Identification of PCR-based markers tightly linked to freezing tolerance in alfalfa.

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Identification of DNA markers closely related to freezing tolerance would greatly assist breeding efforts towards the development of alfalfa (*Medicago sativa* L.) cultivars with improved winter hardiness. Our group has devised a laboratory-based screening protocol for freezing tolerance that allowed us to develop a series of populations more tolerant to freezing (TF) through recurrent phenotypic selection within different cultivars (Nadeau et al. 2002). We achieved up to seven cycles of selection (one cycle per year) within the cultivar Apica that show progressive and continuous improvement of freezing tolerance.

We used a bulk segregant analysis (BSA) approach in combination with the sequence-related amplified polymorphism (SRAP) technique (Li and Quiros, 2001) to uncover DNA polymorphisms associated to variation in freezing tolerance among Apica-derived TF populations. SRAP is a highly reproducible PCR amplification technique that preferentially targets coding regions. As an initial step, we applied the SRAP technique on the original population (ATF0) and populations derived from cycles of selection (ATF2, ATF4, ATF5 and ATF6). Pooled DNA extracts from ≈ 45 genotypes of each of these populations were used for amplifications using 42 combinations of primer sets. We identified several positive and negative polymorphisms related to variation in freezing tolerance among TF-derived populations.

To further assess the relationship between DNA polymorphisms and freezing tolerance, each of the ≈ 45 genotypes belonging to ATF0, ATF2 and ATF5 were subsequently screened for their tolerance to exposure to a single stress temperature (-12 °C) and scored for the presence or absence of bands that were previously found to be polymorphic between the bulk populations. Four set of primers generated polymorphic bands that were closely related to freezing tolerance. Our results illustrate that recurrently selected TF-derived populations in combination with an effective DNA fingerprinting approach can be used to uncover genetic polymorphism closely associated with superior freezing tolerance in alfalfa.

References:

Li, G. and Quiros, C.F. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor. Appl. Genet. 103: 455-461.

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