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**Roles of a major *O*-acetylserine (thiol) lyase
(OASTL) in cysteine biosynthesis, innate immunity
and disease resistance in *Arabidopsis***

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

O-Acetylserine (thiol) lyases (OASTLs) are evolutionary conserved proteins among many prokaryotes and eukaryotes that carry out sulphur acquisition and synthesis of cysteine. OASTL catalyses cysteine biosynthesis using *O*-acetylserine (OAS) and sulfide as substrates. OASTL also interact with another enzyme Serine acetyltransferase (SERAT) to facilitate the production of OAS. Cysteine-derived thiols and metabolites play an important function in regulating cellular redox conditions and modulate abiotic and biotic stress responses. The *Arabidopsis thaliana* genome encodes multiple OASTL isoforms that are targeted to different sub-cellular compartments. The cytosolic OASTL-A1 or known as **ONSET OF LEAF DEATH3** (OLD3) is the major OASTL isoform due to its high OASTL activity. The *old3-1* mutation causes a dysfunctional oastl-a1/old3-1 protein *in vitro* and was previously shown to cause autonecrosis in specific *Arabidopsis* accessions. To investigate why a mutation in a major OASTL isoform causes cell death and necrosis in some but not other accessions different mutations in OASTL-A1 were characterised in *Arabidopsis* accessions. Here it is shown that the *old3-1* mutation causes an autoimmune syndrome and metabolic disorder, in the *Ler-0* accession (parent) genetic background, but not in the reference accession Col-0. This is not the result of lack of functional OASTL-A1 or impaired cysteine biosynthesis. A *Recognition of Peronospora Parasitica 1* (*RPP1*)-like disease resistance *R* gene, from an evolutionary divergent *R* gene cluster in *Ler-0*, shows a negative epistatic interaction to *old3-1* and activates autonecrosis. The severity of autonecrosis was found to be dependent upon variations in temperature and day length. Next, the role of OASTL-A1 was also identified in resistance against infection with virulent and non-virulent *Pseudomonas syringae* pv. *tomato DC3000* strains. Since OASTL also interacts with SERAT, *old3-1* was found to negatively affect the interaction with SERAT *in vivo*, highlighting that the release of R-mediated immunity is associated with the loss of key functions associated with OASTL. Finally various mutations were generated in OASTL-A1 isoforms to identify the relevance between the loss of OASTL functions and R-mediated immunity. These results indicated that motifs in close proximity of *old3-1* mutation play an important role in cysteine biosynthesis and therefore likely an important interface to affect R-mediated immunity. The study indicates a novel cross-talk between cysteine procuring a major OASTL isoform and components of plant immunity and further support emerging evidence that cysteine-derived metabolites function in immune signalling across kingdoms.

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Abbreviations

ABA	Abscisic Acid
ACD6	Accelerated Cell Death 6
AOX	Alternative Oxidase
APK	APS Kinase
APS	Adenosine 5'-PhosphoSulfate
APR	Adenosine 5'-Phosphosulfate Reductase
ATPS	ATP Sulfurylase
Avr	Avirulence
BSAS	β -Substituting Alanine Synthase
Cys	Cysteine
Col-0	Columbia-0
COX	Cytochrome Oxidase
CAS	β -Cyanoalanine Synthase
CLT	Chloroquine-resistance Transporter
cad-2	cadmium sensitive -2
CgS	Cystathionine γ -Synthase
CFU	Colony Forming Units
CamV	Cauliflower Mosaic Virus
CAPS	Cleaved Amplified Polymorphic Sequences
DES	DESulfhydrase
DM	Dobzhansky and Muller
DAB	Di-amino benzidine
EF-Tu	Elongation Factor-Tu
EMS	Ethylmethane sulphonate
EDS1	Enhanced Disease Susceptibility 1
ETI	Effector-Triggered Immunity
ETS	Effector-Triggered Susceptibility
γ -EC	Gamma-glutamylcysteine.
GC-MS	Gas Chromatography- Mass spectrometry
GSH	Glutathione
γ -ECS	γ -glutamyl-cysteine ligase
GSH2	Glutathione Synthetase

GR	Glutathione Reductase
GSNO	S-Nitrosoglutathione
GMO	Genetically Modified Organisms
GSBs	Genetic Sequence Blocks
GABA	γ -AminoButyric Acid
HCA	Hierarchical Clustering Analysis
HPLC	High-Performance Liquid Chromatography
HEPES	4-2-Hydroxyethyl-1-piperazineethane sulfonic acid
Hom	Homozygous
Het	Heterozygous
HR	Hypersensitive response
hrpA-	hypersensitive reaction and pathogenicity A gene
IDT	Integrated DNA Technologies
ICSI	Isochorismate synthase 1
I3M	Indole-3-yl-methyl glucosinolate
JA	Jasmonic Acid
LC-MS	Liquid Chromatography- Mass Spectrometry
L.D.	Long Days
<i>Ler-0</i>	Landsberg <i>erecta-0</i>
LRR	Leucine-Rich Repeat
mETC	mitochondrial Electron Transport Chain
MAMPs	Microbe-Associated Molecular Patterns
MHC	Major Histocompatibility Locus
4MI3M	4-Methoxy-Indol-3-ylMethyl-glucosinolate
PTI /MTI	PAMPS / MAMPs-Triggered Immunity
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NIL	Near-Isogenic Line
NBS	Nucleotide Binding Site
NPR1	Non-expresser of Pathogenesis Related 1
PPi	inorganic Pyrophosphate
PAD4	Phytoalexin deficient 4
PR-1	Pathogenesis Related-1
3PGA	Glyceraldehyde 3-Phosphate
PEP	Phosphoenolpyruvate

OAS	O-acetylserine
ORF	Open Reading Frame
Os	<i>Oryza sativa</i> / Rice
OPT	Oligopeptide Transporters
OASTL	O-acetylserine (thiol) lyase
OLD3/old3	Onset of Leaf Death3
PRR	Pattern Recognition Receptors
PCD	Programmed Cell Death
PAPS	3'-phosphoadenosine at 5'-phosphosulfate
PLP	Pyridoxal-Phosphate
PPDB	Plant Proteome Database
CS26	S-sulfocysteine synthase 26
Pst	<i>Pseudomonas syringe</i>
PCRs	Polymerase Chain Reactions
qRT-PCR	Quantitative real-time PCR
QTL3	Quantitative Trait Locus 3
R	Resistance
RCR3	Required for full <i>Cladosporium. fulvum</i> resistance3
Cf-R	<i>Cladosporium. fulvum</i> Resistance
R.H	Relative Humidity
RD-19	RESPONSIVE TO DEHYDRATION19
RPM1	RESISTANCE TO <i>PSEUDOMONAS SYRINGAE</i> 1
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPP1	RESISTANCE TO <i>PERENOSPORA PARASITICA</i> 1
rim1	<i>root meristemless</i> 1
RNAi	RNA interference
SULTRs	Sulfate Transporters
SLIM1	Sulfur LIMitation1
SiR	Sulphite Reductase
SA	Salicylic Acid
SERAT	Serine acetyltransferase
STAS	Sulfate Transporters and AntiSigma-factor Antagonists
SAR	Systemic Acquired Resistance

S.D.	Short Days
SAM	S-adenosylmethionine
SLIM	Site-Directed Ligase Independent mutagenesis
SSLP	Simple Sequence Length Polymorphism
SID2	Salicylic acid (SA) induction-deficient mutant
TIR	Toll-Interleukin-1-Resistance
TAIR	The Arabidopsis Information Research
TTSS	Type-III Secretion System
TCA	Tricarboxylic acid
UTRs	Un-Translated Regions
WRKY	<u>WRKY</u> GQK (amino acid sequence in the transcription factors)
WT	Wild-type
Ws	Wassilewskija
ZM	<i>Zea Mays</i> /Maize

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