Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/2/10/e1601087/DC1

# Supplementary Materials for

## Sulfur deficiency–induced repressor proteins optimize glucosinolate biosynthesis in plants

Fayezeh Aarabi, Miyuki Kusajima, Takayuki Tohge, Tomokazu Konishi, Tamara Gigolashvili, Makiko Takamune, Yoko Sasazaki, Mutsumi Watanabe, Hideo Nakashita, Alisdair R. Fernie, Kazuki Saito, Hideki Takahashi, Hans-Michael Hubberten, Rainer Hoefgen, Akiko Maruyama-Nakashita

Published 7 October 2016, *Sci. Adv.* **2**, e1601087 (2016)
DOI: 10.1126/sciadv.1601087

**The PDF file includes:**


- fig. S1. Alignment of SDI family proteins in *Arabidopsis*.
- fig. S2. Phylogenetic relationships of SDI family proteins in some monocot and dicot species.
- fig. S3. Histochemical staining of *Arabidopsis* plants transformed with $SDI1_{pro}$:*GUS* and $SDI2_{pro}$:*GUS*.
- fig. S4. Transcript levels of *CYP79F1* and *CYP83A1* were influenced by *SDI1* and *SDI2* similar with other mGSL synthesis genes.
- fig. S5. Perturbation of *SDI1* and *SDI2* influences on the transcript levels of genes involved in primary sulfur metabolism.
- fig. S6. Frequency plot of genes according to *P* values.
- fig. S7. Contribution of each PC.
- fig. S8. Distribution of PC1 (A) and PC2 (B) scores.
- fig. S9. PC1 shows linear correlation with −S-responsive gene expression.
- fig. S10. Keywords that frequently appeared in genes with high scores in PC2.
- fig. S11. Effects of the manipulation of MYB28, MYB29, and MYB76 on gene expression of *SDI1*, *SDI2*, and other factors.
- table S12. Oligonucleotides used for the vector construction.
- table S13. Oligonucleotides used for the isolation of the T-DNA insertion lines.
- table S14. Oligonucleotides used for qRT-PCR analysis.
- References (*77*, *78*)


**Other Supplementary Material for this manuscript includes the following:**
(available at advances.sciencemag.org/cgi/content/full/2/10/e1601087/DC1)

- table S1 (Microsoft Excel format). MT, MS, indolic, and total GSL contents in shoot and root tissues of WT, *sdi1*, *sdi2*, and *sdi1sdi2*.
- table S2 (Microsoft Excel format). Fold changes of GSL contents in shoot and root tissues of *sdi1*, *sdi2*, and *sdi1sdi2* versus WT.
- table S3 (Microsoft Excel format). MT, MS, indolic, and total GSL contents in shoot and root tissues of WT and *SDI1*- and *SDI2*-overexpressing lines.
- table S4 (Microsoft Excel format). Fold changes of GSL contents in shoot and root tissues of *SDI1ox* and *SDI2ox* versus WT.
- table S5 (Microsoft Excel format). Sulfate, Cys, and GSH contents in shoot and root tissues of sdi knockouts and overexpressing lines.
- table S6 (Microsoft Excel format). Transcript levels of genes highly contributed to PC1.
- table S7 (Microsoft Excel format). Transcript levels of genes highly contributed to PC2.
- table S8 (Microsoft Excel format). Transcript levels of GSL-related genes in root tissues of WT, *sdi1sdi2*, and *SDI1ox* detected by microarray analysis.
- table S9 (Microsoft Excel format). Transcript levels of sulfur assimilatory genes in root tissues of WT, *sdi1sdi2*, and *SDI1ox* detected by microarray analysis.
- table S10 (Microsoft Excel format). Transcript levels and gene PCs of genes selected for PCA.
- table S11 (Microsoft Excel format). Transcript levels of GSL-related genes in root tissues of parental, *slim1-1*, and *slim1-2* detected by microarray analysis.

## Supplementary Materials

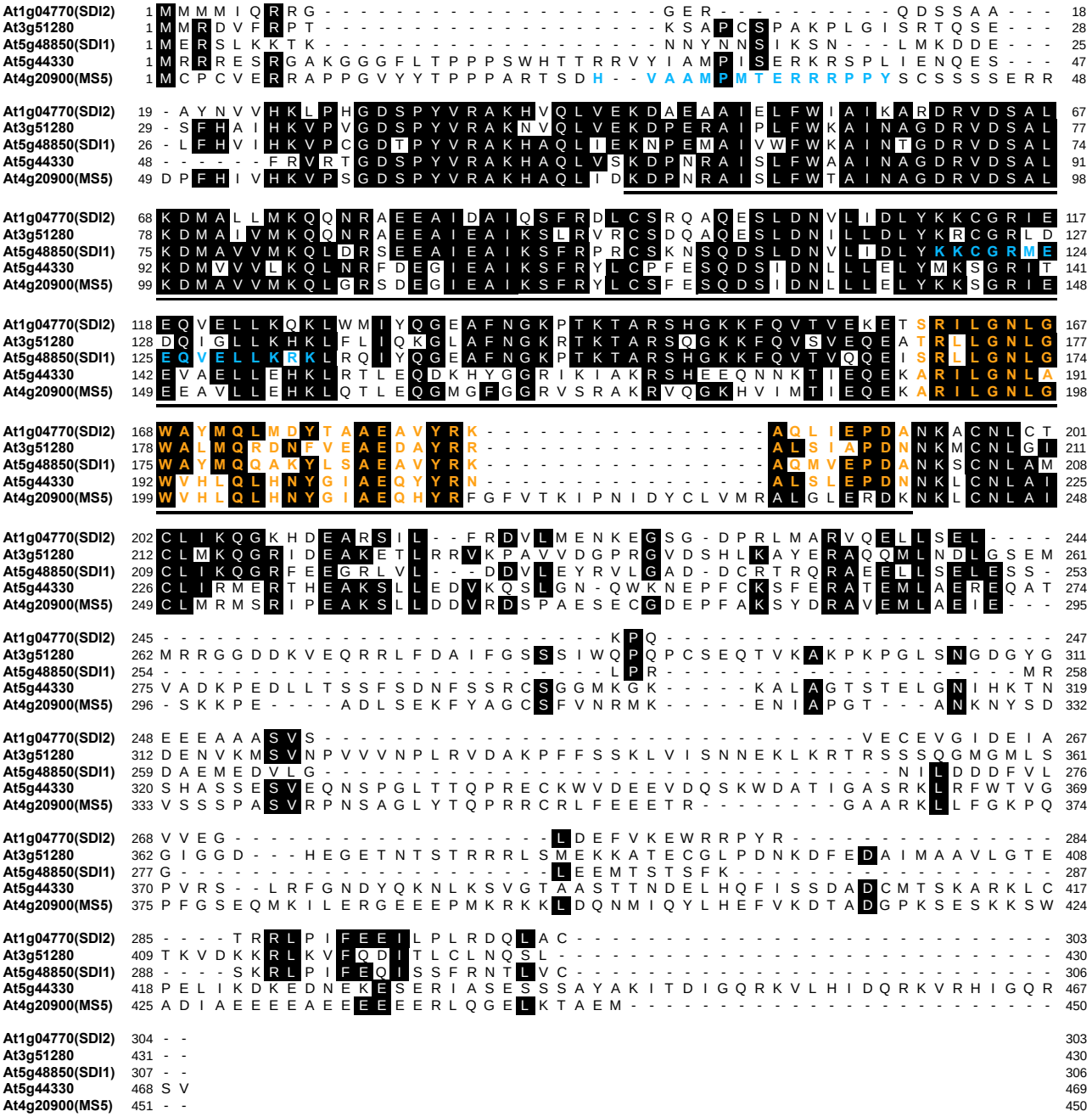


**fig. S1. Alignment of SDI family proteins in *Arabidopsis*.** Alignment of full protein sequences was performed by ClustalW program at DNA Data Bank of Japan (DDBJ) (http://www.ddbj.nig.ac.jp/search/clustalw-j.html). TPR-like helical domains detected by InterPro scan (IPR011990, *38*) are underlined. Orange characters indicate TPR motifs (TPR 1, PF00515) and blue characters indicate nuclear localization signals (NLS_BP, PS50079) detected by Motif Scan (*64*). Amino acid residues conserved in more than 50% of all 5 SDI-family proteins are highlighted in black.

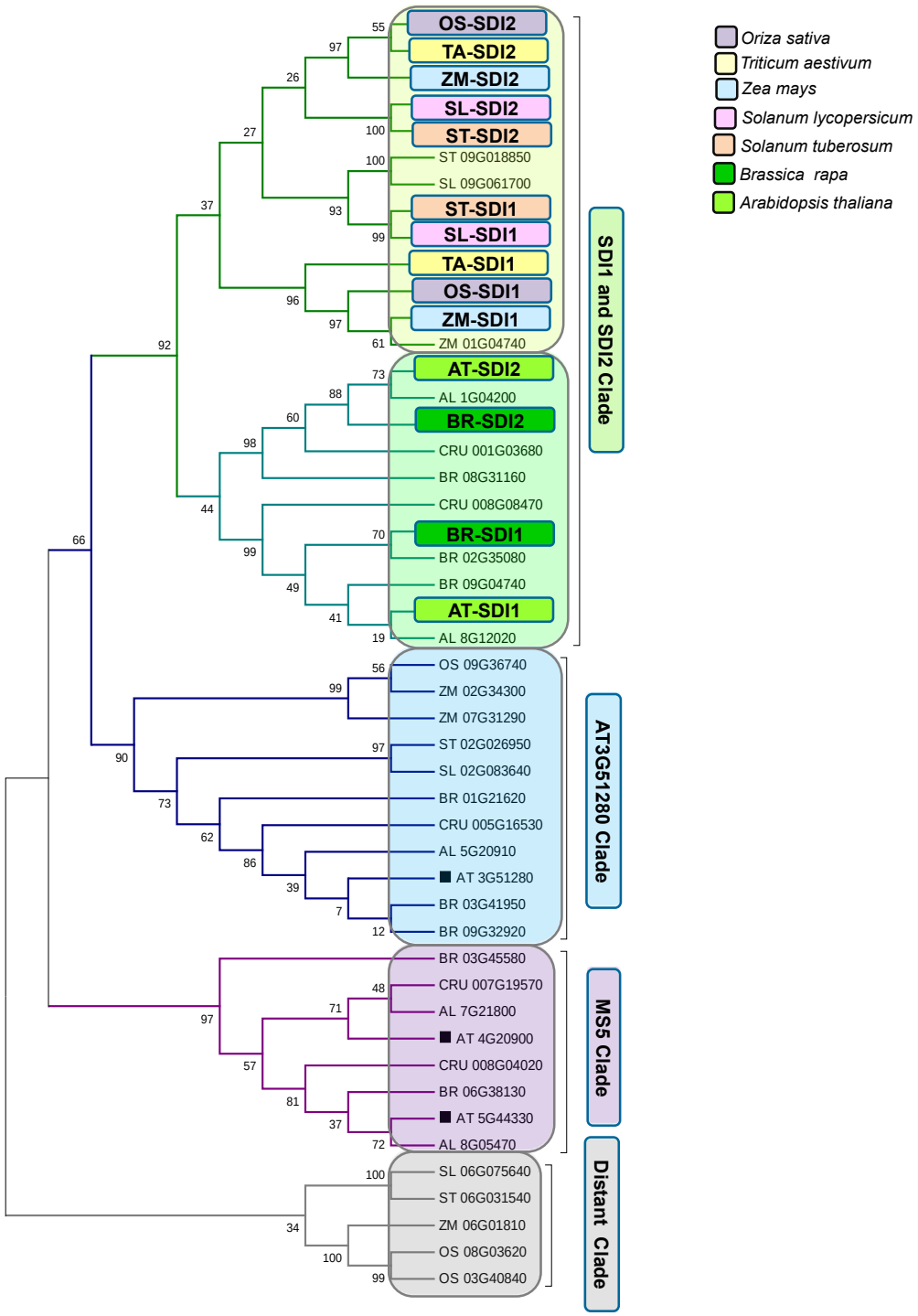


**fig. S2. Phylogenetic relationships of SDI family proteins in some monocot and dicot species.** The phylogenetic tree was constructed by MEGA6 using a statistical neighbor-joining method and parameters as described in the methods. Dicots: AT (*Arabidopsis thaliana),* AL (*Arabidopsis lyrata*), CRU (*Capsella rubella*), BR (*Brassica rapa*), SL (*Solanum lycopersicum*), ST (*Solanum tuberosum*). Monocots: TA (*Triticum aestivum*), ZM (*Zea mays*), OS (*Oriza sativa*). Black squares show *Arabidopsis thaliana* proteins. Abbreviations: AT-SDI1, AT5g48850; AT-SDI2, AT1G04770; BR-SDI1, BR 06G31000; BR-SDI2, BR 10G03040; TA-SDI1, wheat SDI1; OS-SDI1, OS 03G06970; ZM-SDI1, ZM 09G28880; SL-SDI1, SL 09G061700; ST-SDI1, ST 06G008080; OS-SDI2, OS 05G43040; ZM-SDI2, ZM 06G27960; SL-SDI2, SL 09G091600; ST-SDI2, ST 09G027950

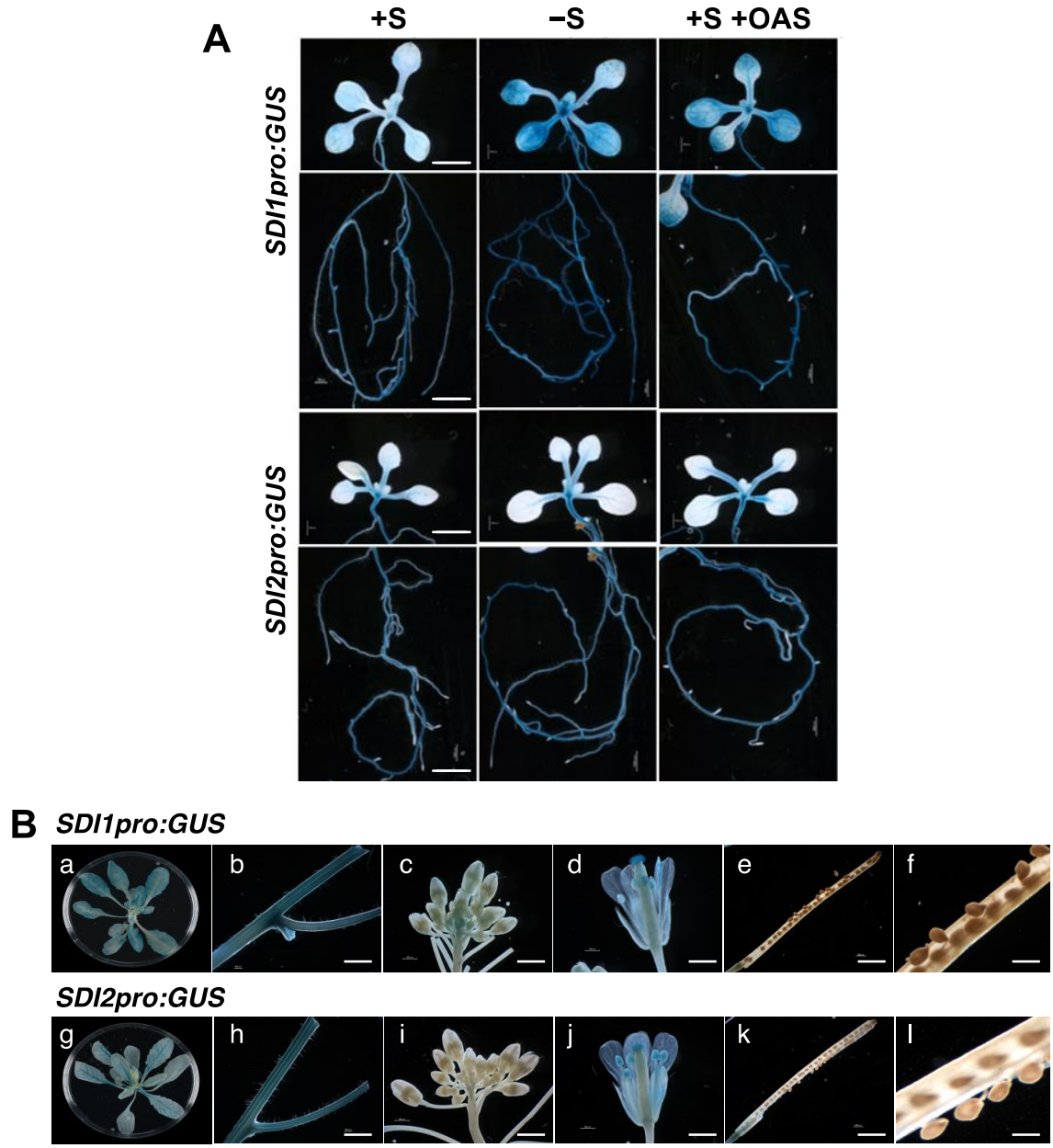


**fig. S3. Histochemical staining of *Arabidopsis* plants transformed with *SDI1*$_{pro}$*:GUS* and *SDI2*$_{pro}$*:GUS*.** (**A**) Seedlings grown under +S, -S and +S (applied with 1 mM OAS) conditions. Scale bars = 1 mm. (**B**) Leaf (a,g), stem (b,h), floral bud (c,i), flower (d,j), silique (e,k) and seeds (f,l) of mature plants are presented. Scale bars in b,c,e,h,i,k = 0.8 mm; in d,j = 0.5 mm.

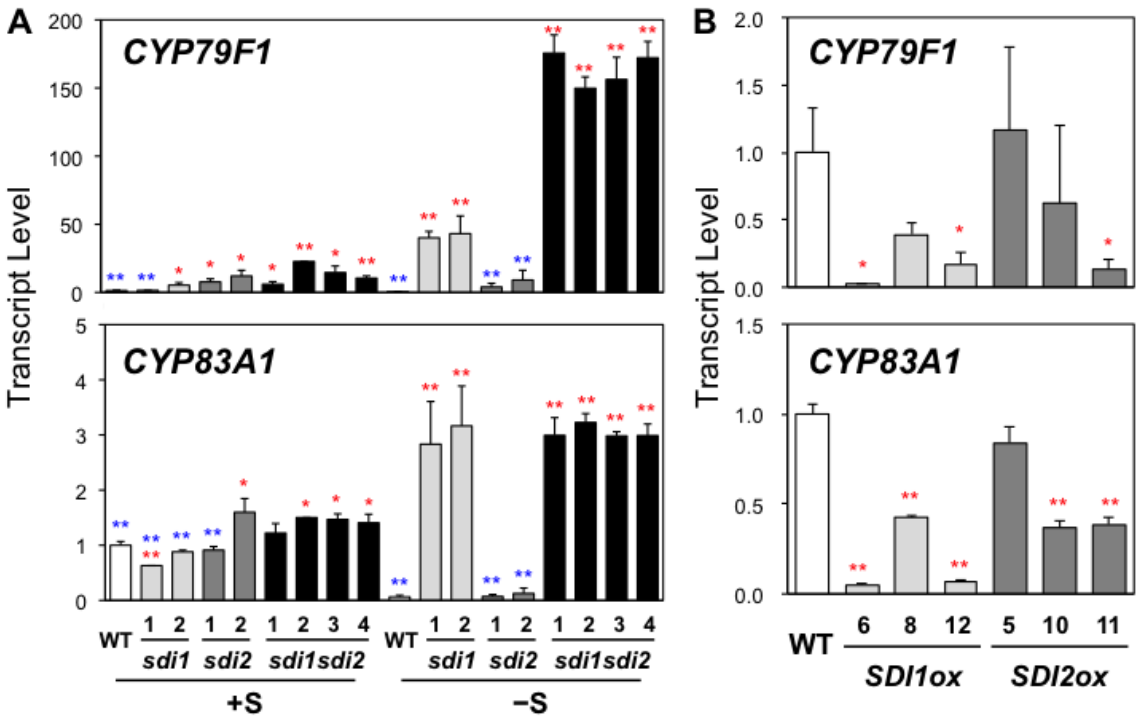


**fig. S4. Transcript levels of *CYP79F1* and *CYP83A1* were influenced by *SDI1* and *SDI2* similar with other mGSL synthesis genes.** (**A**) (**B**) Transcript levels of *CYP79F1*and *CYP83A1* in plant roots analyzed by qRT-PCR. In (**A**), WT (white bars), *sdi1* (pale gray bars), *sdi2* (dark gray bars) and *sdi1sdi2* (black bars) plants grown under +S or −S conditions were used. In (**B**), WT (white bars), *SDI1ox* (pale gray bars) and *SDI2ox* (dark gray bars) plants grown under +S condition were used. Bars and error bars show mean values and SE of triplicates. Double and single asterisks show the significant differences ($P<0.01$, $P<0.05$) detected by Student's *t*-test between WT and T-DNA insertion mutants (red) and those between 4 lines of *sdi1sdi2* and other plant lines (blue) in (**A**), and those between WT and overexpression lines in (**B**).

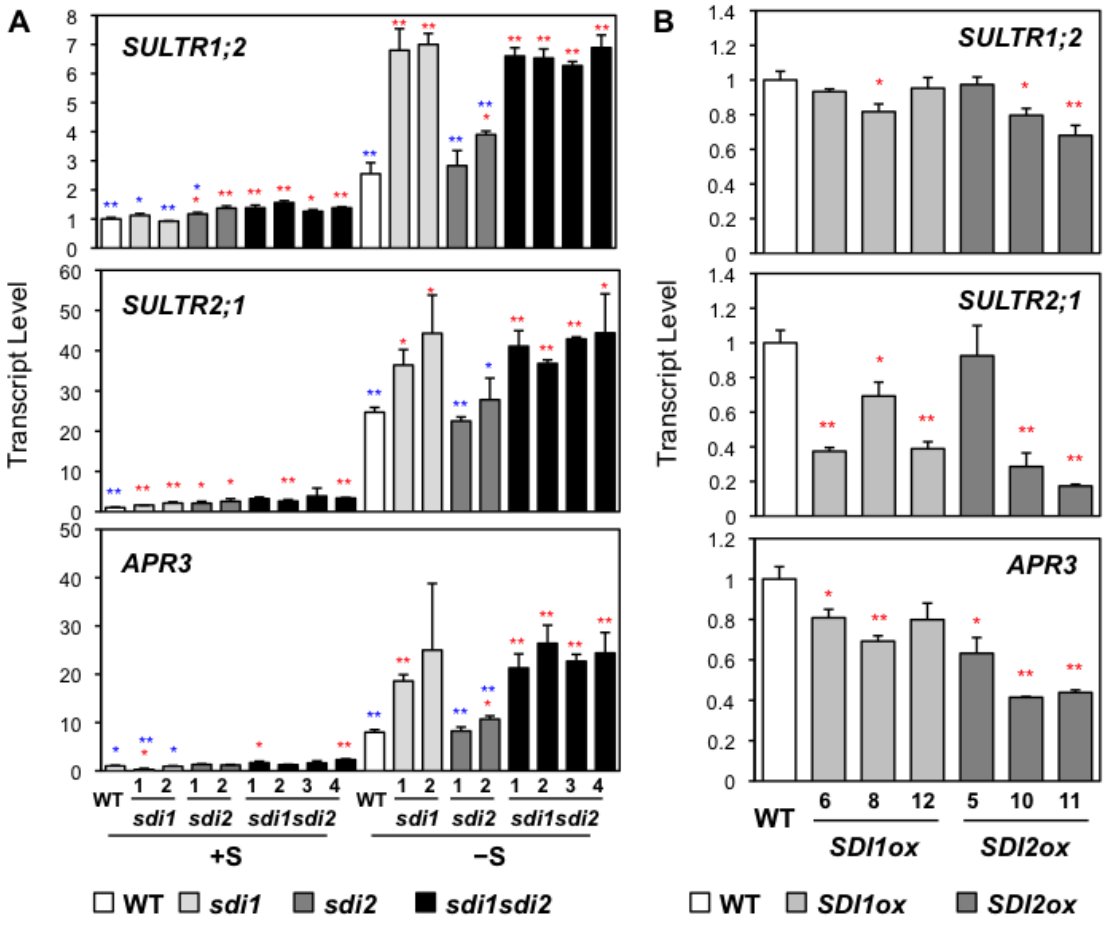


**fig. S5. Perturbation of *SDI1* and *SDI2* influences on the transcript levels of genes involved in primary sulfur metabolism.** (**A**) (**B**) Transcript levels of *SULTR1;2*, *SULTR2;1* and *APR3* in plant roots analyzed by qRT-PCR. In (**A**), WT (white bars), *sdi1* (pale gray bars), *sdi2* (dark gray bars) and *sdi1sdi2* (black bars) plants grown under +S or −S conditions were used. In (**B**), WT (white bars), *SDI1ox* (pale gray bars) and *SDI2ox* (dark gray bars) plants grown under +S condition were used. Bars and error bars show mean values and SE of triplicates. Double and single asterisks show the significant differences ($P<0.01$, $P<0.05$) detected by Student's *t*-test between WT and T-DNA insertion mutants (red) and those between 4 lines of *sdi1sdi2* and other plant lines (blue) in (**A**), and those between WT and over-expression lines in (**B**).

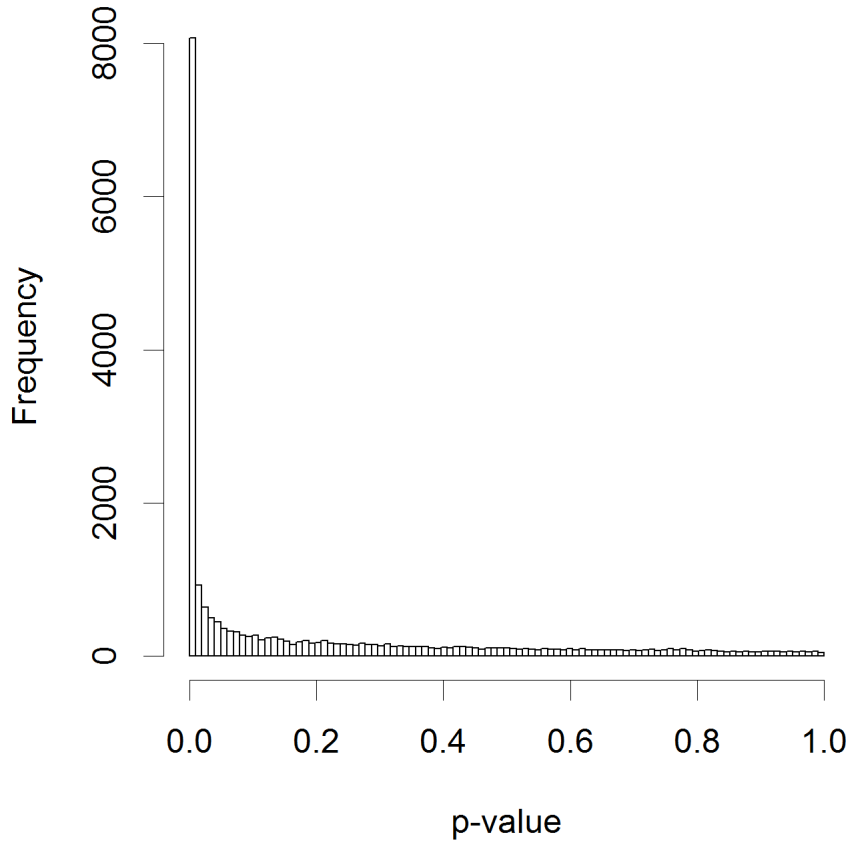


**fig. S6. Frequency plot of genes according to *P* values.** *P*-values among groups were calculated by two-way ANOVA. Six thousands genes with *P* values less than 0.001 were selected for further analysis.

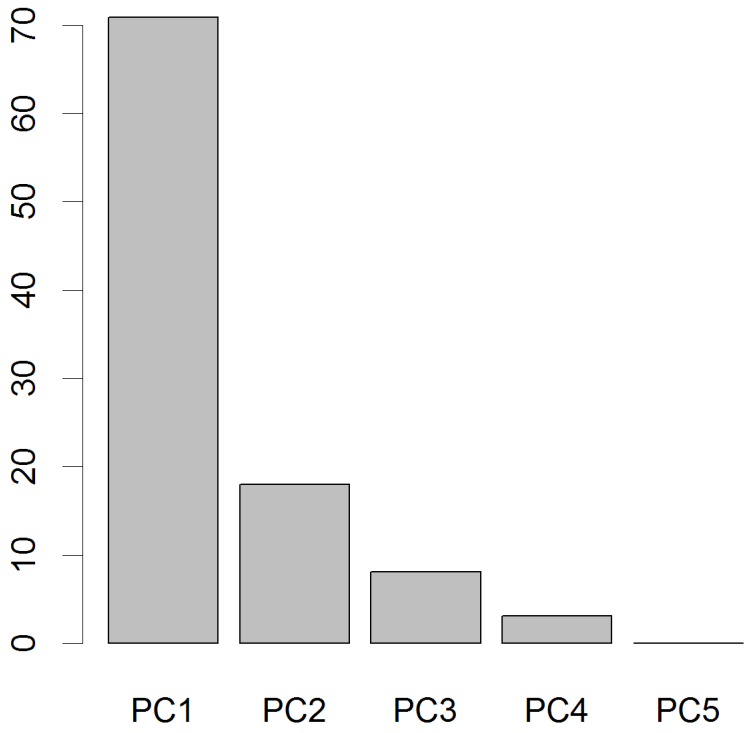


**fig. S7. Contribution of each PC.**

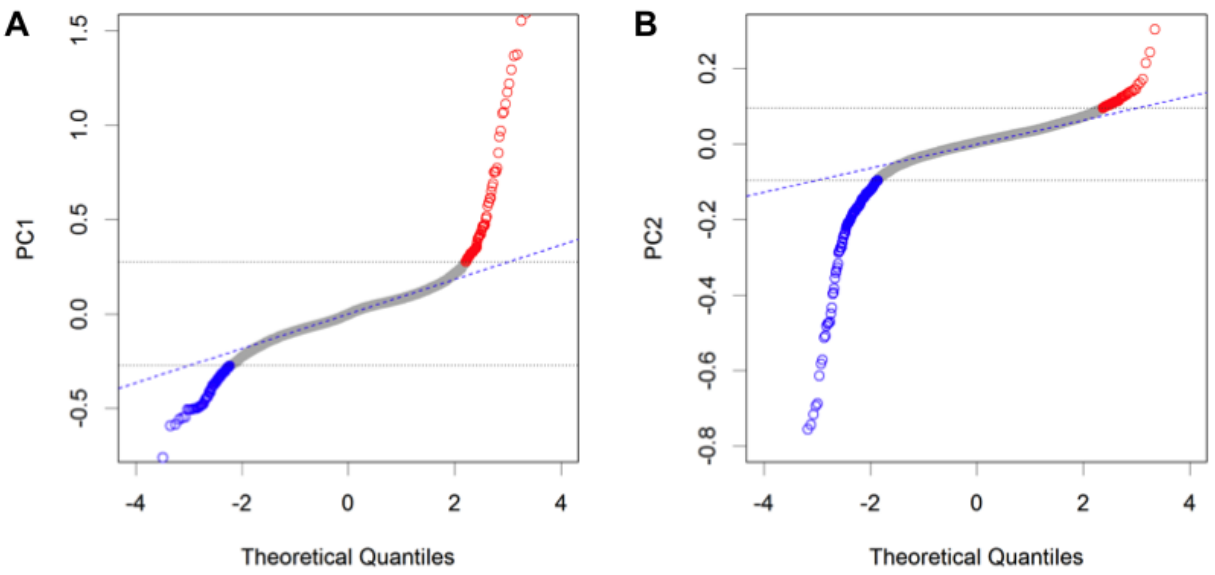


**fig. S8. Distribution of PC1 (A) and PC2 (B) scores.** Sorted scores are compared with theoretical values for the normal distribution. In this plot, linear relationship shows normality of the distribution. The slanted blue line has threshold of zero and slope of the mad. Gray dot lines show the threshold of gene selection, three times of the mad. Selected outlying genes with positive and negative scores were colored with red and blue, respectively.

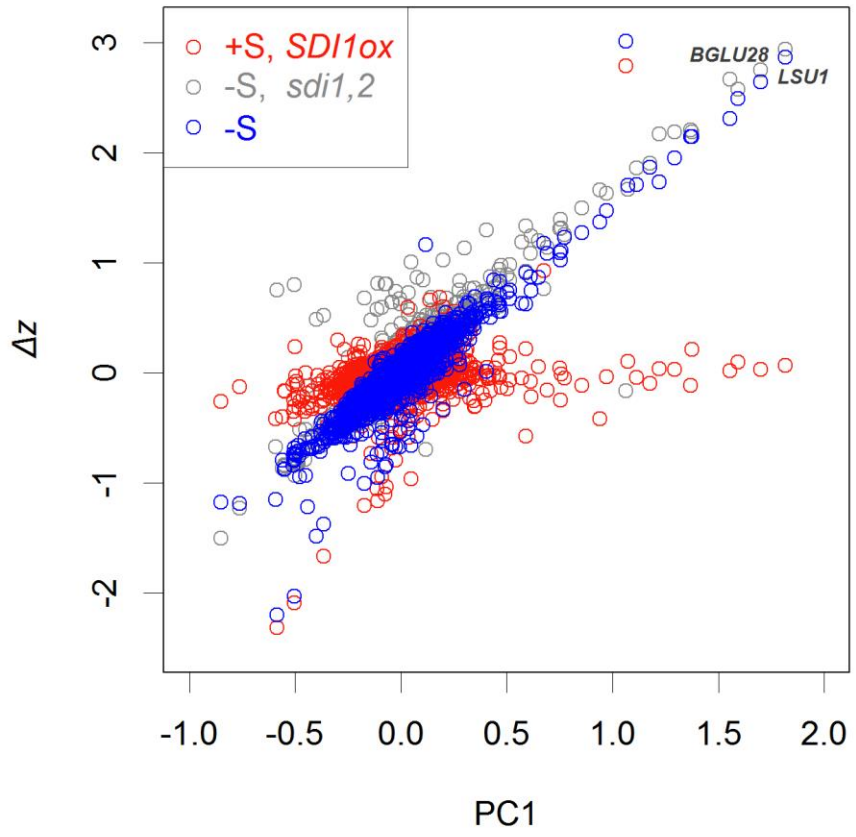


**fig. S9. PC1 shows linear correlation with −S-responsive gene expression.** Comparisons between PC1 and expressional changes ( $\Delta z$) from the control groups. $\Delta z$ of genes selected for PCA in WT grown under –S (blue), *sdi1sdi2* grown under –S (gray) and *SDI1*ox grown under +S (red) compared to Col grown under +S were spotted. Position of the genes that take top two PC1 scores, *BGLU28* and *LSU1*, were indicated.

**A**

| | Gene Ontology Biological Process | selected | contents | p-value | p-value |
|---|---|---|---|---|---|
| PC2 negative | metabolic process | 57 | 2668 | 7.07E-12 | 7.07E-12 |
| | **glucosinolate biosynthetic process** | 37 | 167 | 0.00E+00 | 0.00E+00 |
| | oxidation-reduction process | 28 | 1580 | 1.10E-04 | 1.10E-04 |
| | **cysteine biosynthetic process** | 27 | 225 | 0.00E+00 | 0.00E+00 |
| | indoleacetic acid biosynthetic process | 26 | 110 | 0.00E+00 | 0.00E+00 |
| | cellular amino acid biosynthetic process | 21 | 188 | 0.00E+00 | 0.00E+00 |
| | toxin catabolic process | 17 | 212 | 3.91E-12 | 3.91E-12 |
| | tryptophan catabolic process | 15 | 82 | 0.00E+00 | 0.00E+00 |
| | transmembrane transport | 15 | 517 | 3.00E-05 | 3.00E-05 |
| | defense response to bacterium | 14 | 362 | 2.34E-06 | 2.34E-06 |
| | response to stress | 13 | 387 | 2.36E-05 | 2.36E-05 |
| | response to cyclopentenone | 12 | 128 | 1.11E-09 | 1.11E-09 |
| | response to cadmium ion | 12 | 484 | 7.60E-04 | 7.60E-04 |
| | response to jasmonic acid | 10 | 260 | 7.01E-05 | 7.01E-05 |
| | **sulfate assimilation** | 9 | 14 | 7.44E-15 | 7.44E-15 |
| | response to toxic substance | 9 | 70 | 9.80E-09 | 9.80E-09 |
| | biosynthetic process | 8 | 167 | 8.56E-05 | 8.56E-05 |
| | leucine biosynthetic process | 6 | 14 | 2.75E-09 | 2.75E-09 |
| | response to insect | 6 | 46 | 2.78E-06 | 2.78E-06 |
| | response to karrikin | 6 | 130 | 8.02E-04 | 8.02E-04 |
| | jasmonic acid biosynthetic process | 6 | 132 | 8.67E-04 | 8.67E-04 |
| | **hydrogen sulfide biosynthetic process** | 5 | 8 | 9.33E-09 | 9.33E-09 |
| | branched-chain amino acid biosynthetic process | 5 | 20 | 8.41E-07 | 8.41E-07 |
| | drug transmembrane transport | 5 | 79 | 5.47E-04 | 5.47E-04 |
| | 2,4,6-trinitrotoluene catabolic process | 4 | 2 | 2.94E-09 | 2.94E-09 |
| | **sulfate reduction** | 4 | 4 | 4.64E-08 | 4.64E-08 |
| | regulation of glucosinolate biosynthetic process | 4 | 7 | 4.27E-07 | 4.27E-07 |
| | threonine biosynthetic process | 4 | 11 | 2.54E-06 | 2.54E-06 |
| | sulfate transmembrane transport | 4 | 14 | 6.53E-06 | 6.53E-06 |
| | cell wall modification involved in abscission | 4 | 16 | 1.10E-05 | 1.10E-05 |
| | **sulfate transport** | 4 | 17 | 1.39E-05 | 1.39E-05 |
| | defense response by callose deposition in cell wall | 4 | 17 | 1.39E-05 | 1.39E-05 |
| | **glucosinolate metabolic process** | 4 | 35 | 2.23E-04 | 2.23E-04 |
| | para-aminobenzoic acid metabolic process | 4 | 37 | 2.75E-04 | 2.75E-04 |
| | cellular amino acid metabolic process | 4 | 51 | 9.08E-04 | 9.08E-04 |
| PC2 positive | response to chitin | 17 | 424 | 0.00E+00 | 0.00E+00 |
| | regulation of transcription, DNA-templated | 17 | 2099 | 4.05E-06 | 4.05E-06 |
| | transcription, DNA-templated | 14 | 1466 | 5.69E-06 | 5.69E-06 |
| | response to wounding | 13 | 334 | 9.00E-13 | 9.00E-13 |
| | ethylene-activated signaling pathway | 11 | 224 | 5.32E-12 | 5.32E-12 |
| | response to mechanical stimulus | 10 | 56 | 0.00E+00 | 0.00E+00 |
| | intracellular signal transduction | 9 | 252 | 8.32E-09 | 8.32E-09 |
| | ethylene biosynthetic process | 7 | 118 | 1.42E-08 | 1.42E-08 |
| | respiratory burst involved in defense response | 7 | 124 | 1.99E-08 | 1.99E-08 |
| | response to cold | 7 | 418 | 5.78E-05 | 5.78E-05 |
| | defense response by callose deposition | 4 | 47 | 5.25E-06 | 5.25E-06 |
| | jasmonic acid biosynthetic process | 4 | 132 | 2.82E-04 | 2.82E-04 |

**B**

| | Pathway | selected | contents | p-value | Name of the metabolic pathway |
|---|---|---|---|---|---|
| PC2 negative | PWYQT-4471 | 20 | 61 | 0.00E+00 | **glucosinolate biosynthesis from dihomomethionine** |
| | PWYQT-4472 | 16 | 34 | 0.00E+00 | **glucosinolate biosynthesis from trihomomethionine** |
| | PWYQT-4473 | 16 | 33 | 0.00E+00 | **glucosinolate biosynthesis from tetrahomomethionine** |
| | PWYQT-4474 | 15 | 32 | 0.00E+00 | **glucosinolate biosynthesis from pentahomomethionine** |
| | PWYQT-4475 | 15 | 35 | 0.00E+00 | **glucosinolate biosynthesis from hexahomomethionine** |
| | PWY-1187 | 12 | 30 | 0.00E+00 | **glucosinolate biosynthesis from homomethionine** |
| | PWY-2821 | 11 | 21 | 0.00E+00 | **glucosinolate biosynthesis from phenylalanine** |
| | PWYQT-4450 | 10 | 12 | 0.00E+00 | **aliphatic glucosinolate biosynthesis, side chain elongation cycle** |
| | PWY-601 | 10 | 27 | 5.31E-14 | **glucosinolate biosynthesis from tryptophan** |
| | PWY-6842 | 8 | 66 | 1.03E-07 | glutathione-mediated detoxification II |
| | PWY-6932 | 7 | 10 | 4.27E-12 | selenate reduction |
| | PWY-5340 | 6 | 10 | 3.75E-10 | **sulfate activation for sulfonation** |
| | PWY-6051 | 6 | 16 | 6.04E-09 | 2,4,6-trinitrotoluene degradation |
| | PWY-5320 | 5 | 16 | 2.83E-07 | kaempferol glycoside biosynthesis (Arabidopsis) |
| | PWY-5321 | 5 | 35 | 1.25E-05 | quercetin glycoside biosynthesis (Arabidopsis) |
| | PWY-1186 | 4 | 8 | 7.24E-07 | L-homomethionine biosynthesis |
| | PWY-702 | 4 | 13 | 4.89E-06 | L-methionine biosynthesis II |
| | LEUSYN-PWY | 4 | 16 | 1.10E-05 | L-leucine biosynthesis |
| | HOMOSERSYN-PWY | 3 | 5 | 1.11E-05 | L-homoserine biosynthesis |
| | SULFMETII-PWY | 3 | 5 | 1.11E-05 | **sulfate reduction II (assimilatory)** |
| | PWY-5097 | 3 | 15 | 2.83E-04 | L-lysine biosynthesis VI |
| | PWY1F-FLAVSYN | 3 | 19 | 5.61E-04 | flavonoid biosynthesis |
| | PWY-5272 | 3 | 21 | 7.49E-04 | abscisic acid glucose ester metabolism |
| | DISSULFRED-PWY | 2 | 2 | 1.34E-04 | **Pathway: sulfate reduction IV (dissimilatory)** |
| | GLUTATHIONESYN-PWY | 2 | 2 | 1.34E-04 | **glutathione biosynthesis** |

**fig. S10. Keywords that frequently appeared in genes with high scores in PC2.** Gene ontology biological processes (**A**) and metabolic pathways (**B**) over-represented in PC2 are listed. In both (**A**) and (**B**), frequencies of genes were compared between the selected genes (selected) and the whole chip contents (contents) for each keyword and the *p*-value was estimated after selecting genes specifically expressed toward positive or negative direction of PC2 based on the Normal QQ plot (fig. S8) as described in the Materials and Methods. There was no metabolic pathway over-represented in positive PC2 (B). The keywords of which the *p*-values are less than 0.001 and the frequencies in selected genes are more than 4 (A) or 2 (B) are listed.

| References | | | (17) | (52) | | | (13) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Array element | AGI | Gene | *myb28myb29/WT* | *MYB28ox/WT* | *MYB29ox/WT* | *MYB76ox/WT* | *myb28/WT* | *myb29/WT* | *MYB28ox3/WT* | *MYB28ox5/WT* |
| 248676_at | At5g48850 | ***SDI1*** | 0.39 | **4.53*** | 2.26 | 0.69 | 0.72 | 3.09 | **30.87*** | **12.12*** |
| 261177_at | At1g04770 | ***SDI2*** | 0.41 | 0.98 | 0.97 | 0.60 | 0.63 | 1.74 | **3.81*** | **2.21*** |
| 247549_at | At5g61420 | ***MYB28*** | **0.06*** | **1.37*** | 1.06 | 0.89 | **0.01*** | 1.02 | 2.36 | 1.13 |
| 250598_at | At5g07690 | ***MYB29*** | **0.43*** | 1.43 | **3.25*** | **1.34*** | 1.14 | 0.30 | 0.64 | 0.54 |
| 250589_at | At5g07700 | ***MYB76*** | 0.86 | 1.03 | 1.18 | **6.70*** | 0.83 | 1.72 | 0.72 | 0.68 |
| 260064_at | At1g73730 | ***SLIM1*** | 1.16 | 1.05 | 0.92 | 1.12 | 1.19 | 1.02 | 0.93 | 0.74 |

**fig. S11. Effects of the manipulation of MYB28, MYB29, and MYB76 on gene expression of *SDI1*, *SDI2*, and other factors.** The values indicate fold changes of the *SDI1*, *SDI2* gene expressions versus the corresponding WTs in the *myb28* KO and *MYB28ox* lines (*13*); in the *MYB28/29/76ox* lines (*52*) and in the *myb28myb29* (*17*). Values reported significantly in each reference were marked with asterisks.

**table S12. Oligonucleotides used for the vector construction.**

| Purpose | Oligo Name | Figure No. | Oligonucleotide (5' to 3') | Vectors | | Constructs |
|---|---|---|---|---|---|---|
| | | | | Cloning | Binary | |
| CDS isolation for over-expression of SDIs | SDI1-oxF | 3, 4 S4, S5 | gagagaacacgggggactctagATGGAGAGAAGCTTGAAGAAGACGA | Topo Blunt | pSMAH621 | ***SDI1ox*** |
| | SDI1-oxR | | aacgatcggggaaattcgagctCTAGCAAACTAATGTATTTCTAAAAGACGAGATCTGT | | | |
| | SDI2-oxF | | gagagaacacgggggactctagATGATGATGATGATTCAGAGAAGAGGA | Topo Blunt | pSMAH621 | ***SDI2ox*** |
| | SDI2-oxR | | aacgatcggggaaattcgagctcTCAACAAGCCAATTGATCTCTCAGTGGT | | | |
| Isolation of 5'-region of SDIs | SDI1-proFSal | 1 | GTCGACCAAATCAATCTTGAATAATGCTGATAAACCGTCT | Topo Blunt | pBI101-GFP | ***SDI1pro:GFP*** |
| | SDI1-proRBam | | GGATCCTCTTTTCTCAATAATTAATTATAAAGATGTCTGTTGACAGGCT | | | |
| | SDI2-proFSal | | GTCGACGTAAGAAAAGAGCTTGCTCTGTCTCTGAGAACA | | | ***SDI2pro:GFP*** |
| | SDI2-proRBam | | GGATCCGCAAACTTCGTGTCTCTGTTTCTAAATGAAGA | | | |
| | SDI1-proF | S3 | caccGCCACTGTTATGTTGAGTTGAACC | pENTR | pKGWFS7 | ***SDI1pro:GUS*** |
| | SDI1-proR | | **R1**:CTTCGTCTTCTTCAAGCTTCTCTC/ **R2**:CTTTTTTTCCTCTGTTTTTCTCTTTTCTC | | | |
| | SDI2-proF | | caccCCGTAGTAGTACTCAATCGAACAC | | | ***SDI2pro:GUS*** |
| | SDI2-proR | | CGCAAACTTCGTGTCTCTGTTTC | | | |
| CDS isolation for GFP-fusion for transient transformation | SDI1-GFP-F | 7A | caatttaaatcatttcttttaaagcaaaagcaattttctgaaaattttcaccatttacgaacgatactcgagATGGAGAGAAGCTTGAAGAAG | pCR2.1 | pA7 | ***SDI1-GFP*** |
| | SDI1-GFP-R | | TAGTACGTCGACGCAAACTAATGTATTTC | | | |
| | GFP-SDI1-F | | CAAGGGTCTAGAGAGAAGCTTGAAGA | | | ***GFP-SDI1*** |
| | GFP-SDI1-R | | ttcgagctCTAGCAAACTAATGTATTTC | | | |
| CDS isolation for Y2H screening | SDI1-BD-F | 7B | CATGGAGGCCGAATTCATGGAGAGAAGCTTGAAGAAGACG | infusion | pGBKT7 | ***SDI1-BD*** |
| | SDI1-BD-R | | GCAGGTCGACGGATCCCTAGCAAACTAATGTATTTCTAAA | | | |
| | MYB28-AD-F | | TGGCCATTATGGCCCGGGATGTCAAGAAAGCCATGTTG | | pGADT7-Rec | ***MYB28-AD*** |
| | MYB28-AD-R | | GACATGTTTTTTCCCGGGTCATATGAAATGCTTTTCAAG | | | |
| | MYB29-AD-F | | TGGCCATTATGGCCCGGGATGTCAAGAAAGCCATGTTGT | | | ***MYB29-AD*** |
| | MYB29-AD-R | | GACATGTTTTTTCCCGGGTCATATGAAGTTCTTGTCGTC | | | |
| | MYB76-AD-F | | TGGCCATTATGGCCCGGGATGTCAAAGAGACCATATTGT | | | ***MYB76-AD*** |
| | MYB76-AD-R | | GACATGTTTTTTCCCGGGTCATAAGAAGTTCTTCTCGTC | | | |
| CDS isolation for YFP-fusion for BiFC Assay | SDI1-nYFP-F | 7C | ATGGAGAGAAGCTTGAAGAAGACGA | infusion | pDESTVYNE(R)GW | ***nYFP-SDI1*** |
| | SDI1-nYFP-R | | CTAGCAAACTAATGTATTTCTAAAA | | | |
| | MYB28-cYFP-F | | ATGTCAAGAAAGCCATGTTGCGTCG | | pDEST-GW-VYCE | ***MYB28-cYFP*** |
| | MYB28-cYFP-R | | TATGAAATGCTTTTCAAGCGAGTCT | | | |
| | MYB29-cYFP-F | | ATGTCAAGAAAGCCATGTTGTGTGG | | | ***MYB29-cYFP*** |
| | MYB29-cYFP-R | | TATGAAGTTCTTGTCGTCGTAATCT | | | |
| | MYB76-cYFP-F | | ATGTCAAAGAGACCATATTGTATCG | | | ***MYB76-cYFP*** |
| | MYB76-cYFP-R | | TAAGAAGTTCTTCTCGTCGGAATCT | | | |
| | MYB75-cYFP-F | | ATGGAGGGTTCGTCCAAAGGGCTG | | | ***MYB75-cYFP*** |
| | MYB75-cYFP-R | | ATCAAATTTCACAGTCTCTCCATC | | | |

**table S13. Oligonucleotides used for the isolation of the T-DNA insertion lines.**

| Purpose | KO Name | Resourses | Oligo Name | Oligonucleotide (5' to 3') |
|---|---|---|---|---|
| genomic PCR | *sdi1-1* | SALK_145035.20.15 | sdi1-1 LB | GGGTTCTTCTCTATCAACTGCAACCAACATT |
| | | | sdi1-1,2 RB | GGATAGAATGTTATTGCATTTCTTGCCTGAAAAGGT |
| | *sdi1-2* | SALK_099766.56.00 | sdi1-2 LB | GGCAACATCTAGACCATAAATTGGAACAACTT |
| | | | sdi1-1,2 RB | - |
| | *sdi2-1* | SALK_091618.45.75 | sdi2-1 LB | GAGATCTTTGTTCAAGACAAGCTCAAGAGTCA |
| | | | sdi2-1 RB | gaatccTCAACAAGCCAATTGATCTCTCAGTGGTAAGA |
| | *sdi2-2* | SALK_110128.48.65 | sdi2-2 LB | CCATCAGTTCACTGATCAAGAGACTTGAGAA |
| | | | sdi2-2 RB | gaattcATGATGATGATGATTCAGAGAAGAGGAGGTGAGA |
| | all | | T-DNA LB-02 | CACCCCAGTACATTAAAAACGTCCGCAA |
| RT-PCR of *SDI1* | | | SDI1-F | ATGGAGAGAAGCTTGAAGAAGACGAAGAAC |
| | | | SDI1-R | CTAGCAAACTAATGTATTTCTAAAAGACGAGATCTGT |
| RT-PCR of *SDI2* | | | SDI2-F | ATGATGATGATGATTCAGAGAAGAGGAGGT |
| | | | SDI2-R | TCAACAAGCCAATTGATCTCTCAGTGGTA |

**table S14. Oligonucleotides used for qRT-PCR analysis.**

| AGI Code | Gene Name | Oligonucleotide (5' to 3') | | References |
|---|---|---|---|---|
| At5g48850 | ***SDI1*** | F | TCAGAGCCAAACATGCTCAGTT | This paper |
| | | R | ACAACAGCCATGTCCTTGAGG | |
| At1g04770 | ***SDI2*** | F | CGAGCGAAGCATGTTCAGTTG | |
| | | R | CGTCAATGGCTTCTTCAGCTCT | |
| At3g19710 | ***BCAT4*** | F | CAGAAGATGGTCGGATTCTGCTA | *29* |
| | | R | GGCAAAAGCTGTGAAGGTGGT | |
| At5g23010 | ***MAM1*** | F | AATTCGGAGAACTCGTGGCCT | |
| | | R | GCTCCCGCACATATACCGGAT | |
| At1g16400 | ***CYP79F2*** | F | CCCATAATAGACGAGAGGGTCGAAA | |
| | | R | CGATCGCTGCTATACAAAATTCG | |
| At1g16410 | ***CYP79F1*** | F | CTTGACGTACTGTCGTTTGTTG | *32* |
| | | R | GCTACTCCGAATGTTTGATCG | |
| At4g13770 | ***CYP83A1*** | F | TGGCAATCGTCTCTCTATCTTTC | |
| | | R | GACATCATGTGAATTTGCTTCC | |
| At3g19710 | ***SULTR1;2*** | F | CAGAAGATGGTCGGATTCTGCTA | *29* |
| | | R | GGCAAAAGCTGTGAAGGTGGT | |
| At5g23010 | ***SULTR2;1*** | F | AATTCGGAGAACTCGTGGCCT | *78* |
| | | R | GCTCCCGCACATATACCGGAT | |
| At1g16400 | ***APR3*** | F | CCCATAATAGACGAGAGGGTCGAAA | *77* |
| | | R | CGATCGCTGCTATACAAAATTCG | |
| At2g36170 | ***UBQ2*** | F | CCAAGATCCAGGACAAAGAAGGA | *29* |
| | | R | TGGAGACGAGCATAACACTTGC | |