Extending the Use of Time-domain ^{1}H-NMR for Rapid and Non-invasive Quantification and Characterization of *In-situ* Lipids in Transgenic Bioenergy Crops

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Project Goals

The main objective of ROGUE (Renewable Oil Generated with Ultra-productive Energycane) project is to engineer the two most productive American crops—energycane and *Miscanthus*—to produce a sustainable supply of biodiesel, biojet fuel, and bioproducts. On successful completion of the project, modified bioenergy crops would generate approximately 15 times more oil per acre than seed crops like soybean. The present work demonstrates the development of a non-invasive technique for rapid and chemical-free quantification and characterization of the total in-situ lipids in transgenic bioenergy crops.

Abstract

Biofuels provide green alternatives to the fossil-fuel. However, for biodiesel, we are still dependent on oilseeds, which also constitute part of human food. Bioenergy crops with high triacylglyceride content have the potential to replace oilseeds. To this end, Andrianov et al (2010), Sanjaya et al (2013), and Zale et al (2016) have successfully reported enhanced TAG accumulation in *Nicotiana tabacum*, *Arabidopsis thaliana*, and sugarcane respectively, (Andrianov et al., 2010; Sanjaya et al., 2013; Zale et al., 2016). For energycane, research efforts are underway (ROGUE project) to accumulate TAG molecules. The analysis of *in-situ* lipid contents during developmental stages of transgenic lines and subsequent to each bioprocessing protocol requires tedious sample preparation and extraction with an organic solvent. Therefore, time-domain ^{1}H-NMR spectroscopy has been successfully adapted for the quantification and characterization of *in-situ* lipid in bioenergy crops that eliminates the steps involved in sample preparation and solvent extraction. Td ^{1}H-NMR was

calibrated to quantify *in-situ* lipids. The measured values with NMR spectroscopy were validated by comparing them with the values obtained from the conventional solvent extraction method. The cross-referenced values were not significantly different. Moreover, the relaxation time distribution spectrum was analyzed to characterize i*n-situ* lipids into bound and free form. It also helped in investigating the suitability of pretreatment protocols for transgenic bioenergy crops by facilitating the resolution of the effect of pretreatment protocols on the local proton-population of the biomass sample upon pretreatment. Biomass pretreated with a two-staged pretreatment protocol showed promising results for recovery of both fermentable sugar and oil and can be directly analyzed by NMR without neutralization steps, unlike dilute acid and alkaline pretreated biomass.

References / Publications

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