

Supplementary Materials: Transcriptome Co-Expression Network Analysis Identifies Key Genes and Regulators of Sweet Cherry Anthocyanin Biosynthesis

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Table S1. Anthocyanin biosynthesis structural genes and TFs.

Gene	NCBI accession	Genome number	Reference	Full name
<i>PacANS</i>	XP_021803569.1 AEO79983.1	<i>Pav_sc0000107.1_g100.1.mk</i>	Liu <i>et al.</i> , 2013	anthocyanidin synthase
<i>Pac4CL2</i>	XP_021816145.1	<i>Pav_sc0000636.1_g260.1.mk</i>		4-coumarate-CoA ligase
<i>PacbHLH13</i>	XM_021959322.1	<i>Pav_sc0000586.1_g190.1.mk</i>		basic helixloop-helix transcription factor
<i>PacbHLH74</i>	XP_021828015.1	<i>Pav_sc0001519.1_g020.1.mk</i>		basic helixloop-helix transcription factor
<i>PacDIV</i>	XP_021814588.1	<i>Pav_sc0000580.1_g150.1.mk</i>		DIVARICATA transcription factor
<i>PacERF109</i>	XP_021824713.1	<i>Pav_sc0000071.1_g750.1.mk</i>		ethylene responsive transcription factor
<i>PacERF115</i>	XP_021814656.1	<i>Pav_sc0000042.1_g600.1.mk</i>		ethylene responsive transcription factor
<i>PacGATA8</i>	XP_021826743.1	<i>Pav_sc0000084.1_g450.1.mk</i>		GATA transcription factor
<i>PacGT2</i>	XP_021810680.1	<i>Pav_sc0000378.1_g010.1.mk</i>		GT transcription factor
<i>PaGTE10</i>	XM_021975465.1	<i>Pav_sc0002130.1_g020.1.mk</i>		GTE transcription factor
<i>PacMYB308</i>	MW310249	<i>Pav_sc0000373.1_g330.1.mk</i>		MYB transcription factor
<i>PacPosF21</i>	XP_021803476.1	<i>Pav_sc0012575.1_g010.1.mk</i>		PosF21 transcription factor
<i>PacWRKY7</i>	XP_021820223.1	<i>Pav_sc0000852.1_g460.1.mk</i>		WRKY transcription factor

The partial sequences were confirmed by QPCR products resequencing with ‘Hong Deng’ fruit cDNA as template.

Table S2. Primers for real-time PCR.

Gene	Forward (5' to 3')	Reverse (5' to 3')
<i>PacANS</i>	GCCAAAGTGTGTTCCAAATTCCA	GATCTTCTCCTTTGGTGGCTCA
<i>Pac4CL2</i>	CCTGTTGCATTTGTGGTTCGAT	GCTTGGCTCTCAGGTCTTTTCT
<i>PacbHLH13</i>	GAGGTTGTTGTAAGGGTGAGCT	CTTTTCCTTCGTCAATGGCTCG
<i>PacbHLH74</i>	GGAATCAGCTCTTCTCACCCAT	TGATTTCAAGCGGCCATTTTGT
<i>PacDIV</i>	GTGACTGGCATGAGTGAACAAC	GCATCTGAAAACGCGCATTGTA
<i>PacERF109</i>	GGGCAAAGTCAATCCAATTACTGT	AGCATGAGGTATTGTAGTTGGCT
<i>PacERF115</i>	ACCATATGTTCAAGGCGGAAGT	CGAACGAGAAGAACCAAACCTGC
<i>PacGATA8</i>	AAAACCCCTATGCAATGCCTGTG	AGATGCTGCTGTTAGTATTCGG
<i>PacGT2</i>	ATGTGGTTATGTTGCGCTGCTC	CCATCTCCATCTGCATTTTCATC
<i>PaGTE10c</i>	CAGGCTGGGTAGTTTCAAGCTA	TCATTTGCTGCTTCTGGTTTGG
<i>PacMYB308</i>	CTTTTCTTGCAAGTTTGGGGCTT	CATCTCCAAGCTTCTGTAATCC
<i>PacPosF21</i>	TGGGCCTATGATGAACTTTGCT	ATCCTCCCATTTTCAAGTCCCC
<i>PacWRKY7</i>	CTGCCACTGTTCCAAGAAAAGG	CAGCATGGTTGGATCGTCTAGA
<i>Actin</i>	CCAGGGCTGTGTTTCCTTCTA	ATGATCTGCGTCATCTTTTCT

Table S3. Sequences (5' to 3') for promoter isolation.

Gene	Promoter Sequence	Length (bp)
<i>PacANS</i>	CCCTTATTAAATATAGGGCCTCTTAAATATATTGACTTTCATATTTCCCTCAATTTATCT TTTAATTTTTTTTACAATTTTCTATTATCTTGTTAAAAAATTAATTTAAAGTGATATAT CATATATTGATGAAGAATCACGTGTGTGGTGAAAAATTCAAATTCACGTGGAAACAA ACATGCATGAACATGCCTGGGTCTTGAATGTATGTTGATGTTCAAATCGTTGGAATGA TGTAGGTGTTCCAAGAATTTTTTTTACCATCCCATCCATTTCCGCCACCTGGGATAAA TTTTAGGTGTGTTTCTGCTGAATATTTTTTTTTTAAATAAAAAATTTCTGTTAAAGA CTAATCCTACGGCAATTGGATTGATTGTAAGTTTGATTTTTACGCTCTTTTAAAAAATT GATGTTTTTTTTTCTTATTTAATTTTTAACGAGATGATAAGAAAGTTGTAACAAAAA TTGATAAATTGAAAGAAACATAAAGGTTAATACATTTAATAAGGGTAAATTAGGAAA ACAAAAGCTAGCTTGTCTCGAGTATTAATGATATTGTTTAAAGTGTAAGTAAAGTGA CACATGTCATTAGAAAAAGCGGGCAGATCCAAAAGAAAAGATTAGAGACAAGGTT GCTAGAATTCCCCTGTATCCAAATGTCACCAACAAAACAAGCTAGCTTGCCTCTGTT TGGTCTTGTTGAACCTTGTTTGCTTACTGGTAGGTAGGAAAAACGTGCTCAACAAA ACAAGCGATATATGGTGGTTTTATGAGAATGGAACCTCAACGCCCCGCTGATTTGTGG CACCAATTCAGGCCCGTAGTGTGGATGAGGAAGGAACTTTCTAGCTATTGATACAAC CTACTTATAACTCTTTAAATTAATATATTAACATGAAAAAGTCAAAGAAGCAATA AATTCGAGATCACAACGTTACTTGTACGGACTTATAGCTCTAGTGGCAGAAAAAATA AACACTGCTTATGACAAGAGTCTAACTCTTGCCCTTTTCTATTGAATACAAAGAACG TGGATTTATGAAACCAAATAAATCATTAGGGTAACTTGGGCTCAAAGTTTGAAATATT AGCAAAATGCTTGTAGCTCAAATGGATAAAAGTATTTACACTTGAGATTGATCGTGT ACTCGATTCTCCCCCTCCCGATAACATCTCTACAGAAAATAAAATAGAGAGTTTGGA TTATTAGTCCAGTGAAATAAGGTATAATTGGAAGTGGTTCTAATGCTACATATGCTCA GGCATAAATTGGAATGCAACTAATAAACATGTAGGTGTAGCCCTTAATCTATCTACCG TGCTTAGGGGCCTCTTTGTTCAAGGTAGCCAAATGTGTGATTAATTATTAGTTTTAG AGTAAAAATATAAACTAATTAGTTTCTAGAACAATGCATTTAAATGCTATAGTTAGG CAATGAAGAACAGAAAGTCTTGTAGCTGGCACTGTCTGAAACTCTGAAAGGACGGAT ATGGTTGTAGCTTAAATCCCATGCATGCTCACTTGCCAGTCAAACCTTCCTTGATGA GAAGTCCGGTGGGTGAGAAGTGTACATGGACAACAGACCCAAAATCACACAAAT TCAACACAAACACATTCTCCACTCTCTCTGTTGGCAAGAAGTTCCCTTGAAAT ATAAGCACCTCCAACCTACTAATTGGGTACACTACTATAAAAGCAACATTACCAATAC ACCATTAACCATTTGCTTCATCAATCCACCATAAGAAAAGAGCATAGGCCTGCACTT TTAGTGTAATAAAGCTATTAAAGAAAGCTAGCCAAGAGTTCAGAGCTTACGAAGAG CCGGCTGCCAAA	1850

Table S4. Primers for vector construction for dual-luciferase assays.

Gene	Forward (5' to 3')	Reverse (5' to 3')
<i>PacANS</i>	TCGACGGTATCGATAAGCTTCCC TTATTAAATATAGGGCCCC	GCTCTAGAACTAGTGGATCCTTTG GCAGCCGGCTCTTCGTAAG
<i>PacbHLH13</i>	GCTCTAGAACTAGTGGATCCATG GTTCCAGTGCCAAAGAAGGAC	TCGACGGTATCGATAAGCTTCTACC CAACCGATGACAATGACTG
<i>PacbHLH74</i>	GCTCTAGAACTAGTGGATCCATG GGTGGTCATGACAGTGAGGA	TCGACGGTATCGATAAGCTTTTATA GCTCTGATTTCAAGCGGC
<i>PacDIV</i>	GCTCTAGAACTAGTGGATCCATG TACAGAGGAATTGGAATTC	TCGACGGTATCGATAAGCTTTTCACT GGTATCGCATCGATTGC
<i>PacERF109</i>	GCTCTAGAACTAGTGGATCCATG CCCTTGACGCCAATCGCAT	TCGACGGTATCGATAAGCTTTTATC CTGGCTTCATCAAAGCAG
<i>PacERF115</i>	GCTCTAGAACTAGTGGATCCATG ATTTCAGCTCTAGCTCAGG	TCGACGGTATCGATAAGCTTTTCATC TTGAGTGACTGCCTTCA
<i>PacGATA8</i>	GCTCTAGAACTAGTGGATCCATG ATCGGAACTTCATCGACG	TCGACGGTATCGATAAGCTTTTCAC ATGTAATCCATGGAGATGC
<i>PacGT2</i>	GCTCTAGAACTAGTGGATCCATG CTCGAAAATTCAAAGTTACC	TCGACGGTATCGATAAGCTTTTCAG CTCATAATTGCCATTGAAG
<i>PaGTE10</i>	GCTCTAGAACTAGTGGATCCATG GCACCAACTGTTCCAATAGAC	TCGACGGTATCGATAAGCTTTTCAAT CAATCTCTCCTTCTTCAAAG
<i>PacMYB308</i>	GCTCTAGAACTAGTGGATCCATG GGAAGATCTCCTTGCTGTG	TCGACGGTATCGATAAGCTTTTCATT TCATCTCCAAGCTTCTG
<i>PacPosF21</i>	GCTCTAGAACTAGTGGATCCATG GATAAGGACAAGTCTCCTGC	TCGACGGTATCGATAAGCTTTTAAC AATCCTTCGTTACAGATTGG
<i>PacWRKY7</i>	GCTCTAGAACTAGTGGATCCATG GCTGTGGAGCTCATGATGGG	TCGACGGTATCGATAAGCTTTTAAG ATGATTCGAGGACGAGAGC

The *PacANS* promoter was constructed to pGreen II 0800-LUC vector, and full-length sequences of TFs were constructed to pGreen II 0029 62-SK vector.

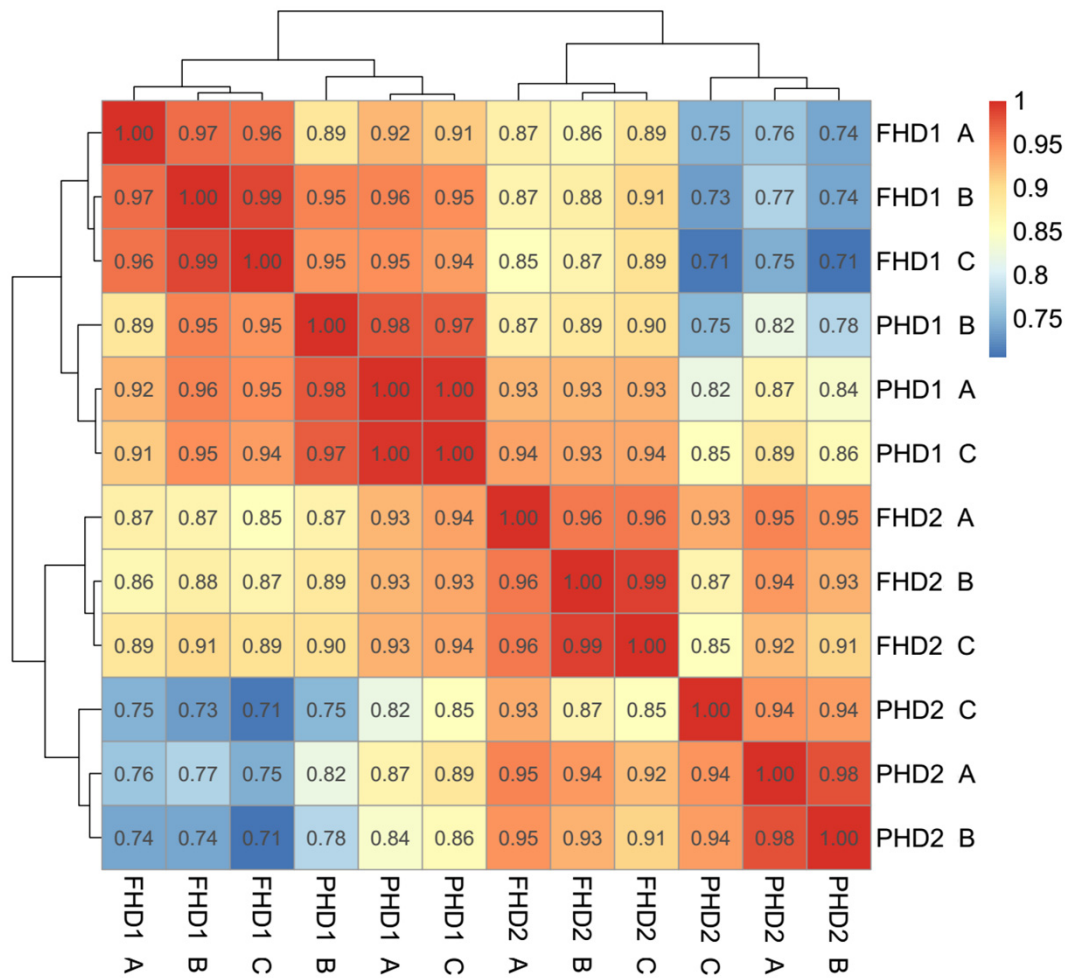
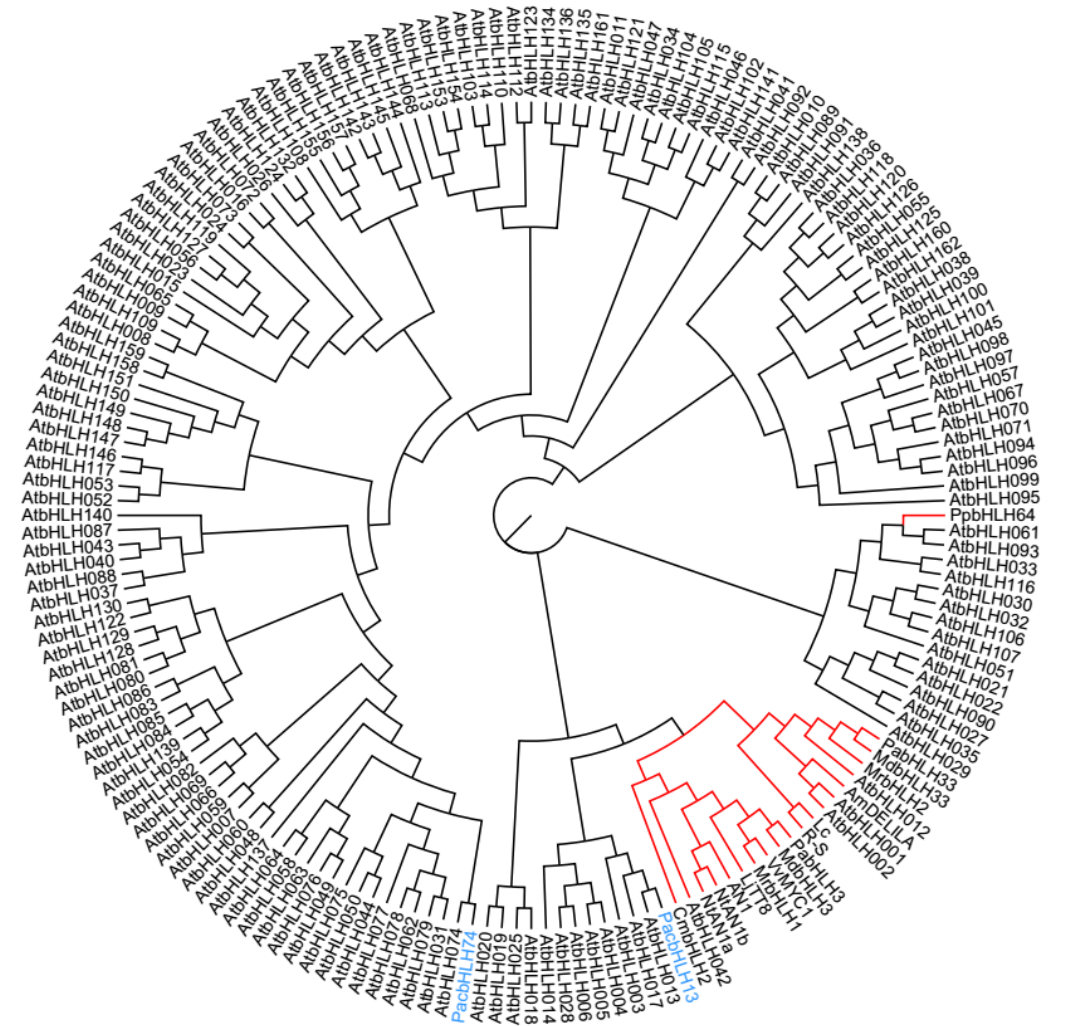


Figure S1. Pearson correlation analysis between three biological replicates.



1.0

Figure S2. Phylogenetic tree analysis of *PacbHLH13/74* with *Arabidopsis thaliana* *bHLH* sequences and anthocyanin synthesis related *bHLHs* in other species (red line). GenBank accession number: *MdbHLH3*, HM122458; *MdbHLH33*, DQ266451; *MrbHLH1*, JX629461; *MrbHLH2*, JX629462; *AmDELILA*, AAA32663; *Lc*, NM001111869; *R-S*, X15806; *VvMYC1*, EU447172; *LjTT8*, BAH28881; *AN1*, AF260918; *NtAN1a*, AEE99257; *NtAN1b*, AEE99258; *CmbHLH2*, KT724056; *PabHLH3*, KP126522; *PabHLH33*, KP126523; *PpbHLH64*, Pbr001646.1). The deduced amino acid sequences of *Arabidopsis thaliana* *bHLHs* were obtained from TAIR. The phylogenetic trees were constructed using FigTree (v1.4.2).