Carbohydrate content of sugarbeet (*Beta vulgaris* L.) transformed with a cytokinin biosynthesis gene

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ABSTRACT

Cytokinins have been shown to regulate a number of physiological and biochemical processes in plants including control of assimilate movement. Elevated levels of cytokinins and auxins have been correlated with cambial initiation and rapid cell division periods in developing sugarbeet taproots. To study the role of cytokinins in carbon partitioning, sugarbeet lines Rel-1 and C69 were transformed with the isopentenyl transferase *ipt* gene fused to a wound-inducible proteinase inhibitor II (Wi) or a tuber-specific patatin (Pa) gene promotor. Isopentenyl transferase is the key enzyme involved in cytokinin biosynthesis. Two transformation methods were used, particle bombardment of embryogenic hypocotyl or cotyledon callus and *Agrobacterium*-mediated cotyledon transformation. Putative transformants were analyzed by PCR and on Southern and Northern blots. Cytokinin concentration was determined by ELISA using monoclonal anti-ZR antibodies. Carbohydrates were extracted and analyzed as described in Ziska et *al.* (1995).

To initiate root development, transformed shoots had to be exposed to high auxin concentrations (50 mg IBA/l) for 24 hours as compared to normal shoots that were maintained on 3 mg IBA/l. *Ipt* shoots rooted in 4-8 weeks and the controls in 2 weeks. Rooting of one of the transformants, Pa-*ipt* 02, that exhibited typical phenotypic cytokinin effects was successful only once on medium with NAA and IBA. The Pa-*ipt* 02 shoots did not root on higher concentrations of auxin (100, 150 or 200 mg IBA or NAA/L) and longer times of exposure (2, 4, 5 days; 2, 3, 4 weeks). Limited success was achieved with vegetative propagation of the Pa-*ipt* 02 plant. All *ipt*-transformed plants had phenotypic characteristics associated with elevated cytokinin levels. Some showed increased adventitious shoot formation while others had reduced apical dominance, a large, proliferative crown and a very small root mass. Others exhibited slower growth and an overall reduction in the number and size of leaves.

PCR analysis of putative transformants confirmed the presence of the *ipt* gene in all tissue culture-grown plants, while the loss of this gene occured in some of the greenhouse-grown plants. Southern blot analysis of three transformants showed a predicted 2.0 or 2.1 Kb fragment coresponding to the *ipt* gene fused to the Wi or Pa gene promotor, respectively. An *ipt*-specific transcript of approximately 2.4 Kb was detected on Northern blots of taproots and leaves. Cytokinin levels were up to 17-fold higher in transformed leaves as compared to levels in untransformed, control leaves. In the taproots, a corresponding increase of 2-fold was observed. Wounding of the taproots appeared to have a negative effect on the endogenous cytokinin level. In one transformant, about a 9 fold increase in leaf sucrose levels was observed while the glucose content was 18 times higher. No corresponding increase in sucrose and glucose levels was observed in the taproots of this plant.

References: Ziska, L.H., R.C. Sicher and D.F. Kremer.1995. Physiologia Plantarum 95: 355-364