Metabolomics for Biological Research- Plant Analytical Platform for Phytohormones and Low Abundant Molecules

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Introduction

Plants produce a massive array of primary and secondary metabolites and plant metabolomics has gained great importance e.g. biosynthetic pathway elucidation, understanding of stress physiology and evaluation of genetically modified crops. Among the metabolites, phytohormones are essential metabolic markers during plant's growth and stress. National Institute of Plant Genome Research (NIPGR) with DBT funding is setting up a platform for metabolomics with advanced and high throughput equipments which will be operational in October 2019. We will be able to quantify targeted metabolites like phytohormones and other low abundant molecules, along with untargeted metabolomics for both primary and secondary metabolites. In this facility, we have TQ-UHPLC-MS/MS, TQ-GC-MS/MS, ICP-MS and HPTLC for analytical purposes along with 15 other equipments. We aim to analyze defense phytohormones (jasmonic acid, abscisic acid, and salicylic acid), and growth phytohormones (cytokinins, auxins and gibberellins), amino acids and low-abundant secondary metabolites through UHPLC coupled to TQ-MS/MS using the multiple reaction monitoring mode (MRM) with internal standards. Primary metabolites (sugars, organic acids) and volatiles (terpenoids and benzenoids) will be analyzed by targeted and untargeted profiling through GC coupled to TQ-MS/MS using NIST metabolite libraries. Specialized secondary metabolites, e.g. anthocyanins and flavonoids will be analyzed through HPTLC and plant ionome profiling by ICP-MS. Thus, this facility will be crucial to accelerate the output of Indian plant science community.

Major Equipments

- **Combined linear ion trap and triple quad MS (SCIEX QTRAP 6500+)**
- **Ultra Performance Liquid Chromatography (1260 Infinity II UPLC)**
- **Gas Chromatography-Mass Spectrometry (GCMS-TQ8050)**
- **Inductively coupled plasma mass spectrometry (Agilent 7800 ICP-MS)**
- **High Performance Thin Layer Chromatography (Camag HPTLC)**

Major Analysis

SCIEX QTRAP 6500+

LC-MS/MS-based analysis and quantification of phytohormones (JA, JA-Ile, SA, ABA, IAA, *trans*-Zeatin)

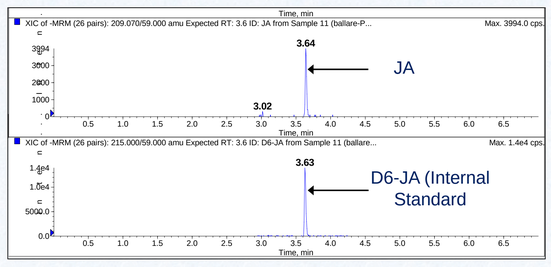
- **Multiple reaction monitoring (MRM) based quantification.**
- **Use of separate labeled internal standard for each phytohormone for quantification.**

LC-MS/MS-based analysis and quantification of amino acids:

- **Multiple reaction monitoring (MRM) based quantification.**
- **Use of separate labeled internal standard for each phytohormone for quantification.**

LC-MS-based untargeted metabolomics

- **Untargeted metabolite profiling through scanning mode of sample extract.**
- **Quantitative profiling through single internal standard.**



Scheduled MRM XIC chromatogram of JA and D6-JA

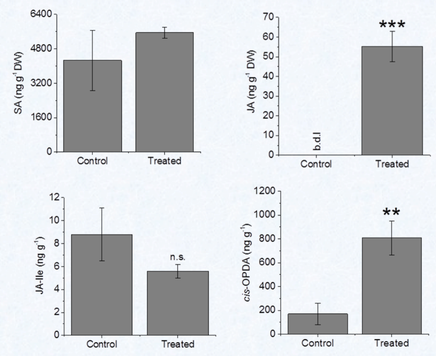
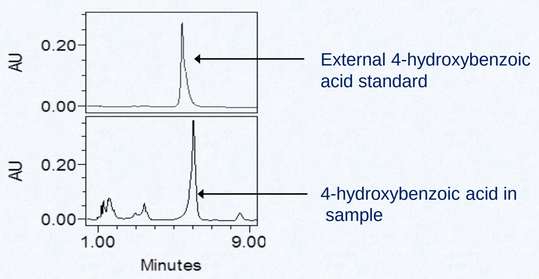


Fig. Phytohormone (SA, Salicylic acid; JA, Jasmonic acid; JA-Ile, (+)-7-isojasmonoyl-L-isoleucine, cis-OPDA, 12-oxophytodienoic acid) quantity in control and *P. indica* treated S. lycopersicum leaf. Each value is mean ± SE of five biological replicates.

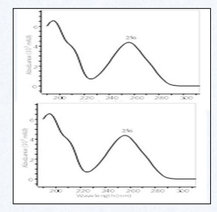
1260 Infinity II UPLC

Reverse phase UPLC-PDA based quantitative profiling of small metabolites

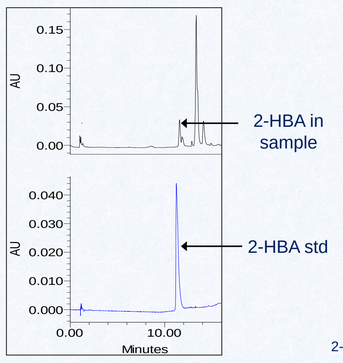
- **Qualitative profiling of metabolites through external standard.**
- **Targeted quantification of metabolite with both internal and external standards.**

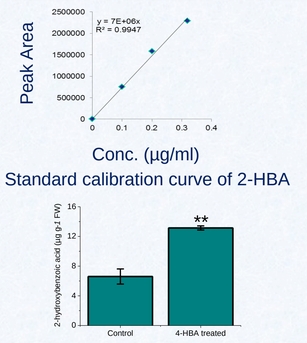


HPLC-chromatogram of 4-hydroxybenzoic acid (4-HBA)



4-hydroxybenzoic acid PDA spectra in standard and sample





Standard calibration curve of 2-HBA

2-HBA quantity in control and 4-HBA treated Arabidopsis. Each value is mean ± SE of five biological replicates.

GCMS-TQ805

GC-MS-based untargeted metabolomics

- **Untargeted metabolite profiling through scanning mode of derivatized sample extract.**
- **Quantitative profiling through single internal standard.**

GC-MS-based analysis and targeted quantification of primary metabolites:

- **Internal standard based relative quantitation.**
- **Use of separate labeled internal standard for each phytohormone for quantification.**

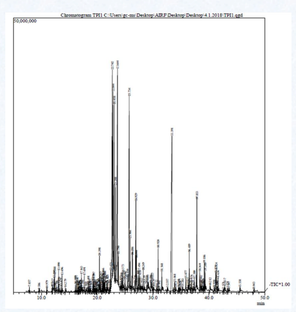


Fig. Representative Total Ion Chromatogram of derivatized tomato leaf extract.

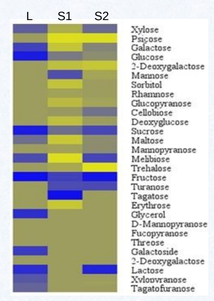


Fig. Representative heat map of comparative untargeted sugar metabolome changes of three different tomato leaves upon herbivory.

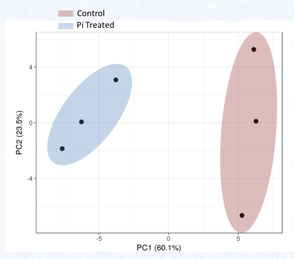


Fig. Principal component analysis of control and *P. indica* treated tomato plants created with the untargeted GC-MS metabolome datasets shows differentiation in clustering.

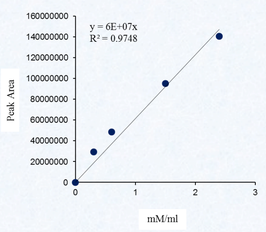


Fig. Standard calibration curve of Sucrose.

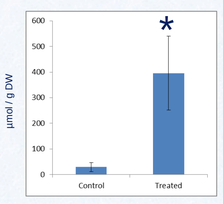


Fig. Targeted quantitation of sucrose in tomato systemic leaf in control and herbivore infested plants.

ICP-MS-based analysis and quantification of inorganics and metal ions (e.g.Na, Mg, Al, K, Ca etc.):

- **Profiling of inorganic metal ions from plants and soil sample.**
- **Quantitation of targeted metal in plant sample in specific condition.**

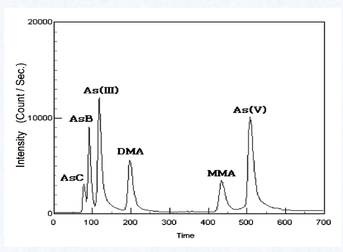


Fig. HPLC-ICP-MS chromatogram for a standard mixture of 6 arsenic species. [As(III): Arsenite, As(V): Arsenate, MMA: Monomethylarsinic acid, DMA: Dimethylarsonic acid, AsC: Arsenocholine, AsB: Arsenobetaine] (Ryu et al., 2009)

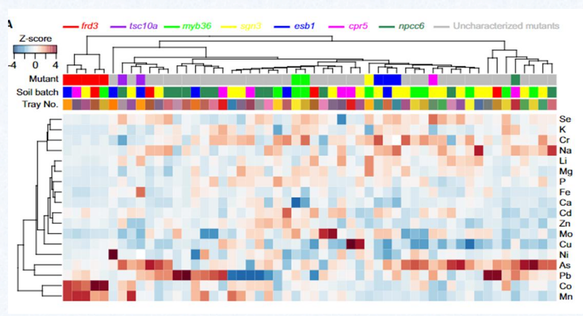


Fig. Hierarchical clustering of Arabidopsis FN mutants. The causal genes for cloned mutants are listed on the top, with the same color highlighting the mutants in the first row. Different soil batches or plant cultivation trays in which the mutants were grown are shown in different colors in the second and third rows, respectively. The remaining rows represent the elements quantified in each mutant. The wild-type Col-0 and thefrd3 mutant were used as controls and grown in all plant cultivation trays. (Hang and Salt, 2016).

Camag **HPTLC**

HPTLC based profiling of plant's secondary metabolites

- **Profiling of phenolic secondary metabolites, e.g. anthocyanin and flavonoids.**
- **Quantitation of targeted secondary metabolites.**

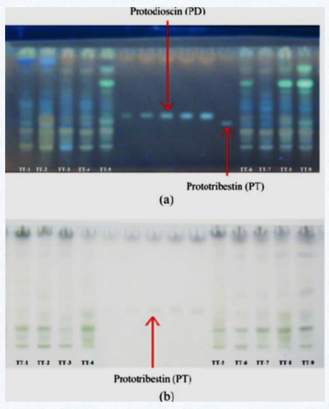


Fig. (a) HPTLC profile of Tribulus terrestris accessions with Protodioscin (PD) and Prototribestin (PT) standard (after derivatization with anisaldehyde-sulfuric acid reagent and visualized under k 366 nm) and (b) HPTLC profile of Tribulus terrestris accessions with Prototribestin (PT) standard (after derivatization with anisaldehyde-sulphuric acid reagent and visualized under normal light).

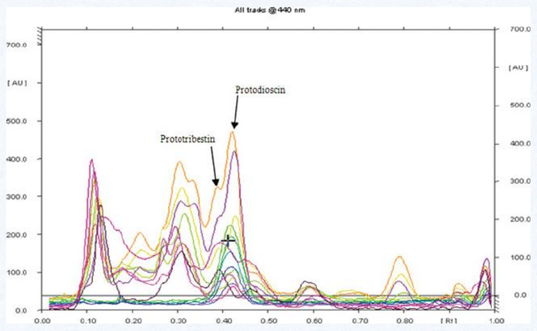


Fig. HPTLC chromatogram of different accessions of Tribulus terrestris fruit samples along with reference standards Prototribestin (PT) and Protodioscin (PD).

Rawat *et* al. 2013

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