

***ENZYME PROFILING OF A RANGE OF SUGARCANE  
TISSUE TYPES WITH DIFFERENT LEVELS OF SUCROSE***

by

**R. ORENDO-SMITH**

Thesis

Presented for the degree

Master of Science

(Plant Biotechnology)

at the

University of Stellenbosch



Promoter: Prof. F.C. Botha  
South African Sugar Research Institute  
Co-Promoter: Mr. J.H. Groenewald  
Institute for Plant Biotechnology  
December 2005

## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted to any university for a degree. Where use was made of the work of others, it is duly acknowledged in the text.

**Signature**

**Date**



## SUMMARY

The study had two main objectives:

- 1) to investigate specific enzyme activity profiles at various developmental stages and to determine possible implications for sucrose metabolism,
- 2) to incorporate enzyme activity data of different internodes to obtain a detailed model of every stage in the tissue maturation process.

The most significant findings of the regulation of sucrose accumulation in this study are centred on three main point controls in sucrose metabolism pathway. Firstly, the maturation of sugarcane internodes coincided with an increase of SPS in most genotypes, and this underlines the key role of this enzyme in sucrose accumulation. Secondly, SuSy activity (cleavage reaction) correlated negatively with sucrose concentration and hence with tissue maturation process, in most of the varieties. This finding indicates that SuSy could well be implicated in sucrose metabolism. Thirdly, *in vitro* PFP activity was found to be negatively correlated to sucrose content in sugarcane varieties differing in amount of sucrose.

In terms of modelling outputs, the steady state concentrations of metabolites (sucrose, glucose and fructose) were calculated by incorporating the  $V_{max}$  values of the enzymatic changes during the increasing maturity on the stem. Cytosolic sucrose concentration declined with tissue maturation in varieties Co331; NCo376 and US6656-15 and simultaneously glucose and fructose concentrations increased. In parallel to that, SPS and SuSy fluxes declined with the internode's age. However, the steady state concentration of sucrose calculated both by the original and corrected model in the younger internodes was to some extent in agreement to the experimental value of sucrose in variety N19.

## OPSOMMING

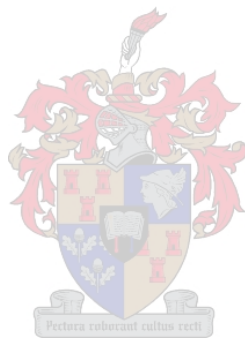
Hierdie studie het twee hoof doelwitte gehad:

- 1) om spesifieke ensieme se aktiwiteite tydens verskillende ontwikkelings stadiums te bepaal, en om moontlike implikasies in terme van sukrose metabolisme te ondersoek,
- 2) om die data van die ensiem aktiwiteite van verskillende internodes te inkorporeer in 'n gedetailleerde model vir elke stadium van weefsel ontwikkeling.

Die mees beduidende bevindings in die regulering van sukrose akkumulاسie wat hierdie studie opgelewer het, is gesentreerd in drie hoof kontrole punte in die sukrose metabolisme weg. Eersens, die ryppwording van suikerriet internodes was ooreenstemmend met 'n verhoging in SPS aktiwiteit in die meeste genotipes. Dit beklemtoon die sleutelrol wat hierdie ensiem speel in sukrose akkumulاسie. Tweedens, SuSy aktiwiteit (sukrose afbraak reaksie) het 'n negatiewe korrelasie getoon met sukrose konsentrasie in meeste van die variëteite - die ensiem speel dus 'n rol in die ryppwordings proses. Derdens, in vitro PFP aktiwiteit was negatief gekorreleerd met sukrose inhoud in suikerriet variëteite met verskillende hoeveelhede sukrose.

In terme van modellerings uitsette, die konsentrasies van metaboliete (sukrose, glukose en fruktose) tydens bestendige toestande is bereken deur die inkorporering van die  $V_{maks}$  waardes van die ensiematiese verandering tydens die ryppwording van die stingel. Sitosoliese sukrose konsentrasies het afeneem met weefsel ryppwording in variëteite Co331, NCo376 en US6656-15, terwyl glukose en fruktose gestyg het. SPS en SuSy flukse het ooreenstemmend gedaal met internode ouderdom in meeste van die genotipes. Die konsentrasie

van sukrose tydens bestendige toestande, bereken met die oorspronklike en aangepaste model, in jonger internodes van variëteit N19 was egter tot 'n sekere mate in ooreenstemming met die eksperimentele sukrose konsentrasies.



## ACKNOWLEDGEMENTS

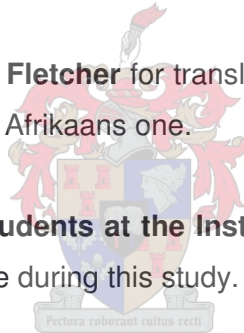
I am deeply thankful to my supervisor, **Prof. FC Botha** whose advice, patience support and encouragement have no doubt enabled me to hurdle the task of completing my degree.

I also express my sincere gratitude to my co-supervisor, **Mr. JH Groenewald** and **Dr. WE Schäfer** for making the time to read and provide important comments to my manuscript.

My appreciation is extended to **Prof. J Kossmann** and **Prof. J Rohwer** for their assistance and for the interest shown in this study.

I am also grateful to **Mr. Hiten Fletcher** for translating the summary of my thesis from the English version to the Afrikaans one.

Thanks go to the **staff and students at the Institute for Plant Biotechnology** for their support and assistance during this study.



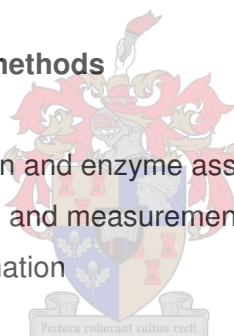
I am very grateful for the financial support received from **Gabonese Government**.

Finally, I would like to thank **my parents, family and friends** for their encouragement and moral support.

## TABLE OF CONTENTS

	page
<b>1 GENERAL INTRODUCTION</b>	<b>1</b>
<b>2 ENZYME ACTIVITIES AND SUCROSE ACCUMULATION</b>	
2.1 Introduction	4
2.2 Sucrose metabolism in internodal tissue and enzyme activities	4
2.2.1 Sucrose metabolism in sugarcane culm	4
2.2.2 Enzyme catalysing sucrose accumulation	4
2.2.2.1 Sucrose phosphate synthase	4
2.2.2.2 Sucrose synthase	5
2.2.2.2.1 Sucrose synthase isoforms	6
2.2.2.3 Invertases	7
2.2.2.3.1 Invertase isoforms	7
2.2.2.3.2 Neutral invertase	7
2.2.2.3.3 Soluble acid invertase	8
2.2.2.4 Fructokinase and Hexokinase	9
2.2.2.4.1 Fructokinase isoforms	9
2.2.2.5 ATP and Pyrophosphate dependent Phosphofructokinase	10

2.2.3	<b>Manipulating sucrose accumulation</b>	11
2.2.4	<b>Kinetic modelling and Metabolic Control Analysis</b>	12
2.2.5	<b>Current limitations of the kinetic model</b>	13
3.	<b>DETERMINATION OF ENZYME ACTIVITY PROFILES IN RELATION TO SUCROSE CONTENT ACROSS SUGARCANE VARIETIES</b>	
3.1	<b>Abstract</b>	15
3.2	<b>Introduction</b>	16
3.3	<b>Materials and methods</b>	18
3.3.1	Materials	18
3.3.2	Protein extraction and enzyme assays	18
3.3.3	Sugar extraction and measurements	20
3.3.4	Protein determination	20
3.3.5	Data analyses	20
3.4	<b>Results</b>	20
3.4.1	Sugar content	20
3.4.2	Enzyme activity	21
3.5	<b>Discussion</b>	28
3.6	<b>Concluding remarks</b>	33
4	<b>VALIDATION OF THE KINETIC MODEL FOR SUCROSE ACCUMULATION IN SUGARCANE INTERNODAL TISSUES</b>	





4.1	<b>Abstract</b>	34
4.2	<b>Introduction</b>	35
4.3	<b>Methods</b>	36
4.3.1	Enzyme activities and modelling	36
4.4	<b>Results</b>	37
4.5	<b>Discussion</b>	40
4.6	<b>Conclusion</b>	44
5	<b>GENERAL CONCLUSION</b>	45
6	<b>LITERATURE CITED</b>	49



## LIST OF FIGURES AND TABLES

**Figure 1** Enzyme activities across varieties in tissues representing young ( $I_{3-4}$ ), maturing ( $I_{6-7}$ ) and mature ( $I_{8-9}$ ) internodes. Each value is the mean of  $\pm$  SD from three distinct measurements.

**Figure 2** Sucrose concentration and SuSy breakdown activity in tissue representing young ( $I_{3-4}$ ), maturing ( $I_{6-7}$ ) and older ( $I_{8-9}$ ) internodes of sugarcane.

**Figure 3** Correlation between mean PFP activity in internodes ( $I_{8-9}$ ) and sucrose content among sugarcane varieties. Each value is the mean  $\pm$  SD of three distinct measurements.

**Table 1** Sugar content across varieties in tissues representing young ( $I_{3-4}$ ) maturing ( $I_{6-7}$ ) and older internodes ( $I_{8-9}$ ). Each value is the mean of  $\pm$  SD from three separate extractions

**Table 2** PFP and PFK activities and PFP/PFK ratios in maturing ( $I_{6-7}$ ) and older ( $I_{8-9}$ ) internodal tissues. Each value is the mean of  $\pm$  SD from 3 separate plants

**Table 3** Measured  $V_{max}$  values of the enzyme-catalyzed reactions. Values are in Mm/min.

**Table 4** Predicted fluxes of the enzyme-catalyzed reactions. Values are in Mm/min.

**Table 5** Calculated metabolite concentrations and futile cycling.

**Table 6** Kinetic model validation: comparison of the calculated and the experimentally determined metabolite concentrations.

## ABBREVIATIONS

ATP	Adenosine triphosphate
BSA	Bovine serum albumin
DTT	1,4 -dithiothreitol
FRK	Fructokinase (EC 2.7.1.4)
FW	fresh weight
HK	Hexokinase (EC, 2.7.1.1)
$K_{eq}$	equilibrium constant
$K_i$	competitive inhibition constant
$K_m$	concentration of substrate that produces half maximal velocity
NI	Neutral invertase (EC 3.2.1.26)
PFK	ATP- dependent phosphofructokinase (EC 2.7.1.11)
PFP	Pyrophosphate-dependent phosphofructokinase (EC 2.7.1.90)
PPi	inorganic Pyrophosphate
SAI	Soluble acid invertase (EC 3.2.1.26)
SPP	Sucrose phosphatase (EC 3.1.3.24)
SPS	Sucrose phosphate synthase (EC 2.4.1.14)
SuSy	Sucrose synthase (EC 2.4.1.13)
SuSy-B	Sucrose synthase – Breakdown direction
SuSy-S	Sucrose synthase – Synthesis direction
UDPG	Uridine 5' diphosphoglucose
Vmax	maximal velocity of reaction at unlimiting substrate concentration