

# Identification and Characterization of Freezing Tolerant Alfalfa (*Medicago sativa* L.) Germplasm

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## Abstract

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Alfalfa is an important forage crop in many countries around the world including the US. Freezing/cold weather is one of the abiotic stresses that limit its production. Improved cultivars with superior performance in cold environments will benefit forage growers and land managers. We conducted a greenhouse screening experiment to compare 14 genotypes for their freezing tolerance. Among these genotypes are several collections that are naturally adapted to the Grand River National Grassland environment in South Dakota. Our hypothesis was that these collections may have developed a tolerance to survive the frigid growth conditions. After three cycles of freezing experiments, two of the collections, RS (Grand Rivers Side) and FR (Foster Ranch) showed greater freezing tolerance in terms of survival rate compared to other 12 genotypes tested. For instance, FR and RS showed 74.5% and 69.5% survival at -10°C. Positive controls Caribou and Apica had 60% and 53% survival, while the negative control CUF-101 had only 20% survival. Electrolyte leakage analysis of leaves and stems following freezing was also conducted to quantify the freezing damage. LT<sub>50</sub>, percentage of cell damage, based on the leakage analysis, was closely correlated with survival rates. Our preliminary data from expression analysis (qPCR) of cold-responsive genes showed that FR and RS exhibited different patterns of cold induction and response. The data suggest that FR and RS may possess different mechanisms from other alfalfa in cold response and adaptation.