



# Elucidation of the Molecular Responses of Sesame (*Sesamum indicum* L.) Leaves to Waterlogging Stress using Transcriptome Profiling

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## Introduction

Sesame (*Sesamum indicum* L.) is a promising oil-rich seeds that have many potential health benefits and have been used in folk medicine for thousands of years. However, most field crops, including sesame, are highly sensitive to waterlogging, which can result in stunted growth and emerge as a major cause of the decrease in sesame production. To date, the molecular mechanisms underlying the responses to waterlogging in sesame remain unclear. Therefore, the present study was conducted to identify the genes related to the stage-specific responses of sesame growth in order to understand the molecular mechanism of sesame seeds against excessive waterlogging stress, and to explore the mechanisms underlying the dynamic metabolic pathways of the related genes.

## Materials and Methods

The seeds of sesame were collected from the Rural Development Administration, Korea. To observe the molecular alterations caused by waterlogging stress in sesame leaves, the present study was carried out in a glass greenhouse under paddy soil conditions. The seedlings were subjected to waterlogging condition maintaining 2 cm of soil surface at 2<sup>nd</sup> and 10<sup>th</sup> leaf stages, and treated for 5-days. Total RNA of the leaves was extracted using RNeasy Plant mini Kit. Through RNA sequencing, five top-enriched differentially expressed genes (DEGs) were considered to validate the reliability of the RNA libraries that were highly related to waterlogging stress.

## Results and Discussions

A total of 2,126 common genes were identified from both 2- and 10-leaf stages. Pathway analysis revealed that the top five metabolic processes related to waterlogging stress were starch and sucrose metabolism, photosynthesis, plant hormone signal transduction, glycolysis/gluconeogenesis, carbon fixation in photosynthetic organisms. Of the 2,126 genes, a total of 37 genes were involved in starch and sucrose metabolism whereas sucrose-synthesizing gene was reduced under waterlogging stress. In addition, genes that produce glycogen by cleaving starch were reduced. In photosynthesis pathway, a total of 30 genes were involved. Reduced expression of the genes related to PS II RC was caused by waterlogging stress. In addition, the expression of plastocyanin, a protein that acts as an electron carrier, was reduced under waterlogging stress. In plant hormone signal transduction pathway, a total of 29 genes were investigated. In this experiment, genes that prevent auxin production and deficiency through the biosynthetic pathway that produces indole, a precursor of auxin, were increased by waterlogging stress. A total of 28 genes were involved in Glycolysis / Gluconeogenesis pathway. Due to the lack of oxygen caused by waterlogging stress, the expression of PDC and ADH -related genes using pyruvate as a substrate was increased. Glycolytic enzymes phosphoglucomutase and enolase-related genes were also reduced. A total of 24 genes involved in carbon fixation in photosynthetic organisms were investigated. Decreased expression of alanine-producing glutamate-- glyoxylate amino -transferase was identified in this study. In addition, fructose-1, 6-bisphosphatase -related genes were decreased as the pH of stroma decreased.

# Results and Discussions

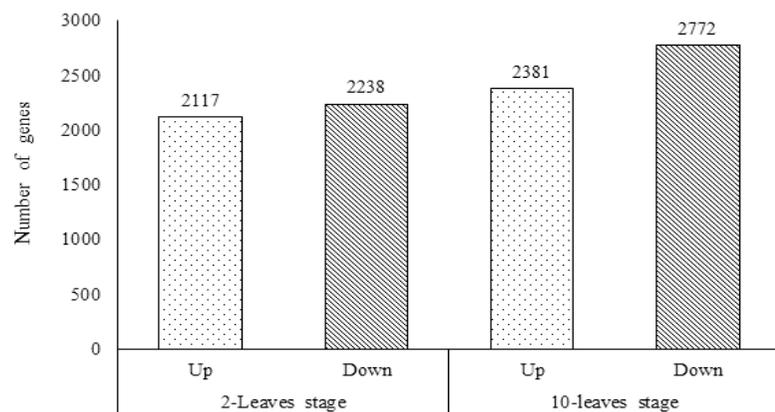


Fig. 1. Differentially expressed genes (DEGs) identified from 2- and 10-leaf stage.

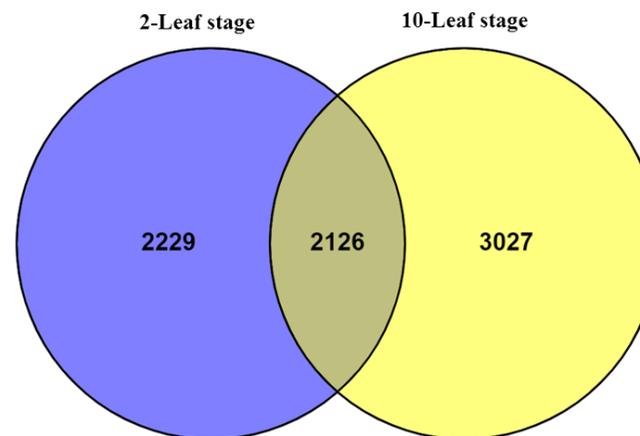


Fig. 2. Venn diagram of DEGs expressed in 2- and 10-leaf stage.

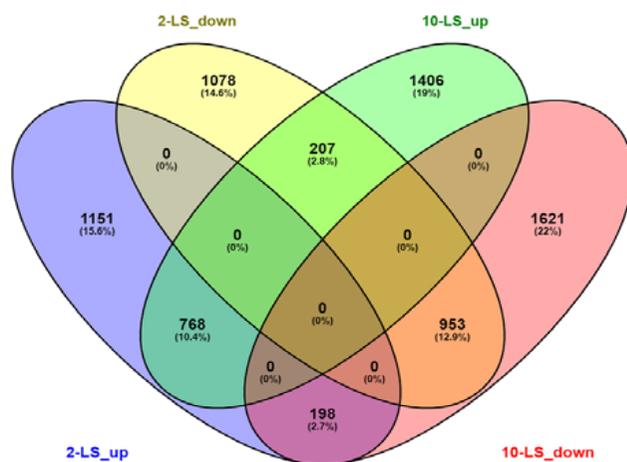


Fig. 3. Venn diagram of common and unique genes in each comparison group expressed in 2- and 10-leaf stage.

Table 5. List of top GO enriched up or down regulated genes and its related pathway.

Pathway	2-leaf stage		10-leaf stage		Gene count
	Up	Down	Up	Down	
<b>Starch and sucrose metabolism</b>	13	24	16	21	37
<b>Photosynthesis</b>	3	27	3	27	30
<b>Plant hormone signal transduction</b>	15	14	17	12	29
<b>Glycolysis / Gluconeogenesis</b>	12	16	16	12	28
<b>Carbon fixation in photosynthetic organisms</b>	7	17	10	14	24
Plant pathogen interaction	14	6	14	6	20
Photosynthesis-antenna proteins	0	18	0	18	18
Oxidative phosphorylation	1	9	2	8	10
Phenylalanine, tyrosine and tryptophan biosynthesis	8	1	1	8	9
Citrate cycle (TCA cycle)	7	1	8	0	8
Phenylalanine metabolism	6	1	3	4	7
Nitrogen metabolism	3	4	2	5	7
Endo cytosol	5	1	4	2	6
Fatty acid biosynthesis	2	3	1	4	5
Basal transcription factors	1	3	2	2	4

## Results and Discussions

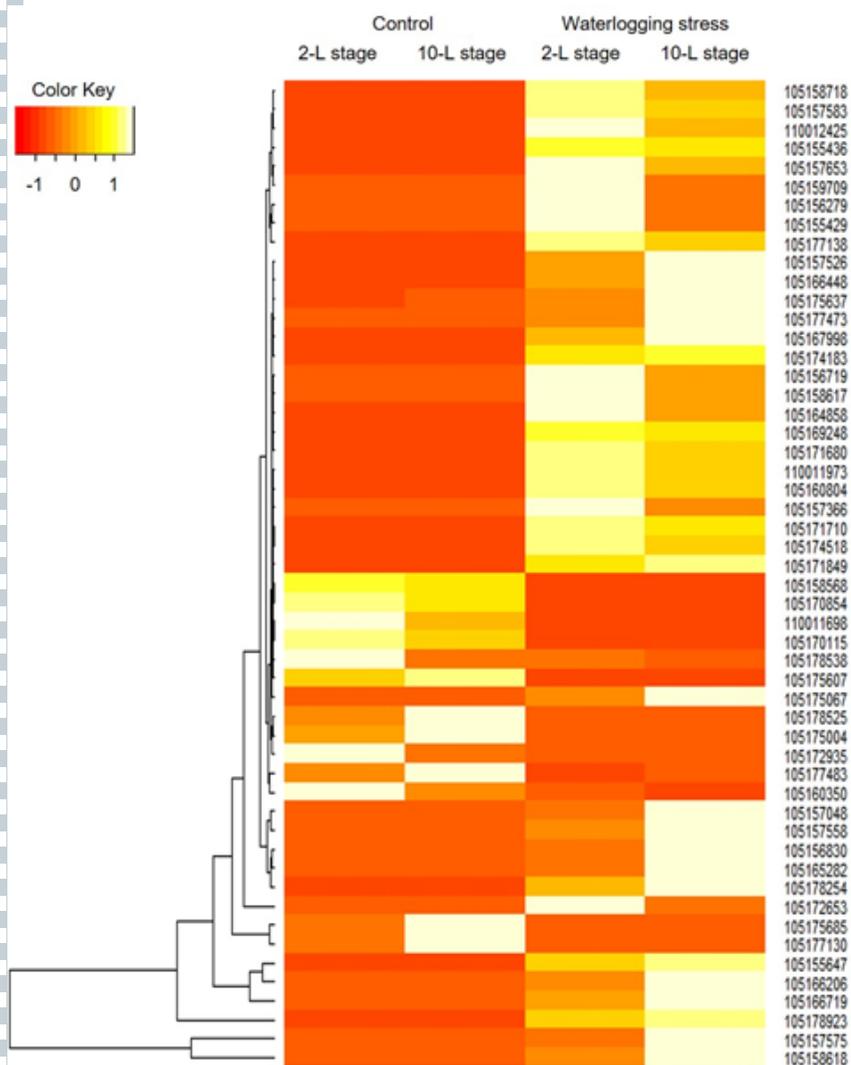


Fig. 4. Heat map analysis of DEGs from 2- and 10-leaf stage. The bar represents the scale of the expression levels of each gene (log<sub>2</sub> FPKM) in each sample, as indicated by red/yellow rectangles.

Table 2. List of DEGs related to starch and sucrose metabolism with FC value and gene regulation.

Enzyme code	Gene ID	Description	2-LS FC value	10-LS FC value	Gene regulation
<b>sucrose-phosphate synthase 2</b>					
2.4.1.14	105174736	probable sucrose-phosphate synthase 4	-3.1720	-5.3376	Down
	105179612	probable sucrose-phosphate synthase 2	-10.5381	-3.4400	Down
<b>1,4-alpha-glucan branching enzyme</b>					
2.4.1.18	105174106	1,4-alpha-glucan-branching enzyme 2-2, chloroplastic/amyloplastic	-5.1111	-5.9014	Down
<b>glycogen phosphorylase</b>					
	105155668	alpha-glucan phosphorylase, H isozyme	-4.5466	-3.3544	Down
2.4.1.1	105160024	alpha-1,4 glucan phosphorylase L-2 isozyme, chloroplastic/amyloplastic	-2.9995	-7.5479	Down
	105162703	4-alpha-glucanotransferase DPE2	-2.8509	-3.4739	Down
<b>4-alpha-glucanotransferase</b>					
2.4.1.25	105162703	4-alpha-glucanotransferase DPE2	-2.8509	-3.4739	Down
<b>phosphoglucomutase</b>					
5.4.2.2	105179246	phosphoglucomutase, chloroplastic	-2.4865	-2.1245	Down
<b>fructokinase</b>					
2.7.1.4	105172020	fructokinase-2	2.4724	2.0545	Up

Table 3. List of DEGs related to Photosynthesis with FC value and gene regulation.

Location	Gene ID	Description	2-LS FC value	10-LS FC value	Gene regulation
<b>photosystem II</b>					
<b>Psb Y</b>	105156667	photosystem II core complex proteins psbY, chloroplastic	-3.6994	-7.9355	Down
	105168347	photosystem II core complex proteins psbY, chloroplastic	-5.3349	-118.6560	Down
<b>Psb 27</b>	105173660	photosystem II repair protein PSB27-H1, chloroplastic	-2.0441	-4.0295	Down
<b>Psb 28</b>	105163467	photosystem II reaction center PSB28 protein, chloroplastic	-2.1640	-5.3032	Down
<b>photosystem I</b>					
<b>PsaD</b>	105156989	photosystem I reaction center subunit II, chloroplastic-like	-3.0261	-9.0825	Down
	105171354	photosystem I reaction center subunit II, chloroplastic-like	-4.2227	-7.9326	Down
<b>PsaE</b>	105163484	photosystem I reaction center subunit IV B, chloroplastic	-3.2848	-5.8813	Down
	105165695	photosystem I reaction center subunit IV A, chloroplastic	-4.0134	-5.3340	Down
<b>PsaF</b>	105167240	photosystem I reaction center subunit III, chloroplastic	-2.5261	-3.8188	Down
<b>PsaG</b>	105175035	photosystem I reaction center subunit V, chloroplastic	-3.6205	-7.0441	Down
<b>PsaH</b>	105157643	photosystem I reaction center subunit VI, chloroplastic-like	-2.6411	-5.5226	Down
	105163925	photosystem I reaction center subunit VI, chloroplastic-like	-5.3460	-7.1749	Down
<b>photosystem I</b>					
<b>PsaK</b>	105175776	photosystem I reaction center subunit psaK, chloroplastic	-8.4785	-25.6665	Down
<b>PsaL</b>	105163786	photosystem I reaction center subunit XI, chloroplastic	-3.0783	-3.7267	Down
<b>PsaN</b>	105178077	photosystem I reaction center subunit N, chloroplastic	-3.9051	-14.0577	Down
<b>PsaO</b>	105170042	photosystem I subunit O	-5.0270	-5.5392	Down
<b>Cytochrome b6-f complex</b>					
<b>PetC</b>	105162660	cytochrome b6-f complex iron-sulfur subunit 1, chloroplastic	-2.7327	-3.0593	Down
	105178615	cytochrome b6-f complex iron-sulfur subunit, chloroplastic	-2.1444	-4.1559	Down
<b>Photosynthetic electron transport</b>					
<b>PetE</b>	105165956	plastocyanin	-3.4235	-6.6926	Down
<b>F-type ATPase</b>					
<b>gamma</b>	105167988	ATP synthase gamma chain, chloroplastic-like	-2.1647	-5.6135	Down
	105175342	ATP synthase gamma chain, chloroplastic	-2.5085	-3.6290	Down
<b>delta</b>	105158931	ATP synthase delta chain, chloroplastic-like	-3.1445	-7.5788	Down
	105165945	ATP synthase delta chain, chloroplastic	-2.1055	-4.7858	Down
<b>b</b>	105165029	ATP synthase subunit b', chloroplastic	-3.8743	-3.6187	Down

## Results and Discussions

**Table 4. List of DEGs related to Plant hormone signal transduction with FC value and gene regulation.**

Location	Gene ID	Description	2-LS FCvalue	10-LS FCvalue	Gene regulation
<b>Auxin</b>					
<b>ARF</b>	<b>auxin response factor</b>				
	105165118	auxin response factor 9	-6.6162	-5.9233	Down
<b>GH3</b>	<b>auxin responsive GH3 gene family</b>				
	105169490	probable indole-3-acetic acid-amido synthetase GH3.1	2.0803	9.3829	Up
<b>Gibberellin</b>					
<b>TF</b>	<b>phytochrome-interacting factor 3</b>				
	105178574	transcription factor PIF4	-2.3528	-2.7786	Down
<b>Ethylene</b>					
<b>EIN3</b>	<b>ethylene-insensitive protein 3</b>				
	105163760	protein ETHYLENE INSENSITIVE 3	2.1828	1.7303	Up
	105170962	protein ETHYLENE INSENSITIVE 3	3.2386	1.6552	Up
<b>Ethylene</b>					
<b>ERF1/2</b>	<b>ethylene-responsive transcription factor 1</b>				
	105158452	ethylene-responsive transcription factor 1B-like	8.6586	3.2002	Up
	105171710	ethylene-responsive transcription factor 1B	17.3441	14.1893	Up
<b>Brassinosteroid</b>					
<b>BAK1</b>	<b>brassinosteroid insensitive 1-associated receptor kinase 1</b>				
	105172594	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1	2.3937	1.6694	Up
<b>BKI1</b>	<b>BRI1 kinase inhibitor 1</b>	<b>BRI1 kinase inhibitor 1</b>	-2.4117	-3.2267	Down
<b>Jasmonic acid</b>					
<b>JAR1</b>	<b>jasmonic acid-amino synthetase</b>	<b>jasmonic acid-amido synthetase JAR1</b>	-1.3874	-15.5728	Down

**Table 5. List of DEGs related to Glycolysis/Gluconeogenesis with FC value and gene regulation.**

Enzyme code	Gene ID	Description	2-LS FC value	10-LS FC value	Gene regulation
<b>5.4.2.2</b>	<b>phosphoglucomutase</b>				
	105179246	phosphoglucomutase, chloroplastic	-2.4865	-2.1245	Down
<b>5.1.3.3</b>	<b>aldose 1-epimerase</b>				
	105157026	aldose 1-epimerase	20.9875	3.7157	Up
<b>3.1.3.11</b>	<b>fructose-1,6-bisphosphatase I</b>				
	105161570	fructose-1,6-bisphosphatase, chloroplastic	-2.9637	-5.7035	Down
	105174519	fructose-1,6-bisphosphatase, chloroplastic-like	-2.2555	-4.4423	Down
	105179554	fructose-1,6-bisphosphatase, cytosolic	-6.5068	-9.7584	Down
<b>1.2.1.12</b>	<b>glyceraldehyde 3-phosphate dehydrogenase</b>				
	105156676	glyceraldehyde-3-phosphate dehydrogenase	7.7557	9.2130	Up
	105158078	glyceraldehyde-3-phosphate dehydrogenase, cytosolic-like	2.4811	2.7757	Up
<b>2.7.2.3</b>	<b>phosphoglycerate kinase</b>				
	105175102	glyceraldehyde-3-phosphate dehydrogenase GAPCP2, chloroplastic	3.5828	2.9481	Up
<b>5.4.2.11</b>	<b>2,3-bisphosphoglycerate-dependent phosphoglycerate mutase</b>				
	105168105	phosphoglycerate kinase, chloroplastic	-3.2020	-14.2607	Down
<b>4.2.1.11</b>	<b>enolase</b>				
	105174126	enolase	2.1189	2.3632	Up
<b>4.1.1.1</b>	<b>pyruvate decarboxylase</b>				
	105175619	pyruvate decarboxylase 1	3.3065	3.2969	Up
<b>1.1.1.2</b>	<b>alcohol dehydrogenase (NADP+)</b>				
	105174487	aldo-keto reductase family 4 member C9	23.9851	13.4210	Up

**Table 6. List of DEGs related to Carbon fixation in photosynthetic organisms with FC value and gene regulation.**

Enzyme code	Gene ID	Description	2-LS FC value	10-LS FC value	Gene regulation
<b>3.1.3.37</b>	<b>sedoheptulose-bisphosphatase</b>				
	105167082	sedoheptulose-1,7-bisphosphatase, chloroplastic	-2.6170	-6.1942	Down
<b>3.1.3.11</b>	<b>fructose-1,6-bisphosphatase I</b>				
	105161570	fructose-1,6-bisphosphatase, chloroplastic	-2.9637	-5.7035	Down
	105174519	fructose-1,6-bisphosphatase, chloroplastic-like	-2.2555	-4.4423	Down
<b>1.2.1.13</b>	<b>glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating)</b>				
	105179554	fructose-1,6-bisphosphatase, cytosolic	-6.5068	-9.7584	Down
<b>1.2.1.12</b>	<b>glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic</b>				
	105158872	glyceraldehyde-3-phosphate dehydrogenase B, chloroplastic	-2.0321	-2.6686	Down
<b>2.7.2.3</b>	<b>glyceraldehyde 3-phosphate dehydrogenase</b>				
	105156676	glyceraldehyde-3-phosphate dehydrogenase	7.7557	9.2130	Up
	105158078	glyceraldehyde-3-phosphate dehydrogenase, cytosolic-like	2.4811	2.7757	Up
<b>5.1.3.1</b>	<b>phosphoglycerate kinase</b>				
	105175102	glyceraldehyde-3-phosphate dehydrogenase GAPCP2, chloroplastic	3.5828	2.9481	Up
<b>5.1.3.1</b>	<b>ribulose-phosphate 3-epimerase</b>				
	105168105	phosphoglycerate kinase, chloroplastic	-3.2020	-14.2607	Down
<b>4.1.1.39</b>	<b>ribulose-bisphosphate carboxylase small chain</b>				
	105179760	ribulose-phosphate 3-epimerase, chloroplastic	-2.0645	-3.0213	Down
	105178030	ribulose bisphosphate carboxylase small chain, chloroplastic-like	-8.1253	-78.5199	Down
<b>1.1.1.37</b>	<b>malate dehydrogenase</b>				
	105178031	ribulose bisphosphate carboxylase small chain, chloroplastic-like	-2.1599	-9.8502	Down
<b>2.6.1.1</b>	<b>aspartate aminotransferase, cytoplasmic</b>				
	105171782	malate dehydrogenase	4.4439	2.2848	Up
<b>2.6.1.2</b>	<b>glutamate-glyoxylate aminotransferase</b>				
	105170896	aspartate aminotransferase, cytoplasmic	3.7690	12.3125	Up
<b>2.6.1.2</b>	<b>glutamate-glyoxylate aminotransferase 2</b>				
	105156639	glutamate-glyoxylate aminotransferase 2	-3.0952	-4.5571	Down

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