

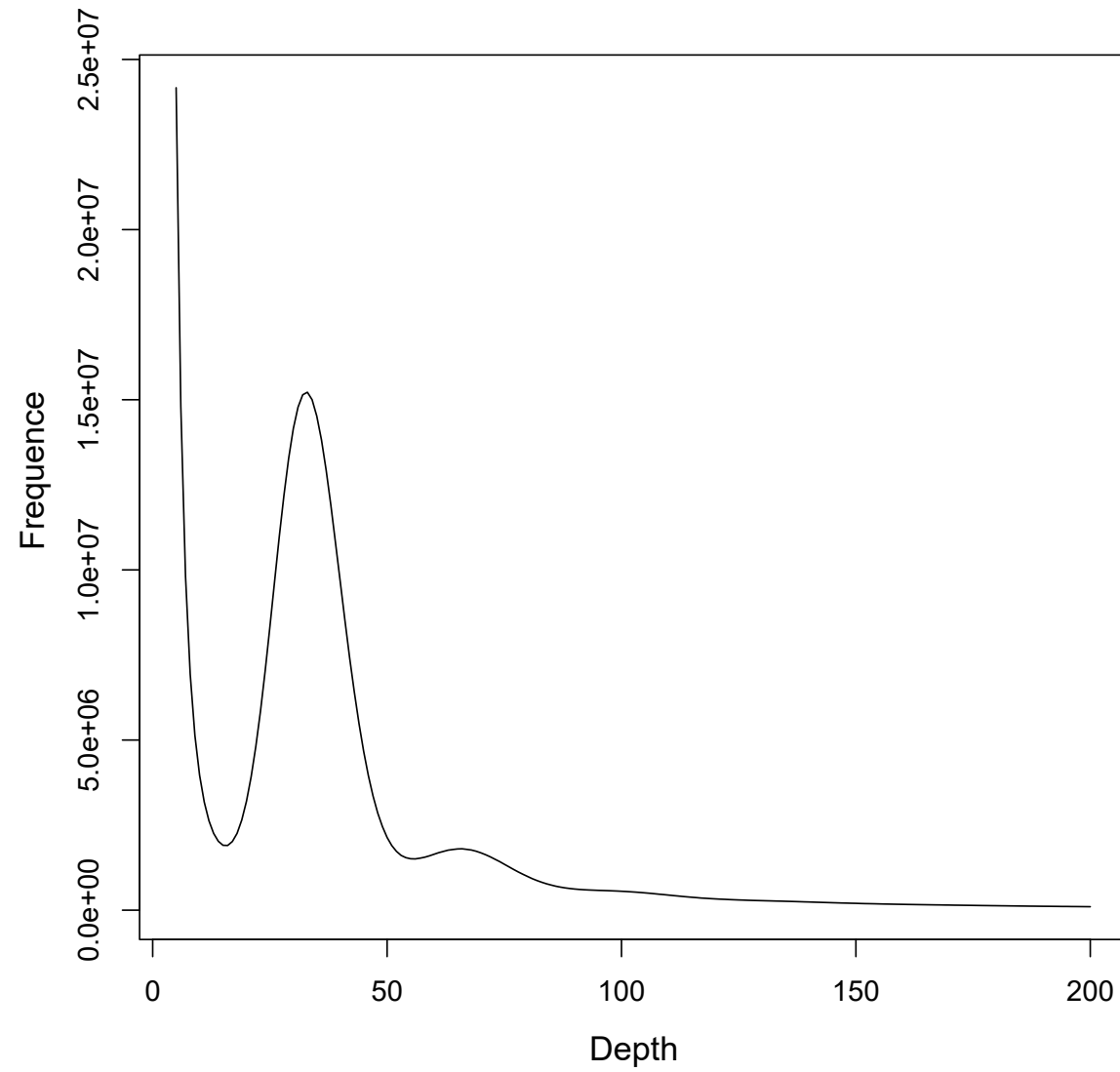
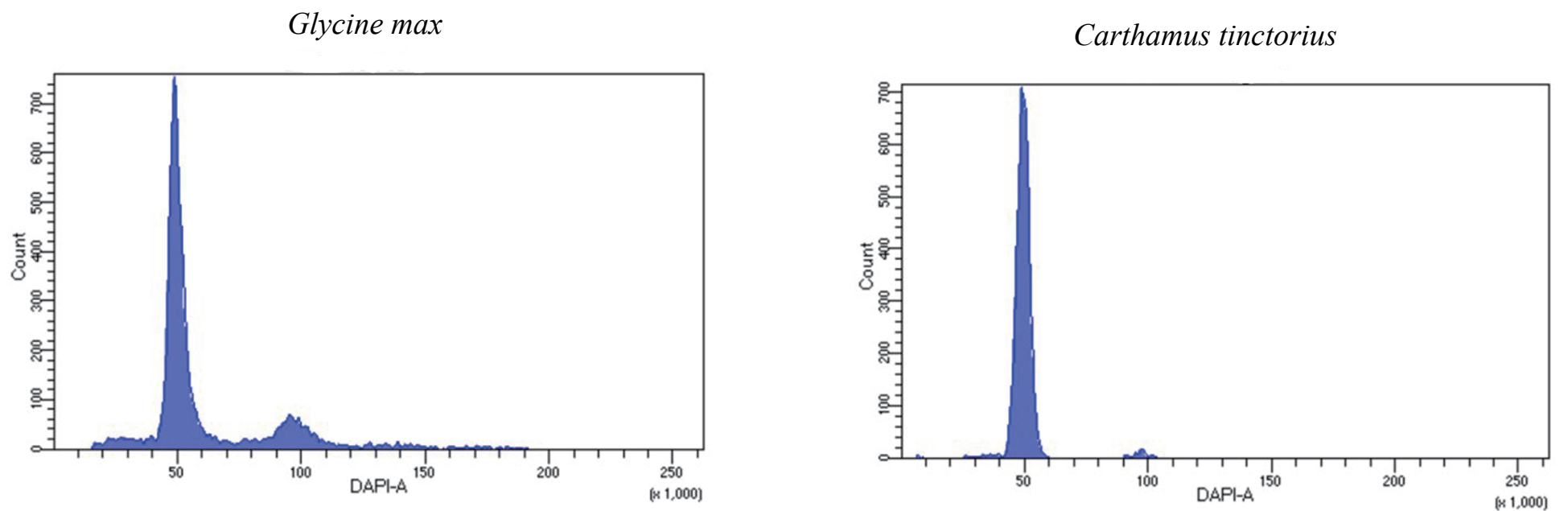
a**b**

Fig. S1 Evaluation of safflower (*Carthamus tinctorius*) genome size estimated using a k-mer frequency analysis **(a)** and flow cytometry using soybean (*Glycine max*) as a control **(b)**. In **(a)**, one main peak was present at a depth of 33, with a minor peak at a depth of 66 where $k = 17$.

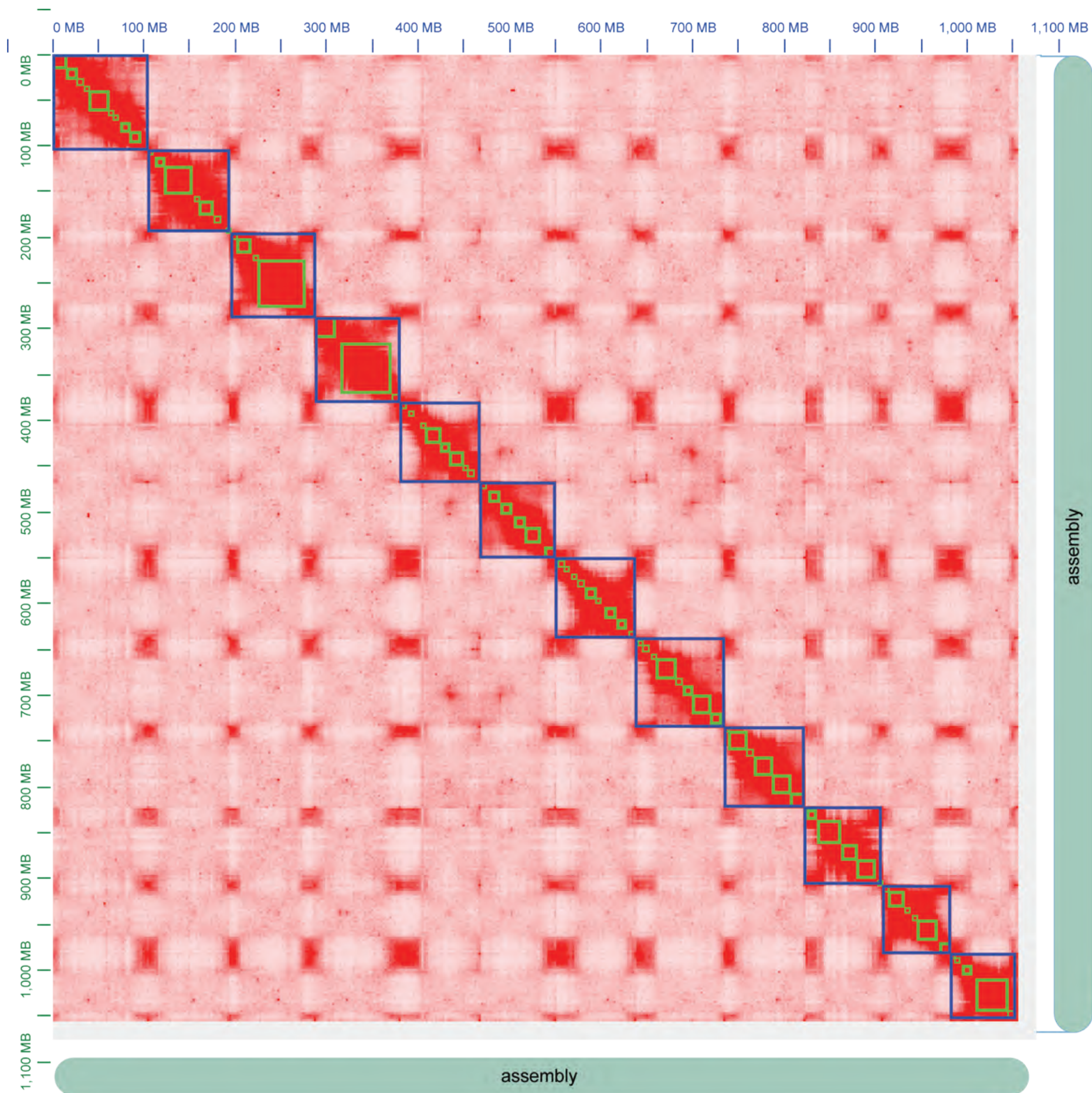


Fig. S2 The Hi-C interacted heatmap for chromosome-scale genome assembly.

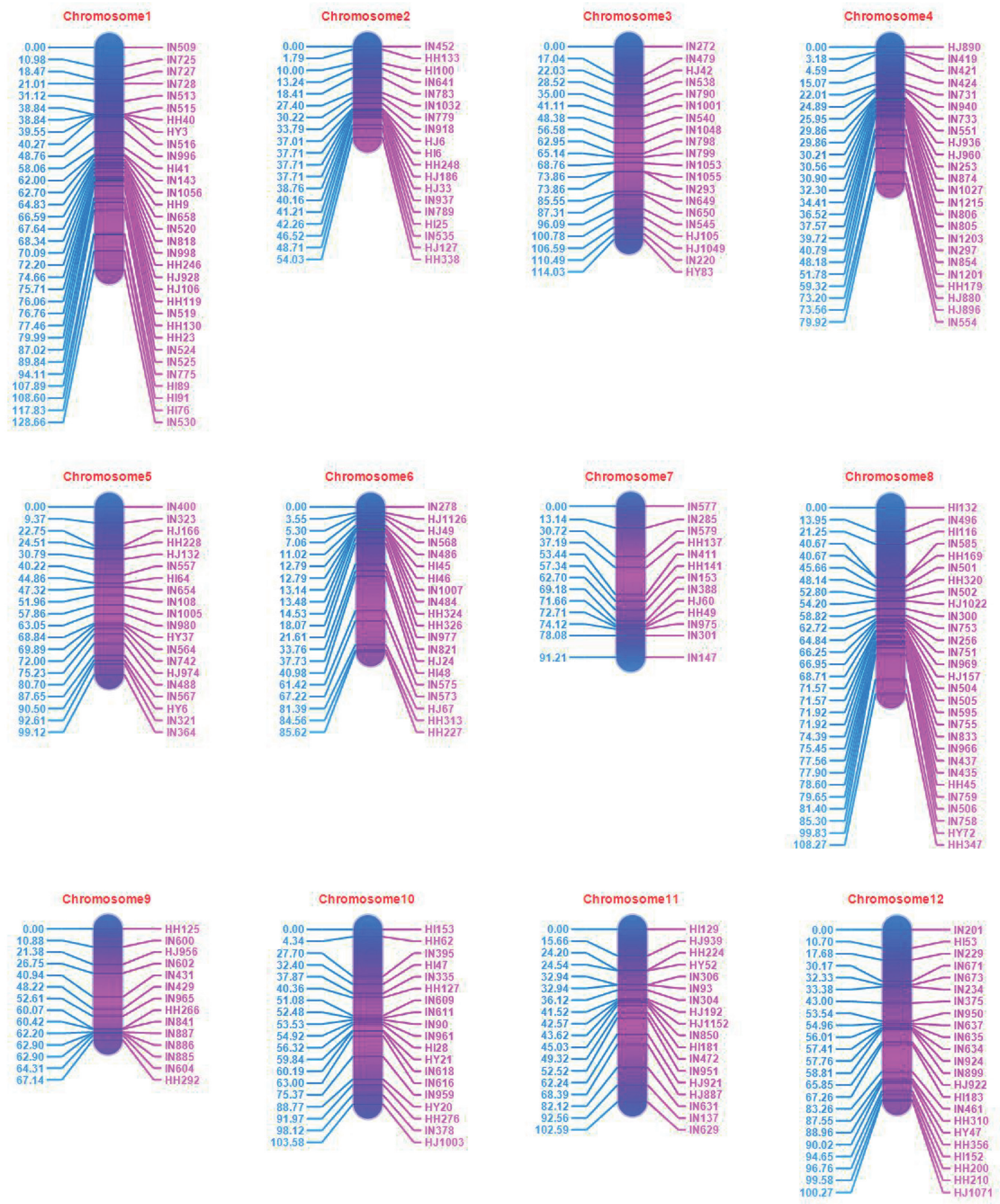


Fig. S3 The high-density genetic linkage map of safflower constructed from the F₂ population of a cross between the parents AH04 and YH04.

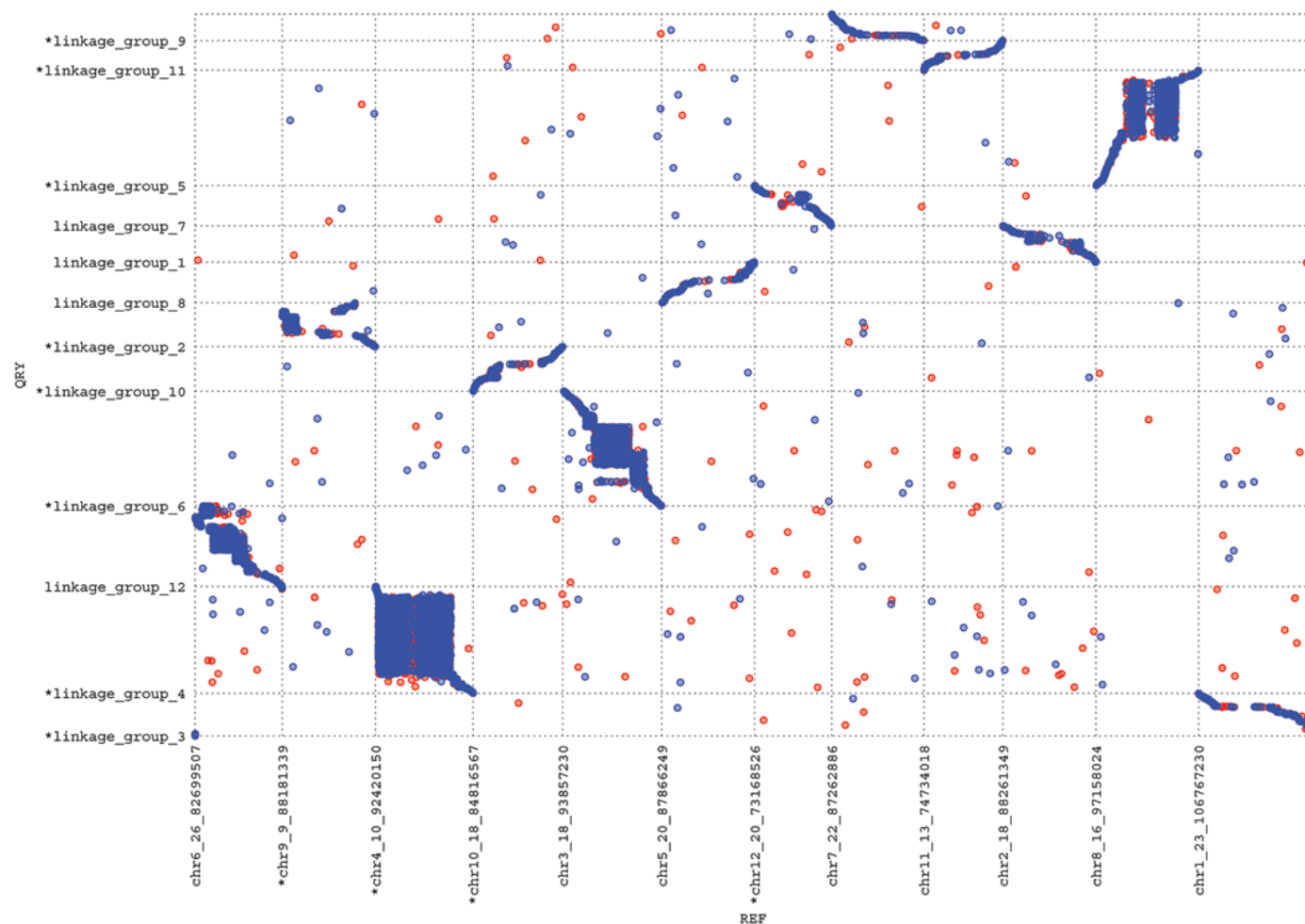


Fig. S4 Synteny plot between our assembled safflower genome using Pacbio and Hi-C, and the published draft genome of safflower generated using Illumina Hi-Seq. The x-axis and y-axis represent the 12 published linkage groups and 12 assembled chromosomes in the study, respectively. Each square represents the region for pairwise syntenic regions among chromosomes and linkage groups. Red dots and blue dots represent reverse mapping sequences and forward mapping sequences between published and our genomes, respectively.

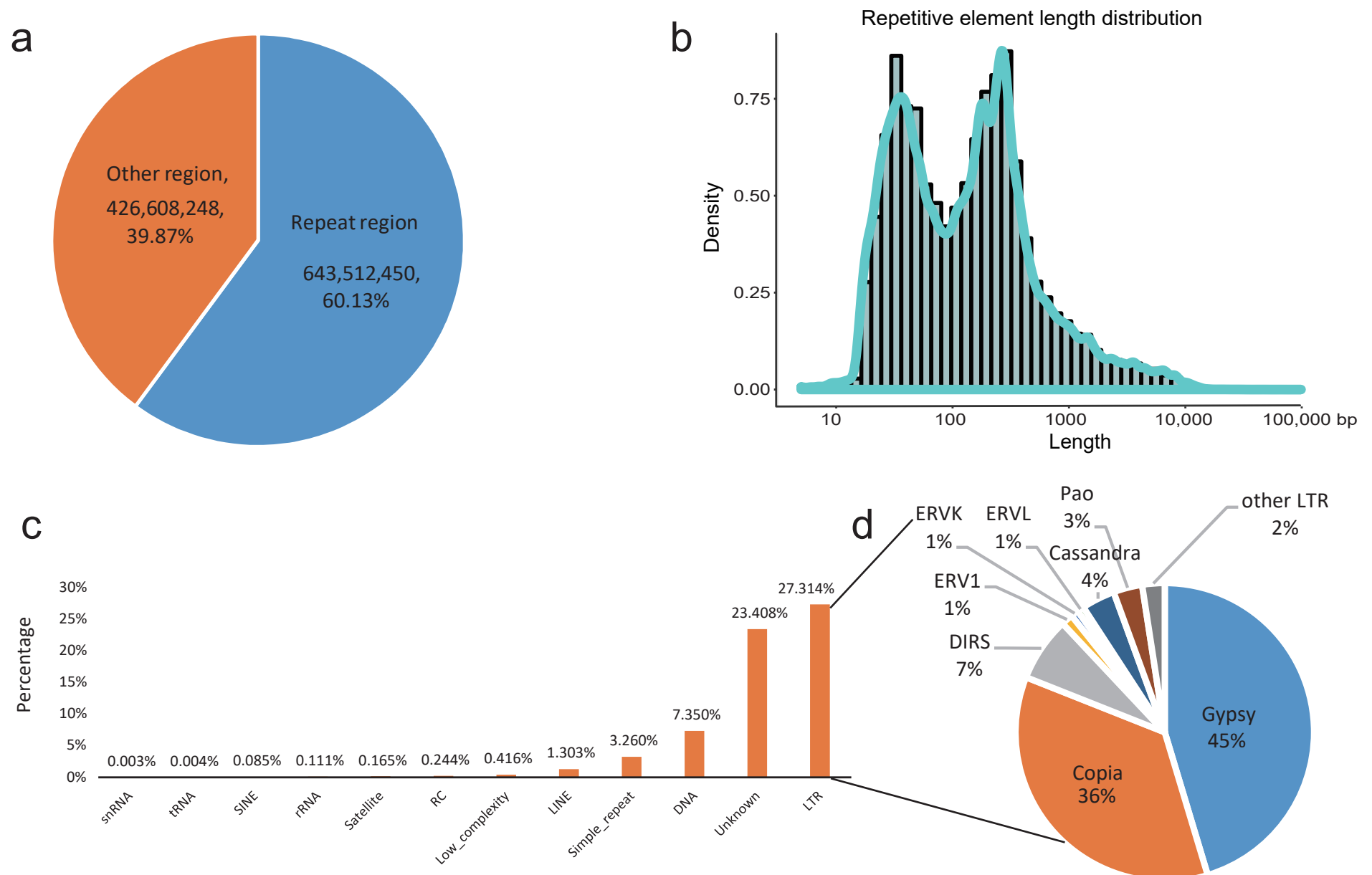


Fig. S5 Characteristics of the repetitive elements in the safflower genome. **(a)** The proportions of repetitive elements in the safflower genome. **(b)** The length distribution of repetitive elements. **(c)** The proportions of different classes of repetitive elements in the safflower genome. **(d)** The proportions of different LTR species.

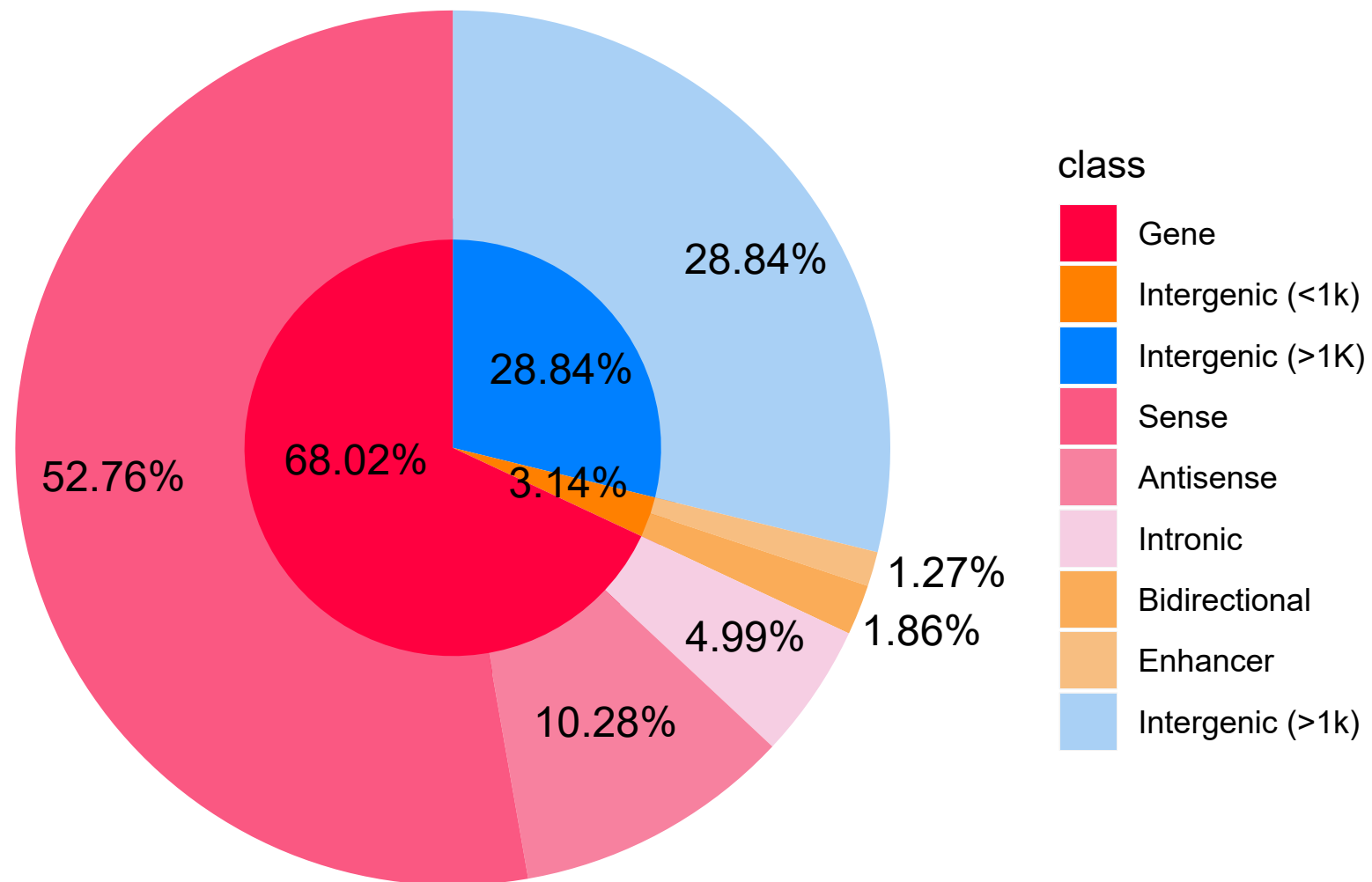


Fig. S6 Identification and classification of long noncoding RNAs according to their position in the safflower genome.

Gene Ontology (GO) component of safflower genome

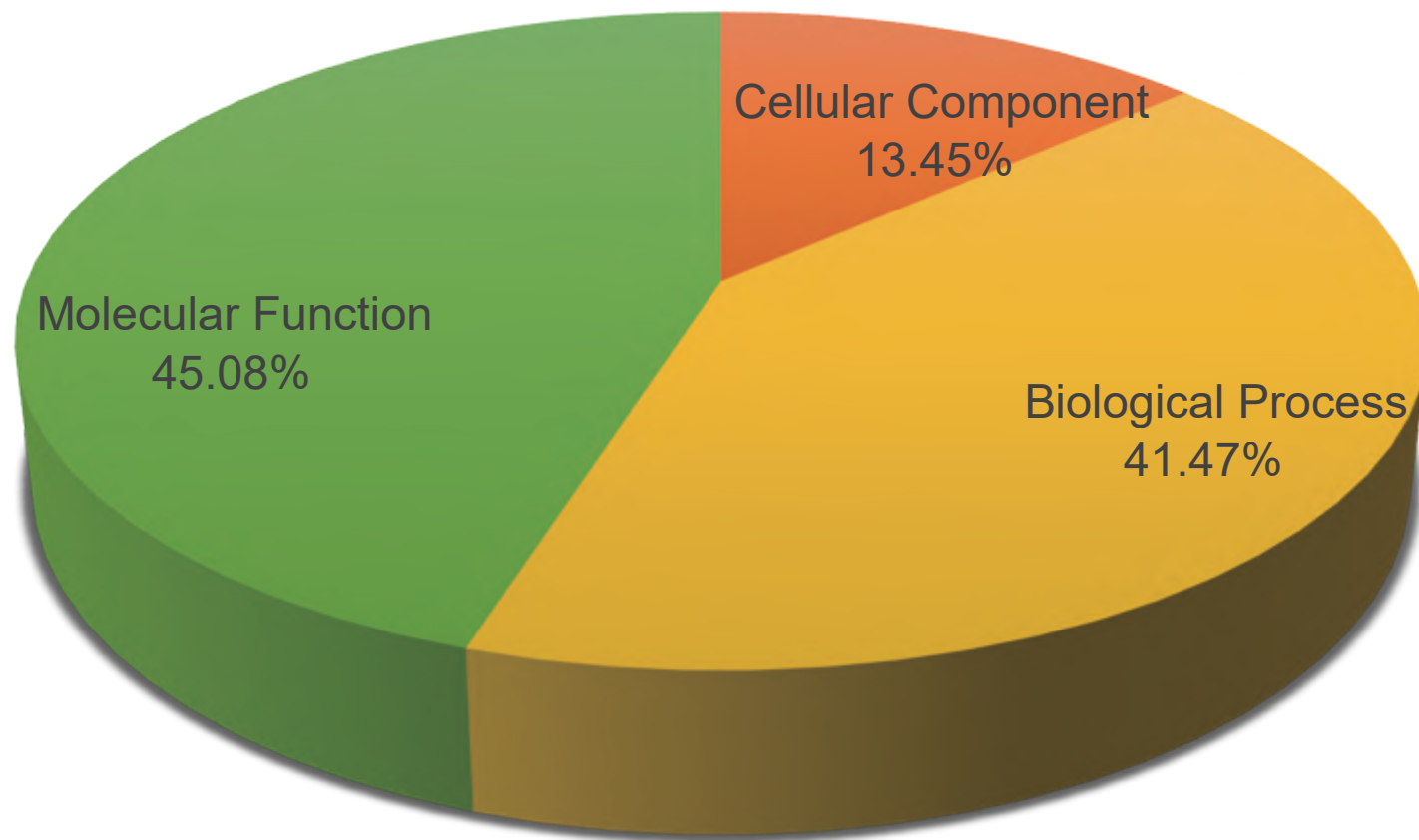


Fig. S7 Gene Ontology categories associated with the annotated genes in the safflower genome.

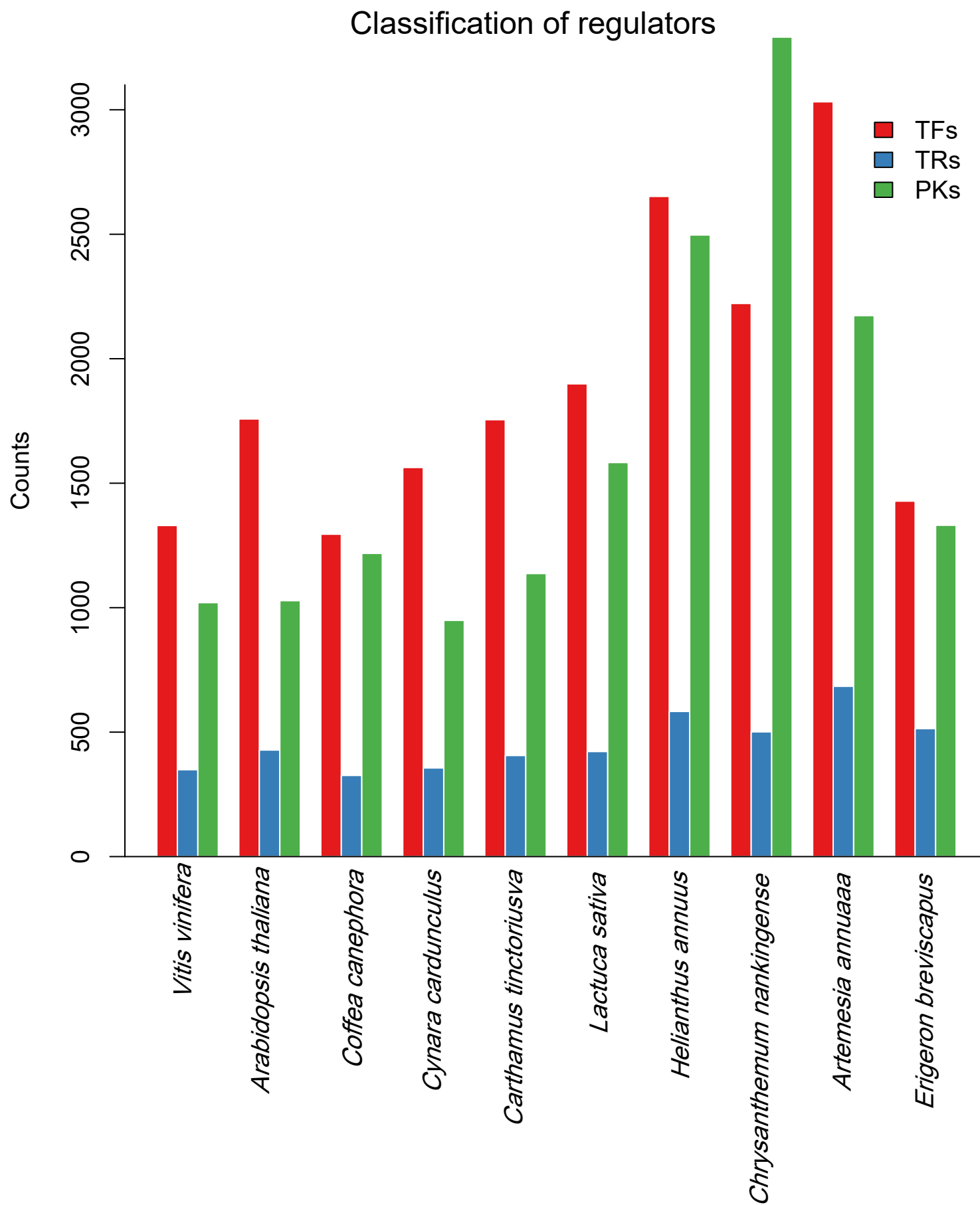


Fig. S8 The number of transcriptional factors (TFs), transcriptional regulators (TRs), and protein kinases (PKs) in the safflower (*Carthamus tinctorius*) and nine other plant genomes.

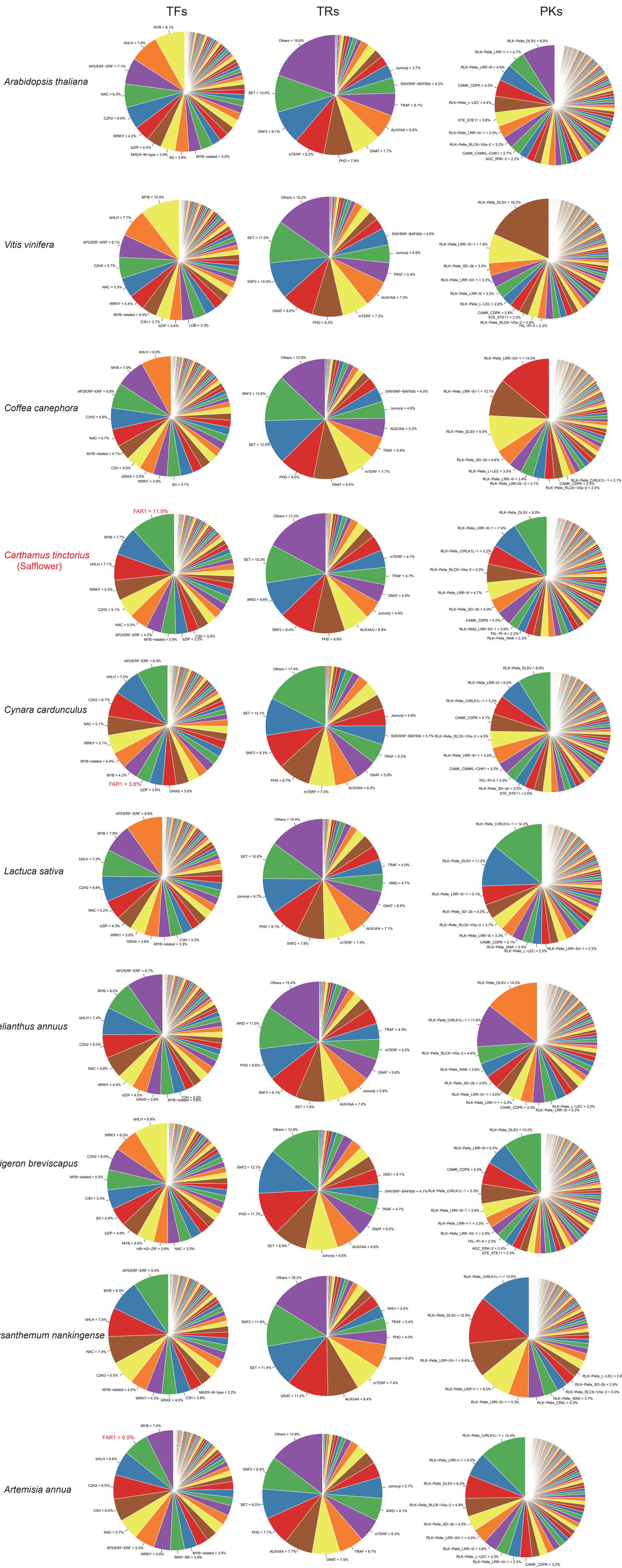


Fig. S9 Proportions of transcriptional factors (TFs), transcriptional regulators (TRs), and protein kinases (PKs) in the safflower and nine other plant genomes.

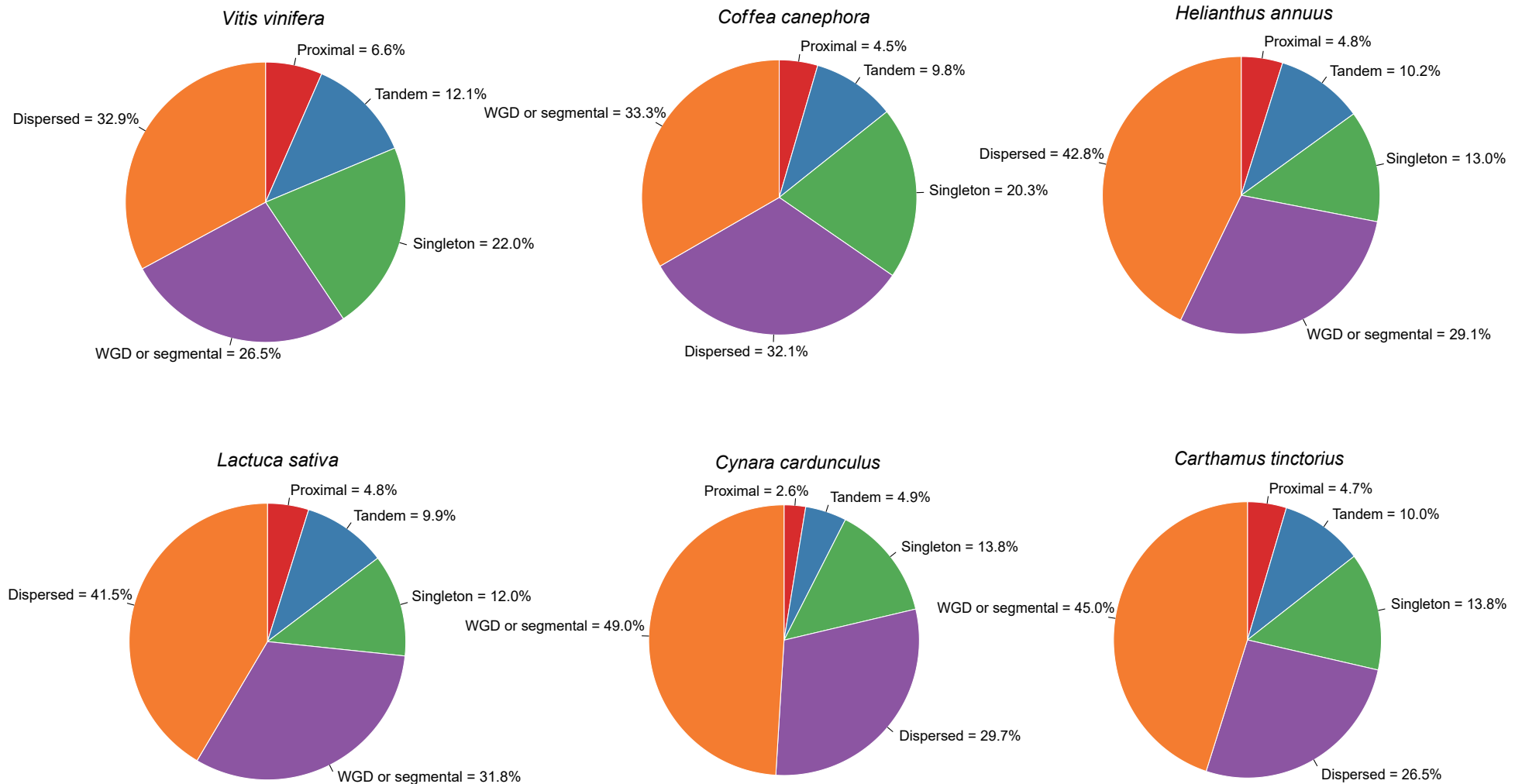
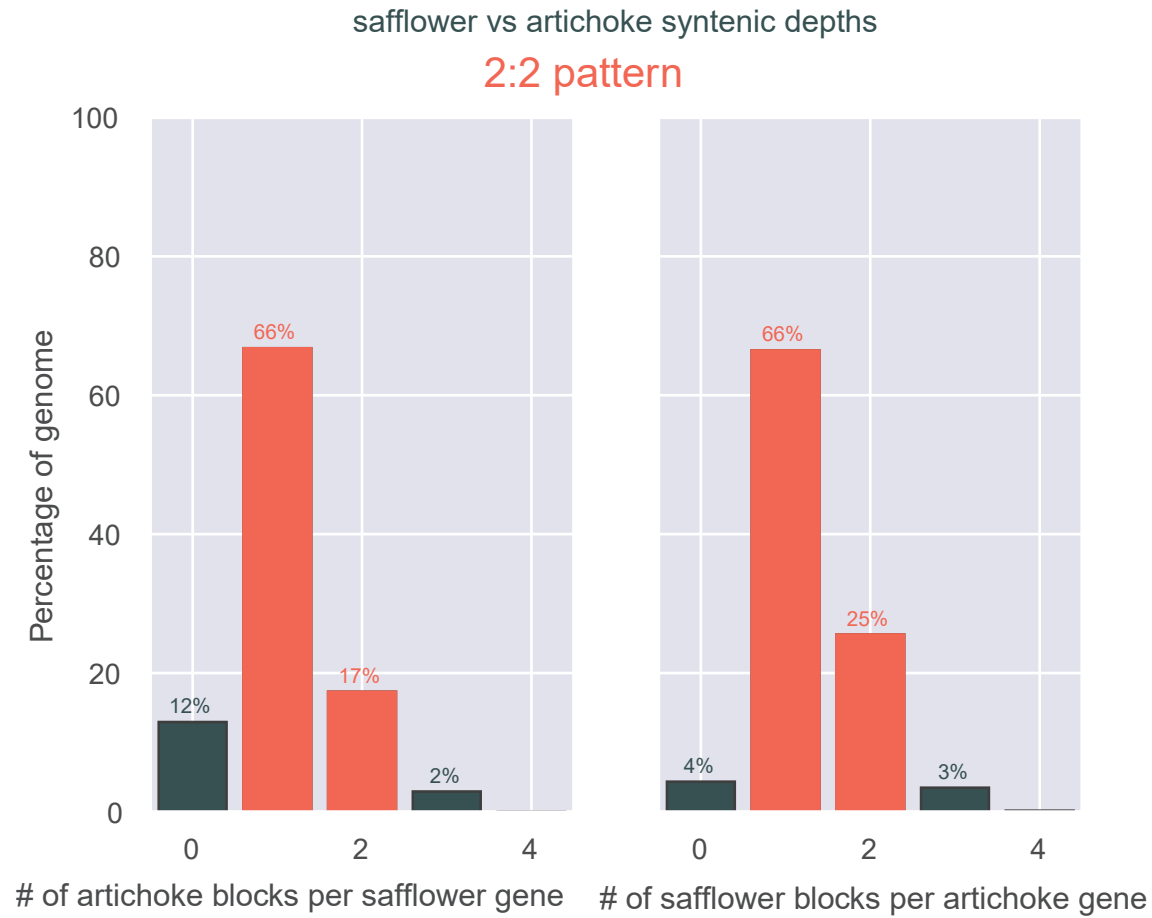


Fig. S10 Types of gene duplication in the safflower (*Carthamus tinctorius*) genome and five other plant species. The distribution of different duplication types was classified using MCScanX as follows: Singleton: no duplication; WGD/segmental: whole-genome or segmental duplications (collinear genes in collinear blocks); Tandem: consecutive duplication; Proximal: duplications in a nearby but not adjacent chromosomal region; Dispersed: duplications using modes other than tandem, proximal, or WGD/segmental. *Vitis vinifera*: grape; *Coffea canephora*: robusta coffee; *Helianthus annuus*: sunflower; *Lactuca sativa*: lettuce; *Cynara cardunculus*: artichoke.

a



b

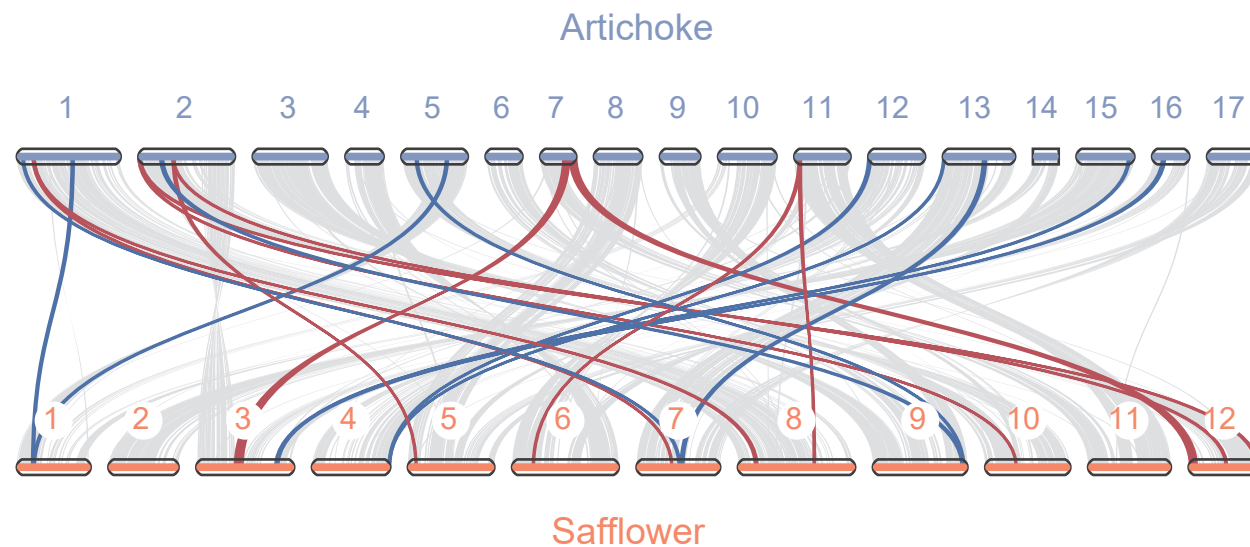
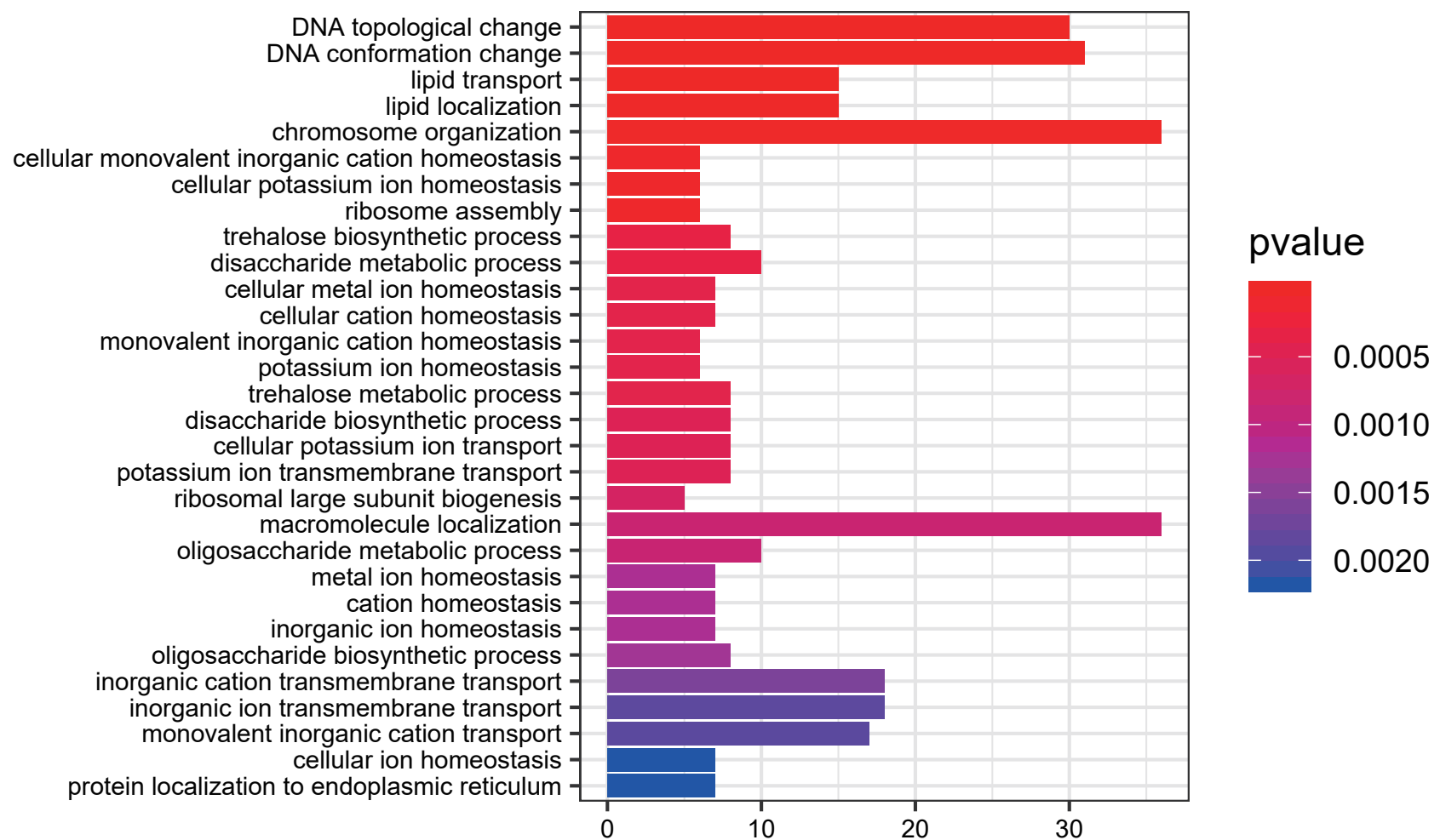


Fig. S11 Syntenic depths in the artichoke versus safflower genome comparison. **(a)** There are two syntenic blocks for both artichoke and safflower, indicating they underwent two common WGD events. **(b)** Macro-synteny between the artichoke and safflower karyotypes. Red lines highlight the two copies of artichoke syntenic blocks per corresponding safflower block. Blue lines highlight the two copies of safflower syntenic block per corresponding artichoke block.

a



b

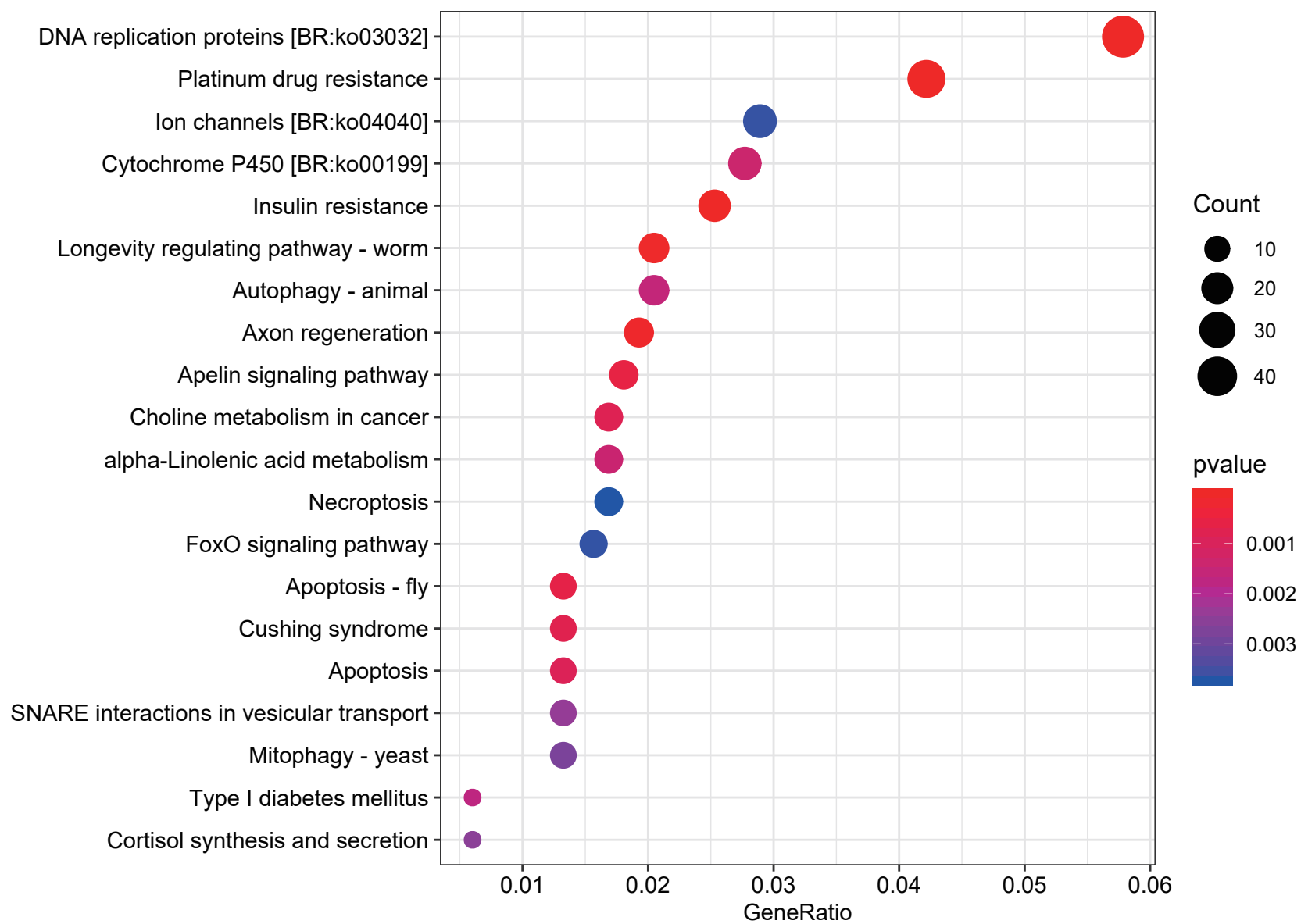
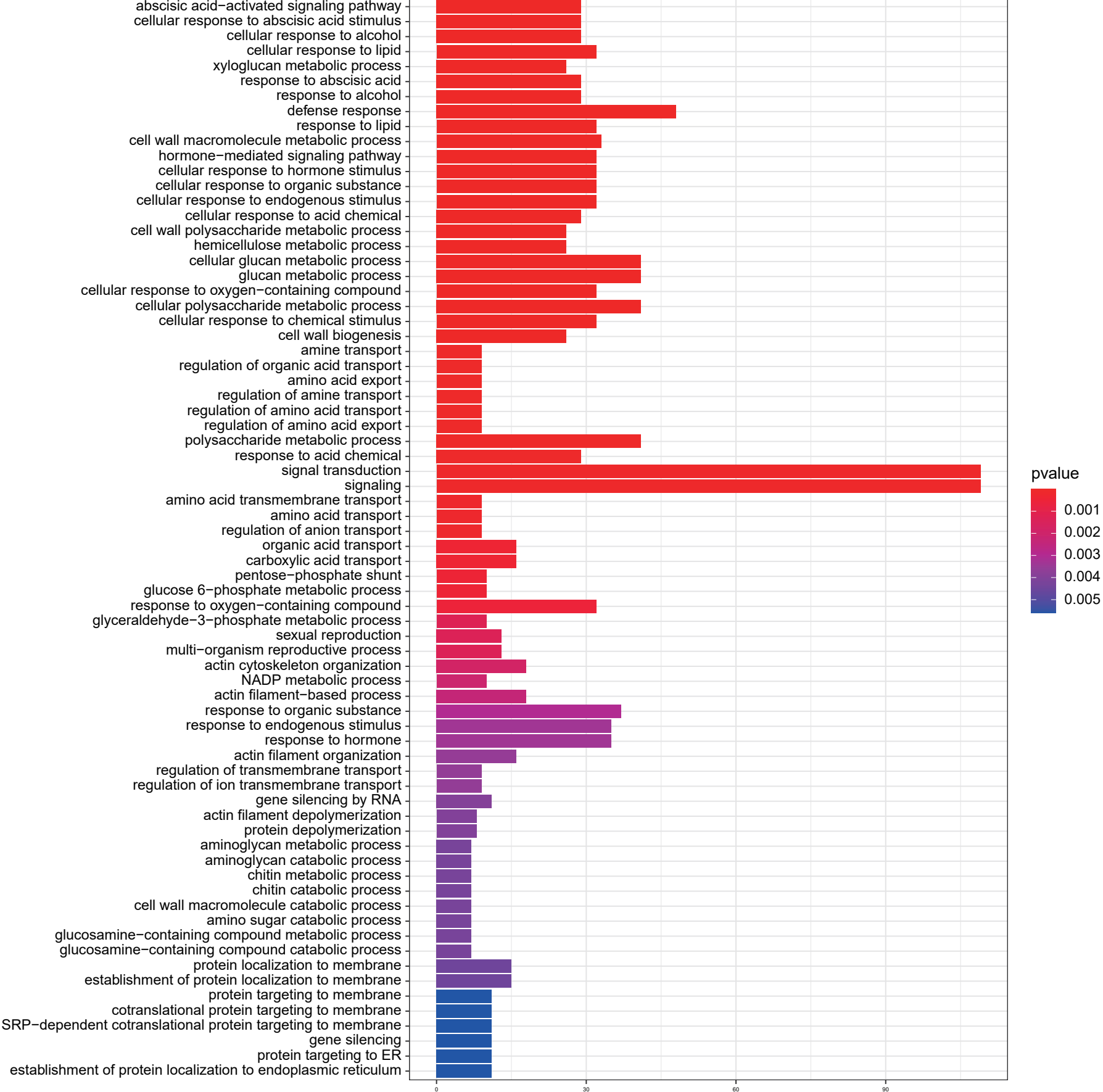


Fig. S12 Enrichment of biological process GO terms **(a)** and KEGG pathways **(b)** associated with the gene families specific to safflower with a q-value <0.05.

a



b

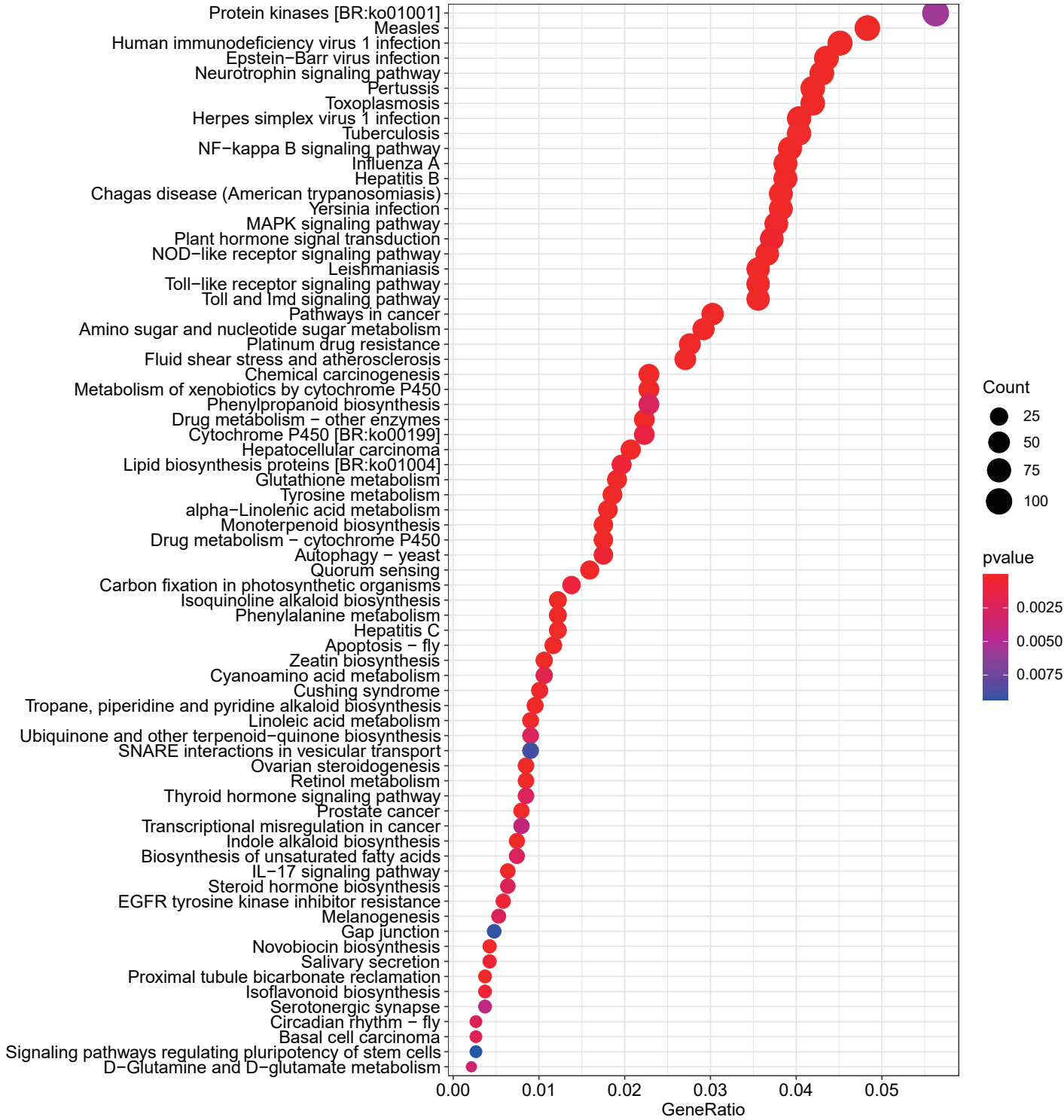
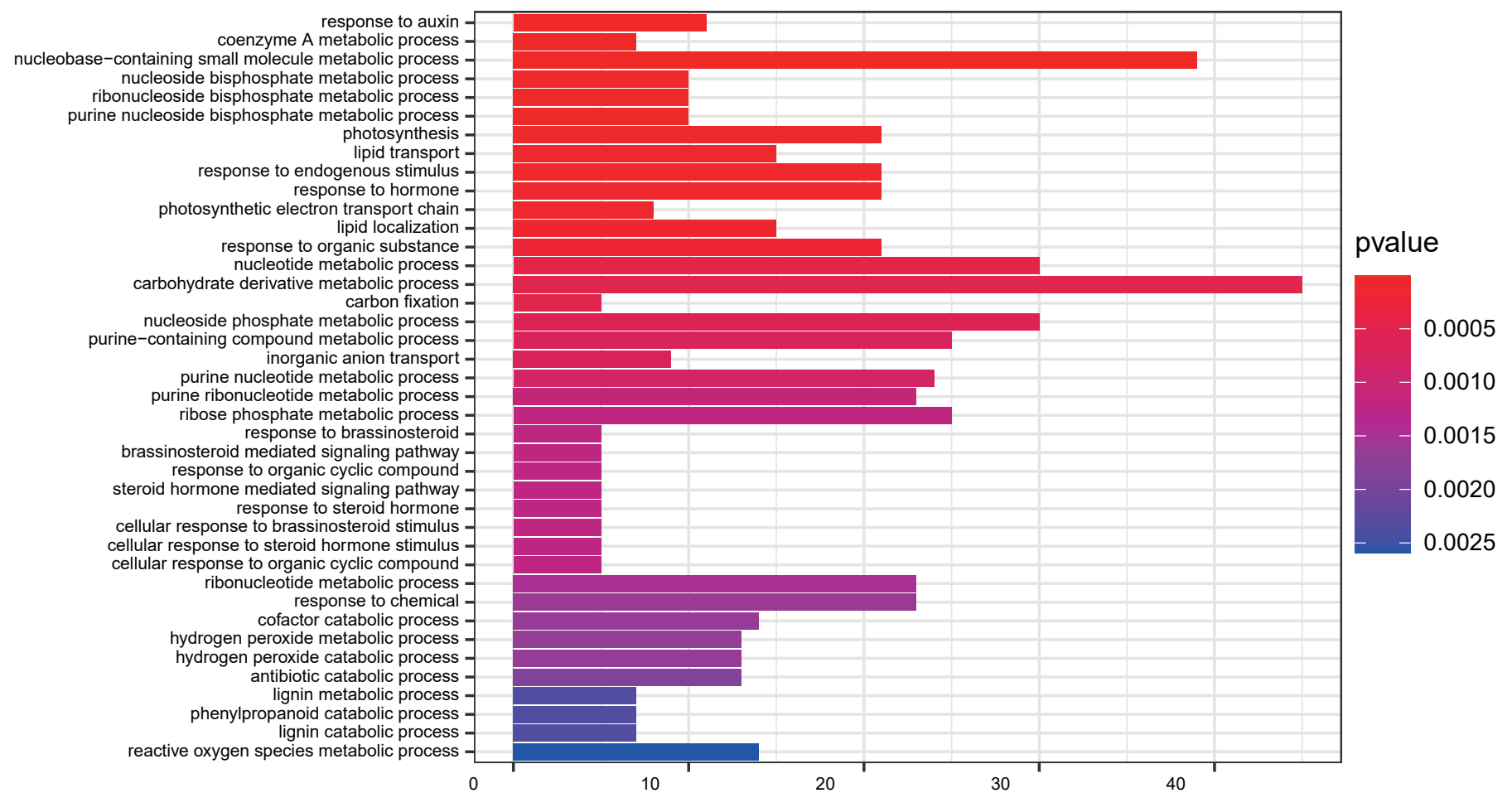


Fig. S13 Enrichment of biological process GO terms **(a)** and KEGG pathways **(b)** associated with the expanded gene families in safflower with a q-value <0.05.

a



b

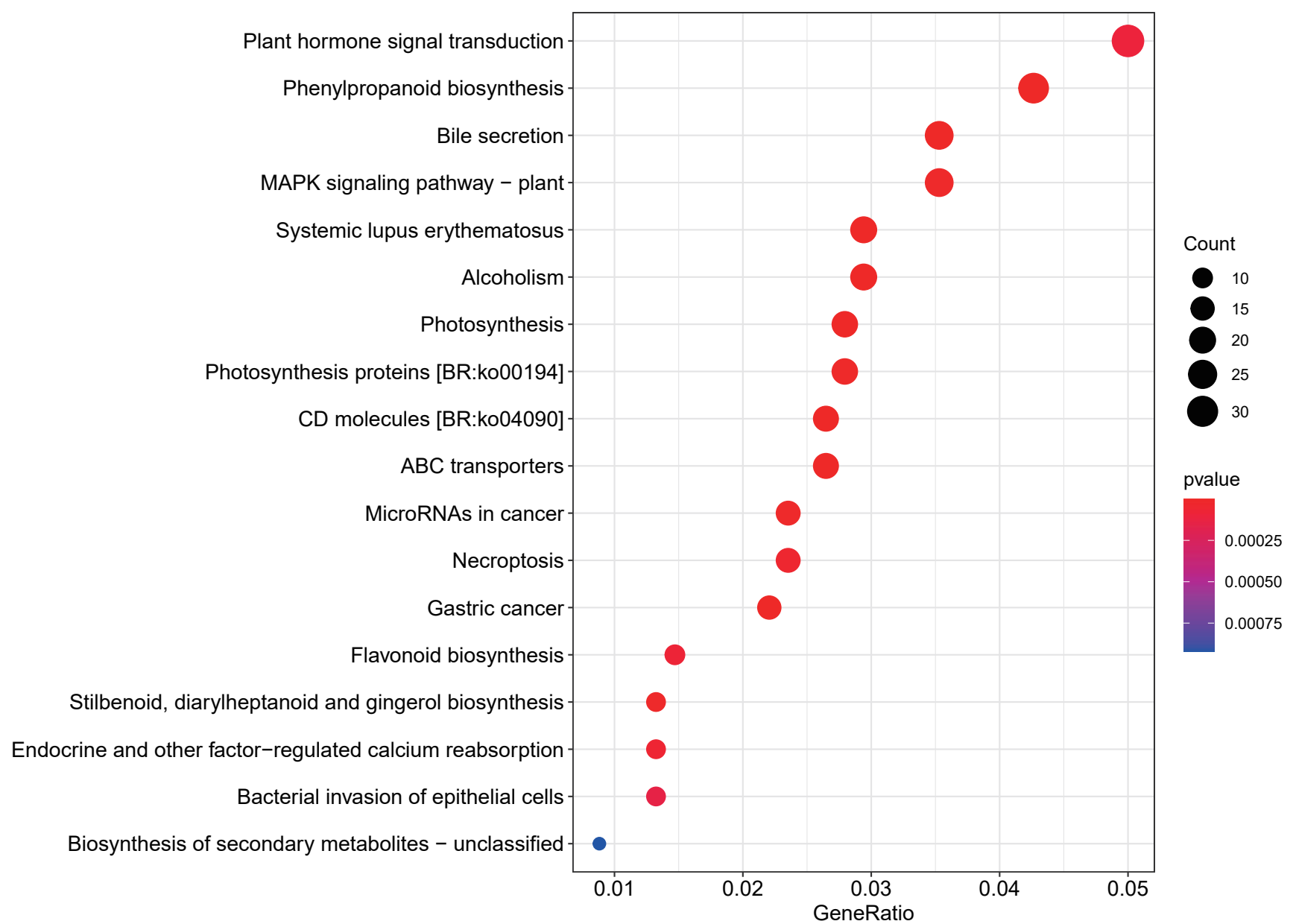


Fig. S14 Enrichment of biological process GO terms (a) and KEGG pathways (b) associated with the contracted gene families in safflower with q-value <0.05.

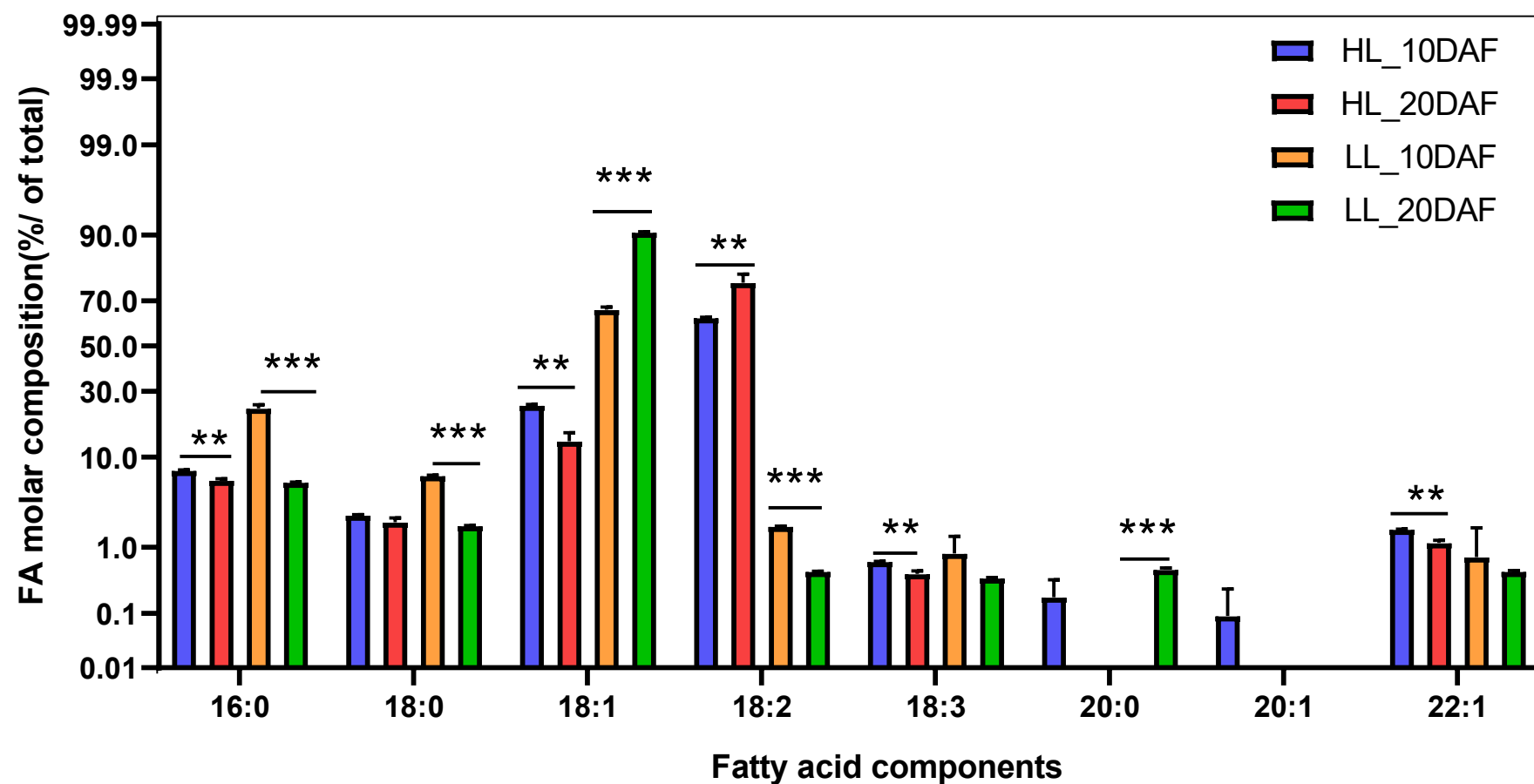


Fig. S15 Safflower seed oil content and fatty acid composition of ‘HL’ (high linoleic acid) and ‘LL’ (low linoleic acid) cultivar plants. DAF: day after flowering. Total oil is analyzed using gas chromatography. Values are means $\pm$ SD from three independent experiments. Student’s t-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

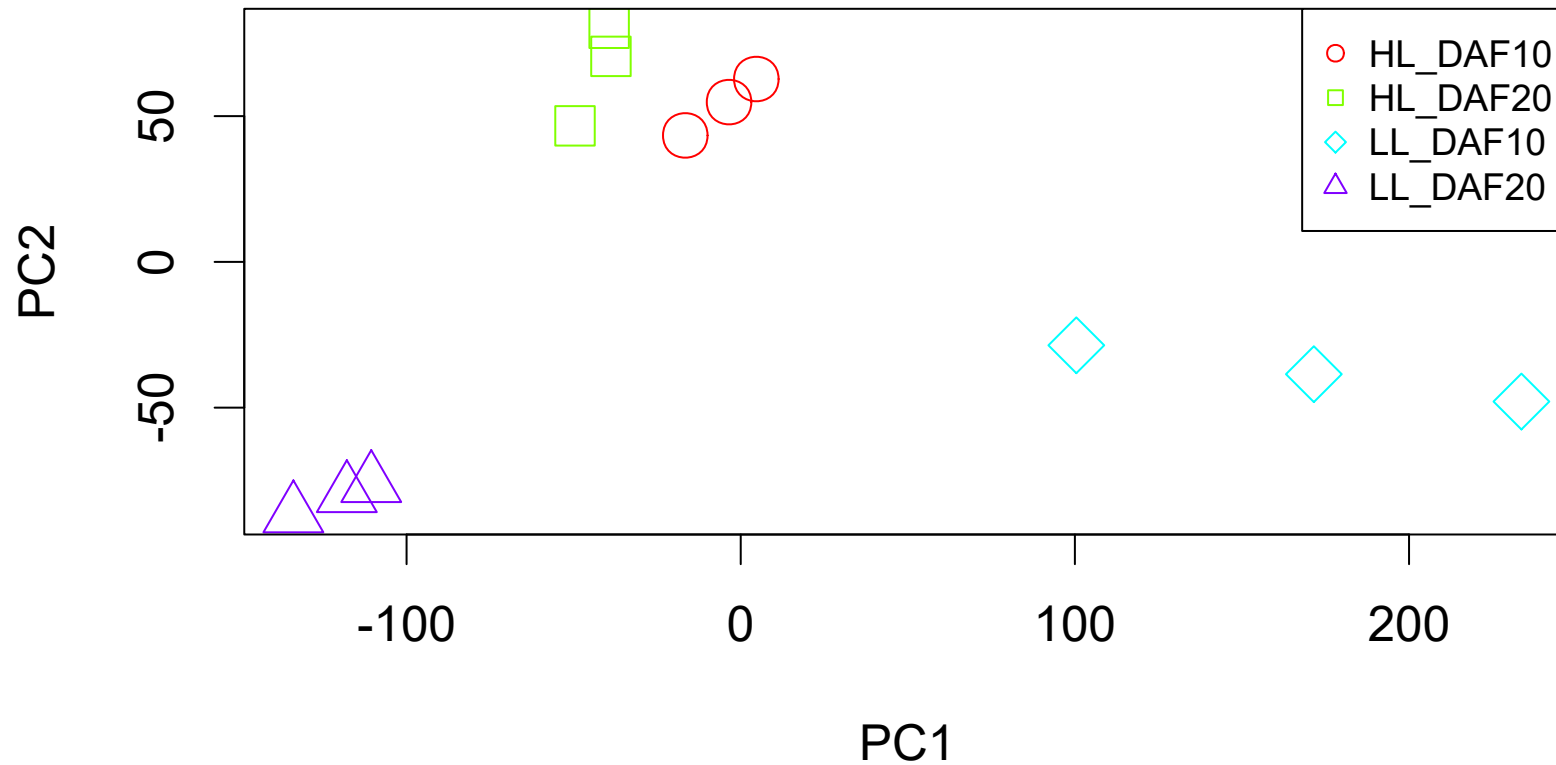


Fig. S16 Sample distance of 12 RNA-seq samples of 'HL' (high linoleic acid) and 'LL' (low linoleic acid) cultivar seeds at 10 days after flowering (DAF) and 20 DAF. The samples are analyzed using a principal component analysis. DAF: days after flowering.

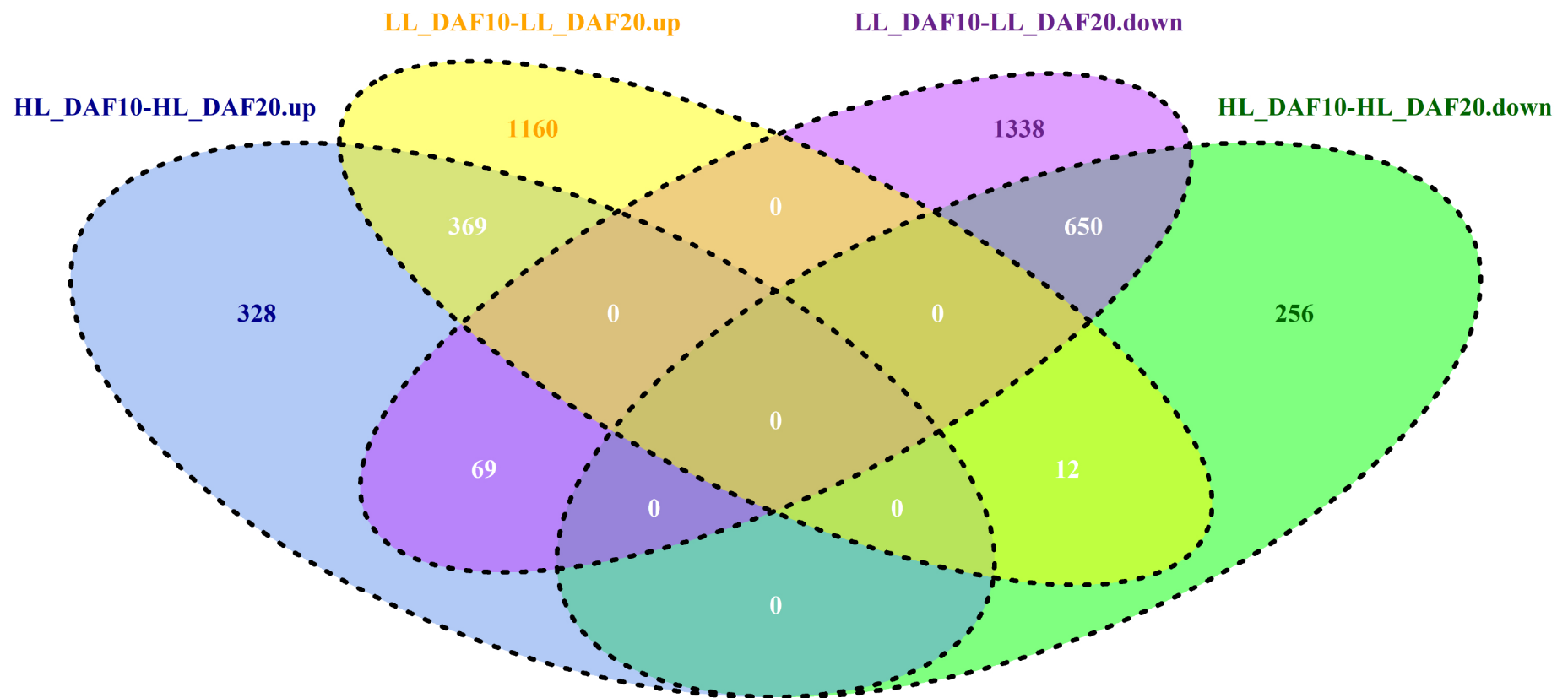


Fig. S17 Venn diagram of four sets of differentially upregulated and downregulated genes in DAF20 versus DAF10 of 'HL' and 'LL' cultivar.

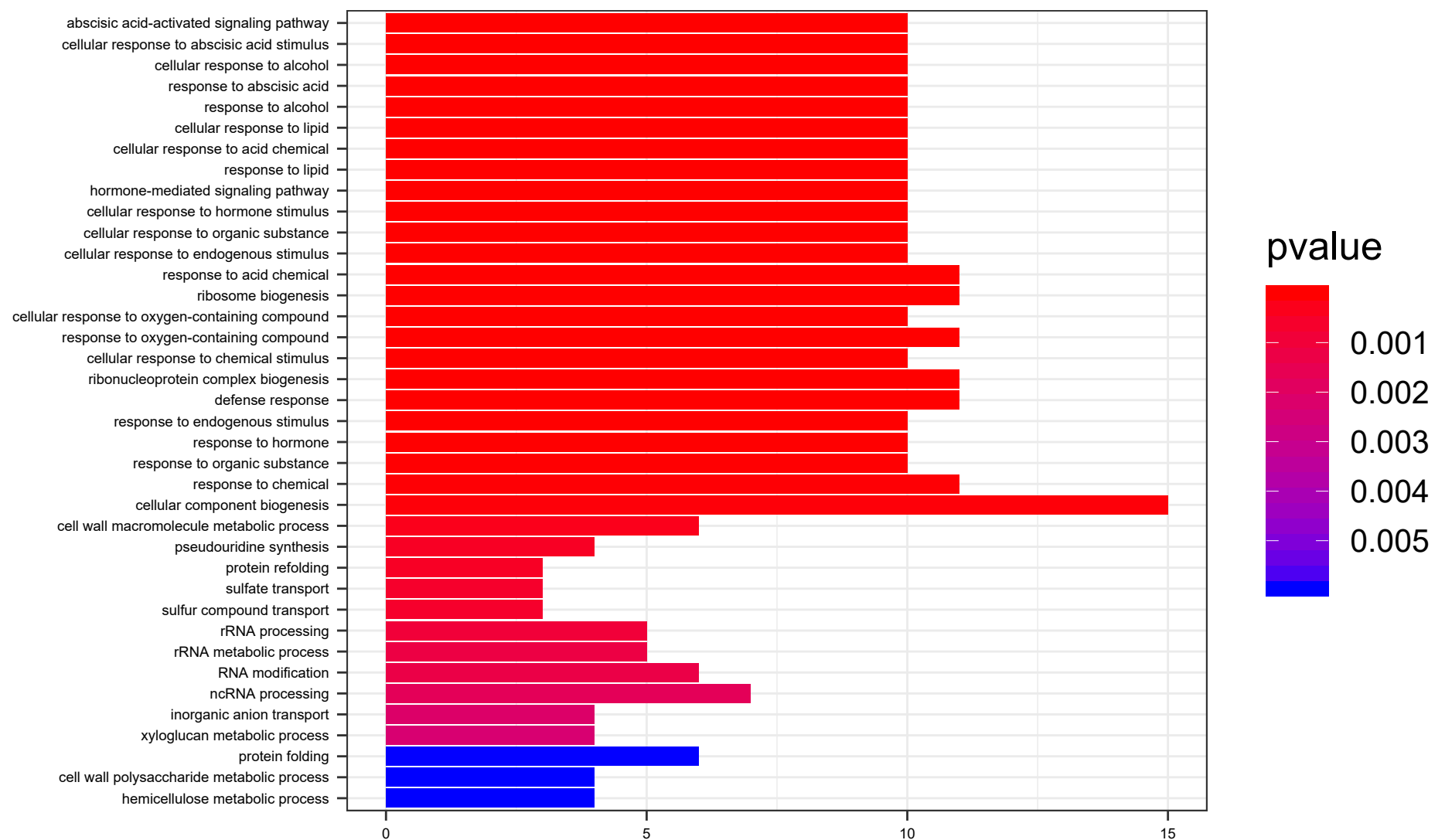


Fig. S18 Enrichment of biological process GO terms of 328 uniquely upregulated genes in DAF20 versus DAF10 in the 'HL' cultivar compared with 'LL' cultivar.

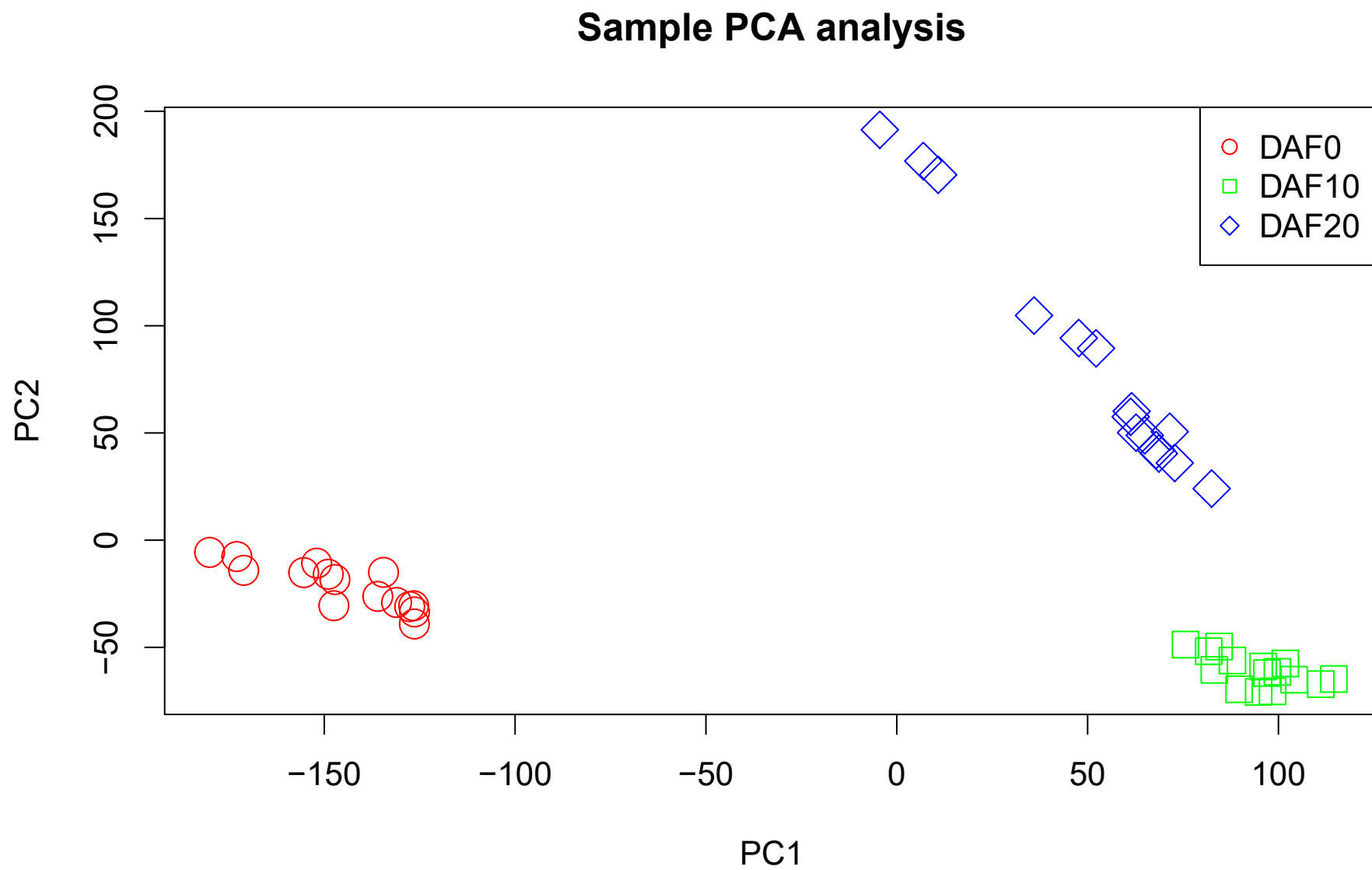


Fig. S19 Sample distance of 45 RNA-seq samples, including 15 ovaries from the ‘HL’ cultivar at 0 DAF, 15 seeds at 10 DAF, and 15 seeds at 20 DAF, determined using a principal component analysis.

a



b

Sample PCA analysis

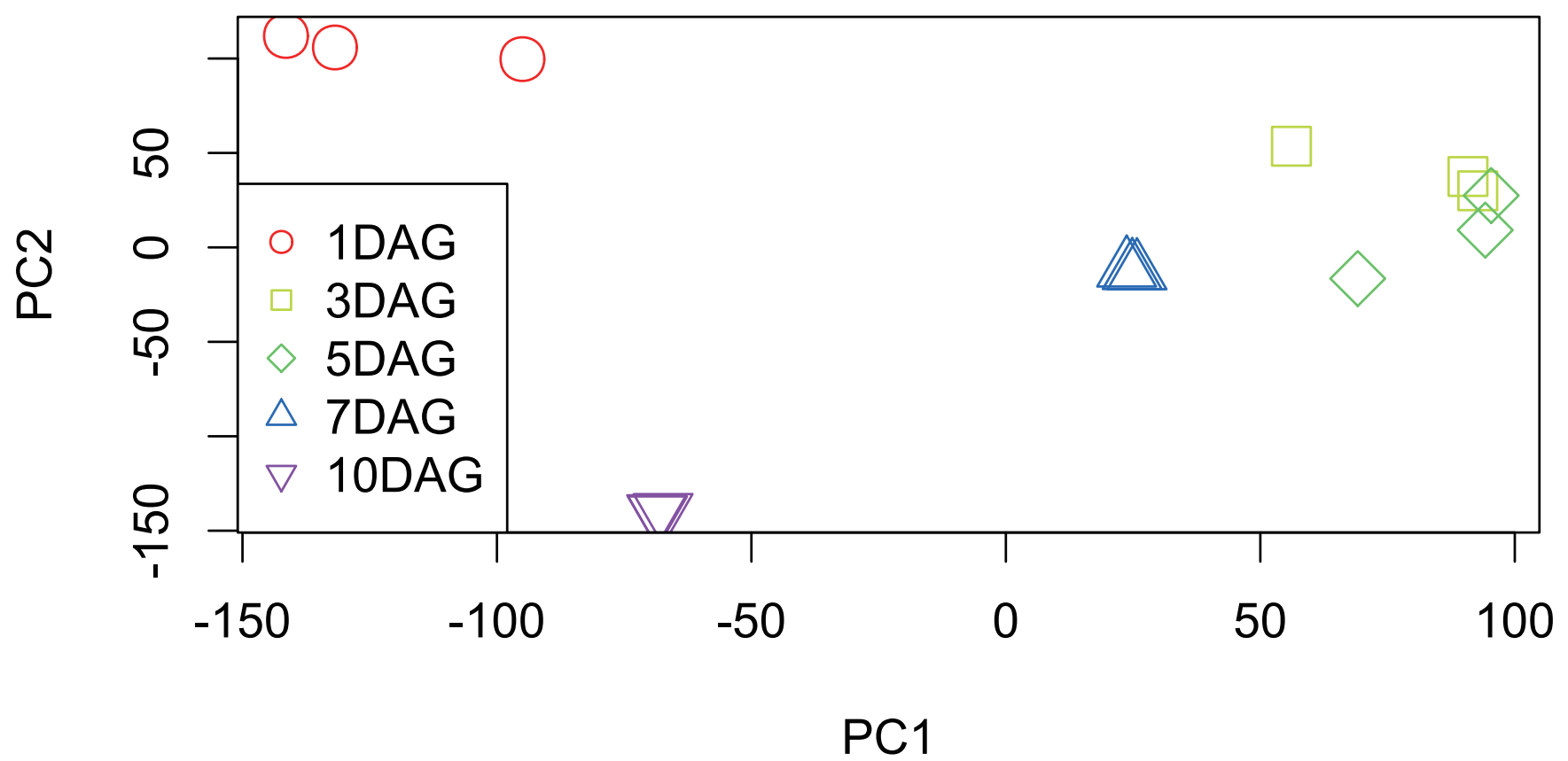
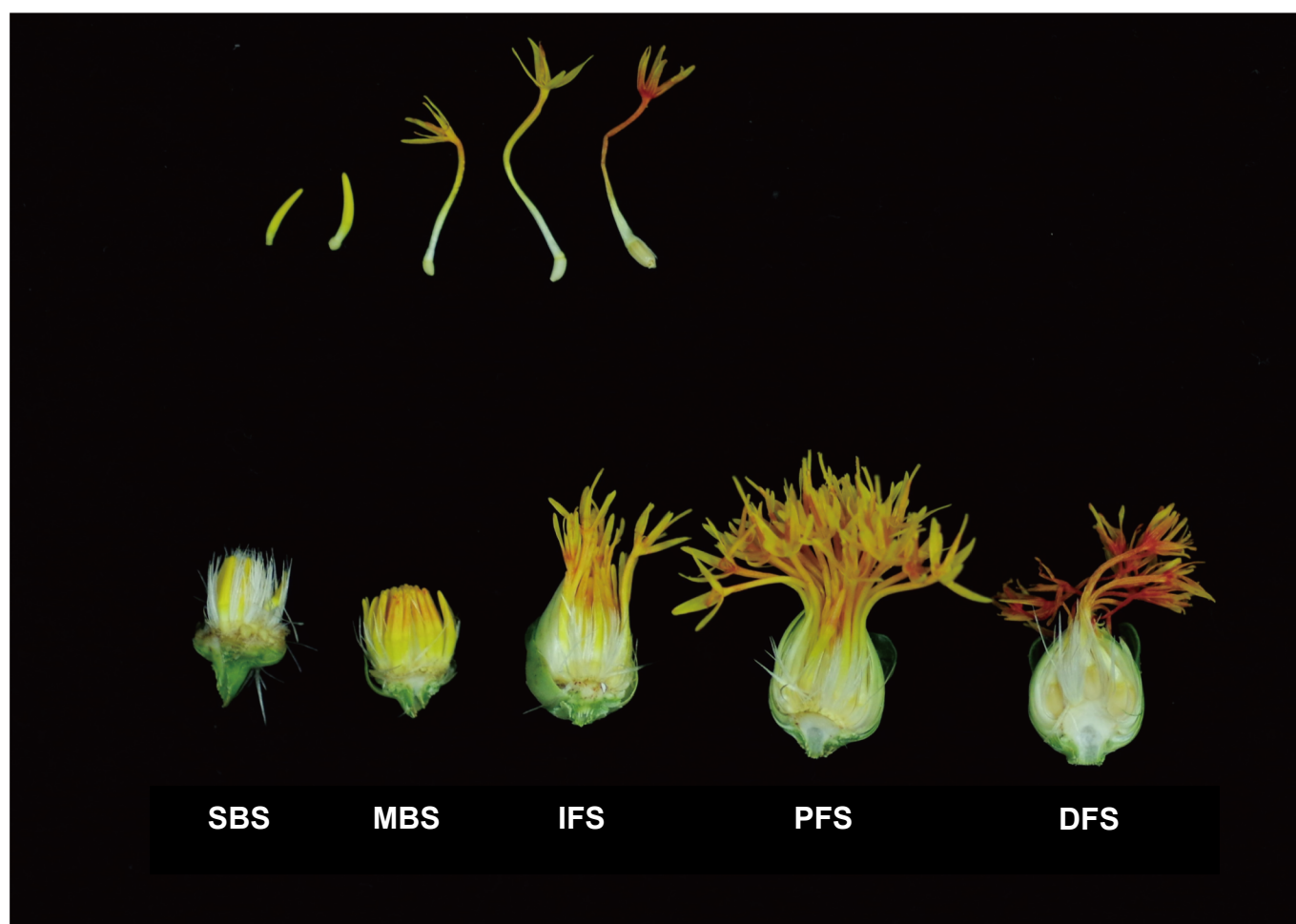


Fig. S20 Cotyledons at different days after germination (DAG; **a**) and sample distances (**b**) of 15 RNA-seq samples at 1 DAG, 3 DAG, 5 DAG, and 10 DAG, as determined using a principal component analysis.

a



b

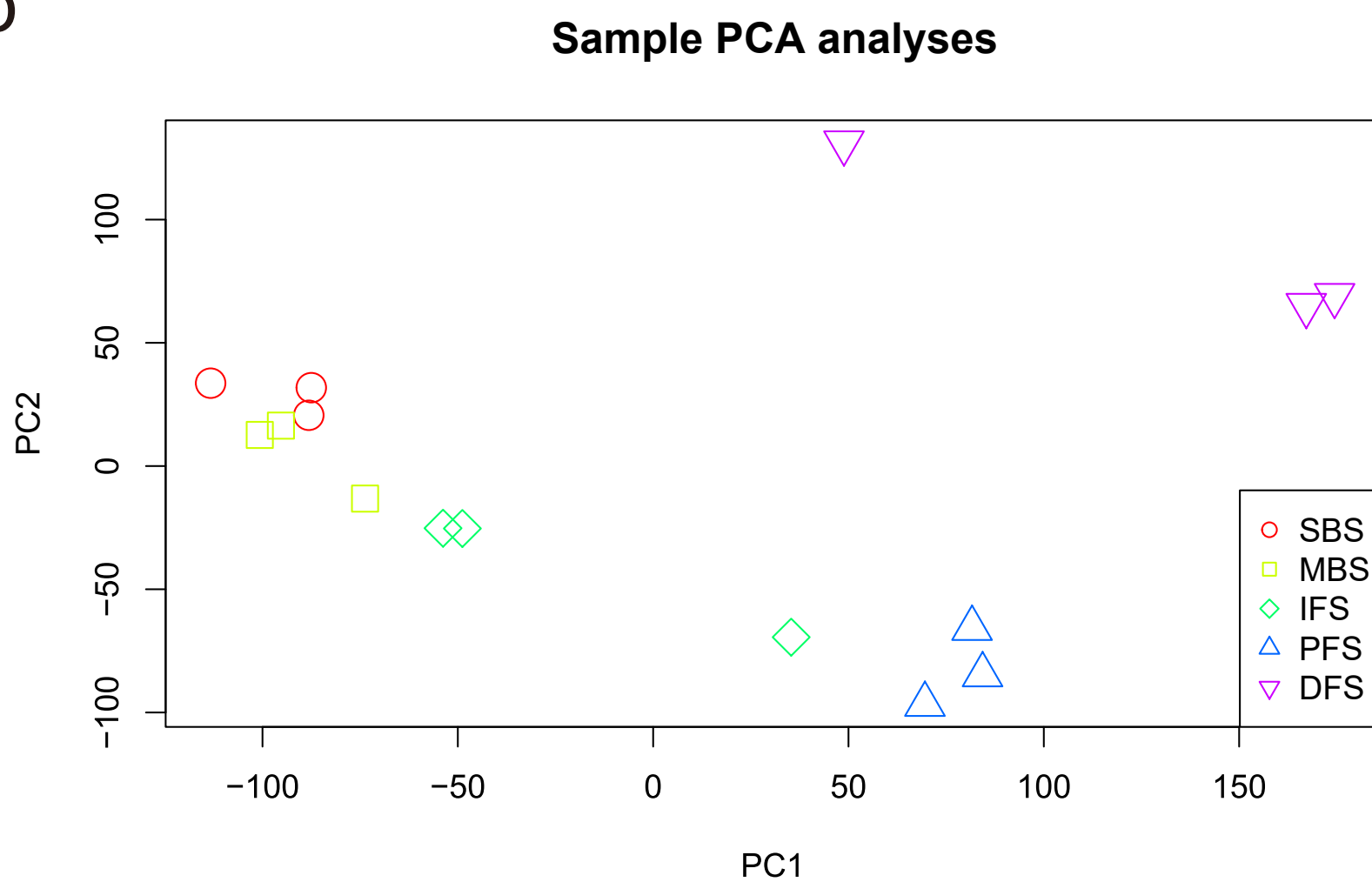


Fig. S21 Filaments (**a**) and sample distances (**b**) of 15 RNA-seq samples at five different stages: small bud stage (SBS), middle bud stage (MBS), initial flowering stage (IFS), peak flowering stage (PFS), and decayed flowering stage (DFS) during flower development, as determined using a principal component analysis.

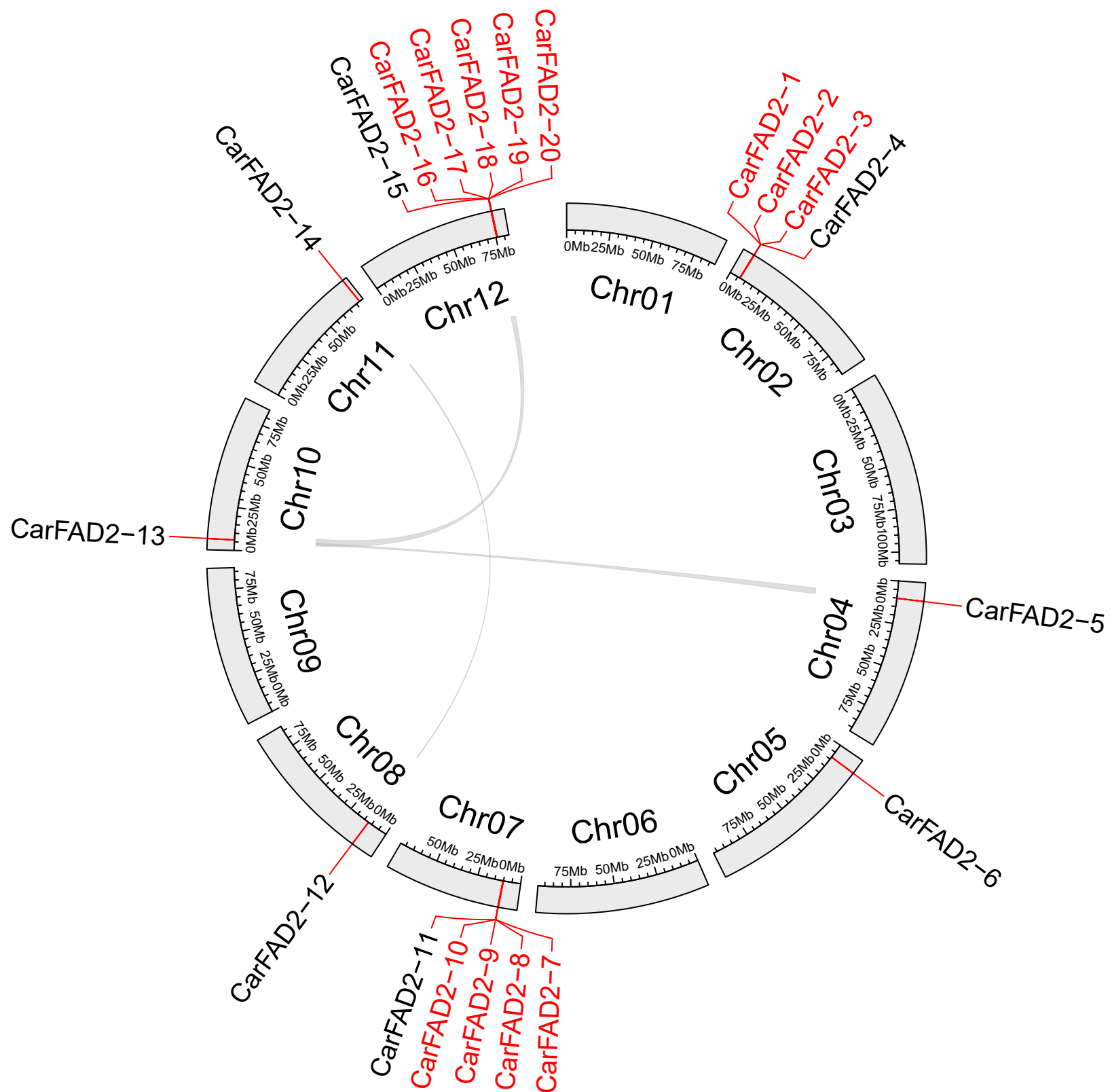


Fig. S22 Distribution of *CarFAD2s* in 12 safflower chromosomes. The links among chromosomes represent collinear blocks, the genes marked with red colors represent the tandem duplication.

a

HL_FAD2-12_5'UTR_1	ACGAAGAAGAAGCACTTCTTCGGTCGCAGAGTGAAGGAGATTTTCAGAGAGCAAGCGCTTC	160
HL_FAD2-12_5'UTR_2	ACGAAGAAGAAGCACTTCTTCGGTCGCAGAGTGAAGGAGATTTTCAGAGAGCAAGCGCTTC	160
HL_FAD2-12_5'UTR_3	ACGAAGAAGAAGCACTTCTTCGGTCGCAGAGTGAAGGAGATTTTCAGAGAGCAAGCGCTTC	160
LL_FAD2-12_5'UTR_1	ACGAAGAAGAAGCACTTCTTCGGTCGCAGAGTGAAGGAGATTTTCAGAGAGCAAGCGCTTC	160
LL_FAD2-12_5'UTR_2	ACGAAGAAGAAGCACTTCTTCGGTCGCAGAGTGAAGGAGATTTTCAGAGAGCAAGCGCTTC	160
LL_FAD2-12_5'UTR_3	ACGAAGAAGAAGCACTTCTTCGGTCGCAGAGTGAAGGAGATTTTCAGAGAGCAAGCGCTTC	160
Consensus	acgaagaagaagcacttcttcggtcgcagagtgaaaggagattttcagagagcaagcgcttc	160
HL_FAD2-12_5'UTR_1	TGTATTTCAGTTATCGTTTTCGATGATTTCCTATTAATAAATCGACGATTATCGTTTGT	320
HL_FAD2-12_5'UTR_2	TGTATTTCAGTTATCGTTTTCGATGATTTCCTATTAATAAATCGACGATTATCGTTTGT	320
HL_FAD2-12_5'UTR_3	TGTATTTCAGTTATCGTTTTCGATGATTTCCTATTAATAAATCGACGATTATCGTTTGT	320
LL_FAD2-12_5'UTR_1	TGTATTTCAGTTATCGTTTTCGATGATTTCCTATTAATAAATCGACGATTATCGTTTGT	320
LL_FAD2-12_5'UTR_2	TGTATTTCAGTTATCGTTTTCGATGATTTCCTATTAATAAATCGACGATTATCGTTTGT	320
LL_FAD2-12_5'UTR_3	TGTATTTCAGTTATCGTTTTCGATGATTTCCTATTAATAAATCGACGATTATCGTTTGT	320
Consensus	tgtatttcagttatcgtttcgatgatttccctataataaatacgacgattatcgtttgttattttt	320
HL_FAD2-12_5'UTR_1	CTTCTGTAATTTTGTGTATCTATCTGTGTCGCATGTAATTTTGTTCCTTTAGATTAT	480
HL_FAD2-12_5'UTR_2	CTTCTGTAATTTTGTGTATCTATCTGTGTCGCATGTAATTTTGTTCCTTTAGATTAT	480
HL_FAD2-12_5'UTR_3	CTTCTGTAATTTTGTGTATCTATCTGTGTCGCATGTAATTTTGTTCCTTTAGATTAT	480
LL_FAD2-12_5'UTR_1	CTTCTGTAATTTTGTGTATCTATCTGTGTCGCATGTAATTTTGTTCCTTTAGATTAT	480
LL_FAD2-12_5'UTR_2	CTTCTGTAATTTTGTGTATCTATCTGTGTCGCATGTAATTTTGTTCCTTTAGATTAT	480
LL_FAD2-12_5'UTR_3	CTTCTGTAATTTTGTGTATCTATCTGTGTCGCATGTAATTTTGTTCCTTTAGATTAT	480
Consensus	cttcctgtaaatTTGTGTATCTatctgtgctgcgatgtaatttTgtttccctttagattataga	480
HL_FAD2-12_5'UTR_1	AACAAAATGATTGTGTTTTTATATTTATGGCTTCTCGGTGGTCGGATTGTGTTTTTA	640
HL_FAD2-12_5'UTR_2	AACAAAATGATTGTGTTTTTATATTTATGGCTTCTCGGTGGTCGGATTGTGTTTTTA	640
HL_FAD2-12_5'UTR_3	AACAAAATGATTGTGTTTTTATATTTATGGCTTCTCGGTGGTCGGATTGTGTTTTTA	640
LL_FAD2-12_5'UTR_1	AACAAAATGATTGTGTTTTTATATTTATGGCTTCTCGGTGGTCGGATTGTGTTTTTA	640
LL_FAD2-12_5'UTR_2	AACAAAATGATTGTGTTTTTATATTTATGGCTTCTCGGTGGTCGGATTGTGTTTTTA	640
LL_FAD2-12_5'UTR_3	AACAAAATGATTGTGTTTTTATATTTATGGCTTCTCGGTGGTCGGATTGTGTTTTTA	640
Consensus	aacaaaatgatttTgtttttatatttatggcttctcggtggtcggatttTgtgttttaattcc	640
HL_FAD2-12_5'UTR_1	ATATAGAAATGATGATATCATTGACAAATGGCAATTGCTTAAAGATACAAGCATTTT	800
HL_FAD2-12_5'UTR_2	ATATAGAAATGATGATATCATTGACAAATGGCAATTGCTTAAAGATACAAGCATTTT	800
HL_FAD2-12_5'UTR_3	ATATAGAAATGATGATATCATTGACAAATGGCAATTGCTTAAAGATACAAGCATTTT	800
LL_FAD2-12_5'UTR_1	ATATAGAAATGATGATATCATTGACAAATGGCAATTGCTTAAAGATACAAGCATTTT	800
LL_FAD2-12_5'UTR_2	ATATAGAAATGATGATATCATTGACAAATGGCAATTGCTTAAAGATACAAGCATTTT	800
LL_FAD2-12_5'UTR_3	ATATAGAAATGATGATATCATTGACAAATGGCAATTGCTTAAAGATACAAGCATTTT	800
Consensus	atatagaaatgatgatatacattgacaaatggcaattTgcttaaagatacaagcatttttcag	800
HL_FAD2-12_5'UTR_1	AGCTGATAGCTGATAAGCTAGCTTATGAATAAATTATGTTTGGTAAAACTAGCTTAT	960
HL_FAD2-12_5'UTR_2	AGCTGATAGCTGATAAGCTAGCTTATGAATAAATTATGTTTGGTAAAACTAGCTTAT	960
HL_FAD2-12_5'UTR_3	AGCTGATAGCTGATAAGCTAGCTTATGAATAAATTATGTTTGGTAAAACTAGCTTAT	960
LL_FAD2-12_5'UTR_1	AGCTGATAGCTGATAAGCTAGCTTATGAATAAATTATGTTTGGTAAAACTAGCTTAT	960
LL_FAD2-12_5'UTR_2	AGCTGATAGCTGATAAGCTAGCTTATGAATAAATTATGTTTGGTAAAACTAGCTTAT	960
LL_FAD2-12_5'UTR_3	AGCTGATAGCTGATAAGCTAGCTTATGAATAAATTATGTTTGGTAAAACTAGCTTAT	960
Consensus	agctgatagctgataagctagcttatgaataaaattatgTTTggTaaaaactagcttatgag	960
HL_FAD2-12_5'UTR_1	ACTAACCGTTTCAATAAGCTTAACTTATGTTCCATCCAAACATGTACTAGCTTATA	1120
HL_FAD2-12_5'UTR_2	ACTAACCGTTTCAATAAGCTTAACTTATGTTCCATCCAAACATGTACTAGCTTATA	1120
HL_FAD2-12_5'UTR_3	ACTAACCGTTTCAATAAGCTTAACTTATGTTCCATCCAAACATGTACTAGCTTATA	1120
LL_FAD2-12_5'UTR_1	ACTAACCGTTTCAATAAGCTTAACTTATGTTCCATCCAAACATGTACTAGCTTATA	1120
LL_FAD2-12_5'UTR_2	ACTAACCGTTTCAATAAGCTTAACTTATGTTCCATCCAAACATGTACTAGCTTATA	1120
LL_FAD2-12_5'UTR_3	ACTAACCGTTTCAATAAGCTTAACTTATGTTCCATCCAAACATGTACTAGCTTATA	1120
Consensus	actaacgTtccaataagcttAActtatgTtccatccaaacatgtactagcttataagcgagct	1120
HL_FAD2-12_5'UTR_1	TAATTATATATTTTGAATCCTTAAGGCTAACGTTTCCTTAGTTTTATTTATGTTGT	1271
HL_FAD2-12_5'UTR_2	TAATTATATATTTTGAATCCTTAAGGCTAACGTTTCCTTAGTTTTATTTATGTTGT	1271
HL_FAD2-12_5'UTR_3	TAATTATATATTTTGAATCCTTAAGGCTAACGTTTCCTTAGTTTTATTTATGTTGT	1271
LL_FAD2-12_5'UTR_1	TAATTATATATTTTGAATCCTTAAGGCTAACGTTTCCTTAGTTTTATTTATGTTGT	1271
LL_FAD2-12_5'UTR_2	TAATTATATATTTTGAATCCTTAAGGCTAACGTTTCCTTAGTTTTATTTATGTTGT	1271
LL_FAD2-12_5'UTR_3	TAATTATATATTTTGAATCCTTAAGGCTAACGTTTCCTTAGTTTTATTTATGTTGT	1271
Consensus	taattatatatttttgaatccttAaggctAACgTtTcccttagTTTTattTatgtTgtggtg	1271
HL_FAD2-12_CDS_1	ATGGGAGGAGGAGGGTGATGCTCGCTCCGAGACCAAAGCTGAAGAAAAGAAGAACCC	160
HL_FAD2-12_CDS_2	ATGGGAGGAGGAGGGTGATGCTCGCTCCGAGACCAAAGCTGAAGAAAAGAAGAACCC	160
HL_FAD2-12_CDS_3	ATGGGAGGAGGAGGGTGATGCTCGCTCCGAGACCAAAGCTGAAGAAAAGAAGAACCC	160
LL_FAD2-12_CDS_1	ATGGGAGGAGGAGGGTGATGCTCGCTCCGAGACCAAAGCTGAAGAAAAGAAGAACCC	160
LL_FAD2-12_CDS_2	ATGGGAGGAGGAGGGTGATGCTCGCTCCGAGACCAAAGCTGAAGAAAAGAAGAACCC	160
LL_FAD2-12_CDS_3	ATGGGAGGAGGAGGGTGATGCTCGCTCCGAGACCAAAGCTGAAGAAAAGAAGAACCC	160
Consensus	atgggaggagagggtgtatgtctgcctccgagaccaaagctgaagaaaagaagaaccactc	160
HL_FAD2-12_CDS_1	CTTACATTGTCTATGACCTTGCCTAGCTTACGCTTGGCACCACCTACATCCACCGCT	320
HL_FAD2-12_CDS_2	CTTACATTGTCTATGACCTTGCCTAGCTTACGCTTGGCACCACCTACATCCACCGCT	320
HL_FAD2-12_CDS_3	CTTACATTGTCTATGACCTTGCCTAGCTTACGCTTGGCACCACCTACATCCACCGCT	320
LL_FAD2-12_CDS_1	CTTACATTGTCTATGACCTTGCCTAGCTTACGCTTGGCACCACCTACATCCACCGCT	320
LL_FAD2-12_CDS_2	CTTACATTGTCTATGACCTTGCCTAGCTTACGCTTGGCACCACCTACATCCACCGCT	320
LL_FAD2-12_CDS_3	CTTACATTGTCTATGACCTTGCCTAGCTTACGCTTGGCACCACCTACATCCACCGCT	320
Consensus	cttacattgtctatgaccttgcctatgacctctactactctggccaccactacatccacgct	320
HL_FAD2-12_CDS_1	CGGCCACCATTGCTTTAGCGATTATCAATGGGTTGACGACACCGTGGGCTTCATAGT	480
HL_FAD2-12_CDS_2	CGGCCACCATTGCTTTAGCGATTATCAATGGGTTGACGACACCGTGGGCTTCATAGT	480
HL_FAD2-12_CDS_3	CGGCCACCATTGCTTTAGCGATTATCAATGGGTTGACGACACCGTGGGCTTCATAGT	480
LL_FAD2-12_CDS_1	CGGCCACCATTGCTTTAGCGATTATCAATGGGTTGACGACACCGTGGGCTTCATAGT	480
LL_FAD2-12_CDS_2	CGGCCACCATTGCTTTAGCGATTATCAATGGGTTGACGACACCGTGGGCTTCATAGT	480
LL_FAD2-12_CDS_3	CGGCCACCATTGCTTTAGCGATTATCAATGGGTTGACGACACCGTGGGCTTCATAGT	480
Consensus	cgGCCaccatgcctttagcgattatcaatgggttgacgacacccgtgggcttcATagTcc	480
HL_FAD2-12_CDS_1	CCCAGATCAAAACTCCCTTGGTACTCGAAATACCTTGAACAACCCGCCCTGGCCGCAT	640
HL_FAD2-12_CDS_2	CCCAGATCAAAACTCCCTTGGTACTCGAAATACCTTGAACAACCCGCCCTGGCCGCAT	640
HL_FAD2-12_CDS_3	CCCAGATCAAAACTCCCTTGGTACTCGAAATACCTTGAACAACCCGCCCTGGCCGCAT	640
LL_FAD2-12_CDS_1	CCCAGATCAAAACTCCCTTGGTACTCGAAATACCTTGAACAACCCGCCCTGGCCGCAT	639
LL_FAD2-12_CDS_2	CCCAGATCAAAACTCCCTTGGTACTCGAAATACCTTGAACAACCCGCCCTGGCCGCAT	639
LL_FAD2-12_CDS_3	CCCAGATCAAAACTCCCTTGGTACTCGAAATACCTTGAACAACCCGCCCTGGCCGCAT	639
Consensus	cccagatcaaaactcccttggTactcgaaatactTgaacaacccgccctggccgcatacagc	640
HL_FAD2-12_CDS_1	GCCCGATTTACAAACATCGCGAACGCCCTCCAGATTGGCTCTCTGACGTGGGGATCGT	800
HL_FAD2-12_CDS_2	GCCCGATTTACAAACATCGCGAACGCCCTCCAGATTGGCTCTCTGACGTGGGGATCGT	800
HL_FAD2-12_CDS_3	GCCCGATTTACAAACATCGCGAACGCCCTCCAGATTGGCTCTCTGACGTGGGGATCGT	800
LL_FAD2-12_CDS_1	GCCCGATTTACAAACATCGCGAACGCCCTCCAGATTGGCTCTCTGACGTGGGGATCGT	799
LL_FAD2-12_CDS_2	GCCCGATTTACAAACATCGCGAACGCCCTCCAGATTGGCTCTCTGACGTGGGGATCGT	799
LL_FAD2-12_CDS_3	GCCCGATTTACAAACATCGCGAACGCCCTCCAGATTGGCTCTCTGACGTGGGGATCGT	799
Consensus	gcccgatttacaaccatcgcgaaagcctccagatttggctctctgacgtggygatctgTgcca	800
HL_FAD2-12_CDS_1	GTTGATCAGGTTCCCTTCAGCATACGCACCCCTTCGTTGGCTCACTACGACGGGT	960
HL_FAD2-12_CDS_2	GTTGATCAGGTTCCCTTCAGCATACGCACCCCTTCGTTGGCTCACTACGACGGGT	960
HL_FAD2-12_CDS_3	GTTGATCAGGTTCCCTTCAGCATACGCACCCCTTCGTTGGCTCACTACGACGGGT	960
LL_FAD2-12_CDS_1	GTTGATCAGGTTCCCTTCAGCATACGCACCCCTTCGTTGGCTCACTACGACGGGT	959
LL_FAD2-12_CDS_2	GTTGATCAGGTTCCCTTCAGCATACGCACCCCTTCGTTGGCTCACTACGACGGGT	959
LL_FAD2-12_CDS_3	GTTGATCAGGTTCCCTTCAGCATACGCACCCCTTCGTTGGCTCACTACGACGGGT	959
Consensus	gttgatcaggttcccttcagcatacgcaccccttcgTtggctcctcactacgacgggtcgga	960
HL_FAD2-12_CDS_1	TTCTCGACAATGCCGCATTATCATGCGATGGAGGCAACGAGGGCGGTGAAGGGGT	1120
HL_FAD2-12_CDS_2	TTCTCGACAATGCCGCATTATCATGCGATGGAGGCAACGAGGGCGGTGAAGGGGT	1120
HL_FAD2-12_CDS_3	TTCTCGACAATGCCGCATTATCATGCGATGGAGGCAACGAGGGCGGTGAAGGGGT	1120
LL_FAD2-12_CDS_1	TTCTCGACAATGCCGCATTATCATGCGATGGAGGCAACGAGGGCGGTGAAGGGGT	1119
LL_FAD2-12_CDS_2	TTCTCGACAATGCCGCATTATCATGCGATGGAGGCAACGAGGGCGGTGAAGGGGT	1119
LL_FAD2-12_CDS_3	TTCTCGACAATGCCGCATTATCATGCGATGGAGGCAACGAGGGCGGTGAAGGGGT	1119
Consensus	ttctcgacaatgccgcattatcatgcgatggaggcaacgagggcggtgaaggggttGctggg	1120
HL_FAD2-12_CDS_1	TGTTTTGGTACAAGATAAA	1139
HL_FAD2-12_CDS_2	TGTTTTGGTACAAGATAAA	1139
HL_FAD2-12_CDS_3	TGTTTTGGTACAAGATAAA	1139
LL_FAD2-12_CDS_1	TGTTTTGGTACAAGATAAA	1138
LL_FAD2-12_CDS_2	TGTTTTGGTACAAGATAAA	1138
LL_FAD2-12_CDS_3	TGTTTTGGTACAAGATAAA	1138
Consensus	tgttttggTacaagataaa	1138

Fig. S23 Sequence alignment of 5’ UTR region (**a**) and coding region (**b**) of *CarFAD2-12* in ‘HL’ and ‘LL’ cultivars.

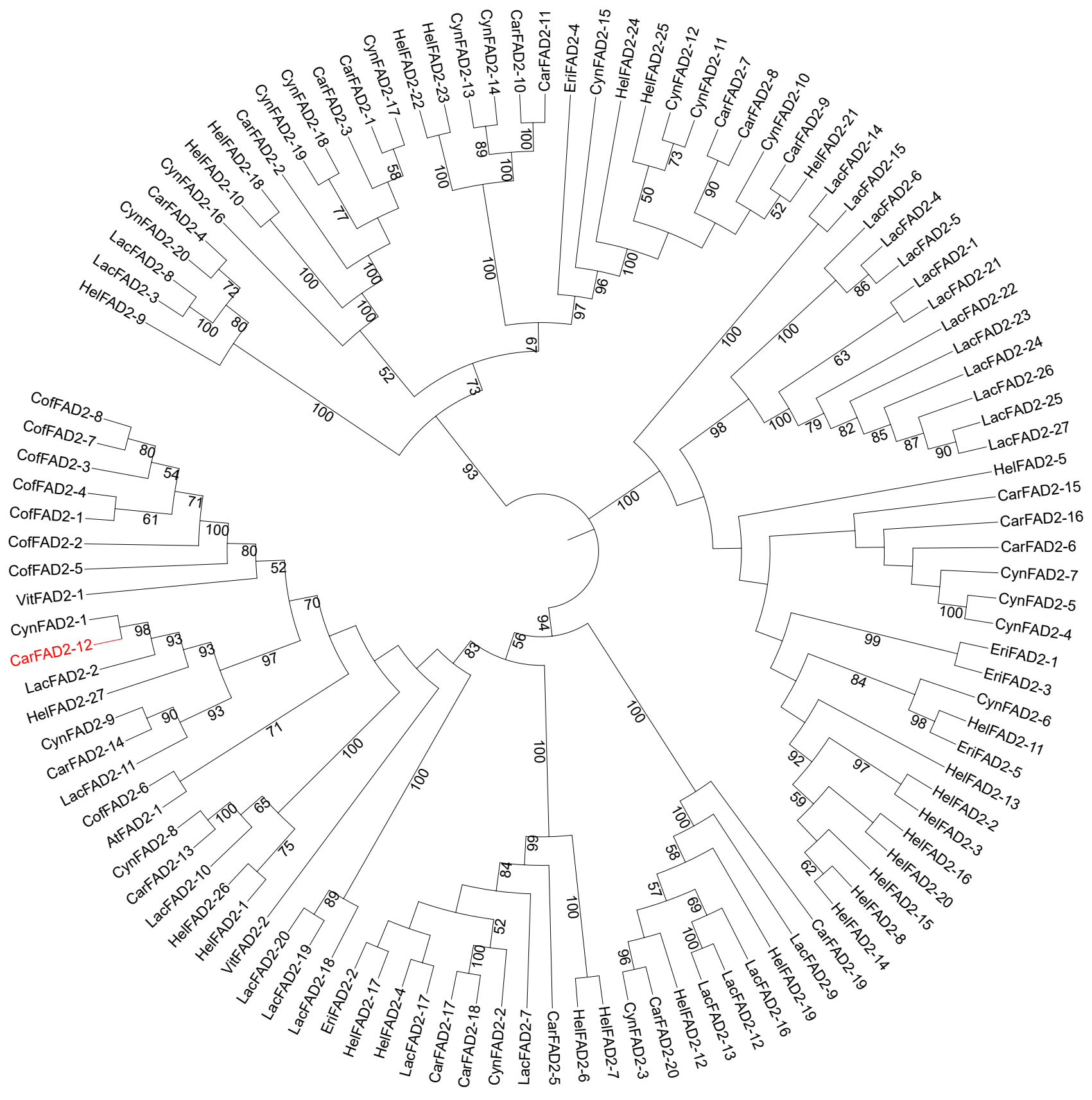


Fig. S24 The phylogenetic tree of *FAD2* (*FATTY ACID DESATURASE 2*) genes of eight species, *Arabidopsis thaliana* (At), *Vitis vinifera* (Vit), *Coffea canephora* (Cof), *Cynara cardunculus* (Cyn), *Erigeron breviscapus* (Eri), *Helianthus annuus* (Hel), *Lactuca sativa* (Lac), and *Carthamus tinctorius* (Car), constructed using Mega X with a Neighbor-Joining method and 500 bootstraps. Only bootstrap values > 50 are shown. The identifier numbers of the *FAD2* members in each species are ordered by their location on the chromosomes. The highly specifically expressed *FAD2* gene in the safflower seeds is *CarFAD2-12*, marked in red.

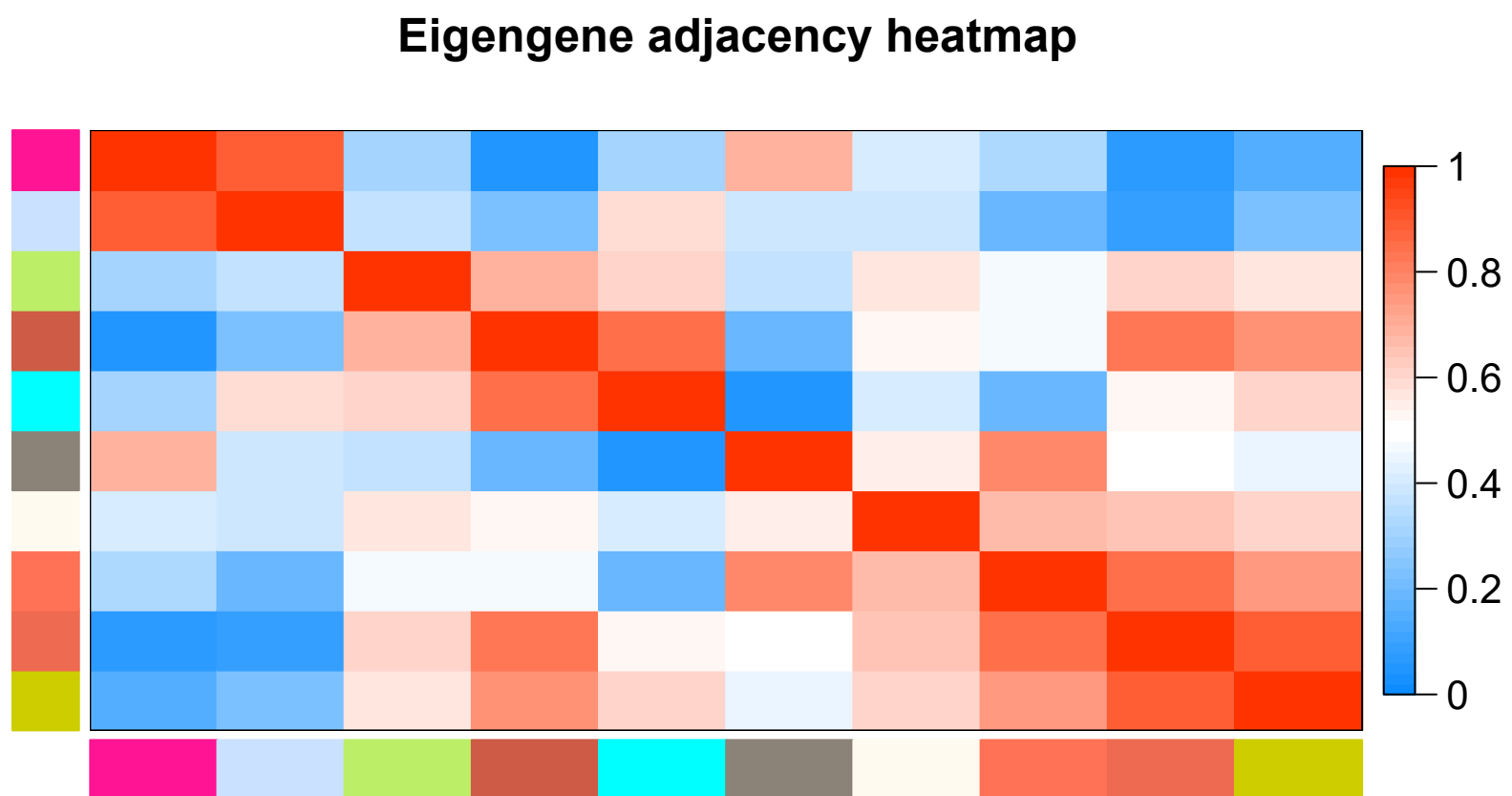
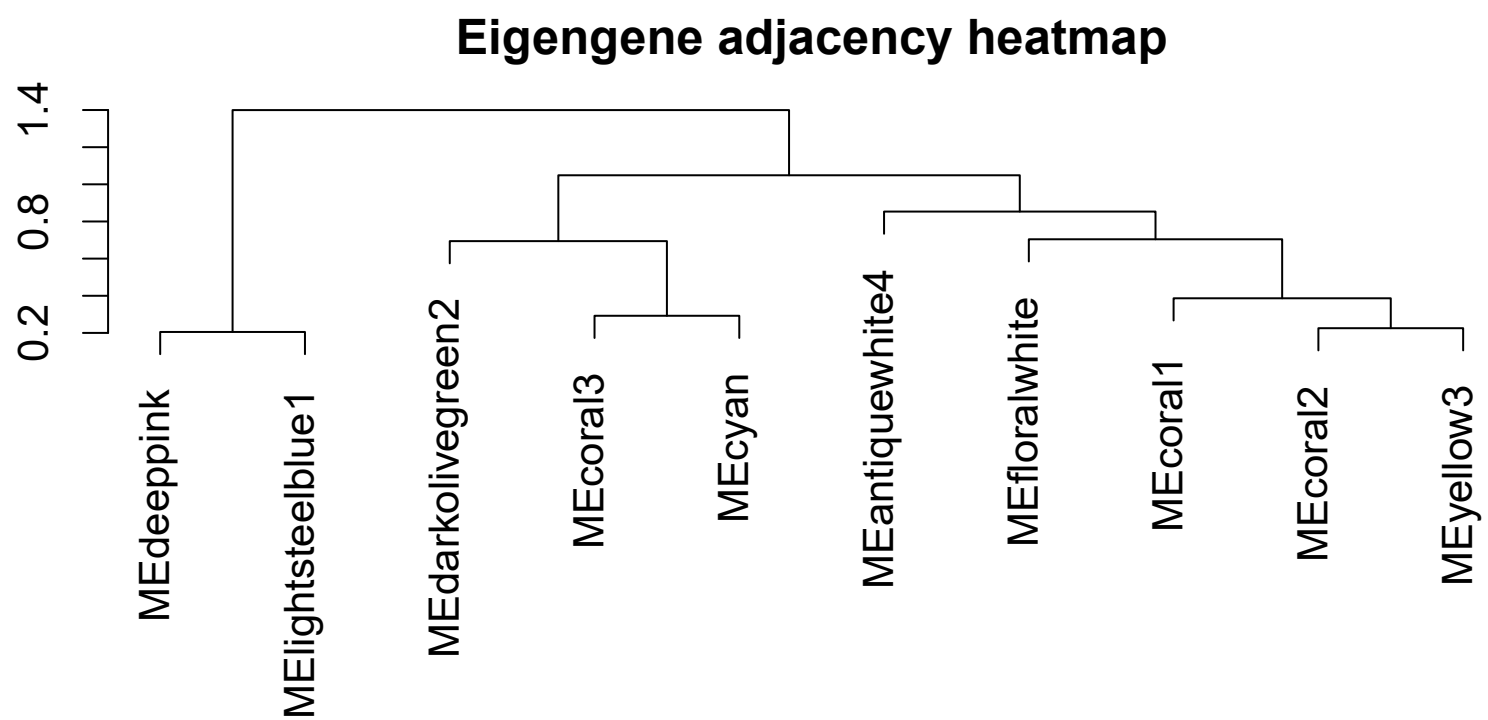


Fig. S25 Relationships of the coexpressed modules from 45 RNA-seq samples in seed development, revealed by the correlation of the module eigengene values (i.e., the first principal component).

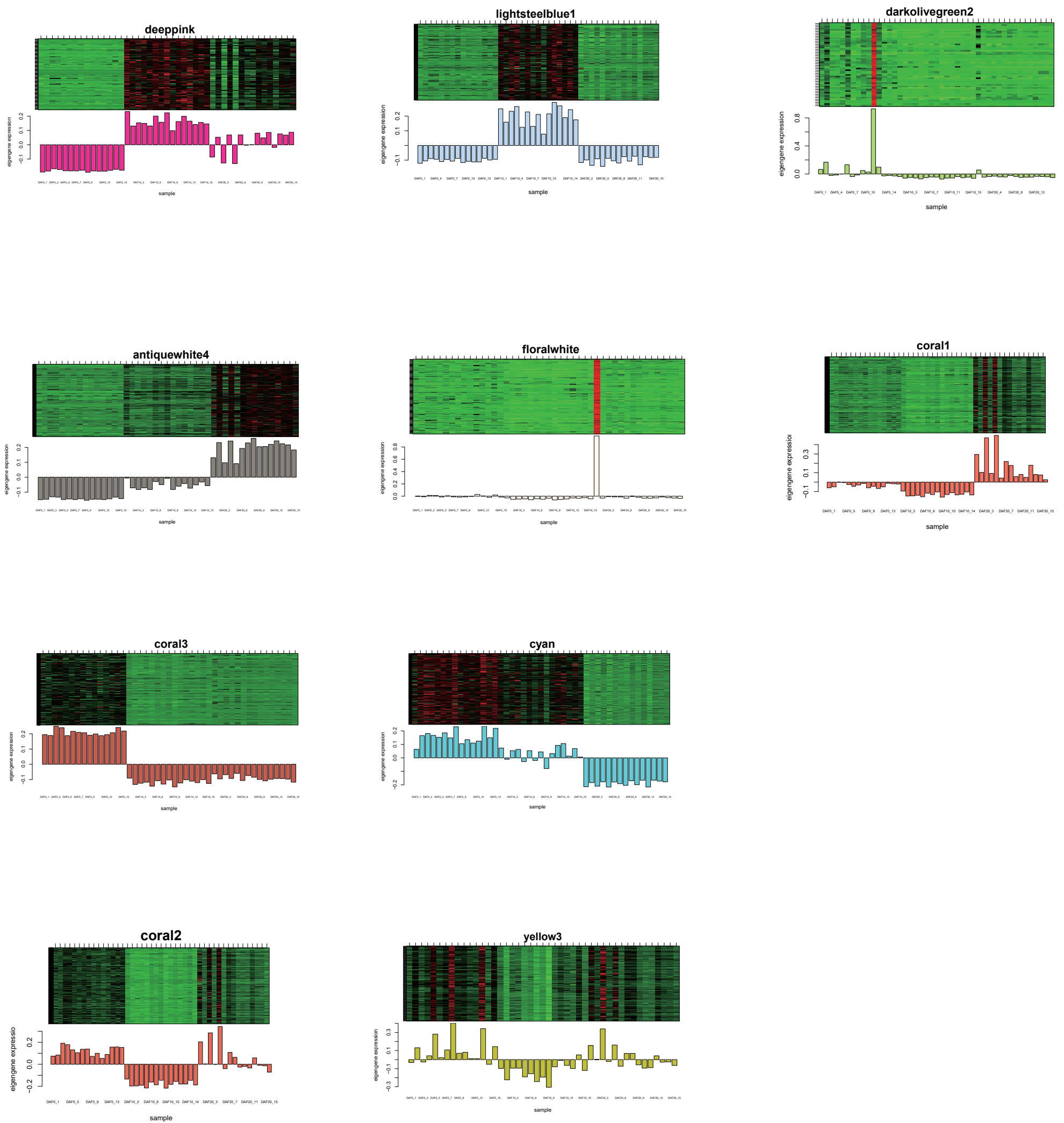


Fig. S26 Expression pattern for each gene module from 45 RNA-seq samples in seed development. The bar plots show eigengene values calculated from the singular value composition.

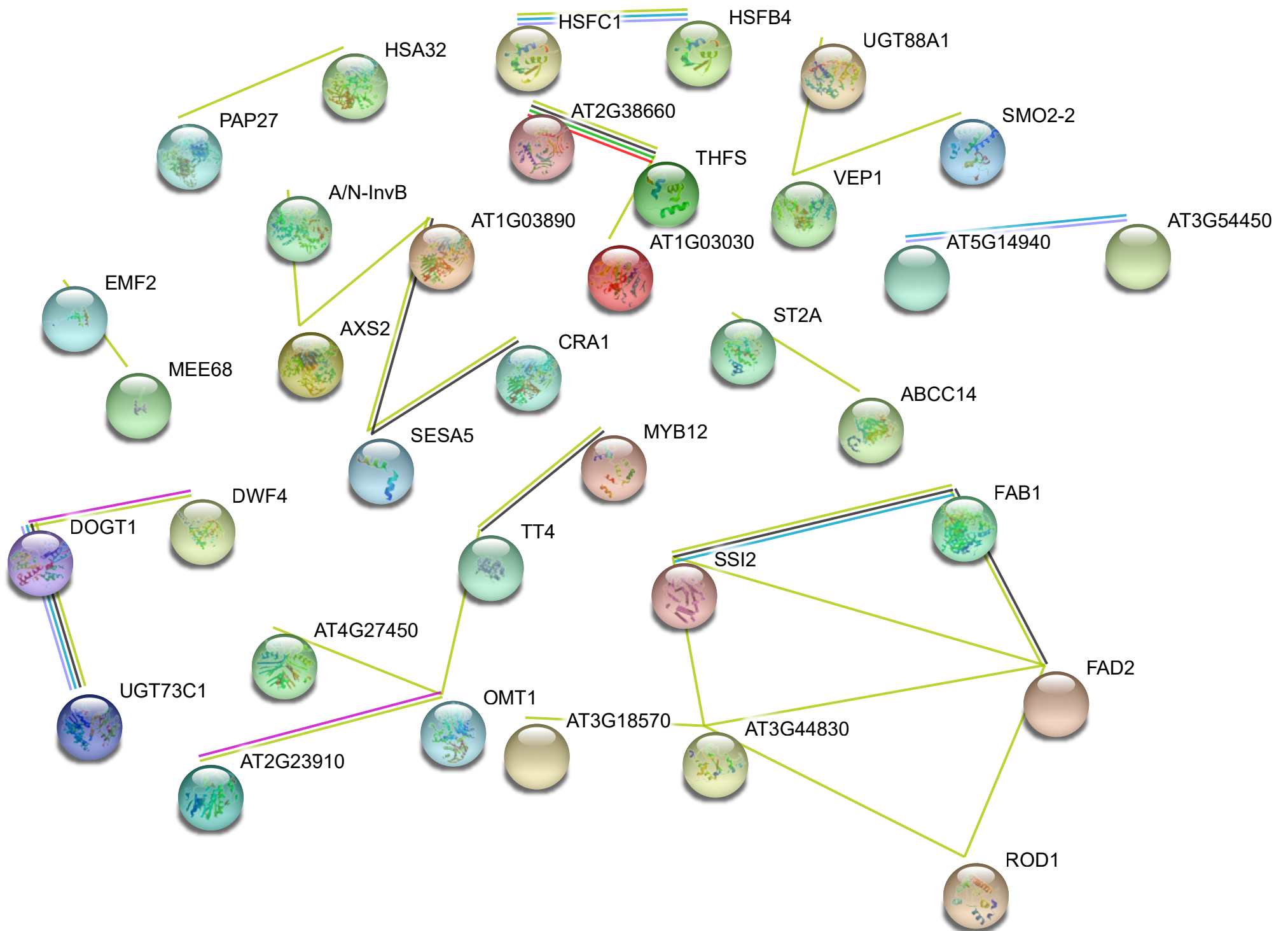


Fig. S27 Associated protein network of *Arabidopsis* homologs of the genes in ‘deeppink’ module including *CarFAD2-12*, as determined using STRING 11.0.

CtAH02G0083800 - *CtAH02* - RI - "-"
Fatty acid biosynthesis[KEGG]

CtAH09G0223800 - CtAH09 - RI - "+"
Fatty acid biosynthesis[KEGG]

CtAH06G0083800 - CtAH06 - RI - "+"
Fatty acid biosynthesis[KEGG],Biosynthesis of unsaturated fatty acids[KEGG],fatty acid metabolic process[GO]

CtAH10G0104700 - CtAH10 - A3SS - "-"
Fatty acid biosynthesis[KEGG]

CtAH03G0131700 - CtAH03 - RI - "+"
Biosynthesis of unsaturated fatty acids/[ECG] fatty acid metabolic process[GO]

CtAH06G0083800 - CtAH06 - RI - "+"
Fatty acid biosynthesis (FAS) Biosynthesis of unsaturated fatty acids (FAS) fatty acid synthetase (FAS)

CtAH09G0223800 - CtAH09 - RI - "+"
Fatty acid biosynthesis[KEGG]

CtAH09G0252900 - CtAH09 - A3SS - "-"
Fatty acid biosynthesis[KEGG]

CtAH12G0091900 - CtAH12 - RI - RI - "+"
Fatty acid biosynthesis[KEGG]

Fig. S28 Differentially alternatively spliced (AS) genes involved in fatty acid biosynthesis. For each gene, AS-covering and total long read counts are shown in AS variants in each stage of seed formation, and differential expression of AS variants is indicated by Δ PSI on the left. The ‘up’, ‘down’, and ‘-’ on the left represent upregulated, downregulated, and no differential expression between adjacent groups, respectively. Each vertical line at the bottom represents continuous DASGs among different stages.

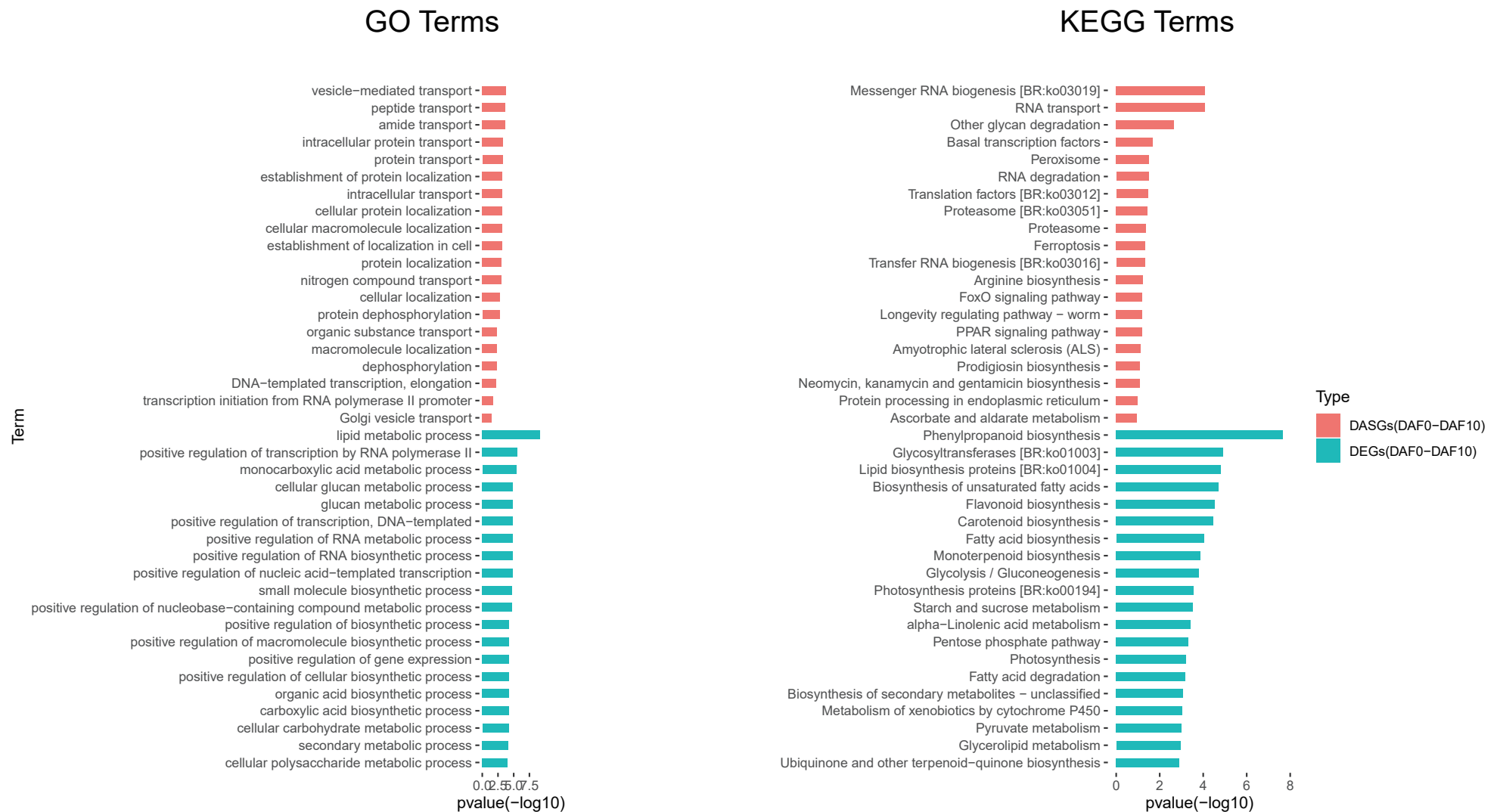


Fig. S29 Top 20 GO and KEGG terms associated with the unique differentially expressed genes and differentially alternatively spliced genes in the comparison of seeds at 10 days after flowering (DAF10) versus DAF0.

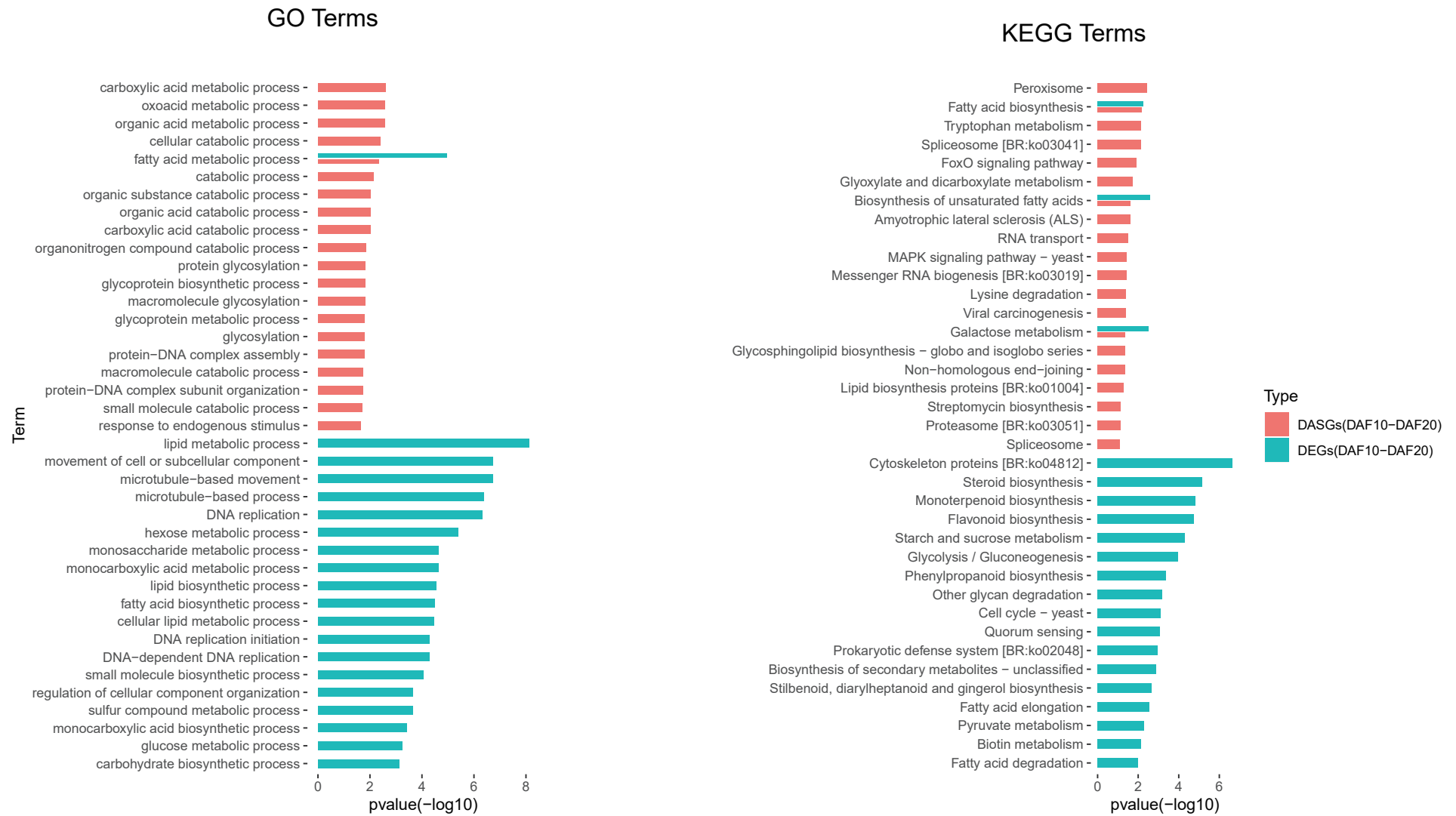


Fig. S30 Top 20 GO and KEGG terms associated with the unique differentially expressed genes and differentially alternatively spliced genes in the comparison of seeds at 20 days after flowering (DAF20) versus DAF10.

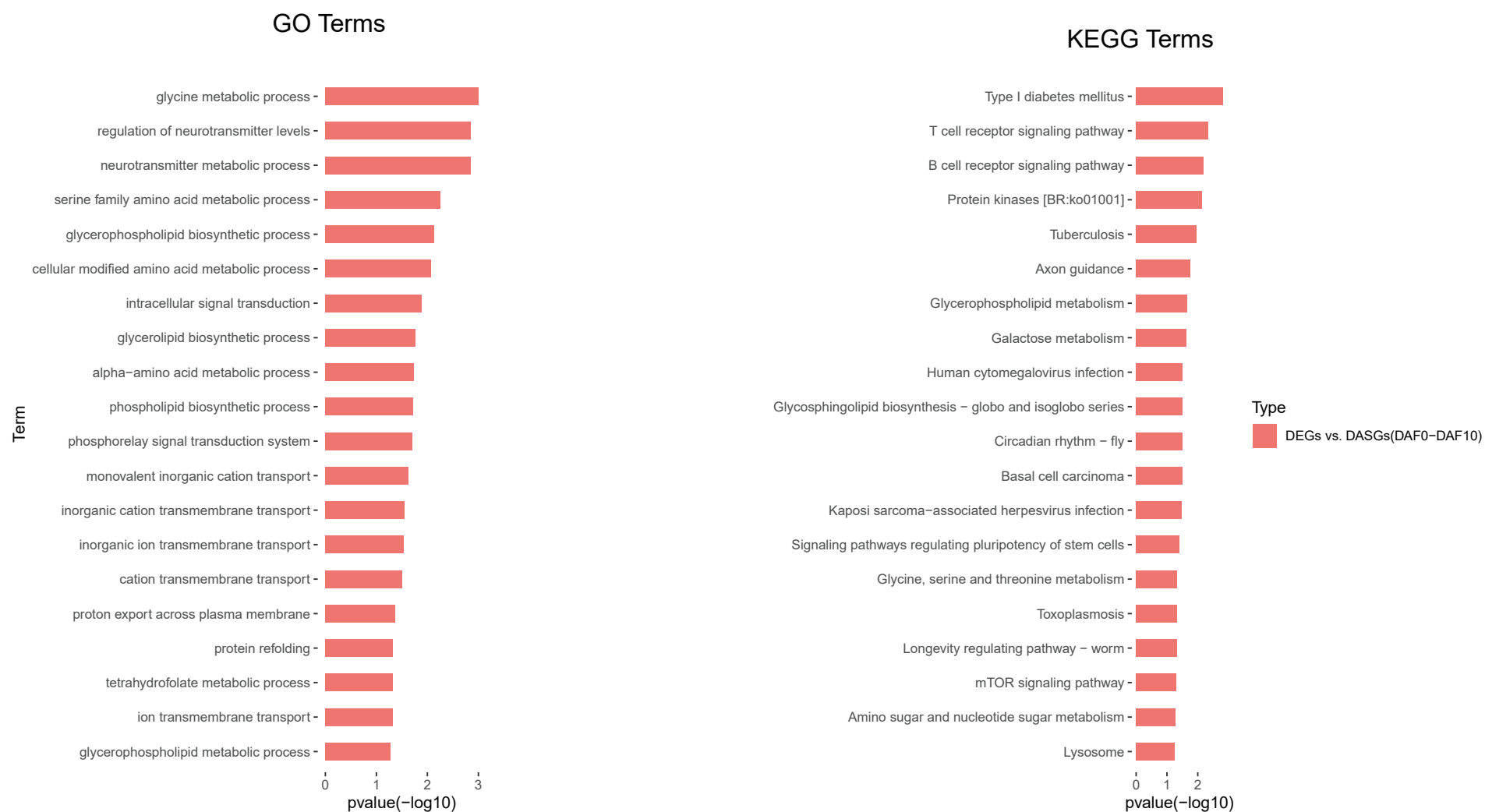


Fig. S31 Top 20 GO and KEGG terms associated with the common differentially expressed genes and differentially alternatively spliced genes in the comparison of seeds at 10 days after flowering (DAF10) versus DAF0.

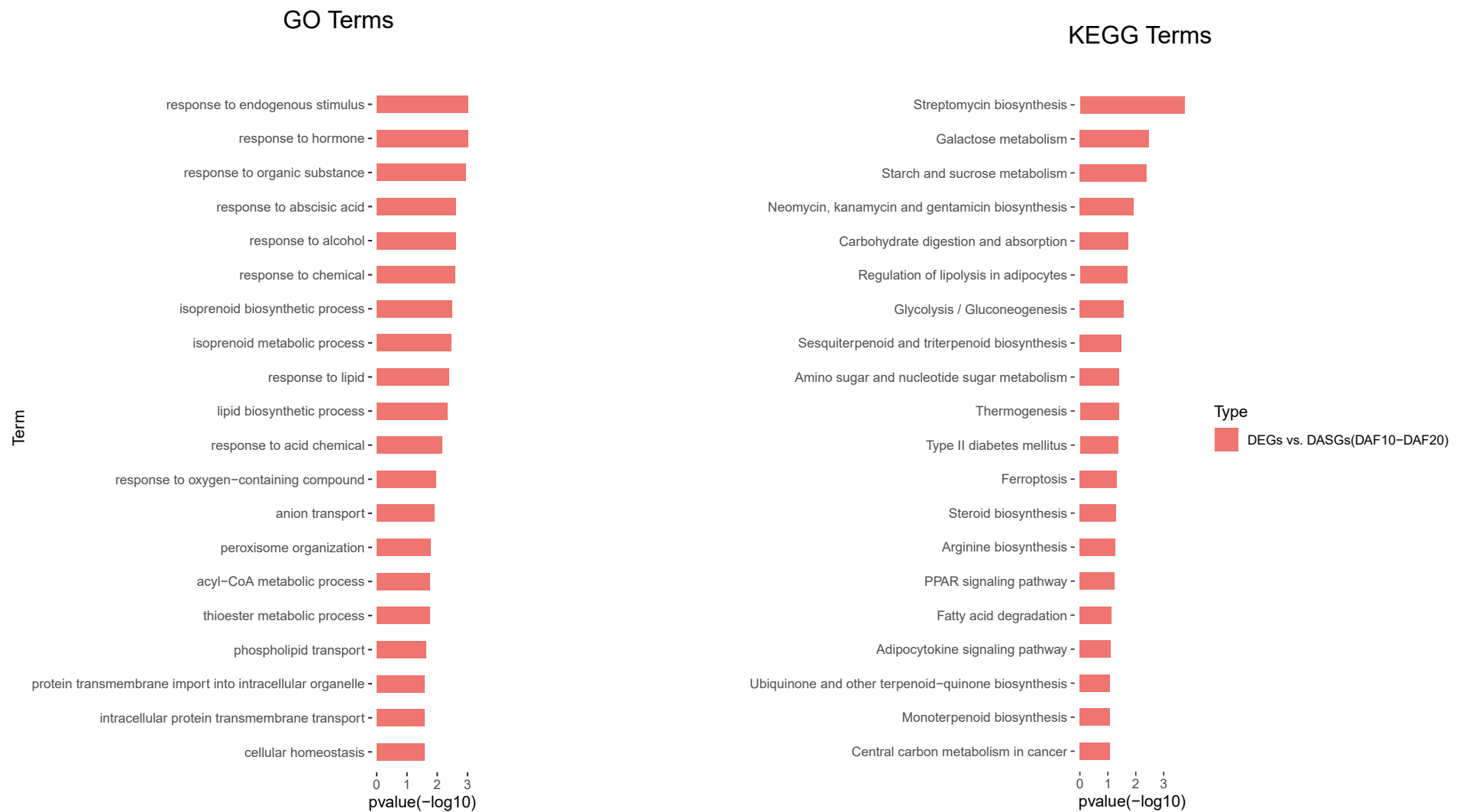


Fig. S32 Top 20 GO and KEGG terms associated with the common differentially expressed genes and differentially alternatively spliced genes in the comparison of seeds at 10 days after flowering (DAF20) versus DAF10.

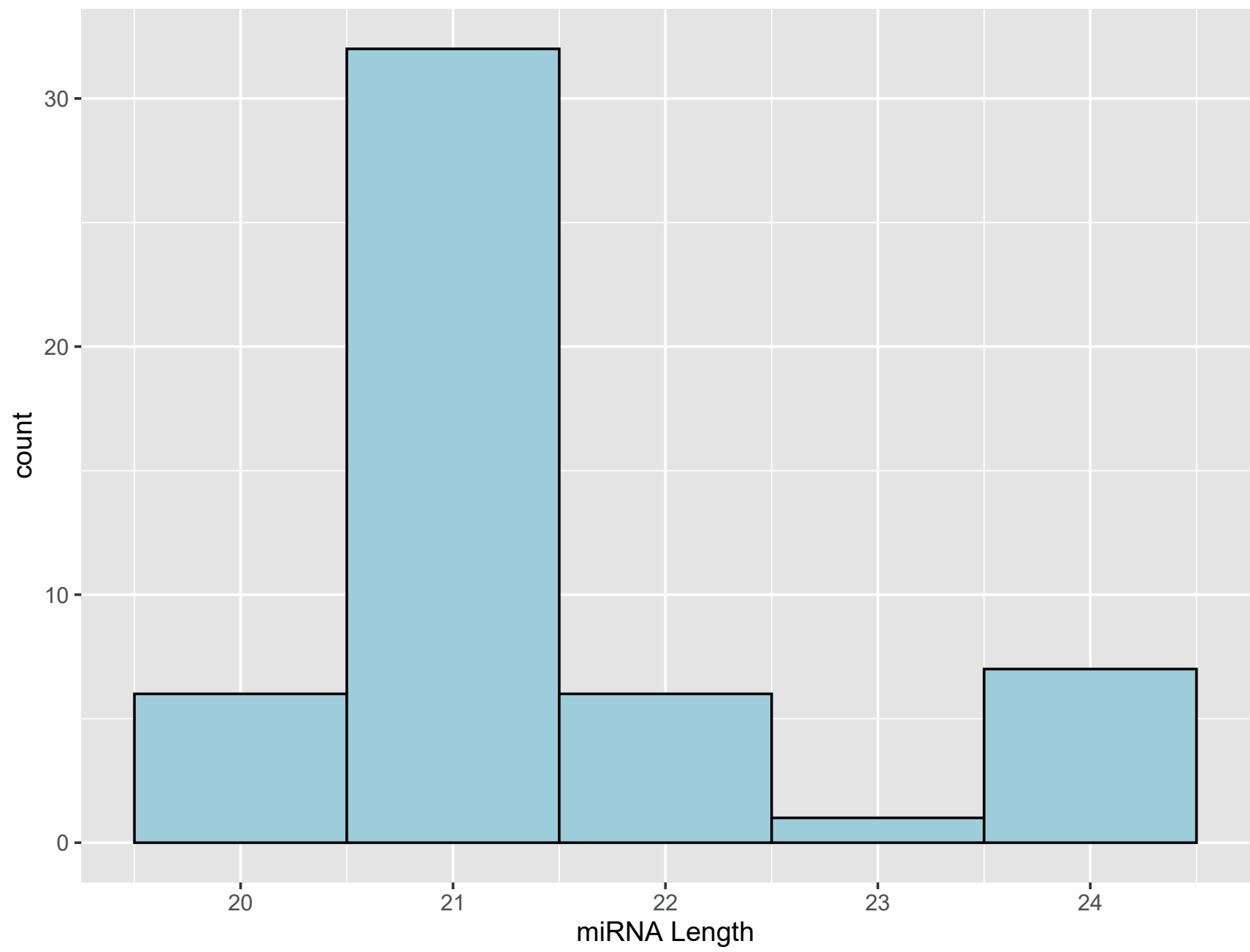


Fig. S33 The length distribution of the 52 identified miRNAs in seed formation.

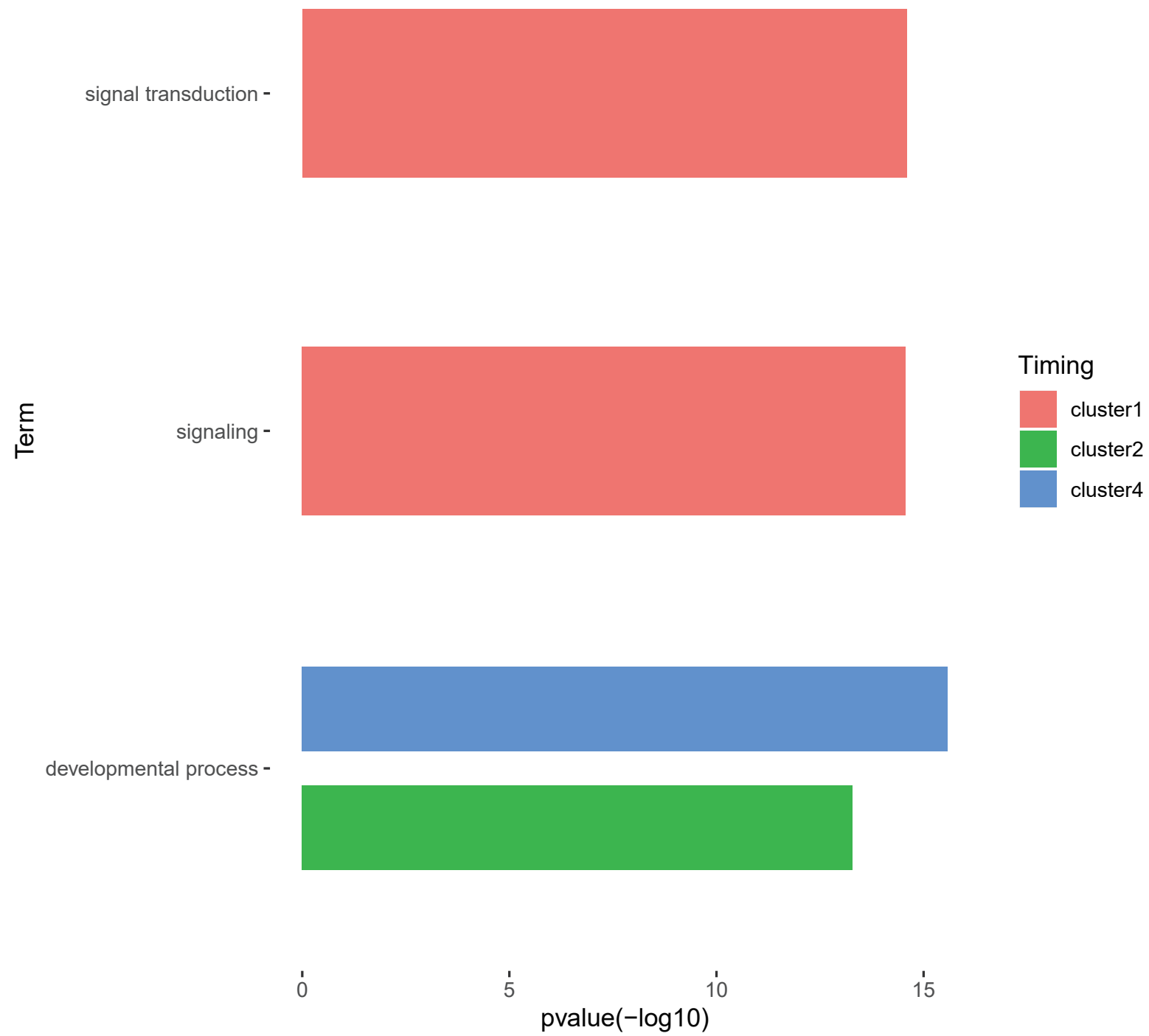


Fig. S34 Enrichment of GO terms in the miRNA-targeted genes for each cluster with a q-value < 0.05.

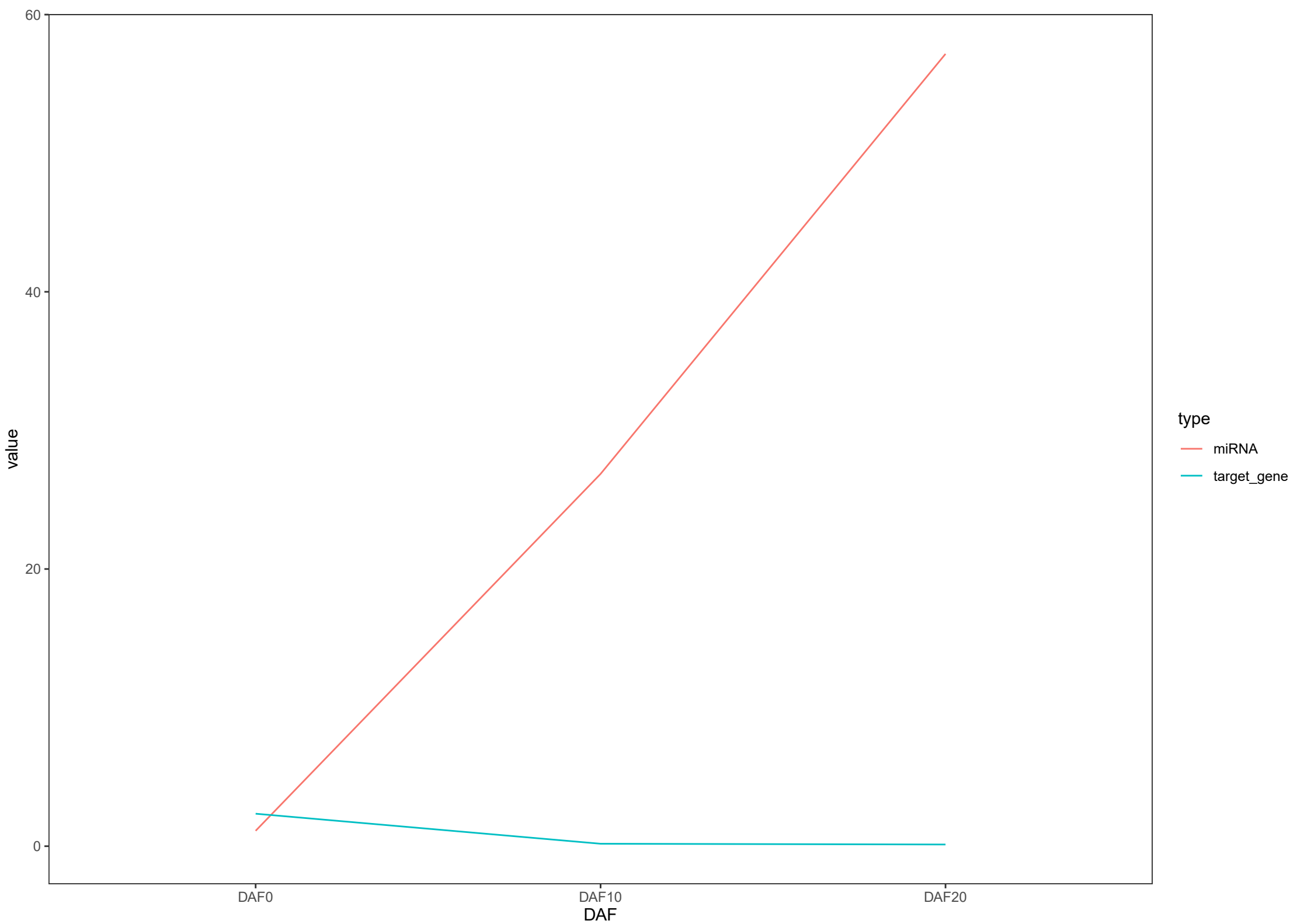


Fig. S35 Expression relationship of Cluster_135896 (ath-miR156h) and its possible target gene *CarFAD2-4* throughout seed development.

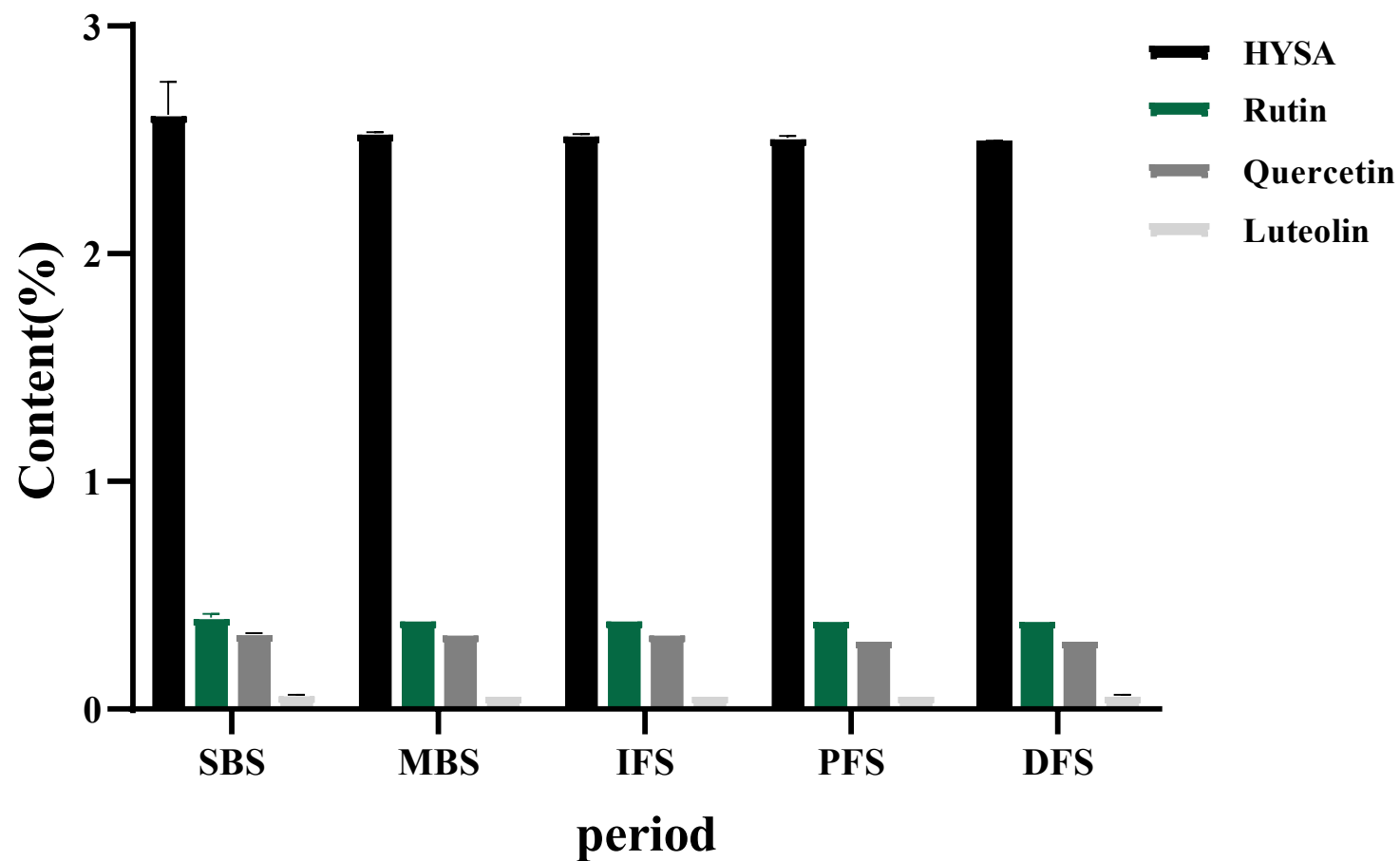


Fig. S36 Contents of HSYA, rutin, luteolin, and quercetin from five different stages: small bud stage (SBS), middle bud stage (MBS), initial flowering stage (IFS), peak flowering stage (PFS), and decayed flowering stage (DFS) during flower development.

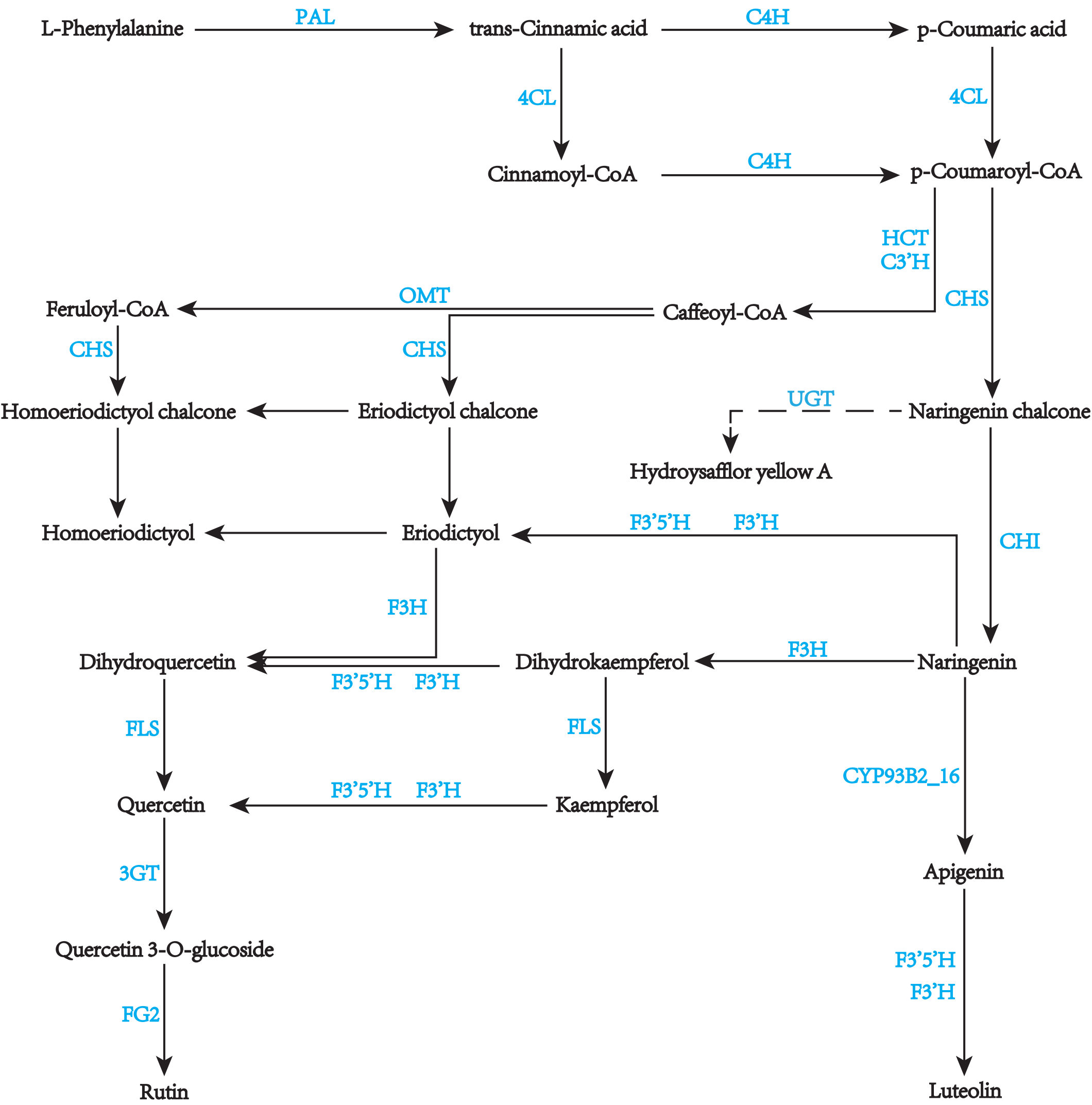


Fig. S37 Schematic diagram of the flavonoid biosynthesis pathway in safflower. Intermediate compounds and enzymes in the flavonoid biosynthesis pathway are represented in black and blue type, respectively.

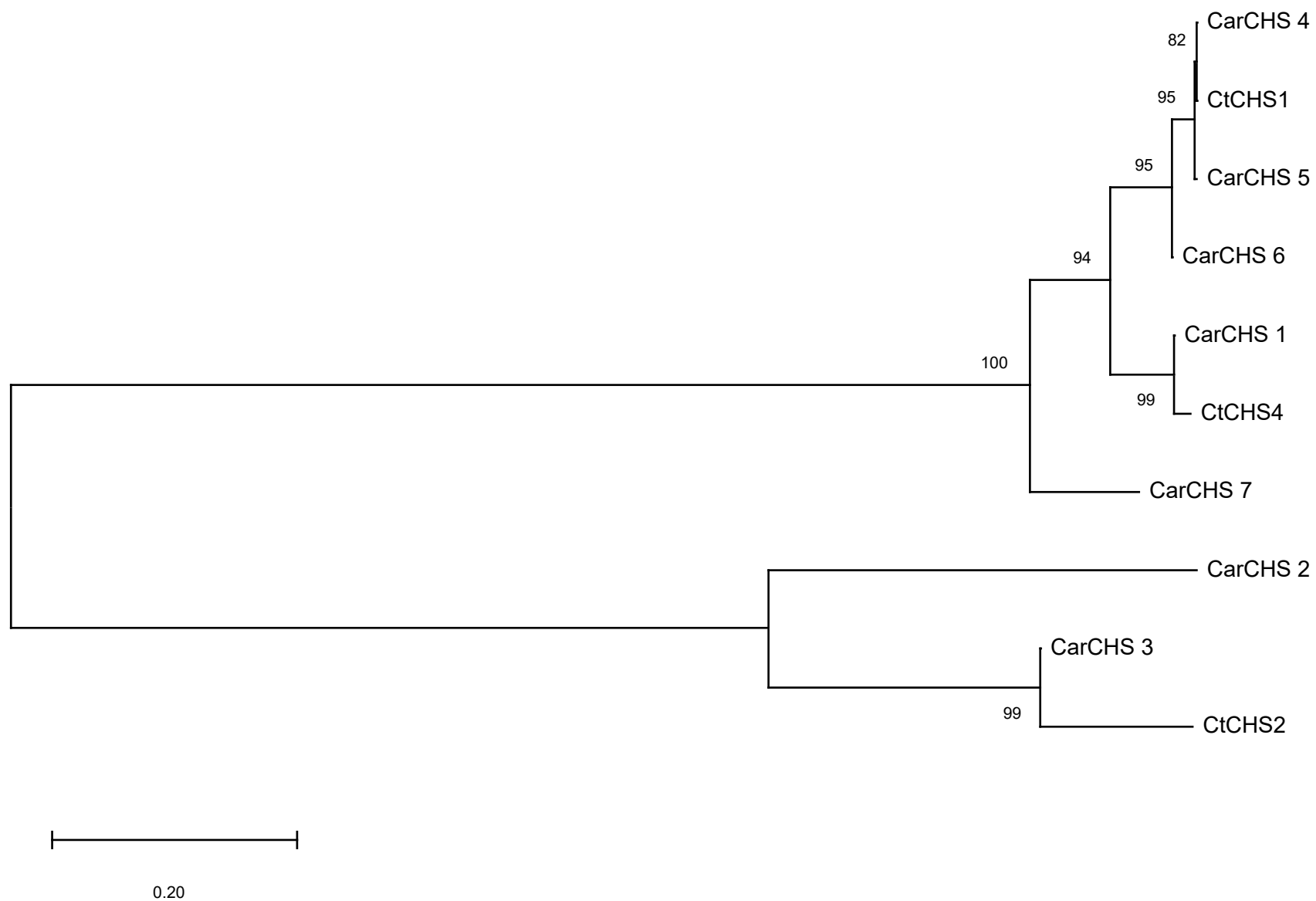


Fig. S38 Gene tree of *CarCHSs* and reported *CtCHS1*, *CtCHS2*, and *CtCHS4* constructed by neighbor-joining method. Only bootstrap values > 50 are shown.

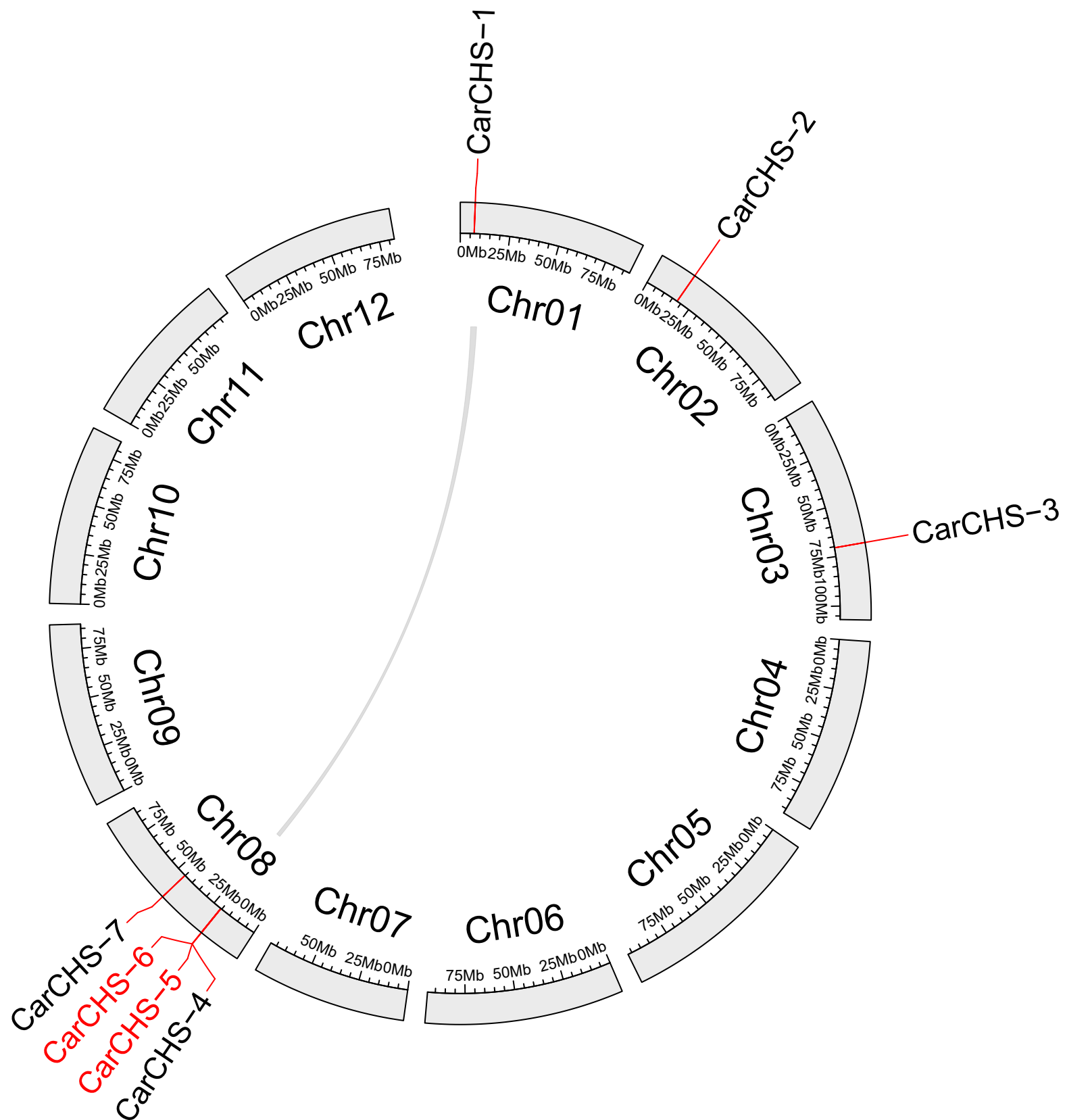


Fig. S39 Distribution of *CarCHS*s in 12 safflower chromosomes. The links among chromosomes represent collinear blocks, the genes marked with red represent the tandem duplication.

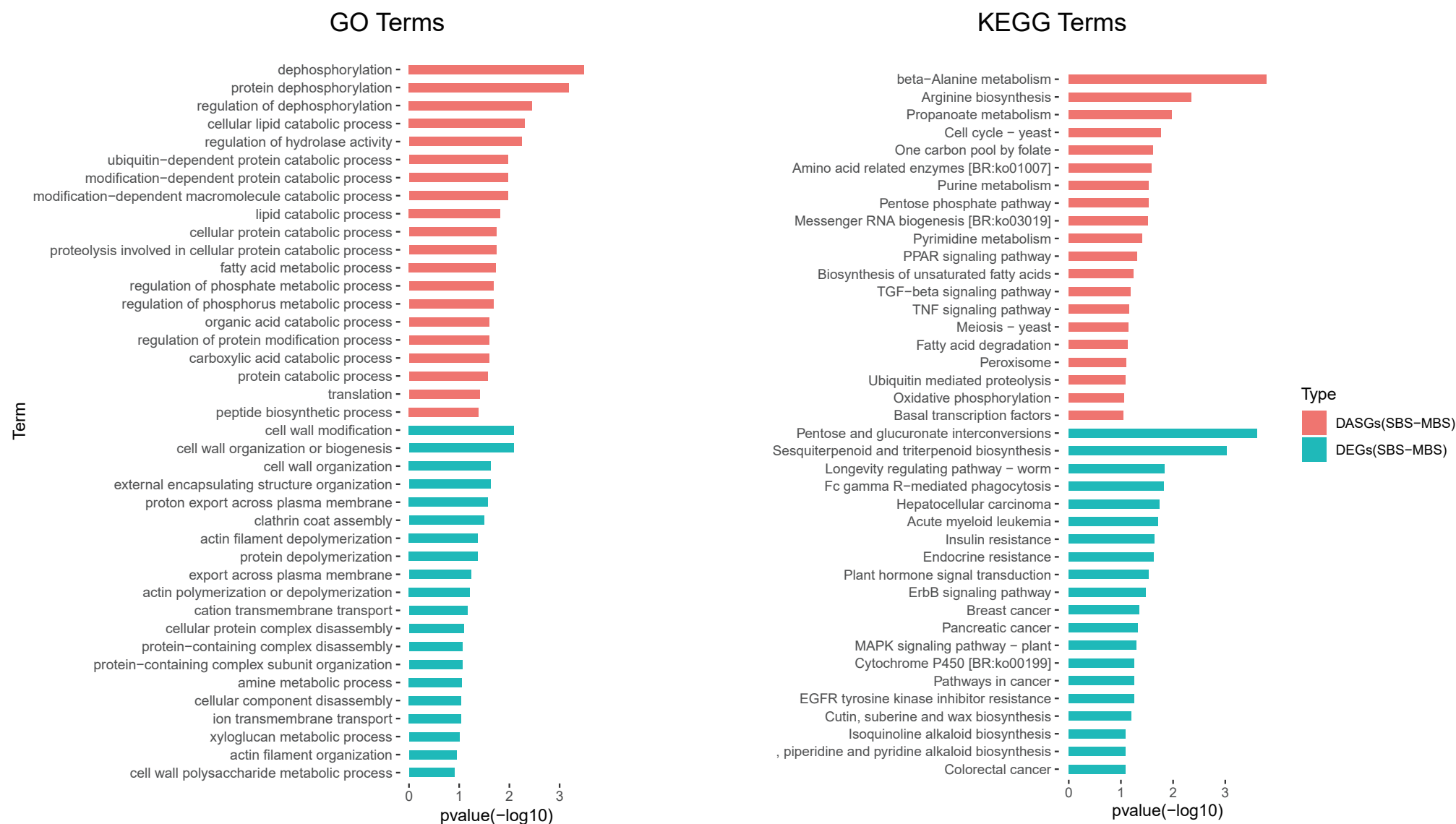


Fig. S40 Top 20 GO and KEGG terms associated with the unique differentially expressed genes and differentially alternatively spliced genes in the comparison of flowers at the small bud stage (SBS) versus middle bud stage (MBS).

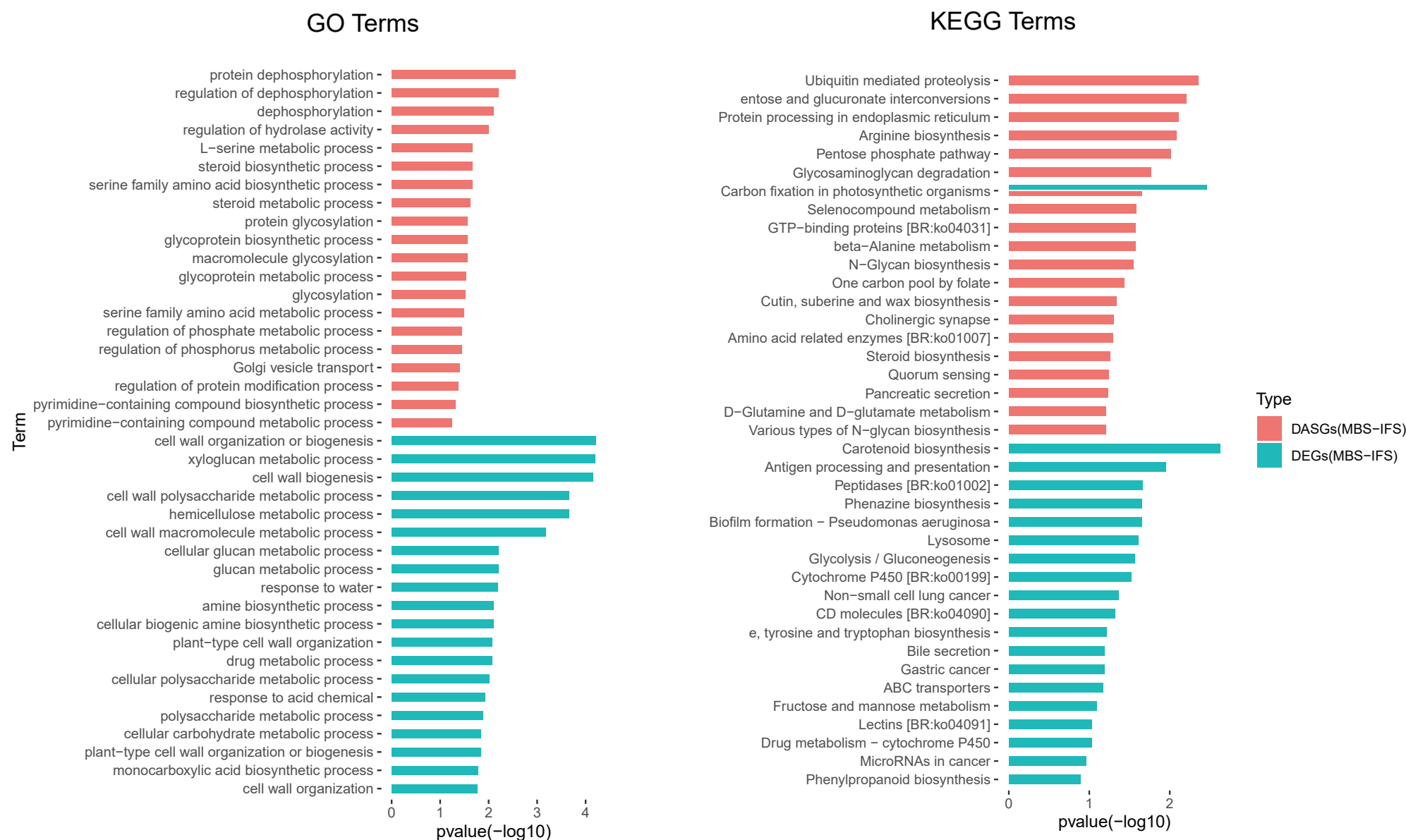


Fig. S41 Top 20 GO and KEGG terms associated with the unique differentially expressed genes and differentially alternatively spliced genes in the comparison of flowers at the middle bud stage (MBS) versus the initial flowering stage (IFS).

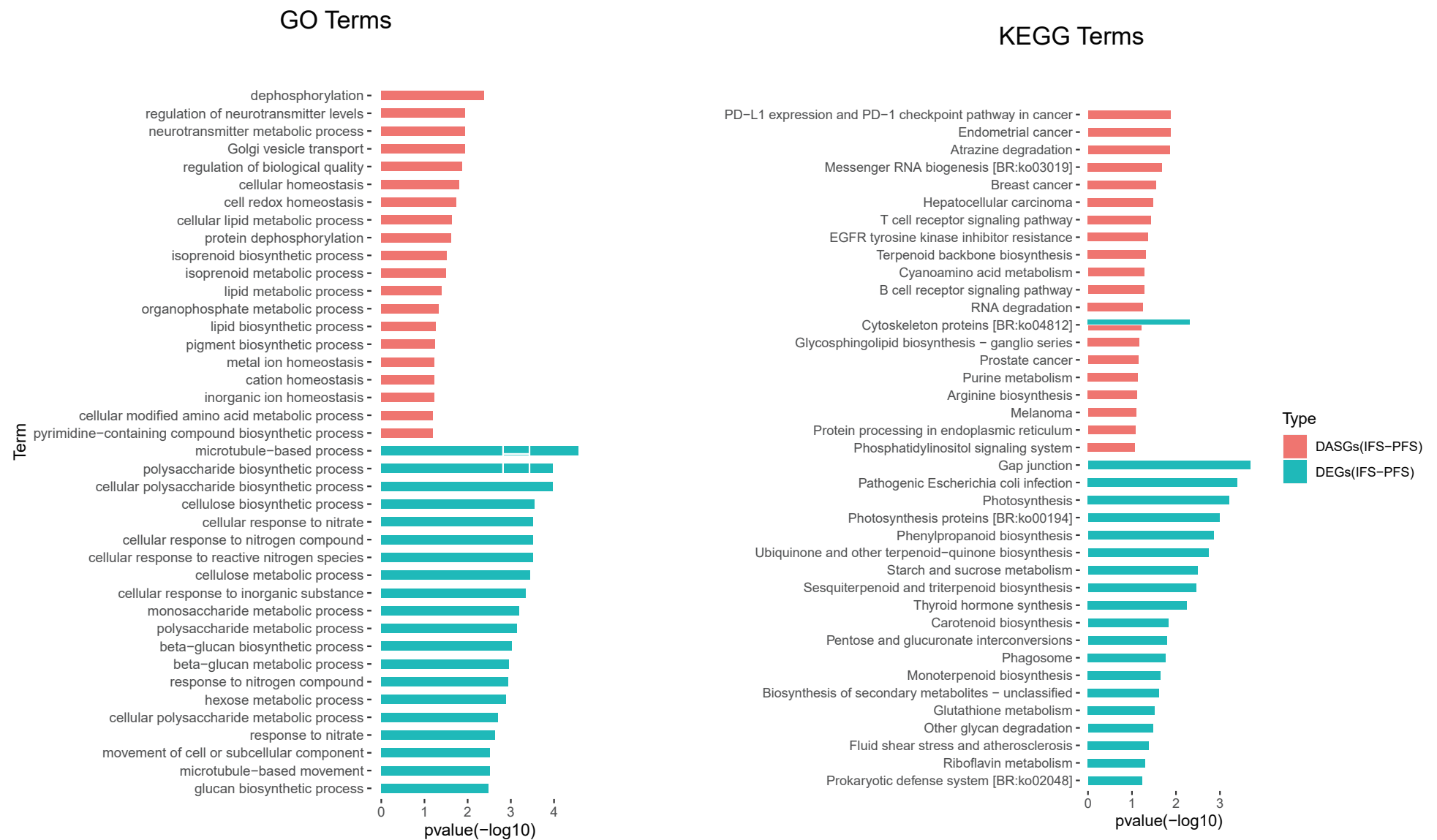


Fig. S42 Top 20 GO and KEGG terms associated with the unique differentially expressed genes and differentially alternatively spliced genes in the comparison of flowers at the initial flowering stage (IFS) versus the peak flowering stage (PFS).



Fig. S43 Top 20 GO and KEGG terms associated with the unique differentially expressed genes and differentially alternatively spliced genes in the comparison of flowers at the peak flowering stage (PFS) versus the decayed flowering stage (DFS).

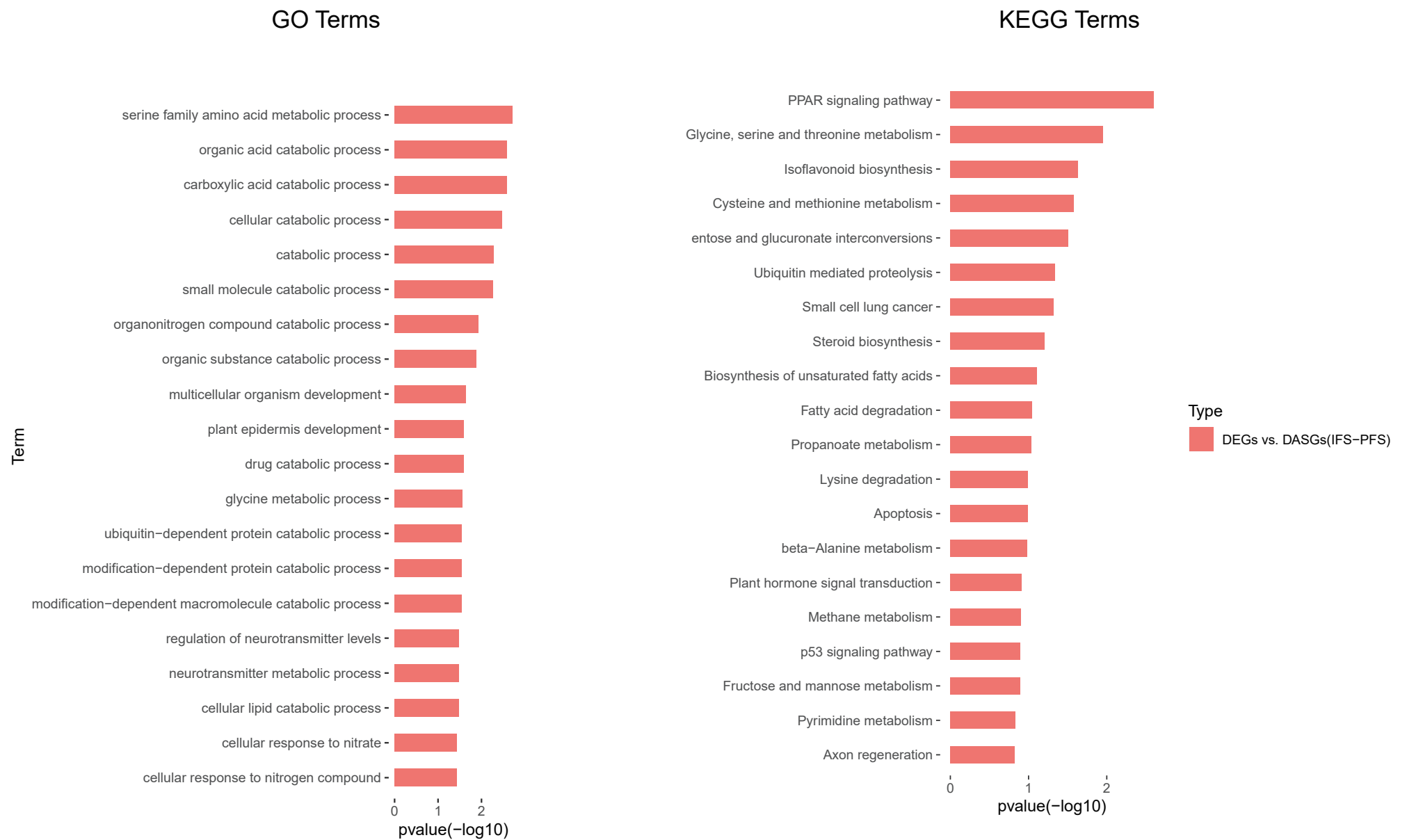


Fig. S44 Top 20 GO and KEGG terms associated with the common differentially expressed genes and differentially alternatively spliced genes in the comparison of flowers at the initial flowering stage (IFS) versus the peak flowering stage (PFS).



Fig. S45 Top 20 GO and KEGG terms associated with the common differentially expressed genes and differentially alternatively spliced genes in the comparison of flowers at the peak flowering stage versus (PFS) the decayed flowering stage (DFS).

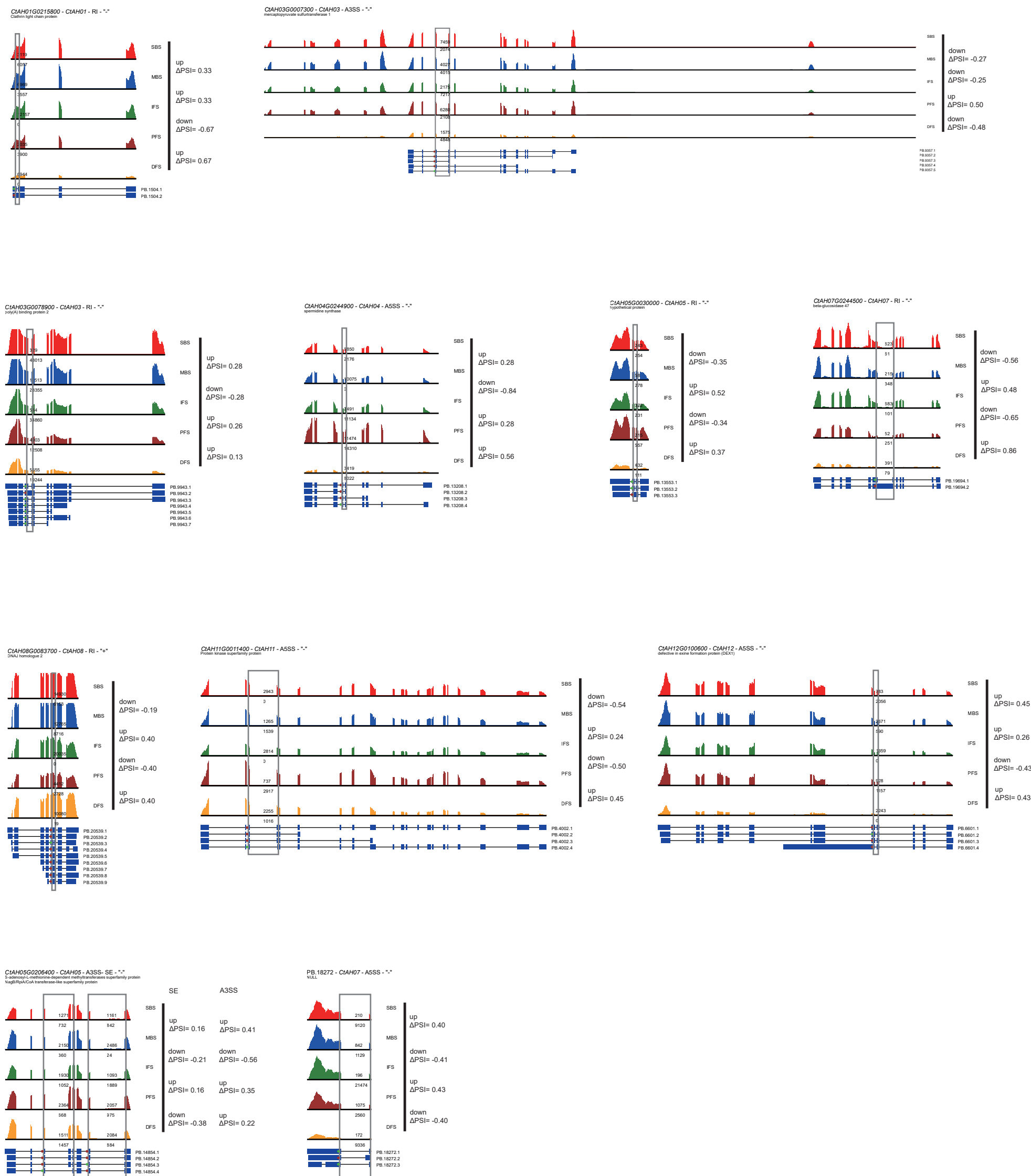


Fig. S46 Expression pattern of the 11 genes common in the differentially alternatively spliced of the four groups (SBS-MBS, MBS-IFS, IFS-PFS, and PFS-DFS). For each gene, AS-covering and total long read counts are shown in AS variants in each stage of flower development, and differential expression of AS variants is indicated by ΔPSI on the left. The ‘up’, ‘down’, and ‘-’ on the left represent upregulated, downregulated, and no differential expression between adjacent groups, respectively. Each vertical line at the bottom represents continuous DASGs among different stages.

CarCHS4	MASLTDIAEIRKAQRAEGPATILAIGTATPNNCIYQADYPDYYFRITNSEHMVELKQKFMRMCDKSMIRKRYMHITEEFL	80
PB.21282.1	MASLTDIAEIRKAQRAEGPATILAIGTATPNNCIYQADYPDYYFRITNSEHMVELKQKFMRMCDKSMIRKRYMHITEEFL	80
PB.21282.2	MASLTDIAEIRKAQRAEGPATILAIGTATPNNCIYQADYPDYYFRITNSEHMVELKQKFMRMCDKSMIRKRYMHITEEFL	80
Consensus	masltdiaeirkaqraegpatilaigtatpnnciyqadypdyyfritnsehmvelkqkfkrmcdksmirkrymwhiteefl	
CarCHS4	KENPNMCEYMAPSLDARQDVVVVEVPKLGKEAATKAKEWGQPKSKITHLIVCTTSGVDMPGADYQITKLLGLRPSVKRF	160
PB.21282.1	KENPNMCEYMAPSLDARQDVVVVEVPKLGKEAATKAKEWGQPKSKITHLIVCTTSGVDMPGADYQITKLLGLRPSVKRF	160
PB.21282.2	KENPNMCASTVTCRVRVCCSLLTRGRSRRRMVAPPPKRWIGVFECLGLVIVRWRWSFTVVSQFPPLPLKIHNYYS..NPL	158
Consensus	kenpnmceymapsldarqdvvvvevpklgkeaatkaikewgqpskithlivcttsgvdmvgadyqitkllglrpsvkrf	
CarCHS4	MMYQQGCFAGGTVIRLAKDLAENNKGARVLVVCSEITAVTFRGPNETHLDSLVGQALFGDGAAAVIVGADPDLATERPLF	240
PB.21282.1	MMYQQGCFAGGTVIRLAKDLAENNKGARVLVVCSEITAVTFRGPNETHLDSLVGQALFGDGAAAVIVGADPDLATERPLF	240
PB.21282.2	CIYNNAELG...FFLYILINIESVVCVFFMLCMILOQWVVPVIVWLLNSMSLICILX.....	210
Consensus	mmyqqgcfaggtvlrlakdlaennkgarvlvvcseitavtfrgpnethldslvgqalfgdgaaavivgadpdlaterplf	
CarCHS4	EMVSAAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIEKALVQAFSPLGISDWNLSFWIAHPGGPAILDQVEQKL	320
PB.21282.1	EMVSAAQTILPDSEGAIDGHLREVGLTFHLLKDVPGLISKNIEKALVQAFSPLGISDWNLSFWIAHPGGPAILDQVEQKL	320
PB.21282.2		210
Consensus	emvsaaqtildpdsegaidghlrevgltfhllkdvpgliskniekalvqafsplgisdwnslfwiahpggpaildqveqkl	
CarCHS4	GLKEEKMRAIRHVLSEYGNMSSACVLFIIDEMRRKSAEDGCATTGEGLDWGVLFSGFGPGLTVETVVLHSVPTTPIA	396
PB.21282.1	GLKEEKMRAIRHVLSEYGNMSSACVLFIIDEMRRKSAEDGCATTGEGLDWGVLFSGFGPGLTVETVVLHSVPTTPIA	396
PB.21282.2		210
Consensus	glkeekmratrhlseeygnmssacvlfiiidemrrksaedgcattgegldwgvlfsgfgpgltvetvvlhsvpttpia	

Fig. S47 Protein sequence alignment of two alternative-splicing variants of *CarCHS4* in flower development.

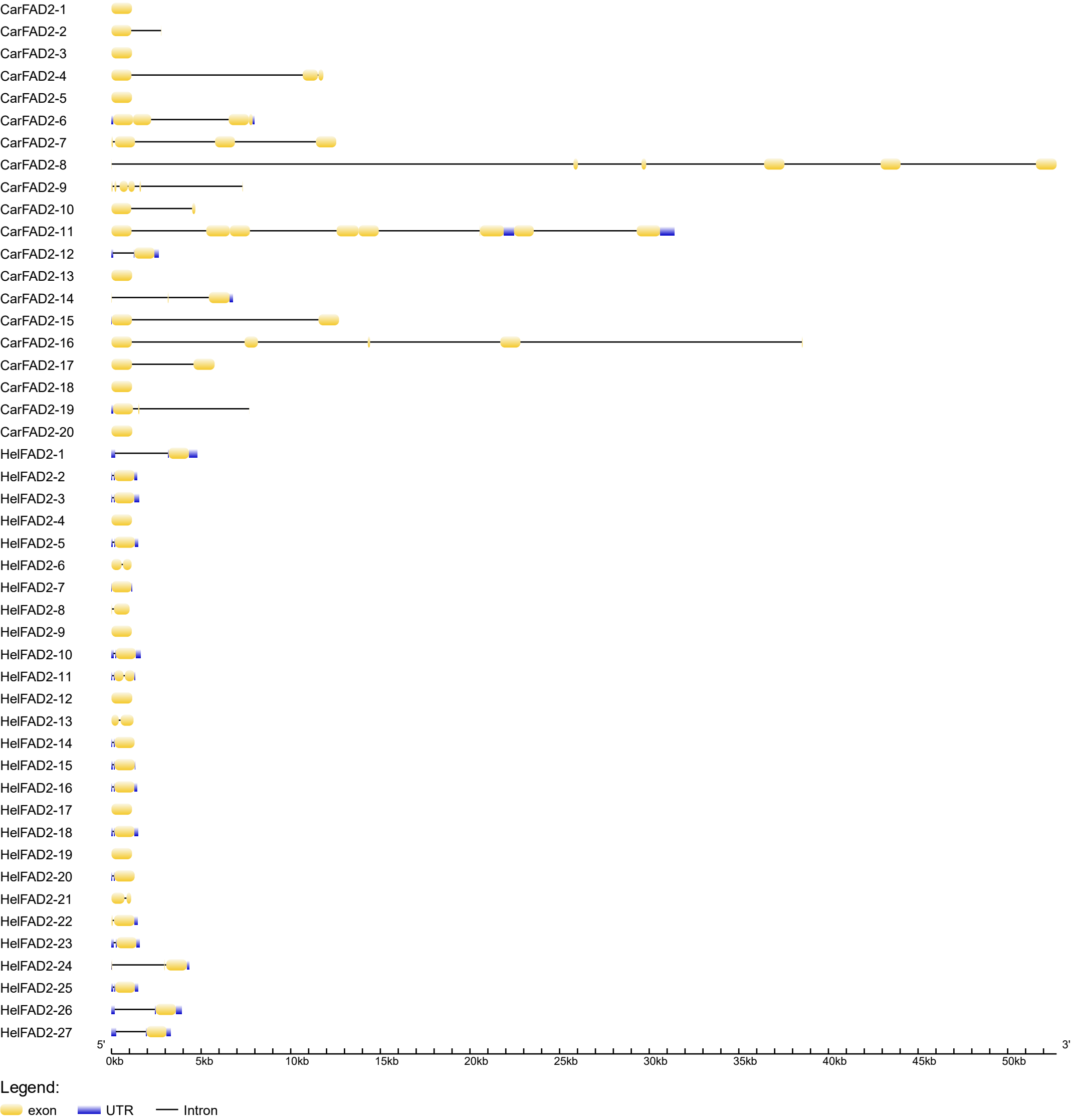


Fig. S48 Gene structure of *FAD2* members identified in safflower and sunflower.

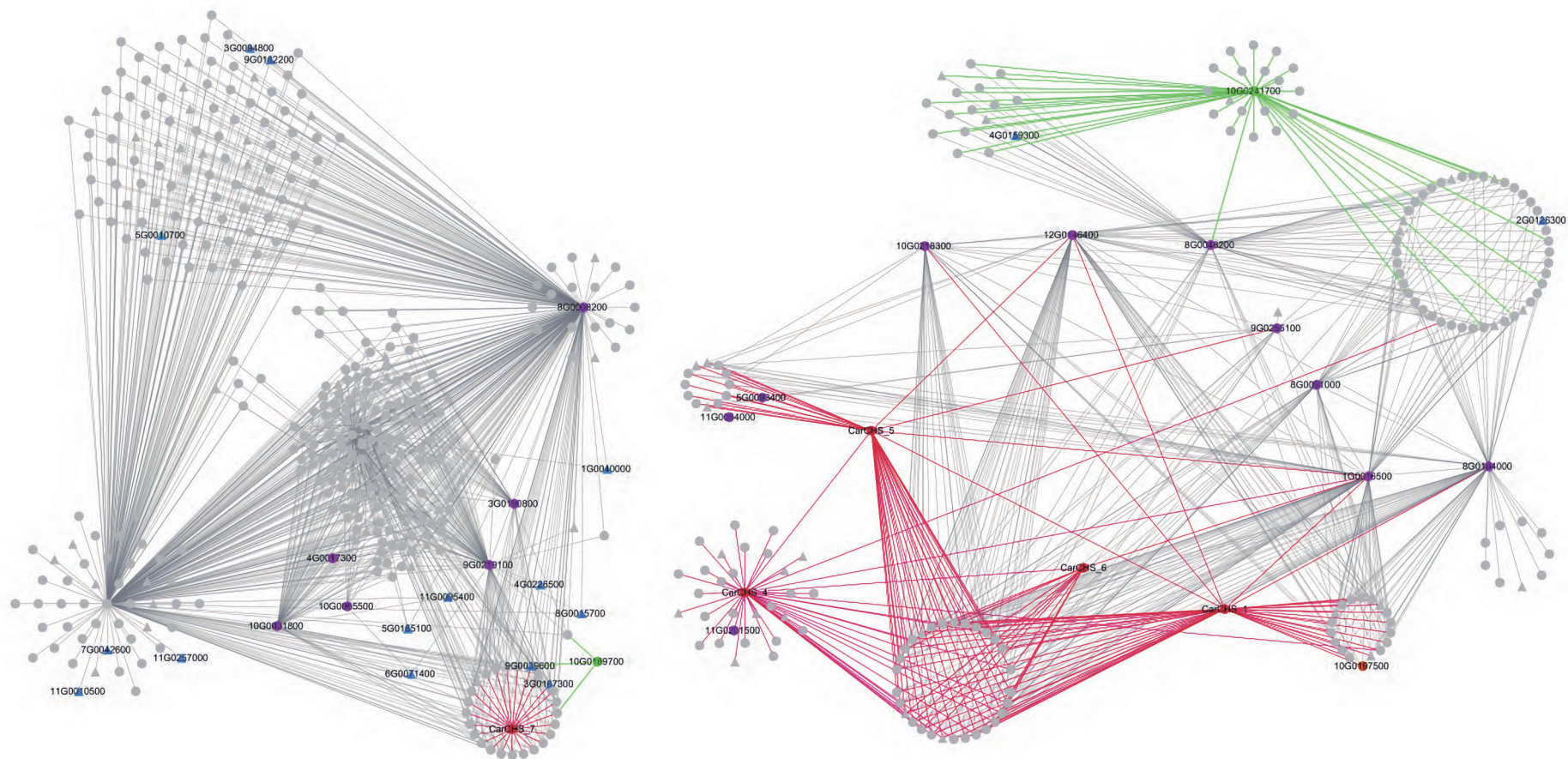


Fig. S49 *CarCHS*s-associated coexpression network in flower. Pink and green dots represent *UGTs* and *ASPs*, respectively; blue triangles represent transcriptional factors, *MYBs* and *bHLHs*.