# Using non-targeted high resolution LC-QTOF profiling to characterize metabolic responses of *Nicotiana attenuata* during infection with *Rhizophagus irregularis*

Exploring the chemical composition of plants is challenging. It is highly dynamic and can be modified by many environmental factors. Knowledge of this modulation is necessary to eventually decode the mechanisms underlying these changes.

## Introduction

Understanding metabolomes as mediators of diverse biological functions is thus decisive for an understanding of a plant's ecological interactions. The mutualistic interaction between plants and root colonizing arbuscular mycorrhizal fungi (AMF) has been shown to result in extensive reprogramming of plant metabolism. However, efficient workflows are required to rapidly analyze metabolite profiles and gain sufficient structural insights to discriminate known compounds from novel/closely related ones. With a software-based metabolite identification method we assessed the metabolic responses of *Nicotiana attenuata* during an infection with the arbuscular mycorrhizal fungus *Rhizophagus irregularis*.

*Keywords: MetaboScape 3.0, LC-QTOF, metabolomics, discovery metabolomics, de-replication, unknown identification*

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## Methods

*N. attenuata* plants were grown in dual-communities, in 2L pots filled with either living or dead inoculum of *R. irregularis*. Tissue of *N. attenuata* was harvested 6 weeks after inoculation and extracted with 80 % methanol. Chromatographic separation of extracts was carried out using a Dionex UltiMate 3000 Rapid Separation LC System (Thermo Fisher) combined with an Acclaim RSLC 120 C18 2.2µ 120 Å (2.1 × 150 mm) column. MS analysis was performed using a high-resolution Q-TOF MS (impact II, Bruker Daltonics) system in electrospray positive ion mode. MetaboScape 3.0 was used for multi pass, region complete feature extraction and alignment, dereplication of known compounds, the identification of novel metabolites and pathway mapping. Custom pathway maps were created using PathVisio 3.2.0.[1,2]

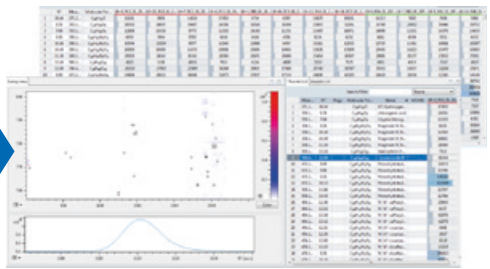

## Workflow

### T-ReX 3D:

**T**ime aligned **Re**gion complete e**X**traction algorithm

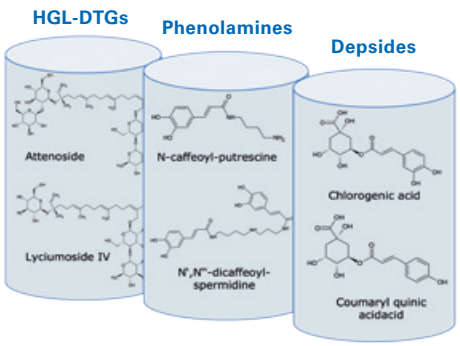
Novel T-ReX 3D algorithm: Ions belonging to the same compound are combined, aligned across all samples and automatically re-extracted in individual samples if the initial features were just below the peak picking threshold.

### Bucketing and Quality Control



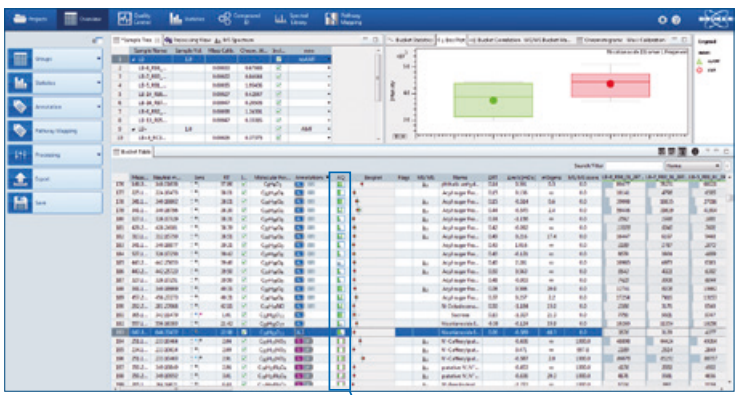
Bucket Table generation by T-ReX 3D addresses the "missing value" problem providing high confidence in the statistical results. Interactive visualizations of chromatograms and extracted features provide optional validation within a dedicated Quality Control perspective.

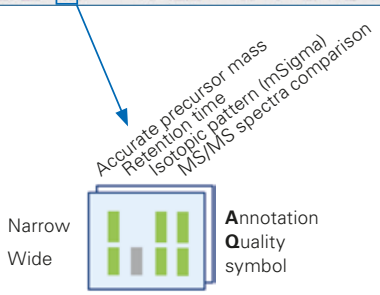
### In-house database



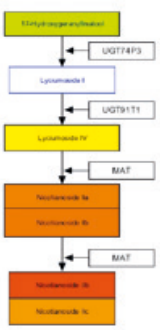

Database (in csv file format) consisting of 553 secondary metabolites identified in *N. attenuata* used as basis to create a target AnalyteList.

### Automatic Compound ID & AQ scoring

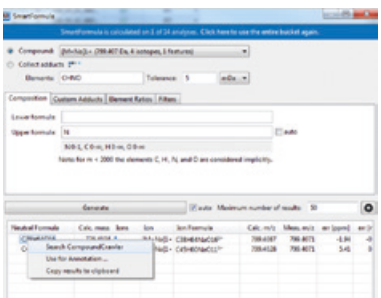





### Pathway Mapping

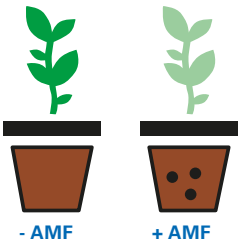


### Molecular formulae

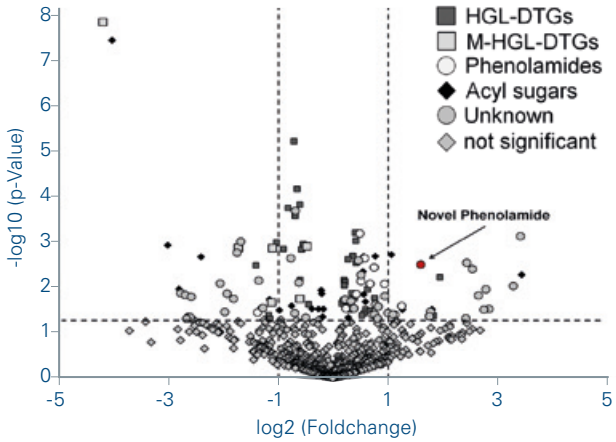


**Automatic annotation** was performed by matching accurate mass, RT, isotopic pattern and MS/MS spectra (if available) contained in the custom AnalyteList against all features extracted and aligned by the T-ReX 3D algorithm. The quality of the annotation was validated using the **AQ score**. For unknowns **automatic molecular formula calculation** was performed by SmartFormula 3D. Subsequently, the formulae were searched with CompoundCrawler for matching structures in public databases.

# Results



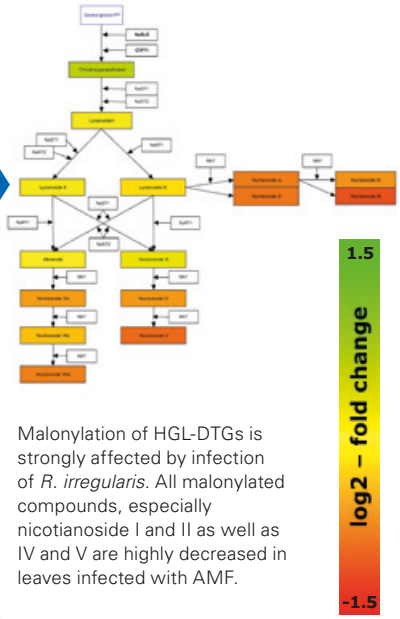

Comparison of *N. attenuata* plants infected with living vs. dead inoculum *R. irregularis*.




A Volcano Plot was calculated using the bucket table generated with MetaboScape 3.0, following T-ReX 3D feature extraction. Shown is the induction respectively the reduction of phenolamides, HGL-DTGs and acyl sugars after infection with living vs. dead *R. irregularis*.

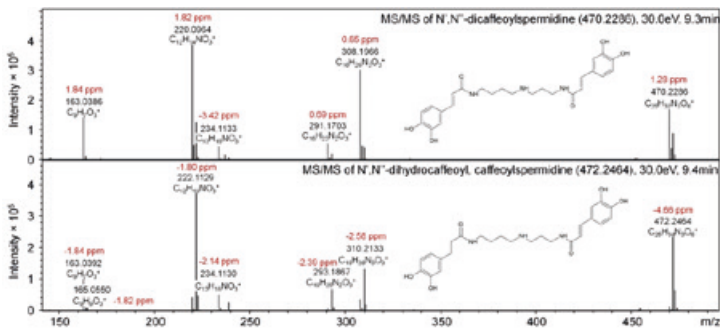
## Pathway analysis

Shown is the fold change in the expression of several diterpene glycosides between wild tobacco leaves infected with AMF and without. Green indicates a major increase of those metabolites and red indicates a strong decrease.



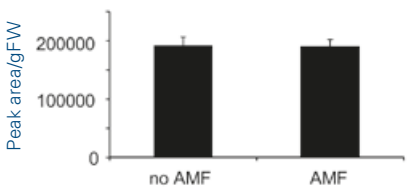

Malonylation of HGL-DTGs is strongly affected by infection of *R. irregularis*. All malonylated compounds, especially nicotianoside I and II as well as IV and V are highly decreased in leaves infected with AMF.

## Compound ID – novel phenolamide

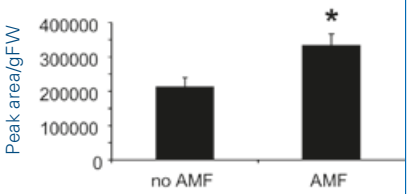



Analyzing the differentially regulated features we annotated a novel compound with high spectral similarity to N',N''-dicaffeoylspermidine: N',N''-dihydrocaffeoyl, caffeoylspermidine, which differs only in the hydration of a double bond in a caffeoyl moiety.

**N',N''-dicaffeoylspermidine**




**N',N''-dihydrocaffeoyl, caffeoylspermidine**




The putative N',N''-dihydrocaffeoyl, caffeoylspermidines was differentially regulated after infection with *R. irregularis* whereas the relative abundance of N',N''-dicaffeoylspermidine did not change.

## Conclusions

- MetaboScape provides a fully integrated workflow for discovery metabolomics.
- Novel T-ReX 3D provides region complete data extraction.
- This workflow enabled us to detect and link diverse effects in the phytometabolome of *N. attenuata* during *R. irregularis* infection.
- AMF infection strongly influences malonylation of HGL-DTGs.
- A novel phenolamide was identified by comparing MS/MS spectral similarities.

# Learn More

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**www.bruker.com/metabolomics**


**References**

[1] Kutmon M., et al.; PLoS Computational Biology **2015**, 11(2): e1004085
[2] van Iersel M.P., et al.; BMC Bioinformatics **2008**, (9): 399


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