Regulatory control of the symbiotic enhanced soybean bHLH transcription factor, *GmSAT1*

A thesis submitted for degree of the Doctor of philosophy

at

The University of Adelaide

School of Agriculture, Food & Wine

Waite Campus

Submitted by

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December 2013

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Abstract

GmSAT1 is a basic Helix-Loop-Helix (bHLH) DNA binding transcription factor expressed in soybean root nodules. *GmSAT1* is a unique protein, in that it is localised on cellular membranes including the symbiosome membrane, which encircles nitrogen-fixing bacteroids in soybean nodules. Its role in the regulation of gene transcription in nodules or in other plant tissues is poorly understood. In this study, *GmSAT1*'s functional activity was investigated through a series of studies that investigated the link between gene activities to functional phenotypes. This analysis included the influence of symbiotic partnerships with rhizobia and AM fungi and non-symbiotic root tissues. In this context, an evaluation of changes in gene transcription with or without *GmSAT1* expression (RNAi-based silencing of *GmSAT1*) was explored at the individual and global gene levels. The data indicates that *GmSAT1;1* and a close relative *GmSAT1;2*, are both expressed in roots and nodules but *GmSAT1;1* displayed an overall enhancement in the symbiotic root nodule. Expression of both genes was reduced with external nitrogen supply to the nodule and inoculated root. Both genes were up-regulated in root and nodule tissues when plants were supplied low levels of phosphate. Using an improved method for transgenic hairy roots, developed as part of this thesis project, *GmSAT1* was silenced using a RNAi construct. Tissues (roots and nodules) were analysed for changes in global gene expression using microarray analysis, the impact on symbiotic relationships (rhizobia and AM fungi) and genetic and biochemical responses to phosphorus supply. Transcriptome analysis identified networks that *GmSAT1;1* may be associated with, including a suite of putatively active circadian clock regulators operating in nodules, phosphorus responsive genes in roots, cell wall maintenance and or stress defence signaling pathways, nitrogen transport and metabolism and genes linked to auxin and gibberellin regulatory pathways.

The influence of phosphorus and the AM fungal symbiosis was investigated in more detail. Loss of *GmSAT1* activity altered AM colonisation, causing a reduction in root colonisation when grown at reduced external P. At higher P levels, colonisation remained unchanged.

Shoot P content was significantly increased at both low and high external P supply in the *GmSAT1* silenced plants, indicating a potential role of *GmSAT1* in mediating P homeostasis.

The impact of gibberellins (GA_3) on *GmSAT1* expression and activity was also investigated. Using both qPCR and native promoter:GUS fusion constructs in transformed soybean hairy roots and nodules the expression of *GmSAT1;1* in roots and nodules decreased with external supply of GA_3. In parallel experiments, RNAi *SAT1*-silenced plants showed similar responses with GA_3 treated plants, where nodule number and weight decreased while plant height significantly increased. Furthermore, microarray analysis indicated *GmSAT1* negatively interacts with known gibberellin-responsive genes, including *GASA6*, *GAMA-TIP*, *CLE2*, *MTO3*, *GIP1*, *TPS11*, and *GBF1*.

The overall findings of this study have shown that *GmSAT1* is an important TF to soybean with a broad transcriptional imprint which influences both root nodule symbiosis and AM fungal symbioses. Its activity appears to be linked to multiple genetic signaling networks that involve phosphorus and nitrogen metabolism, hormone activity and regulation of the circadian clock.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

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December 2013

Statement of Authorship

1. **Manijeh Mohammadi-Dehcheshmeh, Esmaeil Ebrahimie, Stephen D. Tyerman, Brent N. Kaiser (2013)** A novel method based on combination of *semi-in vitro* and *in vivo* conditions in *Agrobacterium rhizogenes*-mediated hairy root transformation of *Glycine* species. In Vitro Cellular and Developmental Biology – Plant, [Published online: 21 November 2013]. Presented in Chapter 3

MM conducted the research. EE helped in *G. canescens* germination and nodulation as well as statistical analysis. MM and BNK designed the experiment. MM, EE, BNK and SDT wrote the manuscript.

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Mohammadi-Dehcheshmeh, M., Ebrahimie, E., Tyerman, S.D. & Kaiser, B.N. (2013) A novel method based on combination of semi-in vitro and in vivo conditions in *Agrobacterium rhizogenes*-mediated hairy root transformation of Glycine species.
In Vitro Cellular and Developmental Biology - Plant, v. 50(2), pp. 282-291

Presentations

1. Manijeh Mohammadi-Dehcheshmeh, David Chiasson, Danielle Mazurkiewicz, Esmaeil Ebrahimie, Steve D. Tyerman and Brent N Kaiser. **Influence of gibberellins on the transcriptional activity of the soybean nodule transcription factor *GmSAT1***. Combio 2012 conference (Presented as Poster)

2. Manijeh Mohammadi-Dehcheshmeh, Esmaeil Ebrahimie, Steve D. Tyerman and Brent N Kaiser. **Phenotypic analysis of soybean root and nodule tissues after RNAi induced silencing of the membrane bound transcription factor *GmSAT1***. Combio 2010 conference (Presented as Poster)

Acknowledgment

Over the course of my PhD, numerous people helped me and here I would like to express my deepest gratitude to all of them. I can't thank them enough as without their help I wouldn't have made it through. First and foremost, I would like to give thanks to my supervisor Dr. Brent Kaiser who was always there when I needed his help and who was always supportive especially when I was facing with most challenging side of my study. Thank you for your continuous help and support in all stages of this thesis. I give thanks to Prof. Steve Tyerman who made few short meetings that I had with him efficient and useful. During the last year of my PhD I have this chance to work with Prof. Sally Smith who introduced me with amazing word of Mychorrhiza. Thank you for helping me to be more precise, and have more patience in research. I feel privileged to have worked with you all.

I would also like to thank all members of the BNK lab (former and present), who have made me feel welcomed, making my PhD more enjoyable specially Karen Francis, Danielle Mazurkiewicz, and Nenah MacKenzie. I also give thanks to Rebecca Stonor for her great help in Phosphorous measurement and David Chiasson for his help in preparing RNA samples for Miscroarray experiment and Ahmad Tahmasebi who taught me working with Flexarray.

I would like to give a special thanks to my husband Dr. Esmaeil Ebrahimie for teaching all bioinformatics skills and helping and supporting me during PhD. Thank you for encouraging me to dream big and pushing the limits which this made me feel strong even when I was sick. I also would like to give thanks to my family for all their support.

I also acknowledge the University of Adelaide, and the Australian Government for scholarships that I received. Last, but by no means least, I also would like to appreciate all nice and friendly staff in Graduate center of University of Adelaide who during difficult time of my sickness were always helpful and supportive. Thank you all. Without your help I wouldn't have made it through.

Abbreviations

ABA	Abscisic acid
AM	Arbuscular mycorrhizal
bHLH	Basic Helix-Loop-Helix
BLAST	Basic local alignment tool
CCaMK	calcium-calmodulin-dependent protein kinase
CRE	cytokinin receptor
DMF	Dimethylformamide
DMI2	Does not Make Infections
EDTA	Ethylenediaminetetraacetic acid
ENOD	Early nodulation gene
ER	Endoplasmic reticulum
ER	Endoplasmic reticulum
GA	Gibberellin
GFP	Green fluorescent protein
GO	Gene Ontology
GUS	β-glucoronidase
H2O2	Hydrogen peroxide
IAA	Indole acetic acid
IPD3	interacting protein of DMI3
kb	Kilobase
kDa	Kilodalton
LB	Luria broth (medium)
LNP	Lectin nucleotide phosphohydrolase
LYK3	LysM receptor kinase 3
µM/M	Macro/ millimolar
MA	Methylammonium (chloride)
MeJA	Methyl jasmonic acid

N	Nitrogen
NFP	Nod factor perception
NFR	Nod factor receptor
NF-YA	Nuclear Factor Y
NIN	Nodule inception
NORK	NODULATION RECEPTOR KINASE
NSP	Nodulation signaling pathway
NUP	nucleoporin
OD	Optical density
P	Phosphorus
PAR	parabolic aluminized reflector
qPCR	quantitative PCR
RNA	Ribonucleic acid
RNAi	RNA interference
RNA-SEQ	RNA Sequencing
RO water	Reverse osmosis water
SYMRK	symbiosis receptor-like kinase
TEM	Transmission electron microscopy
TF	Transcription factor
TMD	Transmembrane domain
TMD	transmembrane domain
wk/d/h	week/ day/ hour
YEM	Yeast extract mannitol (medium)