

**Chromosome-Scale Genome Assembly Provides Insights into the Molecular
Mechanisms of Tissue Development of *Populus wilsonii***

**Chaofeng Li^{1*}, Haitao Xing^{2*}, Can Li³, Yun Ren², Honglei Li², Xue-Qin Wan⁴, Chunlan Lian⁵,
Jia-Xuan Mi⁴, Shengkui Zhang³**

¹Maize Research Institute, Southwest University, Chongqing, 400715, PR China

²College of Landscape Architecture and life Science/Institute of special Plants, Chongqing
University of Arts and Sciences, Chongqing 402168, PR China

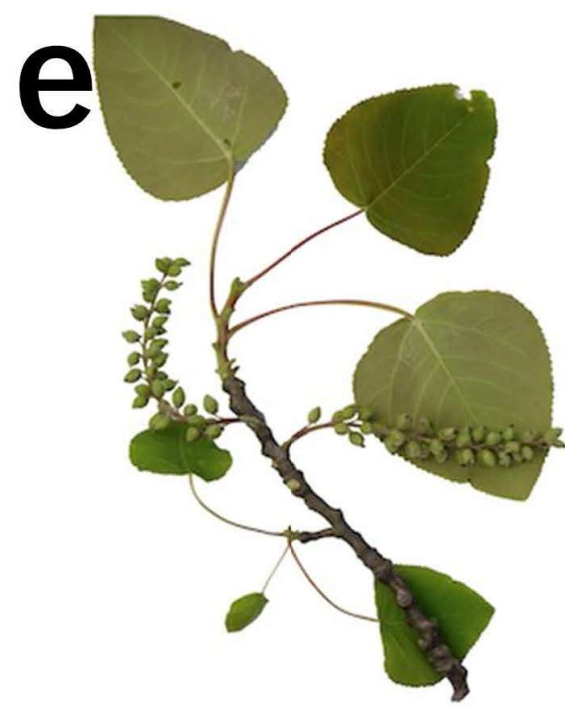
³School of Bioengineering, Qilu University of Technology, Jinan 250353, Shandong, PR China

⁴College of Forestry, Sichuan Agricultural University, Chengdu 611130, PR China

⁵Asian Research Center for Bioresource and Environmental Sciences, Graduate School of
Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Midori-cho, Nishitokyo, Tokyo
188-0002, Japan

Chaofeng Li and Haitao Xing contributed equally to this work.

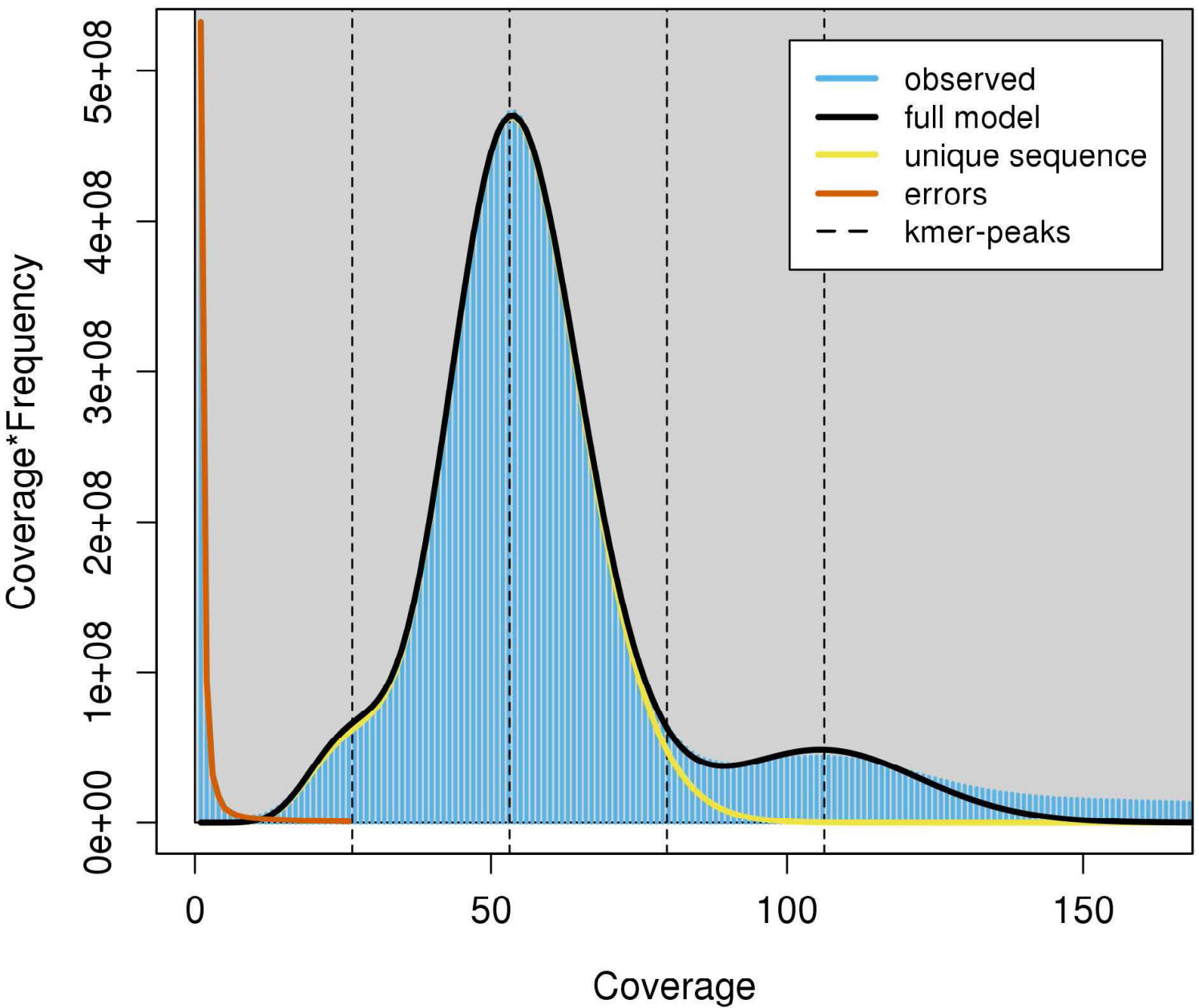
Corresponding author: Shengkui Zhang Email: zsk8920@gmail.com



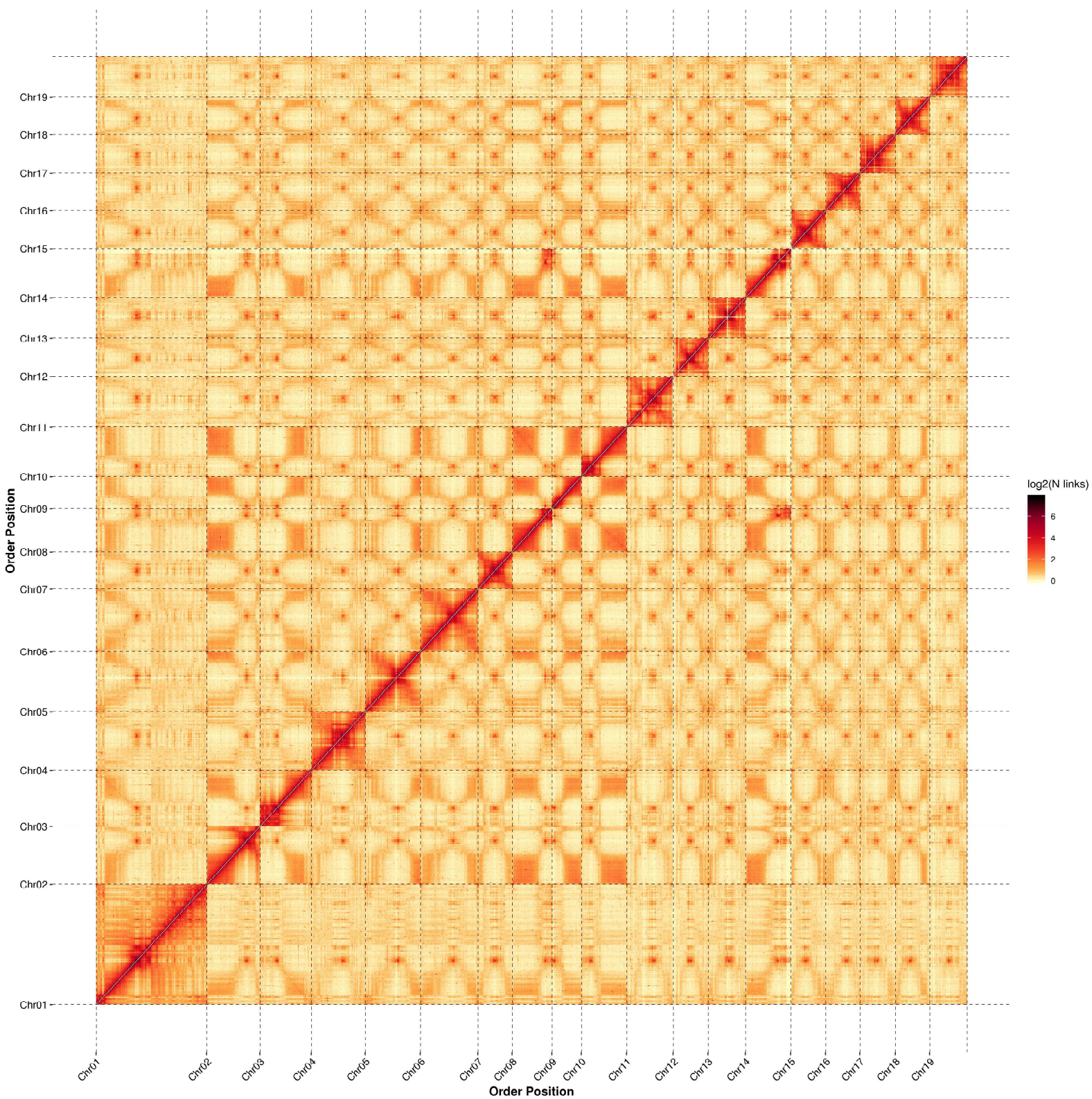
Supplementary Figure 1. Phenotype of the reference genome accession, “*Populus wilsonii*”.
(a) *Populus wilsonii* in their natural habitat. (b) Branches. (c) Male flowers. (d) Leaf bud. (e) Female flowers.

GenomeScope Profile

len:439,392,517bp uniq:59.8%
aa:99.6% ab:0.422%
kcov:26.6 err:0.164% dup:1.25 k:19 p:2



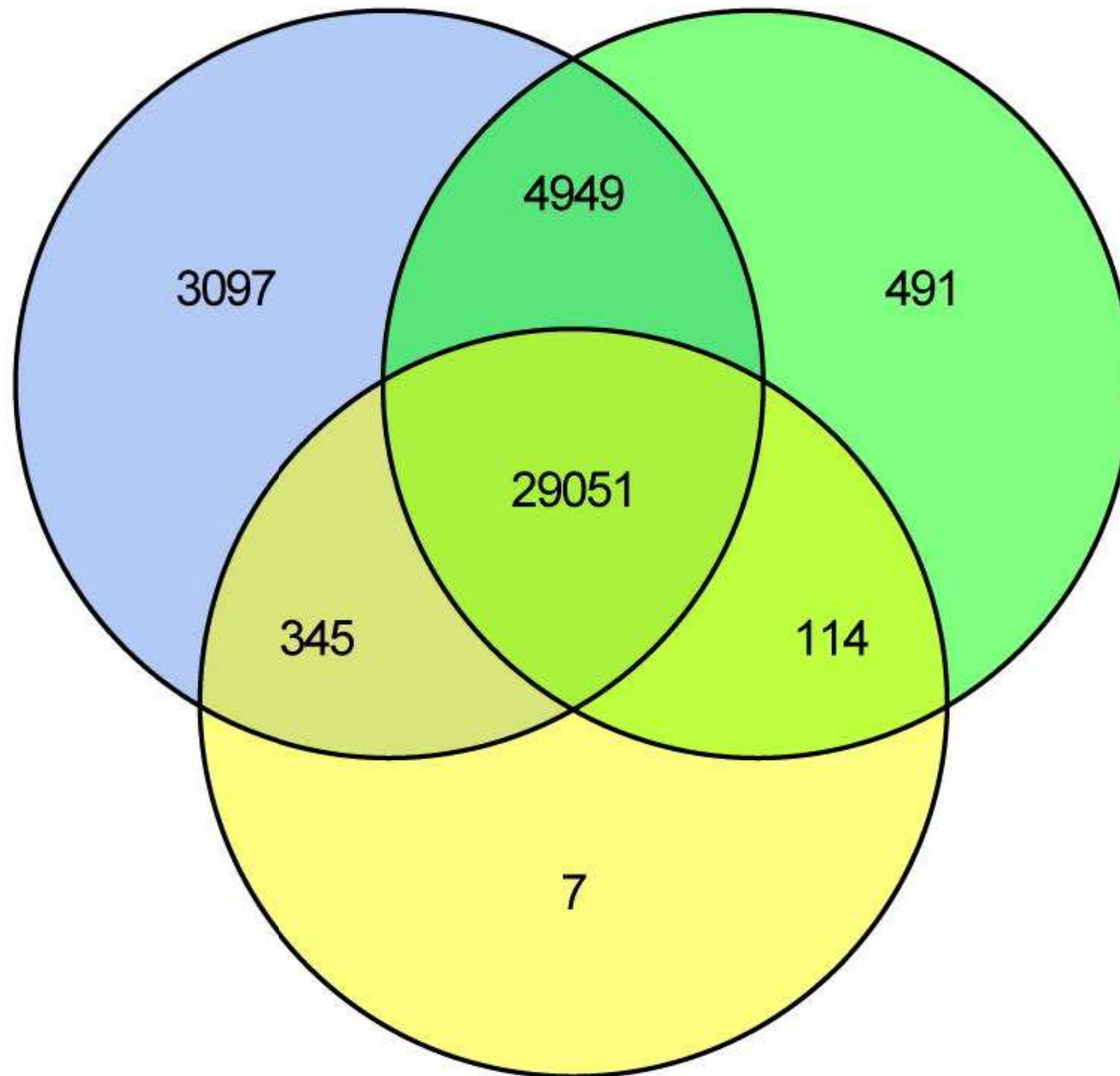
Supplementary Figure 2. The size estimation of the *Populus wilsonii* genome. The genome size was estimated by calculating the distribution of 19-mer frequency in the sequencing reads. The x-axis is the depth (X), and the y-axis is the proportion of sequences that represent the frequency at that depth divided by the total frequency of all depths.



Supplementary Figure 3. The Hi-C chromatin interaction map for the 19 pseudomolecules of *Populus wilsonii* genome.

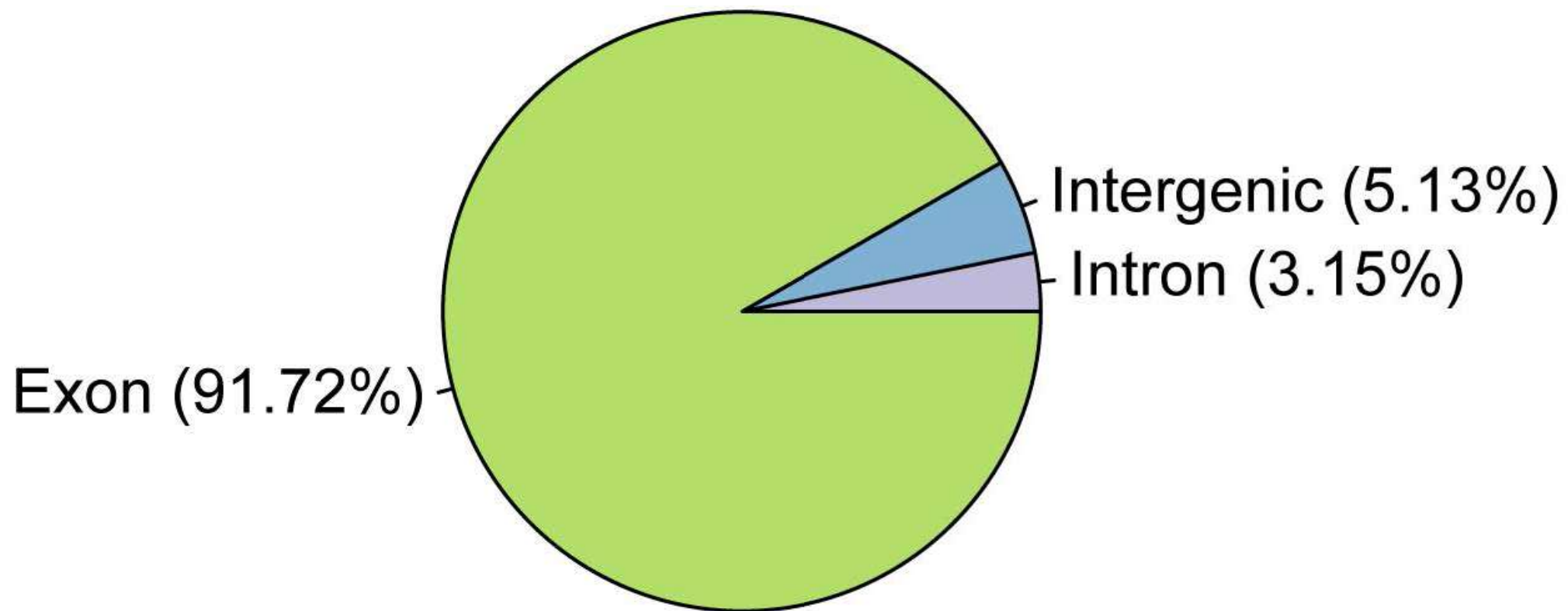
Ab initio

Homology

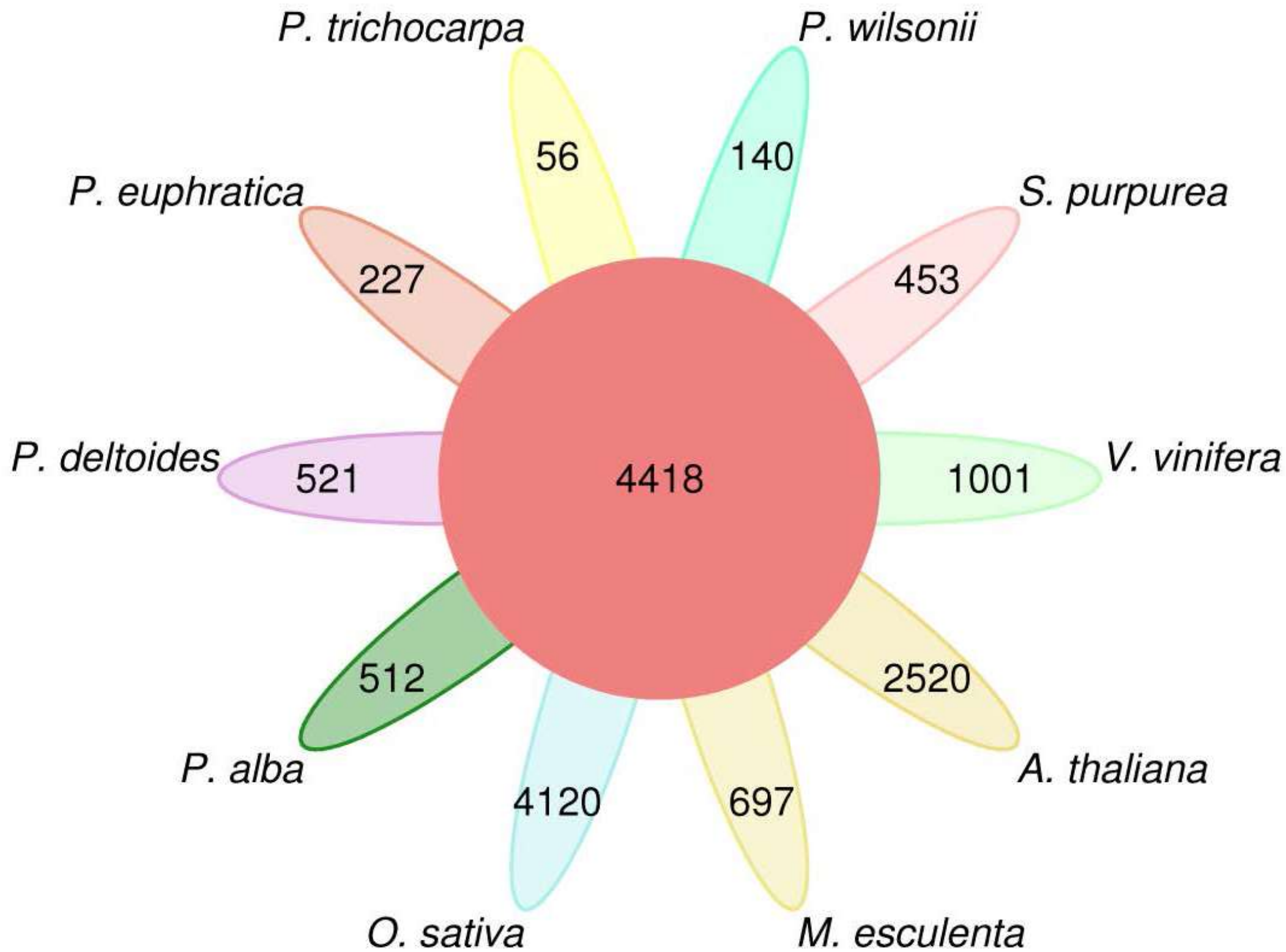


Transcriptome

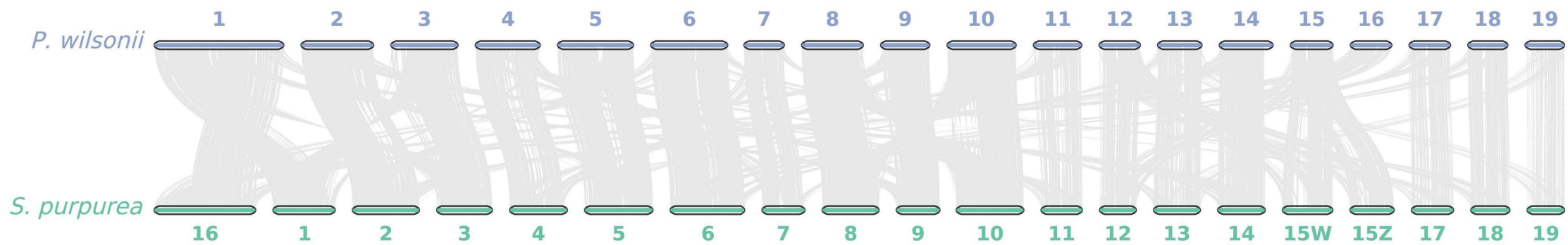
Supplementary Figure 4. The number of protein-coding gene models predicted by the homology-based, de novo and RNA-Seq based methods.



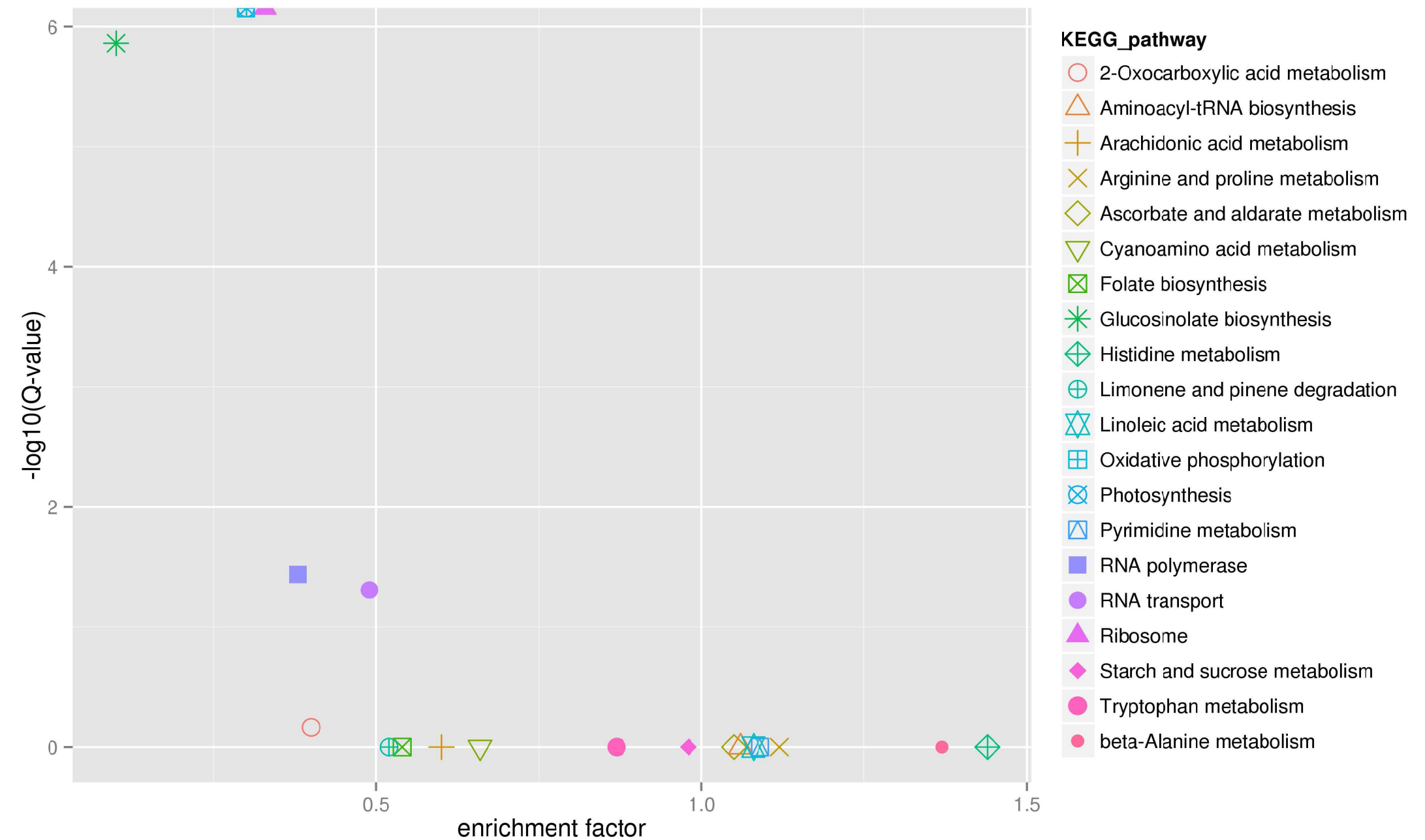
Supplementary Figure 5. Statistical results of RNA-seq clean data map to *Populus wilsonii* genome.



Supplementary Figure 6. Syntenic blocks between *Populus wilsonii* and *Salix purourea*.

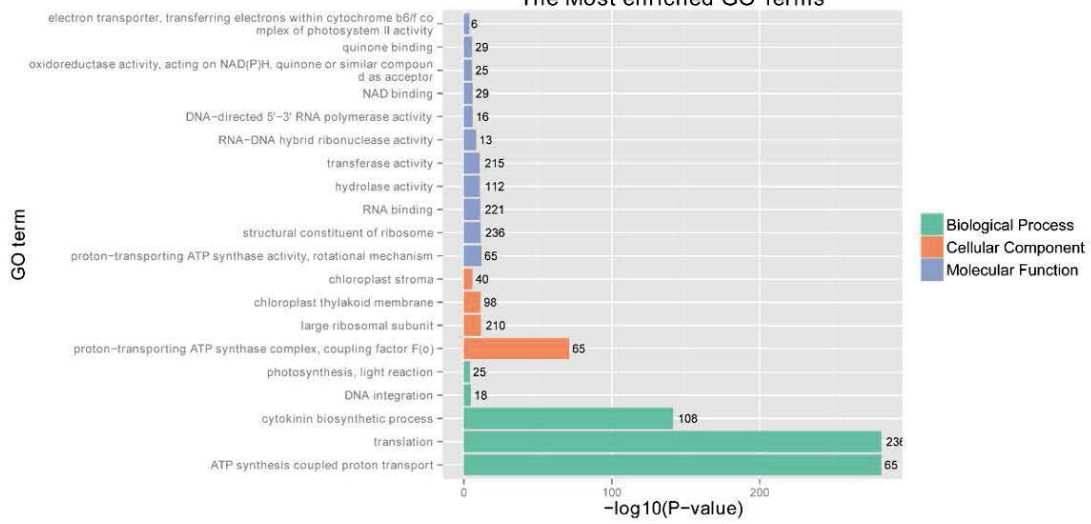


Supplementary Figure 7. The number of gene families shared among all species shown in Venn diagrams.

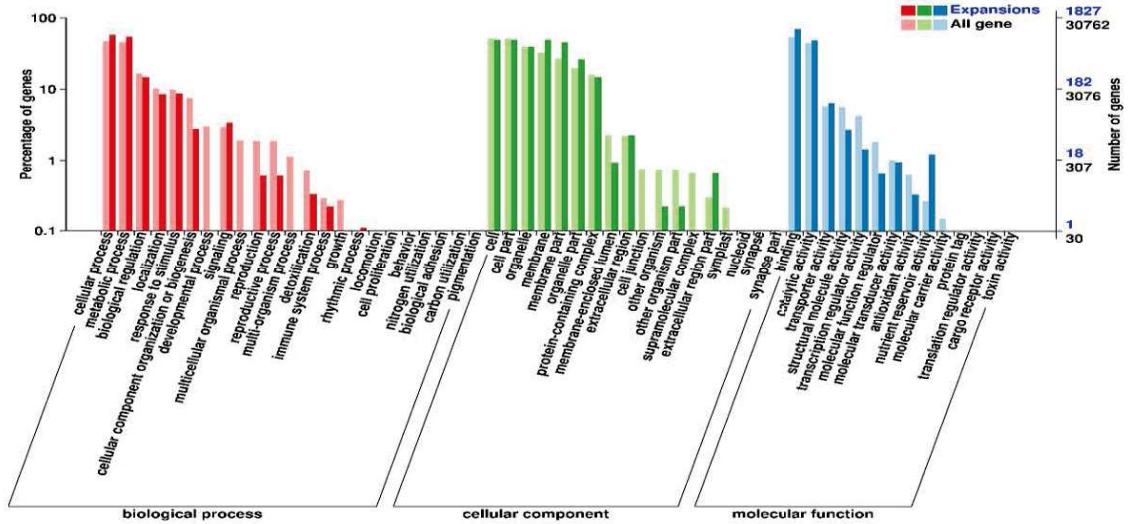


Supplementary Figure 8. KEGG enrichment analysis of unique gene families in *Populus wilsonii*.

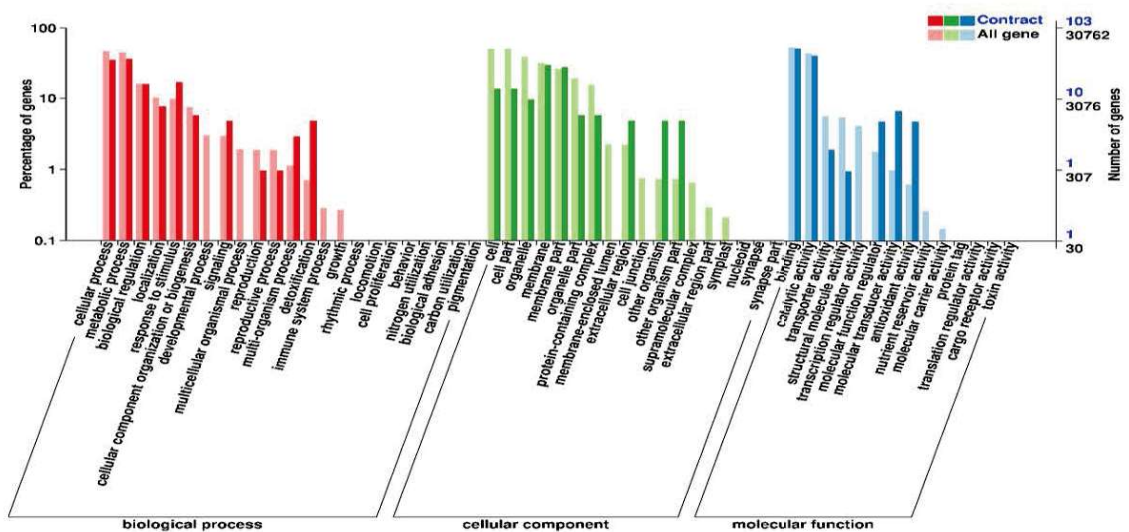
a



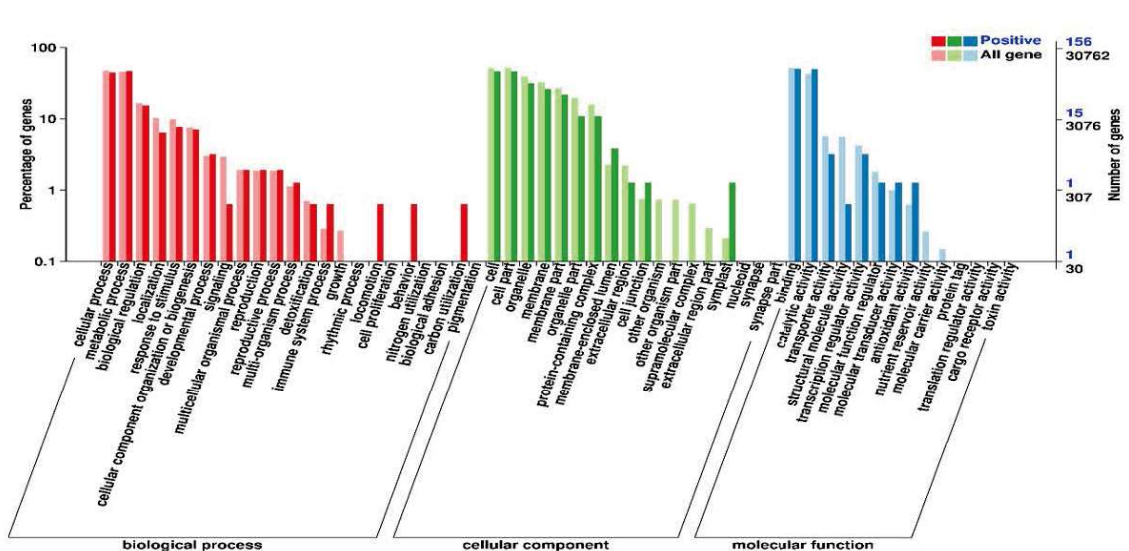
b



c

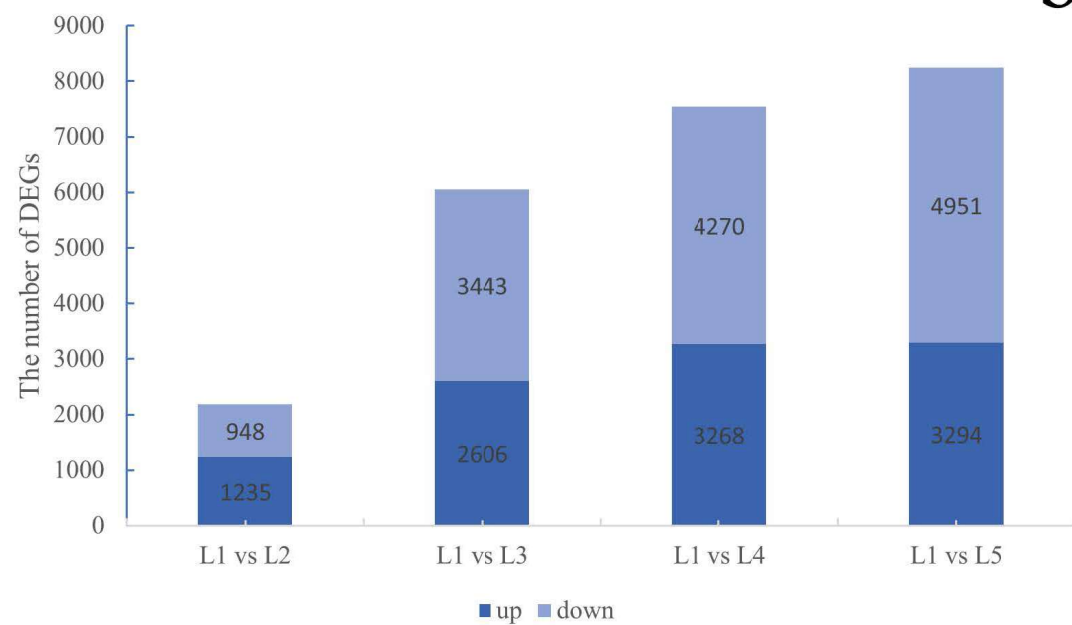


d

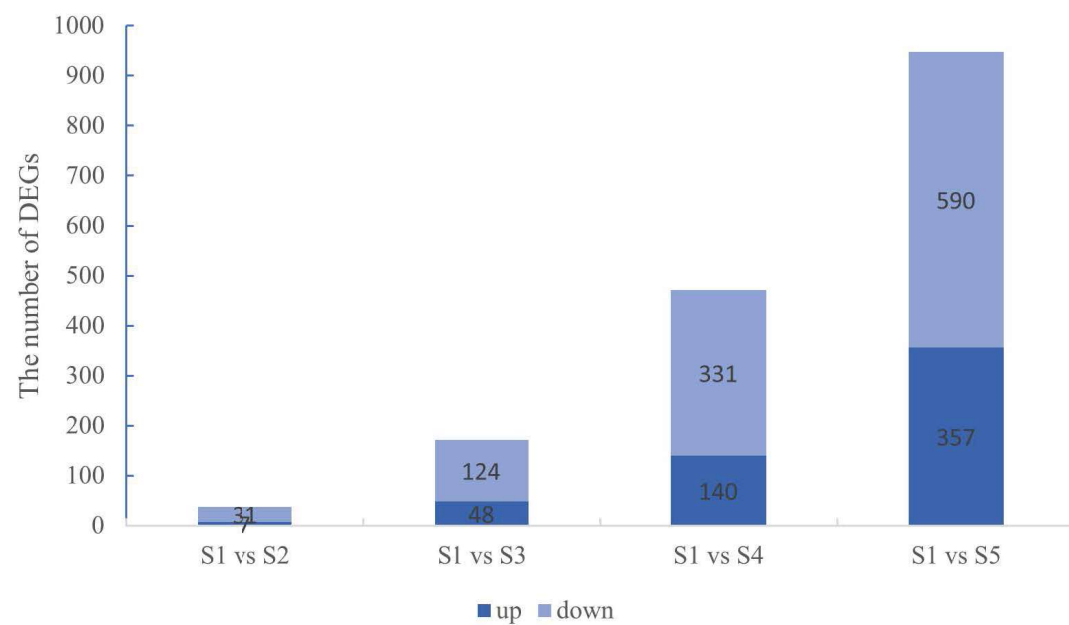


Supplementary Figure 9. GO enrichment analysis of unique gene families(a), expansion gene families(b), contraction gene families(c) and positive selected gene families(d) in *Populus wilsonii*.

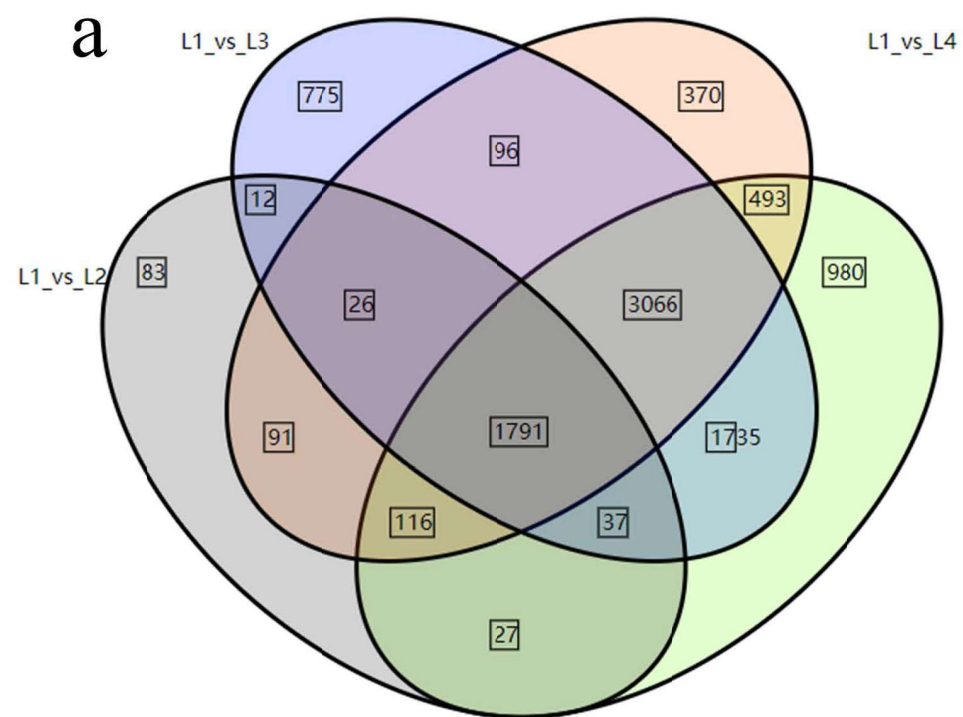
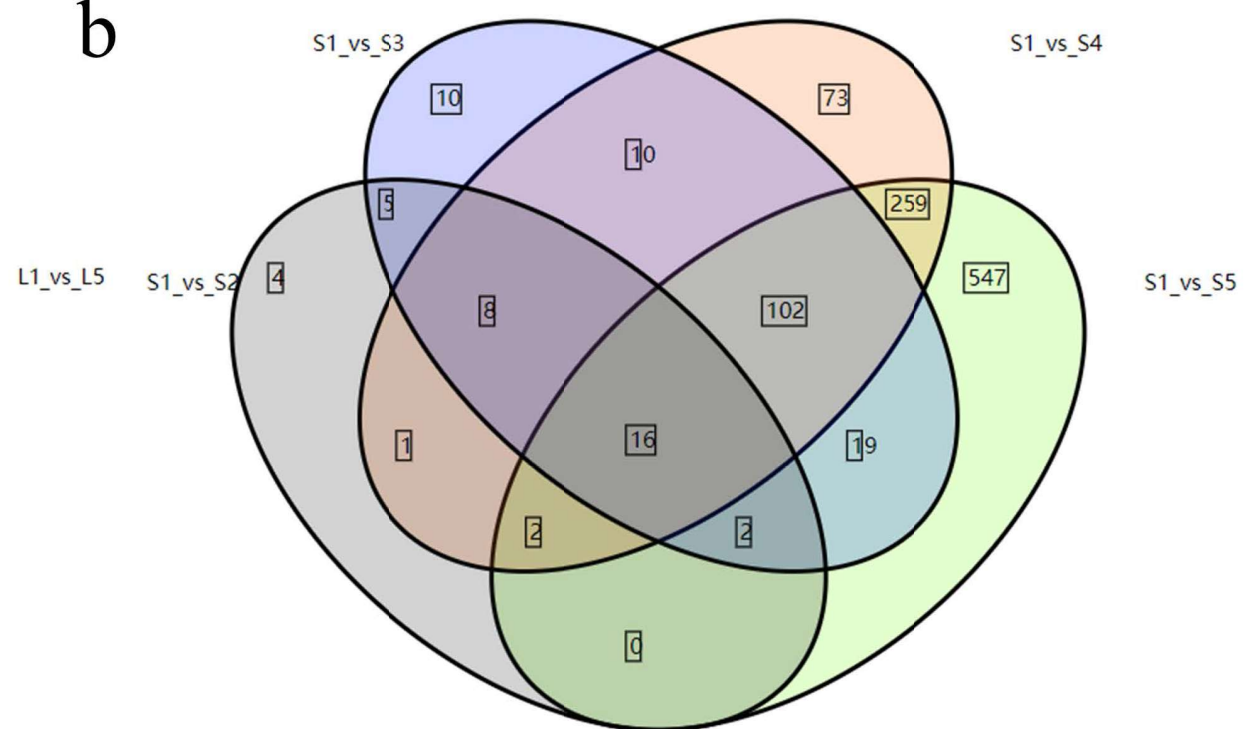
a The DEGs in leaf development



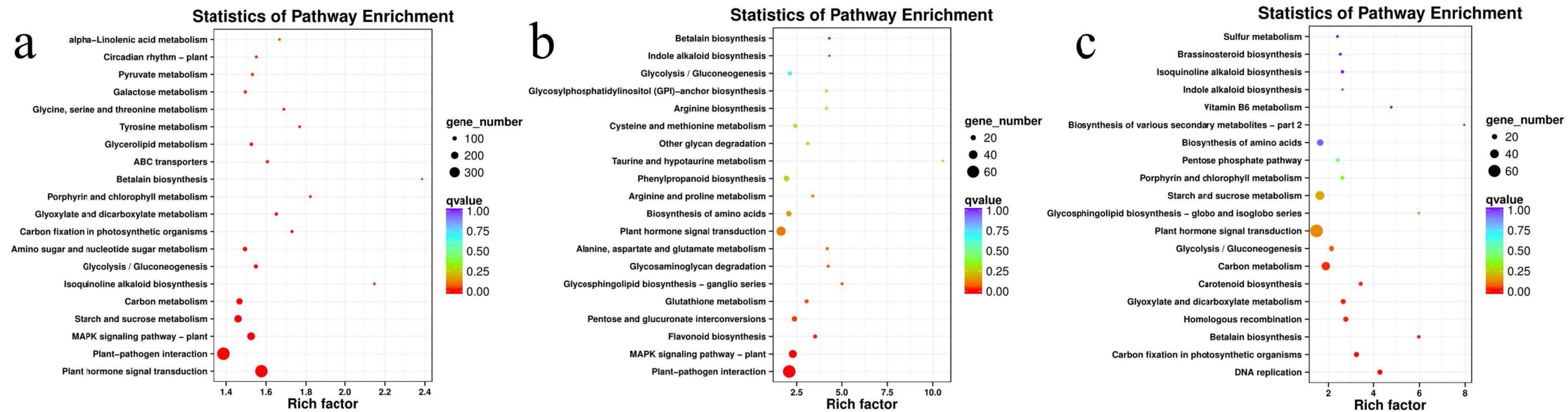
b The DEGs in stem development



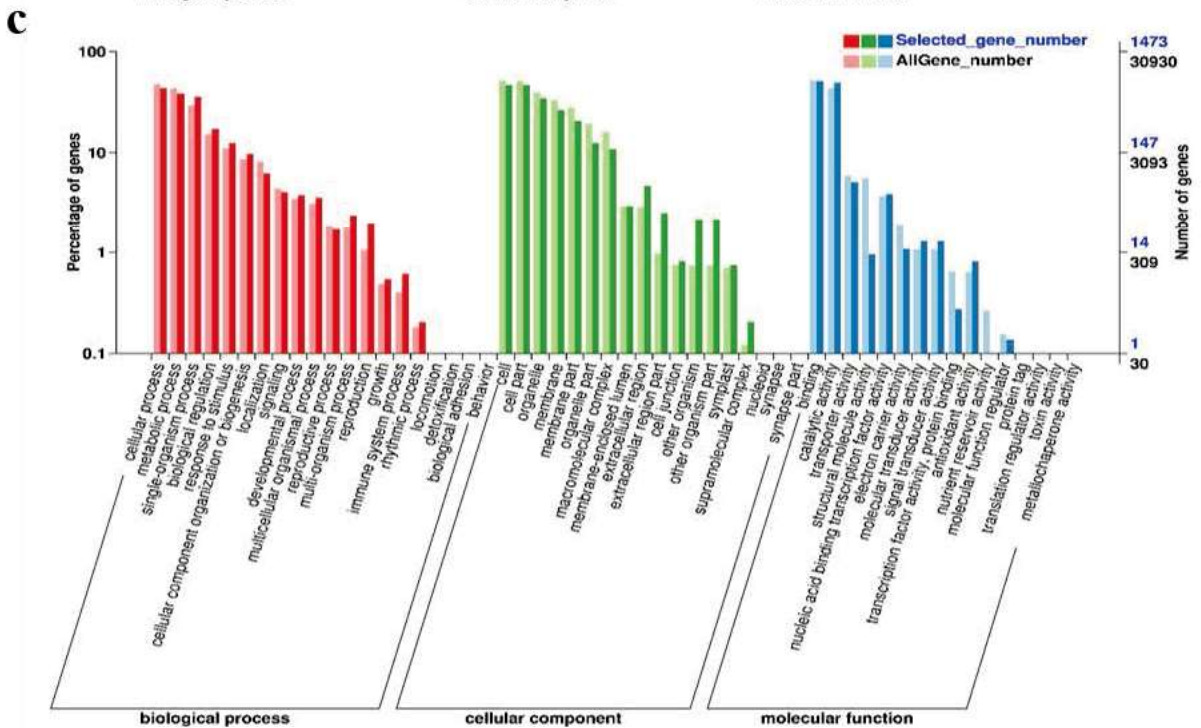
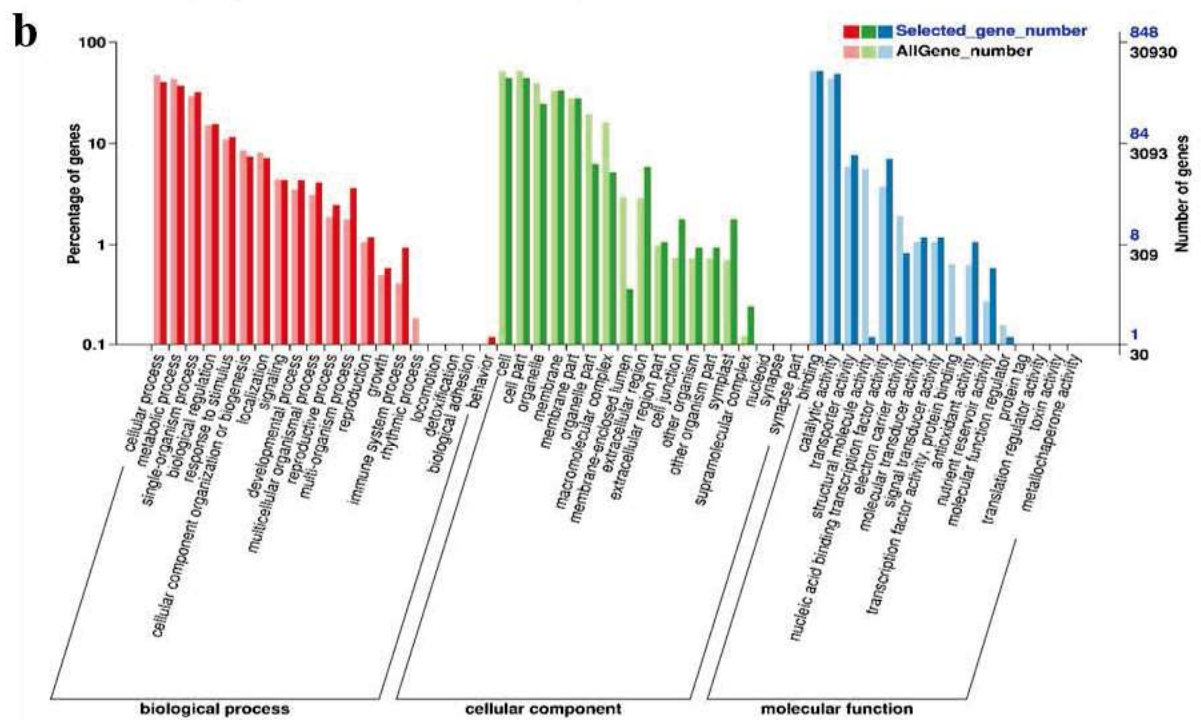
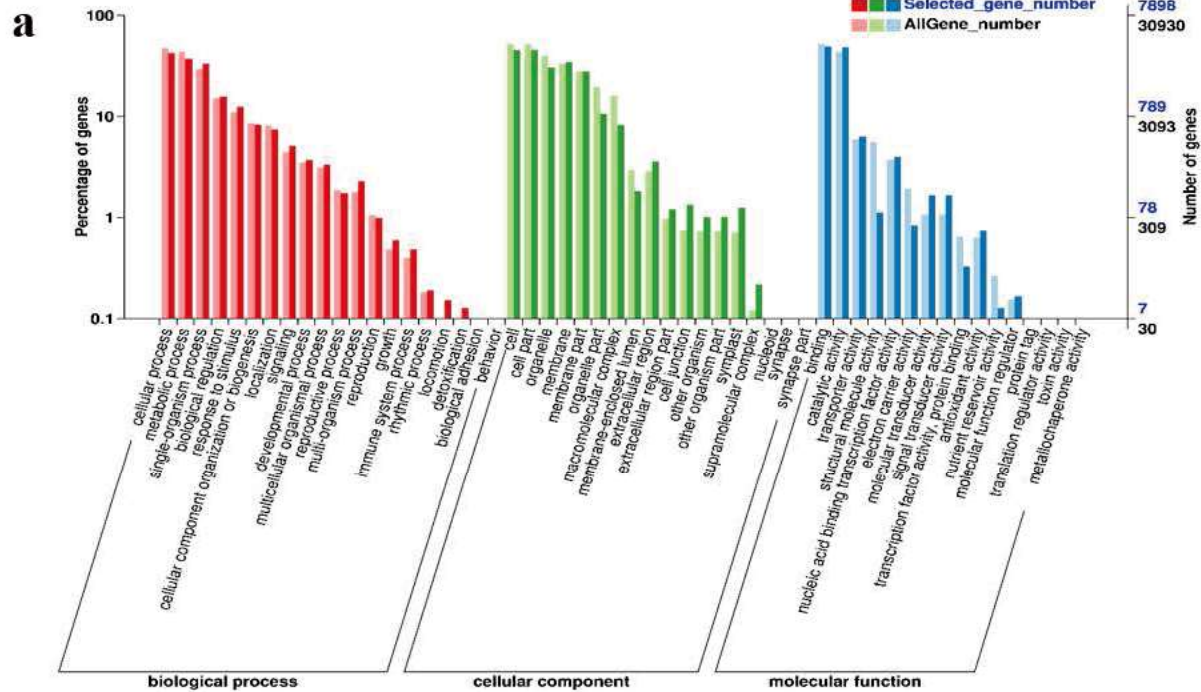
Supplementary Figure 10. The DEGs in leaf(a) and stem (b) development.

a**b**

Supplementary Figure 11. The shared and unique DEGs between the five adjacent stages in leaf(a) and stem (b) development.

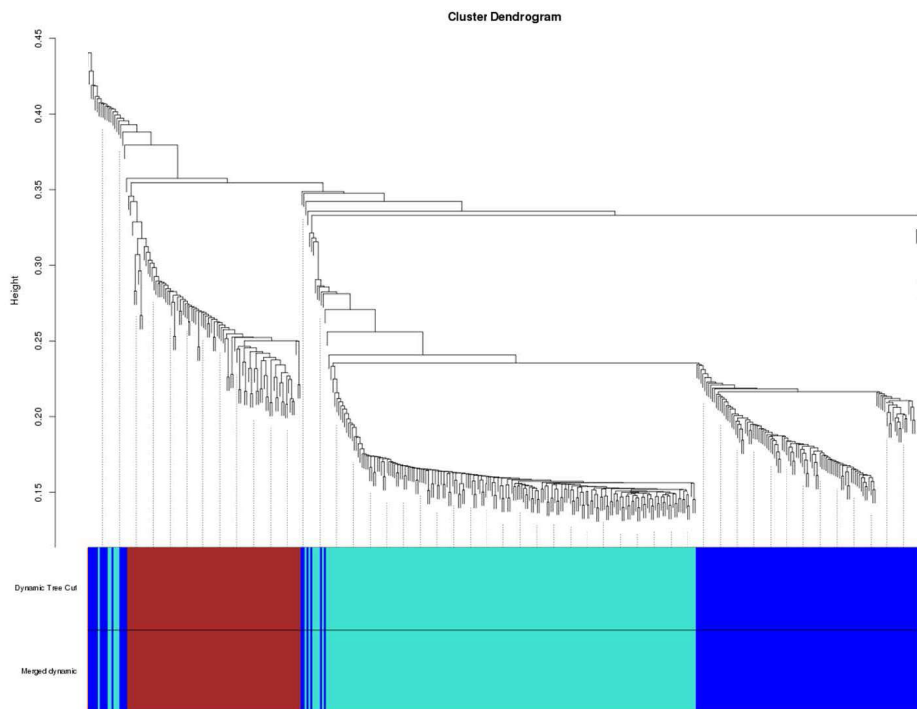


Supplementary Figure 12. KEGG enrichment analysis of all DEGs in leaf(a) and stem(b) development, and shared DEGs in leaf development(c).

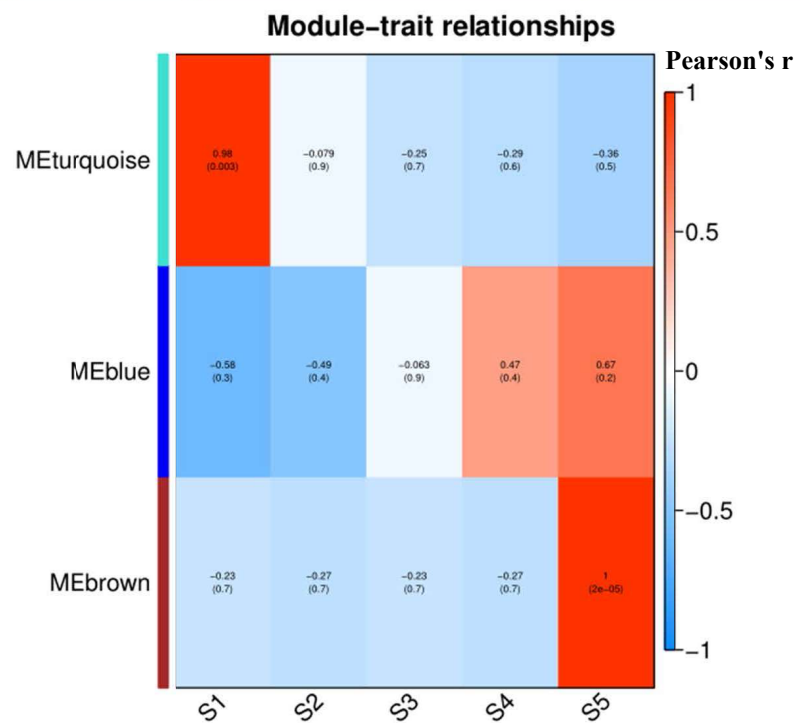


Supplementary Figure 13. GO enrichment analysis of all DEGs in leaf(a) and stem(b) development, and shared DEGs in leaf development(c).

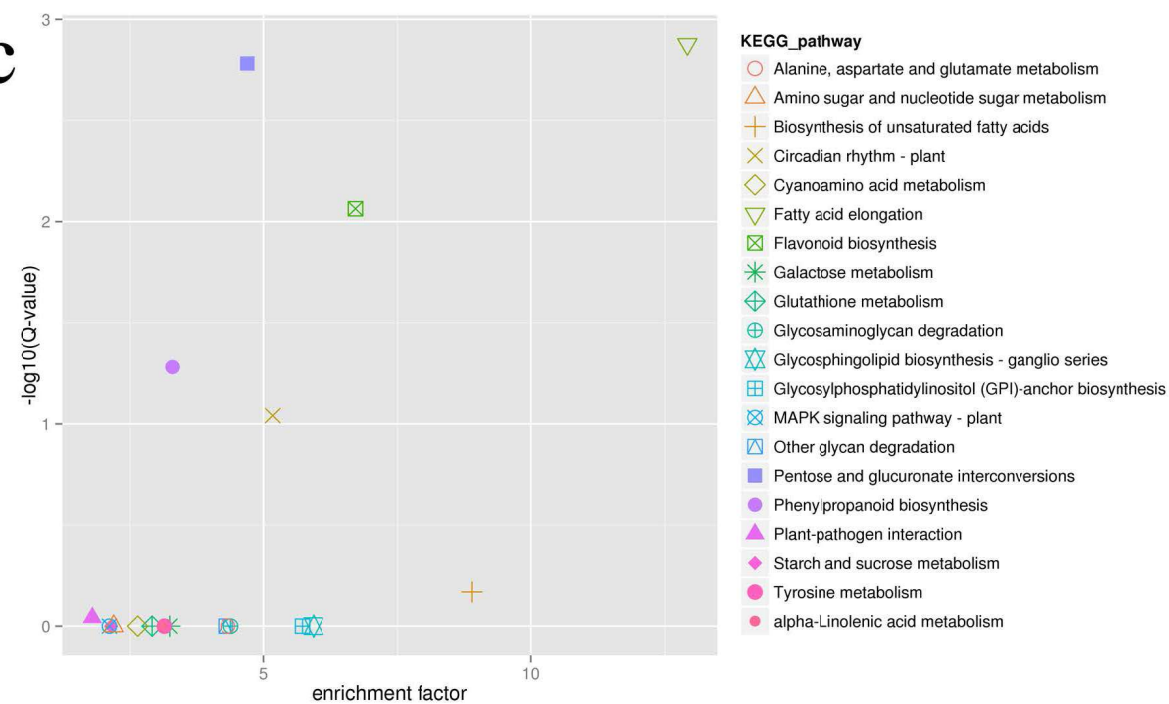
a



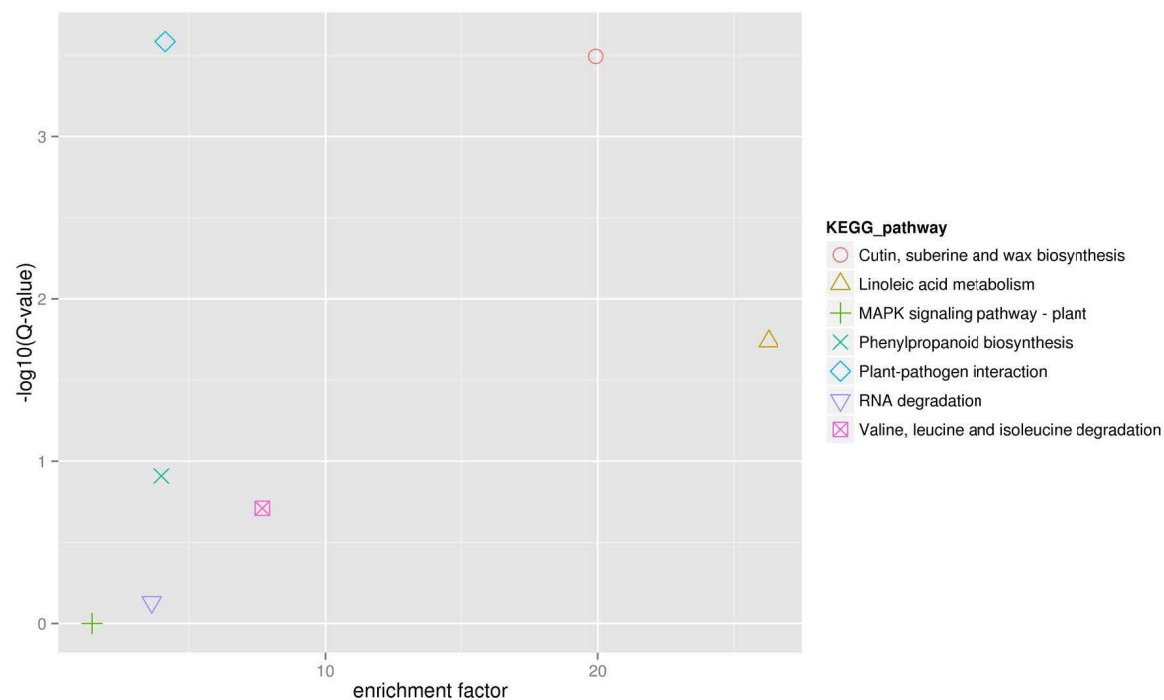
b



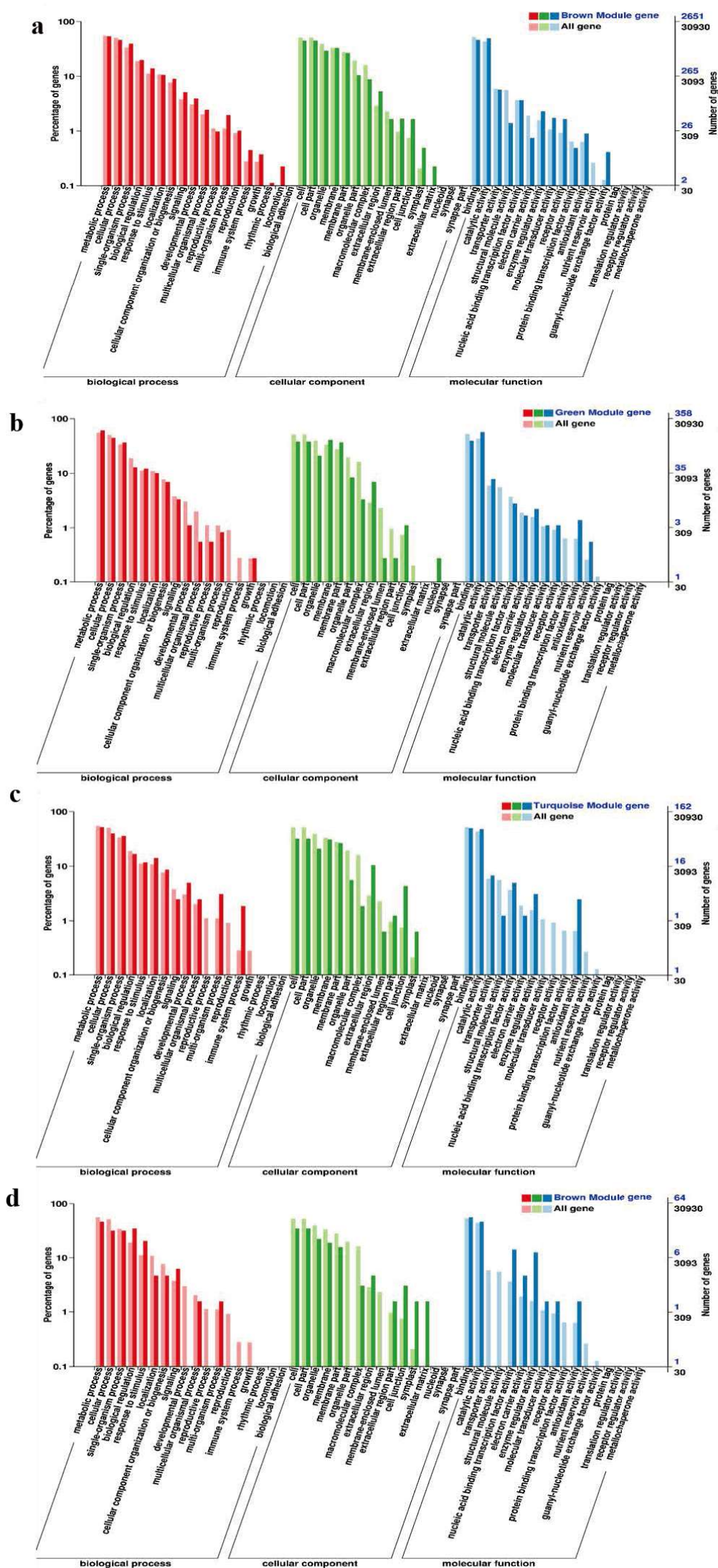
c



d

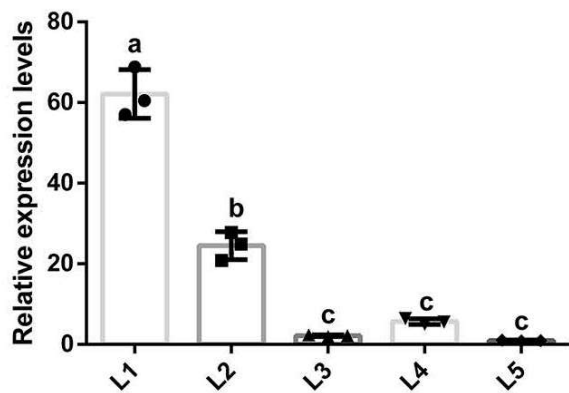


Supplementary Figure 14. Weighted gene co-expression network analysis (WGCNA) of genes during the stem development. a, hierarchical cluster tree showing co-expression modules identified by WGCNA. b, module-stage association (each row corresponds to a module, and each column represents a specific stage. The color of each cell at the row column intersection indicates the Pearson correlation coefficient (Pearson's r) between a module and the stage), positive and negative correlations are shown in red and blue, respectively. c, KEGG enrichment analysis of genes in turquoise module. d, KEGG enrichment analysis of genes in brown module. Each sample for WGCNA had three biological replicates.

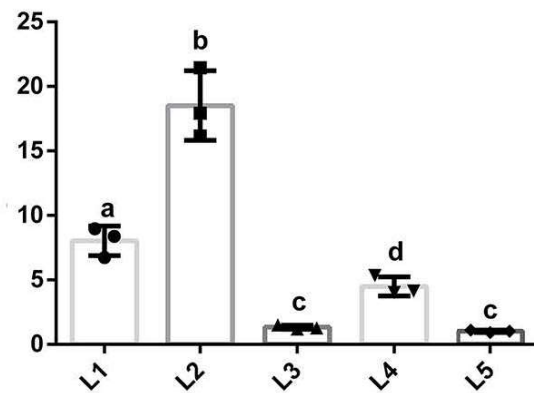


Supplementary Figure 15. GO enrichment analysis of the genes in brown(a) and green(b) module during the leaf development; and the genes in turquoise(c) and brown(d) module during the stem development.

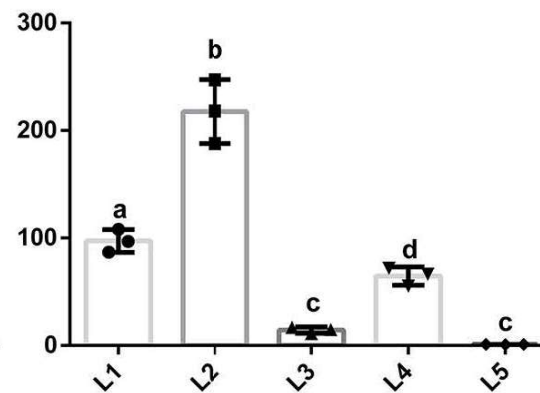
CEPR1



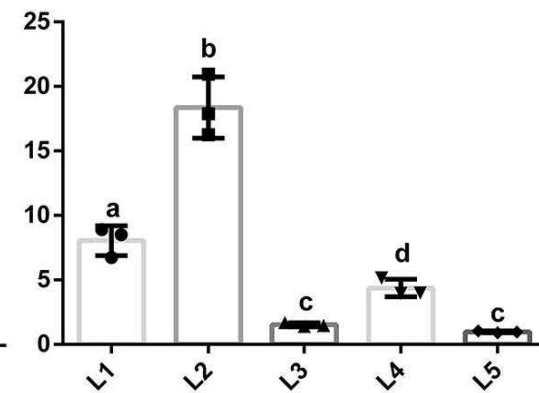
bHLH80



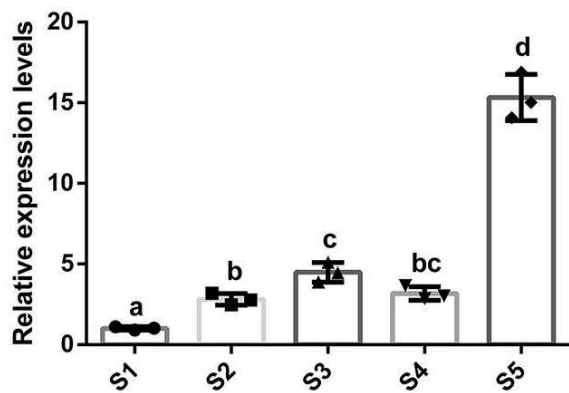
SAUR21



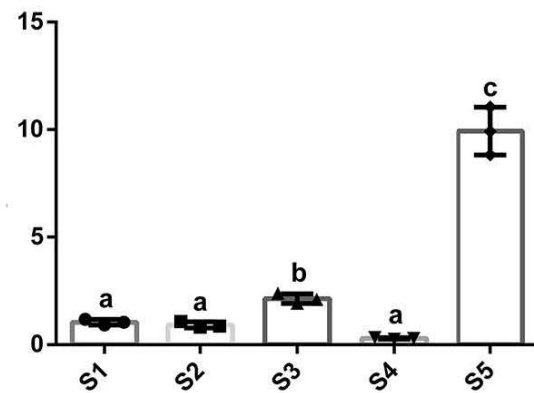
GDSL-3



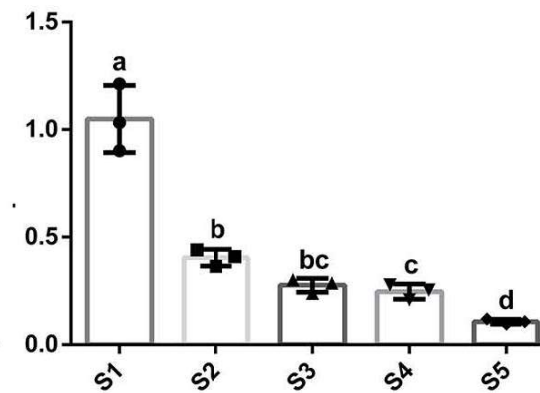
CCR4



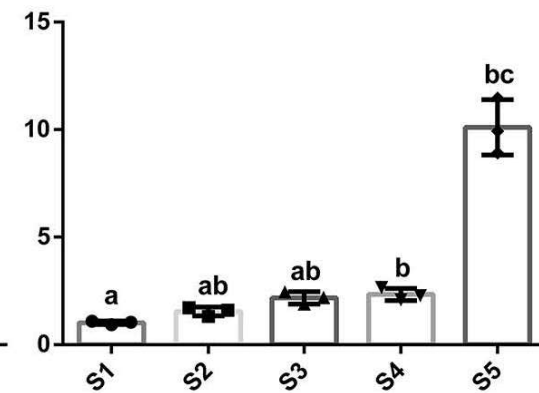
ERF109



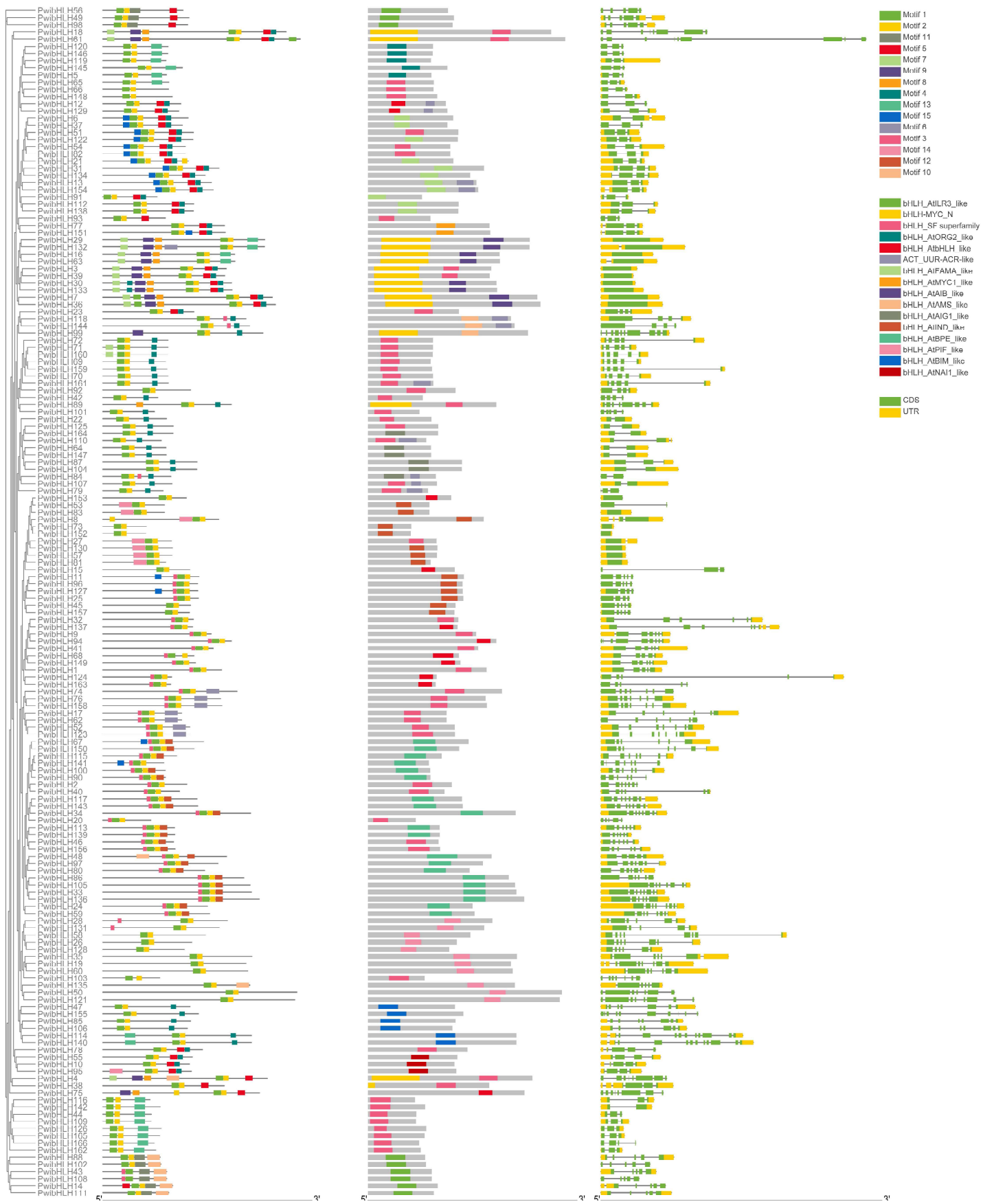
LBD15



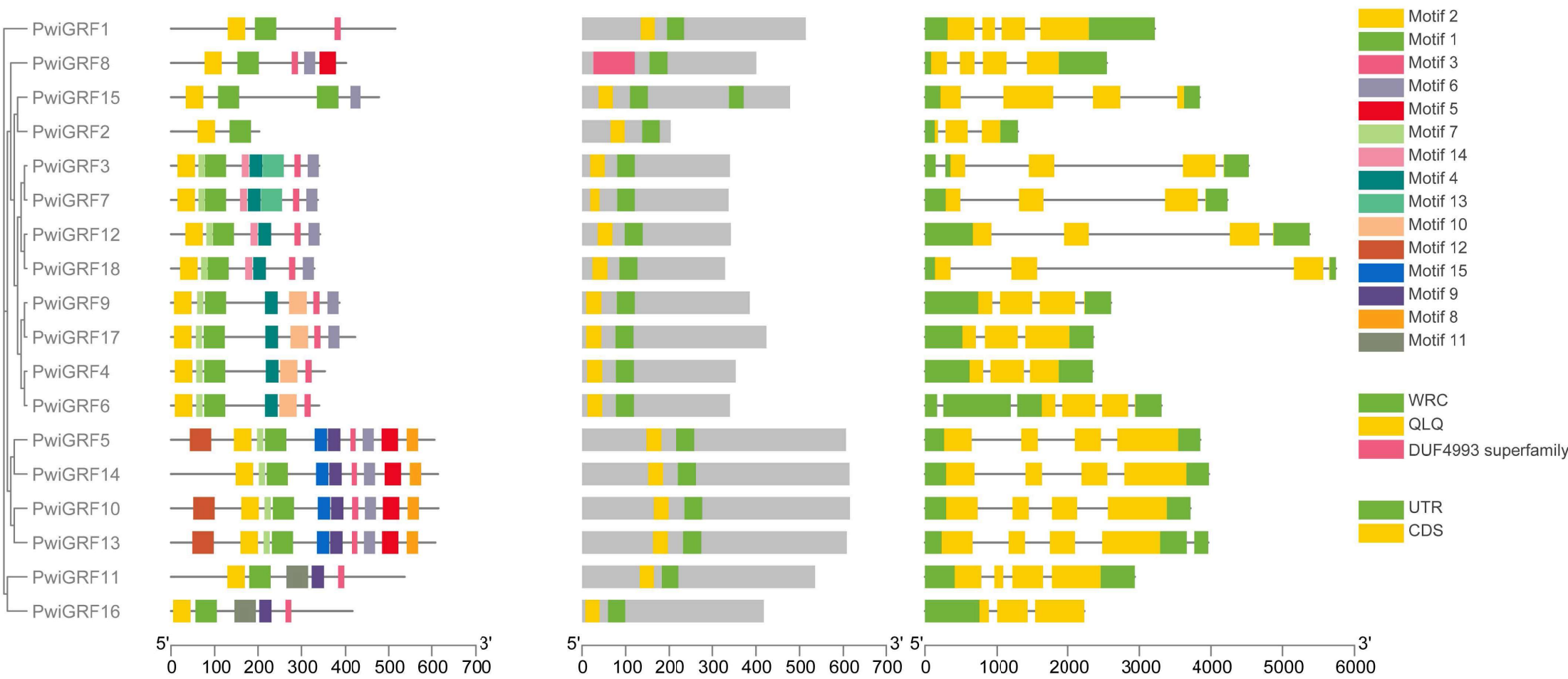
WRKY76



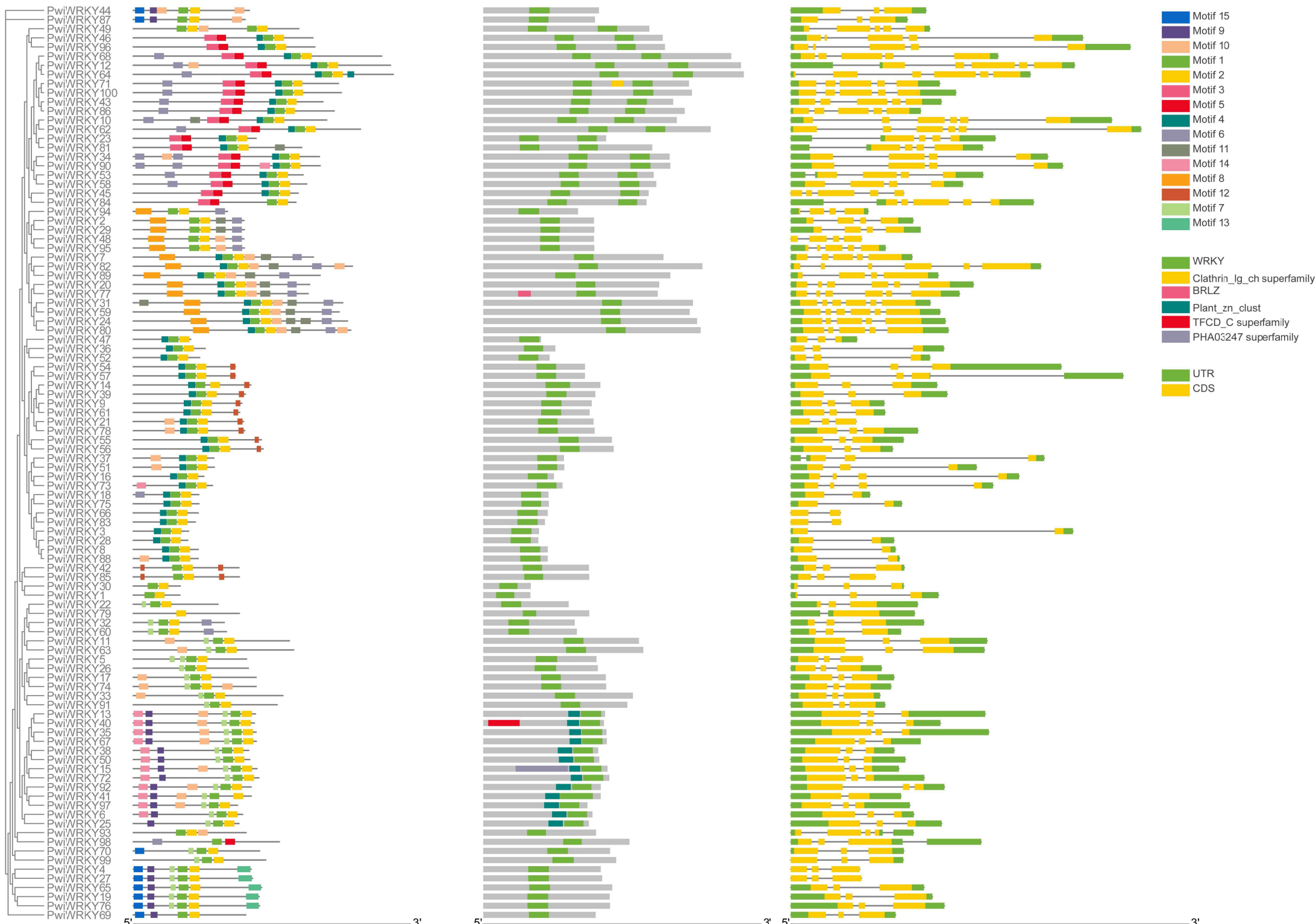
Supplementary Figure 16. Relative expression levels of some identified genes of WCGNA in different development stages of leaf (L1-L5) or stem (S1-S5). Expression profiles of these genes were analyzed using RT-qPCR analysis. The poplar *Ubiquitin (UBQ)* gene was used as an internal control and gene expression profiles were evaluated using the $2^{-\Delta\Delta C_t}$ method. Three biological replicates for each tissue were analyzed. Error bars represent the SD of the mean (n=3). Different letters above bars represent statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA followed by Dunnett's test. In these groups, the same letter indicates that there is no significant difference between the two groups, different letters indicate that there is a significant difference between the two groups. For *CEPR1*, *bHLH80*, *SAUR1*, and *GDSL-3*, the relative expression level of L5 stage was set to be 1. For *CCR4*, *ERF109*, *LBD15*, and *WRKY76*, the relative expression level of S1 stage was set to be 1.



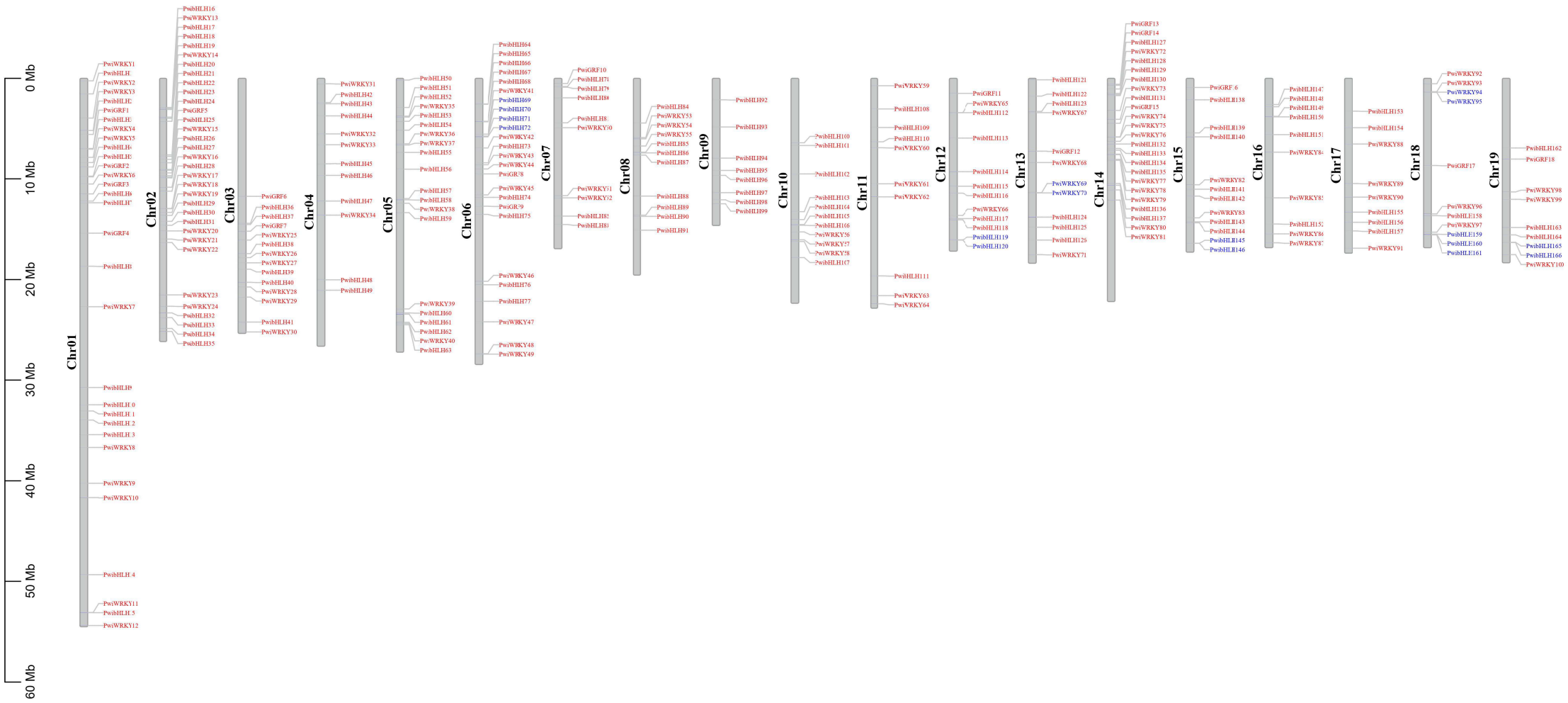
Supplementary Figure 17. The gene structures and conserved motifs of *Populus wilsonii* bHLH family members. From left to right of this panel are phylogenetic trees, conserved motifs, conserved domains, and gene structures of the PwibHLH members.



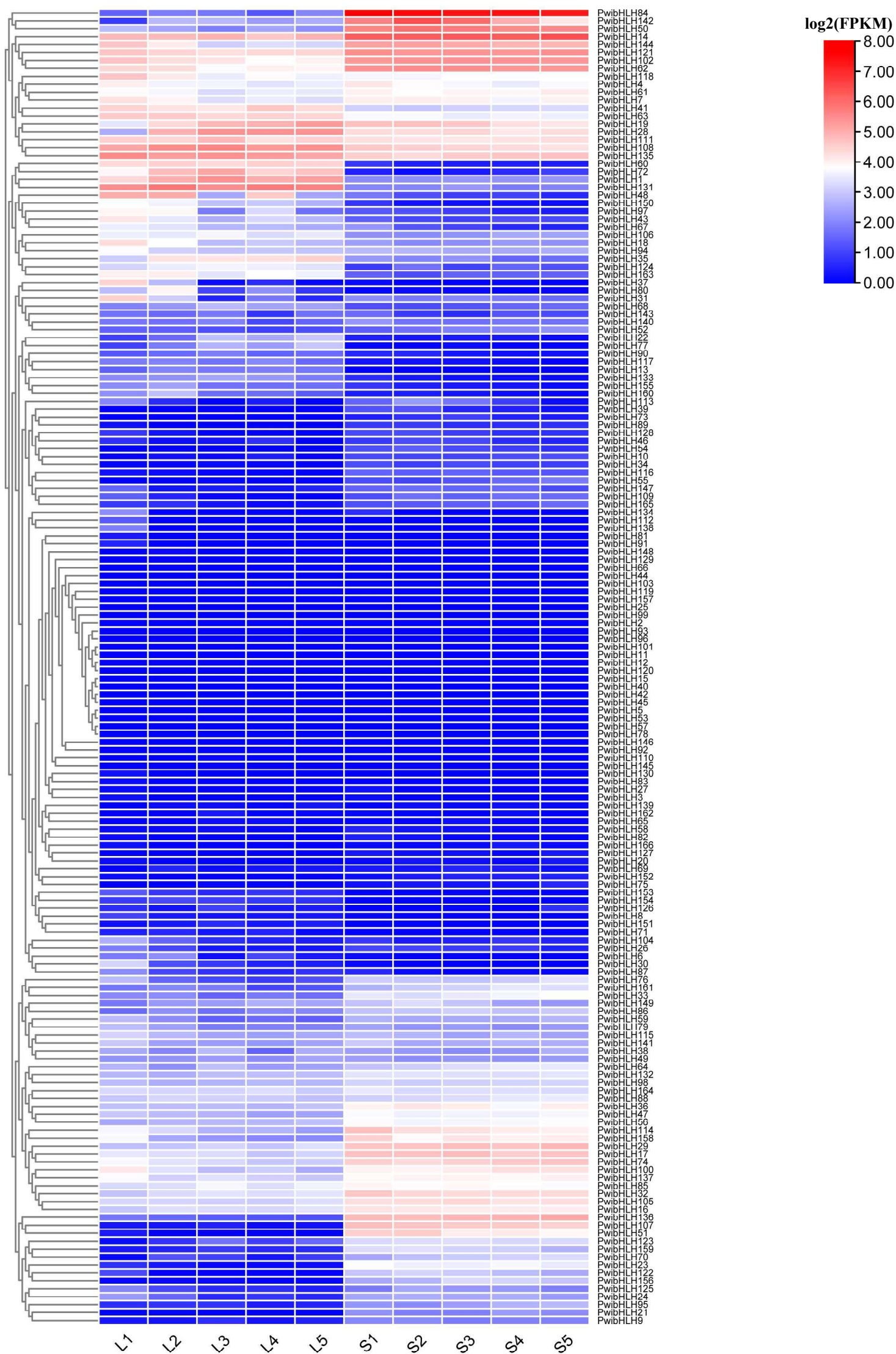
Supplementary Figure 18. The gene structures and conserved motifs of *Populus wilsonii* GRF family members. From left to right of this panel are phylogenetic trees, conserved motifs, conserved domains, and gene structures of the PwiGRF members.



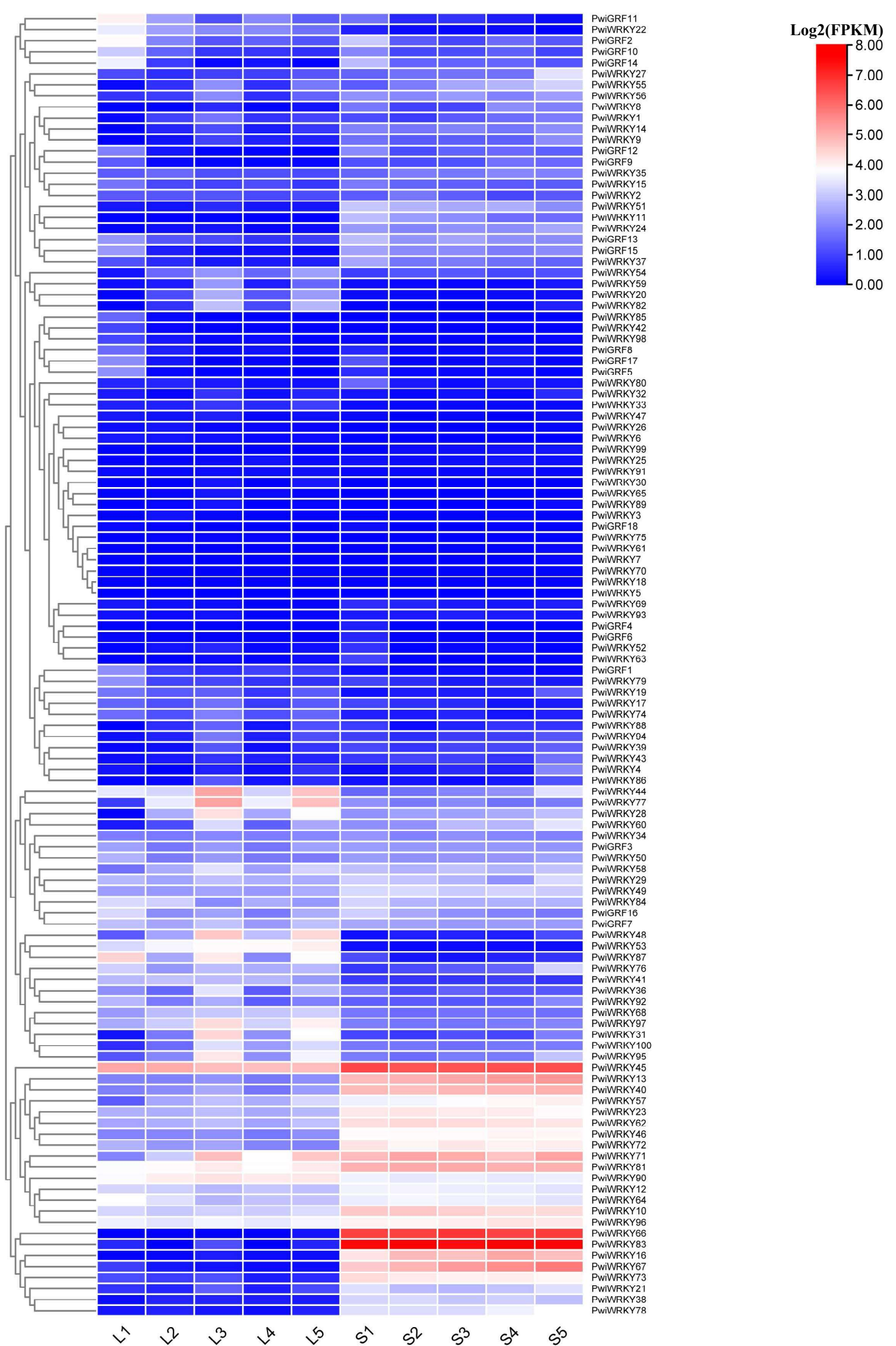
Supplementary Figure 19. The gene structures and conserved motifs of *Populus wilsonii* WRKY family members. From left to right of this panel are phylogenetic trees, conserved motifs, conserved domains, and gene structures of the PwiGRF members.



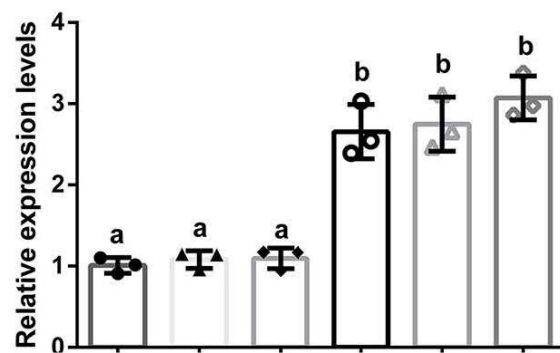
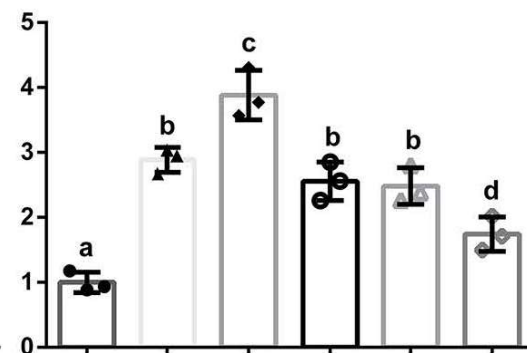
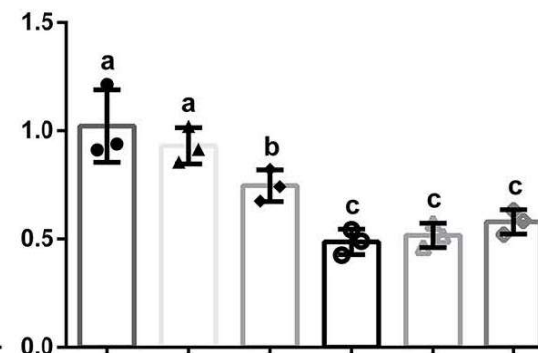
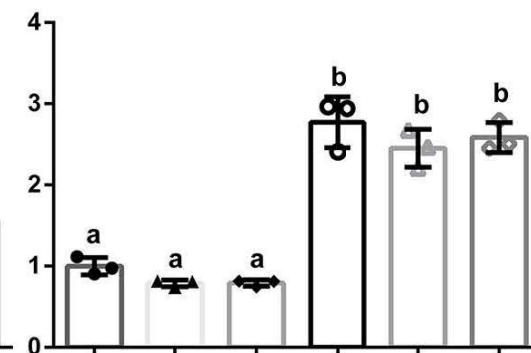
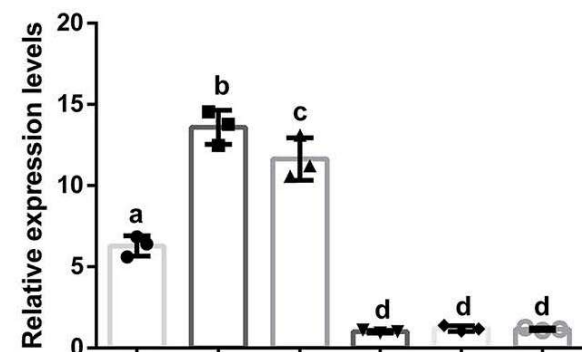
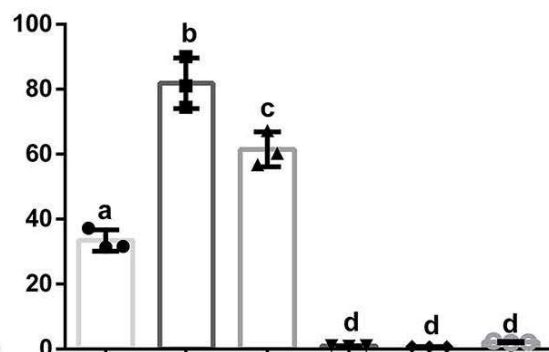
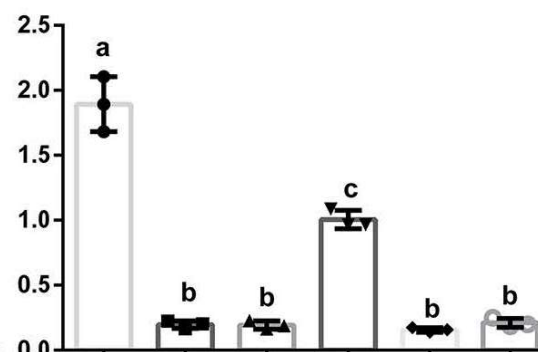
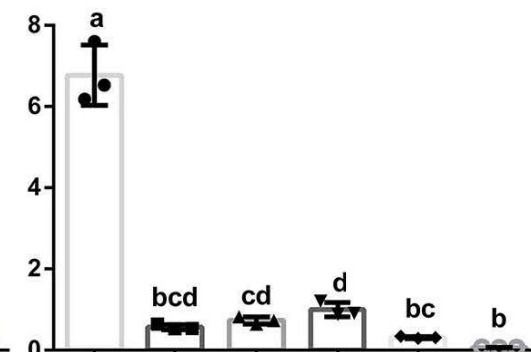
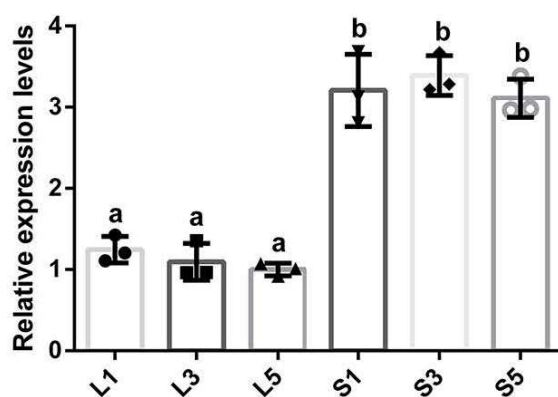
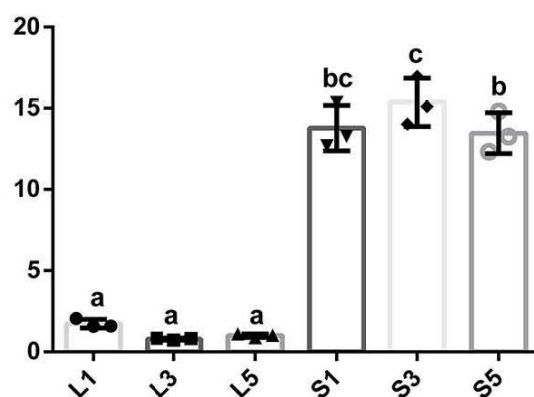
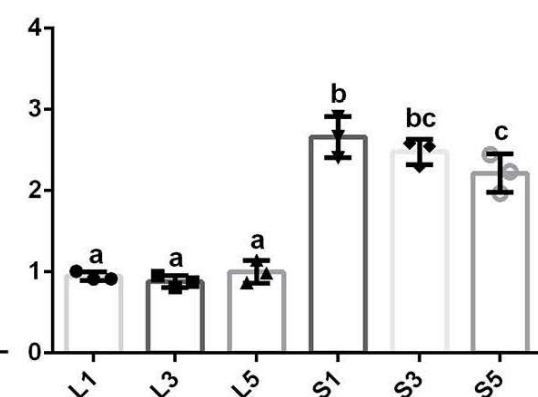
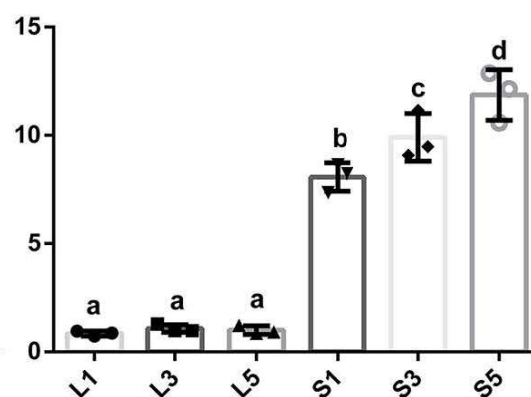
Supplementary Figure 20. Chromosomal locations of *Populus wilsonii* bHLH (PwibHLH), GRF (PwiGRF) and WRKY (PwiWRKY) gene families. Tandem duplicates are represented by blue font.



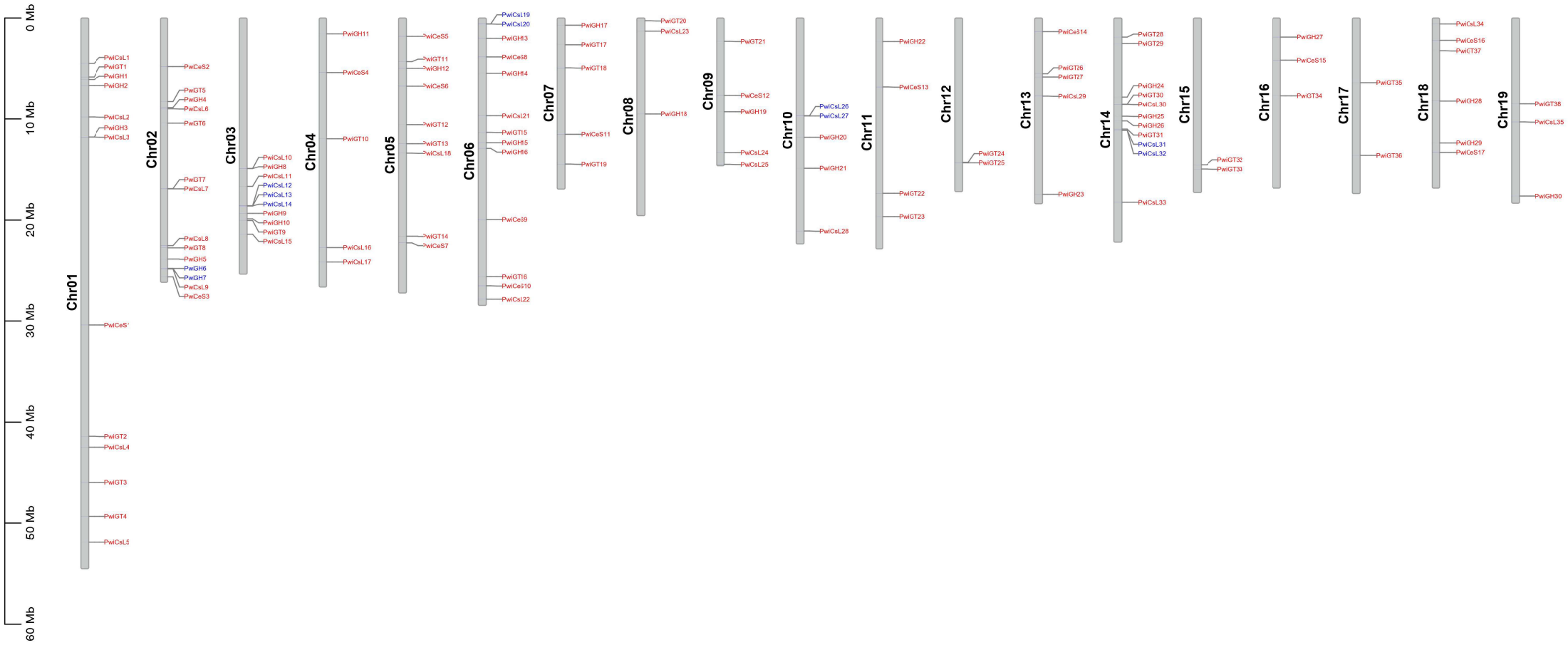
Supplementary Figure 21. Heatmap showing the expression level of bHLH gene members in different development stages of leaf and stem in *Populus wilsonii*. The FPKM values are log₂-based. Red and blue indicate high and low expression levels, respectively. Each sample for every development stage had three biological replicates.



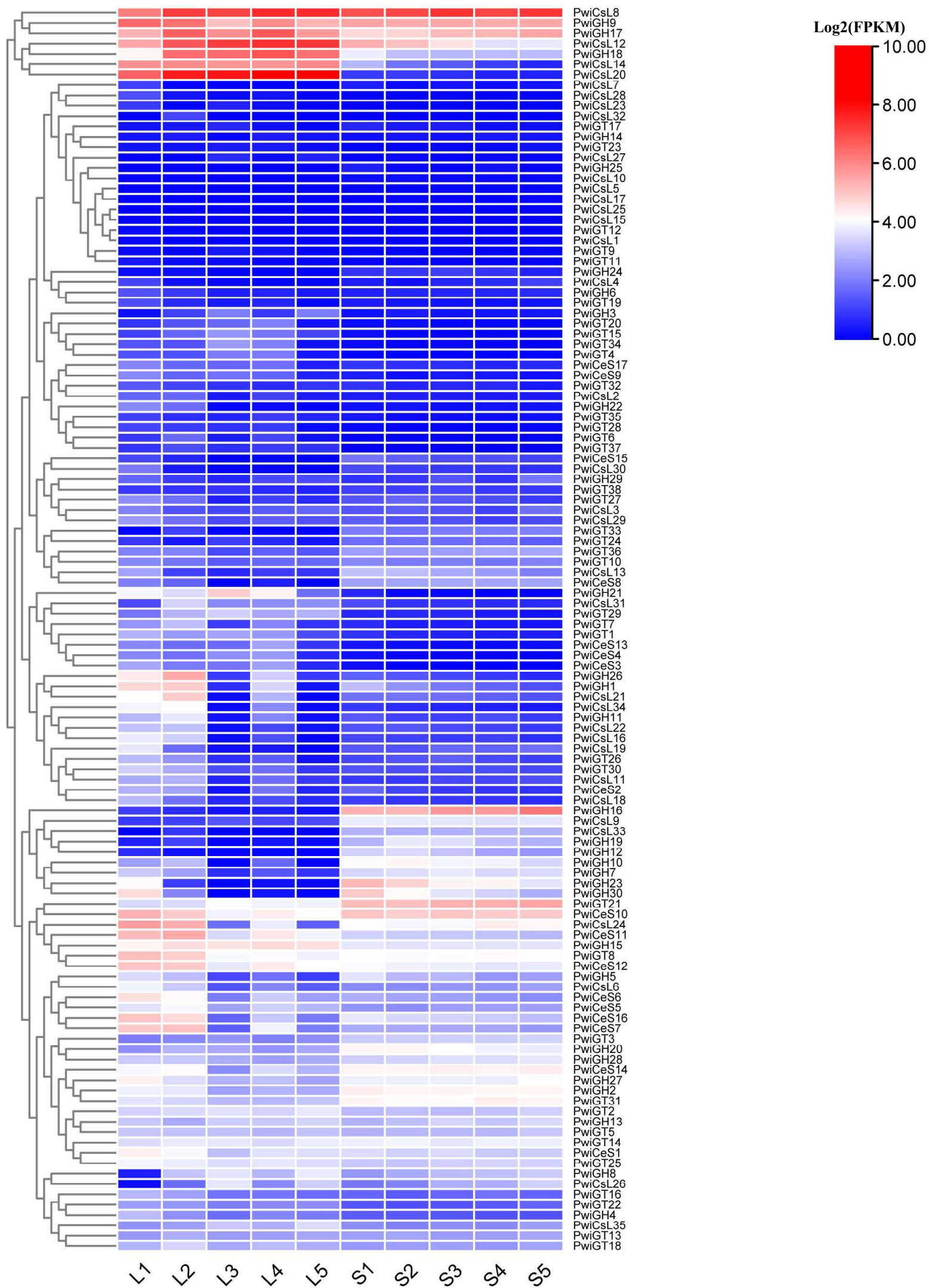
Supplementary Figure 22. Heatmap showing the expression level of GRF and WRKY gene members in different development stages of leaf and stem in *Populus wilsonii*. The FPKM values are log2-based. Red and blue indicate high and low expression levels, respectively. Each sample for every development stage had three biological replicates.

bHLH14*bHLH19**bHLH135**WRKY45**bHLH1**bHLH72**GRF2**GRF11**bHLH17**bHLH50**WRKY10**WRKY13*

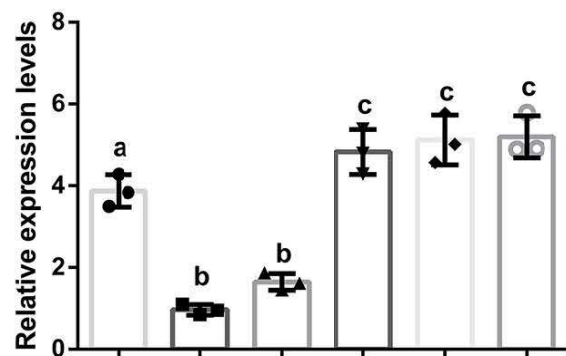
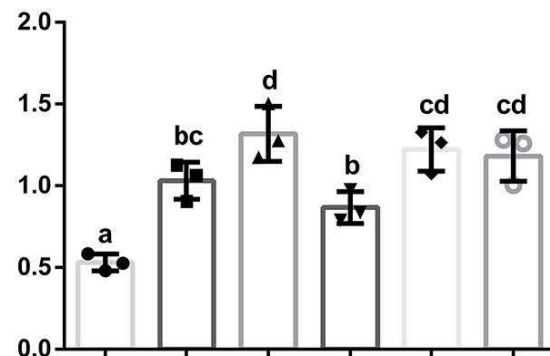
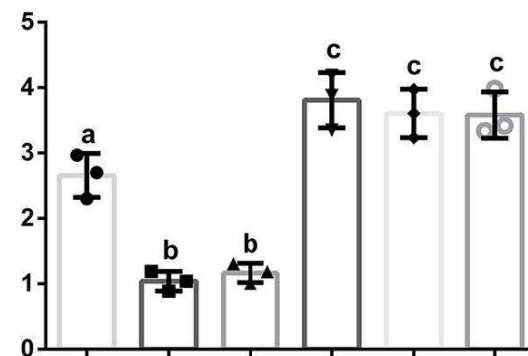
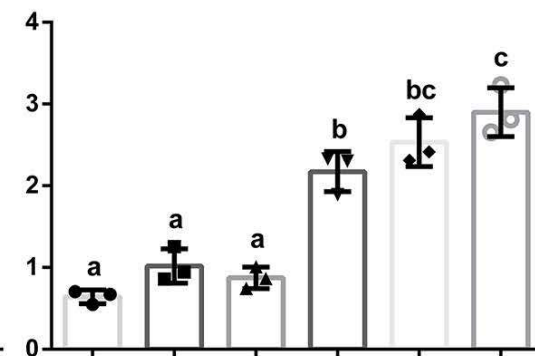
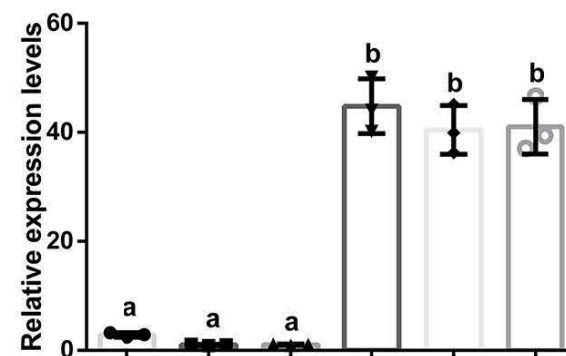
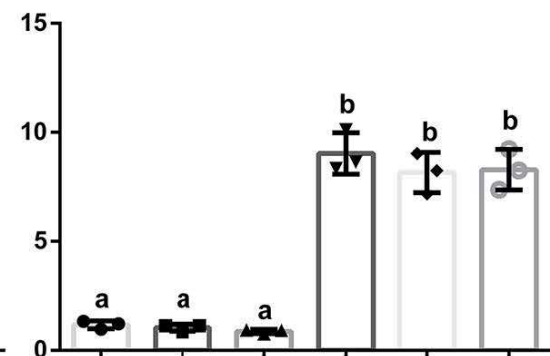
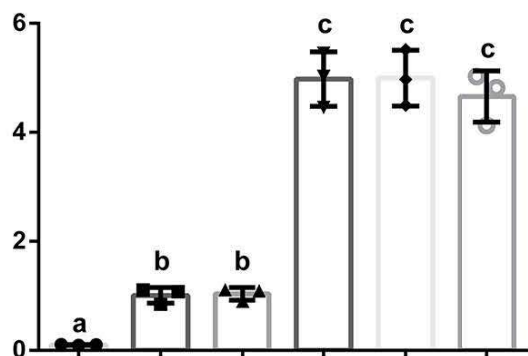
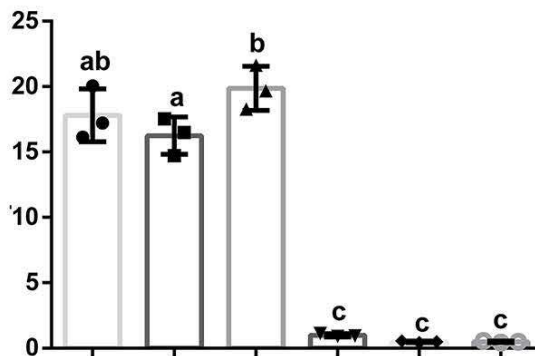
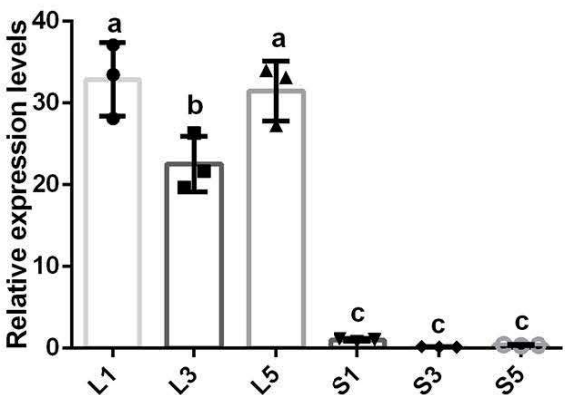
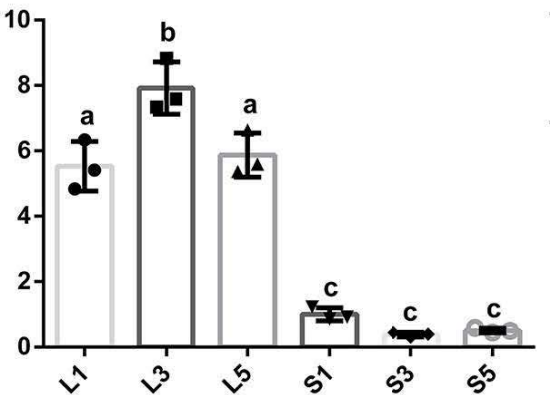
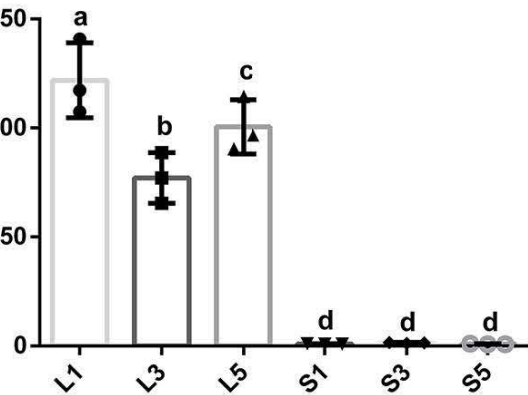
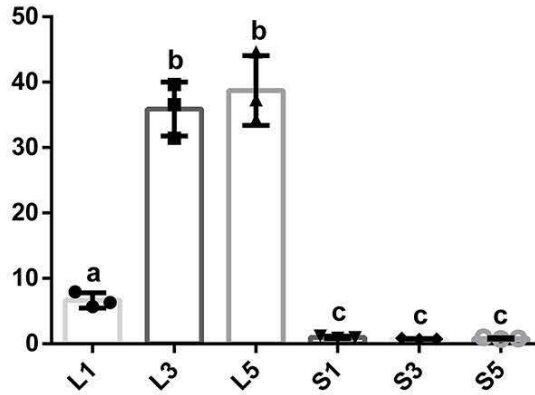
Supplementary Figure 23. Relative expression levels of some bHLH, WRKY and GRF transcription factors in different development stage of leaf (L1, L3 and L5) or stem (S1, S3 and S5). Expression profiles of these genes were analyzed using RT-qPCR analysis. The poplar *Ubiquitin (UBQ)* gene was used as an internal control and gene expression profiles were evaluated using the $2^{-\Delta\Delta Ct}$ method. Three biological replicates for each tissue were analyzed. Error bars represent the SD of the mean (n=3). Different letters above bars represent statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA followed by Dunnett's test. In these groups, the same letter indicates that there is no significant difference between the two groups, different letters indicate that there is a significant difference between the two groups. For *bHLH14*, *bHLH19*, *bHLH135*, and *WRKY45*, the relative expression level of L1 stage was set to be 1. For *bHLH1*, *bHLH72*, *GRF2*, and *GRF11*, the relative expression level of S1 stage was set to be 1. For *bHLH17*, *bHLH50*, *WRKY10*, and *WRKY13*, the relative expression level of L5 stage was set to be 1.



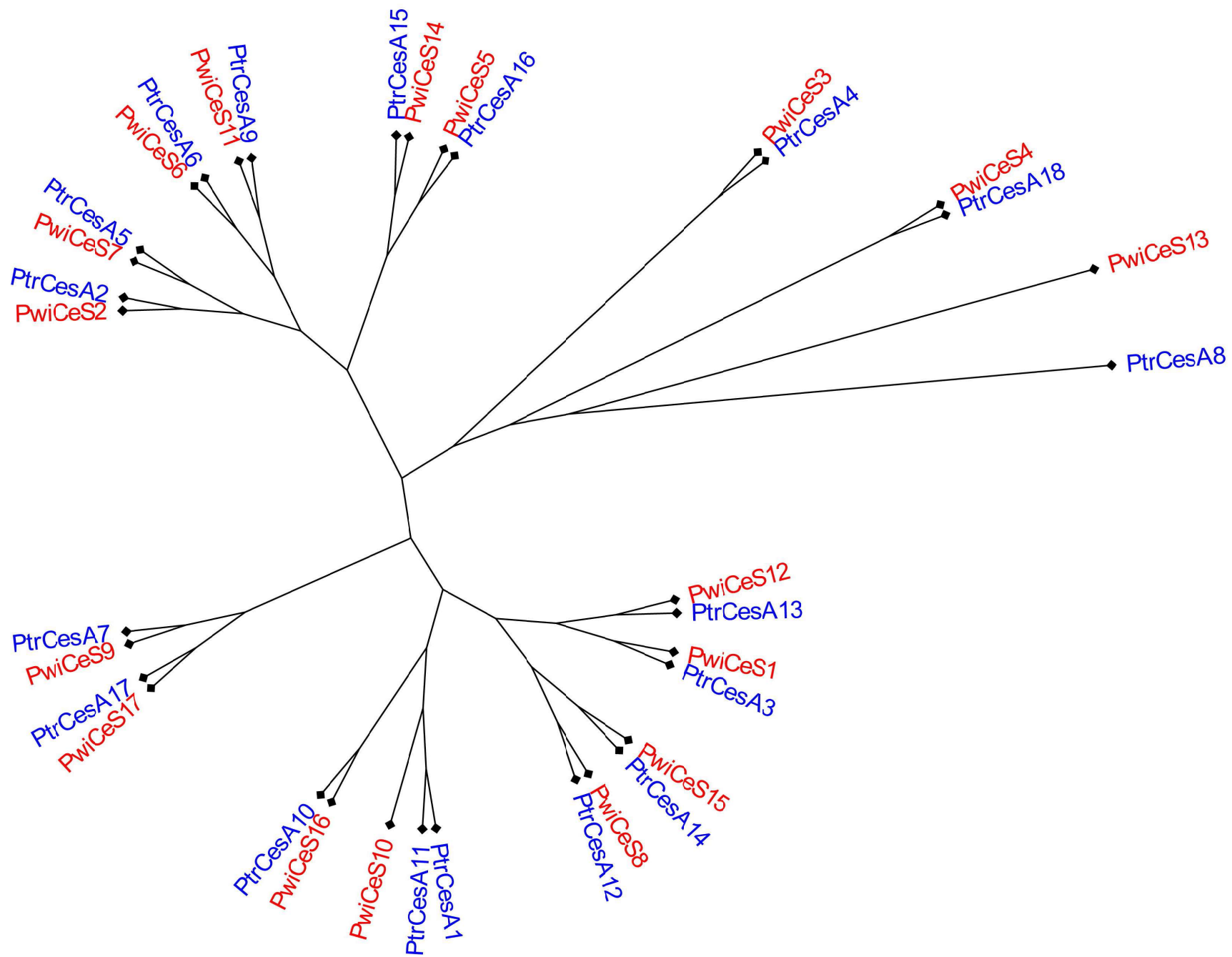
Supplementary Figure 24. Chromosomal locations of *Populus wilsonii* Ces (Pwi Ces), Csl (PwiCsl), GH (PwiGH) and GT (PwiGT) gene families. Tandem duplicates are represented by blue font.



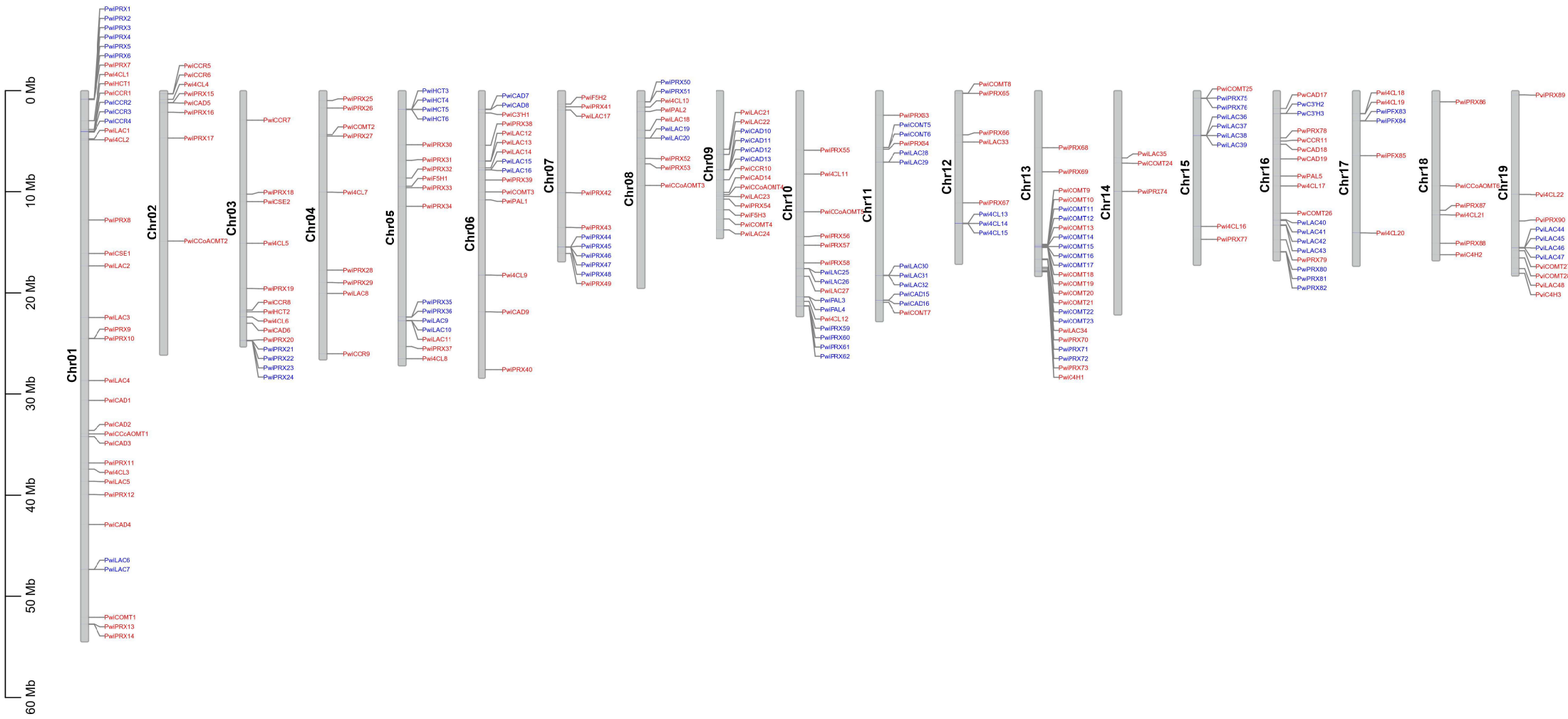
Supplementary Figure 25. Heatmap showing the expression level of four Cellulose synthesis gene in different development stages of leaf and stem in *Populus wilsonii*. The FPKM values are log2-based. Red and blue indicate high and low expression levels, respectively. Each sample for every development stage had three biological replicates.

CesA14*CsL8**GH2**GT21**CAD19**CCR10**PRX30**CHS5**CHS13**CHI1**FNSII3**UFGT15*

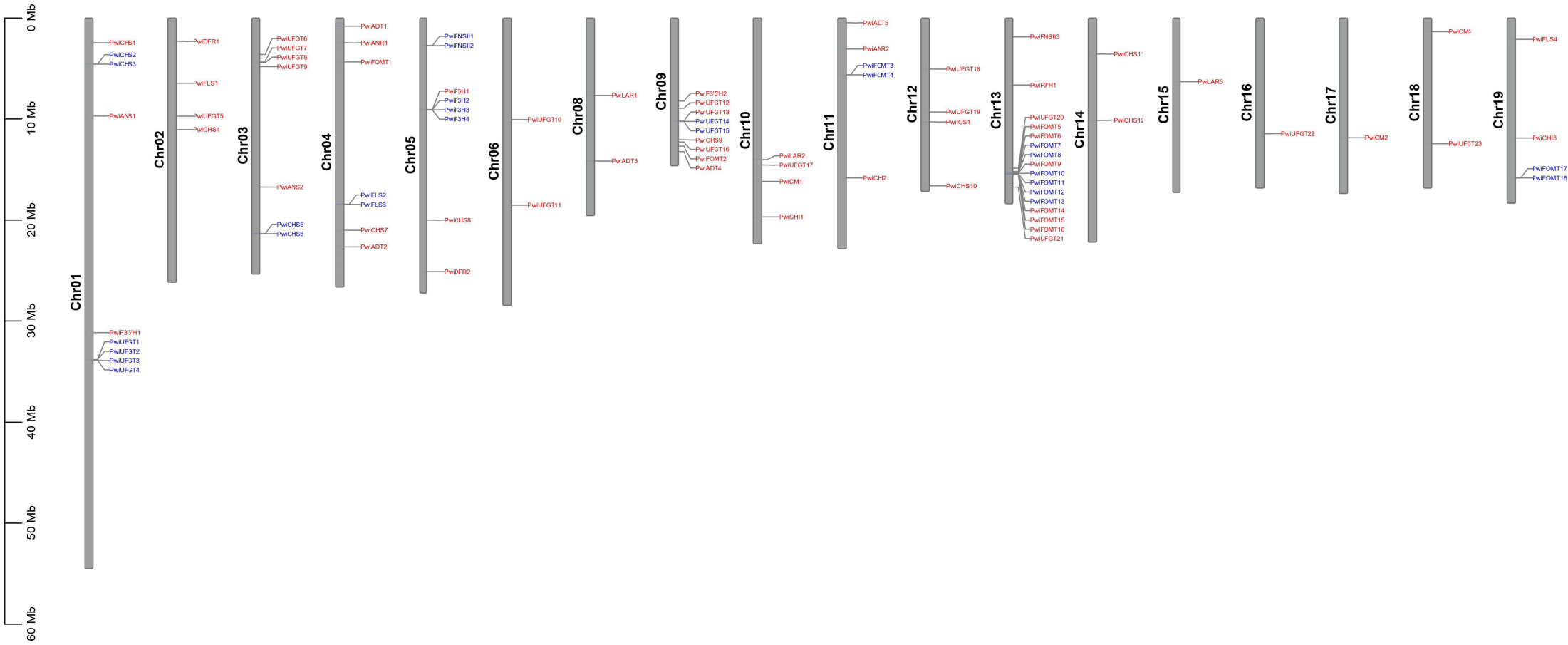
Supplementary Figure 26. Relative expression levels of some key enzyme genes involved in cellulose, hemicellulose, lignin and flavonoid biosynthesis in different development stage of leaf (L1, L3 and L5) or stem (S1, S3 and S5). Expression profiles of these genes were analyzed using RT-qPCR analysis. The poplar *Ubiquitin (UBQ)* gene was used as an internal control and gene expression profiles were evaluated using the $2^{-\Delta\Delta Ct}$ method. Three biological replicates for each tissue were analyzed. Error bars represent the SD of the mean (n=3). Different letters above bars represent statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA followed by Dunnett's test. In these groups, the same letter indicates that there is no significant difference between the two groups, different letters indicate that there is a significant difference between the two groups. For *CesA14*, *CsL8*, *GH2*, and *GT21*, the relative expression level of L3 stage was set to be 1. For *CAD19*, *CCR10*, and *PRX30*, the relative expression level of L3 stage was set to be 1. For *CHS5*, *CHS13*, *CH11*, *FNSII3*, and *UFGT15*, the relative expression level of S1 stage was set to be 1.



Supplementary Figure 27. Phylogenetic analysis of Cellulose genes in *Populus wilsonii* (red) and *Populus trichocarpa* (blue).



Supplementary Figure 28. Chromosomal locations of Lignin synthesis gene families in *Populus wilsonii*. Tandem duplicates are represented by blue font.



Supplementary Figure 29. Chromosomal locations of flavonoids synthesis gene families in *Populus wilsonii*. Tandem duplicates are represented by blue font.

Supplementary Tables

Supplementary Table 1. Overview of sequencing data in *Populus wilsonii*.

Reads Type	Total bases (Gb)	Total reads	Reads N50 (Bp)	Mean Length (bp)	Longest Read (bp)	Function
Subreads	475.88	33,348,682	15,645	14,270	387,426	Assembly
CCS read	30.4	2,019,886	15,075	15,049	36,256	
Read length (bp)	Clean data (Gb)	Total Read Pairs	Mapped Reads	Unique Mapped Read Pairs	Q30(%)	Function
150_150	54.30	181,253,818	320,850,736.00	148,575,876	93.24	Assembly
Read length (bp)	Library	Data (Gb)	Filtered data (Gb)	Depth (×)	Q30 (%)	Function
150_150	350 bp	32.10	27.37	62.28	93.08	Survey

Note: Assuming the genome size of *Populus wilsonii* is 440 Mb.

Supplementary Table 2. Estimation of *Populus wilsonii* genome size based on 19 K-mer statistics.

Clean data	Q30 (%)	Sequencing depth (X)	GC content (%)
27.37Gb	93.08	62.28	35.14
k-mer	Main peak depth of k-mer	Estimated genome Size	Heterozygote frequencies
19	26	439.39 Mb	0.42%

Supplementary Table 3. Overview of the type of Hi-C data.

Type	Number	Ratio (%)
Total Read Pairs	181,253,818	100
Mapped Reads	160,425,368	88.5
Unique Paired Alignments	148,575,876	100
Valid Interaction Pairs	83,433,287	56
Dangling End Pairs	50456221	34
Re-ligation Pairs	3,318,277	2
Self-cycle Pairs	992,030	1
Dumped Pairs	10,376,061	6.98

Supplementary Table 4. Evaluation of Benchmarking Universal Single-Copy Orthologs (BUSCO) and Gene Space Coverage Using Core Eukaryotic Gene Mapping Approach (CEGMA) in *Populus wilsonii* genome.

Type	Number	Percent (%)
Complete BUSCOs (C)	1,591	98.57
Complete and single-copy BUSCOs (S)	1338	82.9
Complete and duplicated BUSCOs (D)	253	15.68
Fragmented BUSCOs (F)	4	0.25
Missing BUSCOs (M)	19	1.18
Total BUSCO groups searched	1,614	-
Number of 458 CEG present in assembly	452	98.69
Number of 248 highly conserved CEGs present	233	93.95

Supplementary Table 5. The clean data of NovaSeq6000 and PacBio mapped to reference genome.

Data Type	Total Reads	Map Reads	Map Rate (%)	Coverage at least 10X (%)	Coverage at least 20X (%)	Coverage at least 30X (%)
NovaSeq 6000	214,444,004	213,643,498	99.63	99.32	99.02	98.63
PacBio CCS	2,019,886	2,018,736	99.94	99.76	99.64	99.43

Supplementary Table 6. Statistics of samples used for RNA-Seq sequencing.

Samples	Organ	Clean reads pair	Clean bases(bp)	Mapped Reads	Rate	Q30(%)
bud	Leaf bud	25782466	7702341298	48,095,328	93.27%	0.9471
flowers	Flowers	23045560	6891330014	43,569,328	94.53%	0.9409
flower_bud	Flower bud	25916157	7748961270	49,085,581	94.70%	0.936
leaf	Leaf	24515553	7312556460	46,805,278	95.46%	0.9416
phloem	Phloem	36645427	10965845302	56,998,075	77.77%	0.9428
root	Root	24735027	7387490240	44,133,181	89.21%	0.942
xylem	Xylem	34560191	10286616988	61,182,923	88.52%	0.9347
L1-1	Leaf	21575061	6441369768	40,721,905	94.37%	0.9411
L1-2	Leaf	24247276	7237877412	45,436,030	93.69%	0.9395
L1-3	Leaf	22712343	6778821166	41,167,377	90.63%	0.9459
L2-1	Leaf	27146929	8097157696	51,268,633	94.43%	0.9427
L2-2	Leaf	23155374	6915249116	43,694,345	94.35%	0.9384
L2-3	Leaf	19902165	5947708884	37,704,181	94.72%	0.9423
L3-1	Leaf	24424927	7296339670	45,941,555	94.05%	0.9418
L3-2	Leaf	19758603	5910762376	37,453,786	94.78%	0.9404
L3-3	Leaf	19639126	5862503642	37,025,030	94.26%	0.9348
L4-1	Leaf	24120701	7194014772	45,692,780	94.72%	0.9429
L4-2	Leaf	25240674	7538611890	47,583,169	94.26%	0.9408
L4-3	Leaf	25578929	7634602964	48,485,165	94.78%	0.944
L5-1	Leaf	23520561	7019809848	44,475,965	94.55%	0.9414
L5-2	Leaf	25735037	7672017466	48,427,848	94.09%	0.942
L5-3	Leaf	20405526	6095447932	38,349,309	93.97%	0.9448
S1-1	Stem	22800715	6812742042	42,980,243	94.25%	0.9106
S1-2	Stem	22836218	6828492916	42,832,487	93.78%	0.9087
S1-3	Stem	22735787	6802845532	43,103,569	94.79%	0.921
S2-1	Stem	19265879	5762734554	35,745,131	92.77%	0.9195
S2-2	Stem	20989232	6280314128	39,433,787	93.94%	0.9196
S2-3	Stem	22355168	6687921290	41,716,552	93.30%	0.9119
S3-1	Stem	22815587	6826112342	43,028,897	94.30%	0.9139
S3-2	Stem	22348949	6673546576	41,673,496	93.23%	0.9125
S3-3	Stem	22312298	6672517446	41,339,946	92.64%	0.911
S4-1	Stem	22410173	6701281464	41,481,410	92.55%	0.9154
S4-2	Stem	23031748	6888293788	43,192,485	93.77%	0.9225
S4-3	Stem	22370476	6686367792	41,527,114	92.82%	0.9142
S5-1	Stem	19240953	5757499444	34,723,306	90.23%	0.9194
S5-2	Stem	19540603	5844492582	36,650,703	93.78%	0.9177
S5-3	Stem	22383929	6696077498	41,118,786	91.85%	0.9144

Supplementary Table 7. Statistics of repeat contents in *Populus wilsonii* genome.

Type	Number	Length(bp)	Rate (%)
Retroelement	213,466	122,441,381	25.65
LTR/Gypsy	91,583	61,579,392	12.9
LTR/Copia	46,293	36,611,814	7.67
LTR/others	50624	18,528,103	3.89
LINE	23,221	5,536,069	1.16
SINE	1,745	186,003	0.04
DNA transposon	176,705	59,967,949	12.56
tandem repeat	318,075	56,056,995	11.74
Total	708,246	238,466,325	49.96

Supplementary Table 8. Statistics on gene information of different species.

Species		<i>P. trichocarpa</i>	<i>P. wilsonii</i>	<i>A. thaliana</i>	<i>P. deltoides</i>	<i>P. euphratica</i>
Gene	Number	34,699	38,054	27,628	44,853	36,426
	Length(bp)	126,544,386	133,688,470	65,552,768	136,044,577	126,214,227
	Average Length(bp)	3646.92	3513.13	2372.69	3033.12	3464.95
Exon	Number	177,938	198,300	141,285	212,920	201,601
	Average Number	5.13	5.21	5.11	4.75	5.53
	Length(bp)	43,737,174	68,941,684	33,622,504	48,528,846	54,848,341
CDS	Average Length(bp)	1260.47	1811.68	1216.97	1081.95	1505.75
	Number	177,938	190,693	141,285	212,920	193,128
	Average Number	5.13	5.01	5.11	4.75	5.3
Intron	Length(bp)	43,737,174	50,284,380	33,622,504	48,528,846	41,373,495
	Average Length(bp)	1260.47	1321.4	1216.97	1081.95	1135.82
	Number	143,239	160,246	113,657	168,067	165,175
Intron	Average Number	4.13	4.21	4.11	3.75	4.53
	Length(bp)	82,807,212	64,746,786	31,930,264	87,515,731	71,365,886
	Average Length(bp)	2386.44	1701.44	1155.72	1951.17	1959.2

Supplementary Table 9. Evaluation the Gene Completeness by Benchmarking Universal Single-Copy Orthologs (BUSCO) in *Populus wilsonii* genome.

Type	Number	Percent
Complete BUSCOs(C)	1,558	96.53%
Complete and single-copy BUSCOs(S)	1,317	81.60%
Complete and duplicated BUSCOs(D)	241	14.93%
Fragmented BUSCOs(F)	37	2.29%
Missing BUSCOs(M)	19	1.18%
Total Lineage BUSCOs	1,614	

Supplementary Table 10. Statistical information of gene function annotation

Annotation Database	Annotated Number	Annotated Ratio (%)
GO Annotation	30,762	80.84
KEGG Annotation	27,948	73.44
KOG Annotation	20,082	52.77
Pfam Annotation	31,963	83.99
Swissprot Annotation	30,446	80.01
TrEMBL Annotation	37,391	98.26
eggNOG Annotation	31,448	82.64
nr Annotation	37,435	98.37
All Annotated	37,547	98.67

Supplementary Table 11. Statistical information of non-coding RNA in *P. wilsonii* genome

Type	Number	Length(bp)
rRNA	8,186	11,428,311
tRNA	5,066	381,283
miRNA	126	14,650
snRNA	101	14,963
snoRNA	474	51,055
Total	13,953	11,890,262

Supplementary Table 12. Species and gene sets used in this study

Species	Abbreviations	Database	Version
<i>Populus euphratica</i>	<i>P. euphratica</i>	BIG Submission	V3.1
<i>Populus alba</i>	<i>P. alba</i>	BIG Submission	V3.1.1
<i>Populus deltoides</i>	<i>P. deltoides</i>	phytozome	v2.1
<i>Populus trichocarpa</i>	<i>P. trichocarpa</i>	phytozome	v4.1
<i>Arabidopsis thaliana</i>	<i>A. thaliana</i>	phytozome	Araport11
<i>Oryza sativa</i>	<i>O. sativa</i>	phytozome	V7_JGI
<i>Manihot esculenta</i>	<i>M. esculenta</i>	phytozome	v7.1
<i>Vitis vinifera</i>	<i>V. vinifera</i>	phytozome	V2.1
<i>Salix purpurea</i>	<i>S. purpurea</i>	phytozome	v5_1

Supplementary Table 13. Orthologous Groups in *P. wilsonii* and other plant species

Item	<i>A. thaliana</i>	<i>M. esculenta</i>	<i>O. sativa</i>	<i>P. alba</i>	<i>P. deltoides</i>	<i>P. euphratica</i>	<i>P. trichocarpa</i>	<i>P. wilsonii</i>	<i>S. purpurea</i>	<i>V. vinifera</i>
Number of genes	27,628	33,849	39,049	40,213	44,853	36,426	34,699	38,054	35,125	31,845
Number of genes in orthogroups	18,385	30,635	21,136	32,537	39,386	30,433	32,862	35,618	33,268	23,665
Number of unassigned genes	9,243	3,214	17,913	7,676	5,467	5,993	1,837	2,436	1,857	8,180
Percentage of genes in orthogroups	66.5	90.5	54.1	80.9	87.8	83.5	94.7	93.6	94.7	74.3
Percentage of unassigned genes	33.5	9.5	45.9	19.1	12.2	16.5	5.3	6.4	5.3	25.7
Number of orthogroups containing species	12,663	18,195	12,589	20,107	22,715	20,499	20,630	21,338	19,011	16,934
Percentage of orthogroups containing species	33.6	48.2	33.4	53.3	60.2	54.3	54.7	56.6	50.4	44.9
Number of species-specific orthogroups	2,520	697	4,120	512	521	227	56	140	453	1,001
Number of genes in species-specific orthogroups	6,823	2,669	11,486	1,211	1,306	504	156	1,072	2,531	3,448
Percentage of genes in species-specific orthogroups	24.7	7.9	29.4	3	2.9	1.4	0.4	2.8	7.2	10.8

Supplementary Table 14. Gene copy number of key genes involved in cellulose synthesis in *Populus wilsonii* and 4 other *Salicaceae* species.

Gene	<i>S. purpurea</i>	<i>P. alba</i>	<i>P. deltoides</i>	<i>P. euphratica</i>	<i>P. trichocarpa</i>	<i>P. wilsonii</i>
CES	16	21	17	23	18	17
CsL	27	41	36	37	36	35
GH	34	36	35	33	33	30
GT	38	42	47	35	40	38

Supplementary Table 15. Gene copy number of key genes involved in lignin and flavonoids synthesis in *Populus wilsonii* and 4 other *Salicaceae* species.

Type	Gene	<i>S. purpurea</i>	<i>P. alba</i>	<i>P. deltoides</i>	<i>P. euphratica</i>	<i>P. trichocarpa</i>	<i>P. wilsonii</i>
Commom	CM	4	5	3	3	3	3
	ADT	8	6	8	5	5	5
	PAL	5	6	6	6	5	5
	C4H	4	4	4	3	4	3
	4CL	16	16	23	21	19	22
Lignin	HCT	3	5	9	4	5	6
	LAC	44	43	45	40	49	48
	PRX	95	98	113	81	97	90
	CCoCAMT	4	6	6	5	6	6
	CCR	15	8	22	12	13	11
	COMT	15	33	42	30	33	28
	CSE	2	2	2	2	2	2
	F5H	4	4	3	2	3	3
	C3H	4	3	3	1	3	3
	CAD	16	18	18	15	18	19
Flavonoids	ANR	1	1	2	2	2	2
	ANS	2	3	2	2	2	2
	CHI	3	5	3	4	4	3
	CHS	10	15	17	10	13	12
	DFR	2	2	4	2	2	2
	FLS	4	5	3	4	4	4
	F3'H	1	1	1	1	1	1
	F3'5'H	1	3	2	1	2	2
	FNS II	2	1	1	0	3	3
	FOMT	12	20	19	16	18	18
	ICS	1	1	1	2	1	1
	LAR	2	3	3	4	3	3
	F3H	3	2	4	1	2	4
	UFGT	22	28	16	25	26	23

Supplementary Table 16. Primers used in this study for qRT-PCR analysis.

Gene Name	Gene ID	Forward Primer sequence (5'-3')	Reverse primer sequence (5'-3')
bHLH1	Pwi01G001590	ACGAATTTGAACCAAAACCCACT	TATGCATGCACACCTTGCAC
GH2	Pwi01G006950	ACAGGCATGATGGTTTCCGT	CCTGACAAAGCCACAAGTGC
GRF2	Pwi01G010250	TGGAACGGAACATGAGCCAG	GCACTACCGATTCCAGGGAG
GDSL-3	Pwi01G017170	CGGGGAGCAGTGACTTTCTT	GGCATCAGCGTTGATCCTTG
WRKY10	Pwi01G032650	CATGCAACCCCAATTACGGC	GCCTGAGATGGATGAACGCT
bHLH14	Pwi01G037250	TCGGTGCAGAAATTGATGGA	AGGCTCCAAGATAGAGCCCA
WRKY13	Pwi02G004060	AGCTGCACACCTACAATGTCA	GAACCTGTATCCGAGGAGCG
bHLH17	Pwi02G004200	AAATGTTGGTGCTGCCATGC	GACGGATCTTCCAAGCACCT
bHLH19	Pwi02G005150	GTGGCAAGATGGGTAAGCCT	ATTGTGCTGGAATCCTCGGG
CsL8	Pwi02G020210	GGTTCATGTCCCGTGATGTAA	CAACCCACCTCTTGACCCC
CHS5	Pwi03G016840	AGCCTAACCGAGGCATTCAA	TTCTCTGGTTTCAGCGCCAA
CEPR1	Pwi04G000150	TATCGGGTGAGTCCCATCA	GCCTCCATACACGCTCTTCA
SAUR21	Pwi04G014630	TGGCCATCCTTCTTAAGGGTA	AAGGAAGTGTAAGACCGCCC
bHLH50	Pwi05G000190	GCTACGGCTGTAGTTCACA	TTGCAAATGCTCACCAGCCT
PRX30	Pwi05G006230	GTTTGTCCAGGCACGGTTTC	GTGACCCTGAAAGGGCTACC
bHLH72	Pwi06G006550	CGTGGTCCCAACATTACCA	TCAGCTTGAATTCTTCTCTTGC
WRKY45	Pwi06G011910	GAACCAGGAGGAGGATCCAA	ACCTTCTTTTCACTGGACAAC
bHLH80	Pwi07G002170	TGGCAGGTGAAGATGTGACC	TGCCCCGACATGAATGTAGT
GT21	Pwi09G001110	AGGAACTCTACTCGACCAGG	TTGGAAGCCTCACGGTCTG
CCR10	Pwi09G007320	TACTAACGGCGGCAAAGGAG	CTGTACCCGGATTACACCAC
UFGT15	Pwi09G009350	AGCTTTATTGGGCGGGGA	CCCCCTTCTTCAACTGCCTTC
ERF109	Pwi09G009600	TTAACACTGCAGAGGAGGCG	AGAACGCATTGTCTTTCCCA
LBD15	Pwi10G015830	GAGGCAAGGCTCAGAGATCC	GGAGGAGAATTGGCAGCGTA
CHI1	Pwi10G018290	TGGCGCAGGGGCGAG	CCGAATATTGGAGGCCCGT
GRF11	Pwi12G001090	AACACCAGCAGCACCCCAG	TGGCAAGAGTTGGACTGCTATT
CesA14	Pwi13G001780	CTTTGCCCCAAGTCCCTCTC	CCACTGCCACCAATAGGAGC
FNSII3	Pwi13G002490	ACGAAGAAAGGAACTGCGCC	GTTATCAGTCCCTGAACACCTGA
WRKY76	Pwi14G007110	GGACTTGGGCTCTAAGGACG	TGGGGTCTGCACATTTTGGT
bHLH135	Pwi14G008540	TGCAACAAGACCAACAAGCG	AGGAACTATCTGACTTGCCGA
CHS13	Pwi14G011620	TCAGCTGCACAAACCATCCT	ACCAGGGTGTGCAATCCAAA
CAD19	Pwi16G007310	AAGCCTCTTGAGCTTCCTGC	CCAAACGTTCCATTGCCGTA
CCR4	Pwi19G008730	ATGTCACACCTCTCACTCCAC	TCAACGACATTCTCGGCAC
UBQ	Pwi01G037400	GTTGATTTTTGCTGGGAAGC	GATCTTGGCCTTCACGTTGT