Development of a Shotgun Lipidomics Approach for Analysis of Lipids in Perennial Ryegrass

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Abstract

Mass Spectrometry is a powerful analytical tool which is used for identification and quantitation of compounds within samples for a variety of sample matrices. One example of this is to look into the lipid profile (lipidome) of perennial ryegrass (*lolium perenne*). The lipid profile tells us many things about the inner workings of rye grass which can lead to better understanding of mechanisms behind desirable traits (such as lipid quantity and composition). Traditional high performance liquid chromatography (HPLC) is the most widely used chromatographic technique when researching into the lipidome of different plants (Burgos et al., 2011; Chen, Markham, & Cahoon, 2012; Degenkolbe et al., 2012). Shotgun lipidomics applies major principles of the traditional methods but differs in the delivery of the sample to the Mass Spectrometer and data analysis; providing considerable advantages, disadvantages and challenges.

A shotgun lipidomics method for analysing the lipids in perennial ryegrass has been developed. This involved first determining the most efficient extraction protocol and then establishing a methodology (based on one found in the literature for animal samples) for shotgun lipidomic analysis of perennial ryegrass. The shotgun data was problematic to analyse using traditional methods so LCMS data was investigated and the results were transferred to the shotgun data. Investigation was conducted to find the limiting factor for the analysis of the shotgun data. This limiting factor was found to be pheophytin a and other chlorophyll derivatives. The high abundance and ion suppression effects attributed to pheophytin a and other chlorophyll derivatives

contributed to unfavourable conditions for analysing the lipidome of perennial ryegrass. The major outcomes of this study are the annotation of 118 lipids in perennial ryegrass using LCMS, with 27 of those being found in the shotgun data also and also the understanding of the limitations of using shotgun techniques for perennial ryegrass. With this understanding further research can be conducted to enhance the methodologies detailed herein.

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List of Abbreviations

BP	Before present
C	Choline
CID	Collision-induced dissociation
CLA	Conjugated linoleic acid
CVD	Cardiovascular disease
d_6-DMBNHS	d_6-*S,S'*-Dimethylthiobutanoylhydroxysuccinimide ester
dd-MS2	Data dependent MS2
DG	Diglycerol
DGDG	Digalactosyldiacylglycerol
DIA	Direct injection analysis
DMF	Dimethyl formamide
E	Ethylamine
EIC	Extracted ion chromatogram
ESI	Electrospray ionisation
Fmoc-Cl	Fmoc chloride
GC	Gas chromatography
HESI	Heated electrospray ionisation
HILIC	Hydrophilic interaction chromatography
HPLC	High performance liquid chromatography
HPLC-MS	High performance liquid chromatography mass spectrometry
I	Inositol
IPA	Isopropyl alcohol
LCMS	Liquid chromatography mass spectrometry
LMSD	LIPID MAPS Structure Database
LPC	Lysophosphotidylcholine

m/z	Mass to charge ratio
MALDI	Matrix-assisted laser desorption ionisation
MG	Monoglyceride
MGMG	Monogalactosylmonoacylglycerol
MS	Mass spectrometry
MS2	Fragmentation of an ion or ions. MS1 is a Full MS scan, where MS2 is the fragmented spectra of ions, which can be targeted or non-targeted depending on experiment and mass spectrometer used.
MTBE	Methyl tert-butyl ether
MUFA	Monounsaturated fatty acid
NPLC	Normal phase liquid chromatography
P680	Chlorophyll P680
PA	Phosphatidic acid
PC	Phosphocholine
PE	Phosphoenthanolamine
PG	Phosphoglycerol
PI	Phosphoinositol
PUFA	Polyunsaturated fatty acid
PS	phosphoserine
PS II	Photosystem II
RPLC	Reverse phase liquid chromatography
S	Serine
SFA	Saturated fatty acid
TEA	Triethylamine
TLC	Thin layer chromatography
TOF	Time of flight
UCD	University of California, Davis