# **Supplementary information**

# Transcriptional identification and characterization of differentially expressed genes associated with embryogenesis in radish (*Raphanus sativus* L.)

Lulu Zhai<sup>1,2</sup>, Liang Xu<sup>1</sup>, Yan Wang<sup>1</sup>, Xianwen Zhu<sup>3</sup>, Haiyang Feng<sup>1</sup>, Chao Li<sup>1</sup>, Xiaobo Luo<sup>1</sup>, Muleke Everlyne<sup>1</sup>, Liwang Liu<sup>1,\*</sup>

#### **1. Supplementary Figures**

**Supplementary Figure S1:** Distribution coverage of DEGs. A: Library 0\_DAP, B: Library 7\_DAP and C: Library 15\_DAP.

**Supplementary Figure S2:** Comparison of gene expression levels among the three libraries based on pairwise comparison. A, B and C showed gene expression level of  $0_DAP$  vs 7\_DAP, 0\_DAP vs 15\_DAP and 7\_DAP vs 15\_DAP, respectively. With an estimated false-discovery rate (FDR) of  $\leq 0.001$  and  $|\log_2Ratio| \geq 1$ , the red shows the proportion of up-regulated genes; the green represents down-regulated genes, and the blue shows the proportion of genes not differentially expressed.

**Supplementary Figure S3:** The related genes involved in pathway of plant hormone signal transduction. The pathway originate from Kyoto Encyclopedia of Genes and Genome data base; A: DEGs between 0\_DAP and 7\_DAP library; B: DEGs between 0\_DAP and 15\_DAP library; C: DEGs between 7\_DAP and 15\_DAP library.

**Supplementary Fig. S4:** Comparison of expression profile by RNA-Seq and RT-qPCR. Comparison of expression profiles of 17 randomly selected genes by RNA-seq and RT-qPCR showing different expression profiles in three comparative groups (0\_DAP\_vs\_7\_DAP, 0\_DAP\_vs\_15\_DAP and 7\_DAP\_vs\_15\_DAP) during embryogenesis. Each data point represent the log2 normalized expression level obtained from RNA-seq (y axis) and RT-qPCR (x axis) analyses.













Supplementary Figure S1: Distribution coverage of DEGs. A: Library 0\_DAP, B: Library 7\_DAP and C: Library 15\_DAP.



1 log10(NAU-DY13\_7DAP <RPKM>)

2

3

4

-2

-1

0

Supplementary Figure S2: Comparison of gene expression levels among the three libraries based on pairwise comparison. A, B and C showed gene expression level of 0\_DAP vs 7\_DAP, 0\_DAP vs 15\_DAP and 7\_DAP vs 15\_DAP, respectively. With an estimated false-discovery rate (FDR) of <0.001 and  $|\log_2 Ratio| \ge 1$ , the red shows the proportion of up-regulated genes; the green represents down-regulated genes, and the blue shows the proportion of genes not differentially expressed.





B



Supplementary Figure S3: The related genes involved in pathway of plant hormone signal transduction. The pathway originate from Kyoto Encyclopedia of Genes and Genome data base; A: DEGs between 0\_DAP and 7\_DAP library; B: DEGs between 0\_DAP and 15\_DAP library; C: DEGs between 7\_DAP and 15\_DAP library.

С



Supplementary Fig. S4: Comparison of expression profile by RNA-Seq and RT-qPCR. Comparison of expression profiles of 17 randomly selected genes by RNA-seq and RT-qPCR showing different expression profiles in three comparative groups (0\_DAP\_vs\_7\_DAP, 0\_DAP\_vs\_15\_DAP and 7\_DAP\_vs\_15\_DAP) during embryogenesis. Each data point represent the log<sub>2</sub> normalized expression level obtained from RNA-seq (y axis) and RT-qPCR (x axis) analyses.

#### 2. Supplementary Tables

**Supplementary Table S1:** The expressed genes with coverage more than 50% (See Excel file).

**Supplementary Table S2:** The up- and down-regulated transcripts based on pairwise comparison among DGE libraries (See Excel file).

**Supplementary Table S3:** The list of DEGs among three cDNA libraries (See Excel file).

**Supplementary Table S4:** GO functional enrichment analysis at early stage of embryogenesis (See Excel file).

**Supplementary Table S5:** KEGG pathways analysis based on pairwise comparison (See Excel file).

**Supplementary Table S6:** The DEGs involved in embryo induction, formation and maturation stages (See Excel file).

**Supplementary Table S7:** Expression profiles of certain interested genes involved during radish embryogenesis (See Excel file).

**Supplementary Table S8:** The correlation analysis between DEGs and miRNAs during radish embryogenesis (See Excel file).

**Supplementary Table S9:** The primers of differentially-regulated genes for RT-qPCR analysis.

Unigene	Gene name	Abbreviation name	Primer sequence		
			Forward sequence (5'-3')	Reverse sequence (5'-3')	(bp)
CL11244.Contig1_CKA	WRKY transcription factor 22	WRKY22	TTTCTCTCTCTGCTTCTTC	TTCTGGTGGTTACTGTTG	123
CL12457.Contig1_CKA	efflux carrier of polar auxin transport	ECPAT	AATGGAGATAGATTGCTGAA	CTGAGAATGGACTGAACA	263
CL14709.Contig1_CKA	Fus3-complementing gene 1	FC1	ATTTCCTTCCTCTCCCTAT	CTGTTGCTCGTAATGATTC	166
CL203.Contig3_CKA	auxin response factor 3-1	ARF3-1	TGGCTCATCATCATCATC	TTCATCAACACCTCTTCG	182
CL5790.Contig1_CKA	WRKY transcription factor 2	WRKY2	ACCAACCATTACCAAGAG	AAACGCTAAACCAGGAAT	174
CL6021.Contig2_CKA	late embryogenesis abundant	LEAHRG	CTTGTCGTAGTGAATGGA	CATCGCTTTAATACTCTATCTC	172
CL6154.Contig1_CKA	Argonaute/Zwille-like protein 2	AG/ZLL2	TCTTCTCCTTCTCCATCA	TTTCTCTCTTCCCGTTTC	184
CL6810.Contig1_CKA	aintegumenta	AIL	CGGAAGCCTATGATGTAG	TCGTTGTTGTTGTTGTTG	172
CL7947.Contig4_CKA	Suppressor of ABI3-5	SUA	GCTCCTCCTATTGACATC	TTATCCTCGTGAACTTCG	283
CL878.Contig7_CKA	ferritin	FERRITIN	GACATAACAGACCGAGAG	CATCATCATCAACAATCACTA	186
CL9728.Contig1_CKA	Class I glutamine amidotransferase	GAT	TGGTGGTTGCTAATGATAA	GAGAGTCCTTGTTGTTGT	292
gi 166138539 gb FD570215.1	AGL9	AGL9	TGAAGATGATAGAGAACAAGAT	GAGAAGATGATGAGAGCAA	124
gi 166144826 gb FD576744.1	F-box/LRR-repeat protein	FBXL	TCTCAACTTCTCCGATTATC	ACACCTTACCAACATCATT	200
gi 167442117 gb FD948074.1 FD948074	4 auxin efflux carrier family protein	EIR	CAGTTGGTGCGATTATTG	CGATGATTGGGAGAAGTAT	243
gi 167451596 gb FD956186.1 FD956186	5 baby boom interacting protein 2	BIP2	GTTCTTCTTCAGGCTGTA	TTCATCGTATTCCGTGTG	104
gi 332783910 gb FY434046.1	ARF8-1	ARF8-1	TGGACAAGGAGGATGATA	AGATAACACAAGGGAAACA	212
gnl UG Rsa#S42001404	Small auxin up RNA protein	SAUR	GAACGGACATCATCTCTC	AATCAGTAGCAACGACAA	270
gnl UG Rsa#S42026394	Late embryogenesis abundant	LEA	CTAACCGCTATGCCATAA	GAATCCTTACTCTCAATCCA	165
gnl UG Rsa#S42049349	Late embryogenesis abundant protein	LEA	TAAGCACAAGGATGAACA	AACCACTATGAACCAGAAA	276
gnl UG Rsa#S43005757	Auxin responsive like protein	ARL	GTGGTAAGAGAAGGTAAGG	ATCAGTTTGGGAGCATTT	170

## Supplementary Table S9: The primers of differentially-regulated genes for RT-qPCR analysis.

Unigene	Gene name	Abbreviation name	Primer sequence		Length
			Forward sequence (5'-3')	Reverse sequence (5'-3')	(bp)
gnl UG Rsa#S43013377	Glutathione S-transferase	GST	TACTTGGTGGGATGAAAC	CCTGACGAACTCTTAACA	299
Unigene13496_CKA	SAUR-like auxin-responsive protein	SAUR	GATGATGATGATGAAGAAGATT	TTCCATATAAACCTCCACAA	170
Unigene18591_CKA	Zwille-like protein 2	ZLL2	ATCCCTCAAACAACCATT	ATCATAACAGCGAACTCTT	185
Unigene1912_CKA	glutamate decarboxylase 1	GAD1	GTCGGATAAGTTGGTAGTAT	AAGTGGAGGATTCATAGC	266
Unigene21980_CKA	Probable LRR receptor-like	LRR-STK	GATACTGTAGGCTCTGAAG	GTTCCTTGACCTTTCCTC	149
Unigene23299_CKA	Argonaute-like protein	AGL	CGAGACACAGTTCAAGAA	ATGCGTTATCACCGTATC	196
Unigene25317_CKA	Leucine-rich repeat-containing	LRRC	ATCTTCATCCACTACCTCT	CCAGCGTTCTTCTTCTTA	164
Unigene25469_CKA	Glutamine synthetase cytosolic isozyme 1-4	GS1-4	CAGGCTTGGATATGAGAA	GCTTGAGGGTATATGATGA	142
Unigene27378_CKA	Nuclear factor Y	NF-Y	AACGGAAGAAGAAGAAGAAGAA	TGGTGAGGTGTAGAAGAT	167
Unigene28056_CKA	CHD3-type chromatin-remodeling	PKL	AGCACAGAATAACCAGAAT	CCATAATAATCCTCCGCATA	192
Unigene31774_CKA	embryonic flower 2_2	EMF2_2	GCATCTCTTCCTCTTCAC	ATCTTCAGTTACTTCAGTCTT	110
Unigene4179_CKA	Leafy cotyledon 1	LEC1-2	CAGGTAGTGGTCTTGTTC	TCTTGATACGCTTTCTTAATAC	195
Unigene446_CKA	Embryo sac development arrest 7	EDA7	CTTCCTCTGACAACATCC	CTTTCCACAACGGTAGAT	262

## Supplementary Table S9: The primers of differentially-regulated genes for RT-qPCR analysis. (Continue)