

Untargeted metabolomics of oat, pea, and faba beans reveal effects of geographic origin and agricultural practice type (conventional vs organic)

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Plant protein sources are rich in diverse classes of small molecules as a response to endogenous and exogenous processes. Better understanding of this chemical space is equally beneficial for transition to plant-based dietary protein sources. Untargeted metabolomics based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful tool to explore small molecular profile of various organisms. In this study, we collected untargeted MS data on grains of oat, pea, and faba beans representing ten different cultivars each and grown in three locations in Denmark – Hammel, Sejet, Hamborg. While traditional agriculture practices were used to harvest crops in Sejet and Hamborg, crops from Hammel were grown organically. For data quality assurance purposes, pooled samples were generated from all biological samples which were run in three technical replicates. As a result, 1,262 features picked by LC-MS data processing software Metaboscape were included in the analysis, of which 99 were annotated. The final feature table included 8 superclasses and 28 classes of compounds annotated using ClassyFire [1] tool in Level II according to Metabolomics Standards Initiative [2]. Crop, location, and cultivar effects were studied using principal component analysis (PCA), analysis of variance (ANOVA), and ANOVA-simultaneous component analysis (ASCA).

Principal component (PC) 1 and PC2 showed a clear separation of crop types where fatty acids (oxo-octadecenoic acid, hydroxyoctadecenoic acid), ceramides, N-ethanolamides (palmitoyl ethanolamide), dipeptides (Leu-Pro) differentiated oat, while choline, abscisic acid, aspartic acid, and uric acid explained the variance of pea metabolites. Pantothenic acid, spermidine, N-phenylacetyl aspartic acid, and indolelactic acid explained the variance for faba beans the most. ASCA results showed distinct clustering of features originating from crops grown organically in Hammel. For oat cultivars grown in Hammel, notably differentiated metabolites were uric acid, umbelliferone, N-phenylacetyl aspartic acid, L-carntine, Leu-Pro; pipercolic acid, arginine, pinolenic acid – for pea cultivars; mandelamide, choline, tyrosine, aspartic acid, and abscisic acid – for faba beans cultivars. ANOVA revealed that N-ethanolamides (palmitoyl, linoleoyl, and oleoyl ethanolamides) were relatively less abundant at all crop types grown in Hammel compared to the ones grown in Sejet and Hamborg. In summary, LC-MS-based untargeted metabolomics distinguished metabolites that are responsible for the effect of crop and geographic origin as well as agriculture practice type.