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Review

Lipidomics *in situ*: Insights into plant lipid metabolism from high resolution spatial maps of metabolites

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ABSTRACT

The emergence of 'omics' technologies (i.e. genomics, proteomics, metabolomics, etc.) have revealed new avenues for exploring plant metabolism through data-rich experimentation and integration of complementary methodologies. Over the past decade, the lipidomics field has benefited from advances in instrumentation, especially mass spectrometry (MS)-based approaches that are well-suited for detailed lipid analysis. The broad classification of what constitutes a lipid lends itself to a structurally diverse range of molecules that contribute to a variety of biological processes in plants including membrane structure and transport, primary and secondary metabolism, abiotic and biotic stress tolerances, extracellular and intracellular signaling, and energy-rich storage of carbon. Progress in these research areas has been advanced in part through approaches analyzing chemical compositions of lipids in extracts from cells, tissues and/or whole organisms (e.g. shotgun lipidomics), and through visualization approaches primarily through microscopy-based methodologies (e.g. fluorescence, bright field, electron microscopy, etc.). While these techniques on their own provide rich biochemical and biological information, coordinated analyses of the complexity of lipid composition with the localization of these lipids at a high spatial resolution will help to develop a new level of understanding of lipid metabolism within the context of tissue/cellular compartmentation. This review will elaborate on recent advances of one such approach – mass spectrometry imaging (MSI) – that integrates *in situ* visualization with chemical-based lipidomics. We will illustrate, with an emphasis on oilseed lipid metabolism, how MS imaging can provide new insights and questions related to the spatial compartmentation of lipid metabolism in plants. Further it will be apparent that this MS imaging approach has broad application in plant metabolic research well beyond that of triacylglycerol biosynthesis in oilseeds.

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Abbreviations: MS, mass spectrometry; MSI, mass spectrometry imaging; MALDI, matrix-assisted laser desorption/ionization; ESI, electrospray ionization; NMR, nuclear magnetic resonance; TAG, triacylglycerol; DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PI, phosphatidylinositol; DGAT, diacylglycerol acyltransferase; PDAT, phosphatidylcholine: diacylglycerol acyltransferase; FA, fatty acids; P, palmitic (16:0); Ln, linolenic (18:3); L, linoleic (18:2); O, oleic (18:1); S, stearic (18:0); G, gadoleic (20:1); numerical designation of lipids indicates number of carbons in acyl chains, number of double bonds.

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1. Overview of mass spectrometry imaging of lipid metabolites

Mass spectrometry imaging (MSI, sometimes also abbreviated IMS) is defined by a group of analytical platforms that map the location and relative abundance of metabolites *in situ*. Each platform operates with a similar overall process by rastering over a tissue surface and producing a set of ions from endogenous metabolites that can be identified and visualized by measuring their mass-to-charge ratios (Fig. 1). The ability to correlate location and metabolite abundance is the key to MS imaging. It is this attribute and improved instrument accessibility [1] that has resulted in a steady increase in number of publications on MS imaging in the last few years (Fig. 2) both in terms of technical advances as well as addressing specific biological questions. In contrast to conventional chemical extracts where spatial information is minimal or absent, in MSI, prepared tissue samples are analyzed directly retaining crucial spatial information for enhanced biochemical characterization. The three primary MS imaging ionization sources adopted commercially to-date include secondary ion MS (SIMS), desorption electrospray ionization (DESI) MS, and matrix-assisted laser desorption/ionization (MALDI) MS. Each platform varies in its mechanism of ionization and therefore offers different levels of spatial resolution (i.e. smallest distance that offers a distinguishable chemical profile) and available imaging applications. Integration of these ionization sources with high-resolution, accurate mass analyzers has enabled high-confidence identification of the ions generated by MSI. Once the raw data is acquired for each MSI method, a spatial map of an ion or set of ions representing a metabolite, protein, or class of desired molecules, can then be reconstructed and visualized using specialized imaging software. Several recent reviews have been published detailing some of the advances in MS imaging [2–4]. Here we will focus mostly on features, considerations, and applications of MALDI-MSI to plant lipid research.

1.1. Secondary ion mass spectrometry (SIMS)

SIMS operates through the emission of secondary metabolite ions from a tissue surface following bombardment by a primary ion beam (1–40 keV) [5,6]. While SIMS was one of first imaging methodologies developed [7–9], recent modifications in SIMS instrumentation now allow imaging of biomolecules up to *m/z* 1000 [10] which has widened its applicability for metabolites but not yet adequate for most proteomics approaches. SIMS sampling resolution of 400 nm to 1–2 μm [11] is superior to comparable imaging platforms and allows potential subcellular imaging and at attomolar concentrations [12]. Unfortunately, SIMS also suffers from extensive fragmentation and low ion yields [5]. Since SIMS requires a strong vacuum, sample preparation is critical to maintaining structural integrity. Sample preparation methods for tissue analysis (similar to those also required for MALDI) including stages of freezing, sectioning, and drying have been adapted from histological protocols. Methods are suitable for cellular or subcellular analysis with minor modifications such as eliminating freeze-drying [6], or through frozen hydration where cells are kept frozen throughout analysis without a drying step, which, incidentally, was found to enhance phospholipid signals [13]. Most of the applications to date for lipid imaging by SIMS [6] have been restricted to animal tissues [14–16] including lipid classes such as fatty acyls, glycerophospholipids, sphingolipids, sterol lipids, and prenol lipids. For plant lipid imaging, the applications are fewer due to the availability of instruments and difficulties in sample preparation but do include a recent report of successfully imaging flavonoids in pea (*Pisum sativum*) and *Arabidopsis thaliana* seed sections [17].

1.2. Desorption electrospray ionization-mass spectrometry (DESI-MS)

DESI operates through the generation of charged secondary droplets containing metabolite ions by directing pneumatically-assisted charged droplets at a tissue surface under atmospheric pressure [18]. DESI requires little sample preparation, offering a simpler alternative to SIMS and MALDI. Tissues are often imprinted on materials such as porous Teflon [19] as an alternative to tissue sections. Unfortunately, due to the ionization mechanism, DESI in most cases is currently limited to spatial resolutions around 100–250 μm [20,21]. A recent report, imaging lipids in brain tissues with morphologically distinct features, demonstrates that spatial resolutions of 35 μm are possible through optimization of DESI experimental parameters [22]. Due to the nature of DESI, much of the experimentation in plant tissues has focused on the surface analysis of lipids [23] in particular on leaves and petals [24], or on secondary metabolites in various tissues [19,25–27].