Effect of plant age on cold acclimation and freezing tolerance of alfalfa

Yves Castonguay, Paul Nadeau and Réal Michaud

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

Fall-seeded plants of alfalfa (*Medicago sativa* L.) acclimated to simulated winter conditions in an unheated greenhouse can achieve very high levels of freezing tolerance with LT_{50} that vary between -20°C and -30°C. This level of resistance is superior to the threshold temperature of -15°C at the crown level generally recognized as critical for winter survival in eastern Canada. The objective of our study was to compare the freezing tolerance and associated cold-induced molecular changes of fall seeded plants with that of plants grown under usual field conditions. Plants of the cv AC Caribou were seeded in spring of 2000 and grown under environmentally-controlled conditions for 8 weeks before being cut and transfered to the field. Plants were subsequently transplanted as hills (8 plants/hill) spaced 90 cm apart in a location adjacent to an unheated greenhouse on an experimental farm located near Quebec City. Assessments were made in two consecutive overwintering periods (2000-2001 and 2001-2002) to evaluate the effect of field plants aging using a new fall seeding in each year for comparison. Fall-seeded plants were established under environmentally-controlled conditions for 5 weeks and were then transfered to the unheated greenhouse for their acclimation to naturally declining photoperiod and temperatures. Field plants were dug out in mid-November and overwintered in the unheated greenhouse under the same environmental conditions than fall-seeded plants. In both years, field-grown plants showed strikingly lower freezing tolerance than their fall-seeded counterparts (LT_{50} of -17°C vs -30°C). We also observed lower levels of freezing tolerance of field-grown plants in their second year (LT_{50}: -16°C) than in the first year (LT_{50}: -18°C). Whether this observation is attributable to seasonal differences or physiological responses is unknown. However, samples taken directly from the field in January confirmed that lower levels of freezing tolerance were the result of plant aging rather than a side effect of rooting out plants in the fall. Reduced freezing tolerance of field-grown plants was consistently associated with markedly lower levels of expression of cold-regulated genes, failed accumulation of the stress-related amino acid proline and lower content of starch reserves. Although levels of the oligosaccharides stachyose and raffinose and the cold-inducible amino acids arginine and histidine were higher in field-grown than in fall-seeded plants in the first year, this response was reversed in the second year indicating a weak relationship between their accumulation and the developmentally-induced differences in cold tolerance. This assay will be pursued for a third year in winter 2002-2003 to further confirm the effects of plant aging on alfalfa adaptation to cold. These results show the importance of stand aging in the determination of freezing tolerance of alfalfa and may have implications for plant improvement program and management practices.