Cold Tolerance Assessment and Biochemical Analysis of Red Clover Populations Selected for Improved Cold Tolerance

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Red clover (*Trifolium pratense* L.) is not reliably persistent in cold-winter regions where it can be winterkilled. Further improvement of cold tolerance in red clover is much needed to expand its reliability and to increase its use in areas experiencing harsh winter conditions.

A new method of selection has recently been developed at AAFC Ste-Foy, for the development of alfalfa populations with improved cold tolerance. The approach relies on the application of successive freezing stresses under environmentally-controlled conditions to progressively eliminate 90% of the less cold tolerant genotypes within the population. This method produces striking increases in cold tolerance that translates into improvement in persistence and yield of alfalfa exposed to stressful conditions in the field.

The goal of the present study was to evaluate the applicability of this selection method for the improvement of cold tolerance of red clover. Freezing stress selection has been achieved within a population from the red clover breeding program at AAFC Charlottetown (CRS15). Two populations potentially more freezing tolerant (TF) have been obtained: CTF1 and CTF2. The three populations (CRS15, CTF1, CTF2) have been characterized for their cold tolerance under controlled conditions and for their persistence under field conditions.

For all populations, the levels of freezing tolerance reached by acclimated plants were unexpectedly low at about -8.0°C. Furthermore, the lethal temperature for 50% of the plants (LT_{50}) did not reveal differences between the selected populations and the original one. This low acclimation potential was confirmed by field persistence assessment. We did however observe typical cold induced biochemical changes such as increases in raffinose and sucrose concentrations as well as starch hydrolysis at low temperature. In some cases biochemical responses differed between the three populations. For instance, starch reserves and the pool of free amino acids were lower in selected populations as compared with the original background. These biochemical changes indicate a response of red clover to selection pressure that did not however translate into superior cold tolerance. These results reflect the multigenic character of cold tolerance requiring concomitant modifications in several parameters to be expressed.

Lack of improvement responses in red clover does not imply that the method can not be applied to this species. Our results rather indicate that the use of populations harboring adaptation alleles in their genetic background is a necessary requirement for a strong response to freezing stress selection.