Expression of CBF-like genes in alfalfa (Medicago sativa L.)

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Plant growth and development is adversely affected by exposure to freezing temperatures. Identification of germplasm with superior freezing tolerance and understanding the molecular biology of the underlying mechanisms would be key to improving freezing tolerance in plants.

As the first step towards the improvement of freezing tolerance in alfalfa, we recently discovered a germplasm, River side (RS), that is naturally adapted to the Grand River National Grassland environment in South Dakota and showed greater freezing tolerance compared to some of the known freezing tolerant germplasm. To understand the molecular basis of freezing tolerance in RS, we examined expression of the *C-repeat binding factor-like* (*CBF-like*) genes in alfalfa. Studies in Arabidopsis and other plants have shown that *CBF* genes play important roles in improving freezing tolerance in plants. In particular, *CBF3* transcripts in Arabidopsis are rapidly upregulated after exposure of plants to a low temperature and *CBF3* is one of the key genes involved in cold acclimation. The objective of this study was to identify the potential functional homolog of *CBF3* in alfalfa and examine whether it is associated with the improved freezing tolerance in RS. We identified 18 *CBF-like* genes after examining the genome of *Medicago truncatula*, a close relative to alfalfa. Phylogenetic analysis showed that *Medicago CBF*s form 5 distinct clusters. Expression profiling of these genes in alfalfa under cold stress revealed diverse induction patterns. Five genes that had a highly overlapping expression pattern, as that of *CBF3* in Arabidopsis under cold stress, were selected for further expression analyses. Three of the five genes showed oscillation in transcript levels under diurnal light-dark cycles. Expression patterns of these genes varied in different tissues and at different developmental stages. Finally, transcript levels of two of these genes showed an early and greater induction in RS when compared to non-freezing tolerant germplasm, suggesting that these two genes are potentially the functional homologs of *CBF3* and may contribute towards the superior freezing tolerance of RS.