DIFFERENTIAL EXPRESSION AND REGULATION OF SUCROSE TRANSPORTERS IN RICE (*Orzya sativa* L. cv Nipponbare) DURING ENVIRONMENTAL STRESS CONDITIONS

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ABSTRACT

Plant productivity is greatly affected by environmental stresses such as drought, salinity and insect herbivory. Plants respond and adapt to these stresses by exhibiting physiological as well as biochemical changes at the cellular and molecular levels in order to survive. Expression of a variety of genes which encode numerous membrane transporters have been demonstrated to be induced by these stresses in a variety of plants. The nutritional status of plants is controlled by these transporters, which are regulated by the transcription of the corresponding genes.

In spite of these adverse stress effects on agricultural yield, only a few studies have focused on gene transcriptional and translational regulation of membrane transporters during environmental stress situations. Rice, like other plants, contains a number of sucrose transporters encoded by a family of genes. However, detailed knowledge of their roles, localization and regulation during environmental stress conditions is lacking.

Bioinformatic tools were used to identify putative *cis*-acting regulatory elements that may be involved in the regulation of rice and *Arabidopsis thaliana* sucrose transporters. The possible *cis*-acting regulatory elements were predicted by scanning genomic sequences 1.5 kbp upstream of the sucrose transporter genes translational start sites, using Plant CARE, PLACE and Genomatix Matinspector professional data bases. Several *cis*-acting regulatory elements that are associated with plant development, plant hormonal regulation and stress response were identified, and were present in varying frequencies within the 1.5 kbp of 5′ regulatory region. The putative *cis*-acting regulatory elements that possibly are involved in the expression and regulation of sucrose transporter gene families in rice and *Arabidopsis thaliana* during cellular development or environmental stress conditions were identified as: A-box, RY, CAT, Pyrimidine-box, Sucrose-box, ABRE, ARF, ERE, GARE, Me-JA, ARE, DRE, GA-motif, GATA, GT-1, MYC, MYB, W-box, and I-box.

Expression analysis was used to elucidate the role of rice (*Oryza sativa* L. cv Nipponbare) sucrose transporter (OsSUT) genes during drought and salinity treatments of three week old rice plants (at four leaf stage) over a 10 days. Among the five rice OsSUT genes identified, only OsSUT2 was observed to be progressively up-regulated during drought and salinity treatments, while OsSUT1, OsSUT4 and OsSUT5 were expressed at low levels, and OsSUT3 showed no detectable transcript expression. Sucrose transport will be essential to meet the cellular energy demands and also for osmoprotectant activities during drought and salinity stresses. It therefore indicates that OsSUT2 which facilitates transport of sucrose from photosynthetic cells will be

essential for rice plants to cope with drought and salinity stresses, and cultivars with a higher OsSUT2 expression should be able to tolerate these environmental stresses better.

The role of OsSUT in assimilate transport during rusty plum aphids (*Hysteroneura setariae*; Thomas) infestation on the leaves of three week old rice (*Orzya sativa* L. cv Nipponbare) cultivar plants, over a time-course of 1 to 10 days of treatments, was also examined by combination of gene expression and β-glucuronidase (*GUS*) reporter gene analysis.

Real Time PCR analysis of the five OsSUT genes revealed that the expression of OsSUT1 was progressively up-regulated during the course of aphid infestation. OsSUT2 and OsSUT4 expression were comparatively low in both the control and treated plants. OsSUT5 showed no clear difference in transcript expression in both control and treated plants, while no detectable transcript expression of OsSUT3 could be found. The up-regulation of OsSUT1 gene was verified at protein level by western blot analysis in both the control and treated plants. OsSUT1 protein expression was found to increase with time during aphid infestation. A similar trend was noticeable in the control plants, however at a lower expression level. These demonstrate that the cellular expression of OsSUT1is regulated by both developmental and environmental factors.

OsSUT1-promoter:::*GUS* reporter gene expression was observed within the vascular parenchyma and/or companion cells associated with phloem sieve elements of the large and small bundles in the phloem tissues of the flag leaf blade regions where feeding aphids were confined, which progressively increased with time of infestation. It is suggested that OsSUT1 may primarily play an essential role in phloem transport of assimilate to wounded tissues from adjacent health tissues or may be involved in the retrieval of assimilate back into the phloem to minimize loss caused by the infestation. Some OsSUT1-promoter:::*GUS* expression was also found in the metaxylem at 10 days after infestation, which could signify a recovery system in which sucrose lost into the xylem as a result of aphids feeding are retrieved back into the phloem through the vascular parenchyma. This was supported by the exposure of cut ends of matured OsSUT1-promoter:::*GUS* rice plant leaf to 2% sucrose solution. OsSUT1-promoter:::*GUS* expression was observed within the protoxylem, xylem and phloem parenchyma tissues. This indicates that sucrose translocating within the xylem tissues are retrieved into the phloem via the OsSUT1 localized within the parenchyma tissues.

In conclusion, the differential expression and regulation of rice (*Orzya sativa* L. cv Nipponbare) sucrose transporters as reported here suggest that OsSUT2 and OsSUT1 were constitutively expressed compared to other rice sucrose transporters during drought and salinity, and rusty plum aphids (*Hysteroneura setariae*; Thomas) infestation stresses respectively. Thus, the expression and regulation of the sucrose transporters could be related to the physiological and

nutritional requirements of the cells during plant developmental or environmental stress state that allows their differential expression.

DECLARATION

I, Omodele IBRAHEEM do hereby declear that this thesis entitled “Differential expression and regulation of sucrose transporters in rice (*Orzya sativa* L. cv Nipponbare) during environmental stress conditions” submitted for the award of the degree PhD (Biochemistry) at the University of Fort Hare, is my own original work and has not been submitted for any degree or examination at any other university. I further declear that all sources of my information have been quoted as indicated in the text and/or reference list.

Omodele IBRAHEEM

DEDICATION

This work is dedicated to him that controls the beginning and the end of all things, the Almighty God, and to my family.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
APS	Ammonium persulphate
AU	Arbitrary Unit
BLAST	Basic Local Alignment Search Tool
Bp	Base Pairs
CC	Companion Cell
cDNA	Complementary Deoxyribonucleic Acid
ESTs	Expressed Sequence Tags
ET	Ethylene
GUS	β-Glucuronidase
HR	Hypersensitive Response
IPG	Immobilised pH Gradient
JA	Jasmonic Acid
kDa	Kilodalton
K_m	Michaelis Constant
mA	Milliampere
MC	Mesophyll Cell
mRNA	Messenger Ribonucleic Acid
NCBI	National Centre For Biotechnology Information
OA	Okadaic Acid
PCR	Polymerase Chain Reaction
PD	Plasmodesmata
PEG	Polyethylene Glycol

pI	Isoelectric Point
PMF	Proton Motive Force
PPU	Pore Plasmodesmata Unit
PR	Pathogenesis Related Proteins
R gene	Resistance Gene
RNA Pol II	Ribonucleic acid Polymerase II
ROS	Reactive Oxygen Species
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SA	Salicylic Acid
SAR	Systemic Acquired Resistance
SC	Sink Cell
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SE	Sieve Elements
SUC	Sucrose Carrier
SUT	Sucrose Transporter
TEMED	N,N,N’N’ tetramethylethylenediamine
TFBs	Transcription Factor Binding Site
TFs	Transcription Factor
TRIS	Tris Hydroxyl Methyl Amino Methane
TSS	Transcription Start Site
ULD	Unloading Domain
Vac	Vacuole
X-Gluc	5-Bromo-4-Chloro-3-Indolyl-β-D-Glucuronide

$\Delta\psi$	Electrical Gradient
ΔH	pH Gradient
5′-UTR	5′-Untranslated Region

BRIEF CHAPTER

SYNOPSIS

CHAPTER 1:

This chapter discusses a concise but comprehensive literature review of sucrose transporters (SUT's) in plants in general. The structural and expressional analysis, mechanism of action, substrate specificities and regulation of SUT's action are discussed.

CHAPTER 2:

The overall rationale and motivation for the present study are discussed in this chapter, followed by the hypothesis and specific objectives which this study seeks to actualize.

CHAPTER 3:

Discussed here is an inclusive comparative analysis of *cis*-acting regulatory elements present within the 5´- untranslated region (5´-UTR) of the sucrose transporter gene families in rice (*Oryza sativa* Japonica cultivar - group) and *Arabidopsis thaliana*, using available bioinformatics tools. The *cis*-acting regulatory elements were predicted by scanning 1500 base pairs upstream of the translational start sites (ATG), using Plant CARE, PLACE and Genomatix Matinspector professional data bases. It is assumed that these *cis*-acting regulatory elements may serve as target binding sites for functional promoters for the expression and regulation of these genes.

CHAPTER 4:

Drought and salinity stresses are two major adverse abiotic stresses that pose a great challenge to survive of plants, most especially commercial crops such as rice. In this chapter, RT-PCR and Real- Time quantitative analyses were carried out to determine the differential expression of individual OsSUT genes, induced by these stress treatments on 3 week 4 leaf rice (*Orzya sativa* L. cv Nipponbare) plants.

CHAPTER 5:

Among plant insect pests, aphids (*Hemiptera, Aphidoidae*) have been reported to cause significant damage to valuable economic cereal crops including rice, wheat, barley and oats. This

chapter is focused on RT-PCR and Real-Time quantitative analyses of the differential expression of individual OsSUT genes, in relation to rusty plum aphid (*Hysteroneura setariae*; Thomas) infestation on 3 week 4 leaf rice (*Orzya sativa* L. cv Nipponbare) plants. Western blotting analysis was used to confirm that the up-regulated OsSUT1 gene is translated into full functional protein. Furthermore, β-glucuronidase (*GUS*) gene expressional analysis was used to confirm in-*vivo*, the up-regulated OsSUT1 gene expression during rusty plum aphid (*Hysteroneura setariae*; Thomas) infestation on 3 week, 4 leaf rice (*Orzya sativa* L. cv Nipponbare) plants, and also to determine the sub-cellular localization of expression during this stress period.

CHAPTER 6:

This chapter briefly discusses future research that should be carried out in order to provide further insight into the role of SUTs during environmental stress conditions, thus providing information that may aid in general crop improvement.