COMPARISON OF SUCROSE CATABOLISM IN ROOTS OF THREE *BETA VULGARIS* L. GENOTYPES WITH DIFFERENT YIELD AND SUCROSE ACCUMULATING CAPACITIES

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ABSTRACT

Sucrose catabolism is a major determinant of sink strength in nearly all plants and affects sucrose partitioning to growing sinks as well as sink size and carbohydrate content. Three enzyme families are responsible for nearly all sucrose catabolism in sugarbeet roots: acid invertase, alkaline invertase and sucrose synthase. Previous work suggested that sucrose synthase may have a role in sink strength and root size in sugarbeet. To examine this observation more thoroughly, sucrose catabolism was compared in three *Beta vulgaris* genotypes with contrasting capacities for root yield and sucrose accumulation. Soluble acid invertase, cell wall acid invertase, alkaline invertase and sucrose synthase activities were compared at five stages of root development in a fodder beet hybrid (high yield, low sucrose content), a commercial sugarbeet hybrid (typical yield and sucrose content) and the sugarbeet breeding line, L19 (low yield, high sucrose content). Sucrose, glucose and fructose concentrations and mass accumulation were also determined. Generally, sucrolytic activity was greatest in the high yielding fodder beet and lowest in the low yielding L19 breeding line at any stage of development. Sucrose synthase activity was the predominant sucrolytic activity at all stages of development examined, and accounted for 90% or more of the total sucrolytic activity in fodder beet and sugarbeet hybrid roots by six weeks after planting and in L19 eight weeks after planting. Total sucrose synthase activity was positively correlated with nonextractable dry matter accumulation. Differences in sucrose concentration between genotypes were observed, although sucrose concentration or accumulation was not highly correlated with any of the major sucrolytic enzymes examined.

INTRODUCTION

Sucrose catabolism in sugarbeet root is a major factor controlling carbon partitioning within the plant, root growth, and sucrose accumulation (GIAQUINTA, R.L., 1979; SUNG, S. *et al.*, 1989; KLOTZ, K.L. & FINGER, F.L., 2002). Three enzyme families, the acid invertases, the alkaline invertases, and sucrose synthases, are responsible for nearly all sucrose catabolism in sugarbeet root. Acid invertases catalyze the hydrolysis of sucrose to fructose and glucose, and occur as soluble and insoluble forms localized to the vacuole

and cell wall, respectively. Alkaline invertases catalyze the same hydrolysis reaction as the acid invertases, but are localized in the cytoplasm and exhibit greatest activity at higher pH values than the acid invertases. The sucrose synthases catalyze the conversion of sucrose to fructose and UDP-glucose, and like the alkaline invertases, are found in the cytoplasm.

The roles of the different sucrolytic activities in carbon partitioning, root growth and sucrose accumulation are largely unknown, although roles for sucrose synthase in carbon partitioning (SUNG, S. *et al.*, 1989) and regulation of root growth (KLOTZ, K.L. & FINGER, F.L., 2002), and a role for acid invertase in sucrose accumulation (GIAQUINTA, R., 1979; BERGHALL, S. *et al.*, 1997) have been proposed. To further investigate the function of these enzymes, acid invertase, alkaline invertase and sucrose synthase activities were determined in three *Beta vulgaris* L. genotypes with differing capacities for mass and sucrose accumulation, and their relationships to carbon partitioning, root growth and sucrose accumulation were determined.

MATERIALS AND METHODS

Beta vulgaris L. genotypes were greenhouse grown in Sunshine Mix #1 (Sun Gro Horticultural Products, Canada) in 15-liter pots with supplemental light under a 16-h light/8–h dark regime. Genotypes included the high yielding, low sucrose accumulating fodder beet variety 'Monovigour' (Danisco, Denmark), the sugarbeet hybrid VDH66156 (Van der Have, Netherlands), and a high sucrose, low yielding inbred sugarbeet line, L19 (PI 590690). Roots were harvested 4, 6, 8, 12, and 16 weeks after seeds were planted. Ten roots of each genotype were harvested at each sampling time. Whole roots or representative longitudinal sections were rapidly frozen in liquid nitrogen at time of sampling. Protein extraction, enzyme activity assays, and carbohydrate determinations were conducted as previously described (KLOTZ, K.L. & FINGER, F.L., 2002).

RESULTS

Significant differences were observed in yield, water content, sucrose concentration, and noncarbohydrate dry matter between the three genotypes (Table 1). At the end of sixteen weeks of growth, the fodder beet variety was more massive, and contained more water and non-carbohydrate dry matter than the sugarbeet hybrid or the breeding line L19. Sucrose concentration was greatest in the breeding line L19. The sugarbeet hybrid was intermediate between the fodder beet variety and L19 in all chemical and physical parameters examined.

The sucrolytic enzymes, soluble acid invertase, alkaline invertase and sucrose synthase exhibited similar patterns of developmental expression (Table 2). Sucrose synthase was the predominant sucrolytic activity at the five stages of development examined and accounted for 90% or more of the total sucrolytic activity in fodder beet and sugarbeet hybrid roots by six weeks after planting and in L19 by eight weeks after planting. Soluble acid invertase activity contributed significantly to total sucrolytic activity only at the earliest stage of development. Alkaline invertase was present at relatively low levels throughout development in

all genotypes. Generally, all sucrolytic enzyme activities were greatest in the fodder beet variety and lowest in L19 at nearly every stage of development. Enzyme activities in the sugarbeet hybrid were generally intermediate between the fodder beet variety and L19 throughout development.

Table 1. Mass, water content, sucrose concentration and non-carbohydrate dry matter concentration of three Beta vulgaris *L. genotypes sixteen weeks after planting.*

	fodder beet	sugarbeet	L19	$LSD_{0.05}$
mass (g)	1237	896	379	114
H_2O content (%)	82.6	80.7	75.2	1.2
sucrose content (g g dry wt^{-1})	0.464	0.695	0.793	0.079
noncarb. dry matter (g g dry wt^{-1})	0.533	0.301	0.206	0.079

Table 2. Sucrolytic enzyme activities of three Beta vulgaris *L. genotypes at five stages of development. Activities are expressed as :mole sucrose cleaved g dry weight^{-1} h^{-1}. n.d., not detected.*

weeks after planting	acid invertase			alkaline invertase			sucrose synthase		
	fodder	sugar beet	L19	fodder	sugar beet	L19	fodder	sugar beet	L19
4	191	108	49.0	52.3	47.3	27.6	908	167	118
6	24.4	16.0	0.63	22.4	18.2	8.62	740	346	33.6
8	10.3	5.19	n.d.	9.47	16.1	1.95	385	426	125
12	0.77	3.40	1.39	16.1	10.3	2.86	377	197	160
16	4.08	0.065	0.59	4.26	2.86	1.71	272	221	66.0
mean	46.0a	26.5b	10.3c	20.9a	19.0a	8.6b	537a	271b	100c
$LSD_{0.05}$	---------- 15.0 ----------			---------- 5.49 ----------			---------- 90.8 ----------		

Total sucrose synthase activity was positively associated with total dry matter, regardless of genotype or stage of development (dry matter = 3.19 x sucrose synthase activity + 9.70; R^2 = 0.86). When total dry matter was divided into its two principle components, sucrose and nonextractable dry matter, total sucrose synthase activity was more closely related to nonextractable dry matter (nonextractable dry matter = 1.62 x sucrose synthase activity – 0.89; R^2 = 0.95) than sucrose content (sucrose content = 1.53 x sucrose synthase activity + 10.7; R^2 = 0.64). Nonextractable dry matter is primarily composed of cell wall materials.

No close association was observed between any of the sucrolytic activities and sucrose concentration or accumulation, suggesting that these enzymes are not major determinants of sugarbeet root sucrose content. Although other studies have suggested a relationship between acid invertase activity and sucrose content in sugarbeet root (GIAQUINTA, R., 1979; BERGHALL, S. *et al.*, 1997), in this study, acid invertase activity did not relate to sucrose concentration (sucrose concentration = -0.0037 x soluble acid invertase activity + 1.86; R^2 = 0.30) or accumulation (sucrose content = 0.066 x soluble acid invertase activity + 23.1; R^2 = 0.15), when means were compared over genotypes and stages of development.

CONCLUSIONS

The high positive association between sucrose synthase activity and nonextractable dry matter suggests a role for sucrose synthase in controlling or limiting cell wall biosynthesis. In such a manner, sucrose synthase may influence root size and mass, and ultimately, the yield of the sugarbeet crop. A role for sucrose synthase in cell wall biosynthesis has previously been demonstrated in cotton where sucrose synthase activity provides substrates for cellulose biosynthesis (AMOR, Y. *et al.*, 1995).

The absence of a relationship between acid invertase activity and sucrose concentration or accumulation questions the importance of this enzyme as a major determinant of sucrose accumulation, and suggests that the factors that regulate sucrose accumulation in sugarbeet root remain to be discovered. The pattern of high soluble acid invertase activity during early sugarbeet root development is similar to that observed in other plant species where soluble acid invertase is thought to have a key role in providing hexose substrates to meet the biosynthetic and metabolic demands of rapidly growing and expanding tissues (RICARDO, C.P.P. & SOVIA, D., 1974; MORRIS, D. & ARTHUR, E., 1985; PFEIFFER, I. & KUTSCHERA, U., 1995).

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