ENGINEERING SUGARBEET FOR IMPROVED DEFENSE MECHANISMS AND CARBON PARTITIONING

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Novel molecular genetic approaches are being utilized to enhance defense properties and improve carbon partitioning in sugarbeet. Two approaches are being used to enhance disease resistance, one of them targets pathogenic fungi and bacteria and the other insect pests. For microbial control, genes coding for proteins with defense properties are being utilized (L.D. Owens, USDA, ARS, Plant Molecular Biology Laboratory, Beltsville). These include the small, cysteine-rich proteins that have been shown in other transgenic plants to display varying levels of toxicity to a broad range of plant pathogenic fungi and to some bacterial pathogens. The cecropin MB39, DB4 thionin, osmotin and PR-S (pathogenesis related protein-S, an osmotin-like protein secreted extracellularly in contrast to the vacuole-targeted osmotin) genes were reconstructed to allow expression in different tissues and under specific inducing conditions. Transgenic sugarbeet plants carrying one to two of these genes have been regenerated and are being grown to maturity for seed production and testing for resistance to *Erwinia carotovora* subsp. *betavasculorum*, *Cercospora beticola* and *Rhizoctonia solani*. For control of insect pests, a novel approach utilizing a gene (*isopentenyl transferase, ipt*) involved in the synthesis of a major class of plant regulating substances, the cytokinins, is being used. The gene was engineered to allow for induction of its expression upon insect feeding or mechanical wounding or to be expressed specifically in the sugarbeet taproot. Using this approach, enhanced resistance to tomato hornworm and a virus-transmitting pest, the green peach aphid, was demonstrated in other transgenic plants. A cytokinin-induced secondary metabolite(s) that appears responsible for the observed enhanced tolerance has been partially purified from transgenic leaves. The compound(s) appears to specifically target certain orders of insects (Lepidoptera, Homoptera, Diptera) but not others (Coleoptera). The most devastating dipteran pest of sugarbeets that feeds along the surface of tap and feeder roots is the root maggot (*Tetanops myopaeformis*). Exposure of gnotobiotic first instar root maggot larvae to the cytokinin-induced insecticidal compound(s) induced a highly atypical wild thrashing and twitching behavior that although reduced was still noted after 4 days of incubation (C.A. Wozniak, USDA, ARS, Northern Crop Science Laboratory, Fargo, ND). A cytokinin biosynthesis gene reconstructed for taproot-specific expression was introduced into sugarbeet for *in planta* evaluation of its effect as a feeding deterrent for the root maggot. Transgenic plants and their progeny will be evaluated for enhanced tolerance to the maggot. Concomitantly, cytokinin's participation in carbon partitioning will be evaluated in these transgenic plants. Transport of assimilates towards site of cytokinin application has been demonstrated in many plant organs but the mechanism behind this transport is not known. Local accumulation of cytokinin in sink organs such as seeds, fruits and roots is speculated to potentially increase plant productivity due to increased assimilate transport. In addition, high cytokinin levels have been correlated with cambial initiation and rapid cell division periods in developing sugarbeet taproots and prior to cytokinesis in synchronized sugarbeet cell suspension cultures derived from the taproot. These results suggest that higher cytokinin levels in the taproot may lead to increased cell division, additional vascular rings and higher sucrose yield. High auxin concentrations were required to stimulate rooting of transgenic shoots carrying the cytokinin gene fused to the taproot-specific promoter presumably to compensate for the elevated cytokinin levels. Rooted transformed plants either appeared normal or exhibited varying degrees of cytokinin effects such as more adventitious shoot development, reduced apical dominance and dark green leaves. Further analysis of transgenic plants for levels expression of the cytokinin gene and genes for sucrose-metabolizing enzymes and sucrose transporters will help correlate the effect of cytokinin on sucrose accumulation and storage root structure and shape. Increased cell division and number of vascular rings in the taproot is anticipated not only to increase the sugar content but also lead to a low-tare sugarbeet with globe-shaped storage root with fewer branches or grooves that would be of benefit to farmers, processing plants and the environment.