SUCROSE METABOLISM IN THE CULM OF TRANSGENIC SUGARCANE WITH REDUCED SOLUBLE ACID INVERTASE ACTIVITY

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Abstract
Transgenic derivatives of sugarcane cultivar Q117, with reduced acid invertase activity, were used to investigate the role of this enzyme in sucrose metabolism in the sugarcane culm. Despite more than 70% reduction in the level of acid invertase activity in the immature internodes, no significant changes in either sucrose load or purity were evident. Radiolabelling experiments indicated no significant influence on sucrose hydrolysis as a result of the reduced acid invertase activity.

Introduction
After numerous studies on carbohydrate metabolism in sugarcane, the metabolic control of sucrose accumulation is still poorly understood (Moore, 1995). Sucrose accumulation occurs despite continuous cleavage of sucrose, when synthesis exceeds breakdown (Batta and Singh, 1986; Hatch et al., 1963; Whittaker and Botha, 1997; Wendler et al., 1990). Many studies have been done on the sucrose breakdown activities, invertases and sucrose synthase (SuSy) (reviewed by Moore, 1995); and more recently on the synthesis activity, sucrose phosphate synthase (SPS) (Ebrahim et al., 1998; Wendler et al., 1990).

Several studies showed a strong correlation between sucrose content and the difference between SPS and soluble acid invertase (SAI) activity, with decreased SAI in tissues accumulating sucrose. Analysis of progeny from crosses between high- and low-sucrose Saccharum clones also indicated a critical role of SAI in regulating sucrose accumulation (Ebrahim et al., 1998; Zhu et al., 1997). However, a decrease in SAI is not always evident during sucrose accumulation (Dendsay et al., 1995; Vorster and Botha, 1999). It is not clear whether there is potential to enhance sucrose accumulation by reduction in SAI below the low levels indirectly selected in current elite sugarcane cultivars.

The efficient sugarcane transformation system allows the production of multiple independent lines altered in the activity of a target gene or enzyme, in the same genetic background. This is a powerful tool to test the metabolic significance of suspected key enzymes in commercial sugarcane varieties.

Here we compare sucrose metabolism in the growing culms of a series of transgenic sugarcane lines with reduced acid invertase activity. Although SAI activity was reduced by more than 70% in some lines, no change in sucrose accumulation or rates of sucrose hydrolysis was evident.

Materials and methods
Transgenic lines of sugarcane cultivar Q117 expressed an antisense fragment from the central conserved region of sugarcane acid invertase, transcribed from the maize Ubil promoter. Lines designated A1, A2, A5 and A8 showed approximately constant antisense transcript levels from internodes 0 to 14. The same (double-stranded) invertase probe reveals a single native transcript, which is detectable only in young internodes and which shows tight seasonal correlation with SAI enzyme activity in field-grown sugarcane. This target invertase transcript in young internodes was reduced by 75% to 90% in the antisense lines, relative to Ubi-GUS and untransformed Q117 control lines (Sawyer, 1999).

For metabolic analysis, approximately five vegetative generations after regeneration of transformed plants, these lines were grown for 9 months at 28 ± 2°C in a glasshouse. These conditions allowed sustained growth, with stalks typically 3 m long at harvest. Stalks were thin (12–25 mm diameter), as commonly observed for regenerated lines under crowded glasshouse conditions. Enzymes were extracted from internodes 3 to 9 and assayed (Botha et al., 1996). Radiolabelling with [U-14C]-glucose and -fructose was done on tissue disks prepared from the same internodes (Bindon and Botha, 2000). Radiolabel in different sugars was quantified by inline isotope detection after HPLC separation (Black and Botha, 2000). Sucrose, glucose and fructose were enzymatically determined in extracts from internodes 3 to 18 (Famiani et al., 2001; Whittaker and Botha, 1997).

Results and discussion
SAI activity was higher in young internodes than in more mature internodes in all lines (Figure 1A). However, the activity varied significantly between lines in the top 7 internodes, with lowest activity in antisense clones A2, A5, and A8. The activity in internode 9 was similar in all lines. It is possible that this low ‘background’ activity arises from apoplastic

KEYWORDS: Antisense, Down-regulation, Vacuolar Invertase.

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invertase released into the crude soluble invertase extracts. Alternatively, there may be different isoforms of SAI, with insufficient homology for detection or inhibition of the form expressed later in internodal development by the gene fragment used in our northern and transformation analyses.

No significant differences were evident in neutral invertase (Figure 1B), and SuSy, UDP-glucose pyrophosphorylase, fructokinase and hexokinase (results not shown).

Despite the large variation in SAI activity, the sucrose levels in young and mature internodes were similar in all the lines (Figure 2A). There was no significant effect on the levels of the two reducing sugars, glucose and fructose. As a result the purity in the internodes of the different lines was similar (Figure 2B). In contrast, a 65% reduction in SAI from higher starting levels (15-20 nmol/mg protein/min) in cell cultures of cultivar H62-4671 doubled cellular sucrose content (Ma et al., 2000).
Label in glucose after a feeding of labelled fructose to the tissue can only occur after hydrolysis of sucrose. We therefore used the ratio of label in glucose and sucrose to estimate the hydrolysis rate. Estimated sucrose hydrolysis varied by up to 8% between the antisense and control lines, but no pattern associated with SAI activity was apparent (Figure 3). Antisense lines A5 and A8, which had the lowest SAI activity, showed opposite apparent effects on sucrose hydrolysis in internode 3, and no apparent reduction in sucrose hydrolysis in older internodes. One interpretation is that most sucrose hydrolysis, or carbon cycling between sucrose and hexose, occurs in the cytosol. A caution is the possibility that the vacuolar sucrose pool is insufficiently labelled during the 3 h labelling time to allow an accurate estimate of vacuolar sucrose hydrolysis.

Collectively these results indicate that in sugar-cane cultivar Q117 the SAI expressed preferentially in young internodes has little direct influence on sucrose accumulation or carbon cycling. Down-regulation of this target appears unlikely to increase sucrose accumulation over most of the commercial growing period. We emphasise that:
1. Q117 is an elite cultivar with low SAI activity relative to 'wild' relatives, and there may be intermediate cultivars with potential to respond differently;
2. our results do not exclude a physiological role for the residual SAI in the transgenic lines, which could be disrupted by further reduction in SAI;
3. the transgenic lines should be analysed under conditions likely to induce SAI expression or 'remobilise' stored sucrose (germination, suckering) to obtain a fuller picture of the physiological and commercial implications of reduced SAI activity.

Acknowledgments
This work was supported by the Universities of Queensland and Stellenbosch, the National Research Foundation of South Africa, and the Queensland Bureau of Sugar Experiment Stations (BSES).

**Fig. 3—Sucrose hydrolysis in several transgenic lines transformed with GUS, or an antisense acid invertase gene. Results are normalised against the hydrolysis observed in Q117 (control).**

**REFERENCES**


