

Table 1 Suppl. Primers used in RT-qPCR.

Primer names	Primers sequences (5'-3')
NCED3-F:	5'-CGGGCAGATTCCCGATTGCC-3'
NCED3-R:	5'-GGATACACGGCGCGCCCGATC-3'
CML2-F:	5'-GGAAGCTCACGATTGAGGAG-3'
CML2-R:	5'-CGATTGCTCCCTCCTCAC-3'
MPK9-F:	5'-CGATCCTTCTACTTTACGGC-3'
MPK9-R:	5'-CTGTGTGAGTGTCAATTGCAG-3'
PRODH-F:	5'-CGAAGATGCAGAGGGATTGTTTC-3'
PRODH-R:	5'-CAGCGCAGAAATGAGAAAACG-3'
TPPD-F:	5'-GAACTTGATCTGGACATGGC-3'
TPPD-R:	5'-CACTGTGGAACGCATCGTATC-3'
PER1-F:	5'-CGATTGCTTCGTTAATGGC-3'
PER1-R:	5'-CGGAATCGCGAGCAGCAATG-3'
LEA8-1-F:	5'-CCAGTACCATCTGCAGAGAC-3'
LEA8-1-R:	5'-GTAACCCTGGGCATTGGCTG-3'
PPR32-F:	5'-CGAAACCTGATGAGTTCACC-3'
PPR32-R:	5'-CGGCATTAAATCAAACATTTGG-3'
ERF1-F:	5'-GGACATGGATATGTTGGAGTTG-3'
ERF1-R:	5'-CTGACGATATTTTGGCATGAGG-3'
NAC-F:	5'-CCTGAGAAGGCATTATTTGG-3'
NAC-R:	5'-GCCTTCCCCTTGTAACACAC-3'
UBC-F:	5'-GATTTATTTTCATTGGCAGGC-3'
UBC-R:	5'-AGGATCATCAGGATTTGGGT-3'

Table 2 Suppl. Output statistics of RNA-Seq of PEG-treated and controls of *Pinus massoniana* (GC- percentage of G and C bases in reads; Q30 - percentage of nucleotides with quality value larger than 30 in reads).

Sample	Raw_reads	Raw_bases	Clean_reads	Clean_bases	Valid_bases	Q30 [%]	GC [%]
Control1	49333204	7399980600	47923134	6925336518	93.59%	93.97	45.36
Control2	49338708	7400806200	47790074	6868448651	92.81%	93.74	45.52
Control3	49044656	7356698400	47679192	6868752033	93.37%	94.10	45.36
PEG1	48920728	7338109200	47355700	6843045183	93.25%	93.85	45.48
PEG2	49773358	7466003700	48485378	6996455491	93.71%	94.27	45.25
PEG3	48960840	7344126000	47468906	6852091815	93.30%	93.97	45.41

Table 3 Suppl. The differentially expressed genes (DEGs) that may be involved in signaling transduction in *Pinus massoniana*.

Pathways	Gene ID	log2FoldChange	Description
Plant hormone signal transduction	TRINITY_DN28017_c0_g1_i2_1	2.8176	abscisic acid receptor PYR/PYL family, PYL
	TRINITY_DN23237_c0_g1_i2_2	1.3290	9- <i>cis</i> -epoxycarotenoid dioxygenase, NCED3
	TRINITY_DN13505_c0_g1_i1_2	1.8478	9- <i>cis</i> -epoxycarotenoid dioxygenase, NCED3
	TRINITY_DN25111_c0_g1_i2_2	-4.3893	protein phosphatase 2C, PP2C
	TRINITY_DN17544_c0_g1_i2_2	-1.0147	protein phosphatase 2C, PP2C
	TRINITY_DN21321_c0_g1_i6_2	-1.6692	protein phosphatase 2C, PP2C
	TRINITY_DN18796_c0_g1_i1_1	1.1113	serine/threonine-protein kinase SRK2, SNRK2
	TRINITY_DN19149_c0_g1_i3_2	1.7711	jasmonate ZIM domain-containing protein, JAZ
	TRINITY_DN27193_c0_g1_i2_2	2.8420	auxin influx carrier (AUX1 LAX family), AUX1
	TRINITY_DN15824_c0_g1_i1_2	2.8520	cytokinin dehydrogenase, CKK
	TRINITY_DN8667_c0_g2_i1_2	4.5033	ethylene-responsive transcription factor 1, ERF1
	TRINITY_DN21968_c0_g1_i1_2	1.3940	SAUR family protein, SAUR
	TRINITY_DN27495_c0_g1_i7_2	-1.0372	SAUR family protein, SAUR
	TRINITY_DN21619_c0_g1_i7_1	-3.9054	coronatine-insensitive protein 1, COI-1
	TRINITY_DN24008_c0_g1_i1_2	1.1326	coronatine-insensitive protein 1, COI-1
	TRINITY_DN22930_c0_g1_i1_2	1.1285	F-box protein, GID2
Calcium signaling pathway	TRINITY_DN22097_c0_g1_i2_2	1.8637	solute carrier family 25, SLC25
	TRINITY_DN17437_c0_g1_i2_2	1.3778	calmodulin, CaM
	TRINITY_DN25729_c0_g1_i4_2	1.4495	calmodulin, CaM
	TRINITY_DN29245_c0_g1_i1_2	5.3493	calmodulin-like protein 2, CML2
	TRINITY_DN14152_c0_g1_i1_2	1.9246	calcium-binding protein KRP1, CARP1
	TRINITY_DN19522_c0_g1_i1_2	1.9307	calcium-binding protein KIC, KIC
	TRINITY_DN25063_c0_g1_i2_1	-1.0555	calcineurin B-like protein 2, CBL2
	TRINITY_DN18683_c0_g2_i2_2	1.0090	calmodulin-binding protein 60 B, CBP60B
MAPK signaling pathway	TRINITY_DN17065_c0_g1_i4_2	2.3316	mitogen-activated protein kinase 9, MPK9
	TRINITY_DN5620_c0_g1_i1_2	1.2221	mitogen-activated protein kinase 6, MPK6
	TRINITY_DN13189_c0_g1_i1_2	3.5080	1-phosphatidylinositol-4-phosphate 5-kinase, PIP5K
	TRINITY_DN18525_c0_g1_i1_1	-2.2089	E3 ubiquitin-protein ligase, SIAH1
	TRINITY_DN25897_c0_g1_i6_1	-1.0449	casein kinase 1, CSNK1

Table 4 Suppl. The differentially expressed genes (DEGs) encoding antioxidant proteins in *Pinus massoniana*.

Gene ID	log2FoldChange	Description
TRINITY_DN26850_c0_g2_i1_1	2.3933	glutathione peroxidase, GPX
TRINITY_DN18471_c0_g1_i1_1	2.2672	glutathione S-transferase, GST
TRINITY_DN24855_c0_g1_i3_2	2.5621	peroxidase 53, PER53
TRINITY_DN26809_c0_g1_i6_2	4.0254	cationic peroxidase 1, PER1
TRINITY_DN26984_c0_g1_i2_2	2.1691	peroxidase 53, PER53

Table 5 Suppl. The differentially expressed genes (DEGs) related to osmotic stress in *Pinus massoniana*.

Gene ID	log2FoldChange	Description
TRINITY_DN24209_c0_g1_i7_2	-1.2205	proline dehydrogenase 1, PRODH
TRINITY_DN8363_c0_g1_i1_1	2.9640	sucrose synthase 1, SUS1
TRINITY_DN26428_c0_g2_i1_2	1.2578	beta-amylase 1, BAM1
TRINITY_DN2854_c0_g1_i1_2	3.0075	glucan endo-1,3-beta-glucosidase 2, E132
TRINITY_DN18317_c0_g1_i2_2	3.7174	probable trehalose-phosphate phosphatase D, TPPD
TRINITY_DN24292_c1_g3_i1_2	4.8789	aquaporin, PIP21
TRINITY_DN29380_c0_g2_i4_1	-1.4263	aquaporin, PIP22

Table 6 Suppl. The differentially expressed genes (DEGs) encoding late embryogenesis abundant (LEA) proteins in *Pinus massoniana*.

Gene ID	log2FoldChange	Description
TRINITY_DN23058_c0_g1_i1_1	1.3907	late embryogenesis abundant protein
TRINITY_DN24970_c0_g1_i3_2	2.1604	late embryogenesis abundant protein
TRINITY_DN5005_c0_g1_i1_2	2.8126	late embryogenesis abundant protein
TRINITY_DN10244_c0_g1_i1_2	7.2890	late embryogenesis abundant protein
TRINITY_DN18834_c0_g1_i1_2	6.3511	late embryogenesis abundant protein, LEA8-1
TRINITY_DN23636_c0_g1_i1_2	1.8864	late embryogenesis abundant protein, LEA4-3
TRINITY_DN27560_c3_g2_i6_2	1.3145	late embryogenesis abundant protein, LEA7-2

Table 7 Suppl. The differentially expressed encoding pentatricopeptide repeat-containing proteins in *Pinus massoniana*.

Gene ID	log2FoldChange	Description
TRINITY_DN11027_c0_g2_i1_1	-2.5819	pentatricopeptide repeat-containing protein, PPR32
TRINITY_DN12872_c0_g1_i2_1	-3.8774	putative pentatricopeptide repeat-containing protein, PPR227
TRINITY_DN2795_c0_g1_i1_1	-7.4250	putative pentatricopeptide repeat-containing protein, PPR108
TRINITY_DN32423_c0_g1_i1_1	-3.4358	pentatricopeptide repeat-containing protein, PPR249
TRINITY_DN34403_c0_g1_i1_1	-3.6704	pentatricopeptide repeat-containing protein, PPR319
TRINITY_DN4397_c0_g1_i1_1	-3.5481	putative pentatricopeptide repeat-containing protein, PPR271
TRINITY_DN5059_c0_g1_i1_1	-3.8970	pentatricopeptide repeat-containing protein, PPR285
TRINITY_DN7851_c0_g1_i1_2	1.4745	pentatricopeptide repeat-containing protein, PPR400

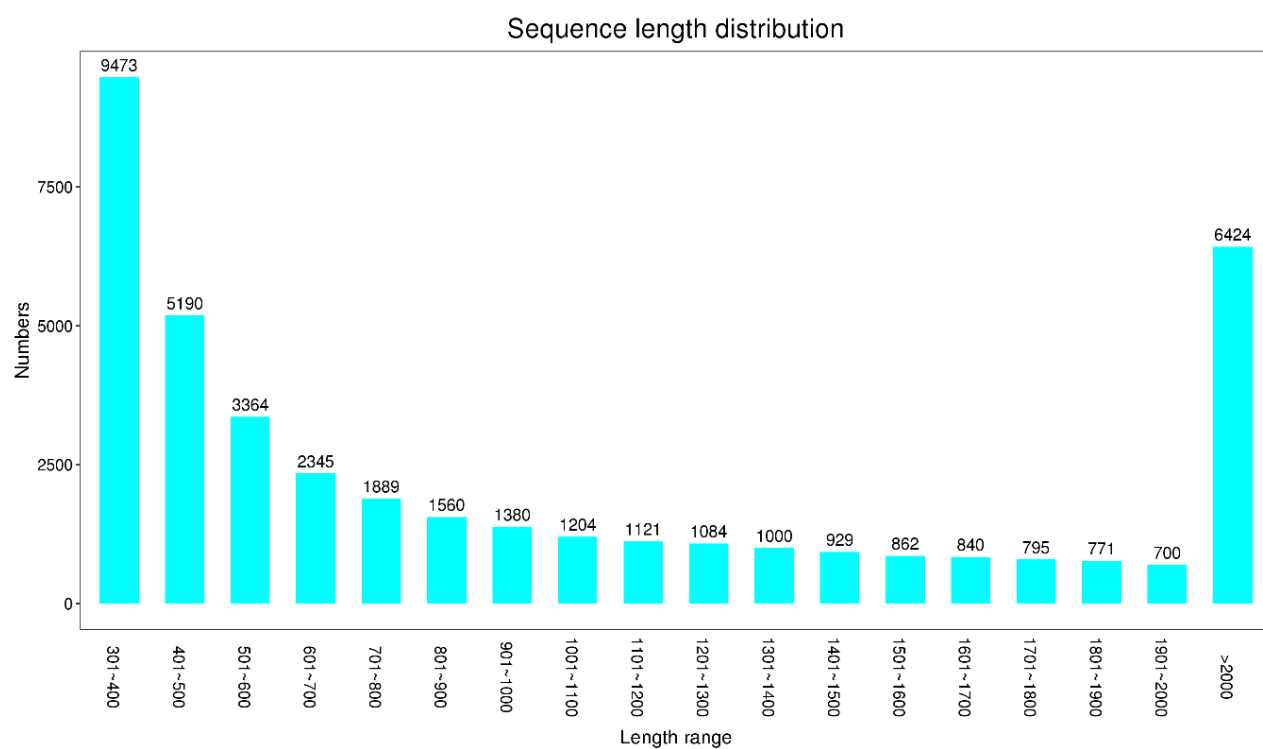


Fig. 1 Suppl. Distribution of assembled unigenes and the number of assembled unigenes of each length.

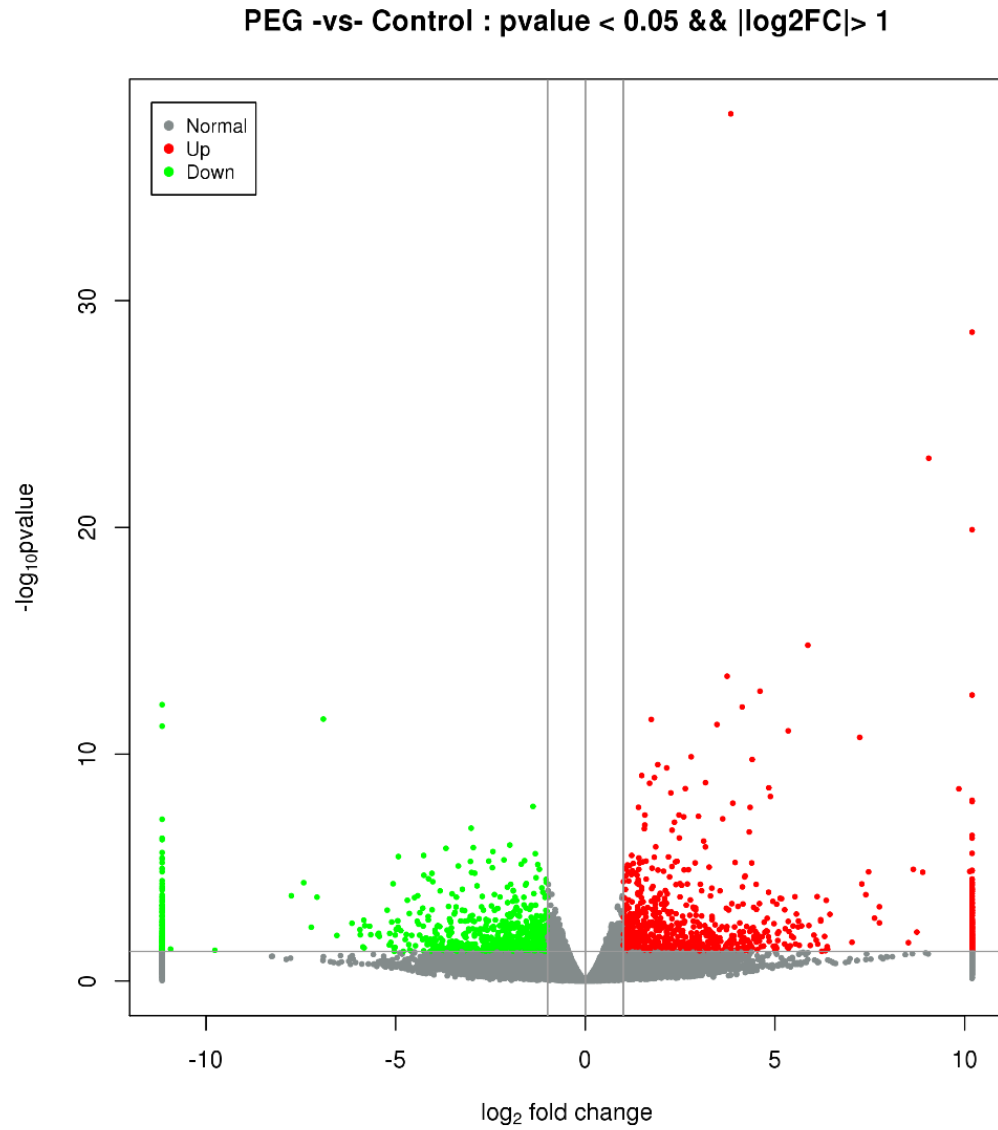


Fig. 2 Suppl. Volcano plot of differentially expressed unigenes of Masson pine seedlings. The x-axis is expressed as log₂ FoldChange. The y-axis is expressed as -log₁₀ (*P* value). *Gray* indicates no significant difference in expression, *red* represents up-regulated unigenes, *green* represents down-regulated unigenes.

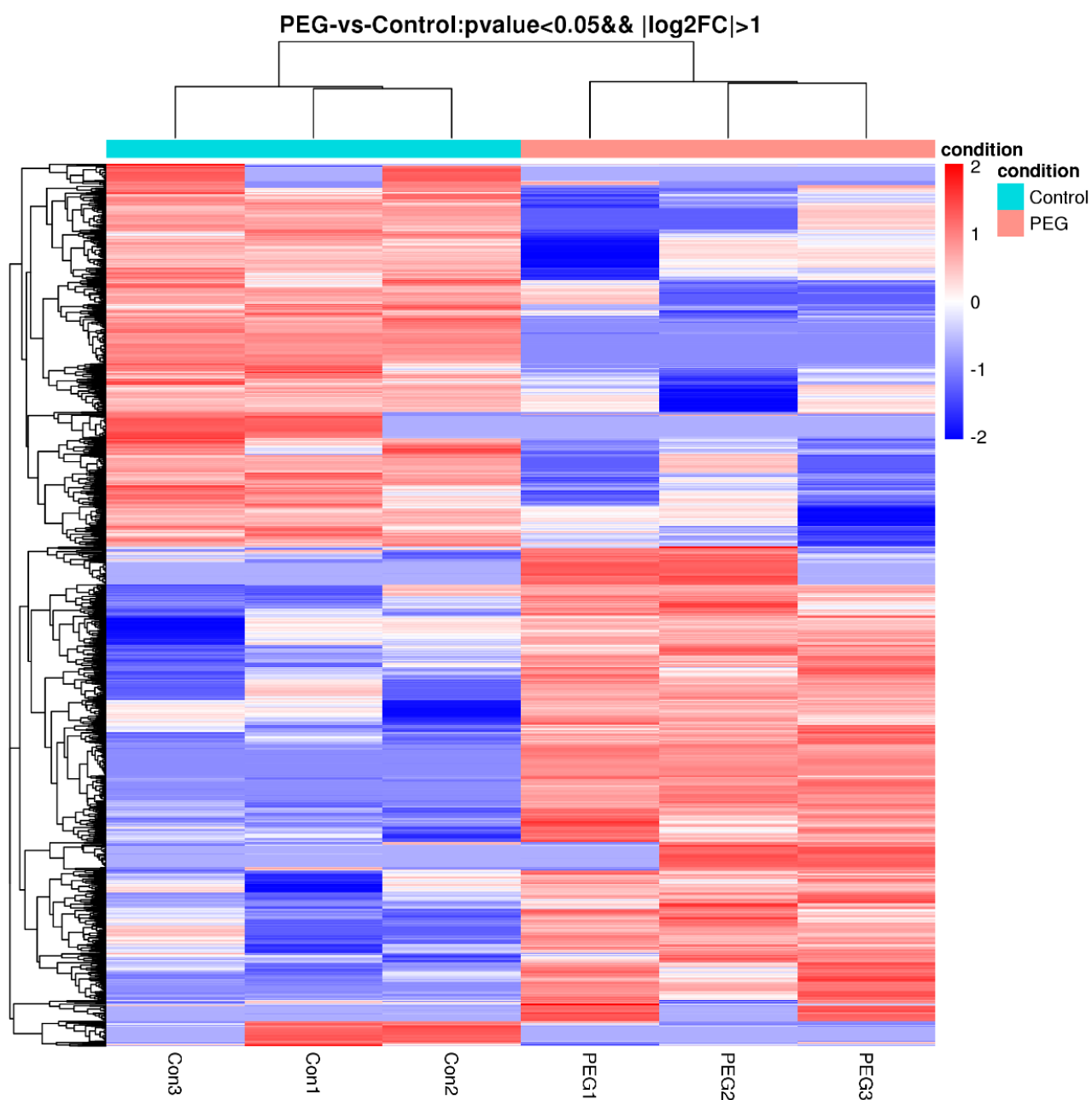


Fig. 3 Suppl. Hierarchical clustering analysis of DEGs of PEG-treated vs. control seedlings. *Red* represents high expressed unigenes, *blue* represents low expressed unigenes.

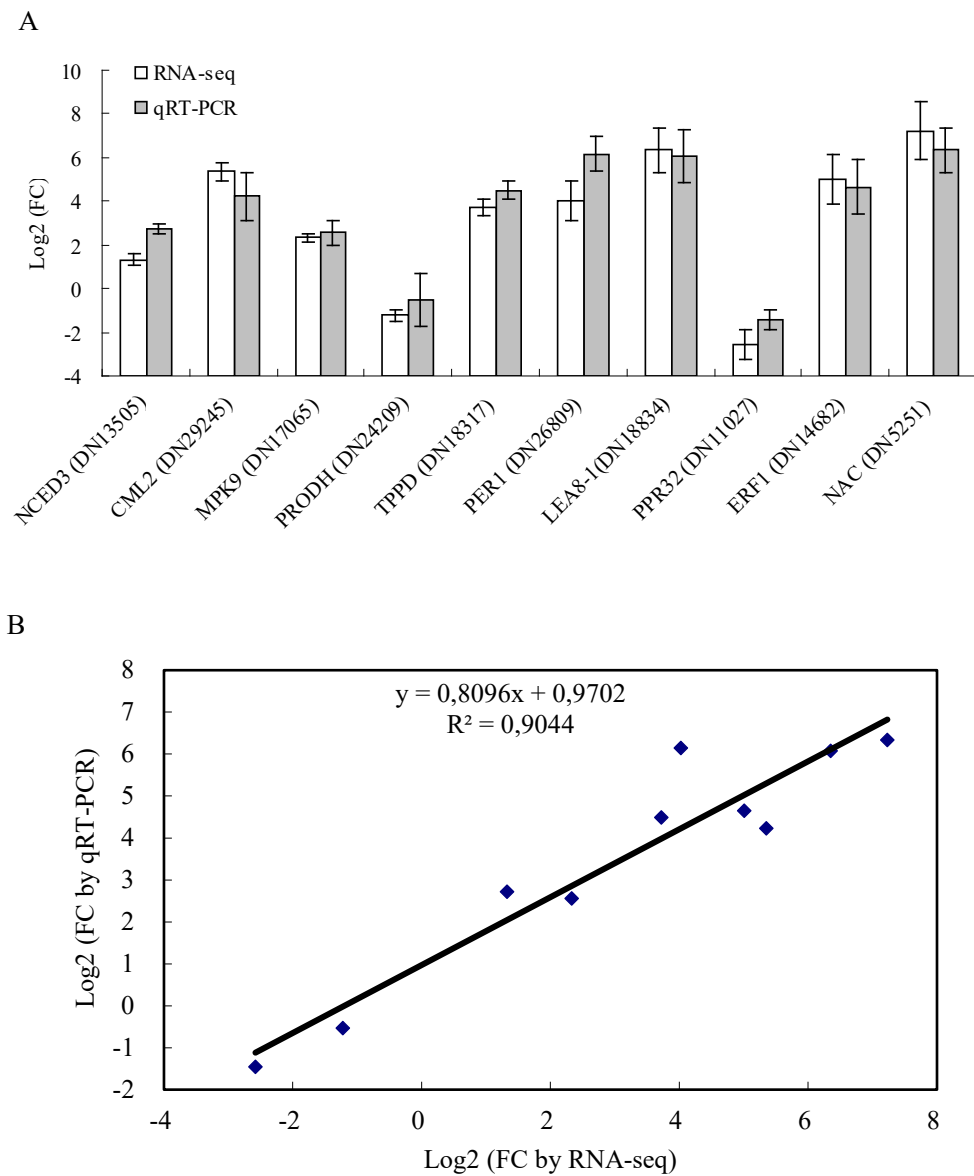


Fig. 4 Suppl. Verification of the RNA-seq results by using RT-qPCR. *A* - Comparison of the results of RNA-seq and RT-qPCR. Ten DEGs identified by RNA-seq were selected for real time RT-PCR analysis. *B* - The correlation between the results of the RT-qPCR and RNA-seq were calculated.

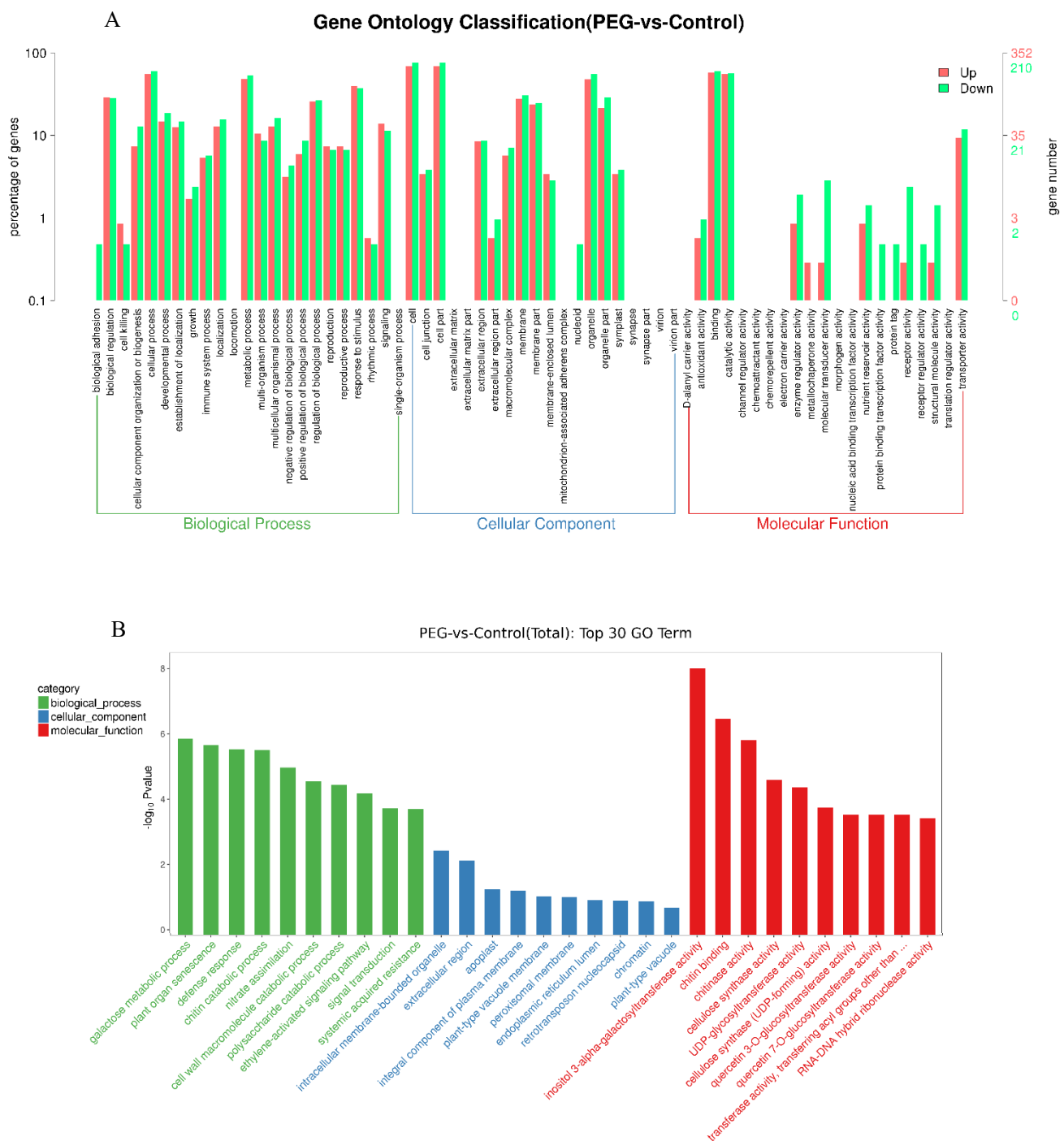
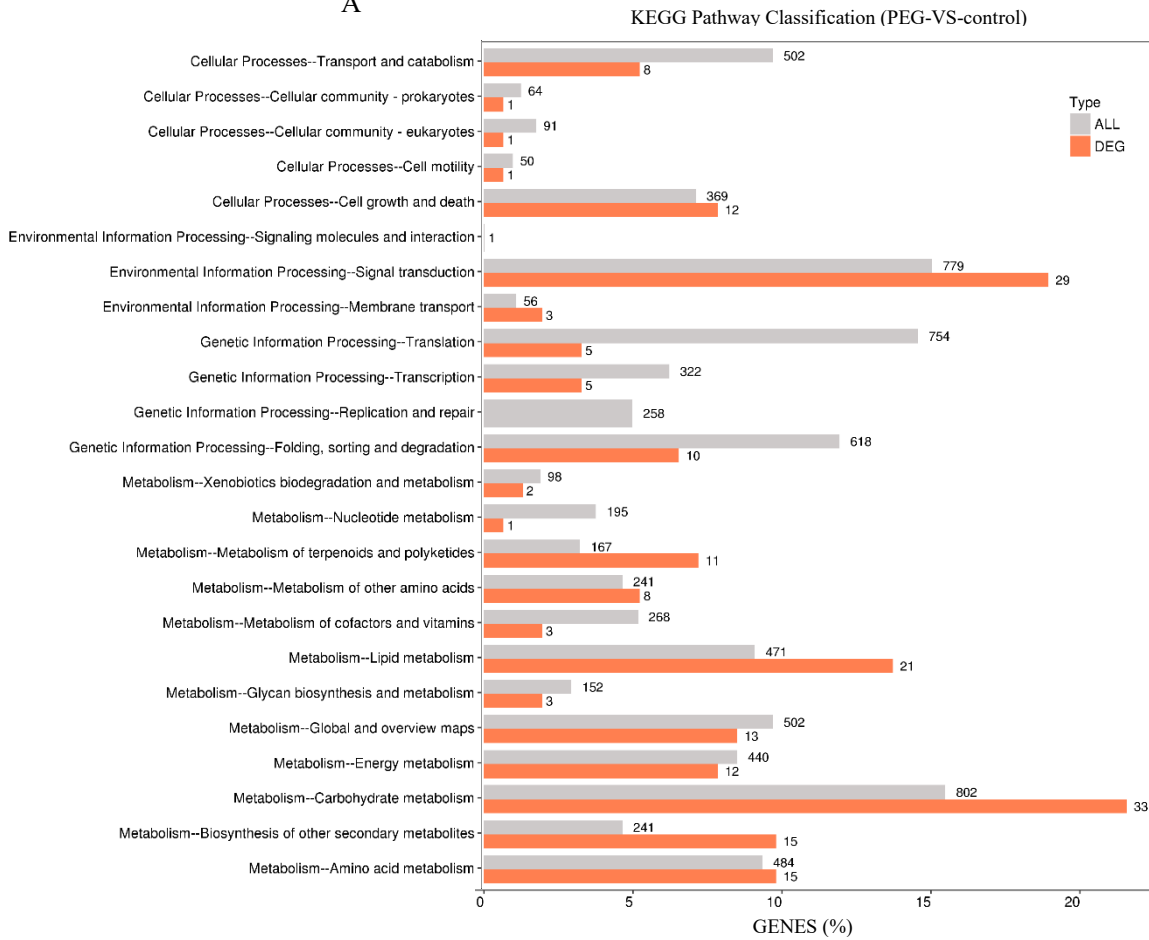
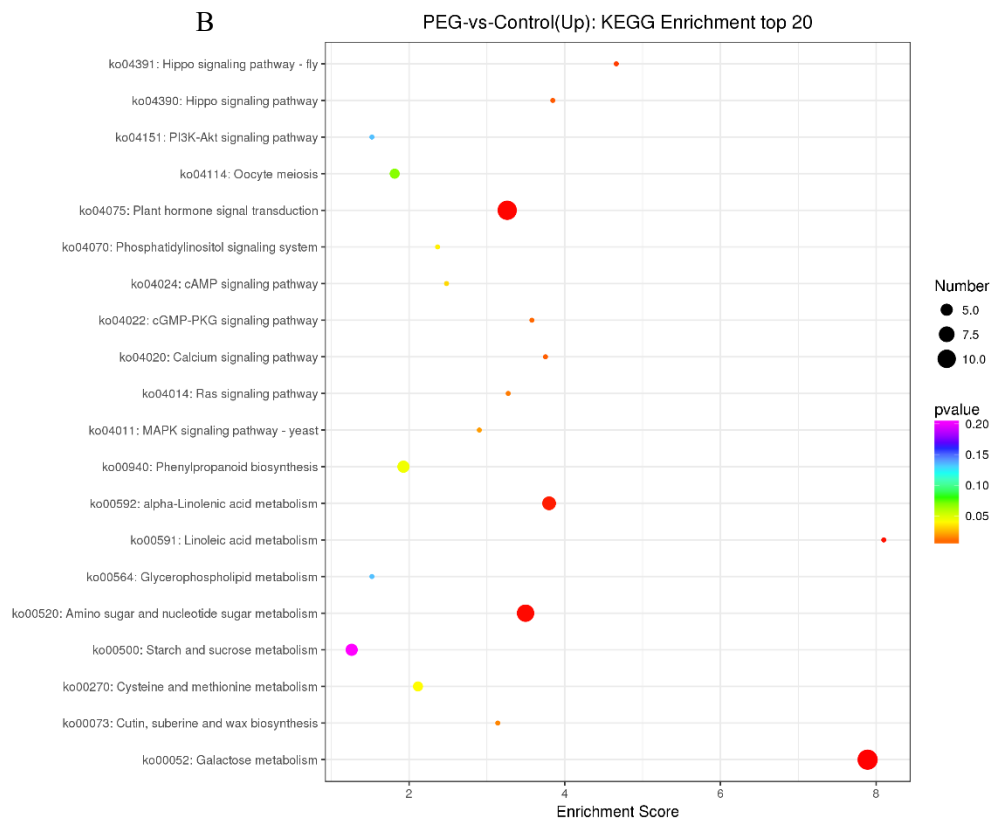


Fig. 5 Suppl. Gene Ontology (GO) classification. *A*. GO classification of upregulated and downregulated differential genes. The X-axis represents different GO terms. The left Y-axis represents the percentage of genes in different GO terms and the right Y-axis represents the number of genes in different GO terms. The upper number in the right Y-axis refers to Up-regulation, and the number below represents Down-regulation. Red columns refer to up-regulated genes, green columns refer to down-regulated genes. *B*. The top 30 GO enrichment classification of DEGs. The X-axis represents different GO terms. The Y-axis is expressed as $-\log_{10}(P \text{ value})$.

A



B



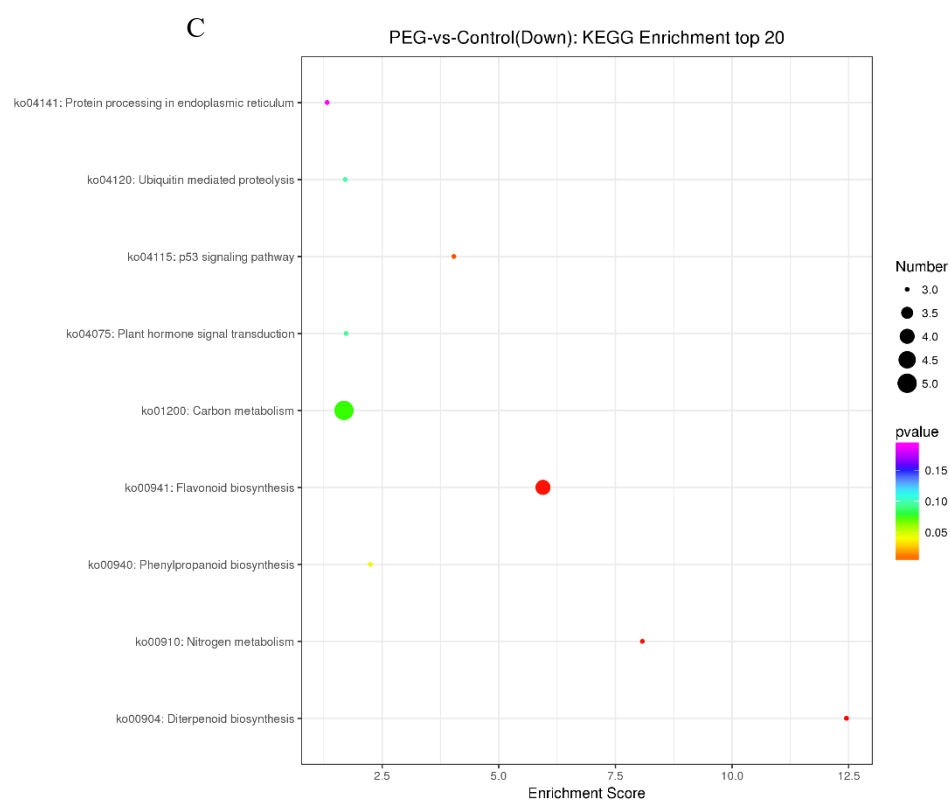


Fig. 6 Suppl. Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway classification. *A*. KEGG pathway classification of DEGs. The X-axis indicates the percentage of genes, The Y-axis indicates the KEGG pathways. “All” indicates all the unigene annotated in KEGG database, “DEG” indicates the differentially expressed unigene annotated in KEGG database. *B,C*. The top 20 upregulated (*B*) and downregulated (*C*) pathways. Different colors denote different P-values, and the size of the bubble indicates the number of DEGs.

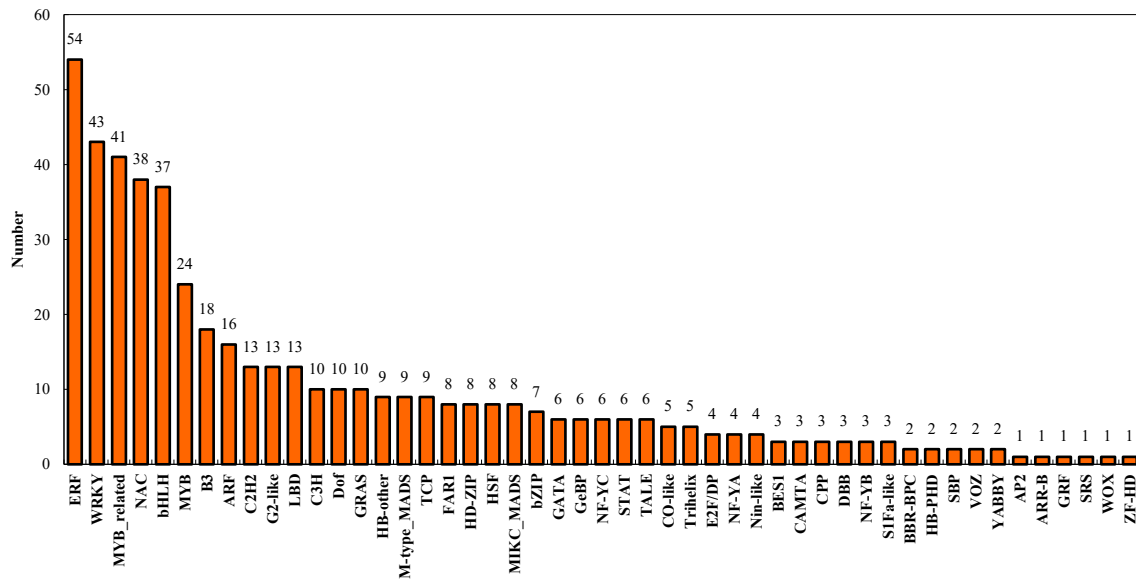


Fig. 7 Suppl. Family of transcription factors differentially expressed after osmotic treatment. The X-axis represents a different family of transcription factors, and the Y-axis indicates the number of transcription factors contained in each transcription factor family. The number above the column indicates the number of transcription factors.