P-024: Untargeted multiplatform metabolomics assay for the analysis of plant-herbivore interactions in broad bean

Mariusz Kowalczyk, Justyna Krzyżanowska-Kowalczyk

Institute of Soil Science and Plant Cultivation, State Research Institute, Puławy, Poland
E-mail: mkowalczyk@iung.pulawy.pl

Orthogonal separation techniques are frequently employed in metabolomics to extend the range of analyzed metabolites. Such approaches are often multi-instrumental because different separation techniques sometimes require different interfaces with the mass spectrometer and not all of them are universally available. In untargeted metabolomics, the use of various instruments and different acquisition modes can introduce undesired variability in the obtained datasets. Also, some of the published strategies for multiplatform metabolomics are based on individual extractions of metabolites for each separation technique, frequently with different sets of internal standards and quality control samples. As a result, multiplatform methods are perceived as difficult and costly, both regarding the financial expenses and labor. However, for applications of untargeted metabolomics in plant sciences, they are usually necessary because of the vast numbers and structural heterogeneity of investigated compounds. Another problem often encountered in plant metabolomics is a broad diversity of observed metabolite concentrations, sometimes spanning a few orders of magnitude, which can generate non-quantitative results due to either saturated or below the detection limit signals.

To address some of these concerns, we developed a method of metabolome analysis based on a single extraction and, at the same time, initial fractionation of metabolites from the 30 mg of powdered plant material. Obtained fractions were then analyzed using a combination of separation techniques: RP-UHPLC, HILIC-UHPLC, GC, and CE. The same detector, high-resolution QTOF mass spectrometer, was used in each case, although with two different ionization modes. LC of polar and semi-polar compounds, as well as CE separations, were interfaced using ESI while APCI was used for GC analyses. Additionally, CE separations were carried out using three different capillaries and buffer systems. Overall, nearly 600 metabolites were annotated and subsequently used as a dataset to investigate the plant-herbivore interactions in broad bean seeds.

This research is supported by the NCN grant 2014/15/B/NZ9/04302.