Characteristics of Alfalfa Populations Selected for Improved Freezing Tolerance.

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Cold tolerance is the most important factor in field survival of alfalfa (*Medicago sativa* L.) grown under severe winter conditions. Selection for winter hardiness in the field has historically been difficult due to the unpredictability of environmental conditions. A method of selection performed under environmentally-controlled conditions has been applied for the development of populations of improved cold tolerance and winter hardiness. Three cultivars of alfalfa have been exposed to multiple cycles of selection. For each selection cycle, 1500 genotypes of each cultivar were subjected to three successive freezing stresses consisting of cold hardening (2 wk at 2°C and 2 wk at -2°C) followed by a step-wise decrease in temperature down to the expected LT_{50}. After the third stress, the 100 most vigorous genotypes were selected and intercrossed to generate potentially more cold tolerant (TF) populations. Between two and five cycles of selection were applied depending on the cultivars. The LT_{50} of the populations maintained althrough winter under unheated greenhouse conditions showed a striking increase in cold tolerance from   -22°C in the original cultivars to -30°C after five cycles of selection. Cold tolerance improvement was not related to any observed changes in gene expression as assessed by in vitro translation and Northern blot hybridization. Levels of metabolites such as amino acids and cryoprotective sugars typically associated with cold acclimation remained unchanged. However, the levels of three thermostable proteins progressively increased with the enhancement in freezing tolerance. Field evaluation of winter hardiness and spring yield at three experimental sites showed marked increases in persistence and yield for some of the selected populations when exposed to stressful conditions. Our results suggest that major increases in winter hardiness of alfalfa can be achieved by cold tolerance-targeted selection.