

**Table S1.** Primer sequence for gene expression analysis.

Primer Name	Primer Sequence (5'-3')
PmMYB4-F	ATGAGCTGCACAACAGGAGGA
PmMYB4-R	TTAGCCAAAATAGATCTGACCT
PmMYB4-Q-F	GCTGTAGGCTGAGGTGGATT
PmMYB4-Q-R	TCATGGTACTGGTACGATTAGGTGC
TUA-F	CAAACCTGGTCCCGTATCCTC
TUA-R	CACAGAAAGCTGCTCATGGTAA
Pro-PmMYB4-F	CATTGCCCATATTAAAAAACTAT
Pro-PmMYB4-R	CCTTTCCTTAGCTTGGGTTTGG
pET28a-PmMYB4-F	CAGCAAATGGGTCGCGGATCCATGAGCTGCACAACAGGAGGA
pET28a-PmMYB4-R	GTGGTGGTGGTGGTGCTCGAGGCTAGGTCAGATCTATTTTGGC

**Table S2.** Physicochemical properties of R2R3-MYBs in *Pinus massoniana*.

Protein name	Number of amino acids	Isoelectric point(pI)	Molecular weight(kDa)	Instability index	Grand average of hydropathicity (GRAVY)	Subcellular localization	Transmembrane structure	Signal peptides
PmMYB1	300	5.18	33496.26	46.12	-0.782	nuclear	none	no
PmMYB2	449	5.64	51034.35	51.77	-0.757	nuclear	none	no
PmMYB3	449	9.2	50840.89	59.4	-0.816	nuclear	none	no
PmMYB4	490	5.43	54207.85	50.23	-0.635	nuclear	none	no
PmMYB5	255	7.61	28728.46	58.17	-0.667	nuclear	none	no
PmMYB6	374	5.56	41873.74	55.09	-0.68	nuclear	none	no
PmMYB7	374	7.72	40782.01	51.89	-0.483	nuclear	none	no
PmMYB8	533	5.59	59059.31	44.57	-0.629	nuclear	none	no
PmMYB9	412	6.62	45039.17	60.88	-0.811	nuclear	none	no
PmMYB10	472	5.91	52926.89	48.4	-0.734	nuclear	none	no
PmMYB11	534	8.99	60021.8	67.85	-0.557	nuclear	none	no
PmMYB12	512	8.3	57632.71	59.98	-0.765	nuclear	none	no
PmMYB13	279	8.82	30678.53	67.32	-0.658	nuclear	none	no
PmMYB14	233	8.93	25982.49	51.21	-0.573	nuclear	none	no
PmMYB15	498	6.31	55156.38	72.48	-1.185	nuclear	none	no
PmMYB16	280	5.76	31314.25	48.39	-0.493	nuclear	none	no
PmMYB17	1502	9.41	160729.68	61.95	-0.741	nuclear	none	no
PmMYB18	113	9.97	13187.29	67.98	-0.681	nuclear	none	no
PmMYB19	294	9.17	32815.75	55.33	-0.716	nuclear	none	no
PmMYB20	1079	5.65	117356.94	54.23	-0.535	nuclear	none	no
PmMYB21	317	8.77	35686.04	51.07	-0.573	nuclear	1	no
PmMYB22	417	5.83	47603.66	49.05	-0.939	nuclear	none	no

Protein name	Number of amino acids	Isoelectric point(pI)	Molecular weight(kDa)	Instability index	Grand average of hydropathicity (GRAVY)	Subcellular localization	Transmembrane structure	Signal peptides
PmMYB23	406	5.47	45895.63	57.64	-0.755	nuclear	none	no
PmMYB24	342	8.96	38909.35	51.64	-0.916	nuclear	none	no
PmMYB25	423	6.77	45278.28	58.15	-0.594	nuclear	none	no
PmMYB26	365	8.67	41565.11	51.71	-0.583	nuclear	none	yes
PmMYB27	312	5.88	35114.92	57.82	-0.796	nuclear	none	no
PmMYB28	383	6.45	43387.42	62.68	-0.638	nuclear	none	no
PmMYB29	573	5.98	61745.9	48.87	-0.521	nuclear	none	no
PmMYB30	241	8.49	27387.03	71.4	-0.747	nuclear	none	no
PmMYB31	218	8.95	23529.61	64.83	-0.277	nuclear	none	no
PmMYB32	107	9.84	12508.47	56.14	-0.715	nuclear	none	no
PmMYB33	442	9.32	50333.72	63.91	-0.912	nuclear	none	no
PmMYB34	483	5.33	53805.48	50.36	-0.629	nuclear	none	no
PmMYB35	415	5.23	45274.92	50.69	-0.624	nuclear	none	no
PmMYB36	506	6.54	56672.97	44.85	-0.723	nuclear	none	no
PmMYB37	405	6.17	45340.94	54.56	-0.619	nuclear	none	no
PmMYB38	443	7.64	48999.75	44.4	-0.715	nuclear	none	no
PmMYB39	317	5.27	35572.35	57.24	-0.611	nuclear	none	no
PmMYB40	380	7.62	42650.66	66.45	-0.693	nuclear	none	no
PmMYB41	232	8.17	25646.6	60.93	-0.906	nuclear	none	no
PmMYB42	232	7.59	26010.4	53.95	-0.715	nuclear	none	no
PmMYB43	300	8.41	33861.35	54.49	-0.607	nuclear	none	no
PmMYB44	386	4.72	43193.95	59.72	-0.585	nuclear	none	no
PmMYB45	438	5.43	48635.51	53.6	-0.787	nuclear	none	no

Protein name	Number of amino acids	Isoelectric point(pI)	Molecular weight(kDa)	Instability index	Grand average of hydropathicity (GRAVY)	Subcellular localization	Transmembrane structure	Signal peptides
PmMYB46	442	5.52	48821.18	52.24	-0.592	nuclear	none	no
PmMYB47	1128	5.05	127035.22	53.48	-0.936	nuclear	none	no
PmMYB48	1727	5.68	189753.5	53.4	-0.758	nuclear	none	no
PmMYB49	386	6.67	43344.46	48.95	-0.703	nuclear	none	no
PmMYB50	846	9.19	94616.39	49.97	-0.933	nuclear	none	no
PmMYB51	245	9.47	28414.28	47.87	-0.801	nuclear	none	no
PmMYB52	1116	6.26	122279.62	56.2	-0.594	nuclear	none	no
PmMYB53	399	6.38	44815.69	50.4	-0.693	nuclear	none	no
PmMYB54	181	9.55	20467.28	44.31	-0.941	nuclear	none	no
PmMYB55	114	9.84	13255.11	62.85	-0.849	nuclear	none	no
PmMYB56	513	7.16	59692.65	42.93	-1.025	nuclear	none	no
PmMYB57	355	8.72	38364.15	58.2	-0.578	nuclear	none	no

**Table S3.** Prediction of phosphorylation sites of R2R3-MYB proteins in *Pinus massoniana*.


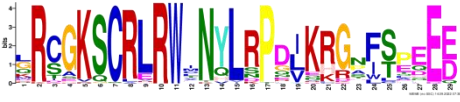
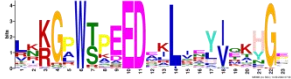


Protein name	Phosphorylation sites number			Protein name	Phosphorylation sites number		
	Serine	Threonine	Tyrosine		Serine	Threonine	Tyrosine
PmMYB1	20	6	2	PmMYB30	20	4	1
PmMYB2	40	9	6	PmMYB31	25	6	1
PmMYB3	35	12	1	PmMYB32	3	1	1
PmMYB4	44	12	7	PmMYB33	51	12	5
PmMYB5	16	11	3	PmMYB34	34	19	11
PmMYB6	31	15	4	PmMYB35	35	12	7
PmMYB7	31	10	1	PmMYB36	32	19	9
PmMYB8	44	21	3	PmMYB37	30	6	4
PmMYB9	38	13	1	PmMYB38	39	10	2
PmMYB10	36	12	7	PmMYB39	32	14	9
PmMYB11	46	20	5	PmMYB40	40	9	3
PmMYB12	46	19	3	PmMYB41	24	6	1
PmMYB13	28	6	3	PmMYB42	16	9	3
PmMYB14	18	12	1	PmMYB43	27	10	4
PmMYB15	49	16	2	PmMYB44	30	12	3
PmMYB16	17	9	3	PmMYB45	34	18	9
PmMYB17	165	46	9	PmMYB46	38	15	6
PmMYB18	7	2	0	PmMYB47	62	33	7
PmMYB19	27	15	1	PmMYB48	165	58	11
PmMYB20	113	34	7	PmMYB49	36	12	2
PmMYB21	39	11	4	PmMYB50	50	22	5
PmMYB22	32	14	5	PmMYB51	10	7	4
PmMYB23	22	9	1	PmMYB52	127	41	10
PmMYB24	26	5	2	PmMYB53	43	13	5
PmMYB25	42	6	4	PmMYB54	11	8	1
PmMYB26	21	11	9	PmMYB55	4	10	0
PmMYB27	25	15	1	PmMYB56	49	19	10
PmMYB28	37	17	4	PmMYB57	35	13	3
PmMYB29	49	18	3				

**Table S4.** Prediction of the Secondary Structure of R2R3-MYB proteins in *Pinus massoniana*.

Protein name	Protein secondary structure(%)			
	Alpha helix (Hh)	Extended strand (Ee)	Beta turn (Tt)	Random coil (Cc)
PmMYB1	38.67%	5.00%	4.00%	52.33%
PmMYB2	29.40%	17.15%	8.91%	44.54%
PmMYB3	32.96%	11.14%	5.35%	50.56%
PmMYB4	29.59%	11.02%	6.73%	52.65%
PmMYB5	25.49%	7.06%	6.67%	60.78%
PmMYB6	38.77%	6.15%	4.81%	50.27%
PmMYB7	30.21%	6.42%	2.94%	60.43%
PmMYB8	33.96%	12.76%	10.13%	43.15%
PmMYB9	32.77%	6.07%	3.16%	58.01%
PmMYB10	27.54%	15.89%	8.26%	48.31%
PmMYB11	48.50%	8.43%	3.37%	39.70%
PmMYB12	36.33%	8.98%	3.52%	51.17%
PmMYB13	20.43%	11.11%	4.30%	64.16%
PmMYB14	24.46%	10.73%	8.15%	56.65%
PmMYB15	26.51%	9.44%	6.22%	57.83%
PmMYB16	37.14%	9.64%	7.14%	46.07%
PmMYB17	20.91%	9.65%	4.06%	65.38%
PmMYB18	38.94%	4.42%	13.27%	43.36%
PmMYB19	22.11%	9.52%	3.40%	64.97%
PmMYB20	31.33%	12.23%	5.93%	50.51%
PmMYB21	38.17%	10.41%	3.15%	48.26%
PmMYB22	35.25%	10.55%	8.15%	46.04%
PmMYB23	30.54%	10.59%	5.67%	53.20%
PmMYB24	37.72%	14.91%	6.73%	40.64%
PmMYB25	37.12%	8.51%	4.02%	50.35%
PmMYB26	26.58%	13.70%	6.58%	53.15%
PmMYB27	23.08%	16.35%	12.50%	48.08%
PmMYB28	28.98%	10.97%	8.36%	51.70%
PmMYB29	26.00%	5.24%	2.97%	65.79%
PmMYB30	31.12%	4.56%	8.30%	56.02%
PmMYB31	16.51%	14.22%	1.83%	67.43%
PmMYB32	26.17%	14.95%	13.08%	45.79%
PmMYB33	43.67%	7.69%	5.20%	43.44%
PmMYB34	29.19%	10.35%	5.18%	55.28%
PmMYB35	34.46%	10.60%	6.02%	48.92%
PmMYB36	21.34%	13.44%	7.11%	58.10%
PmMYB37	27.16%	9.63%	5.43%	57.78%
PmMYB38	36.79%	5.42%	2.71%	55.08%
PmMYB39	28.08%	6.31%	3.47%	62.15%
PmMYB40	32.63%	10.00%	5.79%	51.58%

Protein name	Protein secondary structure(%)			
	Alpha helix	Extended strand	Beta turn	Random coil
	(Hh)	(Ec)	(Tt)	(Cc)
PmMYB41	37.93%	10.78%	5.60%	45.69%
PmMYB42	30.60%	6.47%	6.03%	56.90%
PmMYB43	28.90%	5.52%	5.52%	61.69%
PmMYB44	32.90%	9.33%	7.25%	50.52%
PmMYB45	24.66%	9.59%	5.94%	59.82%
PmMYB46	27.15%	11.31%	4.98%	56.56%
PmMYB47	50.62%	6.21%	3.19%	39.98%
PmMYB48	34.34%	12.97%	6.49%	46.21%
PmMYB49	30.05%	13.21%	5.70%	51.04%
PmMYB50	28.01%	14.18%	6.62%	51.18%
PmMYB51	37.96%	9.39%	6.94%	45.71%
PmMYB52	32.08%	14.78%	6.27%	46.86%
PmMYB53	33.33%	13.28%	6.52%	46.87%
PmMYB54	35.36%	6.08%	4.97%	53.59%
PmMYB55	47.37%	7.02%	7.02%	38.60%
PmMYB56	49.32%	4.29%	2.53%	43.86%
PmMYB57	24.79%	8.73%	4.23%	62.25%

**Table S5.** Conserved Motif Analysis of R2R3-MYB proteins in *Pinus massoniana*.

Motif	Logo	E-value	Sites	Width
1		3.9e-1055	44	41
2		2.1e-924	49	29
3		4.3e-498	49	23
4		1.8E-138	34	11
5		2.5E-95	22	11



**Table S6.** Functional annotation of R2R3-Myb subfamily in *Pinus massoniana*.

Subgroups of R2R3-MYB	Function annotation		The group members
S1	Abiotic stress response	Biotic stress response	PmMYB21
S2	Brassinosteroid pathway	Hypocotyle elongation	PmMYB10, PmMYB44
S4	Phenylpropanoid pathway	Sinapateester biosynthesis	PmMYB5, PmMYB14, PmMYB30, PmMYB6, PmMYB43, PmMYB26, PmMYB18, PmMYB51
S5	Phenylpropanoid pathway, Proanthocyanindinsbio synthesis		YB26, PmMYB18, PmMYB51
S9a	Cell fate	Conical epidermal cell outgrowthTrichome branching	PmMYB49
S11	Abiotic stress response	Osmotic, ABA-mediated	PmMYB53
S13	Mucilage deposition and extrusion	Phenylpropanoidpathway Lignin biosynthesis Stomatal closure	PmMYB8, PmMYB37
S14	Axillary meristem regulation	Lateral organformation Root elongation	PmMYB35
S18	Stamen and anther development	Abiotic stress response ABA sensitivity	PmMYB29
S20	Stamen development	Abiotic/biotic stress response Pollen maturation ABA – GA- or JA-mediated	PmMYB55
S21a	Cell wall thickening	Axillary meristem regulation Lateral organ formation	PmMYB24
S21b	Cell wall thickening	Axillary meristem regulation Lateral organ	PmMYB3
S22	Abiotic stress response	Growth regulation	PmMYB7, PmMYB57
S28	secondary wall biosynthesis		PmMYB16
S37	Mucilage synthesis, seed coat development, and trichome morphogenesis Lignin Biosynthesis		PmMYB23, PmMYB32, PmMYB45, PmMYB46

**Table S7.** Cis-acting element analysis of *PmMYB4* promoter in *Pinus massoniana*.

Element	Core sequence	Function	Amount
G-Box	CACGTT	cis-acting regulatory element involved in light responsiveness	4
AE-box	AGAAACAA	part of a module for light response	1
ATCT-motif	AATCTAATCC	part of a conserved DNA module involved in light responsiveness	2
ARE	AAACCA	cis-acting regulatory element essential for the anaerobic induction	2
CAAT-box	CCAAT	common cis-acting element in promoter and enhancer regions	40
TATA-box	ccTATAAAaa	core promoter element around -30 of transcription start	77
CGTCA-motif	CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness	4
ABRE	TACGTGTC	cis-acting element involved in the abscisic acid responsiveness	4
GT1-motif	GGTTAA	light responsive element	2
TGACG-motif	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness	3
Box 4	ATTAAT	part of a conserved DNA module involved in light responsiveness	6
TCCC-motif	TCTCCCT	part of a light responsive element	1
MBS	CAACTG	MYB binding site involved in drought-inducibility	1
MBSI	aaaAaaC(G/C)GTTA	MYB binding site involved in flavonoid biosynthetic genes regulation	1

>PmMYB4

ATGAGCTGCACAACAGGAGGACTCTCCTCTCCCATCTCCAAACCAAGCTAAGGAAAGGCCTCTGGTCGCCT  
GAGGAGGATGATAAACTCATCAACTACATGATGAAAAACGGCCAGGGTTGCTGGAGCGATGTCGTAAGCA  
AGCTGGTCTGCAGAGATGCGGAAAAAGCTGTAGGCTGAGGTGGATTAATACTATTTAAGGCCCCGACCTCAAAC  
GCGGTGCATTTTCACCCCAGGAAGAACAATTGATCATACACTTGCATTCCATTCTGGGCAACAGGTGGTCTCA  
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CAAGTACAGAGTCAGAATCAATTTCAATTGATCAGGACTCGGCCACCAACAATCTCATTTCCCACCTGTGGA  
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CAGATCTATTTTGGCTAA

>PmMYB4-optimization

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>Pro-PmMYB4

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TCTTTAATTTCTTAATTTGAGATAAATTAATTAAGAGCATAATTAATCGGAAGATAAATCTTAACCCTAGATCT  
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TAAATTAATTTTAAATTAACAATATATTTTAGTTATCTCACTAAATGTCCTTATAAAAAACAAACATCGTCATTA  
TTTATATGAAAAAAACCCTTATATTTTTTAAACTATATTATAATTTTTTATAAATGCTTTTGATAACTACATATAA  
ACCTACATATTAATAATATGTATATATATAAAATAAAATATATATTGTTATAGCATCGCTTGTTTCGAGAAACAATTG  
GGTAGTTAGCTTAGACATTGACTTTGAAAGAATGAAGTTGTTGAAGATAGACCTTGACATGTACATGGCATAA  
TGAAACCACTTTGTTATAAACTCTCTTTACTTCGTTCTATATAAGATTATAAAAGATTAGTTGTTATACCTTATA  
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AATGAGCTGCACAACAGGAGGACTCTCCTCTCCCATCTCAAACCCAAGCTAAGGAAAGG