Selection for Improved Saccharification Efficiency in Alfalfa Stems Assessed by Enzyme-Released Glucose

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Introduction

Alfalfa (*Medicago sativa* L.) has a high potential for sustainable bioethanol production, particularly because of its low reliance on N fertilizer (Samac *et al*. 2006). Genetic improvement for the accumulation of readily fermentable non-structural carbohydrates (NSC) and the saccharification of structural carbohydrate (SC) could significantly increase ethanol conversion rate. Genetic gains for these traits are tributary to the availability of screening techniques for the precise identification of superior genotypes with increased potential for the production of fermentable carbohydrates.

When assessing the genetic variability of parameters linked to cellulosic ethanol production (concentrations of NSC and SC), our results showed a large genetic variability within and among winter hardy- and biomass-type alfalfa cultivars (Duceppe *et al*. 2012). We also developed an efficient enzymatic assay to measure alfalfa stem degradeability, based on the quantity of glucose released by a customized commercially available enzyme cocktail. Despite its robustness, this test is labour intensive, thus limiting analytical capabilities. Near-infrared reflectance spectroscopy (NIRS) was previously shown to successfully predict enzyme released glucose in corn stover (Lewis *et al*. 2010). This approach allowed us to screen a large number of lignified alfalfa stem samples and to identify superior genotypes. Our objective was to determine if it is possible to develop alfalfa cultivars with superior cell wall (CW) degradability.

Method

Plant material, genotypes selection and crosses

Genotypes of alfalfa of the winter-hardy cv. 54V54 and the biomass-type cv. Orca were established in a field nursery in 2008. Genotypes were pre-selected based on high biomass yield and persistence. Plants were individually harvested in September 2009 at the green pod maturity stage. For both cultivars, 20 genotypes with high CW degradability (D+) and 20 genotypes with low CW degradability (D-) were intercrossed in a greenhouse to generate D+1 and D-1 populations obtained after one cycle of divergent selection for CW degradability. Orca and 54V54 D+1 and D-1 populations (1000 genotypes for each population) were seeded and grown in the field throughout the summer.

Assessment of parameters linked with production of cellulosic ethanol

Dried and ground samples of the bottom part of the stems (25 cm) were analyzed for NSC and SC composition by High Pressure Liquid Chromatography. Cell wall degradeability was assessed by enzyme saccharification as described in Duceppe *et al.* (2012). Near-Infrared Reflectance Spectroscopy (NIR-Systems 6500 mono-chromator) was used for the prediction of concentrations of NSC, lignin and enzyme-released glucose.

Results and discussion

NIRS was successfully applied to accurately predict CW degradability in four different alfalfa cultivars (R^2=0.94),

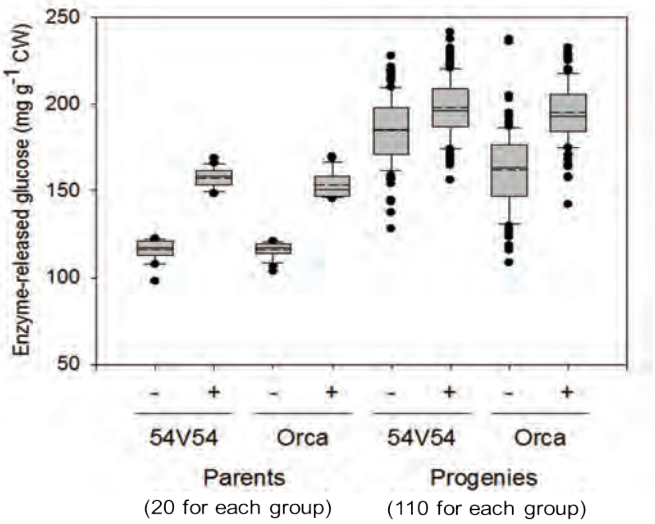

Figure 1. NIRS predictions of glucose released from alfalfa stems by enzyme saccharification in parent plants (20 plants for each group of parents) and in progenies obtained after one cycle of divergent selection (110 plants for each group of progenies). Parent plants were field-grown and the 20 most (D+) and 20 least (D-) degradable genotypes of both 54V54 and Orca cultivars were selected. Progenies were derived from crosses within the 20 genotypes of each 54V54 D-, 54V54 D+, Orca D- and Orca D+ and were greenhouse grown. The full line in boxes represents the median and the dotted lines represent the mean. A box represents the range in which 50% of the data are found while dots identify outliers.

measured by glucose released following enzyme saccharification. NIRS predictions showed that there was a large genetic diversity for enzyme-released glucose. The 10 genotypes with the highest (D+) and the lowest (D-) amounts of enzyme-released glucose of a biomass-type (Orca) and a winterhardy-type (54V54) cultivar were further characterized. D+ genotypes were at least 35% more degradable than D- genotypes. Determination of CW composition by chemical analyses showed that a higher lignin content of the D- genotypes was closely related to their lower enzyme-released glucose (R=-0.83).

For each cultivar tested, 20 D+ and 20 D- genotypes were intercrossed to generate D+1 and D-1 progenies (Fig. 1). Assessment of CW enzyme-released glucose in the progenies showed that this trait is genetically inherited. Our results show that it is possible to exploit the heterogeneity of enzyme-released glucose to produce a new germplasm more prone to enzyme saccharification. Assessment of CW degradability (enzyme-released glucose) of progenies, after one cycle of divergent selection, clearly demonstrated the possibility to create a selection program based on this trait. Progenies with contrasting CW degradability characteristics are currently being used to develop molecular markers for this trait.

Conclusion

The large genetic diversity of enzyme-released glucose and its potential for selection support the potential of alfalfa in the sustainable production of bio-ethanol.

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