Abstract: The resilience of cell walls (CW) to enzymatic hydrolysis of carbohydrates is a major impediment to the economical production of ligno-cellulosic ethanol. The identification of DNA polymorphisms associated to increased CW degradability will accelerate the development of alfalfa (Medicago sativa L.) cultivars with superior ethanol conversion yields. Genotypes with high (D+) and low (D-) CW degradability were identified within a biomass-type population and three winterhardy-type populations. Screening was based on near-infrared reflectance spectroscopy (NIRS) predictions of CW glucose released by enzymatic saccharification. We used sequence-related amplified polymorphism (SRAP) to search for DNA variations associated to differences in enzyme released glucose. A bulk segregant analysis (BSA) of D+, D- and randomly chosen genotypes (20 plants/bulk) was performed using 42 SRAP primer pair combinations. Several polymorphisms that varied in intensity between high and low CW degradability were uncovered. Both increased and decreased intensity in D+ and D- polymorphisms were observed. The Me4/R14 primer pair generated a positive fragment in the biomass-type population (increased intensity in the D+ bulk), and showed a reverse response in the D- bulks for two of the winterhardy-type populations. Assessment of the genotypic occurrence of this fragment confirmed that polymorphism detected with BSA reflects changes in the frequency of occurrence within the different populations. Sequence analysis of the polymorphic Me4/R14 fragment in all populations is currently under way to search for homologies with sequences in gene databases. Our results show that DNA regions associated to CW degradability can be identified. Future studies will assess the value of these polymorphisms in marker-assisted breeding. We would like to thank the Agricultural Bioproducts Innovation Program of Agriculture and Agri-Food Canada for financial support.

alfalfa (medicago sativa l.), bulk segregant analysis, cell wall degradability, srap polymorphisms

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Biochemical and Molecular Analysis of Bioenergy Sorghums for Variation in Lignin Content

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Abstract: Large scale production of lignocellulosic biofuels is yet to become a reality due to technological problems, which include the interfering effect of cell wall lignin in the biomass conversion process. Therefore, selecting dedicated crops containing cell wall compositional traits favorable to superior biomass conversion is expected to make biofuel production economically feasible. Sorghum has the potential to be one of these dedicated crops because of its high biomass yield, low water and fertilizer requirements and tolerance to mineral, drought and heat stress. In this work we applied molecular and biochemical analysis to identify sorghum genotypes showing lower lignin content, which has been shown to increase biomass conversion efficiency.

A genetically diverse sorghum panel comprising 100 accessions was screened for lignin content and other cell wall components, using standard biochemical methods. The panel showed a wide range of phenotypic variation for cell wall composition. Klason lignin content varied from 2 to 12% on the basis of total dry matter and averaged 5.8%. In order to better understand lignin synthesis in sorghum, we have identified sorghum homologs of key genes involved in the lignin biosynthesis pathway, and used Real-Time PCR to study their expression. Five of these genes, C3H1, C3H2, HCT, COMT and F5H, appear to be co-regulated as suggested by highly correlated expression levels in a subset of the diversity panel. In addition, the genes C3H and HCT showed high and positive correlation coefficients. Among the panel accessions were bmr-6 (brown midrib) mutants. One of them (TX2784R bmr-6) and its corresponding non-mutant line (TX2784R) were also analyzed for their fiber and lignin content, as well as gene expression related to the lignin pathway. The bmr-6 gene encodes the CAD enzyme, which acts at a later step within the pathway. The mutant and non-mutant materials were significantly different for lignin and fiber content, with a 50% reduction in lignin content in the mutant, as reported previously. The gene expression results support the hypothesis that the genes are co-regulated since the mutation in the CAD gene is followed by a down-regulation of other genes in the pathway, such as C3H, HCT and COMT. Expression analysis of five other bmr-6 lines showing different genetic backgrounds supports these findings. Future studies will evaluate the remaining genes of the lignin pathway for their expression. Those showing differential expression among accessions that have contrasting lignin content will be validated by association analysis in order to identify superior alleles involved in lignin content.

lignin, bioethanol, sorghum, gene expression, real time PCR

Area: Advances in plant biology and biochemistry for improvement of bioenergy production and quality

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Carbohydrate Degradation of Sugarcane Straw in the Field

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Abstract: Due to the growing demand for renewable energy in the world, more investments on new alternatives are necessary, as the cellulosic ethanol, which could be produced by cell wall enzymatic degradation from different feedstocks. The cell wall is composed of cellulose, lignin, hemicelluloses and pectins. Sugarcane main celluloses are the arabinoxylans and the â-glucans. Sugarcane straw is used to obtain electricity or is left in the field for degradation. Although the straw is important as it decreases impact on land, part of it could still be used to increase the pro-