

The effect of enhanced sucrose-phosphate synthase (SPS) activity on the low-temperature survival of alfalfa (*Medicago sativa* L.)

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Alfalfa (*Medicago sativa*) is a major forage crop for Ontario livestock. However, the winter persistence of this perennial plant limits economic returns in northern climates. Winter survival is determined by a plant's aptitude for dealing with stresses such as freezing, desiccation, ice-encasement, flooding, and disease. Certain carbohydrates, including sucrose, are believed to play a cryoprotective role; they also play a nutritive role in crown buds, promoting spring regrowth. It is proposed that freezing tolerance can be enhanced by raising the levels of sucrose in the plant. Sucrose-phosphate synthase (SPS) shunts carbohydrate away from starch production and into sucrose accumulation. SPS has been found to become increasingly active during cold acclimation of alfalfa. Expression of a constitutively active form of SPS is expected to result in an increase in the proportion of carbohydrate stored as sucrose rather than as starch. Augmented sucrose levels are anticipated to result in improved winter hardiness, and could also enhance the palatability and nutritive value of the forage. To date, the properties of a mutant form of alfalfa SPS lacking a regulatory phosphorylation site have been examined in yeast. Nearly one hundred transgenic lines containing native, mutant, or antisense constructs of SPS cDNA under the control of the 35S promoter have been generated through *Agrobacterium*-mediated transformation. Empty vector and untransformed controls have also been generated. The carbohydrate partitioning, low temperature survival, and regrowth of the transgenics will be examined. Northern dot-blot analysis has shown that SPS mRNA expression increases in the shoot under cold shock (4°C) but not under heat shock (41°C) in alfalfa cultivars of varying dormancy. SPS mRNA levels in the root and crown bud were found to increase under field conditions during the fall acclimation period. Carbohydrate levels in the crown buds and roots of cultivars sampled during the fall acclimation period will be examined.