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Simultaneous untargeted and targeted profiling of underivatized primary metabolites on sulfur-deficient barley

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► **To cite this version:**

Adrián Schwarzenberg, Hikmat Ghosson, Frank Jamois, Jean-Claude Yvin. Simultaneous untargeted and targeted profiling of underivatized primary metabolites on sulfur-deficient barley. European Mass Spectrometry Conference (EMSC) 2018, Mar 2018, Saarbrücken, Germany. , EMSC 2018 Program - Abstracts Book, P062, pp.274, 2018, Tuesday Posters. hal-02713411

HAL Id: hal-02713411

<https://hal.science/hal-02713411>

Submitted on 1 Jun 2020

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Overview

Metabolomics based-mass spectrometry are increasingly applied in diverse scientific domains, notably agronomy and plant biology, to understand plants' behaviors under biotic/abiotic stress conditions. In fact, these stress conditions are able to disrupt many biosynthetic pathways that include mainly primary metabolites. Profiling and quantifying primary metabolites remain a challenging task because they are poorly retained in reverse phase columns due to their high polarity. Two fast and accurate methods were developed to detect and quantify amino acids, organic acids, sulfur metabolites, and secondary metabolites without derivatization using an UPLC coupled to a QTOF mass spectrometer. These methods were successfully applied to sulfur deficient and sulfur controlled barley enabling the identification of several primary metabolites.

Material and Methods



Figure 1. Sulfur controlled (left) and sulfur deficient (right) Barley used in this study

Plant material

Barley plants (*Hordeum vulgare*) were growth under sulfur controlled and sulfur deficient conditions (Fig. 1). Samples (shoots and roots) were harvested and frozen in liquid nitrogen. Samples were grinded under liquid nitrogen using a CryoMill (Retsch, Haan, Germany).

Chemical material

HPLC quality standards, amino acids, organic acids and sulfur metabolites (Figure 3) were purchased from Sigma-Aldrich (Saint Quentin, France).

UPLC Conditions		
	Method A	Method B
Column	Luna Omega PS C18 100x2,1mm, 1,6 μm	HSS T3 100x2,1mm, 1,7μm
T° (°C) Column	35°	25°
Phase A	H ₂ O + 0,5 % FA	H ₂ O + 0,5 % FA
Phase B	MeOH/H ₂ O (70:30) + 0,5 % FA	ACN/MeOH (50:50) + 0,1 % FA
QTOF Conditions		
ESI (kV)	Negative (2,5 kV)	Positive (0,5 kV)
Resolution	20000	20000



Figure 2. UPLC acuity H-Class-Xevo G2-S QTOF MS.

Data analysis

Untargeted data analysis, peak alignment, peak picking, normalization and multivariate data analysis were performed by Progenesis Q1 software (nonlinear dynamics) and Ezinfo 3.0 software (Umetrics AB, Sweden). Targeted analysis was achieved using TargetLynx software (Waters).

Results and discussion

The chromatographic separation was performed in less than 8 min for method A (ESI -) and 4 min for method B (ESI +) as shown in Fig 3. The limits of quantification (LOQ) ranged from 1,5 to 500 ng/mL (method A) and 0,5 to 7,5 ng/mL (method B). Intra-day repeatability and inter-day reproducibility of quantification showed a RSD ≤ 10%.

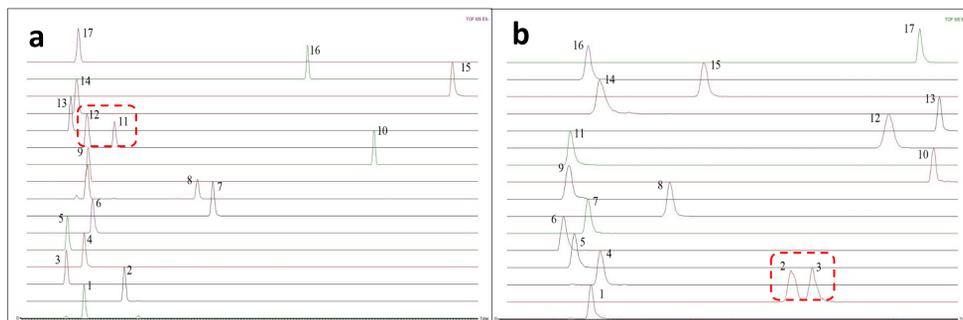


Figure 3. (a) Extracted ion chromatogram of organic acids from method A : (1) Fumaric acid, (2) Succinic acid, (3) Aspartic acid, (4) Malic acid, (5) Glutamic acid (6) Phospho(enol)pyruvic acid, (7) Gallic acid, (8) Cis-Aconitic acid, (9) Shikimic acid, (10) Azelaic acid, (11) Citric acid, (12) Isocitric acid, (13) Gluconic acid, (14) D-Glucose 6-Phosphate/Fructose-6-Phosphate, (15) Kaempferol, (16) Chlorogenic acid, (17) Trehalose 6-Phosphate. (b) Extracted ion chromatogram of amino acids and sulfur metabolites from method B: (1) Pro, (2) Ile, (3) Leu, (4) Asn, (5) Gln, (6) Lys, (7) O-Acetyl-Serine, (8) Met, (9) His, (10) Phe, (11) Arg, (12) Tyr, (13) Trp, (14) Thiamine, (15) GSH, (16) SAM, (17) GSSG

Extraction and multivariate data analysis

20 mg of barley were used for extraction of polar metabolites using two steps, first extraction 1 mL 70% H₂O/29% MeOH/1% Perchloric acid (PA) and the second extraction 0,5 mL H₂O + 0,1% PA. PA was used to avoid the degradation of sulfur containing metabolites¹. Unsupervised principal component analysis (PCA) showed a discrimination between sulfur controlled (+S) and sulfur deficient (-S) barley (Fig. 4).

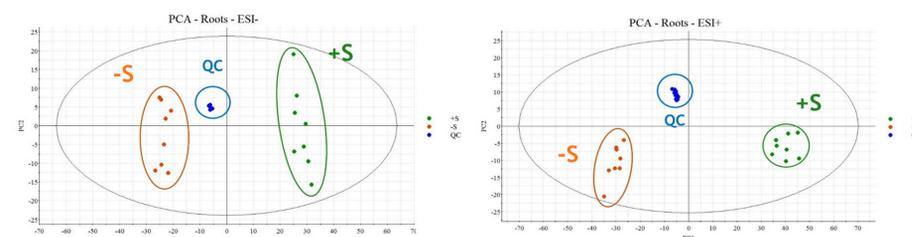


Figure 4. Principal component analysis of roots analyzed with method A (left) and method B (right).

Supervised Orthogonal Projection to Latent Structures Discriminant Analysis (OPLS-DA) was employed to find significant features showing several primary metabolites (Fig. 5) confirmed by the simultaneous targeted analysis (retention time, exact mass and MS/MS) as shown in figure 6.

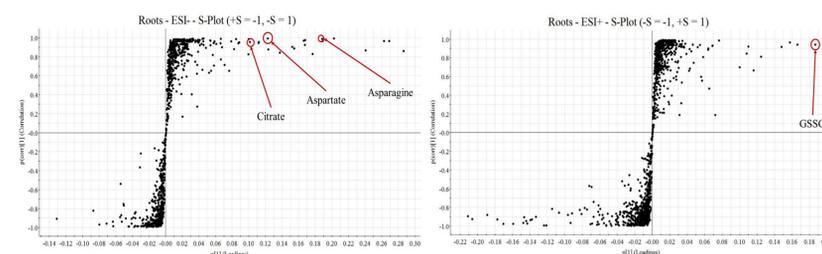


Figure 5. S-Plot from OPLS-DA analysis of roots using the method A and B.

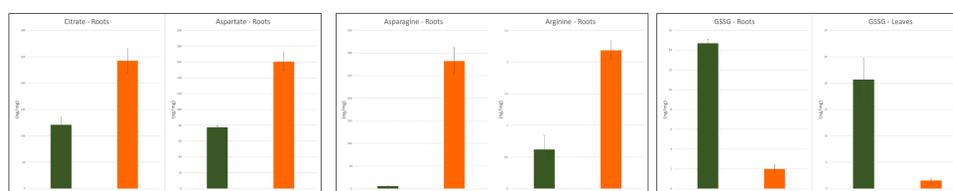


Figure 6. Quantification of significant metabolites in negative ionization mode (citrate and aspartate) and positive ionization mode (arginine, asparagine and GSSG).

Conclusion

The developed methods allow to quantify 33 underivatized polar metabolites including amino acids, organic acids, and sulfur metabolites in complex samples. The simultaneous untargeted and targeted analysis at high resolution enabled the discrimination between two plant batches under sulfur controlled/deficient conditions. Additionally, significant primary metabolites were validated by the targeted analysis that will give us a better understanding of sulfur deficiency in barley plants.

References

- Lafaye, A.; Labarre, J.; Tabet, J.-C.; Ezan, E.; Junot, C., Liquid Chromatography–Mass Spectrometry and 15N Metabolic Labeling for Quantitative Metabolic Profiling. *Analytical Chemistry* **2005**, *77* (7), 2026-2033.