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Review

# The potential of nuclear magnetic resonance to track lipids in planta☆

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ABSTRACT

Nuclear Magnetic Resonance (NMR) provides a highly flexible platform for non invasive analysis and imaging biological samples, since the manipulation of nuclear spin allows the tailoring of experiments to maximize the informativeness of the data. MRI is capable of visualizing a holistic picture of the lipid storage in living plant/seed. This review has sought to explain how the technology can be used to acquire functional and physiological data from plant samples, and how to exploit it to characterize lipid deposition *in vivo*. At the same time, we have referred to the current limitations of NMR technology as applied to plants, and in particular of the difficulty of transferring methodologies optimized for animal/medical subjects to plant ones. A forward look into likely developments in the field is included, anticipating its key future role in the study of living plant.
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*Abbreviations:* NMR, nuclear magnetic resonance; MRI, magnetic resonance Imaging; CSI, Chemical Shift Imaging; CSSI, Chemical Shift Selective Imaging; PRESS, Point-Resolved Spectroscopy; PFG, Pulsed Field Gradient; RF, Radio Frequency; MAS, Magic Angle Spinning; HSQC, Hetero-nuclear single quantum coherence; MALDI, matrix-assisted laser desorption/ionization; TD-NMR, Time-Domain NMR; TR, Repetition Time; TE, Echo-Time; SNR, Signal to Noise Ratio; MS, Mass Spectrometry; GC, Gas Chromatography.

☆ In memoriam of our former colleague Dr. Markus Rokitta. His creativity in plant NMR paved the way for current developments.

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## 1. Introduction: where and why do plants accumulate lipids?

Through photosynthesis, plants convert light into chemical energy, in the form of the complex organic compounds carbohydrates, proteins and lipids. Carbohydrates are accumulated in the plant cell in the form of polysaccharides (in particular starch within the plastids, and cellulose in the cell wall), while storage proteins and lipids are typically compartmentalized within specialized inclusions, isolated from the cytoplasm by a single layer membrane [1–3]. The partitioning of fixed carbon between distinct metabolic pathways [4], cell compartments [5] and between plant organs [6] is a dynamic process, the regulation of which remains far from being resolved [7,8].

The fully reduced carbon bonds present in lipid molecules are associated with a higher energy density than the partially oxidized ones common in carbohydrate and protein molecules; this is because lipid molecules contain few carbon-oxygen bonds, making them weight for weight more energy dense than carbohydrates. Since a smaller volume is therefore required to store the equivalent amount of energy, many plants have evolved the capacity to store energy in the form of lipids, particularly in the seed. Most of the storage lipid content of plants exists in the form of triacylglycerides [9], although waxes are also encountered (for example crystalline wax *Myrica pensylvanica* (bayberry) [10], liquid wax in *Simmondsia chinensis* (jojoba) and others [11].

The proportion of a seed represented by lipid varies tremendously, but can reach as high as 70% in some nut species. Oils tend to be abundant only in the seed or fruit, but a few examples of other oil-rich organs have been documented, notably the tubers of *Cyperus esculentus* [12]. A ready source of available energy is particularly important for the germinating seed, supporting it until seedling has established its own photosynthetic apparatus.

## 2. The advantages of nuclear magnetic resonance (NMR) as an imaging and analytical tool

Plant “lipidomics” emerged with the initial aim to characterize lipids extracted from plant tissues, and has evolved into a broad-ranging study which comprises various aspects of lipid metabolism and functionality. Unfortunately, most of conventional techniques for tracking lipids rely on a destructive assay, which can only provide a static measurement of the lipid content of a specific set of cells or plant organs. The task of data integration appears to be limited, as evidenced in most of the performed studies, and the next challenge is to understand how these molecular, biochemical and structural characteristics work together in a living organism. In contrast, NMR offers a means to non-destructively (and therefore dynamically) assay lipids in living tissue, and thereby facilitates following the plant's growth and development, and its response to its exogenous environment.

Unlike the assays required for optical microscopy (where lipid detection is based on either staining or immuno-labeling), NMR does not require any labeling, nor does it entail complex sample preparation. In addition to detecting lipids, it can in principle also measure a number of physical and other chemical parameters, allowing an increasingly comprehensive picture of the *in vivo* status of the subject to be captured. A particularly powerful variant of NMR is magnetic resonance imaging (MRI), which, unlike X-rays, is based on the use of non-ionizing radiation, and is therefore non-harmful to the subject. In addition, MRI generates three dimensional images, thus overcoming the major drawbacks of both Fourier Transform Infrared Spectroscopy (FT IR) imaging and optical microscopy.

The capability of MRI to separate chemical compounds is much lower, as compared to gas chromatography [13], for $^{1}$H-Lipid-Imaging it is restricted to the compounds shown in Fig. 1. Nevertheless, MRI is the only method which allows for both visualization and quantification of lipid in living plant.

## 3. The value of imaging of plant lipids *in vivo*

The need to develop the means to non-invasively detect lipids has been driven by the medical diagnostics sector, and has led to the elaboration of a number of NMR based protocols [14]. In the plant sciences, where destructive sampling has long dominated, advances in technology have concentrated on tissue dissection and extraction methodology. Although some of these analytical methods have achieved impressive levels of chemical resolution and sample throughput, reconstructing a living environment from data derived from destructive sampling can be extremely difficult or at best be based on a set of suggestions.

With the growing demand for oil, the modern requirements in plant biology and biotechnology are changing faster than ever before [15]. Various metabolic engineering strategies are developed for manipulation of oil content and composition in vegetative and seed tissues of plant [16,17]. The pressure to implement the non-invasive measurement of lipids in plants reflects progress in at least three disparate areas. The first relates to technical improvements in the productivity of established oil crops, in particular oil palm (*Elaeis guineensis*), soybean (*Glycine max*), oilseed rape

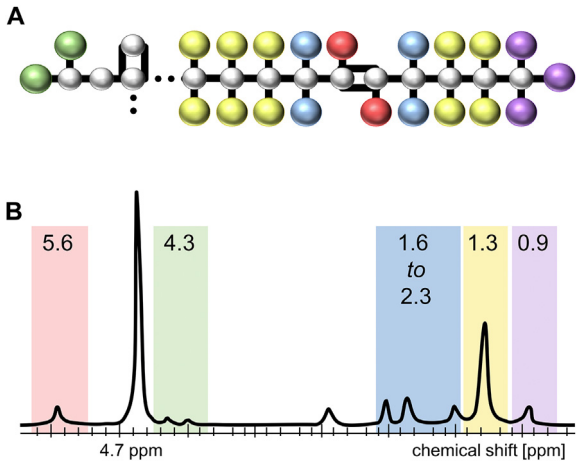


**Fig. 1. Interpretation of 1H NMR spectrum.** (**A**) Scheme shows fragment of lipid molecule made up from carbon (white) and hydrogen (variegated). (**B**) $^{1}$H NMR spectrum (simplified) displays the signals acquired from protons of shown lipid molecule. The NMR frequency of the signal of protons varied corresponding to their molecular environment, as shown by the same color-code. Red: –**H**C=C**H**–; green: glycerol backbone; blue: $(CH_2)_n$–C**H**$_2$–HC=CH, C**H**$_2$–COOH, C**H**$_2$–$CH_2$COOH; yellow: $(CH_2)_n$; purple: $(CH_2)_n$-C**H**$_3$; The water peak appears at 4.7 ppm. The intensity of the individual peaks is informative for quantitative interpretation of the spectrum.

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