Interaction between sulfur (S) and nitrogen (N) assimilation pathways in response to S and N supply in onion (*Allium cepa* L.)

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Abstract

This thesis investigates the extent of interdependency between the sulfur (S) and nitrogen (N) assimilation pathways in the commercially important, S-accumulating, monocot species, onion CUDH2107 (*Allium cepa* L.), to elucidate some of the regulatory points of cross-talk between these two pathways.

To test the interactions between the two pathways, a factorial S x N depletion experiment was set up. Plants were grown in short day conditions to maintain the pre-bulbing stage after which they were transferred to long day conditions to promote bulbing. At the end of the short day conditions, the plants were harvested as leaf, pseudo stem and root and at the end of the long day conditions, as leaf, bulb and roots, for each of the four treatments. The four treatments comprised of control treatment (designated C; comprising 14 mM N and 2 mM S), low S treatment (designated –S; comprising 14 mM N and 0.25 mM S), low N treatment (designated –N; comprising 3.5 mM N, 2 mM S) and coupled low S and N treatment (designated –S-N; comprising 0.25 mM S and 3.5 mM N).

In terms of changes in biomass, both the root and the shoot biomass tended to be higher under the –S treatment at the bulbing stage, although these changes were only significant for the root biomass. Under the – N and the –S-N treatments, the shoot biomass was much lower when compared with the control plants at both the pre-bulbing and the bulbing stage, although no change in the root biomass was observed. The exception was at the bulbing stage under the –S-N treatment where the root biomass was significantly higher when compared with the control plants.

At the transcriptional level in response to the -S treatment, the relative transcript abundance of commonly used S-starvation marker genes, *AcHAST1;1LIKE1* and *AcAPSR1* increased in both root and the leaf tissue and was more marked at bulbing. In contrast, transcript abundance of *AcAPSK1,* which marks a bifurcation in the S-assimilation pathway, decreased. At bulbing, a decrease in the relative transcript abundance of *AcATPS1, AcSIR1* and *AcOASTL3* in the leaf tissue and *AcATPS1, AcAPSK1, AcOASTL2, AcNRT2;1LIKE1* and *AcNiR1* in the root tissue was observed in response to the –S treatment. However, in response to N deprivation, under the –N as well as –S-N treatment, the transcript abundance of *AcHAST1;1LIKE1* and *AcAPSR1* was dramatically reduced in the roots with a significant induction in the leaf tissue at both the stages. In addition, relative transcript abundance of *AcATPS1 , AcAPSK1* and *AcSOX1* also increased whereas *AcOASTL2, AcNR1*

and *AcNiR1* decreased under the –N and the –S-N treatments in the leaf tissue, at pre-bulbing. However, at bulbing, transcript levels of *AcOASTL2 and AcNR1* also increased under both, the –N and the –S-N treatments. In the roots, at pre-bulbing, the relative transcript abundance of *AcHAST1;1LIKE1, AcNRT2;1LIKE1* and all the down-stream reductive S and N assimilation genes investigated declined, while the transcript abundance of *AcAPSK1* increased. A similar response was observed at the bulbing stage for most genes except *AcSOX1* and *AcOASTL3* which increased and *AcOASTL1, AcOASTL2* and *AcNRT2;1LIKE1* showed no change. Similar to the leaf under the –S-N treatment, the transcriptional profile of the genes investigated in the roots under the –S-N treatment also showed a dominant response to N depletion.

In terms of protein accumulation and enzyme activity, AcSiR1 declined in the –S treatment but accumulated in the –N and the –S-N treatment in the leaf tissue at pre-bulbing whereas at bulbing, a decline in protein accumulation was observed under all three treatments. The AcSiR1 enzyme activity declined under the –S and the –N treatment but remained unchanged under the –S-N treatment in the leaf tissue at the pre-bulbing as well as the bulbing stage. In the roots, AcSiR1 accumulated under the –S treatment in both the stages whereas activity remained unchanged. No AcSiR1 protein could be detected under the –N treatment at both stages and in the –S-N treatment at pre-bulbing, whereas the activity increased under these treatments at both stages. Under the –S treatment in the leaf tissue, AcNiR1 accumulated slightly at both pre-bulbing and bulbing whereas the activity remained unchanged. Under the –N and the –S-N treatments, AcNiR1 declined in the leaves at pre-bulbing but accumulated at the bulbing stage. However, the activity remained unchanged at the pre-bulbing stage and was below the assay detection limit at bulbing. In the roots, the AcNiR1 accumulation response was similar to that in the leaf tissue under each treatment at both the stages, whereas the activity declined under all treatments at both stages except at the pre-bulbing stage under the –S treatment where it remained unchanged.

The accumulation of a set of targeted metabolites was also compared over the four treatments. A decline in the S containing flavour precursors, including the lachrymatory factor, thiopropanal-S-oxide, was observed in all tissues in response to low S supply. However, glutathione only declined in the leaf at the bulbing stage. An effect of the –S treatment on the accumulation of N-containing metabolites was observed as an accumulation of the amino-acids in the pseudo-stem and the bulb. In contrast, a decline in the accumulation of the amino-acids and derivatives was observed in the leaf at bulbing. In

response to the –N treatment, most of the N-containing metabolites declined systemically, including the N-pathway cysteine precursor, *O*-acetylserine and serine. Flavonol glucosides accumulated in a tissue-specific manner in the pseudostem at the pre-bulbing stage but declined in the bulb tissue. Generally, sugars accumulated systemically at both developmental stages whereas sugar phosphates accumulated only in the leaf and root tissue at the pre-bulbing stage. The lachrymatory factor thiopropanal-S-oxide, accumulated in the leaf at the pre-bulbing stage but declined at the bulbing stage in response to the –N treatment. The metabolite accumulation profile in the plants under the –S-N treatment was similar in all tissues to that of the –N treatment at both the stages.

The results from the factorial experiment suggest a hierarchy of N nutrition over S nutrition in *A. cepa,* where the incorporation and accumulation of S metabolites as well as bulb formation is regulated by N availability.

A putatively novel point of interaction between the S-assimilation and the N-assimilation pathways *via* sulfite reductase (AcSiR1) and nitrite reductase (AcNiR1) was also investigated. Recombinant AcSiR1 and AcNiR1 were each able to reduce both sulfite and nitrite, although with a higher specific activity for the physiological substrate in each case. Further, solid phase binding assay indicated a positive interaction between the two recombinant proteins, although this could not be confirmed by Isothermal titration calorimetry (ITC). In addition to this, in a short term S x N depletion experiment with *Arabidopsis, AtSiR1* transcripts only declined in the –S-N treatment in the leaves whereas *AtNiR1* transcripts declined in the –S, -N as well as –S-N treatment in wild type plants. In the roots, *AtSiR1* transcripts decline in both the –N and the –S-N treatment in the roots whereas no significant change was observed in the *AtNiR1* transcripts. In a *sir1* T-DNA knock-down line of *Arabidopsis,* the *AtSiR1* and the *AtNiR1* transcripts did not change in response to any treatment in both leaf and the roots.

Substrate redundancy between AcSiR1 and AcNiR1, *in vitro,* along with the other interaction studies suggest that although both AcSiR1 and AcNiR1 can reduce both substrates, the possibility of this being a direct point of cross-talk between the two pathways is not conclusively established.

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Abbreviations

1-PRENCSO	Trans-S-1-propenyl-$_L$-cysteine sulfoxide
2-CPGTH	*S*-2-carboxypropyl glutathione
APSK1	Adenosine-5´-phosphosulfate kinase
APSR/APR	Adenosine-5´phosphosulfate reductase
ATPS/APS	Adenosine-5´-triphosphate sulfurylase
BLAST	Basic logical alignment search tool
bp	Base-pair
bZIP	Basic leucine zipper
cDNA	Complimentary DNA
DMF	*N,N*-dimethyl formamide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	2´-deoxynucleotide 5´ triphosphate
DTT	Dithiothretiol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
FW	Fresh weight
g	Acceleration due to gravity
gDNA	Genomic DNA
g	Gram
h	Hour
HAST1;1/ SULTR1;1	High affinity sulfate transporter
IPTG	Isopropyl-β-D-thiogalactopyranoside
ITC	Isothermal titration calorimetry
kb	Kilo base-pair
kD	Kilo daltons
L	Litre
LB	Luria-Bertani (media or broth)
LF	Lachrymatory factor
M	Molarity (moles per litre)
MCSO	Methyl cysteine sulphoxide
mg	Miligram
Milli-Q-water	Water purified by Milli-Q-ion exchange chromatography
min	Minute
ml	Millitres
mol	Mole (Avagadro's number)
Mpa	Mega Pascal
mRNA	Messenger RNA
NBT	*P*-nitro blue tetrazolium chloride
NCBI	National Centre for Biotechnology Information
ng	Nanogram
NiR	Nitrite reductase
NR	Nitrate reductase
NRT2;1	High affinity nitrate transporter 2;1
*O*AS	*O*-acetylserine
*O*ASTL/*O*AS-TL	*O*-acetylserine(thiol) lyase
°C	Degree celsius
PAGE	Polyacrylamide gel electrophoresis
PAPS	3´- phosphoadenosine-5´-phosphosulfate
PBS	Phosphate buffer saline

PCR	Polymerase chain reaction
pH	-Log (H+)
qRT-PCR	Reverse transcriptase-polymerase chain reaction
Rnase	Riboxynuclease
RO	Reverse osmosis
SAT	Serine acetyltransferase
sec	Second
SEM	Standard error mean
SiR	Sulfite reductase
SOX	Sulfite oxidase
Tm	Melting temperature at which DNA strands separate prior to annealing
Tris	Tris (hydroxymethyl) aminomethane
Tween-20	Polyoxyethylenesorbitan monolaurate
U	Unit (based on enzyme activity)
V	Volt
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
X-Gal	5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside
µg	Microgram
µl	Microlitre
µM	Micromolar

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