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Plant lipidomics at the crossroads: From technology to biology driven science☆

Vladimir Shulaev, Kent D. Chapman *

Department of Biological Sciences, University of North Texas, Denton, TX 76203, United States

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ABSTRACT

The identification and quantification of lipids from plant tissues have become commonplace and many researchers now incorporate lipidomics approaches into their experimental studies. Plant lipidomics research continues to involve technological developments such as those in mass spectrometry imaging, but in large part, lipidomics approaches have matured to the point of being accessible to the novice. Here we review some important considerations for those planning to apply plant lipidomics to their biological questions, and offer suggestions for appropriate tools and practices. This article is part of a Special Issue entitled: BBALIP_Lipidomics Opinion Articles edited by Sepp Kohlwein.

1. Introduction

Lipids play essential roles in plants, serving as structural components of membranes, major storage reserve in seeds, pigments for energy capture in leaves, and signaling molecules for local and long-distance communication.

Lipidomics, or global analysis of lipid composition, has emerged as a powerful technique complementing other global approaches, like transcriptomics, proteomics and metabolomics, that are focused on simultaneous analysis of all or large number of cellular metabolites. Lipidomics can be considered as branch of metabolomics focused on the analysis of the non-water-soluble metabolites [1–3].

In the last few decades progress in lipidomics has been largely driven by improvements in analytical techniques. As with other heavily technology driven disciplines, early lipidomics studies were largely focused on instrumentation and methodology developments with less attention to a particular biological question. The more each discipline matures, the more focus is shifted from technology to biology. We believe that lipidomics is already becoming a mature field where biology is moving to a forefront and technology is mature enough to be routinely used by a wide range of scientists with less specialized training in mass spectrometry or other analytical disciplines.

Although current technology often allows mass spectrometry novices to use lipidomics and other global approaches in their research, caution should be exercised by scientists inexperienced in "omics" technologies to carefully design their experiments and pose the correct question that can be addressed with current methodology. Similar to other metabolomics subdisciplines, biologists often have higher expectations than current lipidomics technology can provide. General expectations from lipidomics studies by the novice in the field is that it will: (1) identify all lipid classes in the sample, (2) identify all lipids within a particular class, (3) identify isobaric species and structural isomers, (4) quantify individual components (using either absolute or relative quantitation methods) and identify differentially expressed lipids using multivariate statistics, (5) identify metabolic networks, (6) identify spatial distribution of the lipids within tissues, and (7) help to formulate hypotheses based on global lipidomics studies or test hypotheses using targeted analysis. Although research efforts continue to make progress in these areas, currently the items in this list are more ambitious than can be achieved in a single study. Limitations exist in technologies and data analysis tools, lack of reference databases and, most important, insufficient knowledge about lipid metabolism in evolutionarily diverse organisms. Knowing these limitations can help to avoid many pitfalls and disappointments and gain the most value from lipidomics studies.

Here we overview the current state of the lipidomics science and point out some limitations with respect to plant lipidomics. A general workflow diagram of the aspects discussed is provided in Fig. 1.

2. Methodology

2.1. Choosing the right toolbox

Generally lipidomics analysis involves sample extraction, one or more analytical technique, data analysis and biological interpretation

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* Corresponding author at: University of North Texas, Department of Biological Sciences, 1155 Union Circle #305220, Denton, TX 76203, United States.
E-mail addresses: vladimir.shulaev@unt.edu (V. Shulaev), chapman@unt.edu (K.D. Chapman).

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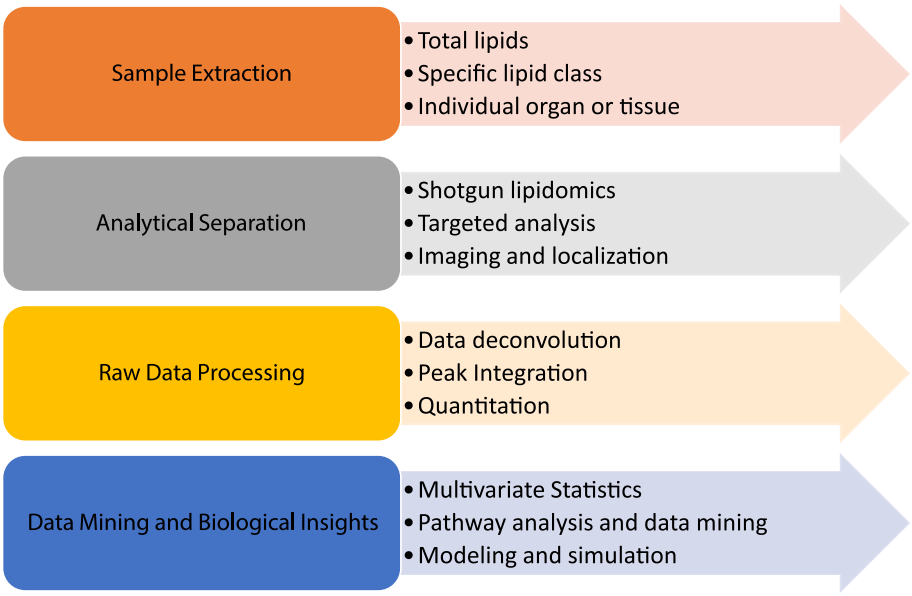

Fig. 1. General workflow for lipidomics studies.

(Fig. 1). Each step is equally important to produce high quality and reproducible data.

The major challenge for lipidomics analysis is to isolate all diverse lipids from the biological sample for subsequent analysis without loss or degradation. Several well-accepted methods for lipid extraction from biological samples exist, including Folch et al. [4], Bligh and Dyer [5] and Matyash [6]. Despite their general applicability and wide use, all of these methods have limitations, mostly in extraction efficiency towards some lipid classes. Sample extraction methods tailored towards specific groups of lipids or more global analysis also exist. For example, plant sphingolipids require more polar extraction procedures, and improvements in extraction procedures have provided new insights into the prevalence and diversity of sphingolipids in plants [7]. Further, certain measures, i.e. adding anti-oxidants to extraction medium or substituting 2-propanol for methanol, have to be taken to preserve lipid integrity and prevent lipid degradation or oxidation, especially for plant tissues.

Today the main techniques for the analysis of lipids are based on mass spectrometry [8]. Different mass analyzers and ionization sources are currently available for lipidomics analysis and their uses can be tailored towards a specific application. Mass analyzers that have been used in metabolomics studies include quadrupole, ion trap, time of flight, Fourier transform (FT) or Fourier transform ion cyclotron resonance (FT-ICR), and Orbitrap. A variety of ion sources can be used with different mass analyzers for lipidomics analysis including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), vacuum or atmospheric, matrix assisted laser desorption ionization (MALDI), desorption ionization (DESI), and direct analysis in real time (DART). Some ionization procedures are more commonly used for certain applications; for example, ESI and to some extent APCI ion sources are predominantly used for shotgun lipidomics while MALDI and DESI ion sources are most useful in imaging applications.

2.2. To aim or not to aim

Broadly speaking, lipidomics can be divided into shotgun and targeted analysis. Shotgun, or global, lipidomics aims at detecting all lipid species within the sample without a priori knowledge about sample composition, while targeted lipidomics target is aimed at analysis of a set of lipid molecules or specific group of lipids. Whether to apply a targeted or untargeted analysis depends on the biological question being addressed and the level of understanding about the lipid composition in the subject organism. For example, it may be unknown which substrate a phospholipase acts upon, and a shotgun lipidomics approach between the specific phospholipase knockout and wildtype could provide clues to the class or molecular species of candidate phospholipid substrate(s). On the other hand, a particular pathway and its metabolites might already be implicated in a biological process, in which case a more targeted analysis of a group of lipids would be warranted, such as oxylipin profiling in pathogen defense responses or triacylglycerol profiling during oilseed maturation. Both shotgun and targeted approaches use similar instrumentation and have inherent limitations, some of which are described below.

Shotgun lipidomics analyses are often performed using direct infusion MS. During direct-infusion MS, the sample is continuously introduced into the electrospray ionization (ESI) source without prior separation. Direct-infusion MS can be performed using either nominal mass instrument such as tandem quadrupole MS [9,10] or using High Resolutions MS (HRMS). Tandem quadrupole methods utilize lipid class specific precursor ion scan (PIS) or constant neutral loss scan (NLS). Using HRMS provides multiple advantages over nominal mass and is becoming the technique of choice for shotgun lipidomics (reviewed in [11]). High mass resolution provides the ability to achieve measurements with ppm and sub-ppm errors which is necessary to reliably identify adducts and assign molecular formulas to detected ions [12].

Despite wide adoption of direct-infusion MS-based shotgun approaches due to its simplicity and speed, there are important limitations to be considered when choosing direct-infusion MS. Direct-infusion MS often suffers from ion co-suppression effects and limited ability to resolve isobaric species. Hence a combination of mass spectrometry with some kind of separation technique is usually needed to overcome these limitations. Liquid chromatography coupled to mass spectrometry (LC-MS) has been used extensively in both global and targeted lipidomics [8,13–19]. Even though the lipids are generally considered hydrophobic compounds many complex lipids are more polar due to phosphorus, sulfur, sugar, and nitrogen atoms in their structure. Because of this polarity range, chromatographic separation of complex lipid mixtures poses a challenge for LC-MS based lipidomics. Typically, a combination of different separation techniques is used to separate different groups of lipids and individual lipid species within each group. Reverse phase (RP) [20–26] and hydrophilic interaction chromatography (HILIC) [13] or their combination [27] are most often used for lipidomics analysis. In LC/MS/MS methods, the runs typically involve

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