Proton NMR and MRI Study of Sub-millimeter Sized Biological Objects

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Introduction

NMR microscopy has been conducted since the onset of MRI of biological applications and significant advances have been made recently [1-4]. MR microscopy has been used for studying seeds complementary to traditional methods and recent studies have largely focused on the monitoring of water uptake and oil distribution during imbibition, which is a crucial process in seed germination [5,6]. Although many NMR and MRI studies have been conducted on various seeds, very "small (<1mm) dry seeds" have not been studied extensively except one study [6]. In our study, the fundamental measurements (NMR spectra, T_1 and T_2) of small dry seeds of four different plant species were conducted by MR imaging and spectroscopy using NMR microscopy system which is capable of investigating miniature objects (up to 750μm in diameter and 2mm in length). In addition, the spectroscopic and anatomical changes of a single *Nicotiana tabacum* (tobacco) seed embedded in wet sand were monitored during an extended imbibition to evaluate the role of MRI to study germination and as a complementary method to the conventional conductivity test.

Methods

Dry seeds of *Oenothera drummondii* (primrose), *Petunia axillaris* (petunia), *Nicotiana tabacum* (tobacco) and *Silene compacta* (silene) were prepared in glass capillaries (750μm inner diameter and 1000μm outer diameter) and sealed by a UV-curing epoxy. All NMR/MRI experiments were conducted on a 9T/10cm magnet controlled by a NTNMR console (TecMag, Inc., Houston, TX, USA) with custom-built RF and gradient coils [5,6]. Proton spectra were acquired with TR of 15s and 16 NSA. T_1 of the seeds were measured with inversion-recovery (IR) spin-echo sequences (TR/TE = 15s/20ms, 8 signal averages). Recovery times were incremented from 50ms to 2500ms. T_2 was measured using Carr-Purcell-Meiboom-Gill (CPMG) and CPMG-FID sequences. CPMG (TR = 15s, interval between echoes = 0.9ms) was used for analysis of overall T_2 and CPMG-FID for T_2 of two major peaks in the spectra. All data were analyzed by mono- or bi-exponential curve-fits using IGOR Pro (WaveMetrics, Inc., Lake Oswego, OR, USA) software. Images were acquired for dry seeds of primrose, petunia, silene and *Begonia subvillosa* (begonia) with a 3D spin-echo sequence (TR/TE = 1000/3.4ms, matrix = 256×32×32, 32 NSA). The imbibition of a single tobacco seed embedded in wet sand was monitored using spectroscopy and 3D imaging with lower spatial resolution (matrix = 256×64×16, FOV = 3.9×2.3×0.75mm^3, voxel = 15×35×47μm^3, 15 NSA) were acquired every 6 hours until no further changes were observed in the spectra. Changes in SNR were calculated for four ROIs using MATLAB software (The Mathworks, Inc., Natick, MA, USA) and plotted by IGOR pro.

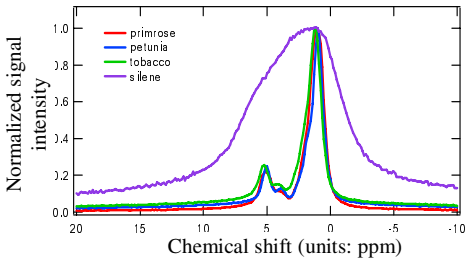

Fig 1. Comparison of normalized NMR signal intensities of four small seeds

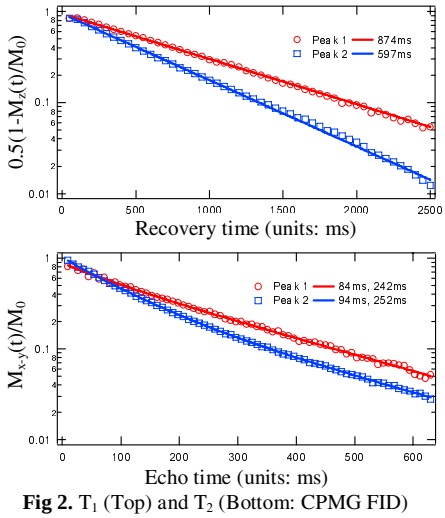

Fig 2. T_1 (Top) and T_2 (Bottom: CPMG FID)

Results

The spectral line widths of oil rich seeds were more defined with two main peaks (Fig 1). The follow-up study revealed the existence of multiple T_1 and T_2 components in all seeds (Fig 2). Silene seeds of broad line-width had much shorter T_2 (not shown here). Images of dry seeds did not show clear internal structures, presumably, due to the broad line width (Images not shown). In the study of imbibition of a tobacco seed embedded in wet sand, apparent chemical shift splitting occurred on the water chemical shift (~4.6 ppm), whereas no significant changes in seed anatomy were observed (Fig 3). Noticeable changes in SNR of exterior region adjacent to seed coat (Fig 4) were observed during imbibition.

Conclusions

Conventional spectroscopy suffers line broadening of spectra caused by solid and viscous seed material; however, it is still useful in distinguishing oil rich seeds from other seeds largely filled with solid material (Fig 1). Multiple functional groups visible with MAS NMR techniques are obscured by the line broadening effect, however, two major peaks are observable and T_1 and T_2 of the peaks could be estimated except for silene seed (Fig 2). Image resolution is impaired due to line broadening by seed material. However, it is still useful in the study of water uptake in the physiological processes in the seeds, because once water content increases mobile water increases MRI visible signal and results in better resolution. Apparent splitting of chemical shift (Fig 3) and noticeable change in SNR (Fig 4) of exterior just next to the dark seed coat implies certain leakage of seed material during the imbibition. This finding (with further study) could be used potentially as a complementary method to conductivity test for assessment of seed quality. Furthermore, the current NMR microscopy system potentially could be used for MR biopsy.

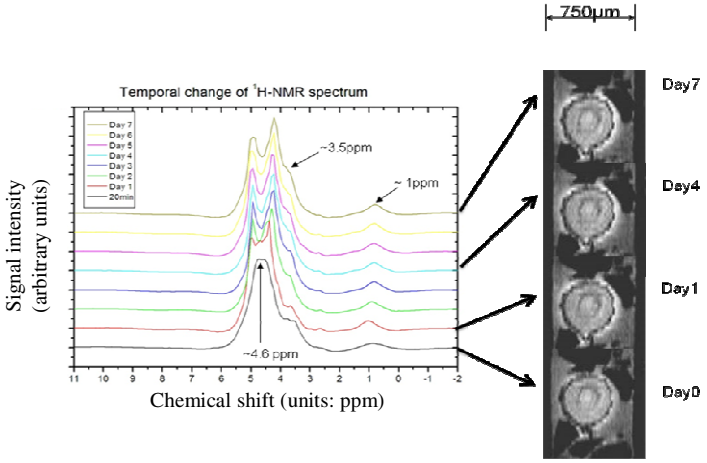

Fig 3. Spectral (left) and anatomical (right) monitoring of a single seed

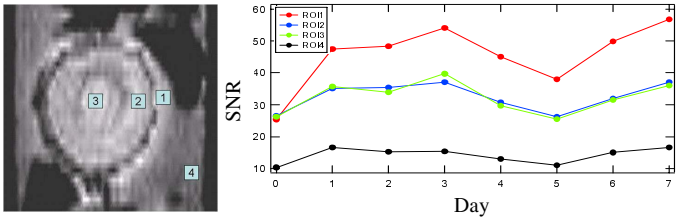

Fig 4. Changes in SNR (right) of ROIs (left) during an extended imbibition of a tobacco seed

References

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