

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

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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 (GA₃) 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 GA₃. In parallel experiments, RNAi *SAT1*-silenced plants showed similar responses with GA₃ 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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Manijeh Mohammadi Dehcheshmeh

December 2013

Statement of Authorship

1. **Manijeh Mohammadi-Dehcheshmeh, Esmail 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 *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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Presentations

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Abbreviations

ABA	Abcisic 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)