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Review

Nuclear magnetic resonance imaging of lipid in living plants

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ABSTRACT

This review highlights technological developments in magnetic resonance imaging (MRI), which are creating opportunities for the three dimensional visualization and quantification of lipids in plant materials. A major feature of MRI is that it is a non-invasive platform, and thus can be used for the analysis of living organisms. An overview of the theoretical aspects of MRI is provided, followed by a description of the various analytical modes available, and an explanation of how MRI can be applied to plant samples and what it can achieve. Various lipid maps and three dimensional models of seeds and fruits are included to demonstrate the potential of MRI and to exemplify recent cutting-edge advances in the field. The importance and prospects of the imaging of lipids in living plants, as well as the integration of lipid imaging with other emerging techniques, are outlined to provide impetus for future plant lipid research.

Contents

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1. Introduction: motivation and objectives

Nuclear magnetic resonance (NMR) has featured in Nobel Prize awards for Physics (Rabi in 1944, Bloch and Purcell in 1952), Chemistry (Ernst in 1991, Wüthrich in 2002) and Medicine (Lauterbur and Mansfield in 2003), highlighting the breakthrough nature of its discovery [1,2]. Its pioneering use to image the human torso in 1977 achieved a resolution of ~0.7 cm but the scan required several hours to complete [3], while current NMR imaging achieves a much higher level of resolution within just seconds. The technology can now display gene expression in living animal tissue [4], track the flow of sugars within a living plant [5] and even image the functioning of the human brain [6].

Lipid research is entering a new and exciting phase. In its infancy, the over-riding aim was to characterize the lipid fraction of the cell, but the discipline has since evolved into a rather broader field [7–9], taking in the analysis of the activity of particular organs such as the brain [10] and specific biological domains such as the macrophages [11], the description of structure and function [12–14], and as a component of genomics and fluxomics [15]. The current challenge is to understand how the molecular, biochemical and structural aspects of a living tissue work together, especially given the dynamic behavior of lipid metabolism, assembly and compartmentalization. The task of data integration is hampered by a lack of either temporal and/or spatial information. The real time imaging and analysis of lipids at various levels of organization (from molecular to the whole organism) may appear to be ambitious, but the thrust of this review is to suggest that progress towards this goal has been significant.

The rising demand for food and fuel has an impact on the size and breadth of the task facing the experimental biologist, the plant breeder and the plant biotechnologist. Plant lipids tend to share a similar fatty acid profile with that of petroleum, but the productivity of lipid-producing crops will need to be considerably raised to provide a viable alternative to mineral oil [16–19]. Production can be enhanced either by improving the yield of established oil crops, primarily oil palm (*Elaeis guineensis*), soybean (*Glycine max*), oilseed rape (*Brassica napus*) and sunflower (*Helianthus annuus*), which together account for >80% of current vegetable oil production. Alternatively, there may be opportunities for developing novel crops which do not compete with conventional crops, such as jatropha (*Jatropha curcas*) and certain algae [20,21]. More radically, it may be possible to convert what are currently considered to be non-lipid crops into lipid producers either by triggering the accumulation of lipid in their vegetative organs [22–24] or by the redirection of carbon storage from carbohydrates (or proteins) into lipids [25]. Such novel strategies demand an holistic approach to characterizing the metabolism of plants, and in particular to their interaction with the environment. While some facets of lipid biology and synthesis, and in particular the regulation, spatial patterning and allelic variation of lipid synthesis-associated genes are well served by available imaging technology, no single platform is yet able to provide a complete picture.

This review begins with a short overview of methods used for lipid analysis, and then focuses on the use of magnetic resonance imaging (MRI) as a non-invasive, highly versatile imaging platform [26,27]. This technology has developed from being only able to deliver a low resolution visualization to one which allows for the fingerprinting MRI [28] and identification of specific compounds at a high level of both spatial and temporal resolution; so a major aim of the review is to provide a clear understanding of what can be achieved by MRI. Since there are many ways to manipulate nuclear spins tailoring an experiment such, that specific information about lipids and other parameters of plants can be extracted, a methodological overview of lipid MRI has been included. Selected examples of MRI-based lipid imaging studies have been used to describe some of the major breakthroughs in the field, and to illustrate current research trends. Finally the future prospects of MRI for the acquisition of quantitative *in vivo* lipid images in living plant tissue are discussed.

2. The state-of-the-art in lipid analysis beyond MRI

2.1. Analysis of lipids in a non-spatially resolved mode

Conventional procedures for lipid analysis rely on a destructive extractive assay [29–32], coupled with mass spectrometry (MS) and/or liquid chromatography [33]. Major technological advances have featured the development of matrix-assisted laser desorption/ionization (MALDI), electrospray ionization (ESI) and tandem MS (MS–MS). Both ^{1}H- and ^{31}P-NMR spectroscopy have made a significant contribution in the characterization of lipids associated with membranes. Thus far, no single analytical platform has been elaborated which is able to measure the full spectrum of lipid components. Sample extraction/preparation is a critical step, requiring strict adherence to standardized and optimized procedures. By linking micro-sampling (e.g., laser dissection) and MS, it has been possible to quantify and localize the lipid fraction present in specific tissues/cells, as demonstrated in oilseed rape [34]. Current MS platforms are capable of a significant level of throughput, delivering sufficient resolution and sensitivity to carry out a "lipidomic" analysis of an individual cell [35] or of a lipid droplet [36]. Destructive sampling is of course undesirable where the aim is to characterize *in vivo* materials such as, prominently, an intact seed, the post analysis viability of which is necessary for any subsequent