

# Plant-Scale Kinetic and Structural Modeling of Sucrose Accumulation in Sugarcane

Johann M. Rohwer<sup>1,\*</sup>, Lafras Uys<sup>1</sup>, Kristy L. Meyer<sup>1</sup>,  
Frikkie C. Botha<sup>2,3</sup>, Jan-Hendrik S. Hofmeyr<sup>1</sup>

1. Dept. of Biochemistry, Stellenbosch University, Stellenbosch, South Africa
2. South African Sugarcane Research Institute, Mount Edgecombe, South Africa
3. Inst. for Plant Biotechnology, Stellenbosch University, Stellenbosch, South Africa

\*E-mail: jr@sun.ac.za

## Background

Sugarcane (*Saccharum officinarum*) produces and stores sucrose at levels in the high millimolar range. Fixated carbon is transported primarily as sucrose from the leaves to the internodal culm where there are three major pathways into which sucrose can be partitioned: transportation into the parenchymal vacuole for storage and accumulation, respiration and insoluble fiber formation. Flux to starch is almost negligible in commercial varieties. The regulation of the complete process is not fully understood and, paradoxically, a large fraction of sucrose is hydrolyzed in a synthesis/breakdown futile cycle.

## Kinetic modeling

To investigate the factors controlling the futile cycle, our group has developed a kinetic model of this process, initially focusing only on medium-mature tissue (internode 5) [1]. Subsequently the model was extended to culm tissue of varying degrees of maturity (internodes 3–10) and now describes the partitioning of carbon to respiration, fiber formation and vacuolar sucrose storage [2]. The model also accounts for sucrose breakdown by neutral invertase, and for sucrose synthesis by all known sucrose synthase isoforms as well as sucrose phosphate synthase. A set of kinetic parameters compiled from the literature allows sugarcane tissue maturation to be modeled by appropriate substitution of maximal velocity values for the enzymes in the different sugarcane internodes.

It became essential to develop software tools for handling large volumes of data, including both input data such as kinetic parameters and output data such as steady-state concentrations and fluxes, as well as coefficients of metabolic control analysis. Using the programming language Python as glue to interface existing tools (the PySCeS simulation software [3] and the Gnumeric spreadsheet program), we created an efficient software environment without having to write new software [4].

The model supports a hypothesis of vacuolar sucrose accumulation against a concentration gradient. A key result from this work is that theoretical sucrose yields increase as a result of a reduction in invertase activity and an increase in the transport activity of sucrose into the vacuole [2]. The model further suggests that the control over the sucrose accumulation process does not change significantly with internode maturity.

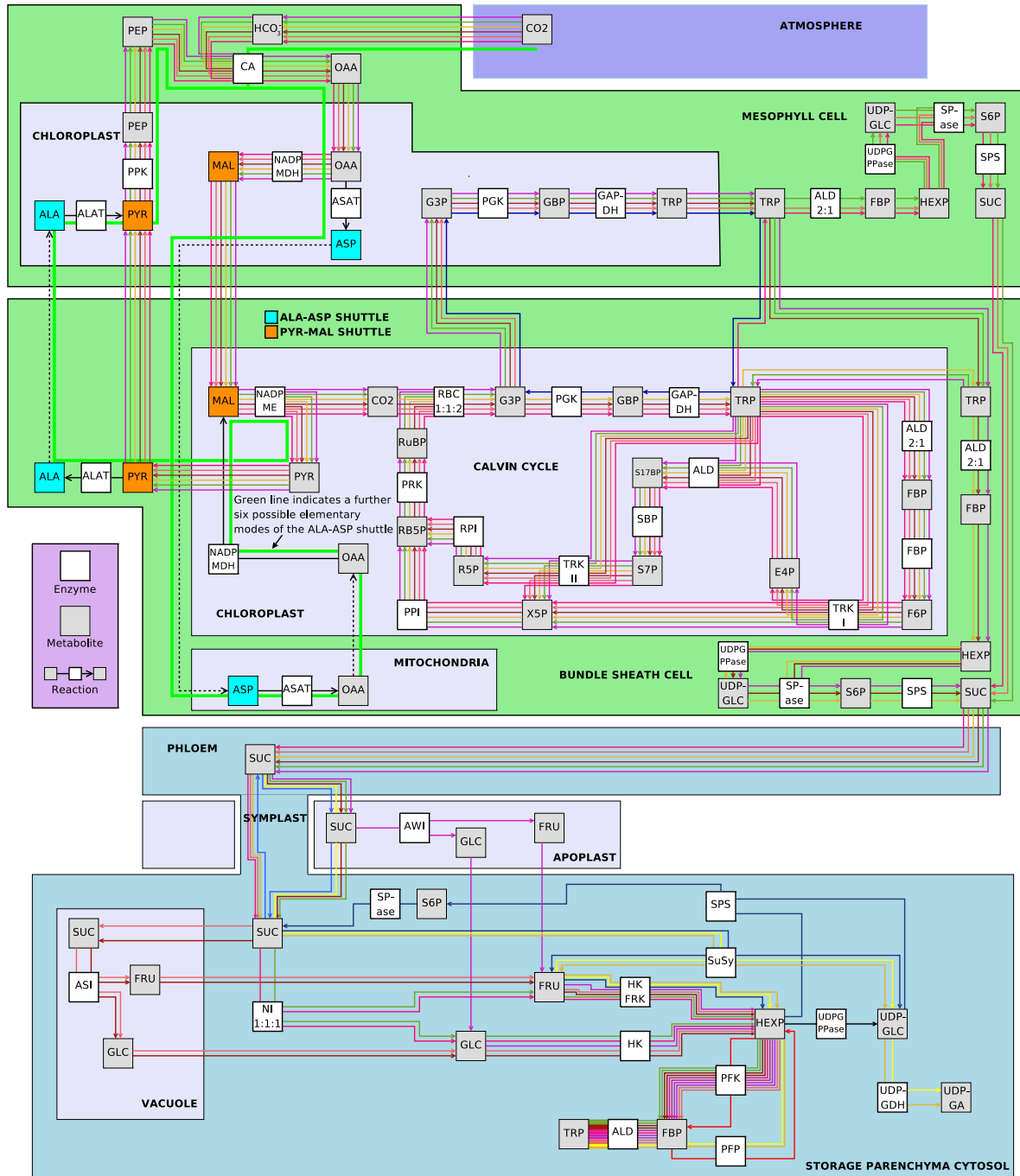


Figure 1: Elementary modes of sucrose accumulation.

**Enzymes:** ALAT, Alanine aminotransferase; ALD, Aldolase; ASAT, Aspartate aminotransferase; ASI, Acid-soluble invertase; AWI, Acid-wall invertase; CA, Carbonic anhydrase; FBP, Fructose biphosphatase; FRK, Fructokinase; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; HK, Hexokinase; NADP-MDH, NADP-malate dehydrogenase; NADP-ME, NADP-malic enzyme; NI, Neutral invertase; PFK, Phosphofructokinase; PFP, Pyrophosphate-dependent PFK; PGK, Glycerate-3-phosphate kinase; PPI, Phosphopentose epimerase; PPK, Pyruvate phosphate dikinase; PRK, Phosphoribulokinase; RBC, Ribulose biphosphatase carboxylase-oxygenase; RPI, Ribose-5-phosphate isomerase; SBP, Sedoheptulose biphosphatase; SPS, Sucrose phosphate synthetase; SPase, Sucrose phosphatase; SuSy, Sucrose synthase; TRK, Transketolase; UDPGPPase, UDP-glucose pyrophosphorylase.

**Metabolites:** ALA, Alanine; ASP, Aspartate; CO<sub>2</sub>, Carbon dioxide; E4P, Erythrose-4-phosphate; F6P, Fructose-6-phosphate; FBP, Fructose-1,6-phosphate; FRU, Fructose; G3P, 3-Phosphoglycerate; GBP, 1,3-Bisphosphoglycerate; GLC, Glucose; HCO<sub>3</sub><sup>-</sup>, Hydrogen carbonate; HEXP, Hexose Phosphate; MAL, Malate; OAA, Oxaloacetate; PEP, Phosphoenolpyruvate; PYR, Pyruvate; R5P, Ribose-5-phosphate; RB5P, Ribulose-5-phosphate; RuBP, Ribulose biphosphate; S17BP, Sedoheptulose-1,7-bisphosphate; S6P, Sucrose-6-phosphate; S7P, Sedoheptulose-7-phosphate; SUC, Sucrose; TRP, Triose Phosphate; UDPGA, UDP-glucuronic acid; UDPGLC, UDP-glucose; X5P, Xylulose-5-phosphate.

## Structural modeling

Our next aim was to extend the model to encompass the complete pathway from carbon fixation in the leaves to sucrose metabolism and accumulation in the culm. Since kinetic detail need not be known for a structural analysis, this allowed construction of the model on a large scale.

To infer properties of pathway fluxes, we performed an elementary mode analysis [5]. This allows one to identify all the alternative pathways that carbon can travel from fixation to storage, fiber formation and respiration (Fig. 1). Moreover, energetically wasteful futile cycles have been identified. The main conclusion from this analysis is that there is no carbon fixation pathway that is more energy efficient than any other.

## Conclusion

This work has implications for the rational design of genetic engineering strategies and improved crop selection. This in turn could improve yields from sugarcane as a food crop and ethanol production from sugarcane fermentation.

## References

- [1] Rohwer, J. M. and Botha, F. C. (2001) Analysis of sucrose accumulation in the sugar cane culm on the basis of *in vitro* kinetic data. *Biochem. J.* **358**: 437–445
- [2] Uys, L., Botha, F. C., Hofmeyr, J.-H. S. and Rohwer, J. M. (2007) Kinetic model of sucrose accumulation in maturing sugarcane culm tissue. *Phytochemistry* doi:10.1016/j.phytochem.2007.04.023
- [3] Olivier, B. G., Rohwer, J. M. and Hofmeyr, J.-H. S. (2005) Modelling cellular systems with PySCeS. *Bioinformatics* **21**: 560–561
- [4] Uys, L., Hofmeyr, J. H. S., Snoep, J. L. and Rohwer, J. M. (2006) Software tools that facilitate kinetic modelling with large data sets: an example using growth modelling in sugarcane. *IEE Proc.-Syst. Biol.* **153**: 385–389
- [5] Schuster, S., Dandekar, T. and Fell, D. A. (1999) Detection of elementary flux modes in biochemical networks: a promising tool for pathway analysis and metabolic engineering. *Trends Biotechnol.* **17**: 53–60