KINETIC MODELLING OF SUCROSE METABOLISM: A POWERFUL PREDICTIVE TOOL FOR GENETIC MANIPULATION OF SUGAR CONTENT IN SUGARCANE

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The apparent failure to make significant progress in increasing the sucrose content of commercial varieties during the past two decades has led to the speculation that a yield plateau has been reached. Several research groups have therefore been investigating genetic manipulation of sucrose metabolism as an alternative approach, to supplement conventional breeding. However, the general lack of knowledge regarding the complexity of sucrose metabolism and control of sugar accumulation makes the selection of candidate genes for manipulation extremely difficult. Over the past few years the Institute for Plant Biotechnology has accumulated a significant amount of information about carbon partitioning, enzyme and metabolite levels, and metabolic fluxes in the sugarcane culm. This information was used to develop a kinetic metabolic model of sucrose synthesis and breakdown in the culm. The model accurately simulates the characteristic sucrose accumulation pattern observed in sugarcane. Surprisingly, the model predicted limited control on sucrose metabolism for some of the current genes being targeted for manipulation. By referring to the example of genetically modified sugarcane with reduced acid invertase, it will be illustrated that this model could become an invaluable tool in developing a strategy to manipulate metabolism in sugarcane.

During the past decade, significant developments in the ability to genetically manipulate plant metabolism have led to an international interest in modifying sucrose accumulation in sugarcane. However, it has become increasingly evident that the successful genetic manipulation of end product accumulation is dependent on a thorough understanding of the metabolism involved and the control of carbon flux.

Although it is well known that significant differences in sucrose content are evident between different sugarcane varieties, the biochemical basis for this is still poorly understood (Moore, 1995). Traditionally, identification of key regulatory reactions in metabolism was based on the assumptions that these would be largely irreversible, that the enzymes would be regulated and that reciprocal changes in flux and substrate concentrations would be evident (Stitt and Sonnewald, 1995). However, it is now obvious that this approach is very limited, as it does not consider the correlation between enzyme activity and actual flux within the system. The severe shortcoming of this traditional approach is evident from the numerous examples of unexpected effects resulting from the genetic manipulation of specific genes in plants.

Current knowledge about sucrose accumulation in sugarcane is almost exclusively based on the traditional approach. It is evident that sucrose accumulation is accompanied by a continuous cleavage and synthesis of sucrose in storage tissue (Gayler and Glasziou, 1972; Batta and Singh, 1986; Whittaker and Botha, 1997) and cell suspension cultures (Wendler *et al*., 1990). Despite numerous studies on enzyme activities that could potentially be important in sucrose accumulation, no consistent pattern has emerged.

A model that predicts that sucrose accumulation is dependent on a system in which sucrose phosphate synthase activity exceeds that of acid invertase is currently favoured by most (Zhu *et al*., 1997). However, the system is complex and all the potential permutations make it virtually impossible to identify the reactions that could be good targets for increasing the sucrose load by genetic manipulation.

Instead of the cumbersome gene-by-gene manipulation strategy currently followed, the authors have been developing a kinetic model that describes sucrose accumulation in sugarcane. This was made possible by the large volume of information gathered over the past five years focusing on this process (Botha *et al*., 1996; Whittaker and Botha, 1997; Vorster and Botha, 1999; Bindon, 2000; Bindon and Botha, 2000). This model could be an invaluable tool for predicting the most likely control points of processes that lead to sucrose accumulation.

The study deals with sucrose accumulation by the sugarcane culm. Initially the model described the cytosolic reactions, which involved hexose uptake, sucrose synthesis and breakdown, and loading of sucrose into the vacuole (Rohwer and Botha, 2001). Current refinements include sucrose uptake from the cytosol and hydrolysis of sucrose in the vacuole, as well as return of sugars from the vacuole.

Values for kinetic constants were taken from the literature where available. Maximal enzyme activities were taken as determined for culm tissue from internode 5, reflecting medium-mature tissue (Botha *et al*., 1996). For conversion, 1 g fresh weight of tissue was assumed to correspond to 700µl intracellular volume (Whittaker and Botha, 1997), comprising 10% cytoplasmic and 90% vacuolar volume (Komor, 1994). For the model to be able to calculate a steady state, the concentrations of the terminal boundary metabolites (source and sink) need to be fixed. These were entered as measured in internode 5 tissue (Whittaker and Botha, 1997). In addition, the cofactors ATP, ADP and UDP, as well as phosphate, were not modelled as explicit system variables, but their concentrations were entered as constants.

The general performance of the model closely matches the data obtained over the years in many studies on sucrose metabolism in sugarcane (Table I) (Whittaker and Botha, 1997; Vorster

and Botha, 1999; Bindon and Botha, 2000). One of the interesting contradictions is that UDP-glucose is much higher than the 0.9 mM previously reported for sugarcane internodal tissue (Whittaker and Botha, 1997). However, the predicted value precisely matches that calculated for sugarcane suspension cultures (Dancer *et al*., 1990). The model predicts an approximate 30% futile cycling of carbon between sucrose and the hexoses, and a partitioning of about 70% carbon into sucrose, compatible with the experimental data. The prediction of the model is that this process of futile cycling of carbon in the cytosol is one of the major controlling factors for sucrose accumulation. According to the model, the steps for fructose uptake and phosphorylation, glucose uptake and neutral invertase all have a significant effect on futile cycling.

The discrepancy between predicted and measured UDP-glucose levels could indicate an inaccuracy in the model. However, it could equally well indicate a problem with the measurement of the metabolite. This is currently being investigated.

Although many have pursued acid invertase as a primary target to increase the sucrose load in sugarcane, the model ascribes a relatively small role to that enzyme. To evaluate this, a series of transgenic clones with reduced acid invertase performance were recently investigated. Despite as much as a 70% reduction in acid invertase activity, the authors could detect no effect on the sucrose load, juice purity, or carbon cycling in these genotypes.

Further refinement and validation of the model is required before it can be used with confidence as a precise predictive tool. However, its accuracy regarding cellular metabolite levels, sucrose cycling and accumulation, and role of acid invertase in sucrose accumulation, clearly indicate that this is an important approach to pursue in future research. More exact prediction of those enzymes which are most likely to have a major influence on sucrose load will increase the efficiency of the transgenic sugarcane programme, and would lead to a major reduction in its associated costs.

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REFERENCES

Batta, SK and Singh, R (1986). Sucrose metabolism in sugar cane grown under varying climatic conditions: synthesis and storage of sucrose in relation to the activities of sucrose synthase, sucrose-phosphate synthase and invertase. *Phytochemistry* 25: 2431-2437.

Bindon, KA (2000). Carbon partitioning in sugarcane internodal tissue with special reference to the insoluble fraction. MSc Thesis, University of Stellenbosch. 77 pp.

Bindon, KA and Botha, FC (2000). Tissue disks as an experimental system for metabolic flux analysis in the sugarcane culm. *South African Journal of Botany* 66: 260-264.

Botha, FC, Whittaker, A, Vorster, DJ and Black, KG (1996). Sucrose accumulation rate, carbon partitioning and expression of key enzyme activities in sugarcane stem tissue. In: *Sugarcane: research towards efficient and sustainable production*: JR Wilson, DM Hogarth, JA Campbell, and AL Garside (Eds), Brisbane, CSIRO Division of Tropical Crops and Pastures, pp 98-101.

Dancer, J, Veith, R, Feil, R, Komor, E and Stitt, M (1990). Independent changes of inorganic pyrophosphate and the ATP/ADP or UTP/UDP ratios in plant cell suspension cultures. *Plant Science* 66: 59-63.

Gayler, KR and Glasziou, KT (1972). Physiological functions of acid and neutral invertases in growth and sugar storage in sugar cane. *Physiol Plant* 27: 25-31.

Komor, E (1994). Regulation by futile cycles: the transport of carbon and nitrogen in plants. In: *Flux control in biological systems:* ED Schulze (Ed), San Diego, Academic Press, pp 153-201.

Moore, PH (1995). Temporal and spatial regulation of sucrose accumulation in the sugarcane stem. *Aust J Plant Physiol* 22: 661-679.

Rohwer, J and Botha, FC (2001). Analysis of sucrose accumulation in the sugarcane culm on the basis of in vitro kinetic data. *Biochem J* (in press).

Stitt, M and Sonnewald, U (1995). Regulation of metabolism in transgenic plants. *Plant Physiol Plant Mol Biol* 46: 341-368.

Vorster, DJ and Botha, FC (1999). Sugarcane internodal invertases and tissue maturity. *J Plant Physiol* 155: 470-476.

Wendler, R, Veith, R, Dancer, J, Stitt, M and Komor, E (1990). Sucrose storage in cell suspension cultures of Saccharum spp. (sugarcane) is regulated by a cycle of synthesis and degradation. *Planta* 183: 31-39.

Whittaker, A and Botha, FC (1997). Carbon partitioning during sucrose accumulation in sugarcane internodal tissue. *Plant Physiol* 115: 1651-1659.

Zhu, YJ, Komor, E and Moore, PH (1997). Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. *Plant Physiol* 115: 609-616.

Table 1. Kinetic model validation: comparison of calculated and experimentally determined fluxes and metabolite concentrations.

Step	Model prediction	Experimental
Flux (mM min^{-1})		
Glucose uptake	0.127	0.195
Hexokinase	0.157	0.197
Glycolysis	0.049	0.041
Sucrose accumulation	0.097	0.127
Metabolite concentration (mM)		
Glucose	30	29
Fructose	37	30
UDP-glucose	2.72	0.9/2.67
Glucose-6-phosphate	0.37	0.21
Fructose-6-phosphate	0.19	0.12