Max-Planck-Institut für chemische Ökologie

Development of an HPLC-TOF-MS screening-platform to assess ppHypSys-dependent metabolic changes in *Nicotiana attenuata* during insect herbivory

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Introduction

A reliable HPLC-TOF-MS-based metabolomics platform to screen for chemical changes in leaf extracts of *Nicotiana attenuata* after simulated insect attack W+OS (W = mechanical wounding, OS = application of *Manduca sexta*'s oral secretions) is presented.

The platform was used to investigate the role of the hydroxyproline-rich glycopeptide systemin precursor (ppHypSys) as a signaling molecule during insect herbivory. After either reducing endogenous ppHypSys transcript levels by RNAi (IRsys) or over-expressing the ppHypSys precursor under the control of a 35S-promotor (OVsys), we compared metabolomic changes of wild-type (WT), IRsys and OVsys in response to W+OS.

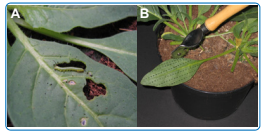

Fig. 1 (A) *Nicotiana attenuata* leaf attacked by *Manduca sexta*. (B) Insect herbivory was simulated by wounding leaves with a fabric pattern wheel and applying insect's oral secretions.

Methods

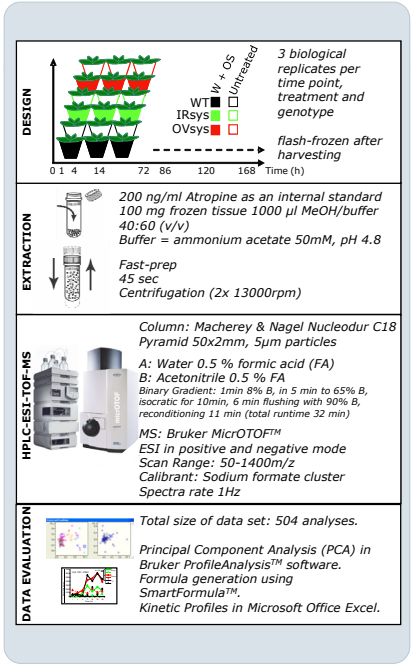

Fig. 2 Experimental design, sample preparation and HPLC-ESI-TOF-MS settings, data evaluation

Results

To filter out the changes influenced by ppHypSys the principal component analysis (PCA) was applied and the impact of specific ions on the grouping of samples was investigated on both a time- and genotype-scale. Prior to PCA, peak finding was performed using the FindAllCompounds peak finder delivering isotopic as well chromatographic information.

Fig. 3 presents an example of the general workflow: All datasets are first screened using PCA. Influential loadings explaining the trends in the scores plot are investigated more closely. Here, *m/z* 251 at 60s, corresponding to caffeoylputrescine, is one of the highest loading on PC1 (A). Kinetic plots for this ion showed that plants silenced for ppHypSys expression failed to accumulate this compound (B) during herbivory.

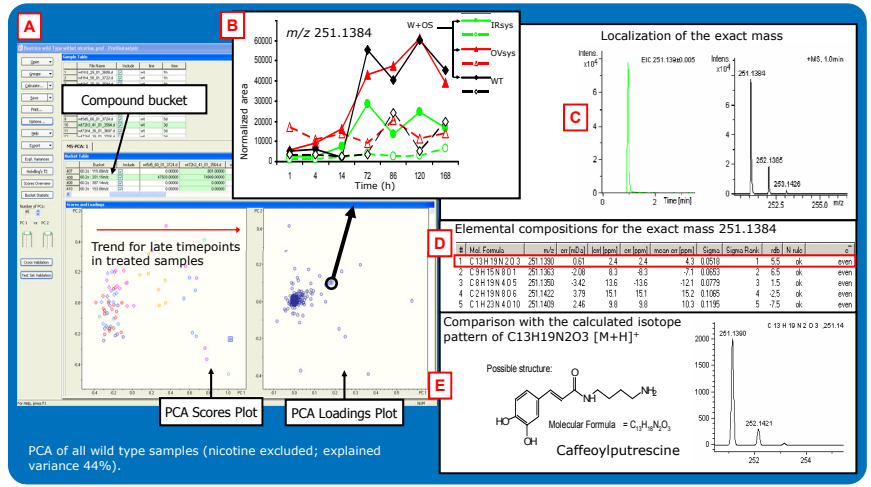

Fig. 3 General work-flow for the filtering and identification of ions of interest using ProfileAnalysis : Example of caffeoylputrescine

The peak of interest was then localized in the original data (C) and the elemental composition was calculated using both the accurate mass and isotopic pattern information (D+E).

Summary

Using an untargeted ESI-TOF-MS combined with PCA, we verified that large reconfigurations of *N. attenuata* metabolome occur during insect attack. By comparing profiles obtained from WT and genetically modified plants, we discovered the regulatory role played by ppHypSys on the biosynthesis of caffeoylputrescine, an alkaloid accumulating during insect feeding[1].

[1]Lou and Baldwin (2004) Nitrogen Supply Influences Herbivore-Induced Direct and Indirect Defenses and Transcriptional Responses in *Nicotiana attenuata*. Plant Physiology

Conclusions

- The proposed method combining an ESI-TOF-MS based metabolic screening with statistical analysis, kinetic profiles and subsequent identification was successfully applied to a large plant metabolomics data set investigating the influence of ppHypSys during insect herbivory.
- The nitrogen containing molecules are currently independently explored using WT plants grown in N^{15} media. In a next step experiments using accurate and high resolution MS/MS data and classical NMR analysis will be carried out.

ESI-TOF