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Investigation into the relationship between
ethylene and sulfur assimilation in *Arabidopsis*
thaliana and onion (*Allium cepa* L.)

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Abstract

The phytohormone ethylene (C₂H₄) mediates the adaptive responses of plants to various nutrient deficiencies including iron (Fe)-deficiency, phosphorus (P)-deficiency and potassium (K)-deficiency. However, evidence for the involvement this hormone in the sulfur (S) deficiency response is limited to date. In this study, the effect of C₂H₄ treatment on the accumulation of the S-assimilation enzymes ATP sulfurylase (ATPS), adenosine-5'-phosphosulfate-reductase (APR), O-acetylserine-(thiol)-lyase (OASTL) and sulfite reductase (SiR) was examined in *A. thaliana* and onion (*A. cepa*). To complement this, the effect of short-term S-depletion on the expression of the 12-member gene family of the C₂H₄ biosynthetic enzyme, 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase (ACS) from *A. thaliana*, designated *AtACSI-12*, was also examined.

Western analyses were used to show that plants of *A. thaliana* pre-treated with the C₂H₄-signalling inhibitor 1-MCP, had elevated levels of ATPS, APR and OASTL protein in leaf tissue at all time points examined, suggesting that C₂H₄ has an inhibitory effect on the accumulation of these enzymes. However, SiR appeared to be under dual regulation by C₂H₄: under S-sufficient conditions C₂H₄ appears to prevent the unnecessary accumulation of SiR and conversely promote the fast accumulation of SiR under S-depleted conditions.

The changes in *AtACSI-12* expression in the root and leaf tissues of S-sufficient and S-depleted plants of *A. thaliana* were examined by RT-PCR using gene-specific, exon-spanning primers. The expression patterns of *AtACS2*, *AtACS6* and *AtACS7* were comparable regardless of S availability and may therefore be housekeeping genes. In contrast, the expression of *AtACS5* in leaf, and *AtACS8* and *AtACS9* in roots was repressed under S-depleted conditions, although the mechanism of this repression cannot be elucidated from this study. The protein products of these closely-related genes are believed to be phosphorylated and stabilised by a CDPK whose activity may be compromised by S-depletion. The inhibition of *AtACS5*, *AtACS8* and *AtACS9*

expression, and the decrease in *AtACS5*, *AtACS8* and *AtACS9* accumulation, and hence less C_2H_4 production, may be part of the plant adaptive response to S-depletion, as the C_2H_4 -mediated repression of root growth is alleviated to allow the plant to better seek out the lacking nutrient. The expression of the MPK-stabilised genes *AtACS2* and *AtACS6* appeared to be similar regardless of S availability, although this may merely be a consequence of the scoring method used in this study, which cannot determine whether there was any difference in the level of expression of these genes. The expression of *AtACS10* and *AtACS12* was repressed in S-deficient plants. Although both *AtACS10* and *AtACS12* isozymes possess the hallmark seven conserved regions found in the ACSes of other plant species, they are also phylogenetically related to alanine and aspartate aminotransferases, and are known to encode aspartate (*AtACS10*) and aromatic amino acid transaminases (*AtACS12*). Therefore, the apparent downregulation of these genes suggests that the downregulation of amino acid metabolism may be part of the plant adaptive response to S-depletion.

The downregulation of several *AtACS* genes, and therefore possibly also C_2H_4 biosynthesis, in S-deficient plants was accompanied by an accumulation of APR protein. The increase in APR protein that also occurred in 1-MCP-treated plants indicates that C_2H_4 may be involved in the plant response to S-depletion, because in both cases the upregulation of the S-assimilation pathway, as manifested by the accumulation of APR protein, occurred when C_2H_4 biosynthesis and signalling was repressed. However, the possible role of other phytohormones in the plant response to S-depletion cannot be excluded, as there is evidence for crosstalk between the C_2H_4 signalling pathway and those of auxin, abscisic acid (ABA), cytokinins and jasmonic acid (JA). Furthermore, because C_2H_4 has been implicated in the response of various plants to Fe-deficiency, P-deficiency, and K-deficiency, in addition to S-deficiency, it may be a regulator of the plant adaptive response to nutrient stresses in general.

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Abbreviations

°C	degrees Celsius
-K	insufficient potassium supply, as determined for the specific study quoted
+N	sufficient nitrogen supply, as determined for the specific study quoted
-N	insufficient nitrogen, supply, as determined for the specific study quoted
+P	sufficient phosphorus supply, as determined for the specific study quoted
-P	insufficient phosphorus supply, as determined for the specific study quoted
1-MCP	1-methylcyclopropene
8L/16D	8 hours light/16 hours dark
A	amps
ABA	abscisic acid
ACC	1-amino-cyclopropane-1-carboxylic acid
ATPS	adenosine triphosphate (ATP) sulfurylase
APR	adenosine-5'-phosphosulfate (APS) reductase
AVG	aminoethoxyvinylglycine
bp	base pair(s)
BSA	bovine serum albumin
CBB	Coomassie [®] Brilliant Blue
CDPK	Ca ²⁺ dependent protein kinase
CS	cysteine synthase complex
Cys	cysteine
DF	dilution factor
DTT	dithiothreitol
g	g force
GFP	green fluorescent protein
Gly	glycine
GUS	β-glucuronidase

hr	hours
IM	isolation medium
JA	jasmonic acid
kb	kilobase(s)
L	litre
M	molar concentration
Met	methionine
MPK6	mitogen-activated protein kinase 6
MPKK5	mitogen-activated protein kinase kinase 5
min	minute(s)
mRNA	messenger RNA
N	nitrogen
NaOAc	sodium acetate
OASTL	<i>O</i> -acetylserine-thiol-lyase
P	phosphorus
PCR	polymerase chain reaction
PBS	phosphate buffered salt
ppm	parts per million
PVDF	polyvinylidene difluoride
PVP-40	polyvinylpyrrolidone
re	refer
RT	reverse transcription
RT-PCR	reverse transcription-polymerase chain reaction
rpm	revolutions per minute
s	seconds
SAM	<i>S</i> -adenosylmethionine synthetase
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate – polyacrylamide gel electrophoresis
Ser	serine
SiR	sulfite reductase
U	unit(s)
V	volt(s)
Wt	wild type