Down-regulation of sorbitol dehydrogenase and up-regulation of sucrose synthase in shoot tips of the transgenic apple trees with decreased sorbitol synthesis

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Abstract

Both sorbitol and sucrose are translocated to, and utilized in, sink tissues of apple (Malus domestica). Considering that antisense suppression of aldose 6-phosphate reductase resulted in lower concentrations of sorbitol and higher concentrations of sucrose in source leaves without altering the vegetative growth of apple trees, it was hypothesized that sorbitol metabolism is down-regulated and sucrose metabolism is up-regulated in shoot tips of the transgenic plants. Carbohydrate measurements indicated that sorbitol concentration was lower whereas sucrose concentration was higher in the shoot tips of transgenic apple plants with decreased sorbitol synthesis compared with the untransformed control. However, the shoot relative growth rate was not altered in the transgenic plants. Sorbitol dehydrogenase (SDH) activity was decreased; acid invertase activity and neutral invertase activity remained the same, whereas sucrose synthase (SUSY) activity was increased in shoot tips of the transgenic plants. The SDH transcript level was lower whereas the SUSY transcript level was higher in shoot tips of the transgenic plants. SDH activity and SDH transcript level were specifically stimulated by exogenous sorbitol fed to the shoot tips via the transpiration stream but were specifically inhibited by sucrose. SUSY activity and SUSY transcript level were dramatically enhanced by sucrose, but decreased by glucose and fructose. Neither acid invertase nor neutral invertase activity responded to sucrose, glucose, fructose, or any other sugars tested. It is concluded that sorbitol dehydrogenase is down-regulated, whereas sucrose synthase is up-regulated in shoot tips of the transgenic apple trees with decreased sorbitol synthesis, leading to homeostasis of vegetative growth. Sorbitol and sucrose act as signal molecules to modulate the expression and activities of sorbitol dehydrogenase and sucrose synthase, both of which play an important role in determining the sink strength of apple shoot tips.

Key words: Apple, carbohydrate metabolism, relative growth rate, shoot tip, sink strength, sorbitol, sorbitol dehydrogenase, sucrose, sucrose synthase.

Introduction

The growth and development of a sink organ depend on its sink strength, which is defined as the ability to import and utilize photoassimilates synthesized in source leaves (Ho, 1988). Sucrose is a major photosynthetic end-product and the translocated carbohydrate in most plants. The channeling of sucrose into sink metabolism requires the cleavage of sucrose by invertase (EC 3.2.1.26) or by sucrose synthase (SUSY) (EC 2.4.1.13) (Sturm and Tang, 1999; Koch, 2004). The former hydrolyses sucrose into fructose and glucose, whereas the latter converts sucrose and UDP into fructose and UDP-glucose. Both enzymes are crucial for plant growth, development, and carbon partitioning in sink tissues (Sturm and Tang, 1999; Koch, 2004). SUSY

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Abbreviations: A6PR, aldose-6-phosphate reductase; SDH, sucrose dehydrogenase; SUSY, sucrose synthase.

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modulates the import of sucrose into tomato fruit during the early stages of fruit development, and its activity is positively correlated with fruit relative growth rate (Sun et al., 1992; Wang et al., 1993, 1994). Antisense inhibition of SUSY leads to a reduction in sucrose unloading and fruit set in tomato fruit (D’Aoust et al., 1999). SUSY is also important for the growth and development of vegetative sink tissues. SUSY is one of the first to show elevated expression as leaf primordia differentiate from the apical meristem (Pien et al., 2001), and high SUSY activity is closely associated with the sink strength in the cladodes of Opuntia ficus-indica during the sink–source transition (Wang et al., 1998). Antisense suppression of SUSY expression in carrot results in a significant decrease in the leaf and root growth (Tang and Sturm, 1999). The sink strength of potato tubers is, to a large extent, determined by SUSY (Zrenner et al., 1995). Acid invertase plays an important role in the early development and sucrose partitioning of carrot plants (Tang et al., 1999). Tuber-specific overexpression of apoplastic acid invertase in transgenic potato plants leads to an increase in potato yield (Hajirezaei et al., 2000).

Carbohydrates not only provide energy and building blocks for plant growth and development, but also regulate all stages of a plant’s life cycle, from seed germination and vegetative growth to reproductive development, as important signal molecules via modulation of the expression of many genes involved (Koch, 1996; Sheen et al., 1999; Smeekens, 2000; Gibson, 2004). Regulation of gene expression by sugars occurs in both source and sink tissues (Koch, 1996; Roitsch, 1999). In general, low sugar status promotes photosynthesis and storage mobilization, whereas high levels of sugar stimulate growth and storage of starch and other carbohydrates (Koch, 1996). Hexoses and sucrose have been recognized as important signal molecules in source–sink regulation (Roitsch, 1999), and sucrose-specific, hexokinase-dependent, and hexokinase-independent signalling pathways have been found in plants (Koch, 1996; Smeekens, 2000; Gibson, 2004).

In addition to sucrose, sorbitol is a major end-product of photosynthesis in many economically important tree fruits in the Rosaceae family, such as apple, pear, peach, cherry, apricot, and plum. Both sorbitol and sucrose are transported from source leaves to sink tissues such as fruit and shoot tips (Bielecki, 1982; Loescher, 1987). In the sink, sorbitol is mainly converted to fructose by sorbitol dehydrogenase (SDH) (EC 1.1.1.14) (Negrn and Loescher, 1979; Yamaguchi et al., 1994; Oura et al., 2000) before entering the central metabolism. SDH activity is high in young leaves of apple (Loescher et al., 1982) and peach (Merlo and Passera, 1991) and the shoot apex of peach (Lo Bianco et al., 1999a, b), and is considered to be a good indicator of sink strength (Lo Bianco et al., 1999a, b). SDH is also actively expressed in developing apple fruit (Nosarszewski et al., 2004), and its transcript level is enhanced by exogenous feeding of sorbitol (Archbold, 1999; Iida et al., 2004). However, the role of sucrose-metabolizing enzymes in regulating sink growth has been largely overlooked in sorbitol-synthesizing species and it is not known how the two metabolic systems, one for sorbitol and the other for sucrose, are co-ordinated in response to sorbitol and sucrose availability in the sink tissues.

In transgenic apple trees with antisense suppression of aldose-6-phosphate reductase (A6PR), sorbitol concentration was significantly decreased whereas sucrose concentration was elevated in the source leaves, yet CO₂ assimilation and plant vegetative growth were not altered (Cheng et al., 2005). Since both sorbitol and sucrose are utilized in sink organs of sorbitol-synthesizing species, it was reasoned that the co-ordination of both sorbitol and sucrose metabolism would determine the overall sink strength. It was hypothesized that sorbitol metabolism is down-regulated and sucrose metabolism is up-regulated in the shoot tips of the transgenic plants. The objective of this work was to test the above hypothesis by monitoring the activity and transcript level of the key enzymes in sorbitol and sucrose metabolism in shoot tips of the transgenic apple trees with decreased sorbitol synthesis and their responses to exogenous sugars fed via the transpiration stream.

Materials and methods

Plant growth conditions

Untransformed ‘Greensleeves’ apple (Malus domestica Borkh.) and antisense lines (A27, A04, and A10) that have decreased expression of A6PR were used in the current study. A27 has ~30% of the control A6PR activity, whereas both A04 and A10 have ~15% of control A6PR activity in mature leaves (Cheng et al., 2005). The untransformed control and the antisense lines were grafted onto M.26 rootstocks.

All the trees were 2 years old and grown in 7.6 l plastic containers in a medium of 1 sand:2 MetroMix 360 (v/v) (Scotts, Marysville, OH, USA) outdoors under natural conditions in Ithaca, NY, USA. They were all pruned to 5–6 vegetative buds during dormancy. Only two shoots were allowed to grow on each tree, and any extra shoots were removed shortly after budbreak. They were supplied with 15 mM N using Plantex® NPK (20–10–20) with micronutrients (Plantex Corp., Ontario, Canada) twice weekly during the growing season. Fungi- cides and pesticides were sprayed at regular intervals throughout the growing season to protect the plants from diseases and insects.

During the period of active shoot growth from late June to mid July, 1 g of shoot tip tissues (apex with 2–3 expanding leaflets) was taken at noon under full sun from one shoot per tree as a sample. Leaf discs (1 cm² in size) were taken at the same time from recently fully expanded, mature leaves fully exposed to sunlight for carbohydrate analysis. The shoot tip samples for SDH activity measurements were immediately analysed; all the other samples were frozen immediately in liquid nitrogen and stored at −80 °C until use.

Analysis of relative shoot growth

Growth analysis was carried out during active shoot growth. The length between the third and the fourth leaflets was recorded twice at an interval of 24 h. The net increase in length was divided by the original length to give the relative growth rate (mm d⁻¹ mm⁻²). Ten shoots of each genotype of apple plants were measured.
Feeding the shoot tips with exogenous sugars

Feeding the shoot tips with exogenous sugars via the transpiration stream was carried out according to Krapp et al. (1991) with minor modification. Actively growing shoots were cut from the trees and immediately inserted into water, and re-cut under water to ~15 cm. Three unfolded leaves at the top were kept on each shoot and the others were gently removed by cutting the petioles with sharp scissors. Shoots were randomly assigned to sugar treatments, and six shoots per replicate (one for SDH activity, one for activities of other enzymes, and one for SDH and SUSY transcripts at each sampling time) were rapidly transferred from water to a beaker containing 200 ml of water or 50 mM sugar as indicated in the Results. Each treatment was replicated five times, and the beakers were randomly arranged in a fume hood (2 m × 1.2 m × 1.5 m) under fluorescent lights at ~20 μmol photons m⁻² s⁻¹ on the leaves at 23 °C. Shoot tip samples (1 g each) were taken as described above just before sugar feeding (0 h) and at 12 or 24 h after sugar feeding was initiated.

Extraction and assay of enzymes

SDH was extracted according to Lo Bianco et al. (1998) with some modifications. The fresh sample was rapidly chopped into small pieces and ground with a precooled mortar and pestle in 4 ml of extraction buffer containing 100 mM TRIS–HCl (pH 9.0), 8% glycerol, 1% (w/v) insoluble polyvinylpolypyrrolidone (PVPP), and 1% bovine serum albumin (BSA) plus 4% (w/v) PVPP. The homogenate was centrifuged at 16 000 g for 10 min at 2 °C and 1 ml of the supernatant was desalted with a PD-10 column (Amersham Biosciences, Piscataway, NJ, USA). The enzyme activity was assayed spectrophotometrically following the protocol of Lo Bianco et al. (1998).

SUSY and soluble invertase activities were measured according to Thévenot et al. (2005). Each shoot tip sample was ground with a precooled mortar and pestle in 5 ml of extraction mixture containing 50 mM HEPES-NaOH (pH 7.0), 2 mM MgCl₂, 1 mM Na₂-EDTA, 2.6 mM dithiothreitol (DTT), and 1% bovine serum albumin (BSA) plus 4% (w/v) PVPP, and acid-washed sand. This process took ~2 min. The homogenate was centrifuged at 16 000 g for 10 min at 2 °C and 1 ml of the supernatant was desalted with a PVPP-containing 8% (w/v) insoluble PVPP. The homogenate was centrifuged and desalted as described above. Neutral invertase activity (50 μl of desalted extract) was measured at 25 °C in a 860 μl final volume reaction buffer containing 50 mM HEPES-NaOH (pH 7.0), 2 mM MgCl₂, 1 mM Na₂-EDTA, 100 mM sucrose, 2.6 mM DTT, 1 mM ATP, 0.44 mM NAD⁺, and a desalted coupling enzyme mixture (3.5 U of phosphoglucose isomerase, 2 U of glucose 6-phosphate dehydrogenase, and 4.2 U of hexokinase), NAD⁺ reduction was recorded at 340 nm over a 3 min period in the linear portion of the time-dependent increase of absorbance. SUSY activity was then measured by adding 1 mM UDP to the same cuvette and further NAD⁺ reduction was monitored. For acid invertase activity assay, the pH of 25 μl of desalted extract was lowered with 25 μl of 200 mM Na acetate buffer (pH 4.8) first, then the reaction was carried out at 30 °C for 15 min after addition of 10 μl of 600 mM sucrose. At the end of the 15 min period, the pH of the mixture was increased by adding 50 μl of 500 mM NaH₂PO₄ buffer (pH 7.0), and the samples were immediately boiled for 3 min to denature the enzyme. Glucose and fructose derived from sucrose hydrolysis were transformed by adding 750 μl of reaction buffer (50 mM HEPES-NaOH pH 7.0, 2 mM MgCl₂, and 1 mM Na₂-EDTA) containing 1 mM ATP, 0.44 mM NAD⁺, and the desalted coupling enzyme mixture described above. After incubation at 25 °C for 30 min and centrifugation at 12 000 g for 3 min, the NADH formed was measured at 340 nm.

Carbohydrate extraction and determination

Soluble sugars were extracted from the samples (with xylitol added as an internal standard) with 80% ethanol three times at 80 °C, and then passed through ion exchange columns to remove charged material. Sorbitol, sucrose, glucose, and fructose were separated and quantified by a Dionex DX-500 series chromatograph system with a pulsed amperometric detector (Dionex, Sunnyvale, CA, USA) as previously described (Cheng et al., 2005).

RNA extraction and gel blotting

The frozen shoot tip tissues were ground in liquid nitrogen to a fine powder using a mortar and pestle. Total RNA was isolated according to Gasic et al. (2004). A 10 μg aliquot of total RNA from each sample was separated on a 0.8% (w/v) agarose gel containing formaldehyde and then blotted to Zeta-probe membrane (Bio-Rad, Hercules, CA, USA) according to Sambrook et al. (1989). DNA probes specific for SDH and SUSY were synthesized by polymerase chain reaction (PCR) using apple fruit SDH cDNA (AY244807) and apple SUSY cDNA (CN943285) as templates, respectively. The primers were: SDH forward primer, 5'-CATGAGTGTGCTGGGATC-3'; SDH reverse primer, 5'-CCAACACTAAGGCTACACA-3'; SUSY forward primer, 5'-ACCCGATCCTTGCACCTTGAGA-3'; and SUSY reverse primer, 5'-GATCAGGCTTGCCATG-3'. The amplified DNA fragments were recovered from the 1% agarose gel with a Zymoclean Gel DNA Recovery Kit™ (Zymo Research, Orange, CA, USA) according to the manufacturer’s protocol. The template was labelled with [32P]dATP using the Promega Prime-a-Gene Labeling System (Promega, Madison, WI, USA). Northern blot hybridization was performed as described in Sambrook et al. (1989).

Results

Sugar concentrations in source leaves and shoot tips

As reported before (Cheng et al., 2005), mature leaves of the transgenic apple trees with antisense suppression of A6PR had lower concentrations of sorbitol but higher concentrations of sucrose (Fig. 1). The sorbitol concentration in antisense line A27 was decreased to ~55% of that in the untransformed control, whereas the other two lines, A04 and A10, had ~25–30% of the sorbitol concentration in the control at noon (Fig. 1A). The sucrose concentration in mature leaves of the transgenic plants was increased to 225–320% of that in the control (Fig. 1B). Both glucose and fructose concentrations were slightly higher in the transgenic lines and the untransformed control, whereas the other two lines, A04 and A10, showed similar changes to those in the mature leaves, but to a lesser extent (Fig. 2). Compared with the untransformed control, the sorbitol concentration was decreased by 68–84% in A27 and to ~50% in both A04 and A10 (Fig. 2A), whereas the sucrose concentration was increased by 20% in A27 and by 68–84% in A04 and A10 (Fig. 2B). There was no significant difference in glucose or fructose concentration in the shoot tips between the transgenic and control plants (Fig. 2C, D).

Shoot relative growth rate

No difference in relative shoot growth rate was observed between the transgenic lines and the untransformed control.
The unchanged growth was also reflected in similar leaf number, leaf size, and shoot length and diameter at the end of growing season (Cheng et al., 2005).

Activities of the enzymes related to sorbitol and sucrose metabolism
SDH activity was decreased from 18.7 μmol h⁻¹ g⁻¹ FW in the untransformed control to 16.0 μmol h⁻¹ g⁻¹ FW in A27 and to 12.5–11.0 μmol h⁻¹ g⁻¹ FW in A04 and A10 (Fig. 3A). Sorbitol oxidase activity was undetectable in apple shoot tips in this study (data not shown). SUSY activity was increased from 9.0 μmol h⁻¹ g⁻¹ FW in the control to 11.8 μmol h⁻¹ g⁻¹ FW in A27 and to ~15.0 μmol h⁻¹ g⁻¹ FW in A04 and A10 (Fig. 3B). Both acid invertase and neutral invertase activities were not significantly altered (Fig. 3C, D).

Transcript levels of SDH and SUSY in shoot tips
To understand whether SDH and SUSY activities were transcriptionally regulated, both SDH and SUSY expression levels in shoot tips of the untransformed control and the transgenic lines were determined by RNA gel blotting. The SDH transcript level was lower whereas the SUSY transcript level was higher in the transgenic lines (Fig. 4), corresponding to lower sorbitol and higher sucrose concentrations in their shoot tips (Fig. 2A, B).

Activities and transcript levels of SDH and SUSY in response to exogenous sugars
SDH activity in the shoot tips fed with water decreased during the 24 h feeding period (Fig 5). Although the initial SDH activities were different between the untransformed control and the transgenic lines, SDH activity increased to a comparable level in all the plants at 12 h in response to sorbitol feeding, then decreased to a similar level at 24 h (Fig. 5). Sucrose feeding significantly decreased SDH activity at 12 h and 24 h.

SUSY activity decreased slightly during the 24 h of water feeding (Fig. 6). Sorbitol feeding did not alter SUSY activity. However, SUSY activity increased in response to sucrose feeding, reaching a similar level after 24 h in all the plants regardless of their initial SUSY activities (Fig. 6).

Neither acid invertase activity nor neutral invertase activity responded to sucrose or sorbitol feeding (data not shown).

Fig. 1. Concentrations of sorbitol (A), sucrose (B), glucose (C), and fructose (D) in mature leaves of the untransformed control (CK) and antisense lines (A27, A04, and A10) of ‘Greensleeves’ apple. Each bar is the mean of five replicates with the standard error. Samples were taken at noon. P-values from analysis of variance for sorbitol, sucrose, glucose, and fructose are <0.0001, <0.0001, 0.02, and 0.001, respectively. Means were separated using least significant difference (LSD) at the 5% significance level.
The expression of SDH and SUSY in the shoot tips also responded to exogenous sorbitol and sucrose fed via the transpiration stream. SDH transcript was remarkably enhanced after 12 h of sorbitol feeding in the untransformed control, whereas it was dramatically reduced after sucrose feeding (Fig. 7A). SUSY expression was strongly enhanced by sucrose feeding (Fig. 7A). Similar results were observed in the transgenic lines A10 (Fig. 7B), A27, and A04 (data not shown).

Various sugars were fed to shoot tips of the untransformed control to determine if the observed effects of exogenous sorbitol and sucrose on SDH and SUSY (Figs 5–7) are sorbitol and sucrose specific. Palatinose and turanose, two non-metabolizable sucrose analogues, did not show any additional effect on SDH activity or gene expression compared with the water control (Figs 8A and 9A). Glucose and fructose, the products of sorbitol oxidation and sucrose hydrolysis, did not alter SDH activity or its transcript level (Figs 8A and 9A). SUSY activity or transcript level did not respond to palatinose or turanose feeding, but both were significantly reduced by glucose and fructose feeding (Figs 8B and 9B). None of the tested sugars caused any change in acid invertase or neutral invertase activities (Fig. 8C, D).

**Discussion**

The data clearly showed that both the enzyme activity and transcript level of SDH were decreased whereas those of SUSY were increased in shoot tips of the transgenic apple trees with decreased sorbitol synthesis (Figs 3A, B, 4). The down-regulation of SDH and up-regulation of SUSY correlated well with the lower sorbitol concentration and higher sucrose concentration in shoot tips of the transgenic plants (Fig. 2A, B). Exogenous sugar feeding experiments demonstrated that SDH activity and SDH transcript level were specifically enhanced by sorbitol but decreased by sucrose, and that SUSY activity and SUSY transcript level were specifically increased by sucrose (Figs 5–9). Collectively, these findings strongly support the hypothesis that sorbitol metabolism is down-regulated whereas sucrose metabolism is up-regulated in shoot tips of the transgenic apple trees with decreased sorbitol synthesis.

The lower SDH activity and transcript level in shoot tips of the transgenic plants with lower sorbitol concentration (Figs 3A, B, 4) and the stimulation of SDH activity and transcript level by exogenous sorbitol feeding (Figs 5, 7, 8A) are consistent with what was observed previously in apple fruit (Archbold, 1999) and Japanese pear fruit (Iida...
et al., 2004). These findings indicate that SDH expression and enzyme activity are regulated by sorbitol availability. Mannitol, another polyol in higher plants, was also reported to induce mannitol dehydrogenase activity in celery cell culture (Pharr et al., 1995). However, the mechanism by which these polyols modulate the gene expression of their metabolizing enzymes remains unknown. It appears that regulation of SDH transcription and activity by sorbitol availability occurs only in sink tissues as SDH activity is undetectable in mature leaves of apple (Loescher et al., 1982) and peach (Merlo and Passera, 1991) although they have very high concentrations of sorbitol. High SDH activity has been associated with high sink strength in young leaves of apple (Loescher et al., 1982) and peach (Merlo and Passera, 1991) and the shoot apex of peach (Lo Bianco et al., 1999a, b). However, in the transgenic apple trees with decreased sorbitol synthesis, relative shoot growth rate, a measure of sink strength (Ho, 1988), remained unchanged although SDH activity of the shoot tips was significantly decreased (Fig. 3A). This strongly suggests that the sink strength of apple shoot tips is not solely determined by SDH. Sorbitol oxidase, which converts sorbitol to glucose (Yamaki, 1980), might play a minor role during the early stages of fruit growth in apple (Yamaki and Ishikawa, 1986) and shoot growth in peach (Lo Bianco et al., 1999a, b), but its function in controlling apple shoot growth appears to be negligible as its activity was undetectable in this study.

It is interesting to note that sucrose feeding specifically decreased both SDH activity and SDH transcript level in shoot tips (Figs 5, 7) and this suppression appears to be unrelated to glucose and fructose as the effect of sucrose on SDH could not be mimicked by exogenous glucose and fructose (Figs 8A, 9A). Sucrose regulates a number of carbohydrate-responsive genes (Smeekens, 2000), and previous studies have demonstrated the existence of sucrose-specific regulatory pathways (Chiou and Bush, 1998; Rook et al., 1998). Although the signal transduction pathway of sucrose on SDH is currently unknown, it might provide an additional mechanism for regulating

Fig. 3. Activities of sorbitol dehydrogenase (A), sucrose synthase (B), acid invertase (C), and neutral invertase (D) in shoot tips of the untransformed control (CK) and antisense lines (A27, A04, and A10) of ‘Greensleeves’ apple. Each bar is the mean of five replicates with the standard error. Samples were taken at noon. P-values from analysis of variance for the data in (A), (B), (C), and (D) are <0.0001, 0.0001, >0.05, and >0.05, respectively. Means were separated using LSD at the 5% significance level.

Fig. 4. Transcript levels of sorbitol dehydrogenase and sucrose synthase in shoot tips of the untransformed control (CK) and antisense lines (A27, A04, and A10) of ‘Greensleeves’ apple.
sorbitol utilization in sink tissues of sorbitol-synthesizing species, which allows sucrose to be integrated into sorbitol metabolism. So, the lower SDH activity and SDH transcript level (Figs 3A, 4) in the transgenic apple trees with decreased sorbitol synthesis may have resulted from a combination of lower sorbitol and higher sucrose concentrations. The effect of exogenous sucrose on SDH found in this study apparently contrasts with the result reported by Iida et al. (2004) that sucrose feeding enhanced SDH expression in slices of Japanese pear. The difference could be related to tissue type, but the exact reason remains to be understood.

The finding that both transcript level and enzyme activity of SUSY were up-regulated in the transgenic apple shoot tips (Figs 3B, 4) and in response to sucrose feeding (Figs 6, 7, 8B) indicates that SUSY may play an important role in regulating apple shoot growth in response to sucrose availability. The up-regulation of SUSY expression by sucrose in shoot tips of the transgenic apple trees is in agreement with the results obtained on potato (Fu and Park, 1995), maize (Koch et al., 1992; Koch, 1996), Arabidopsis (Ciereszko and Kleczkowski, 2002), and the suspension culture of rice cells (Liao and Wang, 2003). SUSY is a key enzyme in regulating sink growth by supplying substrate for respiration (Hänggi and Fleming, 2001), generating UDP-glucose for cell wall biosynthesis in association with cellulose synthase (Haigler et al., 2001; Ruan et al., 2003) and providing substrate for starch synthesis in storage sinks (Sun et al., 1992; Zrenner et al., 1995). Multiple SUSY genes have been found in a number of plant species and some of them are sugar responsive (Koch, 1996, 2004). However, the response to sugars is gene dependent. For example, the shrunken 1 gene in maize is maximally expressed under conditions of limited carbohydrate supply, whereas Sus1 is up-regulated when sugars are abundant (Koch et al., 1992). Apparently, apple shoot SUSY is responsive to sucrose (Fig. 7), glucose, and fructose (Fig. 9B). Since the concentrations of glucose and fructose are not altered (Fig. 2C, D), the higher SUSY activity and SUSY transcript level in shoot tips of the transgenic plants (Figs 3B, 4) are apparently caused by the elevated sucrose level (Fig. 2B).

Apple shoot tips have high activities of both acid invertase and neutral invertase, but neither responded to the elevated sucrose level in the transgenic plants (Fig. 3C, D) or exogenous sucrose, or any other sugar (Fig. 8C, D). Like SUSY, acid invertase has multiple isoforms and these isoforms show contrasting responses to sugar stimulus,
and the expression of the same gene can differ markedly in various tissues (Xu et al., 1992; Koch, 1996; Roitsch et al., 2003). The unchanged invertase activity in response to sugar feeding observed in this study suggests that the apple invertase gene(s) in the shoot tips may not be sugar responsive. As reported in other species (Sung et al., 1989a, b), they might function as maintenance enzymes for the growth of apple shoot tips.

It seems that regulation of carbohydrate metabolism by sorbitol and sucrose availability provides a mechanism for the transgenic apple plants to maintain their vegetative growth. As sorbitol synthesis is decreased by antisense inhibition of A6PR, sucrose accumulates in the source leaves (Fig. 1; Cheng et al., 2005). Consequently, less sorbitol and more sucrose is transported to the shoot tips (Fig. 2). Low concentrations of sorbitol, probably together with high levels of sucrose, in the shoot tips have led to down-regulation of SDH expression and the enzyme activity. On the other hand, SUSY expression and the enzyme activity are up-regulated by high concentrations of sucrose. As a result, the total capacity for metabolizing the imported carbohydrates remains unaltered and the vegetative growth of the transgenic plants is maintained. It appears that the existence of metabolic systems for both sorbitol and sucrose and their regulation by sorbitol and sucrose availability in the sink tissues brings extra plasticity to the growth of the sink tissues in sorbitol-synthesizing species compared with the plants that use sucrose as the sole

Fig. 6. Sucrose synthase activity in shoot tips of the untransformed control (CK) and antisense lines (A27, A04, and A10) of ‘Greensleeves’ apple in response to sugar feeding. Sorbitol (50 mM, open circle), water (filled circle), or sucrose (50 mM, filled triangle) was fed via the transpiration stream. Each bar is the mean of five replicates with the standard error. Analysis of variance was performed separately for the data collected at 12 h and 24 h. P-values for CK, A27, A04, and A10 are <0.0001, 0.0002, <0.0001, and 0.001 at 12 h, and <0.0001, <0.0001, 0.0006, and <0.0001 at 24 h, respectively. Means were compared separately at each sampling time using LSD at the 5% significance level.

Fig. 7. Steady-state transcript levels of sorbitol dehydrogenase and sucrose synthase in shoot tips of the untransformed control (A) and antisense line A10 (B) of ‘Greensleeves’ apple in response to sugar feeding. Sorbitol (50 mM), water, or sucrose (50 mM) was fed via the transpiration stream.
imported sugar. Significant reduction in shoot growth was observed in Arabidopsis plants when sucrose synthesis was decreased by antisense inhibition of sucrose phosphate synthase or cytosolic fructose 1,6-bisphosphatase, the two key enzymes for sucrose synthesis (Strand et al., 2000). Interruption of sucrose transport by antisense inhibition of a sucrose transporter delayed the development of sink organs of potato (Kühn et al., 1996, 2003), tobacco (Bürkle et al., 1998), and tomato (Hackel et al., 2006) plants.

In conclusion, SDH is down-regulated whereas SUSY is up-regulated in the shoot tips of the transgenic apple trees with decreased sorbitol synthesis, leading to homeostasis of vegetative growth. Sorbitol and sucrose act as signal molecules to modulate the expression and activities of SDH and SUSY, both of which play an important role in determining the sink strength of apple shoot tips.

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$P$-values for the data in (A), (B), (C), and (D) are 0.0001, <0.0001, >0.05, and >0.05 at 12 h, and 0.0005, <0.0001, >0.05, and >0.05 at 24 h, respectively. Means were compared separately at each sampling time using LSD at the 5% significance level. For (C) and (D), all the means were not significantly different at 12 h and 24 h and the letters were omitted for clarity.


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