Global metabolite profiling of leaves and flowers of *Ocimum basilicum* L. grown in greenhouse under different light wavelengths

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INTRODUCTION

Megatrends such as increasing human population and urbanization, water scarcity and climate change have resulted in a decline in fertility and availability of arable land around the world. As a result, plant breeders are increasingly investigating indoor plant farming. Growing plants indoors, however, can be challenging since growth conditions such as soil type, air flow, water and light exposure must be properly provided for optimal plant health. In fact, growing conditions heavily affect many plant characteristics including size, nutrient profile, flowering capacity and disease resistance. When observing the impact of these conditions at the molecular level, differences in primary and secondary metabolites can be directly linked with specific growth conditions. For example, plants undergoing drought conditions sense water stress and produce signaling molecules such as abscisic acid.[1]

In the last decade, many agricultural technologies have been developed to meet the need for growing plants indoors. In the current study, basil plants (*Ocimum basilicum* L.) were grown from seedling to flowering stages in a new concept microcosm[2] (European patent 3236741/2019) in which the different light wavelengths were supplied to plants to study the effects on the growth and metabolome of different plant parts. SWATH DIA was used to generate a comprehensive digital map of the metabolome of each sample. MarkerView software was then used to pinpoint differentiating metabolites that were subsequently identified using library matching to the Natural Products HR-MS/MS and NIST spectral libraries using SCIEX OS software.

METHODS AND MATERIALS

Sample preparation: The plants were cultivated for approximately 60 days under either W or B/R light condition. The top leaves (apical, Ap L), middle leaves (median, Me L) and inflorescences (flower) were harvested and extracted with a solution of 1:1, ethanol/water. A Sep-Pak C18 was used to extract chlorophyll. Two batches were run ("I" and "IV"), corresponding to 2 different plant source , and each sample was prepared in triplicate. Quality control samples ("QC") were prepared in triplicate by mixing the extracts from all the plant parts. The QC samples were used to develop and optimize the SWATH DIA method with variable windows. The final optimized method was then used to acquire all sample data.

Chromatography: An ExionLC system equipped with an HSS T3 column (2.1 × 100 mm, particle size 1.8 µm, Waters) was used for metabolite separation at a flow rate of 0.45 mL/min. The column temperature was 40°C. Injection volume was 5 µL in positive ion mode and 8 µL in negative ion mode. Gradient conditions are shown in Table 2.

Mass spectrometry: Data were acquired in positive and negative modes using a TripleTOF 6600 system using the data-independent SWATH DIA approach. The following settings were used: CUR 30; GS1 40; GS2 40; ISV 4500; TEM 500. SWATH DIA used the following parameters: 25 variable windows, TOF mass range 150-1000 Da; TOF accumulation time 50 ms; MS/MS mass range 50-1000 Da; MS/MS accumulation time 25 ms.

Data processing: Data were processed using SCIEX OS software 1.6 and the Natural Products HR-MS/MS and NIST spectral libraries. Statistical analyses were performed using MarkerView software.

RESULTS

To find differentially regulated metabolites between the plant samples, the TOF MS data from the SWATH DIA were first processed using statistical tools in the MarkerView software. These tools enable fast discrimination among different sample groups to immediately pinpoint features of interest.

Figure 1 (left) displays the Scores plot for the Partial Least Squares Discriminant Analysis (PLS-DA), using the TOF MS data from all samples. As shown here, samples are differentiated into groups, with leaf samples segregated below the horizontal line and flower samples above the line. Biological replicates are shown for all sample types and tend to group together with some replicates more closely associated with one another than with other replicates. For example, the flower samples extracted from plants grown under blue/red light ("Flower_B/R") are tightly grouped and segregated further from other flower samples in the upper right quadrant of the Scores plot (circled in red, Figure 1). These samples are very similar to each other but contain features with abundances that highly differentiate them from other samples.

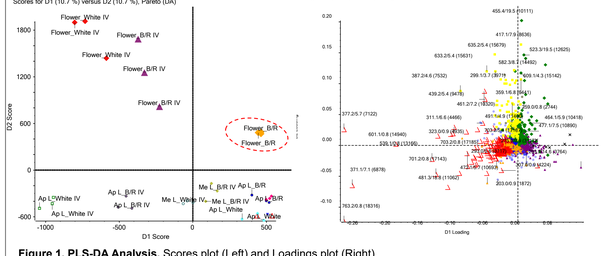
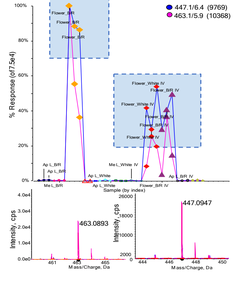


Figure 1. PLS-DA Analysis. Scores plot (Left) and Loadings plot (Right).

To understand which features are most responsible for the differentiation and separation of sample groups and replicates, the PLS-DA loadings plot was examined. Features with higher D1 and D2 loadings are more differentiating. Closer examination of the upper right quadrant shows several features (Figure 1, right) are differentiating for the B/R flower.



The responses for all sample types for 2 of these peaks ("447.1/6.4 (9769)" and "463.1/5.9 (10368)") are shown in Figure 2. Both peaks are observed with much higher abundance in flower than in leaf samples, with the highest response observed for flowers extracted from plants grown under blue/red light in batch 1. Differentiating features identified from the various PLS-DA quadrants were added to an interest list for further analysis.

Whereas the PLS-DA groups samples into categories and finds differentiating features that contribute to these groupings, the pairwise t-test finds features that are discriminatory between 2 groups of samples.

Figure 2. Differential regulation found with principal component analysis. (Top) Responses for *m/z* 447.1 and 463.1 are plotted for all samples. (Bottom) TOF MS data for both features.

Figure 3 shows all data obtained from pairwise t-test for the apical leaf samples grown under blue/red light (AP L_B/R) and the flower samples grown under blue/red light (Flower_B/R). The right panel shows features with a low p-value and high fold change which are ones that differ in abundance between the groups with high confidence; these can be selected and added to an interest list for identification.

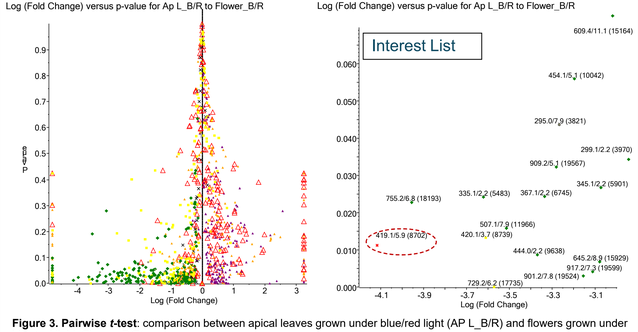


Figure 3. Pairwise *t*-test: comparison between apical leaves grown under blue/red light (AP L_B/R) and flowers grown under blue/red light (Flower_B/R).

The profile plot for one of these features, "419.1/5.9 (8702)," (Figure 3, purple circle) is shown in Figure 4 (left) for all sample types and growth conditions. This peak is much more abundant in flower samples grown using either blue/red light or white light, than leaf samples grown under any conditions.

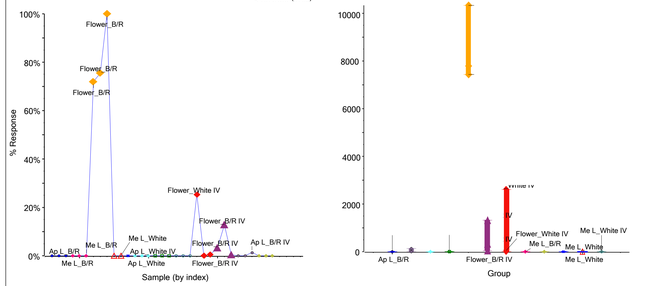


Figure 4. Profile plot (left) and bar (right) for the peak with m/z 419.1 Data are shown across all samples.

Figure 5 shows the identification of hyperin (isoquercitin) as one of the features that exhibited discriminatory properties between flowers and leaves based on the PLS-DA (Figure 2). Library matching of the 463.1/5.9 (10368) feature found in PLS-DA using SWATH DIA data isoquercitrin with high confidence. Multiple levels of matching are used, including the retention time. Figure 5 shows identification based on time (left), the precursor mass, isotope data and low mass error (center and bottom left), and the MS/MS spectrum and library match (right).

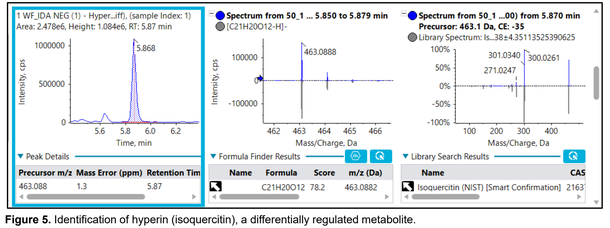


Figure 5. Identification of hyperin (isoquercitin), a differentially regulated metabolite.

Hyperin is a bioflavonoid, and studies have shown that it may have antioxidant, antiinflammatory, anticancer, antiviral, antibacterial, antiparasitic, cardioprotective and hepatoprotective activity. Higher levels of hyperin were found in flowers as opposed to leaves in this study.

CONCLUSIONS

- SWATH DIA for untargeted plant metabolomics provides complete datasets with high-resolution MS and MS/MS data for every precursor in a sample using a single injection workflow
- MarkerView software provides flexibility in the usage of various multivariate statistical analysis and plot generation tools (PCA, PLS-DA, t-test) to distinguish classes, find interesting features/biomarkers and highlight trends
- Creating an interest list directly from statistical graphs provides effortless transfer of interesting features from MarkerView software to SCIEX OS software for library matching
- Library matching of the target list to the Natural Products HR-MS/MS and NIST spectral libraries uses multiple lines of evidence embedded in the SWATH DIA data for compound identification

REFERENCES

1. Xinyi Y *et* al. (2021) Response Mechanism of Plants to Drought Stress.
2. "Innovation: ENEA "Microcosm" arrives on the market thanks to FOS" 3/18/2021

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