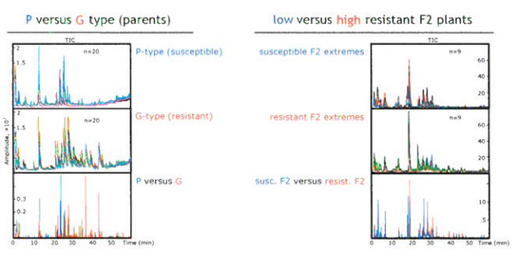
Experimental setup & data flow



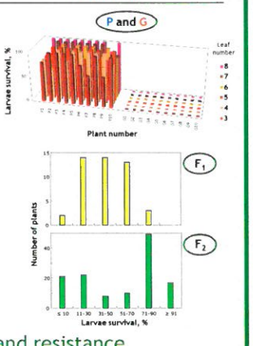
Producing hybrids between P & G



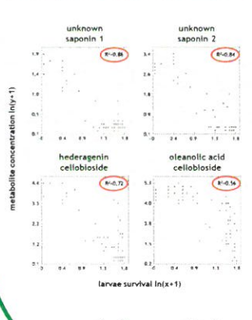
Untargeted metabolite profiling by LC-MS



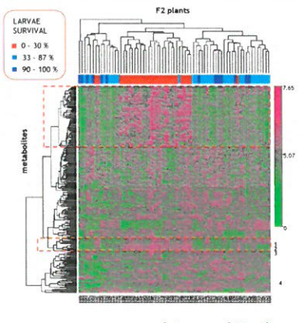
Bioassays



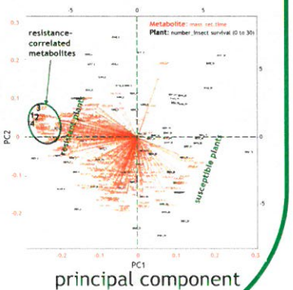
Correlation and co-variation between metabolite composition, metabolite profile and resistance



correlation analysis



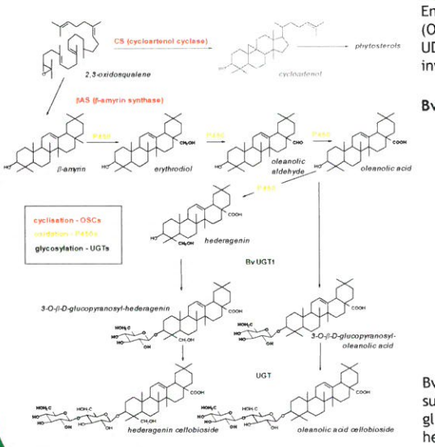
two-way hierarchical clustering



principal component analysis

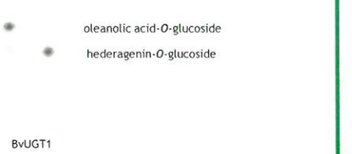
BIOCHEMISTRY

Proposed biosynthetic pathway of saponins in *Barbarea vulgaris*



Saponins are derived from the phytosterol pathway. Enzymes belonging to the classes of oxidosqualenecyclases (OSCs), cytochrome P450 monooxygenases (P450s) and UDP-glycosyltransferases (UGTs) are expected to be involved in their biosynthesis.

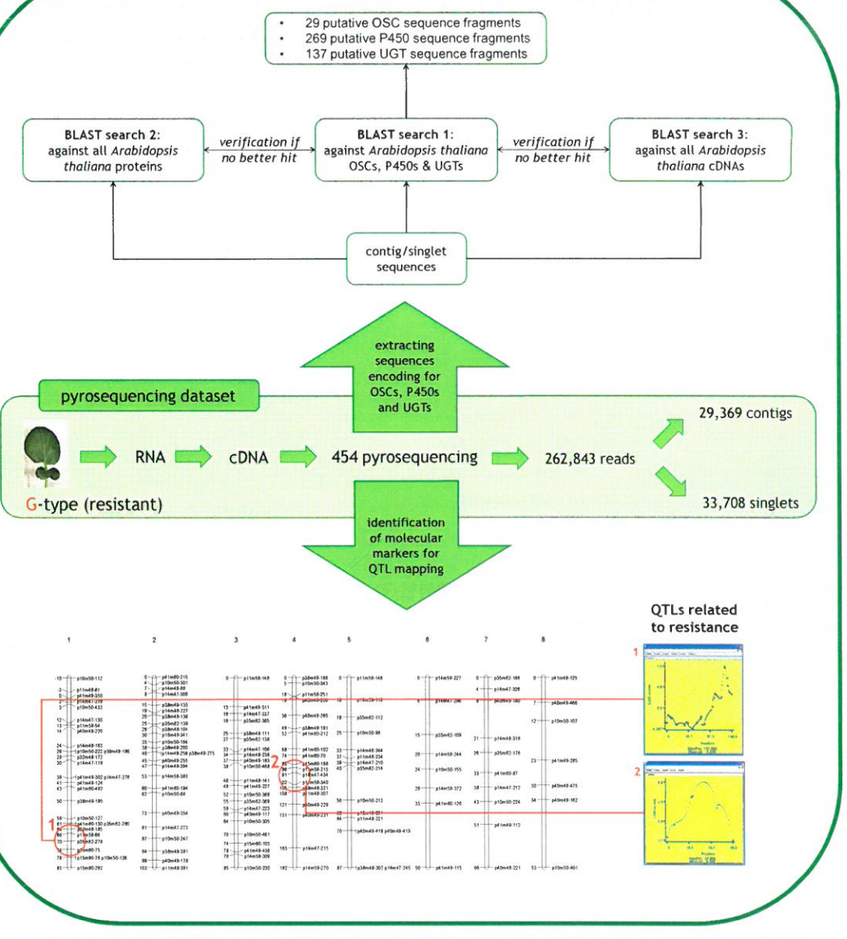
BvUGT1 catalyses the first glycosylation step



TLC-scan of an BvUGT1 activity assays using [^{14}C]-UDP-Glucose and oleanolic acid or hederagnin as substrates.

BvUGT1, isolated from a japanese *Barbarea vulgaris* subspecies, was shown to catalyze the first 3-*O*-glucosylation of the saponin aglycons oleanolic acid and hederagenin. Putative orthologs of this gene could be found in both the G- and P-**type**.

GENOMICS



CONCLUSIONS:

- an untargeted metabolomics approach identified four saponins as most correlating with resistance to herbivory in *Barbarea vulgaris*
- pyrosequencing provided sequence information about homlogs of genes of interest as well as SSRs for QTL mapping

PERSPECTIVES:

- Search for genes involved in saponin biosynthesis by:
  - screening for homologs of enzymes known to catalyze similar steps of related pathways in the pyrosequencing dataset
  - setup and screen microsome preparations for relevant activities & identification of enzymes in active fractions by MALDI-TOF-MS
  - studies of metabolite profile co-segregation with QTLs
  - exploitation of the synteny to *Arabidopsis thaliana* for QTL saturation mapping
- Use knowledge of the saponin pathway for bioengineering or molecular breeding of crop plants with increased anti-insecticidal properties