

Table 1 Suppl. List of primers used for isolation of the flavonoid biosynthesis-related genes of *Tricyrtis* spp.

Gene	Analyzes	Position	Sequence (5'→3')
<i>TrMYB1</i>	3'RACE	forward	GATGAGGAGGACCTCATTATCAGG
	5'RACE (reverse transcription)	reverse	GGTCGAGCTTGTGGTTTG
	5'RACE (1 <sup>st</sup> PCR)	forward	GAACTACTGGAACTCACATTGG
	5'RACE (1 <sup>st</sup> PCR)	reverse	GTCCTACCGGGAATTCGACCGG
	5'RACE (2 <sup>nd</sup> PCR)	forward	GCTGGAGATAAGATTGATGG
<i>TrbHLH2</i>	5'RACE (2 <sup>nd</sup> PCR)	reverse	GGACCACCTGTTCCTAAAAG
	3'RACE (1 <sup>st</sup> PCR)	forward	GTGACAAGGAGCAGTCCAC
	3'RACE (2 <sup>nd</sup> PCR)	forward	AGTCTTCTGCAGAACCATTCTCG
	5'RACE (reverse transcription)	reverse	GTCACACTAGTAG
	5'RACE (1 <sup>st</sup> PCR)	forward	AGTCTTCTGGCAGAACCATTCTCG
	5'RACE (1 <sup>st</sup> PCR)	reverse	TCGACAGTCACCTCCATTGG
	5'RACE (2 <sup>nd</sup> PCR)	forward	AGTCTTCTGCAGAACCATTCTCG
<i>TrWDR1</i>	5'RACE (2 <sup>nd</sup> PCR)	reverse	AGAACCACACTTGACCTCTCGTG
	3'RACE (1 <sup>st</sup> PCR)	forward	GTGACAAGGAGCAGTCCAC
	3'RACE (2 <sup>nd</sup> PCR)	forward	GTTGTGGTGCTGGATATACG
	5'RACE (reverse transcription)	reverse	ATCGCAATCCAATCG
	5'RACE (1 <sup>st</sup> PCR)	forward	GTTGTGGTGCTGGATATACG
	5'RACE (1 <sup>st</sup> PCR)	reverse	CTGGACTTACTGTTGTTCCAGCACG
	5'RACE (2 <sup>nd</sup> PCR)	forward	GTTGTGGTGCTGGATATACG
<i>TrCHI</i>	5'RACE (2 <sup>nd</sup> PCR)	reverse	GTGGAACATGAGCTTGGTGG
	3'RACE	forward	CTTCAAGGCGGAGACC
	5'RACE (reverse transcription)	reverse	CCTTTCTAGCATCCTC
	5'RACE (1 <sup>st</sup> PCR)	forward	CGATAACAAAGCTCTTTCAGAC
	5'RACE (1 <sup>st</sup> PCR)	reverse	CGAACCATCCTTCGAGAATCC
<i>TrF3H</i>	5'RACE (2 <sup>nd</sup> PCR)	forward	GTCCATCATTGGTGAGCACGGCGTG
	5'RACE (2 <sup>nd</sup> PCR)	reverse	GTGAACCGGAAGGGGAGTGGGTG
	3'RACE	forward	GGTGAAGCAGTCAAGATTGG
	5'RACE (reverse transcription)	reverse	CTTAAGCTTGGCAAGCTCGATG
	5'RACE (1 <sup>st</sup> PCR)	forward	TCAAGAATGCCGACCACCAG
<i>TrF3'H</i>	5'RACE (1 <sup>st</sup> PCR)	reverse	GGTATGAGAAGTACGCTACTATCTCC
	5'RACE (2 <sup>nd</sup> PCR)	forward	AGCCGATTACTTTCAGCGAGATG
	5'RACE (2 <sup>nd</sup> PCR)	reverse	TGAAATATCCCCCAGTCCTCG
	3'RACE	forward	GAGGAGGAGGATGAGGCAAAC
	5'RACE (reverse transcription)	reverse	TGGGTGGCGGATCAA
<i>TrFLS</i>	5'RACE (1 <sup>st</sup> PCR)	forward	CCCAACCTCCCATTCCCTAC
	5'RACE (1 <sup>st</sup> PCR)	reverse	CGTGTCTGTTCCCTGCTGTG
	5'RACE (2 <sup>nd</sup> PCR)	forward	AACTCTCCTGGTTAACGTTTGG
	5'RACE (2 <sup>nd</sup> PCR)	reverse	CAAGAAACGCATCCATCCTC
	3'RACE	forward	CTSRABYTGRTCDCCRATRTC
	5'RACE (reverse transcription)	reverse	ATATGCTGAGCAAAGGTTTCG
	5'RACE (1 <sup>st</sup> PCR)	forward	TTGAGCAATGGGAGGTACAAG
<i>TrDFR</i>	5'RACE (1 <sup>st</sup> PCR)	reverse	GTCACCTATGTGGATGACAAGAG
	5'RACE (2 <sup>nd</sup> PCR)	forward	CAAGAATGTCATGGCCTGTG
	5'RACE (2 <sup>nd</sup> PCR)	reverse	GACATGTCAGTATGTGCCAC
	3'RACE	forward	CGTCAAGATGACTGGATGGATG
	5'RACE (reverse transcription)	reverse	GTCACACTAGTAG
<i>TrANS</i>	5'RACE (1 <sup>st</sup> PCR)	forward	TCAAGTATGAGTACACCGTGGAG
	5'RACE (1 <sup>st</sup> PCR)	reverse	GTCCTACCGGGAATTCGACCGG
	5'RACE (2 <sup>nd</sup> PCR)	forward	TGTTTGATGACGGAATCCAG
	5'RACE (2 <sup>nd</sup> PCR)	reverse	GCGGTTATCATACTGGGTGG
	3'RACE	forward	GGGCAGTGTTTCGTGAGCCGCAAAGG
<i>TrANS</i>	5'RACE (reverse transcription)	reverse	ATATGCTGAGCAAAGGTTTCG
	5'RACE (1 <sup>st</sup> PCR)	forward	GCTCACCTTCCTGCTCACC
	5'RACE (1 <sup>st</sup> PCR)	reverse	GGCTAGCTCTGGTTGTGG
	5'RACE (2 <sup>nd</sup> PCR)	forward	AGCGGAGCCTGCTAGGTTTC
	5'RACE (2 <sup>nd</sup> PCR)	reverse	CTCCTTTGTCCTCCGTATAC
	common primer for 3'RACE	reverse	CTGATCTGAGGTACCGGTCC

Table 2 Suppl. List of genes used for the phylogenetic analysis.

Group	Gene	Plant species	Accession No.	Identity [%]	Similarity[%]	
R2R3-MYB	<i>AmROSEA1</i>	<i>Antirrhinum majus</i>	DQ275529	41.4	54.4	
	<i>AtPAP1</i>	<i>Arabidopsis thaliana</i>	AF325123	38.8	50.8	
	<i>AtTT2</i>	<i>Arabidopsis thaliana</i>	NM_122946	33.7	46.6	
	<i>GhMYB10</i>	<i>Gerbera</i> sp.	AJ554700	39.5	52.5	
	<i>InMYB2</i>	<i>Ipomoea nil</i>	AB234211	42.4	56.8	
	<i>LhMYB6</i>	<i>Lilium hybrid</i>	AB534587	42.4	56.5	
	<i>LhMYB12</i>	<i>Lilium hybrid</i>	AB534586	43.5	57.3	
	<i>LjTT2a</i>	<i>Lotus japonicus</i>	AB300033	31.5	45.3	
	<i>OgMYB1</i>	<i>Oncidium</i> sp.	EF570115	36.8	48.9	
	<i>PhAn2</i>	<i>Petunia hybrida.</i>	AF146702	42.4	51.9	
	<i>VvMYBA1</i>	<i>Vitis vinifera</i>	AB097923	40.4	50.2	
	<i>ZmPI</i>	<i>Zea mays</i>	L19495	35.4	45.5	
	<i>ZmC1</i>	<i>Zea mays</i>	X52201	35.5	44.0	
	bHLH	<i>AtTT8</i>	<i>Arabidopsis thaliana</i>	AJ277509	40.9	54.1
		<i>AtEGL3</i>	<i>Arabidopsis thaliana</i>	NM_105042	30.8	50.0
		<i>InbHLH1</i>	<i>Ipomoea nil</i>	AB232774	32.5	51.2
<i>InbHLH2</i>		<i>Ipomoea nil</i>	AB232775	46.1	61.8	
<i>LhbHLH1</i>		<i>Lilium</i> sp.	AB222075	31.8	50.1	
<i>LhbHLH2</i>		<i>Lilium</i> sp.	AB222076	59.6	70.1	
<i>LjTT8</i>		<i>Lotus japonicus</i>	AB490778	49.4	63.7	
<i>LjGL3</i>		<i>Lotus japonicus</i>	AB492284	32.7	50.2	
<i>PhAN1</i>		<i>Petunia hybrida.</i>	AF260919	46.2	62.1	
<i>PhJAF13</i>		<i>Petunia hybrida.</i>	AF020545	30.1	49.7	
<i>TjbHLH1</i>		<i>Tulipa fosteriana</i>	KC256778	32.7	51.1	
<i>TjbHLH2</i>		<i>Tulipa fosteriana</i>	KF924736	56.8	70.1	
<i>ZmIN1</i>		<i>Zea mays</i>	U57899	42.1	54.6	
<i>ZmLc</i>		<i>Zea mays</i>	M26227	28.3	47.4	
WDR		<i>AaTTG1</i>	<i>Anthurium amnicola</i>	GDJX01022041	74.6	85.2
		<i>AtTTG1</i>	<i>Arabidopsis thaliana</i>	NM_180739	66.9	81.1
	<i>InWDR1</i>	<i>Ipomoea nil</i>	AB232779	69.3	82.8	
	<i>LjTTG1</i>	<i>Lotus japonicus</i>	AB490777	67.3	81.1	
	<i>PhAN11</i>	<i>Petunia hybrida.</i>	U94748	70.9	84.9	
	<i>PeWDR1</i>	<i>Phalaenopsis equestris</i>	KF769483	59.8	73.3	
	<i>RrTTG1</i>	<i>Rosa rugose</i>	JX878895	71.0	83.2	
	<i>VvWDR1</i>	<i>Vitis vinifera</i>	DQ517913	72.4	84.6	
	<i>ZmPAC1</i>	<i>Zea mays</i>	AY115485	63.5	75.7	
	CHI	<i>AmCH11</i>	<i>Antirrhinum majus</i>	AB861648	63.2	76.1
<i>AtCHI</i>		<i>Arabidopsis thaliana</i>	AJ418046	54.9	70.3	
<i>CgCHI</i>		<i>Canna generalis</i>	DQ160232	62.6	75.7	
<i>CmCHI</i>		<i>Chrysanthemum morifolium</i>	JF834891	57.6	72.5	
<i>DpCHI</i>		<i>Dahlia pinnata</i>	AB591827	60.4	76.2	
<i>GmCHI2-A</i>		<i>Glycine max</i>	NM_001249839	50.4	68.8	
<i>LhCHI</i>		<i>Lilium</i> sp.	KJ784468	70.3	83.1	
<i>NtCHI</i>		<i>Narcissus tazetta</i>	JN227884	62.6	76.8	
<i>PhCHI</i>		<i>Petunia hybrida.</i>	Y00852	57.4	73.1	
<i>TjCHI</i>		<i>Tulipa fosteriana</i>	JX878398	69.9	81.8	
<i>VvCHI</i>		<i>Vitis vinifera</i>	NM_001281104	60.4	79.6	
<i>ZmCHI1</i>		<i>Zea mays</i>	NM_001150530	64.0	77.5	
<i>AtF3H</i>		<i>Arabidopsis thaliana</i>	AF064064	63.3	68.9	
<i>CaF3H</i>		<i>Curcuma alismatifolia</i>	GU140081	62.8	69.4	
<i>EgF3H</i>		<i>Eustoma exaltatum</i>	AB078956	64.7	70.0	
<i>GmF3H</i>		<i>Glycine max</i>	NM_001249868	58.1	67.2	
<i>LhF3H</i>		<i>Lilium</i> sp.	AB699160	65.0	69.9	
<i>NtF3H</i>		<i>Narcissus tazetta</i>	JQ388493	63.0	69.3	
<i>PhF3H</i>		<i>Petunia hybrida.</i>	LQ275617	60.9	68.3	
<i>PhF3H</i>		<i>Phalaenopsis</i> sp.	JX162553	60.5	67.3	
<i>TjF3H</i>	<i>Tulipa fosteriana</i>	JX863898	65.8	71.6		
<i>VvF3H</i>	<i>Vitis vinifera</i>	EF192467	62.8	70.2		

F3'H	<i>ZmFHT1</i>	<i>Zea mays</i>	NM_001136803	61.7	67.8	
	<i>AtF3'H</i>	<i>Arabidopsis thaliana</i>	AF271650	62.1	77.2	
	<i>CmF3'H</i>	<i>Chrysanthemum morifolium</i>	AB523844	64.1	77.6	
	<i>DpF3'H</i>	<i>Dahlia pinnata</i>	FJ216428	65.0	79.0	
	<i>GmSF3'H1</i>	<i>Glycine max</i>	NM_001250086	63.2	77.9	
	<i>InF3'H</i>	<i>Ipomoea nil</i>	AB113261	64.2	77.4	
	<i>LhF3'H-1</i>	<i>Lilium sp.</i>	AB699161	78.2	84.1	
	<i>PhF3'H</i>	<i>Petunia hybrida.</i>	AF155332	64.1	78.1	
	<i>PeF3'H</i>	<i>Phalaenopsis equestris</i>	KF769465	50.5	63.2	
	<i>SbF3'H</i>	<i>Sorghum bicolor</i>	AY675075	62.9	76.7	
	<i>ThF3'H</i>	<i>Torenia sp.</i>	AB057672	60.5	75.6	
	<i>TjF3'H</i>	<i>Tulipa fosteriana</i>	JX878399	75.4	85.0	
	<i>VvF3'H</i>	<i>Vitis vinifera</i>	KT216254	67.5	79.2	
	DFR	<i>ApDFR</i>	<i>Agapanthus praecox</i>	AB099529	70.1	82.8
		<i>AtDFR</i>	<i>Arabidopsis thaliana</i>	AK221622	59.2	76.2
		<i>CaDFR</i>	<i>Curcuma alismatifolia</i>	GU126388	66.7	81.2
		<i>CgDFR</i>	<i>Cyclamen graecum</i>	AB517921	60.7	77.3
<i>ChDFR</i>		<i>Cymbidium sp.</i>	AF017451	66.7	77.8	
<i>GhDFR</i>		<i>Gossypium hirsutum</i>	FJ713480	58.3	76.3	
<i>IhDFR</i>		<i>Iris hollandica</i>	AB332098	69.8	82.0	
<i>LhDFR</i>		<i>Lilium sp.</i>	AB058641	78.0	87.0	
<i>PhDFR</i>		<i>Petunia hybrida.</i>	AF233639	58.1	75.2	
<i>RhDFR</i>		<i>Rosa sp.</i>	AY780885	60.4	77.7	
<i>TjDFR</i>		<i>Tulipa fosteriana</i>	JX401219	65.4	75.8	
<i>ZmDFR</i>		<i>Zea mays</i>	NM_001158995	65.1	79.5	
FLS		<i>AcFLS1</i>	<i>Allium cepa</i>	AY221247	74.0	86.9
		<i>AmFLS</i>	<i>Antirrhinum majus</i>	DQ272591	66.4	80.7
		<i>AtFLS1</i>	<i>Arabidopsis thaliana</i>	NM_001203337	63.4	76.8
		<i>DaFLS1</i>	<i>Dioscorea alata</i>	KJ022640	73.2	84.5
		<i>EgFLS</i>	<i>Eustoma grandiflorum</i>	AB078965	66.3	81.2
	<i>GmFLS1</i>	<i>Glycine max</i>	NM_001250490	64.7	77.2	
	<i>NtFLS</i>	<i>Narcissus tazetta</i>	JX467719	71.9	85.1	
	<i>PhFLS</i>	<i>Petunia hybrida.</i>	Z22543	64.4	79.0	
	<i>RrFLS1</i>	<i>Rosa rugosa</i>	KM099095	62.4	76.7	
	<i>TjFLS</i>	<i>Tulipa fosteriana</i>	KF924735	80.5	88.6	
	<i>VvFLS1</i>	<i>Vitis vinifera</i>	AB086055	63.6	76.4	
	<i>ZmFLS1</i>	<i>Zea mays</i>	NM_001147443	57.1	72.3	
	ANS	<i>AcANS</i>	<i>Allium cepa</i>	AY585678	63.3	78.4
		<i>AaANS</i>	<i>Anthurium andraeanum</i>	EF079869	65.7	82.1
		<i>AtANS</i>	<i>Arabidopsis thaliana</i>	JF681791	63.5	79.1
		<i>EgANS</i>	<i>Eustoma grandiflorum</i>	AB551412	61.7	77.7
		<i>GhANS</i>	<i>Gerbera sp.</i>	AY997842	63.0	77.6
<i>GmANS</i>		<i>Glycine max</i>	AY382828	67.7	81.2	
<i>InANS</i>		<i>Ipomoea nil</i>	AB073920	64.1	78.6	
<i>LhANS</i>		<i>Lilium sp.</i>	KP857572	81.2	88.4	
<i>PeANS3</i>		<i>Phalaenopsis equestris</i>	KF769463	57.4	75.1	
<i>TfANS</i>		<i>Tulipa fosteriana</i>	JX401218	76.8	86.5	
<i>VvANS</i>		<i>Vitis vinifera</i>	EU156063	66.0	80.1	

Table 3 Suppl. List of primers used for the real-time semi-quantitative PCR analysis.

Gene	Position	Sequence (5'→3')	PCR conditions
<i>TrMYB1</i>	forward	TCTATTACTTGATGAGGAGTCGAAC	annealing temperature: 60°C cycle number: 32
	reverse	GTTCTTAGTGCCAGTTCTTCTTG	
<i>TrbHLH</i>	forward	CTACTCCCGGAGATGCGTTGAGAACC	annealing temperature: 60°C cycle number: 32
	reverse	CAGTCCTACCGGGAATTCGACCGGC	
<i>TrWDR</i>	forward	CTACTCCCGGAGATGCGTTGAGAACC	annealing temperature: 60°C cycle number: 32
	reverse	CAGTCCTACCGGGAATTCGACCGGC	
<i>TrCHS</i>	forward	TGGAGGTTAAGTTGGCGTTG	annealing temperature: 60°C cycle number: 30
	reverse	CGCTTCCTCATCTCATCCAG	
<i>TrCHI</i>	forward	CCCTCCTGGTGCCTCCATCCTCTTC	annealing temperature: 60°C cycle number: 30
	reverse	CGCTAATGGCAATTGGCTCCTTAGC	
<i>TrF3H</i>	forward	GGCGCTCGCATGCAAGCTCTGGG	annealing temperature: 60°C cycle number: 30
	reverse	GGCACCACGACGGGCTTGACAG	
<i>TrF3'H</i>	forward	GCGGCGGAGGAGTTAAGGAGATGG	annealing temperature: 60°C cycle number: 30
	reverse	GGCCATCTACTGTGCTCGAGG	
<i>TrFLS</i>	forward	ATGGAGGTCGAGAGAGTCCAAG	annealing temperature: 60°C cycle number: 30
	reverse	ACTCTATGGCAGCCTCAGTTATG	
<i>TrDFR</i>	forward	CAATGCCACCCAGTATGATAAACC	annealing temperature: 60°C cycle number: 32
	reverse	CATGAAAAGAACACATATATCTCCC	
<i>TrANS</i>	forward	GACAATTGTGTTGAAGCCGTTGG	annealing temperature: 60°C cycle number: 30
	reverse	GGGCTCCACCGAAGATTTTATTG	
<i>TrAct2</i>	forward	TGCCATGTATGTTGCCATTGAG	annealing temperature: 60°C cycle number: 30
	reverse	AGGGAGTCGGTCAGGTCTCTG	

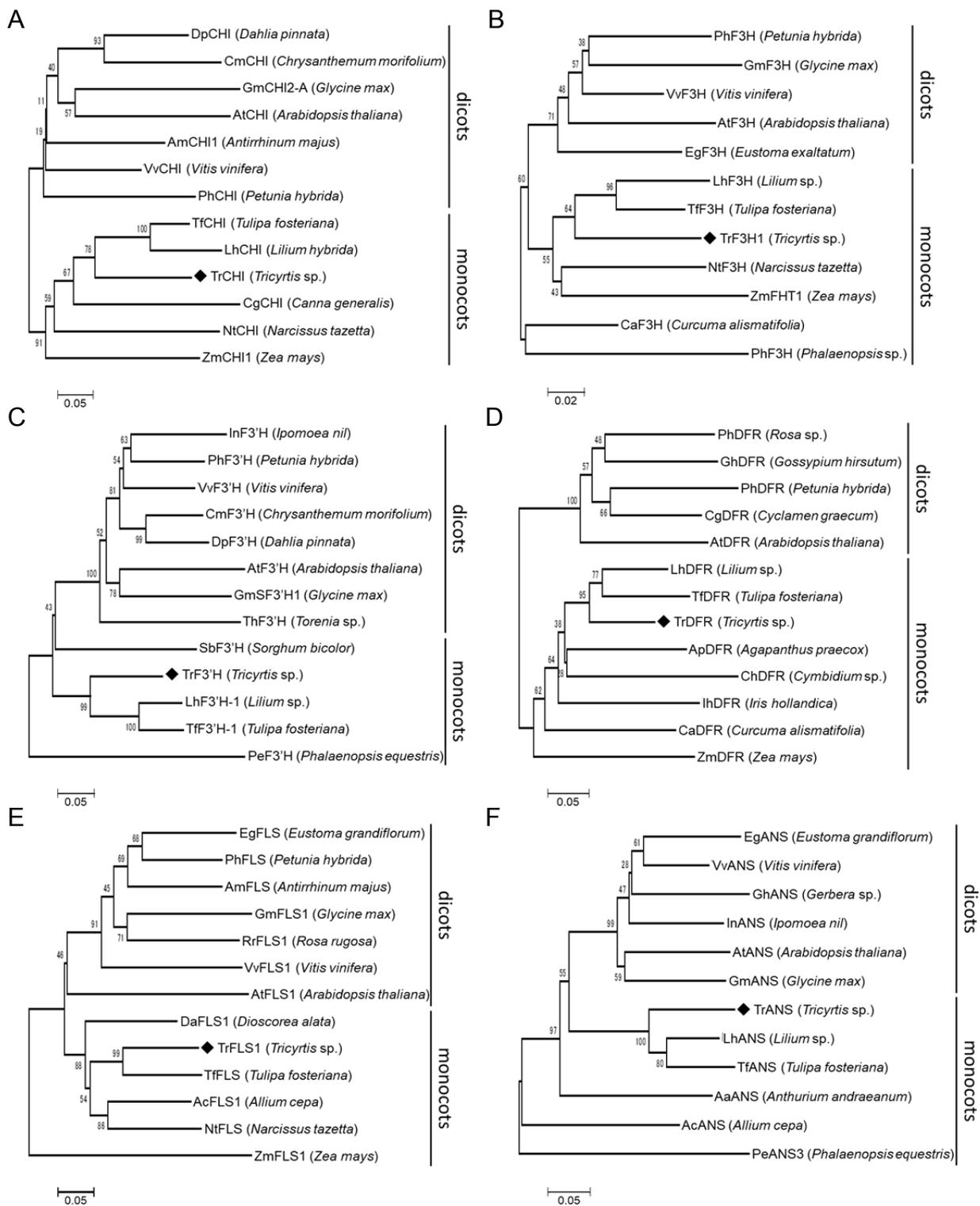


Fig. 1 Suppl. Molecular phylogenetic trees of the deduced amino acid sequences of flavonoid biosynthesis genes. CHI, chalcone isomerase (A); F3H, flavanone 3-hydroxylase (B); F3'H, flavonoid 3'-hydroxylase (C); DFR, dihydroflavonol 4-reductase (D); FLS, flavonol synthase (E); ANS, anthocyanin synthase (F).

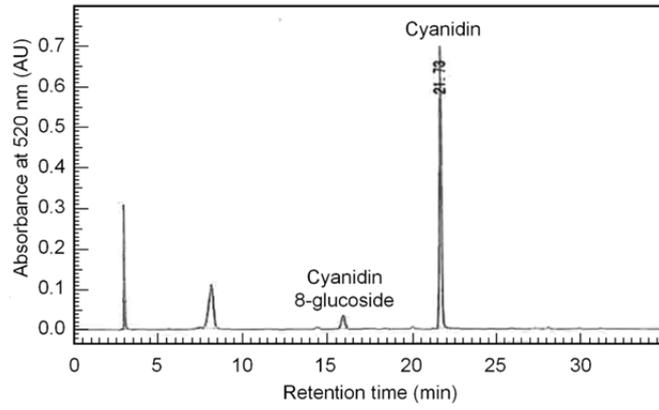


Fig. 2 Suppl. HPLC profile for the accumulation of anthocyanidins in outer tepals of stage 5 flowers.