A bulk segregant approach to identify genetic polymorphism associated with cold tolerance in alfalfa

Y. Castonguay, J. Cloutier, S. Laberge, A. Bertrand and R. Michaud.

Agriculture and AgriFood Canada, 2560 blvd.
Hochelaga, Sainte-Foy, QC, Canada, G1V 2J3, castonguayy@agr.gc.ca

Cold tolerance is a determinant factor in the survival of alfalfa (*Medicago sativa* L.) exposed to severe winter conditions. Selection for winter hardiness in the field has historically been hindered by the unpredictability of test winters. Our group has recently devised a method of selection performed under environmentally-controlled conditions for the improvement of freezing tolerance. Using this approach, we proceeded with recurrent selections in two commercial cultivars (Apica and Evolution) to generate populations potentially more tolerant to freezing (TF). Six and four cycles of selection were respectively performed in Apica and Evolution. Assessment of freezing tolerance of plants maintained throughout winter under unheated greenhouse conditions showed striking increases in cold tolerance ranging from 3 to 8^{o}C depending on the population of origin, the number of cycles of selection and the acclimation conditions. Field evaluation of winter hardiness confirmed marked improvement in persistence and superior spring regrowth for the TF populations when exposed to stressful conditions.

Bulk segregant analysis (BSA) is an efficient approach for the identification of genes having major effects on quantitative traits (1,2). It allows candidate genes potentially associated to a trait to be tested for differences in allele frequency between populations derived from a given genetic background and selectively improved for that trait of interest. Pooled DNA extracts from 45 genotypes from each of the two original alfalfa cultivars and their derived TF populations selectively improved for superior freezing tolerance were hybridized with candidate genes typically associated with cold acclimation. Polymorphisms that intensified with the number of selection cycles were uncovered for homologs of galactinol synthase (GS), dehydrins and chitinases. The likelihood that these polymorphic markers are genetically linked to a QTL controlling freezing tolerance is high considering that selection was performed within a single genetic background and was solely targeted towards the improvement of that trait. Although an identical polymorphism was observed in the two genetic backgrounds (Apica and Evolution) with the GS gene, distinct profiles were obtained with the dehydrin and chitinase genes for the two populations. These observations hint at the commonalities and differences in the adaptative gene complements that are present in the two genetic backgrounds. Our results illustrate that the combination of unique genetic material and BSA can help elucidate molecular and genetic bases of superior cold tolerance in alfalfa and identify molecular markers to be used in breeding programs.

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(2) Quarrie, S.A., V. Lazic-Jancic, D. Kovacevic, A. Steed and S. Pekic. 1999. Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize. J. Exp. Bot. 50: 1299-1306.