Supplementary Information

Impact of overexpression of cytosolic isoform of *O*-acetylserine sulfhydrylase on soybean nodulation and nodule metabolome

Hari B. Krishnan[1,2]*, Bo Song[1,3], Nathan W. Oehrle[1], Jeffrey C. Cameron[4], Joseph M. Jez[5]

[1]USDA-ARS, Plant Genetics Research Unit, 105 Curtis Hall, University of Missouri, Columbia, MO 65211, USA

[2]Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA

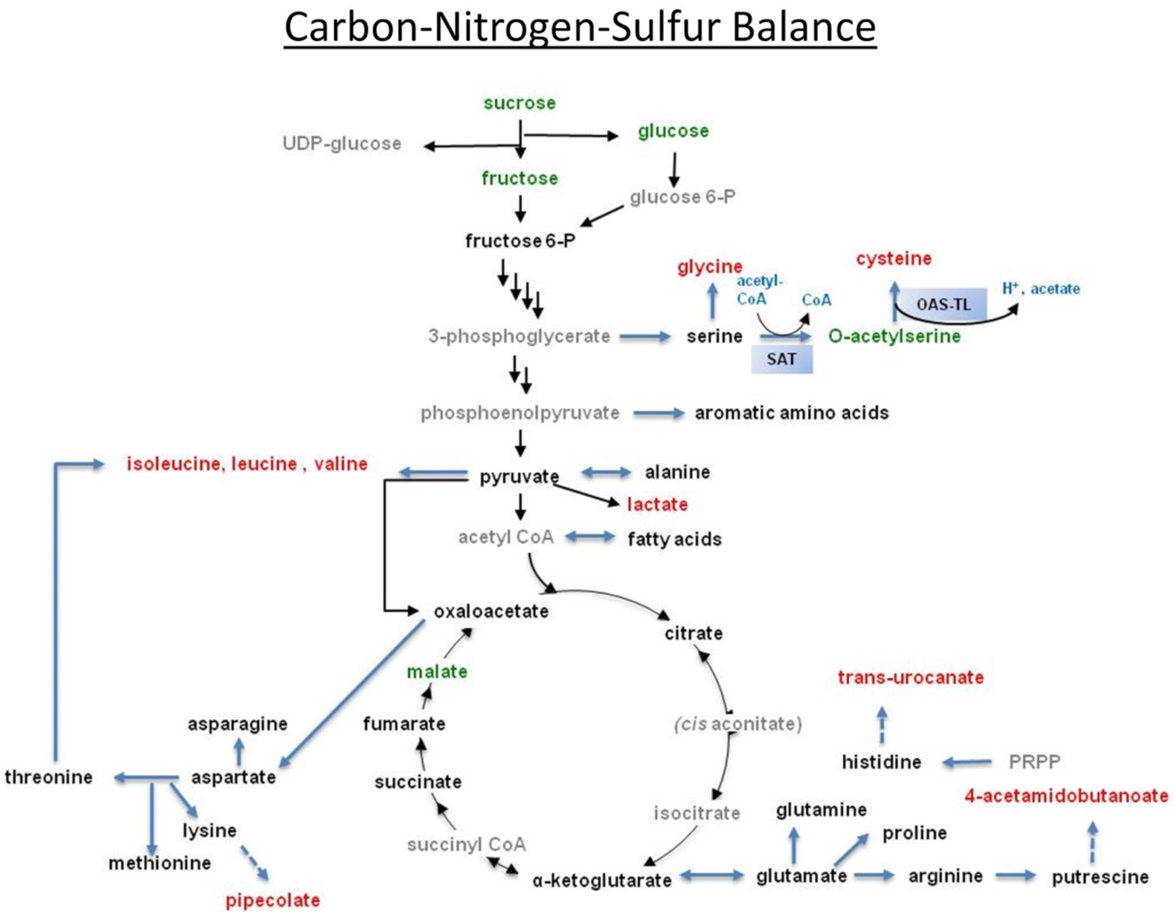
[3]Key Laboratory of Soybean Biology at the Chinese Ministry of Education, Northeast Agricultural University, Harbin 150030, China

[4]Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0596

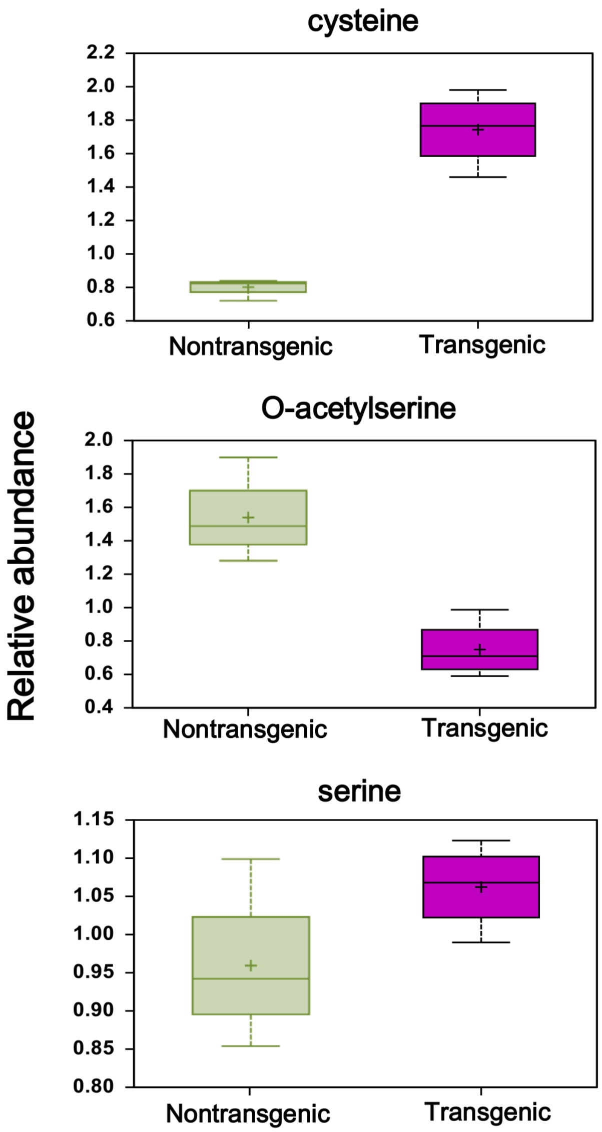
[5]Department of Biology, Washington University in St. Louis, St. Louis, Missouri 63130, USA

*Corresponding author's E-mail: Hari.Krishnan@ARS.USDA.GOV

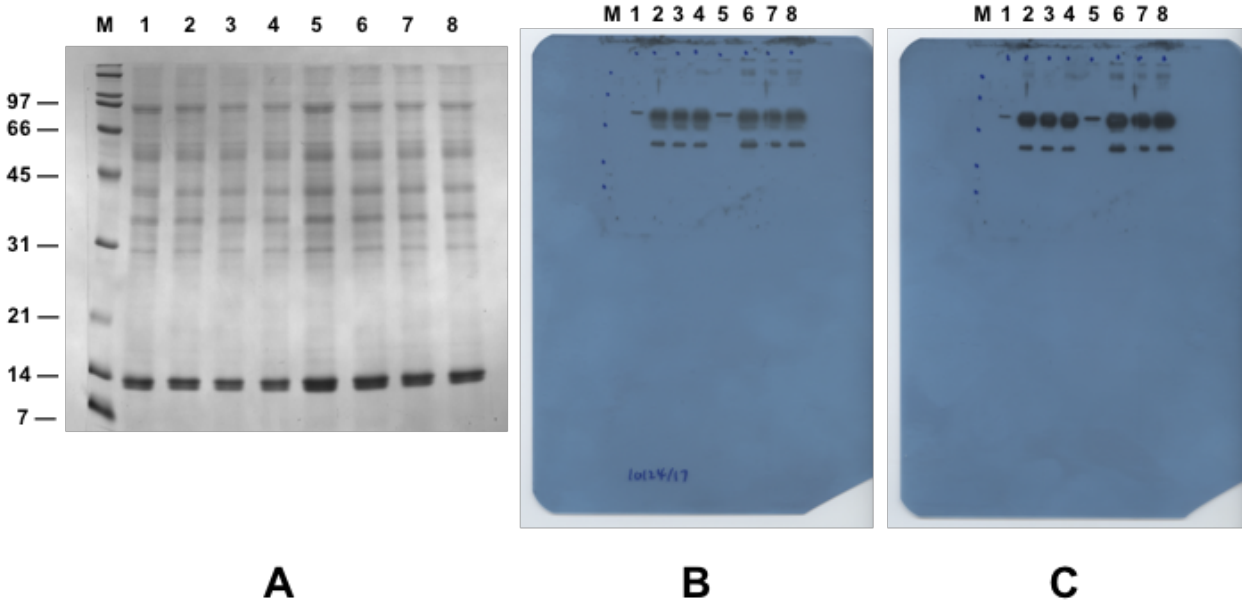
Supplemental Fig. 1. Carbon-Nitrogen-Sulfur Balance in soybean nodules. Metabolites that are elevated in OASS overexpressing transgenic soybean nodules are shown in red and those that are reduced when compared to non-transformed wild-type nodules are shown in green fonts.



Supplemental Fig. 2. Changes in the abundance of key metabolites involved in sulfur assimilatory pathway in non-transformed wild-type and transgenic soybean nodules. Relative abundance of metabolites is shown on y-axis. Box plots were generated for compounds with significant increase or decrease using both t-test and FDR, with $p<0.05$ and $q<0.10$ as significant values.



Supplemental Fig. 3. Uncropped digital images of full-length gel and immunoblot. Total nodule proteins from 20 day old nodules of nontransgenic and transgenic plants were separated on 15% SDS-PAGE gels and either stained with Coomassie Blue (A) or transferred to nitrocellulose membrane and probed with antiserum against soybean OASS (B and C). Antibody reactivity was detected by using anti-rabbit IgG-horseradish peroxidase conjugate followed by chemiluminescent detection. Lanes (1 and 5) nodule protein from non-transformed wild-type; lanes (2 and 6), (3 and 7) and (4 and 8) nodule protein from OASS transformed transgenic plants CS02, CS022, and CS027, respectively. Lanes 1 to 4 contain 10 μg and lanes 5to 8 contain 20 μg of nodule protein. Autoradiograph B and C was exposed for 5 and 10 seconds, respectively.



Supplemental Table 2. Antioxidant enzyme activities in 15 and 30 day old soybean nodules*.

	Ascorbate peroxidase	Catalase	Superoxide dismutase
Nontransgenic 15 day	2.41 μmol min^{-1} mg^{-1} ± 0.06	128 μmol min^{-1} mg^{-1} ± 10.39	42.33 U mg^{-1} ± 0.87
Transgenic 15 day	2.31 μmol min^{-1} mg^{-1} ± 0.04	141 μmol min^{-1} mg^{-1} ± 8.78	45.67 U mg^{-1} ± 1.03
	F stat: 2.0773 p-value: 0.1688 Tukey HSD: 0.1688	F stat: 1.0090 p-value: 0.3301 Tukey HSD: 0.3301	F stat: 6.0964 p-value: 0.0252 Tukey HSD: 0.0252
Nontransgenic 30 day	2.27 μmol min^{-1} mg^{-1} ± 0.07	82 μmol min^{-1} mg^{-1} ± 2.89	51.33 U mg^{-1} ± 0.68
Transgenic 30 day	2.44 μmol min^{-1} mg^{-1} ± 0.05	75 μmol min^{-1} mg^{-1} ± 5.86	62.00 U mg^{-1} ± 1.41
	F stat: 4.2503 p-value: 0.0570 Tukey HSD: 0.0571	F stat: 1.2590 p-value: 0.2784 Tukey HSD: 0.2784	F stat: 46.6020 p-value: 0.0000004 Tukey HSD: 0.0010

*Enzyme activity error is reported as standard deviation of the mean. One-way ANOVA with post-hoc Tukey HSD test was performed (n=9). p-values > 0.05 demonstrate no significant difference among enzyme activities.

Supplemental Table 1. Identification of nodule cytosolic proteins. Following 2D-PAGE separation, protein spots were identified using peptide mass fingerprinting (MALDI-TOF-TOF). Spot identifiers correspond to those proteins labeled in Figure 6. MOWSE scores represent those searches performed via Mascot. Peptides matched, % coverage and accession numbers resulted from searches confined to plant databases. Database used was National Center for Biotechnological Information (NCBIprot).

Spot	**Protein ID**	**MOWSE**	**Peptides Matched (Sequences) ---- % Coverage**	**NCBIprot**	**(Position) Peptide Matched**	**Peptide Score**
A	Cysteine synthase (*Glycine max*)	681	7 (6) ---- 32 %	NP_001235628.1	(71-92) K.SVLIEPTSGNTGIGLAFMAAAR.G (71-92) K.SVLIEPTSGNTGIGLAFMAAAR.G + Ox.(M) (176-194) K.IDAFVSGIGTGGTITGAGK.Y (205-225) K.LIGVEPVESPVLSGGKPGPHK.I (265-285) K.EGLFVGISSGAAAAAAFQIAK.R (293-304) K.LIVAVFPSFGER.Y (305-315) R.YLSSVLFESVR.R	100 98 121 170 168 55 67
B	Phosphinothricin acetyl transferase (synthetic construct)	275	4 (4) ---- 32 %	AAR86773.1	(38-52) R.TEPQEPQEWTDDLVR.L (81-96) R.NAYDWTAESTVYVSPR.H (100-112) R.TGLGSTLYTHLLK.S (121-135) K.SVVAVIGLPNDPSVR.M	57 63 74 82