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應用手冊

Metabolomics of Broccoli Sprouts Using UPLC with Ion Mobility Enabled LC-MS^E and TransOmics Informatics

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Abstract

The Waters Omics Research Platform with TransOmics Informatics, featuring UPLC and HDMS^E technologies, enables researchers to improve how they screen and differentiate molecular phenotypes of plants exposed to different environmental stimuli. This highthroughput approach has applications in agricultural, food, and nutritional, as well as natural product research.

Benefits

Use of the Waters Omics Research Platform, which combines UPLC with ion mobility LC-MS^E and TransOmics Informatics, allows for the rapid characterization of molecular phenotypes of plants that are exposed to different environmental stimuli.

Introduction

Cruciferous vegetables, such as broccoli, cabbage, kale, and brussels sprouts, are known to be anti-carcinogenic and possess antioxidant effects. They are widely consumed in the world, and represent a rich source of bioactive metabolites.[1] Young broccoli plants are an especially good source of chemoprotective metabolites, with levels several times greater than mature plants. Growth conditions and environmental stresses exert a significant influence on the metabolism of broccoli sprouts.[2]

The aim of this work is to study how the complete set of small-molecule metabolites, the "metabolome," of broccoli sprouts is modulated under different growth conditions. As the metabolome reflects both genetic and environmental components (e.g., light conditions and nutrients), comprehensive metabolite profiles can describe a biological system in sufficient depth to closely reflect the ultimate phenotypes, as shown in Figure 1.

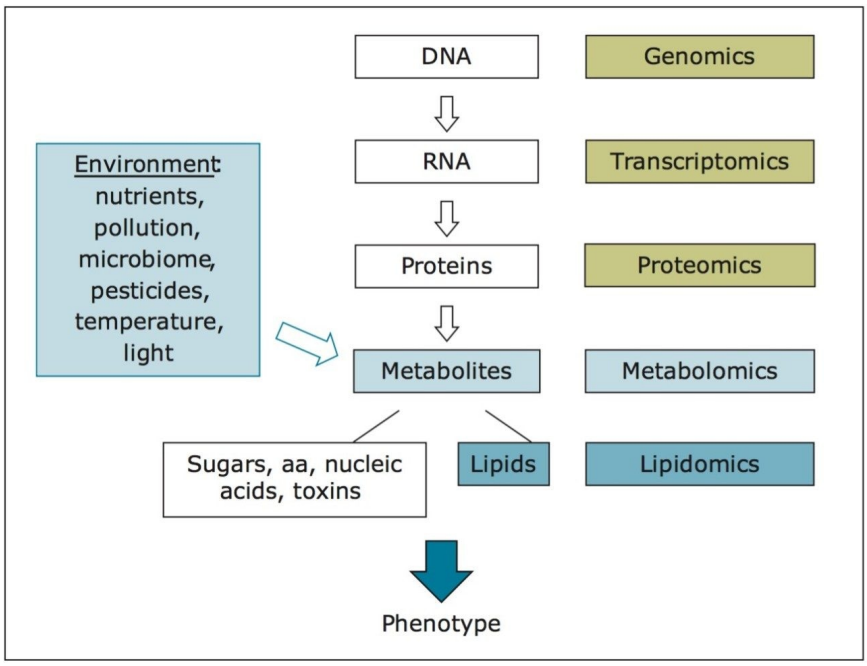

Figure 1. Metabolomics aims to screen all the metabolites present in biological samples. Metabolites can derive from both the generic imprint and from the environment (e.g., different growth conditions). Metabolites are counted in the order of thousands and have a wide range of chemical complexity and concentration. The profiling of the entire set of metabolites, or metabolome, characterizes the molecular phenotype of the biological system.

Experimental

Sample Description

Broccoli seeds (*Brassica oleracea* L. var. *botrytis* subvar. *cymosa*) were germinated in the germination cylinder of Vitaseed sprouter, and grown hydroponically for five days at 21 °C in a plant growth chamber (Clf Plant Climatics, Wertingen, Germany) equipped with PHILIPS Master TL-D 36W/840 cool-white fluorescent tubes providing a photosynthetic photon flux density of 110 mmol m-2 s-1, under three different light regimes: a) dark (achieved by covering the sprouting device with a cardboard box), b) continuous light, and c) continuous light plus two days of treatment with sucrose 176 mM.

Sprout samples, collected from the germination cylinder, were immediately frozen in liquid nitrogen and stored at -80 °C. Frozen sprouts were ground to a fine powder in a Waring blendor cooled with liquid nitrogen. Each sample of broccoli sprouts was extracted with 100% methanol (sample to solvent ratio 1:25 w/v) at 70 °C for 30 minutes under vortex mixing to facilitate the extraction. The samples were successively centrifuged (4000 rpm, 30 minutes, 4 °C), the supernatants were collected, and the solvent was completely removed using a rotary evaporator under vacuum at 40 °C. The dried samples were dissolved in methanol with the same volume of extraction, and filtered through 0.20-µm syringe PVDF filters.[3]

UPLC conditions

System:	ACQUITY UPLC
Column:	ACQUITY CSH C_{18} 2.1 x 100 mm, 1.7 µm
Mobile phase A:	60:40 10 mM NH_4HCO_2 in ACN/H_2O
Mobile phase B:	90:10 10 mM NH_4HCO_2 in IPA/ACN
Flow rate:	0.4 mL/min
Column temp.:	55 °C

Injection volume:	5.0 µL

Elution gradient:

Min	A%	B%	Curve
Initial	60.0	40.0	Initial
2.0	57.0	43.0	6
2.1	50.0	50.0	1
12.0	46.0	54.0	6
12.1	30.0	70.0	1
18.0	1.0	99.0	6
18.1	60.0	40.0	6
20.0	60.0	40.0	1

MS conditions

UPLC analytical column was connected to the ESI probe using PEEK Tubing, 1/16 inch, (1.6 mm) O.D. x 0.004 inch. (0.100 mm) I.D. x 5 ft (1.5 m) length, cut to 400 mm in length.

Mass spectrometer:	SYNAPT G2-S HDMS
Mode of operation:	Tof HDMS^{E}
Ionization:	ESI +ve and -ve

Capillary voltage:	2.0 KV (+ve) and 1.0 KV (-ve)
Cone voltage:	30.0 V
Transfer CE:	Ramp 20 to 50 V
Source temp.:	120 °C
Desolvation temp.:	550 °C
Cone gas:	50 L/h
MS gas:	Nitrogen
IMS T-Wave velocity:	900 m/s
IMS T-Wave height:	40 V
Acquisition range:	50 to 1200

Data acquisition and processing:

TransOmics Informatics and HDMS Compare for SYNAPT Systems

Results and Discussion

We applied an untargeted metabolomics approach (Figure 1) to identify molecular alterations induced by different growth conditions in broccoli sprouts (Figure 2). Metabolites were extracted from the sprouts, and analyzed using UltraPerformance LC (UPLC) coupled with an ion mobility enabled QTof mass spectrometer, the SYNAPT G2-S HDMS (Figure 3), as reported in the Experimental conditions.

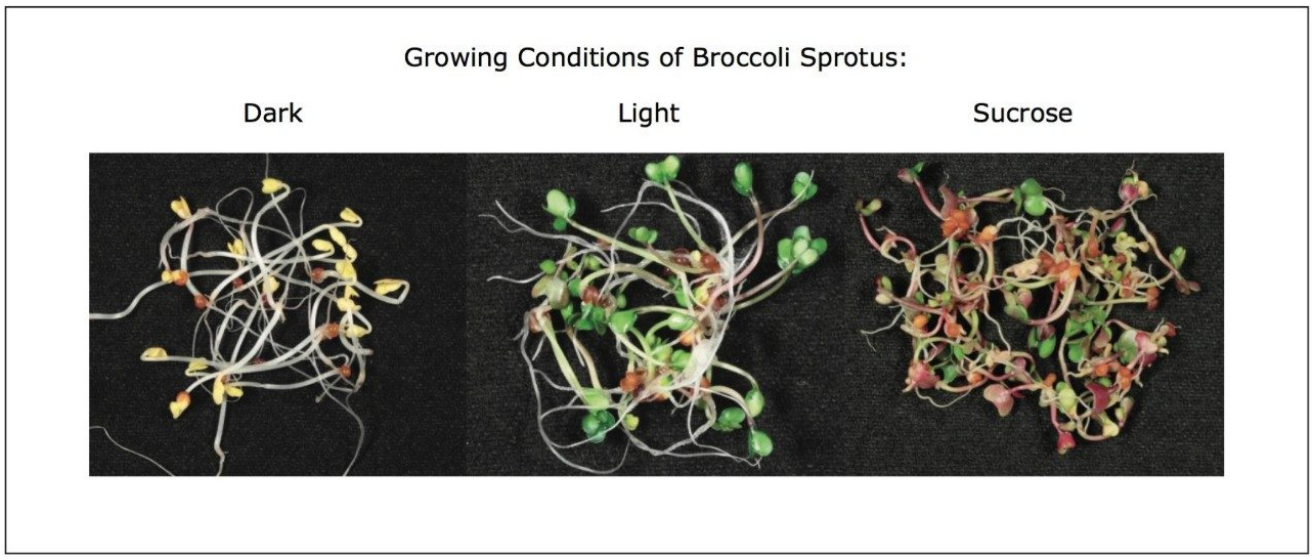

Figure 2. Broccoli sprout samples grown under different conditions.

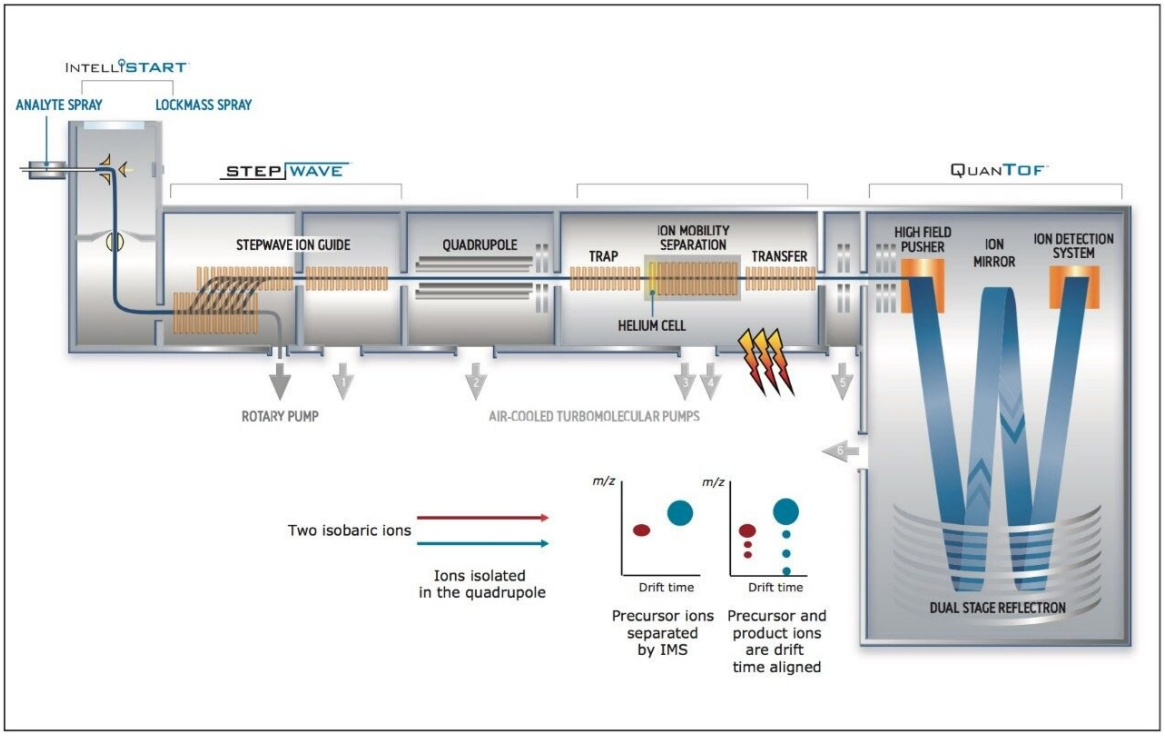

Figure 3. Schematic of the SYNAPT G2-S HDMS System configuration showing the ion mobility cell and the collision cell used to fragment the metabolites in HDMS^E mode.

UPLC maximized the separation of a wide range of chemical complexity present in the broccoli sprouts (Figure 4). Metabolites were ionized using ESI and, subsequently entered into the vacuum region of the MS system where they passed through the tri-wave ion mobility separation (IMS) cell (Figures 3, 4, and 5).

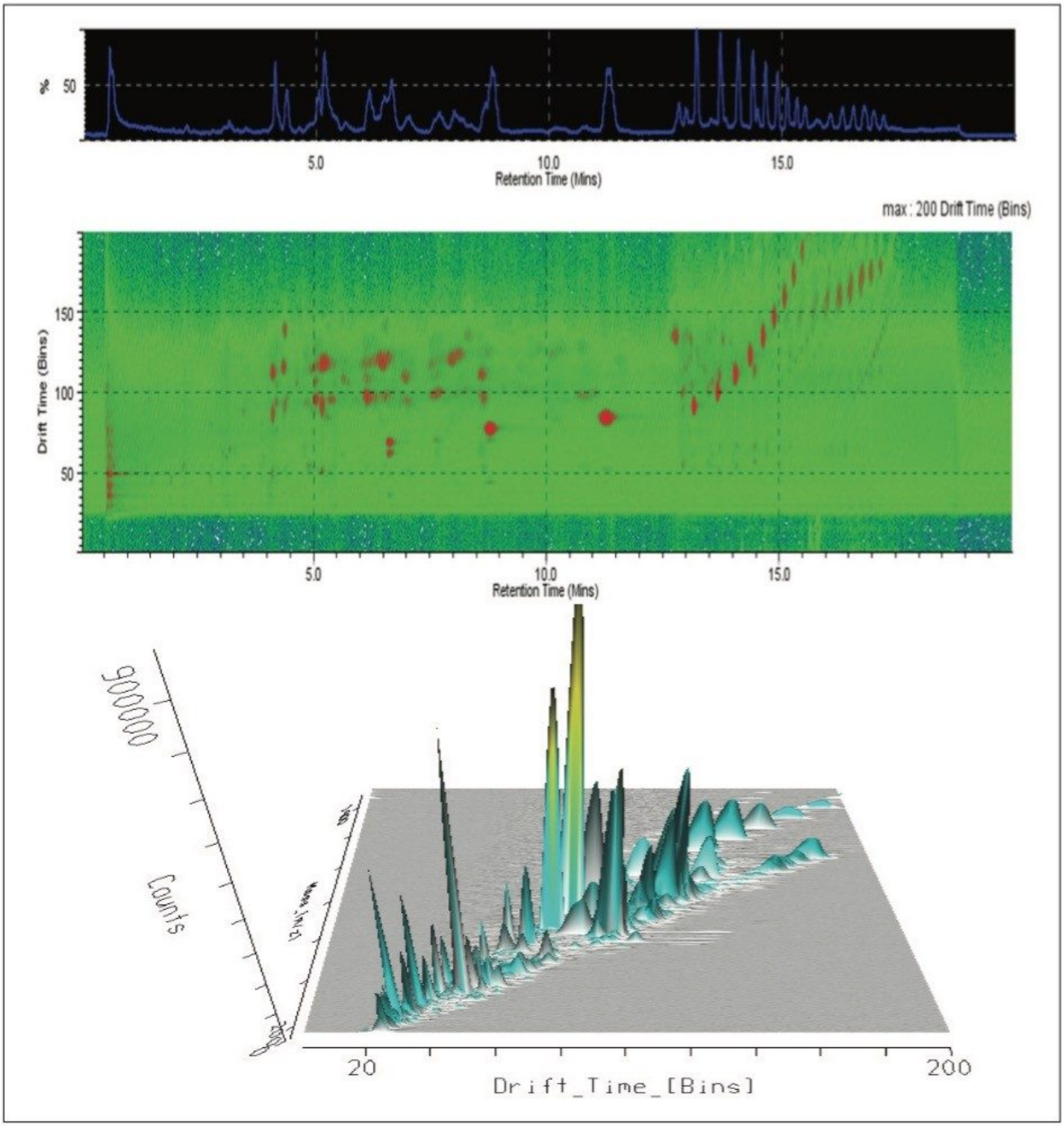

Figure 4. After UPLC separation, metabolites can be further separated in another dimension using ion mobility cell before MS detection This mode of acquisition is named High Definition Mass Spectrometry (HDMS). Metabolites show characteristic drift times according to their size, shape and charge. The combination of UPLC

and ion mobility increase peak capacity and specificity in the quantification and identification process.

The T-Wave IMS device uses RF-confining fields to constrain the ions in the radial direction, while a superimposed repeating DC voltage wave propels ions in the axial direction through the dense gas-filled cell. The height and speed of the wave can be used to separate ions by their ion mobility[1]. As such, metabolites migrate with characteristic mobility times (drift times) according to their size and shape (Figures 3, 4, and 5). Therefore, IMS provides an additional degree of separation to chromatography, improving peak capacity over conventional UPLC-MS techniques (Figure 5).

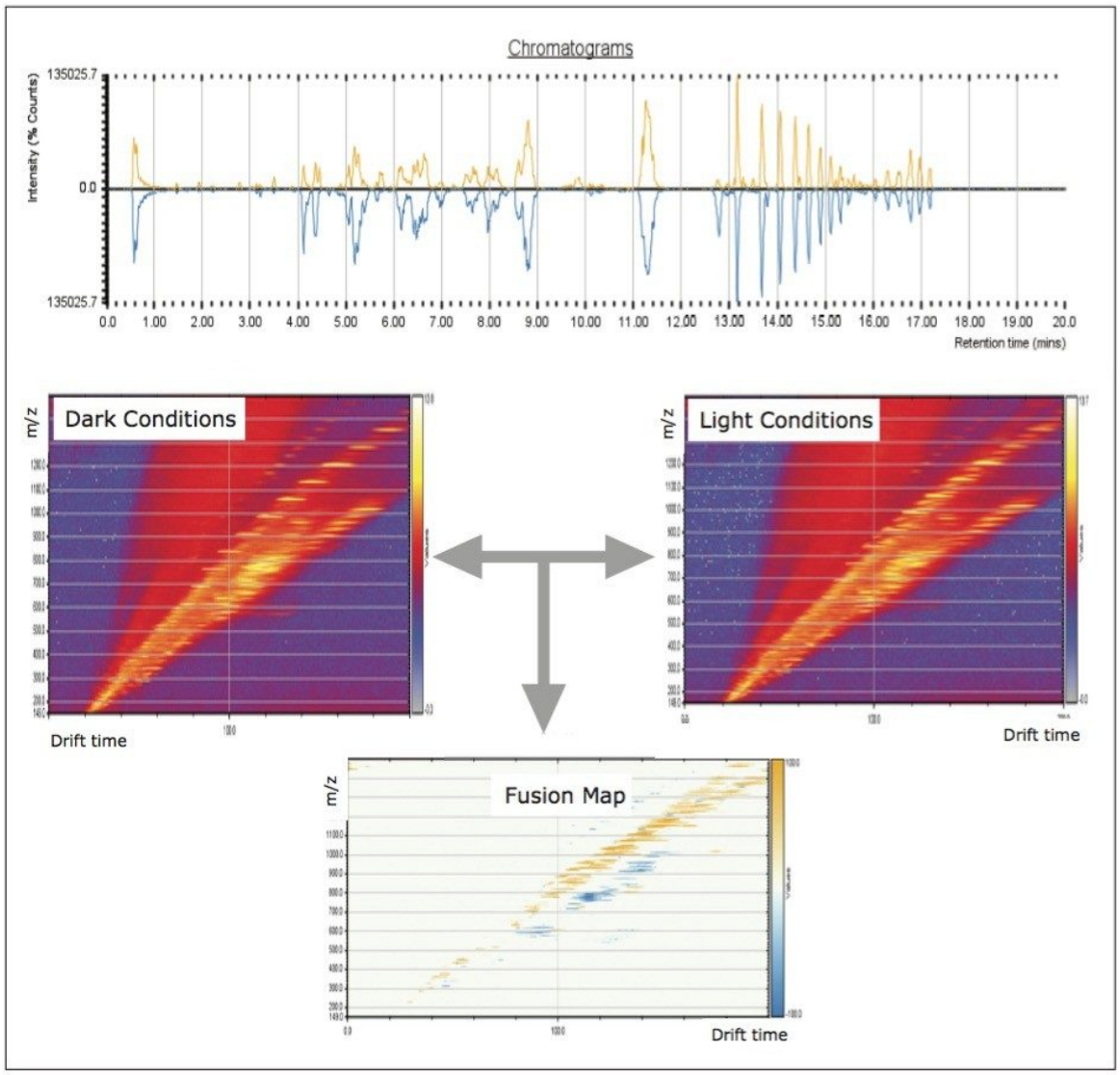

Figure 5. Representative UPLC/UPLC-MS chromatograms showing qualitative differences between broccoli sprout samples grown under light or dark conditions (upper panel). HDMS Compare Software was used to compare condition-specific molecular maps, highlighting key areas of significant differences between two samples with two different colors (bottom panel).

To aid in the identification and structural elucidation of metabolites, collision induced dissociation (CID) of metabolite precursor ions after IMS separation is performed using a particular mode of operation named HDMS^E. This approach utilizes alternating low and elevated collision energy in the transfer cell, thus recording all of the precursor and fragment ions in a parallel and continuous manner (Figure 6). The alternating scans acquire low

collision energy data, generating information about the intact precursor ions, and elevated collision energy data, that provides information about associated fragment ions (Figure 6). The incorporation of ion mobility separation of coeluting precursor ions before CID fragmentation produces a cleaner MS/MS product ion spectra, facilitating easier metabolite identification, as shown in the bottom panel of Figure 6.

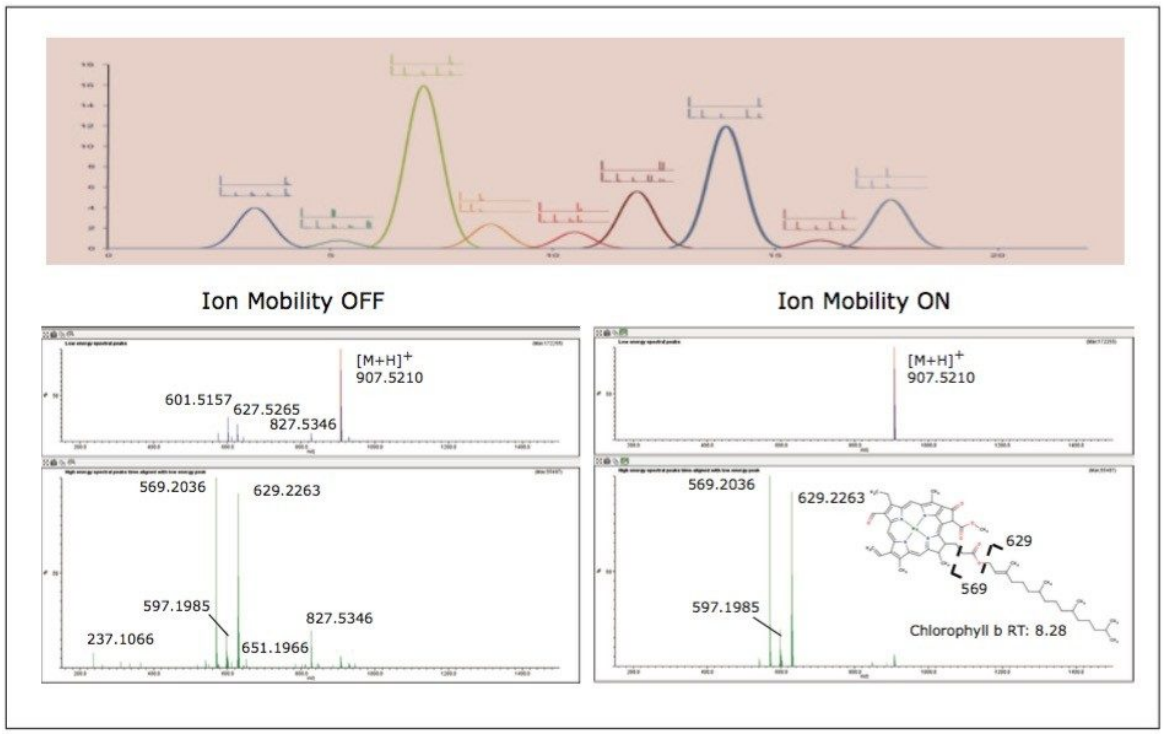

Figure 6. Representative UPLC/HDMS^E chromatogram showing the acquisition of both precursors and fragment spectra information along one single chromatographic run (upper panel). Applying high collision energy in the transfer collision cell, precursor molecules can be broken down into constituent parts, to deduce the original structure (bottom panel). In this example, the identification of the chlorophyll structure is based on the observation of characteristic fragments generated with high energy, using MS^E which matched with a compound search (Figure 8) and previously published results.

The analysis provided a metabolite profile, which represents a biochemical snapshot of the metabolite inventory for each sample analyzed. Differences at the metabolite level between groups were analyzed using TransOmics Informatics which provided multivariate statistical analyses tools, including principal component analysis (PCA)

(Figure 7A), correlation analysis (Figure 7B), review compound (Figure 8A), and database search functionalities (Figure 8B) for metabolite identification.

Preliminary results suggest that growth conditions induce specific alterations in the "metabolome" of broccoli sprouts, some of which are strictly related to photosynthetic processes.

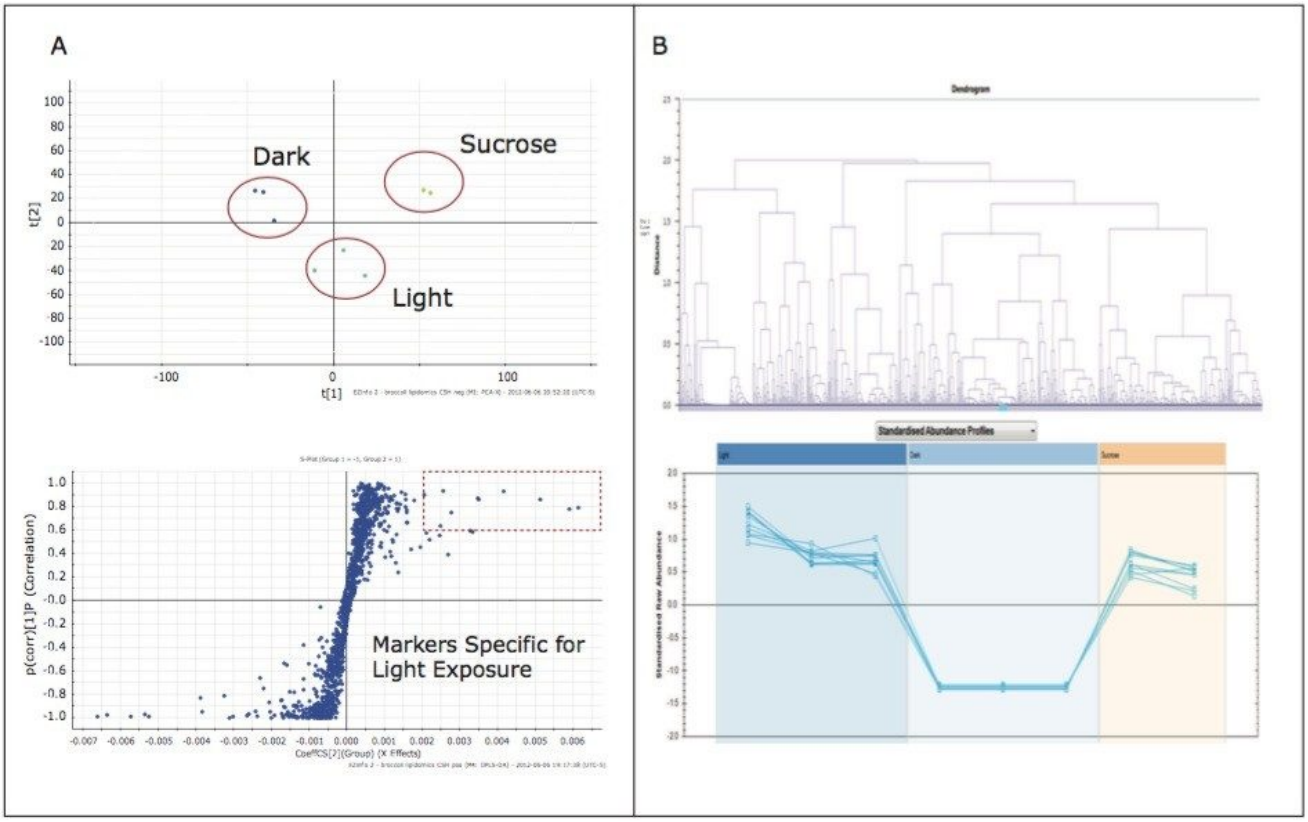

Figure 7. TransOmics multivariate statistical analysis of the UPLC-HDMS^E runs (A) allowed to separate samples into clusters, isolating the metabolites that contributed most to the variance among groups. Correlation analysis (B) helped to identify similar patterns of alterations among metabolites

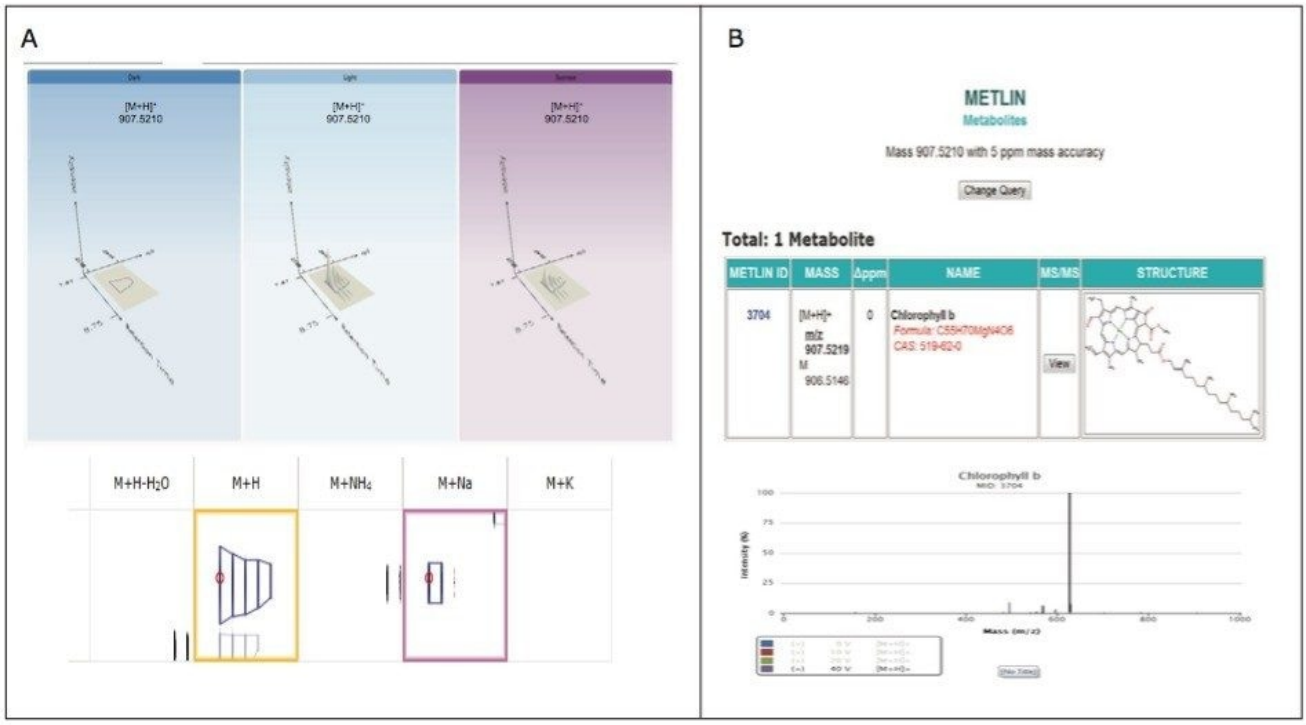

Figure 8. The statistical identification of metabolic alterations was followed by a review of the measurements (e.g., 3D montage and adducts deconvolution, (A) and a search on local or online databases (e.g., METLIN, (B)) for structural identification. In this example, a database search lead to a putative structure of a chlorophyll metabolite, which was only detected in broccoli sprouts grown in light conditions (A).

Conclusion

The Waters Omics Research Platform with TransOmics Informatics, featuring UPLC and HDMS^E technologies, enables researchers to improve how they screen and differentiate molecular phenotypes of plants exposed to different environmental stimuli. This highthroughput approach has applications in agricultural, food, and nutritional, as well as natural product research.

References

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