



CHAPTER 16

Increasing Winter Hardiness in Alfalfa

Annick Bertrand and Yves Castonguay

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Alfalfa (*Medicago sativa* L.) is the most important forage legume cultivated in North America. Despite its broad adaptation to various climates, harsh winter conditions cause recurrent winterkill to alfalfa in Canada and in other cold regions of the world. Exposure to extreme low temperatures coupled with insufficient levels of freezing tolerance is the main cause of winter injury and associated yield reduction. Perennial plants undergo extensive physiological and molecular modifications during fall which increase their tolerance to subsequent exposure to subfreezing temperatures. For example, unacclimated alfalfa will not tolerate a mild freeze of -5°C (23°F) during summer but, after an acclimation period, it will withstand winter temperatures of -15 to -20°C (5 to -4°F). Climatic factors and crop management practices can reduce freezing tolerance by either interfering with the cold acclimation process or by exposing the plants to injurious, sub lethal temperatures. However, freezing tolerance of alfalfa can be improved through breeding.

Climatic factors affect freezing tolerance

Declining temperatures in the fall trigger acclimation of alfalfa to cold, with temperatures below 5°C (41°F) accelerating the acquisition of freezing tolerance. Thus, erratic

increases of air temperature, particularly temperatures far above the optimal 5°C (41°F), can slow the process of cold acclimation. Overwintering plants must withstand the direct effects of subfreezing temperatures and secondary stresses that reduce the capacity of the plants to tolerate exposure to subfreezing temperatures. Among the most stressful factors is encasement of plants in ice, usually the result of freezing rain or freeze-thaw cycles. This may cause anoxic conditions that impair the process of cold acclimation (Bertrand et al. 2001). Furthermore, plants encased in ice are often exposed to very low air temperatures because ice has a higher thermal conductivity than snow. Ice encasement as well as soil heaving can cause direct physical damages to plants. An adequate level of freezing tolerance is maintained throughout winter in fully acclimated alfalfa if crown temperature remains slightly below the freezing point. Conversely, exposure to temperatures above 0°C (32°F) during winter can cause a loss of freezing tolerance. This process, called cold de-acclimation, can compromise winter survival of alfalfa if plants are subsequently exposed to a deep frost.

Management

The capacity of alfalfa to both withstand winter and to resume growth vigorously in spring relies on reserve carbon

(C) and nitrogen (N) stored in the perennial taproots and crowns. Thus, management and other factors affecting the accumulation of reserves will affect winter survival and regrowth of alfalfa in the following spring. In Québec, cutting strategies must include a rest period of at least 500 growing degree-days (GDD; 5°C/41°F basis) in the fall to ensure the replenishment of taproot C and N reserves. During the second and third year after establishment, spring yield of alfalfa can be reduced if a fall harvest is taken after a rest period of less than 400–500 growing degree-days due to poor reserves of C and N in the root (Fig. 1). Taking a fall harvest can even result in total mortality during a harsh winter (Fig. 1). Therefore fall cutting should be managed based on the following index, from low to high risk: 1) no fall harvest; 2) fall harvest taken 600 growing degree-days after the last summer harvest; 3) fall harvest taken 500 growing degree-days after the last summer harvest; 4) fall harvest taken less than 400 growing degree-days after the last summer harvest (Bélanger et al. 1999).

Elevated soil moisture, unbalanced phosphorus (P) and potassium (K) fertilization, and root diseases are additional factors interfering with the accumulation of reserves in taproots in fall, all contributing to reduced alfalfa persistence. The age of alfalfa stands also affects winter hardiness; freezing tolerance is very high for young seedlings (around -30°C/-22°F) but decreases drastically in the second winter (around -15°C/5°F) and declines further in the third and fourth winters (Fig. 2). This could be linked with a greater capacity of young plants to undergo molecular changes endowing freezing tolerance, such as the accumulation of organic reserves, and because younger plants have lower disease infestations and have been exposed to less physical damage. Strategically balanced age distribution of alfalfa stands on the farm would minimize the risks of winter losses.

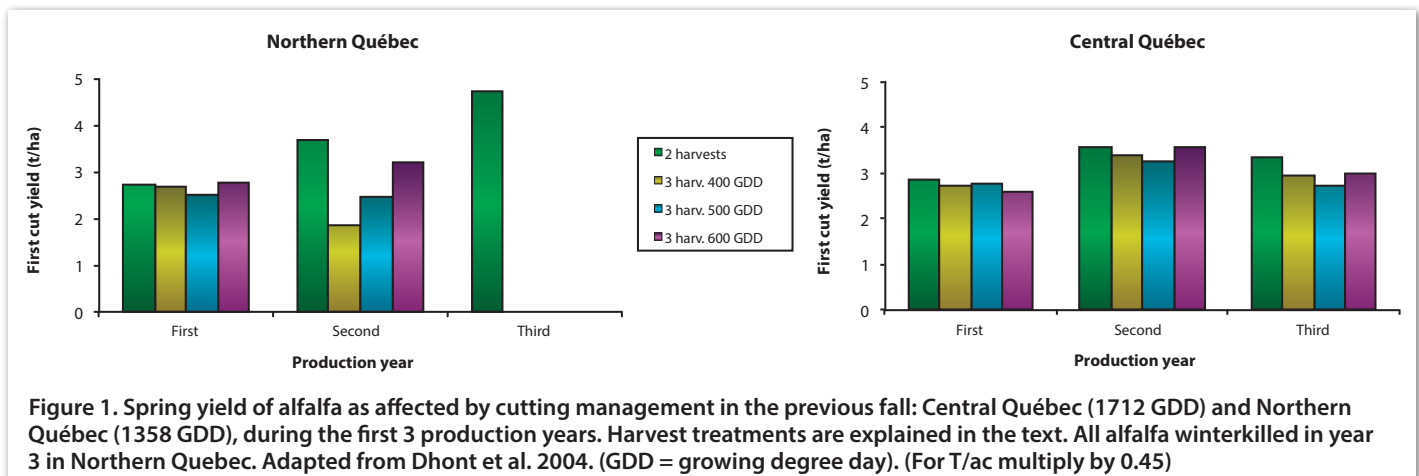
The choice of cultivars also impact winter survival and yield. Fall-dormant alfalfa cultivars, well adapted to harsh winter conditions of Canada, exhibit much better winter survival than less dormant cultivars but at the cost of lower forage yield. This is explained by the faster shoot regrowth

after cutting of less dormant cultivars in fall. Generally, choice of cultivar is a trade-off between winter survival and high yield. However, research suggests that it might be possible to dissociate the genetic components of dormancy, and that it is possible to select for superior freezing tolerance without increasing plant dormancy (Castonguay et al. 2006a).

Breeding

Improvement of alfalfa winter hardiness has been achieved traditionally through selection of genotypes that survive severe winter conditions within populations with good agronomic performance. This is a tedious process that relies on the chance occurrence of challenging winters and the costly maintenance of multi-year tests. Improvements in winter hardiness of alfalfa have been achieved through the conventional approach in eastern Canada but significant winter damage is still a frequent occurrence. This is due both to fall and winter climatic conditions that do not allow the expression of the genetic potential for freezing tolerance or to exposure of plants to extreme subfreezing temperatures.

Breeding for superior winter hardiness is difficult due to the multi-gene nature of freezing tolerance. Plant acclimation to low temperatures involves a cascade of molecular events to evoke the adaptive responses. Plants need to sense low temperatures and to transduce that signal to activate the expression of cold-regulated genes or to repress it in the case of genes that interfere with the acquisition of freezing tolerance. These initial responses must then be translated into biochemical and ultra-structural modifications in cells that will confer superior tolerance-to-freezing at the cellular level. Most of these modifications aim at the maintenance of structural and functional membrane integrity of cells due to both cell dehydration caused by extracellular freezing and to direct damage caused by the growth of ice crystal inside or outside the cells. Because hundreds of genes are involved in the process of freezing tolerance acquisition and because of a strong genotype by environment interaction, breeding for this trait is very complex.



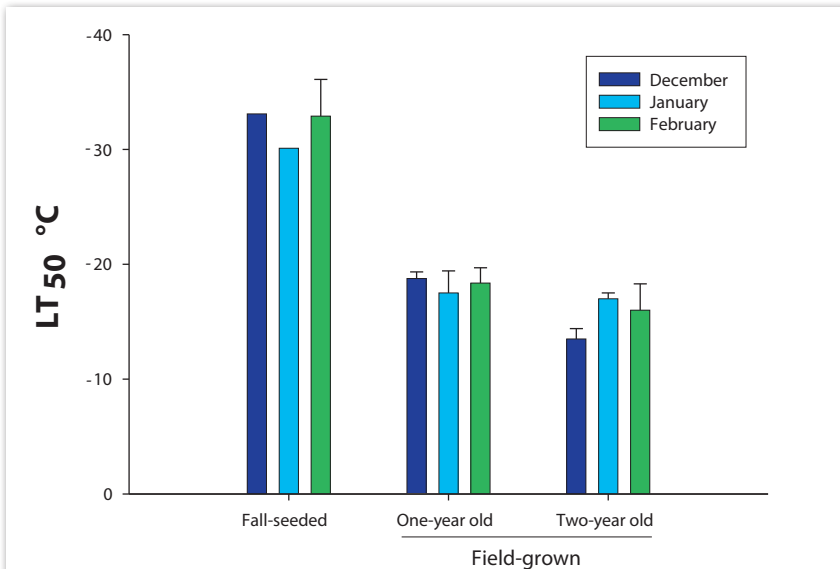


Figure 2. Effect of stand age on the freezing tolerance of alfalfa in an unheated greenhouse; LT50 is expressed as the temperature killing 50% of alfalfa plants in freezing tests conducted three times during winter. Fall-seeded alfalfa was seeded under controlled conditions and acclimated, while field grown alfalfa was transferred from the field for overwintering. See text for explanation. Data from Castonguay Y., Nadeau P. and Michaud R. (Unpublished).

Breeding approaches

Conventional

Conventional breeding of open pollinated species exploits natural genetic variability between and within populations. The freezing tolerance (LT50: lethal temperature for 50% of the plants) of a small subset of 10 genotypes randomly selected within the cultivar Apica which is well adapted in Québec ranged from -11.9 to -17.7°C (10.6 to 0°F) (Castonguay et al. 2006b). This illustrates the significant genetic variability for this trait within a cultivar. It also highlights the large unexploited reservoir of genetic material harbouring superior potential for freezing tolerance in alfalfa.

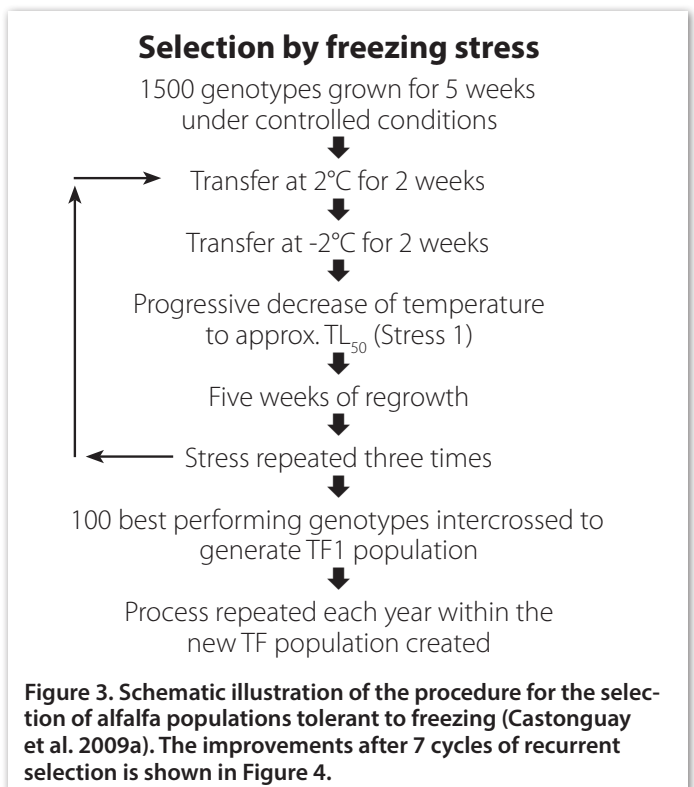
The efficiency of freezing tolerance improvement is dependent on the availability of reliable phenotyping assays or tests that minimize genotype by environment interactions. Selection of genetic material with superior freezing tolerance is best achieved using standardized indoor tests performed under environmentally-controlled conditions that preclude irregular fluctuations occurring in the field. A method of recurrent selection, based on identifying and then intercrossing the 10% most vigorous genotypes after three controlled freezing stresses, was employed towards the development of alfalfa populations with improved tolerance-to-freezing (Fig. 3). The freezing tolerance of these new tolerance-to-freezing populations was improved by around 5°C (9°F) compared to the original populations after only a few cycles of selection (Fig. 4). Moreover, these tolerance-to-freezing populations survived better and yielded more than the original cultivars at field sites that experienced severe winter conditions (Castonguay et al. 2009 a).

Field assessments of tolerance-to-freezing populations showed that there was no negative impact on other agronomic traits. Although the selection for freezing tolerance under environmentally-controlled conditions is more predictable than under field selection, the process remains lengthy and requires several cycles of selection to achieve the desired level of tolerance. However, this method of recurrent selection produces genetic material enriched with genes associated with superior freezing tolerance.

Marker-assisted selection

Marker-assisted selection is a process whereby a marker, based on variation in protein or DNA, is used for the indirect selection of a trait of interest. The identification of molecular markers and their subsequent integration into marker-assisted selection approaches could greatly accelerate conventional breeding approaches in the improvement of multigenic

traits such as freezing tolerance. A DNA marker is typically derived from a small region of DNA that differs in its nucleotide sequence between individuals within a species. These differences are called DNA polymorphisms and they could be considered as a signpost for the presence of a gene linked to the trait of interest. Bulk segregant analysis of pooled DNA samples is a simple but powerful approach to identify genetic differences associated to cold adaptation



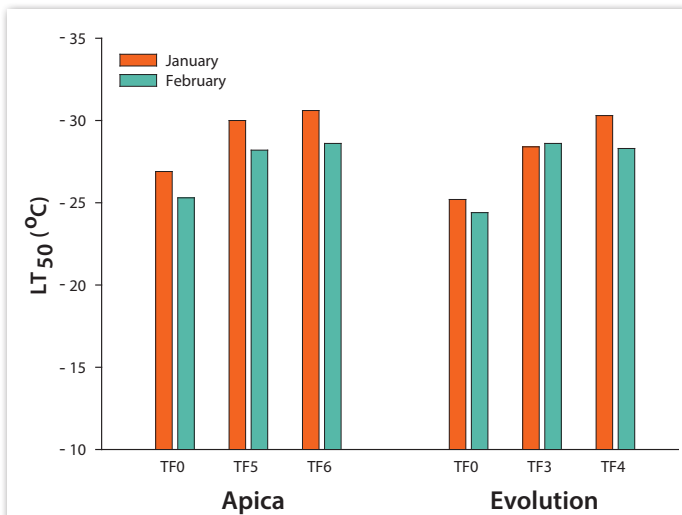


Figure 4. Freezing tolerance of the Apica and Evolution populations (TF0) compared to recurrently selected populations (T 3–6, where number indicates cycles). Freezing tolerance is expressed as LT₅₀ during two freezing tests undertaken in January and February. (TF = tolerance-to-freezing).

among tolerance-to-freezing populations recurrently selected for increased freezing tolerance (Castonguay et al. 2010).

Using bulk DNA samples from the cultivar Apica (TF0), and populations derived from that initial background after two (TF2) and five (TF5) cycles of selection, alfalfa was screened for DNA polymorphisms using a polymerase chain reaction based gene amplification technique targeting preferentially coding sequences at random. Polymerase chain reaction is a molecular biology technique consisting of amplifying a specific DNA segment to generate a high amount of copies. It is then possible to characterize the

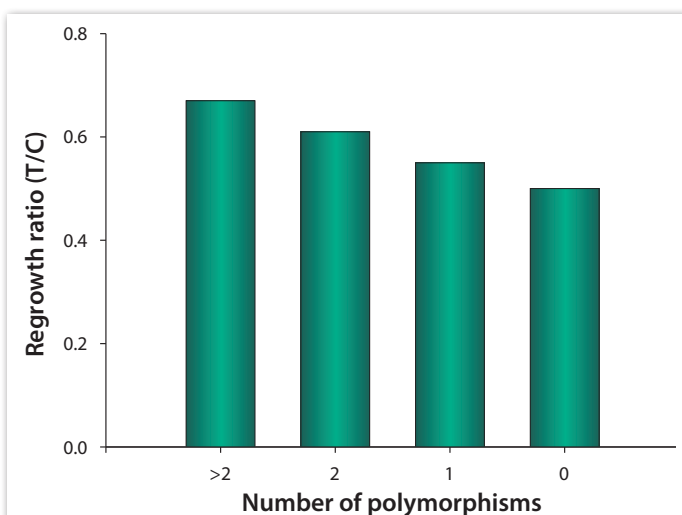



Figure 5. Relationship between the level of freezing tolerance and the number of polymorphisms in genotypes of cultivar Apica. Genotypes were grouped by the number of polymorphisms that they accumulate (0 to 4). Freezing tolerance was rated as the ratio of regrowth of 6 clonal propagules exposed to -12°C (T) to regrowth of 6 unstressed controls (C). A ratio value of 1 reflects almost complete tolerance to the freezing test. Adapted from Castonguay et al. 2010.

source of the observed polymorphism by sequence analysis.

DNA polymorphisms that increase in frequency in response to selection pressure were uncovered by Castonguay et al. (2010). Assessment of individual genotypes confirmed a close relationship between the number of polymorphisms and the level of freezing tolerance; 34% more genotypes survived -12°C (10°F) when they possessed more than two polymorphisms compared to those that had none (Fig. 5). Recently polymorphic sites within dehydrin genes were shown to be closely linked with superior freezing tolerance in alfalfa (Rémus-Borel et al. 2010). Dehydrins belong to a family of proteins induced by environmental stresses whose accumulation has been linked to superior adaptation to cold in many plant species. These novel findings could pave the way to the development of functional markers to screen for adaptive alleles in breeding populations.

Alfalfa winter hardiness in a changing climate

The changes in climatic conditions that have been recorded since the beginning of the twentieth century are likely to accelerate due to increases in anthropogenic greenhouse gas emissions. Such changes are likely to impact perennial field crop growth and development as well as winter survival. For instance, elevated temperatures have been shown to promote autumn growth and delay the acquisition of maximum frost tolerance. The warmer autumn and winter conditions predicted will likely increase the risk of winter damage through suboptimal autumn acclimation, dehardening temperatures in winter and lack of insulating snow cover (Bélanger et al. 2002).

Moreover, elevated atmospheric CO_2 has been shown to stimulate growth at low temperature and to reduce the freezing tolerance of alfalfa (Bertrand et al. 2007). The acquisition of freezing tolerance is also influenced by the strain of N-fixing rhizobium bacteria in symbiosis with the plant and it has been shown that it may be possible to select rhizobium strains to improve alfalfa freezing tolerance under future climatic conditions (Bertrand et al. 2007). The extent of increase in damage due to these future winter conditions varies with region. Efforts toward the understanding of the molecular and genetic bases of alfalfa adaptation to subfreezing temperature and the development of new molecular technologies must be sustained to allow Canadian farmers to face the challenge of increasing alfalfa winter survival under future climatic conditions. 

References available online at www.farmwest.com

Annick Bertrand Agriculture and Agri-Food Canada, Soils and Crops Research and Development Centre, Québec, QC, Canada | annick.bertrand@agr.gc.ca

Yves Castonguay, Agriculture and Agri-Food Canada, Soils and Crops Research and Development Centre, Québec, QC, Canada