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Improved saccharification efficiency of alfalfa for bioethanol production: From phenotypic characterization to marker-assisted selection

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Alfalfa (*Medicago sativa L.*) has a high potential for sustainable bioethanol production. Genetic improvement for the saccharification of structural carbohydrates could significantly increase ethanol conversion rate. Genetic gains for this trait are tributary to the availability of screening techniques for the precise identification of superior genotypes. We developed an efficient enzymatic assay to measure alfalfa stem saccharification, based on the quantity of glucose released by a customized commercially available enzyme cocktail. Using that new assay, we observed a large genetic diversity for saccharification within and among cultivars. To increase the analytical throughput, we used near-infrared reflectance spectroscopy (NIRS) to predict cell wall (CW) saccharification in hundreds of lignified stem samples. Twenty (20) genotypes with high (S+) and 20 genotypes with low (S-) saccharification (S) expressed as the levels of enzyme-released glucose were selected within each of abiomass-type (Orca) and a winter hardy-type (54V54) cultivar. These genotypes were intercrossed to generate a first cycle of recurrent selection for high (S+1) and low (S-1) saccharification. Assessment of CW enzyme-released glucose after a second cycle of recurrent phenotypic selection confirmed that this trait is genetically inherited. Populations recurrently selected for saccharification were used to identify DNA polymorphisms associated with this trait using the sequence-related amplified polymorphism (SRAP) PCR-based technique. Polymorphisms positively or negatively related to saccharification were identified in each genetic background using a bulk analysis of pooled DNA (50 genotypes/population). Subsequent scoring of these polymorphisms within each genetic backgroundled to the identification of genotypes that combine two or more polymorphisms associated to saccharification. These elite genotypes were intercrossed to generate a first cycle of marker-assisted selection with potentially higher saccharification (MAS S+1). A second cycle of MAS selection was performed to further increase the frequency of these markers in MAS S+2 populations. Comparative assessment of populations obtained with phenotypic recurrent selection and marker-assisted selection is underway to assess the complementarity of these new selection methodologies and establish their performance for the development of populations with significantly higher ethanol conversion rates in alfalfa.

Biography

Annick Bertrand has internationally recognized expertise on physiology and biochemistry of perennial forage crops. She developed new selection methods for the improvement of complex trait such as saccharification potential and abiotic stress resistance of perennials.

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