

Supplementary Information

Table S1. Length of epimedii R2R3-MYB coding sequences and introns with their predicted splice junctions ^a.

Gene name	Length (bp)		Intron I (phase 1)		Intron II (phase 2)			
	Coding Sequence (bp)	Protein Sequence (aa)	Intron I (bp)	Intron II (bp)	5'Splice site	3'Splice site	5'Splice site	3'Splice site
<i>EsMYB1</i>	888	295	112	90	GCAG:GTATTC	ATTCAG:GT	AACAA:GTAAGA	TTTC ITG :GTGG
<i>EsMYB2</i>	1323	440	121	89	GCAG:GTAAGG	TTTCAG:GT	AATAG:GTATTA	CACCAG:GTGG
<i>EsMYB3</i>	927	308	none	none	none	none	none	none
<i>EsMYB4</i>	1068	355	141	140	ACTG:GTGAAA	TTGAAG:GA	AACAA:GTAATT	GAGCAG:ATGG
<i>EsMYB5</i>	735	244	85	88	GCAG:GTAAAT	CTTCAG:GT	AACAA:GTGAGT	TCGCAG:GTGG
<i>EsMYB6</i>	1101	366	92	98	GCAG:GTAAC	TTGCAG:GA	AACAG:GTATTG	TTGTAG:GTGG
<i>EsMYB7</i>	804	267	272	93	GCCG:GTAAGA	GTGCAG:GT	AACAG:GTGCTT	GTTTAG:ATGG
<i>EsMYB8</i>	897	298	none	280	none	none	CACAA:GTAAGT	TTCCAG:GCTC
<i>EsMYB9</i>	873	290	none	107	none	none	AACAG:GTGATT	TTGCAG:ATGG
<i>EsMYB10</i>	903	300	83	135	GCAG:GTAAAG	ACGTAG:GT	AACAG:GTATTA	TTGTAG:ATGG
<i>EsMYB11</i>	801	266	117	66	GCAG:GTAAAT	TTTCAG:GT	AGCAA:GTGAGT	TTACAG:GTGG
<i>EsMYB12</i>	783	260	109	159	GCAG:GTTGGT	CCTCAG:GT	AACAG:GTGCGA	TTATAG:GTGG
<i>EsMYB13</i>	1053	350	none	none	none	none	none	none

^a Coding sequences indicate the length in nucleotides from the translation start codon to the stop codon and protein sequences correspond to the deduced amino acid length of coding sequence. Intron I and II represent the first and second introns, respectively. Intron phase refers to the position in a codon where the intron is inserted: after the first nucleotide (phase 1) or after the second nucleotide (phase 2) of a codon. Italic nucleotide pairs GT and AG (except *EsMYB1* intron II, bold and italic TG shown) represent the beginning and the end of the introns, respectively.

Table S2. Blast analysis of epimedii R2R3-MYB proteins in *Arabidopsis* nr protein database.

Protein name	Amino acid	Top blastp in <i>Arabidopsis</i>	Accession number	Score	Query coverage	E value
EsMYB1	295	MYB4	NP_195574.1	277	92%	2.00×10^{-74}
EsMYB2	440	MYB61	NP_172425.2	317	99%	2.00×10^{-86}
EsMYB3	308	MYB73	NP_195443.1	249	94%	6.00×10^{-66}
EsMYB4	355	MYB60	NP_172358.1	255	58%	8.00×10^{-68}
EsMYB5	244	MYB3	NP_564176.2	177	60%	3.00×10^{-44}
EsMYB6	366	MYB86	NP_850879.1	249	43%	4.00×10^{-66}
EsMYB7	267	TT2 (MYB123)	NP_198405.1	181	59%	2.00×10^{-45}
EsMYB8	298	MYB	NP_200698.1	295	89%	7.00×10^{-80}
EsMYB9	290	MYB5	NP_187963.1	270	81%	2.00×10^{-72}
EsMYB10	300	TT2 (MYB123)	NP_198405.1	195	55%	7.00×10^{-50}
EsMYB11	266	MYB102	NP_567626.1	177	53%	3.00×10^{-44}
EsMYB12	260	MYB4	NP_195574.1	237	83%	2.00×10^{-62}
EsMYB13	350	MYB44	NP_201531.1	272	97%	1.00×10^{-91}

Table S3. Blast analysis of epimedii *R2R3-MYB* proteins in nr protein database excluding *Arabidopsis*.

Protein name	Amino acid	Top blastp out <i>Arabidopsis</i>	Accession number	Score	Query coverage	<i>E</i> value
EsMYB1	295	<i>Gossypium hirsutum</i> , MYB1	AAA33067.1	302	99%	7.00×10^{-80}
EsMYB2	440	<i>Betula luminifera</i> , MYB-like	ACJ38663.1	433	99%	2.00×10^{-119}
EsMYB3	308	<i>Glycine max</i> , MYB112	ABH02852.1	292	93%	7.00×10^{-77}
EsMYB4	355	<i>Vitis vinifera</i> , MYB60	XP_002271738.1	306	95%	3.00×10^{-81}
EsMYB5	244	<i>Vitis vinifera</i> , MybPA2	ACK56131.1	178	78%	1.00×10^{-42}
EsMYB6	366	<i>Ricinus communis</i> , MYB	XP_002511336.1	327	99%	2.00×10^{-87}
EsMYB7	267	<i>Lotus japonicus</i> , TT2a	BAG12893.1	221	86%	2.00×10^{-55}
EsMYB8	298	<i>Antirrhinum majus</i> , DIV	AAL78741.1	358	99%	1.00×10^{-96}
EsMYB9	290	<i>Vitis vinifera</i> , MYB5b	AAX51291.1	303	91%	5.00×10^{-80}
EsMYB10	300	<i>Gossypium hirsutum</i> , MYB10	AAK19615.1	258	90%	1.00×10^{-66}
EsMYB11	266	<i>Malus x domestica</i> , MYB11	AAZ20431.1	181	62%	1.00×10^{-43}
EsMYB12	260	<i>Vitis vinifera</i> , MYBC2-L1	ABW34393.1	332	85%	9.00×10^{-89}
EsMYB13	350	<i>Glycine max</i> , MYB50	ABH02824.1	358	97%	9.00×10^{-125}

Table S4. List of primers used for conserved domain and the full-length cDNA amplification of epimedii *R2R3-MYB* genes.

Gene name	Degenerate primers for conserved domain (Forward/Reverse) ^a	Gene specific primers for full-length cDNA (Forward/Reverse)	Length of FLC (ORF) ^b (bp)
<i>EsMYB1</i>	5' TGY TGY GAR AAR GCN CAY AYN AA 3' 5' TT CCA RTA RTT YTT NAY YTC RTT 3'	5' GACATTTCCCCCAGTTTTCCT 3' 5' AATGATTACAAGTAGGTATTTGAACA 3'	1122 (888)
<i>EsMYB2</i>	5' CAY TCN TGY TGY TWY AAR CAR AA 3' 5' CAY AGY TGY TGY TWY AAR CAR AA 3' 5' AR RTT YTT DAT YTC RTT RTC NGT 3'	5' GAGACTCTTCTGCTCTTGCTGAT 3' 5' GCCTTTATTCAAGCCTTTCAAATT 3'	1460 (1323)
<i>EsMYB3</i>	5' AAR GGN CCN TGG TCN CCN GAR GA 3' 5' AAR GGN CCN TGG ASN CCN GAR GA 3' 5' AT NGT NGC CCA YTT RTT NCC RAA 3'	5' GCTCACAACCAACCCTCCCCTT 3' 5' CTCAAAACCCCAAACTATCTCCATCA 3'	1085 (927)
<i>EsMYB4</i>	5' AAR GGN CCN TGG ASN CCN GAR GA 3' 5' CCA RTA RTT YTT DAT RTC RTT RTC 3'	5' AGCTAGCTGGTGCAAAAGGAATTG 3' 5' TGAAGATACCCAAAGGTCCCAGAAC 3'	1297 (1068)
<i>EsMYB5</i>		5' GCAAAACAGAGGGAAAAAATAAT 3' 5' CTGCAGTTTATTGAAAATTTCACA 3'	822 (735)
<i>EsMYB6</i>		5' AGATTTGCGTAAAAACCACTTGAT 3' 5' CGCAGAATAGAAGAATCCATTTCAC 3'	1563 (1101)
<i>EsMYB7</i>		5' GACAGTAGTCTTTCAAGCCTAAGAAATG 3' 5' TACTCTGCAGGAACGGAAGACAC 3'	820 (804)
<i>EsMYB8</i>		5' GGGATGGAAATTCAGTCACCAAG 3' 5' ATGATTCGGGTGCAATACATAATTGC 3'	1216 (897)
<i>EsMYB9</i>		5' CTGCCGTGCTGTGTAACATAGA 3' 5' TCAATGTAACCTATGACAACAAGAAGC 3'	1095 (873)
<i>EsMYB10</i>		5' GGAAATGGGAAGAAGCCCCTGTTGT 3' 5' TGATCTATTTACCAAACATTGAATC 3'	986 (903)

Table S4. Cont.

Gene name	Degenerate primers for conserved domain (Forward/Reverse) ^a	Gene specific primers for full-length cDNA (Forward/Reverse)	Length of FLC (ORF) ^b (bp)
<i>EsMYB11</i>		5' CAGAGAAAGGAAAAGAAAATGGTT 3' 5' AGAAAAAATTTTGATTTTATTACACC 3'	878 (801)
<i>EsMYB12</i>		5' AGTCTGTCTGTTGGGTGTGGATG 3' 5' TCACTGCTTCAGAAATGCTTACCAC 3'	1199 (783)
<i>EsMYB13</i>		5' CCTCCTCTATACAAACCTCCTACCTT 3' 5' CGATTGCTCTCTGATTTCCTGC 3'	1303 (1053)

^a two forward degenerate primers of *EsMYB2* and *EsMYB3* are mixed as the forward primers for conserved domain PCR, respectively. ^b FLC indicates the full-length cDNA, ORF in the bracket indicates the open reading frame.

Table S5. List of primers used for qPCR assay of *epimedi* *R2R3-MYB* genes.

Gene name	Primers (forward/reverse)	Length of amplicon (bp)
<i>EsMYB1</i>	5' CCTTCCATCATCTTCTTCTTC 3' 5' TTCTTCTTCTGTTGCTGTTG 3'	127
<i>EsMYB2</i>	5' AATCCTTCCAACCACAATG 3' 5' CTGCTTCCACTATTACTTCTG 3'	144
<i>EsMYB3</i>	5' CCTACTCCTACTGCTGTG 3' 5' TGCTTCTCCTTCTCCTTATC 3'	125
<i>EsMYB4</i>	5' AAGTGCGAGGAGTTAGTC 3' 5' ATGGTAGGTAGTTTCAGTTG 3'	141
<i>EsMYB5</i>	5' AAAATAATGGGTAGGAAGCC 3' 5' AGCAACAGATTTCAGCAAG 3'	96
<i>EsMYB6</i>	5' TCATCTCCTTCTCCTCCATTC 3' 5' CCCATTTTACGACGACAG 3'	106
<i>EsMYB7</i>	5' CAGACATCAAGAGAGGAAAC 3' 5' CAGCAATCAGAGACCATC 3'	93
<i>EsMYB8</i>	5' GTCTCTGGAACAATCTGC 3' 5' CCGTAAGGTGGAACAAAC 3'	147
<i>EsMYB9</i>	5' ATACCAACAACCCAAACC 3' 5' CTTAGCAATGACTCCAGAAC 3'	77
<i>EsMYB10</i>	5' TGATAGCAGGACGACTTC 3' 5' TGGTATAGGTTGTGGTTGG 3'	173
<i>EsMYB11</i>	5' TATGGGCGACTTTGCTAC 3' 5' CCTCTCCTCATTTTCATCTCC 3'	94
<i>EsMYB12</i>	5' TCCTTCATCTTCATCCTCTTC 3' 5' CATCCTTCGCCATTTAGTTG 3'	170
<i>EsMYB13</i>	5' GGTTTCGTTTCCTAAGACTG 3' 5' GATGAGATGATTGTTGTTGTTG 3'	148
<i>EsActin</i>	5' CACCACAACCTGCTGAACG 3' 5' AATCGCTCTGCTCCAATG 3'	165