Screening of perennial grasses and a mutant maize collection by Fourier-Transformed InfraRed (FTIR) spectroscopy for improved biofuel traits

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Introduction Currently the potential of biomass crops, including grasses, is limited because most species have not been bred for this purpose. However traits such as lignification, phenolic cross-linking and carbohydrate accessibility, which are also important for nutritive quality in forage grasses, can affect potential biofuel quality in applications such as combustion, fast-pyrolysis or fermentation. A collection of *Lolium* and *Festuca* species known to exhibit a range of lignin, cell wall phenolic and carbohydrate concentrations have been used to test optimum characteristics for biofuel processing. This collection formed a "calibration" set for subsequent high through-put FTIR chemical screening of additional plant lines: (1) A set of *Lolium-Festuca* substitution lines, in which *L. perenne* chromosomes or chromosome segments are substituted by homoeologous regions of *F. pratensis,* that provide the potential to physically map biofuel traits to an individual chromosome or chromosome segment; (2) A maize transposon (Robertson's *Mutator*) induced mutant collection, which provides the potential to identify gene sequences underlying important biochemical traits linked to biofuel as determined by FTIR analysis.

Materials and methods. High through-put biochemical analyses of plant material were undertaken using FTIR spectroscopy followed by some molecular genetic analysis. Plant material was finely ground and subjected to the Attenuated Total Reflectance (ATR) FTIR. Alternatively leaf discs or sectioned material were analysed by transmission mode FTIR. Maize PCR was performed using *Mutator* and gene specific PCR primers to screen candidate plants identified by FTIR analysis.

Results *Lolium* and *Festuca* species were analysed by FTIR to produce calibration sets, e.g. for lignin content (Figure 1). After appropriate data correction, the application of simple Principle Component Analysis (PCA) to the complete FTIR spectra indicates that different grass genotypes can be readily distinguished by these techniques. Data on the spectral "signatures" from detailed FTIR analyses of known cell wall biochemical mutants will be compared to newly characterised plants along with the available genetic characterisation data.

Conclusions The data from this study indicates that FTIR is a sensitive, robust and rapid screening method that following the application of appropriate calibrations can be used to detect differences in a broad range of cell wall biochemical components. When coupled to rapid methods for identifying the associated genes this provides a powerful tool for integrating trait analysis with the underlying molecular biology. This work has the potential to identify the biochemical and molecular bases of a number of quality traits which affect the suitability of grasses for use as livestock or biofuel based feedstocks.

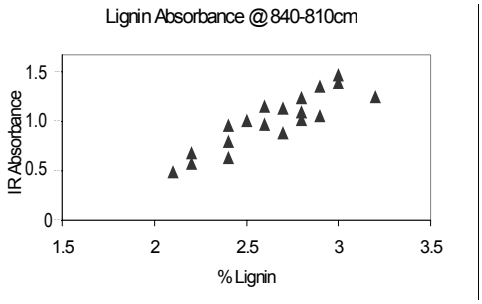

Figure 1 The sum FTIR absorbance values at 840 and 810cm^{-1} specific for G- and S-lignin respectively, plotted against %Lignin derived from Klason lignin analysis