NON-DESTRUCTIVE QUANTIFICATION OF OIL AND PROTEIN IN COTTONSEED BY TIME-DOMAIN NUCLEAR MAGNETIC RESONANCE

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Abstract

Modification of cotton seed quality traits is likely to be achieved through a combination of genetic modification, manipulation of nutrient allocation and selective breeding. Oil and protein stores comprise the majority of mass of cottonseed embryos. A more comprehensive understanding of the relationship between fiber quality, fiber yield and embryo reserve accumulation will help assist breeders in their efforts to improve seed value. Here we report a method for the rapid, non-destructive quantification of oil and protein levels within cottonseed by ^{1}H time-domain nuclear magnetic resonance (TD-NMR). This approach is suitable for a minimal amount of seed and represents an accurate, non-invasive alternative to conventional, time consuming chemical extractions.

Introduction

Manipulation of the hydrogen nuclei within oilseeds using radio-frequency pulse sequences (TD-NMR, time-domain nuclear magnetic resonance) produces characteristic relaxation signals for protein, carbohydrate, moisture, and oil. The Bruker Minispec mq20 uses a low-field magnet designed to non-destructively obtain and process these liquid and solid NMR signals.

Here we show the value of this approach by surveying seed reserve content in diverse germplasm. Previous research has established a weak negative correlation between protein and oil for several *Gossypium* varieties [Pandey 1973; Kohel 1978,1983] with weak correlations among fiber yield and oil content or fiber yield and protein content [Turner 1976; Hake 2005]. The goal is to evaluate and estimate diversity in seed oil content and seed protein content to identify lines with extremes of either. A re-evaluation of the correlations underlying these seed quality parameters will provide current breeders with valuable feedback for future germplasm development [Liu 2009].

Methods

Oil and protein content is quantified on the Bruker Minispec mq20 (Bruker Optics, Billerica, MA) in pooled representative samples from diverse cottonseed lines (≈1.5 grams composed of 10 to 25 seeds). The samples are warmed in specially designed 18-mm NMR tubes to 40^{o}C by a Techne Hybridizer HB-1D forced air oven to minimize relaxation variability within replicates.

Oil content in seeds is non-invasively measured by isolating the oil signal component through a spin-echo pulse sequence [Todt et al., 2006]. Signal intensity was directly proportional to the amount of oil. Reference oil values were obtained by gravimetric determination following total lipid extractions.

Protein content in seeds is non-invasively measured by using a Crelax pulse sequence (Fig 1 left panel) that extracts both the spin-lattice and spin-spin relaxation properties using TD-NMR. Reference protein values (41 lines) were obtained by AOCS Ba 4e-93 total nitrogen combustion (POS Pilot Plant, Canada). A chemometric model was used

to statistically correlate the signal intensities to reference protein amount (Fig 1 right panel). The protein content in the cottonseed samples was then measured non-invasively applying the chemometric algorithm.

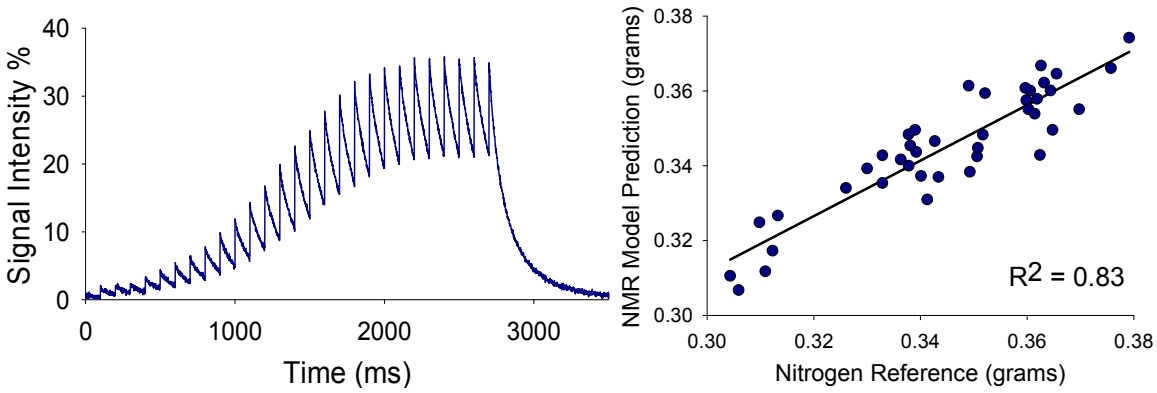

Figure 1: The TD-NMR Crelax pulse sequence (left panel) for quantifying protein by applying a chemometric algorithm to statistically correlate the signal intensities to reference protein amount (right panel).

Results

Germplasm Survey for Seed Protein and Oil

A germplasm survey of a genetically diverse Pee Dee germplasm (Fig 2) yielded a broad range of oil and protein content, 15.3 - 21.2% and 16.6 - 25.2% respectively. Across the germplasm, a very weak positive correlation (r = 0.19) between protein and oil was measured that deviates from a very weak positive correlation described in previous literature [Pandey 1973; Kohel 1978,1983]. Variability within plot replicates and across multiple harvesting years (data not shown) provides breeders with quantitative trait information that can be used to select for future germplasm development.

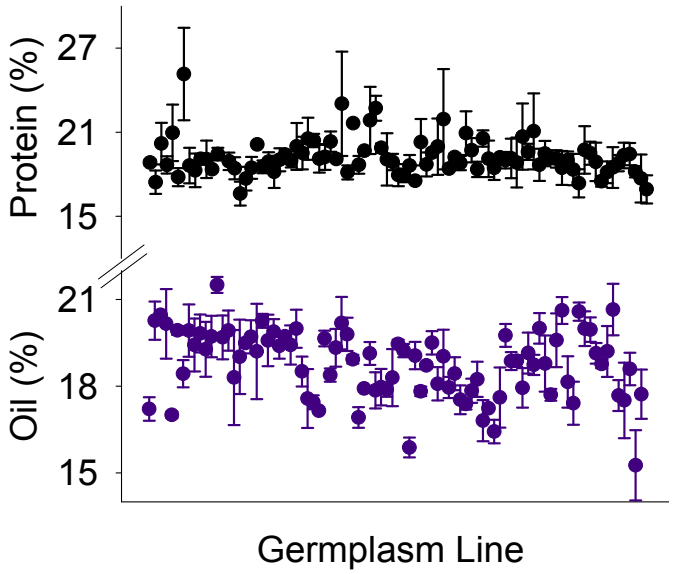

Figure 2: Representative mean protein and oil content (n=3) of a genetically diverse Pee Dee germplasm replicated across three plots. Sorted by line identification.

Conclusions

TD-NMR provides a non-destructive alternative to chemical extractions for quantifying protein and oil within cottonseed. Estimates of protein content were less accurate than for oil, especially across diverse germplasm, but future improvements in chemometric modeling are planned to reduce this variability. Reproducibility was better

within varieties suggesting that signal interference across diverse germplasm must be accounted for. Germplasm surveys provide oil and protein quantities to breeders that can be used for the selection of desired quantitative traits.

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