Supplementary material

Assessing Transcriptomic Responses to Oxidative Stress: Contrasting Wild-Type Arabidopsis Seedlings with *dss1(I)* and *dss1(V)* Gene Knockout Mutants

Ivana Nikolić, Mira Milisavljević and Gordana Timotijević *

Group for Plant Molecular Biology, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, 11042 Belgrade, Serbia;
ivana.nikolic@imgge.bg.ac.rs (I.N.);
milisavljevicm@imgge.bg.ac.rs (M.M.)
* Correspondence: timotijevic@imgge.bg.ac.rs

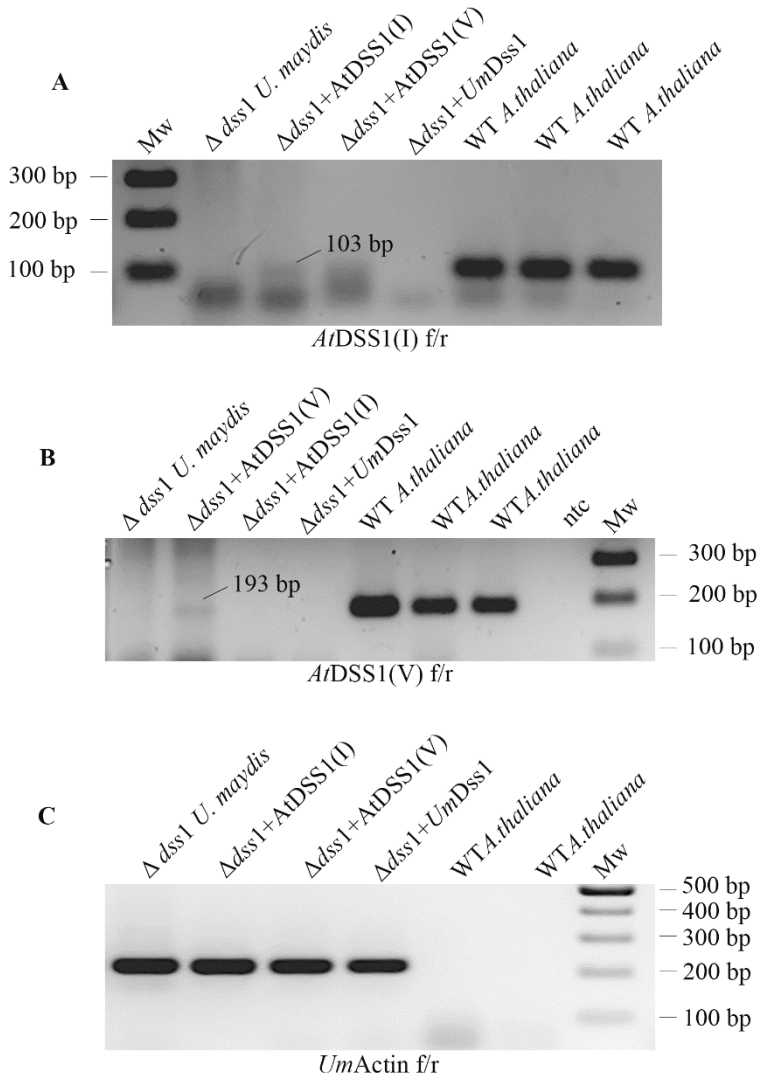


Figure S1: PCR detection of transcripts *AtDSS1(I)* and *AtDSS1(V)* isolated from *U. maydis* Δ*dss1* mutant transformants. **A** – Gel electrophoresis of PCR reactions with gene-specific primers for *A. thaliana DSS1(I)* cDNA. **B** – Gel electrophoresis of PCR reactions with gene-specific primers for *A. thaliana DSS1(V)* cDNA. **C** – Gel electrophoresis of PCR reactions with gene-specific primers for *U. maydis* actin. Δ*dss1* –*dss1* mutant of *U. maydis*; Δ*dss1*+AtDSS1(I) – Δ*dss1* mutant *U. maydis* complemented with the *AtDSS1(I)* WT sequence of *A. thaliana*; Δ*dss1*+AtDSS1(V) – Δ*dss1* mutant of *U. maydis* complemented with the *AtDSS1(V)* WT sequence of *A. thaliana*; Δ*dss1*+UmDss1 – Δ*dss1* mutant of *U. maydis* complemented with WT *UmDss1* from the genome of *U. maydis*; WT – wild strain of *A.thaliana* – positive control reaction with gene-specific primers for *AtDSS1 A. thaliana*.

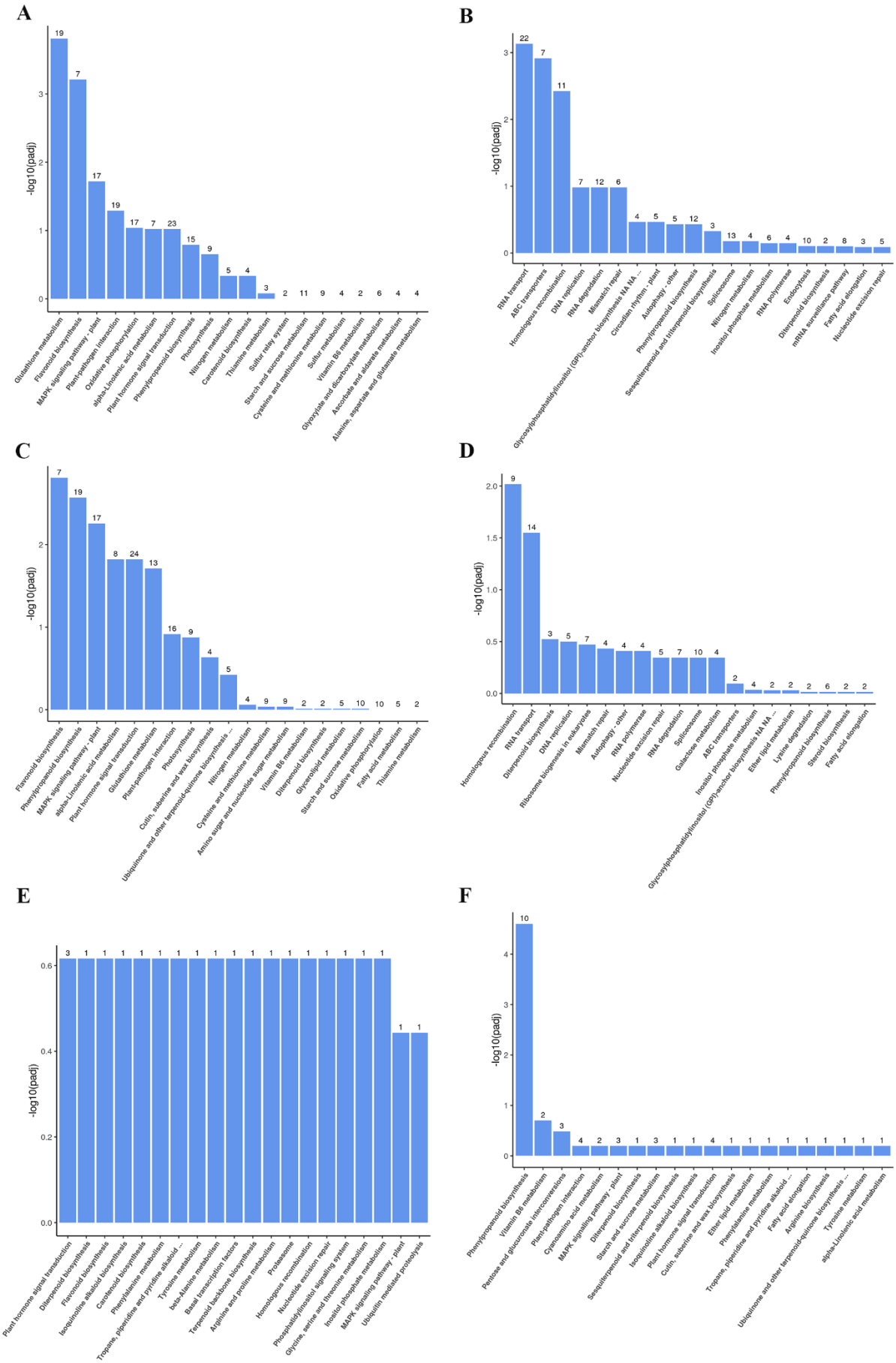

A
-log10(padj)
Glutathione metabolism
Flavonoid biosynthesis
MAPK signaling pathway - plant
Plant-pathogen interaction
Oxidative phosphorylation
alpha-Linolenic acid metabolism
Plant hormone signal transduction
Phenylpropanoid biosynthesis
Photosynthesis
Nitrogen metabolism
Carotenoid biosynthesis
Thiamine metabolism
Sulfur relay system
Starch and sucrose metabolism
Cysteine and methionine metabolism
Sulfur metabolism
Vitamin B6 metabolism
Glyoxylate and dicarboxylate metabolism
Ascorbate and aldarate metabolism
Alanine, aspartate and glutamate metabolism
B
-log10(padj)
RNA transport
ABC transporters
Homologous recombination
DNA replication
RNA degradation
Mismatch repair
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis NA NA ...
Circadian rhythm - plant
Autophagy - other
Phenylpropanoid biosynthesis
Sesquiterpenoid and triterpenoid biosynthesis
Spliceosome
Nitrogen metabolism
Inositol phosphate metabolism
RNA polymerase
Endocytosis
Diterpenoid biosynthesis
mRNA surveillance pathway
Fatty acid elongation
Nucleotide excision repair
C
-log10(padj)
Flavonoid biosynthesis
Phenylpropanoid biosynthesis
MAPK signaling pathway - plant
alpha-Linolenic acid metabolism
Plant hormone signal transduction
Glutathione metabolism
Plant-pathogen interaction
Photosynthesis
Cutin, suberine and wax biosynthesis
Ubiquinone and other terpenoid-quinone biosynthesis ...
Nitrogen metabolism
Cysteine and methionine metabolism
Amino sugar and nucleotide sugar metabolism
Vitamin B6 metabolism
Diterpenoid biosynthesis
Glycerolipid metabolism
Starch and sucrose metabolism
Oxidative phosphorylation
Fatty acid metabolism
Thiamine metabolism
D
-log10(padj)
Homologous recombination
RNA transport
Diterpenoid biosynthesis
DNA replication
Ribosome biogenesis in eukaryotes
Mismatch repair
Autophagy - other
RNA polymerase
Nucleotide excision repair
RNA degradation
Spliceosome
Galactose metabolism
ABC transporters
Inositol phosphate metabolism
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis NA NA ...
Ether lipid metabolism
Lysine degradation
Phenylpropanoid biosynthesis
Steroid biosynthesis
Fatty acid elongation
E
-log10(padj)
Plant hormone signal transduction
Diterpenoid biosynthesis
Flavonoid biosynthesis
Isoquinoline alkaloid biosynthesis
Carotenoid biosynthesis
Phenylalanine metabolism
Tropane, piperidine and pyridine alkaloid ...
Tyrosine metabolism
beta-Alanine metabolism
Basal transcription factors
Terpenoid backbone biosynthesis
Arginine and proline metabolism
Proteasome
Homologous recombination
Nucleotide excision repair
Phosphatidylinositol signaling system
Glycine, serine and threonine metabolism
Inositol phosphate metabolism
MAPK signaling pathway - plant
Ubiquitin mediated proteolysis
F
-log10(padj)
Phenylpropanoid biosynthesis
Vitamin B6 metabolism
Pentose and glucuronate interconversions
Plant-pathogen interaction
Cyanoamino acid metabolism
MAPK signaling pathway - plant
Diterpenoid biosynthesis
Starch and sucrose metabolism
Sesquiterpenoid and triterpenoid biosynthesis
Isoquinoline alkaloid biosynthesis
Plant hormone signal transduction
Cutin, suberine and wax biosynthesis
Ether lipid metabolism
Phenylalanine metabolism
Tropane, piperidine and pyridine alkaloid ...
Fatty acid elongation
Arginine biosynthesis
Ubiquinone and other terpenoid-quinone biosynthesis ...
Tyrosine metabolism
alpha-Linolenic acid metabolism

Figure S2: Graphical representation of the results of KEGG analysis of metabolic pathways involving proteins whose genes are down- or upregulated in seedlings treated with hydrogen peroxide. **A** – Downregulated in *dss1(I)* vs. WT; **B** – upregulated in *dss1(I)* vs. WT; **C** – downregulated in *dss1(V)* vs. WT; **D** - upregulated in *dss1(V)* vs. WT; **E** - downregulated in *dss1(I)* vs. *dss1(V)*; **F** – upregulated in *dss1(I)* vs. *dss1(V)* pathways. Descriptions of the functions of KEGG pathways are shown on the x-axis; y-axis – p-values (-log10(padj)); the order of the pathways is based on the p-value (column height). A statistically significant value of $p<0.05$ was taken for the KEGG pathways. The numbers indicate the counts of genes with an altered expression that are associated with the described pathways.

Table S1: List of primers.

Gene ID	Primer name	Primer sequence	Product size (bp)
AT5G63960	GIS5_F GIS5_R	5′- TCCTTCGAATACCCCACCAC -3′ 5′- CGCCGTACAAGAGAGTTTCG -3′	137
AT2G01440	RECG_F RECG_R	5′- GGCATCAAAGAAGTCTACTCAATG -3′ 5′- CAACGTATACTCTGCCTCCAGAC -3′	64
AT4G21070	BRCA1_F BRCA1_R	5′- CCATGTATTTTGCAATGCGTG -3′ 5′- TGTGGAGCACCTCGAATCTCT -3′	105
AT5G45400	RPA70_F RPA70_R	5′- GACGCTGGGAGATATGGACA -3′ 5′- CGGACGGGAATAAGCAGTTG -3′	171
AT1G10930	RECQ4A_F RECQ4A_R	5′- TCATCACTCGCTCCCAAAGT -3′ 5′- CATGGGGCTTTGGTCAACTC -3′	160
AT1G05490	CLSY3_F CLSY3_R	5′- GGGATCAGTCTGGTAGGAGC -3′ 5′- CTGCCCGATTCTGTAAGCAC -3′	102
AT2G22140	EME1B_F EME1B_R	5′- TTATACGGTGTGCTGCCTCA -3′ 5′- GCCTCGTCTACACAGTGTCT -3′	168
AT1G60930	RECQ4B_F RECQ4B_R	5′- CCCTTCCAATGGCAAAAGCT -3′ 5′- TTGCTCACCTTGGCTTTTCC -3′	223
AT5G07990	CYP75B_F CYP75B_Rr	5'-AGGCGTCGCTGGTAAAATGA -3' 5′- ATAGCCCAGTCCACCGTACT -3′	249
AT4G22880	LDOX_F LDOX_R	5'-TGGGGAGTGATGCATTTGATCA -3' 5′- ATCTTTCCAGTGGCTTGATCGT -3′	137
AT4G11280	AOCS6_F AOCS6_R	5'- TGTAGACGAGTTTATCCGCGAG -3' 5'- CCGAATCAAACGTTGCTGTCTT -3'	164
AT2G44460	BGLU28_F BGLU28_R	5'- GAACCATCGATGACCCTCTACC -3' 5'- AGCCCGCAACAGTTATGACATA -3'	187
AT2G14610	PR1_F PR1_R	5'- TGTAGGTGCTCTTGTTCTTCCC -3' 5′- GTTGCCTCTTAGTTGTTCTGCG -3′	160
AT5G64110	PRX70_F PRX70_R	5'- CGGACTGGGTATTACGGTAGTG- 3' 5'- GTCACAGCCTTGAACGAAACAA- 3'	144
UMAG_11232	UmActin f UmActin r	5′- CTGGAAGCTGCGGGTATTCA -3′ 5′- GAGTACTTTCGCTCGGGAGG -3′	209
AT1G64750	UmNdeI_F 3UTR DI_R	5′- TCCATATGGCGGCAGAACCG -3' 5′- GCAAAGGAAAAGCAGAACCG -3'	347
AT5G45010	UmNdeI_F 3UTR DV_R	5′- TCCATATGGCGGCAGAACCG -3' 5′- GAGAGAAGATGGTGATTATCAGAATAG-3'	431
AT1G64750	AtDSS1(I) f AtDSS1(I) r	5′- CTGAAGTAGTAAAGATGGATCTGTTT -3' 5′- TGGCTAACTTCCTTCACTTCT -3'	103
AT5G45010	AtDSS1(V) f AtDSS1(V) r	5′- AAGTGGTGAAGGTGGATCTATTC -3' 5′- CATTTCTTCTCACTAGCATTCTCAAG -3'	193
	M13_F M13_R	5′- TCCCAGTCACGACGTCGT -3′ 5′- GGAAACAGCTATGACCATG -3′	

Table S2: List of analyzed genes.

Gene ID	Gene name	Protein name	Function description
AT5G63960	*GIS5*	Gigantea suppressor 5	The catalytic subunits of DNA polymerase δ
AT2G01440	*RECG*	ATP-dependent DNA helicase homolog RECG	DNA repair and HR
AT4G21070	*BRCA1*	Breast cancer susceptibility 1 homolog	DNA repair and cell-cycle control by homologous recombination (HR)
AT5G45400	*RPA70*	Replication protein A 70 kDa DNA-binding subunit C	HR, DNA repair, and replication
AT1G10930	*RECQ4A*	ATP-dependent DNA helicase Q-like 4A	DNA repair and suppression of HR
AT1G05490	*CLSY3*	Chromatin remodeling 31	Gene silencing
AT2G22140	*EME1B*	Essential meiotic endonuclease 1b	DNA repair and HR
AT1G60930	*RECQ4B*	ATP-dependent DNA helicase Q-like 4B	DNA repair and HR
AT5G07990	*CYP75B1*	Cytochrome P450 75B1	Biosynthesis of flavonoids
AT4G22880	*LDOX*	Leucoanthocyanidin dioxygenase	Biosynthesis of anthocyanin and protoanthocyanidin
AT4G11280	*AOCS6*	1-aminocyclopropane-1-carboxylate synthase 6	Biosynthesis of ethylene
AT2G44460	*BGLU28*	Beta glucosidase 28	Glucosinolate catabolic process
AT2G14610	*PR1*	Pathogenesis-related gene 1	Defense response
AT5G64110	*PRX70*	Peroxidase 70	Biosynthesis and degradation of lignin