KLOTZ, KAREN L.[1]*, DARRIN M. HAAGENSON[1], and J. MITCHELL McGRATH[2], [1]USDA-ARS, Northern Crop Science Laboratory, P.O. Box 5677, University Station, Fargo, ND 58105, and [2]USDA-ARS, Sugar Beet and Bean Research Unit, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824. **Sucrose synthase gene expression is tissue-specific, developmentally regulated, and influenced by abiotic stresses.**

ABSTRACT

Sucrose synthase is the predominant sucrose degrading activity in sugarbeet root and is believed to have roles in carbohydrate partitioning to the root during production and sucrose loss during storage. To improve our understanding of sucrose synthase expression and the factors that control it, and to provide the molecular basis for future studies into sucrose synthase function, the genes responsible for sucrose synthase expression were compared, their tissue and developmental specific patterns of expression were described, and their response to environmental factors determined.

Two genes are responsible for sucrose synthase expression, sugarbeet sucrose synthase I (SBSS1; GenBank accession X81974) and sugarbeet sucrose synthase II (SBSS2; GenBank accession AY457173). SBSS1 was identified and cloned from a root cDNA library by Hesse and Willmitzer in 1996 (Plant Mol. Biol., 30:863-872). SBSS2 was identified in 2003 from an EST database generated from a cDNA library made from four day old osmotically stressed seedlings subtracted against four day old water-germinated seedlings (Haagenson, Klotz, and McGrath, unpublished data). Based on cDNA sequence, SBSS1 is predicted to encode a protein of 822 amino acids with a molecular mass of 93.7 kDa. SBSS2 is predicted to encode a protein of 806 amino acids with a molecular mass of 93.2 kDa. The two genes share 62% nucleotide identity and are 80% similar in deduced amino acid sequence.

Northern analysis revealed that both genes exhibited high expression in roots and low expression in leaves. In floral tissue, low expression of SBSS1 and moderate expression of SBSS2 was observed. Developmentally, SBSS1 was expressed throughout root development with greatest expression during midseason growth (5 to 10 weeks after planting). In contrast, SBSS2 was most highly expressed during early development (3 to 6 weeks after planting), and exhibited very low levels of expression during late season development (12 to 16 weeks after planting). Western analysis demonstrated that protein levels within organs and throughout development were similar to transcript levels, although developmental changes in protein levels were delayed from transcriptional changes. This suggests that organ and developmental specific expression of sucrose synthases is likely to be regulated primarily at the level of transcription, but influenced by protein stability.

Environmental stresses including harvest, wounding, cold and anaerobic conditions affected steady state transcript levels for the two sucrose synthase genes. Harvest was associated with a decline in the steady state mRNA levels of both SBSS1 and SBSS2. The decrease in mRNA was observed during seven days storage at 20°C or 10°C and was greatest for SBSS2. Wounding caused a transient increase in both SBSS1 and SBSS2 transcript levels, with SBSS1 and SBSS2 transcript levels elevated for three days and one day after injury, respectively. Cold (2°C) had no apparent effect on SBSS1 transcript levels, but was associated with elevated SBSS2 transcript levels beginning three days after the inception of the cold treatment. Anaerobic conditions were associated with a large increase in SBSS1 transcript levels and a decrease in SBSS2 transcript levels. The effect of harvest, wounding, cold and anaerobic conditions on

SBSS1 and SBSS2 transcript levels, however, did not correspond to similar alterations in sucrose synthase protein levels or enzyme activity. The lack of relationship between transcript and protein levels suggest that protein stability and posttranscriptional regulation of expression are important determinants of sucrose synthase activity in sugarbeet root in response to abiotic stresses.