

Table S1. Primers used in the study

		Forward(5'-3')	Reverse(5'-3')
cDNAs/ DNAs	<i>FhTPS1</i>	ATGGCTCTCTTGCCGTGTCTTC	TTAGAGGGGAATGGGTTC
	<i>FhTPS2</i>	ATGGCGTGTCTTCCATTCCA	TTAGAGGGGAAACAGGTTGTA
	<i>FhTPS3</i>	ATGGGGACAGAAATGGCACTC	CTATTTCATGGGCTTAAAA
	<i>FhTPS4</i>	ATGACTACCTTCTCAAAGAT	TTATATGCTCTTGAAAAGCA
	<i>FhTPS5</i>	ATGGCTTTCTTGCCGTGCCT	TTAGAGGGGAATAGGTTCAAT
	<i>FhTPS6</i>	ATGGAGTCAGTGCTGCTGGG	CTAGAAATAGTCATCTTCGA
	<i>FhTPS7</i>	ATGGAGTCAGTACTGCTGAG	CTAGTAGTAGTCCCCCGGGA
	<i>FhTPS8</i>	ATGGAGGTTGTGAGTGCTGGA	TCAATTCTGAATTGGCCAAAC
Generation of constructs used in subcellular localization	<i>FhTPS1</i>	GATTACGCTCATATGATGGCTCTCT TGCCGTGTCT	GCTCACCATGAGCTCGAGGGGAAT GGGTTC
	<i>FhTPS2</i>	GATTACGCTCATATG ATGGCGTGTCTTCCATTCCA	GCTCACCATGAGCTC TTAGAGGGGAAACAGGTTGTA
	<i>FhTPS3</i>	GATTACGCTCATATGATGGGGACA GAAATGGCAC	GCTCACCATGAGCTCTTTCATGGG CTTAAAA
	<i>FhTPS4</i>	GATTACGCTCATATG ATGACTACCTTCTCAAAGAT	GCTCACCATGAGCTC TTATATGCTCTTGAAAAGCA
	<i>FhTPS5</i>	GATTACGCTCATATGGCTTTCTTGC CGTGCCTTTC	GCTCACCATGAGCTCGTTGACTTG CTCATTCCCCA
	<i>FhTPS6</i>	GATTACGCTCATATG ATGGAGTCAGTGCTGCTGGG	GCTCACCATGAGCTC CTAGAAATAGTCATCTTCGA
	<i>FhTPS7</i>	GATTACGCTCATATGATGGAGTCAG TACTGCTGAG	GCTCACCATGAGCTCGTAGTAGTC CCCCGGGA
	<i>FhTPS8</i>	GATTACGCTCATATGATGGAGGTTG TGAGTGCTGG	GCTCACCATGAGCTCATTCTGAATT GGCCAA
Generation of constructs used in Heterologou s expression in <i>Escherichia coli</i>	<i>FhTPS1</i>	CAAGGATCC ATGGCTCTCTTGCCGTGTCTTC	CAAGAGCTC TTAGAGGGGAATGGGTTC
	<i>FhTPS2</i>	CAAGGATCC ATGGCGTGTCTTCCATTCCA	CAAGAGCTC TTAGAGGGGAAACAGGTTGTA
	<i>FhTPS3</i>	CAAGGATCC ATGGGGACAGAAATGGCACTC	CAAGAGCTC CTATTTCATGGGCTTAAAA
	<i>FhTPS4</i>	CAAGGATCC ATGACTACCTTCTCAAAGAT	CAAGAGCTC TTATATGCTCTTGAAAAGCA
	<i>FhTPS5</i>	CAAGGATCC ATGGCTTTCTTGCCGTGCCT	CAAGAGCTC TTAGAGGGGAATAGGTTCAAT
	<i>FhTPS6</i>	CAAGGATCC ATGGAGTCAGTGCTGCTGGG	CAAGAGCTC CTAGAAATAGTCATCTTCGA
	<i>FhTPS7</i>	CAAGGATCC ATGGAGTCAGTACTGCTGAG	CAAGAGCTC CTAGTAGTAGTCCCCCGGGA

<i>FhTPS8</i>		<u>CAAGGATCC</u> ATGGAGGTTGTGAGTGCTGGA	<u>CAAGAGCTC</u> TCAATTCTGAATTGGCCAAAC
qRT-PCR	<i>FhTPS1</i>	CACTACAAACCTTACCGCCAACA	GCTTTACAAAGATCTGCCCCACGA
	<i>FhTPS2</i>	CGTCTCTGCCTTCTTCTCACT	ATGGTTTCCTCATCTTCCTGC
	<i>FhTPS3</i>	CTTCTTTCAACACTCCCTAC	TAACACCACTGCTCCTAACT
	<i>FhTPS4</i>	GAGGTTTCTCTTTCTTTTCG	GAGTCCAGTTTTCTTTTGTC
	<i>FhTPS5</i>	CTGTCTCTTCGCTCTTCCCG	GTTGACTTGCTCATTCCTCA
	<i>FhTPS6</i>	TGGAGGGAGGAAGGGTATGT	GCACGATTTTTGGAATGTTG
	<i>FhTPS7</i>	ACGACATCTTCTCTGCCG	CACAACCTTCCATCCATC
	<i>FhTPS8</i>	TCCGTTTCCGATTGTTGAGAC	ATTGAAGTAAGCCGATGATGT
Generation of constructs used in Heterologous expression in tobacco	<i>18s</i>	TCCTGATACGGGGAGGTAGTGACA	ACTTGCCCTCCAATGGATCCTCG
	<i>FhTPS1</i>	<u>CGGGATCC</u> ATGGCTCTCTTGCCGTGTCTTC	<u>CGAGCTC</u> TTAGAGGGGAATGGGTTCAA
	<i>FhTPS2</i>	<u>CGGGATCC</u> ATGGCGTGTCTTCCATTCCA	<u>CGAGCTC</u> TTAGAGGGAAACAGGTTGTA
	<i>FhTPS6</i>	<u>CGGGATCC</u> ATGGAGTCAGTACTGCTGAG	<u>CGAGCTC</u> CTAGTAGTAGTCCCCCGGGA
	<i>FhTPS7</i>	<u>CGGGATCC</u> ATGGAGTCAGTACTGCTGAG	<u>CGAGCTC</u> CTAGTAGTAGTCCCCCGGGA

Table S2. TPS proteins from other plant species used in phylogenetic analysis

Protein	Protein ID in NCBI	Note
AtTPS2	NP 193406.3	(E)-bate-ocimene/myrcene synthase
AtTPS14	NP 001185286.1	(±)-3S-linalool synthase
AtTPS24	NP 189209.2	1,8-cineole synthase
SITPS5	NP 001233805.1	linalool synthase
SITPS24	NP 111307929.1	ent-kaurene synthase
SITPS40	NP 001234008.2	copalyl-diphosphate synthase
SITPS8	XP 004231365.1	1,8-cineole synthase
AgPIN1	O24475.1	pinene synthase
MtTPS15	XP 003621227.1	ocimene synthase
MtTPS23	XP 003619707.1	nerolidol synthase
ZmTPS6	NP 001105674.1	(S)-beta-macrocarpene synthase
ZmTPS10	NP 001105850.1	(E)-beta farnesene synthase
ZmTPS23	ABY79213.1	(E)-beta caryophyllene synthase
VGwGerA	ADR66821.1	germacrene A synthase
VvGwBer	ADR74195.2	(E)-alpha-bergamotene synthase
VvGwgCad	ADR74199.1	Gamma-cadinene synthase
HcTPS8	AGY49283.1	linalool synthase
HcTPS7	AHJ57305.1	sabinene synthase
LaCARS	AGL98419.1	caryophyllene synthase
LaGERDS	AGL98420.1	germacrene D synthase
LaLINS	Q2XSC5.1	linalool synthase

Table S3. Composition and contents of volatile compounds released from flowers in different developmental stages

Compounds	Red River [®]					Ambiance				
	R1	R2	R3	R4	R5	A1	A2	A3	A4	A5
Monoterpenes										
α -Pinene	n.d	n.d	n.d	24.15 \pm 3.86	33.57 \pm 4.16	n.d	n.d	n.d	n.d	n.d
β -Pinene	n.d	n.d	n.d	42.07 \pm 5.09	25.71 \pm 2.99	n.d	n.d	n.d	n.d	n.d
Myrcene	n.d	n.d	n.d	57.33 \pm 2.61	69.21 \pm 4.26	n.d	n.d	n.d	14.83 \pm 3.42	28.54 \pm 2.52
1,8 cineole	n.d	n.d	11.38 \pm 0.92	76.54 \pm 12.15	334.28 \pm 23.30	n.d	n.d	n.d	n.d	n.d
D-Limonene	n.d	n.d	17.09 \pm 1.38	45.67 \pm 7.95	143.57 \pm 16.79	n.d	n.d	n.d	n.d	n.d
cis-Ocimene	n.d	n.d	26.17 \pm 3.68	200.67 \pm 28.5	736.42 \pm 59.13	n.d	n.d	n.d	n.d	n.d
trans-Ocimene	n.d	n.d	50.64 \pm 6.74	8	125.62 \pm 10.2	n.d	n.d	n.d	n.d	n.d
				8	617.86 \pm 62.09					
cis-Linaloloxide	n.d	n.d	n.d	79.67 \pm 13.14	152.14 \pm 23.59	n.d	n.d	n.d	30.67 \pm 5.19	33.63 \pm 4.61
Terpinolene	n.d	n.d	n.d	8.72 \pm 3.29	41.43 \pm 7.03	n.d	n.d	n.d	n.d	n.d
Linalool	n.d	31.25 \pm 5.38	176.32 \pm 32.1 2	7134.39 \pm 109 6.23	18345.13 \pm 22 47.65	80.50 \pm 5 .33	163.41 \pm 9.01	325.76 \pm 45 .98	14971.67 \pm 13 75.14	24341.88 \pm 15 27.14
1,2-Dihydrolinalool	n.d	n.d	n.d	n.d	21.43 \pm 4.67	n.d	n.d	n.d	121.87 \pm 12.65	162.51 \pm 20.94
(-)-4-Terpineol	n.d	n.d	n.d	22.34 \pm 4.26	35.58 \pm 2.14	n.d	n.d	n.d	n.d	n.d
Hotrienol	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	98.27 \pm 7.83	170.25 \pm 8.75

α -Terpineol	10.13 \pm 1.84	26.31 \pm 5.38	164.21 \pm 11.12	6981.34 \pm 891.36	8876.42 \pm 291.71	n.d	n.d	n.d	n.d	n.d
Sesquiterpenes										
Cyclosativene	n.d	n.d	n.d	13.29 \pm 6.22	35.83 \pm 13.85	n.d	n.d	n.d	n.d	5.38 \pm 2.15
Copaene	n.d	n.d	n.d	n.d	n.d	9.76 \pm 0.25	60.58 \pm 7.36	116.08 \pm 10.38	404.15 \pm 30.27	542.53 \pm 36.57
Elemene	n.d	n.d	n.d	n.d	n.d	1.83 \pm 0.09	10.03 \pm 1.21	15.02 \pm 1.09	40.17 \pm 2.18	58.09 \pm 8.08
α -Gurjunene	n.d	n.d	n.d	n.d	n.d	1.83 \pm 0.83	13.08 \pm 2.19	26.50 \pm 2.96	101.28 \pm 9.54	127.63 \pm 17.07
Caryophyllene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	2.68 \pm 0.74	4.25 \pm 2.00
α -Guaiene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	6.58 \pm 0.99	13.17 \pm 2.58	20.13 \pm 3.81
α -Patchoulene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	6.83 \pm 1.01	15.75 \pm 4.50
Sativene	n.d	n.d	n.d	n.d	n.d	n.d	6.77 \pm 2.08	11.08 \pm 0.97	35.34 \pm 5.21	43.25 \pm 4.08
γ -Cadinene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	21.54 \pm 2.59	93.88 \pm 11.73
γ -Gurjunene	n.d	n.d	n.d	23.47 \pm 7.32	75.57 \pm 14.67	n.d	n.d	14.78 \pm 3.28	28.67 \pm 5.28	26.63 \pm 6.96
Selinene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	7.17 \pm 2.88	8.38 \pm 3.04
α -Bulnesene	n.d	n.d	n.d	n.d	n.d	n.d	11.42 \pm 3.63	20.09 \pm 2.85	65.43 \pm 9.82	96.10 \pm 9.29
Vatirenene	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	6.25 \pm 1.71
Nerolidol	n.d	n.d	n.d	19.33 \pm 8.23	36.21 \pm 9.35	n.d	n.d	n.d	n.d	n.d

Carotenoid derivatives

Dihydro-ionone	n.d	n.d	5.27±0.43	601.26±35.9	913.02±21.23	n.d	11.09±4.21	7.27±2.94	19.67±4.51	16.50±3.47
e				7						
β-Ionone	n.d	n.d	11.61±4.76	2622.19±89.02	4350.29±102.53	n.d	14.12±1.26	10.37±1.38	12.84±1.72	18.75±1.53
α-Cyclocitral	n.d	n.d	n.d	n.d	74.28±10.05	n.d	n.d	n.d	n.d	n.d

R1-R5 and A1-A5 were the flower developmental stages defined as previously studies (Li *et al.*, 2016; Sun *et al.*, 2016; Sun *et al.*, 2016)

n.d represented tentatively undetected

Data were the means ± SD of three independent experiments, and the unit was ng g⁻¹ FW (fresh weight).

Table S4. Composition and contents of volatile compounds released from different flower tissues

[illegible]

Linalool	56372.75± 1389.89	69562.78± 1686.61	17362.45± 749.32	3906.67±1 97.34	2284.48±1 39.32	79726.54± 2185.37	98650.26± 2098.67	12500.67± 1098.72	4238.56±2 09.98	3109.67±3 93.19
1,2-Dihydroli nalool	83.38±12. 97	793.57±26 .59	n.d	n.d	n.d	197.38±10 .24	n.d	n.d	n.d	n.d
(-)-4-Terpineo l	60.39±9.6 8	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Hotrienol	n.d	n.d	n.d	n.d	n.d	63.96±6.9 1	n.d	n.d	n.d	n.d
α-Terpineol	8772.33±5 03.38	21687.38± 729.96	8975.48±3 05.21	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Sesquiterpen es										
Cyclosativene	29.68±3.8 9	n.d	n.d	n.d	n.d	11.78±1.9 8	n.d	n.d	n.d	n.d
Copaene	n.d	n.d	n.d	n.d	n.d	1135.73±1 06.38	2353.83±1 02.39	637.51±67 .93	n.d	n.d
Elemene	n.d	n.d	n.d	n.d	n.d	137.67±13 .94	1093.71±4 9.92	140.09±15 .37	n.d	n.d
α-Gurjunene	n.d	n.d	n.d	n.d	n.d	286.67±16 .32	1207.28±5 9.01	220.72±23 .01	n.d	n.d
Caryophyllene	n.d	n.d	n.d	n.d	n.d	13.73±2.9 8	n.d	n.d	n.d	n.d
α-Guaiene	n.d	n.d	n.d	n.d	n.d	23.45±3.2 9	n.d	n.d	n.d	n.d
α-Patchoulene	n.d	n.d	n.d	n.d	n.d	12.09±2.6 3	n.d	n.d	n.d	n.d

Sativene	n.d	n.d	n.d	n.d	n.d	80.93±10.37	596.28±9.12	186.41±15.23	n.d	n.d
γ-Cadinene	n.d	n.d	n.d	n.d	n.d	45.02±6.29	n.d	210.14±10.29	n.d	n.d
γ-Gurjunene	74.97±3.09	n.d	n.d	n.d	n.d	67.37±4.29	n.d	n.d	n.d	n.d
Selinene	n.d	n.d	n.d	n.d	n.d	21.38±1.31	n.d	n.d	n.d	n.d
α-Bulnesene	n.d	n.d	n.d	n.d	n.d	119.38±10.23	665.12±30.12	322.45±20.91	n.d	n.d
Vatirenene	n.d	n.d	n.d	n.d	n.d	25.03±3.01	n.d	n.d	n.d	n.d
Nerolidol	60.67±4.78	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Carotenoid derivatives										
Dihydro-Ionone	1002.67±34.78	1078.67±46.89	1524.96±39.78	n.d	n.d	n.d	n.d	n.d	n.d	n.d
β-Ionone	4667.69±397.97	2263.18±148.98	4274.88±127.97	n.d	n.d	23.17±2.39	n.d	n.d	n.d	n.d
α-Cyclociral	335.46±30.78	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d

n.d represented tentatively undetected

Data were the means ± SD of three independent experiments, and the unit was ng g⁻¹ FW (fresh weight).

Table S5. Information of *FhTPS* genes isolated from flowers of *Freesia* cultivars

Candidate transcripts	<i>FhTPS</i> genes	Protein sequence length	Top <i>Arabidopsis</i> BLAST match	Top BLAST match excluding <i>Arabidopsis</i>	Homology (%)	Function prediction
Unigene_125076	<i>FhTPS1</i>	592 residues	NP_179998.1 terpene synthase 10 [<i>Arabidopsis thaliana</i>]	AMT81307.1 myrcene synthase [<i>Lilium sp.BT-2016</i>]	44 ^a ,53 ^b	Monoterpene synthase
Unigene_125270	<i>FhTPS2</i>	595 residues	NP_189212.1 terpene synthase-like sequence-1.8-cineol e [<i>Arabidopsis thaliana</i>]	AMT81307.1 myrcene synthase [<i>Lilium sp.BT-2016</i>]	44 ^a ,54 ^b	Monoterpene synthase
Unigene_125191	<i>FhTPS3</i>	590 residues	NP_567511.3 terpene synthase 03 [<i>Arabidopsis thaliana</i>]	OAY64179.1Alph a-terpineol synthase,chloropla stic [<i>Ananas comosus</i>]	43 ^a ,55 ^b	Monoterpene synthase
Unigene_116598	<i>FhTPS4</i>	566 residues	NP_176361.2 terpene synthase14[<i>Arabido psis thaliana</i>]	ADR74212.1(3S)- linalool/(E)-neroli dol synthase [<i>Vitis vinifera</i>]	45 ^a ,50 ^b	Monoterpene synthase/Sesquiterpene synthase
Unigene_110102	<i>FhTPS5</i>	607 residues	NP_189212.1 terpene	AMT81307.1 myrcene synthase	45 ^a ,53 ^b	Monoterpene synthase

			synthase-like sequence-1.8-cineol e protein [<i>Arabidopsis</i> <i>thaliana</i>] NP_197784.2	[<i>Lilium</i> <i>sp.BT-2016</i>] AAC315702.2		
Unigene_122329	<i>FhTPS6</i>	566 residues	terpene synthase 21[<i>Arabidopsis</i> <i>thaliana</i>] NP_197784.2	sesquiterpene synthase [<i>Elaeis</i> <i>oleifera</i>] AAC315702.2	36 ^a ,55 ^b	Sesquiterpene synthase
Unigene_105518	<i>FhTPS7</i>	570 residues	terpene synthase 21[<i>Arabidopsis</i> <i>thaliana</i>] NP_197784.2	sesquiterpene synthase [<i>Elaeis</i> <i>oleifera</i>] AAC315702.2	35 ^a ,51 ^b	Sesquiterpene synthase
Unigene_80141	<i>FhTPS8</i>	566 residues	terpene synthase 21[<i>Arabidopsis</i> <i>thaliana</i>] NP_197784.2	terpene synthase [<i>Populus</i> <i>trichocarpa</i>] All32473.1	35 ^a ,44 ^b	Sesquiterpene synthase

a% Similarity to *Arabidopsis*.

b% Similarity to other plant sequence

Table S6. Correlation analysis between gene expression and volatiles in different flower tissues of Red River[®] and Ambiance

	<i>FhTPS1</i>		<i>FhTPS2</i>		<i>FhTPS4</i>		<i>FhTPS6</i>		<i>FhTPS7</i>		<i>FhTPS8</i>	
	Red River [®]	Ambiance	Red River [®]	Ambiance	Red River [®]	Ambiance	Red River [®]	Ambiance	Red River [®]	Ambiance	Red River [®]	Ambiance
α -Pinene	---	---	0.374	NA	---	---	---	---	---	---	---	---
β -Pinene	---	---	---	---	---	---	---	---	---	---	---	---
Myrcene	---	---	0.623*	-0.306	---	---	0.296	0.477	NA	0.445	---	---
1,8 cineole	---	---	0.377	NA	---	---	---	---	---	---	---	---
D-Limonene	---	---	0.438	NA	---	---	0.114	NA	NA	NA	---	---
cis-Ocimene	---	---	---	---	---	---	0.838**	NA	NA	NA	---	---
trans-Ocimene	---	---	---	---	---	---	0.837**	NA	NA	NA	---	---
cis-Linaloloxide	---	---	---	---	---	---	---	---	---	---	---	---
Terpinolene	---	---	---	---	---	---	0.479	NA	NA	NA	---	---
Linalool	0.700**	0.692**	---	---	0.763**	0.929**	0.514*	0.703**	NA	0.482	---	---
1,2-Dihydrolinalool	---	---	---	---	---	---	---	---	---	---	---	---
(-)-4-Terpineol	---	---	---	---	---	---	---	---	---	---	---	---
Hotrienol	---	---	---	---	---	---	---	---	---	---	---	---
α -Terpineol	---	---	0.553*	NA	---	---	---	---	---	---	---	---
Cyclosativene	---	---	---	---	---	---	---	---	NA	0.437	---	---
Copaene	---	---	---	---	---	---	NA	0.693**	NA	0.516*	---	---
Elemene	---	---	---	---	---	---	NA	0.423	NA	0.345	---	---
α -Gurjunene	---	---	---	---	---	---	---	---	---	---	NA	0.929**
Caryophyllene	---	---	---	---	---	---	NA	0.521*	NA	0.431	---	---
α -Guaiene	---	---	---	---	---	---	---	---	NA	0.440	---	---
α -Patchoulene	---	---	---	---	---	---	---	---	---	---	---	---
Sativene	---	---	---	---	---	---	---	---	NA	0.378	---	---

γ -Cadinene	---	---	---	---	---	---	---	---	NA	0.709**	---	---
γ -Gurjunene	---	---	---	---	---	---	---	---	---	---	---	---
Selinene	---	---	---	---	---	---	NA	0.544*	---	---	NA	-0.306
α -Bulnesene	---	---	---	---	---	---	---	---	---	---	---	---
Vatirenene	---	---	---	---	---	---	---	---	---	---	---	---
Nerolidol	---	---	---	---	---	---	0.837**	NA	NA	NA	-0.301	NA

--- represented that volatile terpenes of *Freesia hybrid* were undetected in enzymatic assay of FhTPS

NA represented no Correlation

** p<0.01 * p<0.05

Table S7. Correlation analysis between gene expression and volatiles for fully opened flowers of Red River[®] and Ambiance

	<i>FhTPS1</i>	<i>FhTPS2</i>	<i>FhTPS6</i>	<i>FhTPS7</i>	<i>FhTPS8</i>
α -Pinene	---	0.997**	---	---	---
β -Pinene	---	---	---	---	---
Myrcene	---	0.985**	0.975**	-0.962	---
1,8 cineole	---	0.998**	---	---	---
D-Limonene	---	0.998**	0.976**	-0.969	---
cis-Ocimene	---	---	0.977**	-0.974	---
trans-Ocimene	---	---	0.977**	-0.971	---
cis-Linaloloxide	---	---	---	---	---
Terpinolene	---	---	0.971**	-0.959	---
Linalool	0.832*	---	-0.819	0.576	---
1,2-Dihydrolinalool	---	---	---	---	---
(-)-4-Terpineol	---	---	---	---	---
Hotrienol	---	---	---	---	---
α -Terpineol	---	0.992**	---	---	---
Cyclosativene	---	---	---	-0.853	---
Copaene	---	---	-0.970	0.983**	---
Elemene	---	---	-0.959	0.981**	---
α -Gurjunene	---	---	---	---	0.871*
Caryophyllene	---	---	-0.848	0.902**	---
α -Guaiene	---	---	---	0.976**	---
α -Patchoulene	---	---	---	---	---
Sativene	---	---	---	0.983**	---
γ -Cadinene	---	---	---	0.982**	---
γ -Gurjunene	---	---	---	---	---

Selinene	---	---	-0.892	---	0.947**
α -Bulnesene	---	---	---	---	---
Vatirenene	---	---	---	---	---
Nerolidol	---	---	0.956**	-0.933	-0.707

--- represented that volatile terpenes of *Freesia hybrid* were undetected in enzymatic assay of FhTPS

NA represented no Correlation

** p<0.01 * p<0.05

Table S8. Summary of FhTPSs in the proposed model to explain volatile terpene biosynthesis in flowers of Ambiance and Red River®

Gene	Subcellular location		Tissue specific expression		Enzymatic products	
	Chloroplast	Cytoplasm	Red River®	Ambiance	Red River®	Ambiance
<i>FhTPS1</i>	Y	---	Petal/Pistil/Stamen	Petal/Pistil/Stamen	Linalool	Linalool
<i>FhTPS2</i>	Y	---	Petal/Pistil/Stamen	---	α -Terpineol/ Myrcene	---
<i>FhTPS4</i>	Y	---	Calyx/Torus	Calyx/Torus	Linalool	Linalool
<i>FhTPS6</i>	---	Y	Petal/Pistil/Stamen	Petal/Pistil/Stamen	cis-Ocimene/ trans-Ocimene/Myrcene / Nerolidol	Selinene / Myrcene/ Elemene
<i>FhTPS7</i>	---	Y	---	Petal/Pistil/Stamen	---	Copaene/ γ -Cadinene/ Caryophyllene/ Cyclosativene/ γ -Gurjunene/ Sativene α -Guaiene/ Elemene/ Myrcene
<i>FhTPS8</i>	---	Y	---	Petal/Pistil/Stamen	---	α -Gurjunene

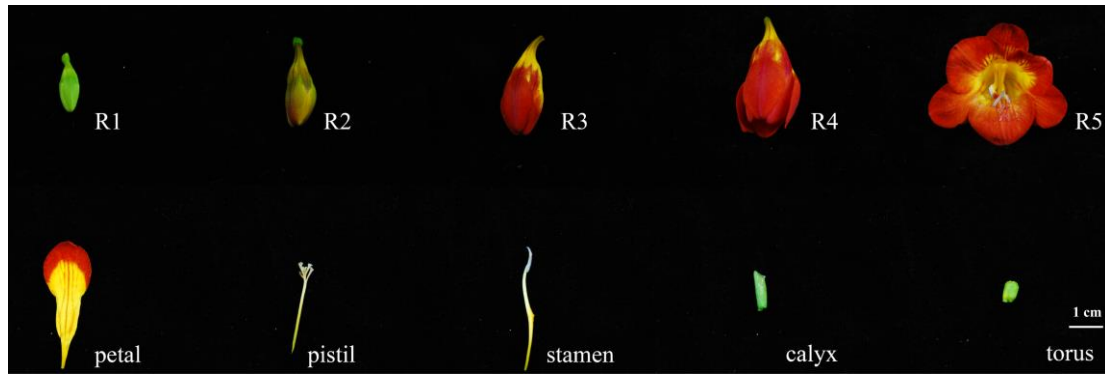


Fig. S1. Flower developmental stages and different tissues of Red River®.

The flower developmental stages and flower tissues were defined as in previous studies (Li *et al.*, 2016; Sun *et al.*, 2016; Sun *et al.*, 2016)



Fig. S2. Flower developmental stages and different tissues of Ambiance.

The flower developmental stages and flower tissues of Ambiance were defined according to that of Red River[®], as in previous studies (Li *et al.*, 2016; Sun *et al.*, 2016; Sun *et al.*, 2016).

FhTPS1 (Red River [®])	MALLPCLPSQFPCSPVTGFVRLPLLSSRRSSKGVQSSNYRFRCCINTGTSVSQPLRRAASYPQNIWDDSYIQALNCGYMGDEQVNEIRKLKEEVGQLFSDSKEILYQIELIDELQQLGVA
FhTPS1 (Ambiance)	MALLPCLPSQFPCSPVTGFVRLPLLSSRRSSKGVQSSNYRFRCCINTGTSVSQPLRRAASYPQNIWDDSYIQALNCGYMGDEQVNEIRKLKEEVGQLFSDSKEILYQIELIDELQQLGVA

FhTPS1 (Red River [®])	YHFQDEIKDKLSTIFCSLEKTSLFMENDLKATSLVFRLLREHGFHASADIFNNFRENKGNFKSCLKNDMEGMINLYEASFFAVEGENQLDEARVFATEHLRHLSESLVEASLRERVAHAL
FhTPS1 (Ambiance)	YHFQDEIKDKLSTIFCSLEKTSLFMENDLKATSLVFRLLREHGFHASADIFNNFRENKGNFKSCLKNDMEGMINLYEASFFAVEGENQLDEARVFATEHLRHLSESLVEASLRERVAHAL

FhTPS1 (Red River [®])	ELPLHFRMSRLHTRWFIDWYEKKVDKNSNLCRLAKLDFNFVQNIYKRELKELSRWWTNLGLGQKLSFARDRLVENYLFVIGWAFEPKLWQNREAMTMANCLVTTLDDIYDVYGSLELEL
FhTPS1 (Ambiance)	ELPLHFRMSRLHTRWFIDWYEKKVDKNSNLCRLAKLDFNFVQNIYKRELKELSRWWTNLGLGQKLSFARDRLVENYLFVIGWAFEPKLWQNREAMTMANCLVTTLDDIYDVYGSLELEL

FhTPS1 (Red River [®])	FTDAVNRWDAAEIEQLPDYMKTCIMALFNTTNLTANKIMYSKGVNIIPQLRRSWADLCKAYLVEAKWYHSGYMPLEEYLDTAWISISGPVVLTAQAYCTSENITDEALKCYNFYDPVVRQ
FhTPS1 (Ambiance)	FTDAVNRWDAAEIEQLPDYMKTCIMALFNTTNLTANKIMYSKGVNIIPQLRRSWADLCKAYLVEAKWYHSGYMPLEEYLDTAWISISGPVVLTAQAYCTSENITDEALKCYNFYDPVVRQ

FhTPS1 (Red River [®])	SSMISRLWNDLATSTAEMERGDVPKSIQCYMHEKGVSEEVAREHIRDMIVSISKKFDYDCISNSSIAESLKSVALDVHRMSQCVYQYEDGYGEQGHQKREQVISLLFEPIPL
FhTPS1 (Ambiance)	SSMISRLWNDLATSTAEMERGDVPKSIQCYMHEKGVSEEVAREHIRDMIVSISKKFDYDCISNSSIAESLKSVALDVHRMSQCVYQYEDGYGEQGHQKREQVISLLFEPIPL

Fig. S3. Alignment of deduced amino acid sequences of FhTPS1 in Red River[®] and Ambiance.

FhTPS2	(Red River [®])	MACLPFHYTTYSRSPAGILFRSSLPSSHCRARRSRSNESANRIRCCNNTQISQPLRRTANYPPTIWENSYIQE ¹ NTDYMQEDEETIEIGKLKEYVMTRLIISNSDQIELIDTLQQLGVAY
FhTPS2	(Ambiance)	MACLPFHYTTYSRSPAGILFRSSLPSSHCRARRSRSNESANRIRCCNNTQISQPLRRTANYPPTIWENSYIQE ¹ NTDYMQEDEETIEIGKLKEYVMTRLIISNSDQIELIDTLQQLGVAY

FhTPS2	(Red River [®])	HFQEEIQNILATIFCSIKKIIP ² TIHNDIYATALLFRLREKGFHVSTNIFNNFKEEGGTFKACLKNDIKGMLSLYEASFLAVEGENELDEARLFATECLKHTMENSLSEPSMKERIVHA
FhTPS2	(Ambiance)	HFQEEIQNILATIFCSIKKIIP ² TIHNDIYATALLFRLREKGFHVSTNIFNNFKEEGGTFKACLKNDIKGMLSLYEASFLAVEGENELDEARLFATECLKHTMENSLSEPSMKERIVHA

FhTPS2	(Red River [®])	LELPLHWRMSRLHSRWFIDQYEKDEKMNP ³ TLLRLAKLDFNFVQTIYKRELKELSRWWSNLDLLGDKLG ⁴ FARDRLVENYLWTVGSAFEPKFWQ ⁵ SREALTKANCLITTIDDIYDVYGT ⁶ LDEL
FhTPS2	(Ambiance)	LELPLHWRMSRLHSRWFIDQYEKDEKMNP ³ TLLRLAKLDFNFVQTIYKRELKELSRWWSNLDLLGDKLG ⁴ FARDRLVENYLWTVGSAFEPKFWQ ⁵ SREALTKANCLITTIDDIYDVYGT ⁶ LDEL

FhTPS2	(Red River [®])	VLFTDV ⁷ AD ⁸ RWDVNAIEQLPDYMK ⁹ TCLLALFNTTNDTAYKILNLKGVIIIPQLKKVWADLC ¹⁰ KAYLVEAKWYHSGYMP ¹¹ TLEEYLDNGWISISGHVALAHAFCTSE ¹² DIYRALQCYNQLSPNL
FhTPS2	(Ambiance)	VLFTDV ⁷ AD ⁸ RWDVNAIEQLPDYMK ⁹ TCLLALFNTTNDTAYKILNLKGVIIIPQLKKVWADLC ¹⁰ KAYLVEAKWYHSGYMP ¹¹ TLEEYLDNGWISISGHVALAHAFCTSE ¹² DIYRALQCYNQLSPNL

FhTPS2	(Red River [®])	LFHSSVIVRLVDDLATSTAE ¹³ LERGDVPKAIQCYMKQNRVSEEVAREKIKEMIVSTWEKLN ¹⁴ GD ¹⁵ LIATSSVVFQSV ¹⁶ ALNFP ¹⁷ MAQCIYQYGDGYGDPTQKT ¹⁸ KDQIVSLLIQPVSL
FhTPS2	(Ambiance)	VFHSSVIVRLVDDLATSTAE ¹³ LERGDVPKAIQCYMKQNRVSEEVAREKIKEMIVSTWEKLN ¹⁴ GD ¹⁵ LIATSSVVFQSV ¹⁶ ALNFP ¹⁷ MAQCIYQYGDGYGDPTQKT ¹⁸ KDQIVSLLIQPVSL
:*****		

Fig. S4. Alignment of deduced amino acid sequences of FhTPS2 in Red River[®] and Ambiance.

Protein	Source	Sequence
FhTPS3	(Red River [Ⓢ])	MGTEMALQHLCSSFNTPYSLTIVSAKTESTRIDTDNKLAKALQVRSSGVGTGTHATVGTGRRRKANYQPRLWDYQSLQCHSTEINCQDEKDAVKI KEAVNHLLYKETKLEDKLEL IDALQRL
FhTPS3	(Ambiance)	MGTEMALQHLCSSFNTPYSLTIVSAKTESTRIDTDNKLAKALQVRSSGVGTGTHATVGTGRRRKANYQPRLWDYQSLQCHSTEINCQDEKDAVKI KEAVNHLLYKETKLEDKLEL IDALQRL *****
FhTPS3	(Red River [Ⓢ])	GLSYQFEEEEKIILGSIKVNSTDPNSLHATALLFRLLRENGFQISQENFNVFKDEKGC FKECLCKDAQGMLSLYEASQLMFRREATLDDARIFTTKHLNDIEAYTDMHLKERVERALE
FhTPS3	(Ambiance)	GLSYQFEEEEKIILGSIKVNSTDPNSLHATALLFRLLRENGFQISQENFNVFKDEKGC FKECLCKDAQGMLSLYEASQLMFRRESTLDAARIFTTKHLNDIKAYMDVHLKEKERVERALE *****:*** *****:*** *:***:*****
FhTPS3	(Red River [Ⓢ])	LPLHWRTPRSDIRWYVEQYNSDMSIQPVLQLAKVDFNVQILHRELGRMVRWWEDLGLAEQLPFVRDRLVECYFYAAGVVSNPNLGYCREGVAKVCNLIITLDDVYDINGFSDELVQLT
FhTPS3	(Ambiance)	LPLHWRTPRSDIRWYVEQYNSDMSIQPVLQLAKVDFNVQILHRELGRMVRWWEDLGLPERLPFVRDRLVECYFYAAGVVSNPNLGYCREGVAKVCNLIITLDDVYDINGFSDELVQLT *****:***** ***** *:*****
FhTPS3	(Red River [Ⓢ])	NAVNRWEISAVEGLPEYMKTSYSALYDTTNELARNILQKGDWDPSSYLKKAWADLCNSFLVEAKWHYSGYVPTIQEYLDNGWISVSGHVLLHAFFLSRQCITKEMVQCLETYPNFVRSS
FhTPS3	(Ambiance)	NAVNRWEIRAVEGLPEYMKTSYSALYDTTNELARNILQKGDWDPSSYLKKAWADLCNSFLVEAKWHYSGYVPTIQEYLDNGWISVSGHVLLHAFFLSRQCITKEIVQCLETYPNFVRSS ***** ***** ***** *:*****.*****
FhTPS3	(Red River [Ⓢ])	STICRLSNDLVTSAAEMERGDSPASIQCYMHEHGVTEEVALEGIKVLINETWKKLNEDVANGCVFPRSLANLAVDLAQTAYCMYRNGDGIGAPDKENKNDISSTFFKPMK
FhTPS3	(Ambiance)	STIFRLCNDLGTAAEIERGDAPTSIQCYMHEHGVTEEVALEGIKVLIDETWKKLNEDVANGCVFPRSLANLADLARTSYCTYVNGDGIGAPDKENKNDISSTFFKPMK *** ** *** * ***:***:*.*****:***** *****:*****:***-*.*** *****

Fig. S5. Alignment of deduced amino acid sequences of FhTPS3 in Red River® and Ambiance.

FhTPS4	(Red River [®])	MTTFSKMSASPPSPFVTRNSGCANKTSTGTISQSNYSPQSARLVQRLGSLLTNVREDQSDMKHAENLMRVKSLFPQLEDPLECMNTIDSLQRLGIDYHFKKEIKDMLGHIYERFRQIEH
FhTPS4	(Ambiance)	MTTFSKMSASPPSPFVTRNSGCANKTSTGTISQSHYSPQSVRLVQRLGSLLTNVREDQSDMKHAENLMRVKSLFPQLEDPLECMNTIDSLQRLGIDYHFKKEIKDMLGHIYERFRQIEH
		*****:****_*****_*****
FhTPS4	(Red River [®])	HLITGDLFEVSLSFRLLRQAGHHVSSDVFYKFIDDKGKLDSSLRTDIEGLLSLHEASYLNTGEDILYRTKEFTIEHLTSCMEHLESDGASLVEQTLKSPIHKTL SKYNSPYYINRRQEKL
FhTPS4	(Ambiance)	HLITGDLFEVSLSFRLLRQAGHHVSSDVFYKFIDDKGKLDSSLRTDIEGLLSLHEASYLNTGEDILYRTKEFTIEHLTSCMEHLESDGASLVEQTLKSPIHKTL SKYNSPYYINRRQEKL

FhTPS4	(Red River [®])	TRYGVLNEVARVDYNQVQTIYQRELFEILSWWKEIGLVQELNFI RDQPLKWTWSMTVLPDPQFSKCRISLTKVIAFVYIIDDFDIYGTLEELSLFTEAIRKWELSGAETLPTMQILY
FhTPS4	(Ambiance)	TRYGVLNEVARVDYNQVQTIYQRELFEILSWWKEIGLVQELNFI RDQPLKWTWSMTVLPDPQFSKCRISLTKVIAFVYIIDDFDIYGTLEELSLFTEAIRKWELSGAETLPTMQILY

FhTPS4	(Red River [®])	KTLYDITNEIAEATYEEHNWNP IGH LKESWARLCDAFLKEAKWFQSKKVPKAD EYLANAIVSSGVYTVLLHAYFLLGEGITQENANFLKTNPTLLSSPATILRLWDDL GNAEDENQEGYD
FhTPS4	(Ambiance)	KTLYDITNEIAEATYEEHNWNP IGH LKESWARLCDAFLKEAKWFQSKKVPKAD EYLANAIVSSGVYTVLLHAYFLLGEGITQENANFLKTNPTLLSSPATILRLWDDL GNAEDENQEGHD
		*****:*
FhTPS4	(Red River [®])	GSYVEYLMQENPNYTMESSRDHVMKMISSWEALNKECFSSSRFAPNLMAGCLNLSRMIEVMYSYDTNQNL PVLEEYITKLLFKSI
FhTPS4	(Ambiance)	GSYVEYLMQENPNYTMESSRDHVMKMISSWEALNKECFSSSQFAPNLMAGCLNLPRMIEVMYSYDTNQNL PVLEEYITKLLFKSI
		*****:***** *****

Fig. S6. Alignment of deduced amino acid sequences of FhTPS4 in Red River[®] and Ambiance.

FhTPS6	(Red River [®])	MESVLLGSPPDGTVTSSVTRRPASANYHPSVWGDYFIKQQFPPSKIQKSEAWIKQRVEELIIKIKIMLTESTDQLQEMQLIDAVQRLGVAYHFEKEIDDKLRRIHNANLDSSDLHFISLR
FhTPS6	(Ambiance)	MESVLLGSPPDGTVTSSVTRRPASANYHPSVWGDYFIKQQFPPSKIQKSEAWIKQRVEELIIKIKIMLTESTDQLQEMQLIDAVQRLGVAYHFEKEIDDKLRRIHNANLDSSDLHFISLR

FhTPS6	(Red River [®])	FRLLRQHGYNVPSDVFNKFKDDEGNFRSSLCEQVRVLLSLYEAYLSIPGEDILDEALEFTKRHLKYYSMESNYLEPALATHISHALQAPLRRLERLEARQYINIEKDDEIRNDYILE
FhTPS6	(Ambiance)	FRLLRQHGYNVPSDVFNKFKDDEGNFRSSLCEQVRVLLSLYEAYLSIPGEDILDEALEFTKRHLKYYSMESNYLEPALATHISHALQAPLRRLERLEARQYINIEKDDEIRNDYILE

FhTPS6	(Red River [®])	FAKLDFHLLQLVHREELKSISGWWKSSGLIEKLNARDRVAECYFWALGVYYPECYSRARKMLTKVLLQFSLMDDTYDAYGTLEELQLYTKAIQRWNLDGVDELEECMKFQYLALYDMAK
FhTPS6	(Ambiance)	FAKLDFHLLQLVHREELKSISEWWKSSGLIEKLNARDRVAECYFWALGVYYPECYSRARKMLTKVLLQFSLMDDTYDAYGTLEELQLYTKAIQRWNLDGVDELEECMKFQYLALYDMAK

FhTPS6	(Red River [®])	DFEDELADDNIQYRVNYLREATKNTTKAWLKEAEWREEGYVPSFEEYFTVSLPSATYPTVACVSYVGMGEIVTKEALDWIFNIPKIVQAATMITRCMDLVSSEFERKRDHVATAIQCYM
FhTPS6	(Ambiance)	DFEDELADDNIQYRVNYLREATKNTTKAWLKEAEWREEGYVPSFEEYFTVSLPSATYPTVACVSYVGMGEIVTKEALDWIFNIPKIVQAATMITRCMDLVSSEFERKRDHVATAIQCYM

FhTPS6	(Red River [®])	KEYEGASSEDACKVIRKMVEDGWKVANQECLNQKISIHLLTKIFNLARVMETMYKEIDSYTQSTTTLKDHITLLFVEPISFEDDYF
FhTPS6	(Ambiance)	KEYEGASSEDACKVIRKMVEDGWKVANQECLNQKIPHLLTKIFNLARVMETMYKEIDSYTQSTTTLKDHITLLFVEPISFEDDYF

Fig. S7. Alignment of deduced amino acid sequences of FhTPS6 in Red River[®] and Ambiance.

FhTPS8	(Red River [®])	MEVVSAGEEVVRPIAKFSPSIWGDFFINHSFPLANDKNAITLIEQRVEELKRTVKELFSTNQYITERLLLIDDLQRLGIDYRFKQEIIDEALKCIHIDNDNIDDNMYLVALRFLLRQQGY
FhTPS8	(Ambiance)	MEVVSAGEEVVRPIAKFSPSIWGDFFINHSFPLANDKNAITLIEQRVEELKRTVKELFSTNQYITERLLLIDDLQRLGIDYRFKQEIIDEALKCIHIDNDNIDDNMYLVALRFLLRQQGY

FhTPS8	(Red River [®])	HVSSDVFSKFKDDNGSFKQEYAGNIIGLLQLYEATGVRIQEDSILDEAFDFAKYHMERSITADTVKGVLAIRHALEMPFHRSRKRLGARYYMSIYEKDEARNVDLLELAKLDFTLQR
FhTPS8	(Ambiance)	HVSSDVFSKFKDDNGSFKQEYAGNIIGLLQLYEATGVRIQEDSILDEAFDFAKYHMERSITADTVKGVLAIRHALEMPFHRSRKRLGARYYMSIYEKDEARNVDLLELAKLDFTLQR

FhTPS8	(Red River [®])	LYQDEIKTFTMWYNQELGPKKLSFSRDRAVENYFWALGIMHFEPELATGRLLLAKLLAYVLVWDDMYDAYGTFDELRLFTDVIERWDLESVDHLPEYMRVCLSSYSNFMREVEDELIKNE
FhTPS8	(Ambiance)	LYQDEIKTFTMWYNQELGPKKLSFSRDRAVENYFWALGIMHFEPELATGRLLLAKLLAYVLVWDDMYDAYGTFDELRLFTDVIERWDLESVDHLPEYMRVCLSSYSNFMREVEDELIKNE

FhTPS8	(Red River [®])	RDQLKPYVAEMMKYIIDGFFQEAKWLNENYIPTMEEYLANGLKTGGQTTLNGFSLLFMSEDKVTKDLEWVLSMPNILKASTLIGRLLNDIKTTKLEHERMHVASSIQIYMNEAGVTEAM
FhTPS8	(Ambiance)	RDQLKPYVAEMMKYIIDGFFQEAKWLNENYIPTMEEYLANGLKTGGQTTLNGFSLLFMSEDKVTKDLEWVLSMPNILKASTLIGRLLNDIKTTKLEHERMHVASSIQIYMNEAGVTEAM

FhTPS8	(Red River [®])	AIAKLNGMVADFWDINKELLDALPFQKDFNTLTNLFARTLEVLYKHEDAFTHGSIQREQIDLMLVWPIQN
FhTPS8	(Ambiance)	AIAKLNRMVADFWDINKELLDALPFQKDFNTLTNLFARTPEVLYKHEDAFTHGSIQREQIDLMLVWPIQN
***** *****		

Fig. S8. Alignment of deduced amino acid sequences of FhTPS8 in Red River[®] and Ambiance.

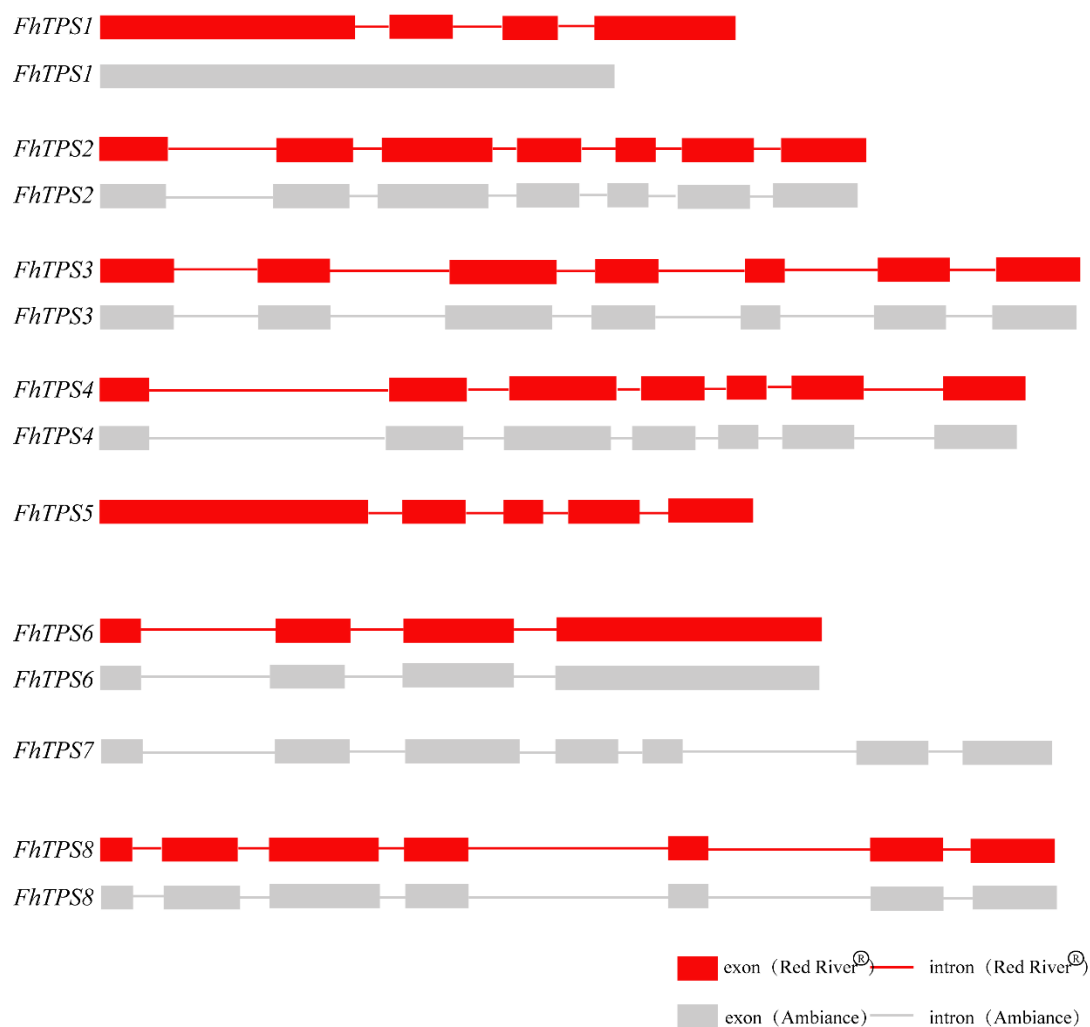


Fig. S9. Genomic structures of the *FhTPS* genes for two cultivars of *Freesia hybrida* (Red River® and Ambiance).

Genomic organization of *FhTPS* genes. The frames and solid lines represented exons and introns, respectively.



Fig. S10. Conserved residues analysis and subcellular localization of FhTPS proteins in two cultivars of *Freesia hybrida*

Full-length protein sequence alignment of *Freesia* TPS proteins (FhTPS1-FhTPS8).

Functionally important conserved residues (RRX₈W, DDXD and DDXSXXXE/NDXXTXXE) are highlighted with a colored background.

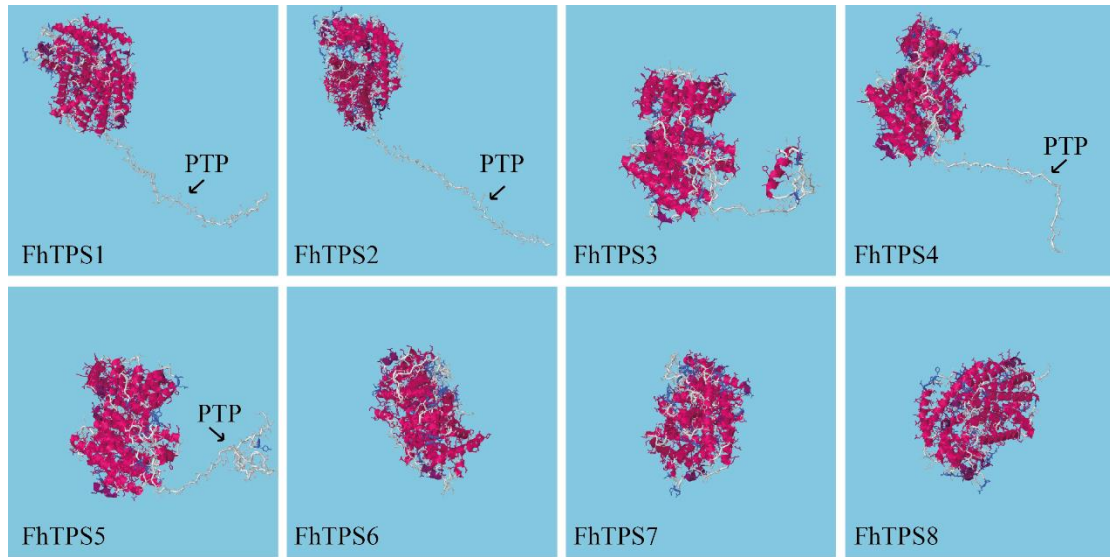


Fig. S11. Three-dimensional model of the structure model of FhTPSs.

The globular protein is shown in pink and the chloroplast transit peptide (PTP) in white.

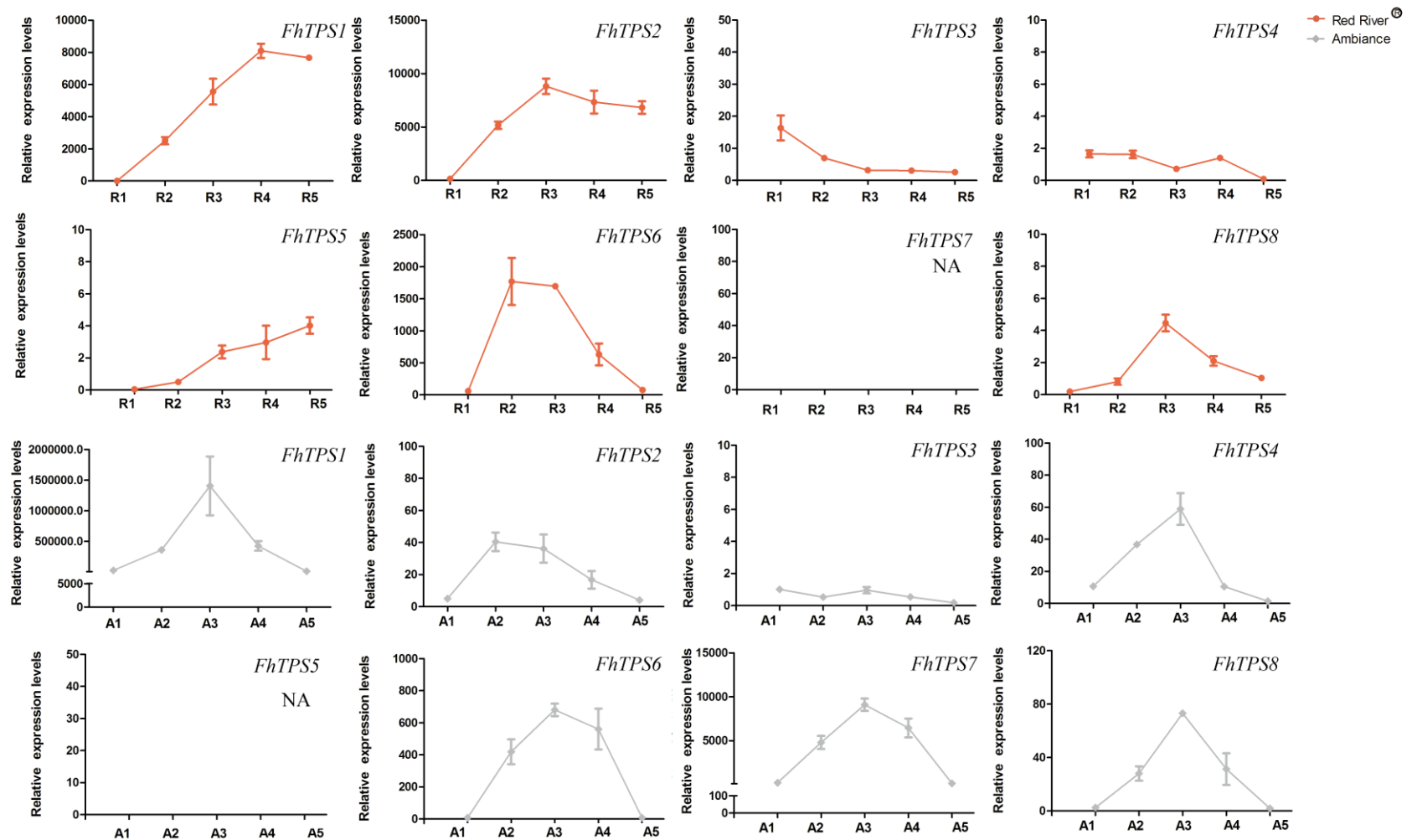


Fig. S12. Expression patterns of *FhTPS* genes in five developmental stages of Red River® and Ambiance

Real-time PCR gene expression analysis of *FhTPS* genes at five development stages of Red River[®] and Ambiance. Relative gene expression levels were calculated with formula $2^{-\Delta\Delta C_T}$. Gene expression levels of *FhTPS* genes in Red River[®] and Ambiance are represented by red and gray lines, respectively. NA indicates no gene expression. The flower developmental stages and tissues were defined as in previous studies (Li et al., 2016; Sun et al., 2016; Sun et al., 2016). All results are presented as means \pm SD of triplicate experiments.

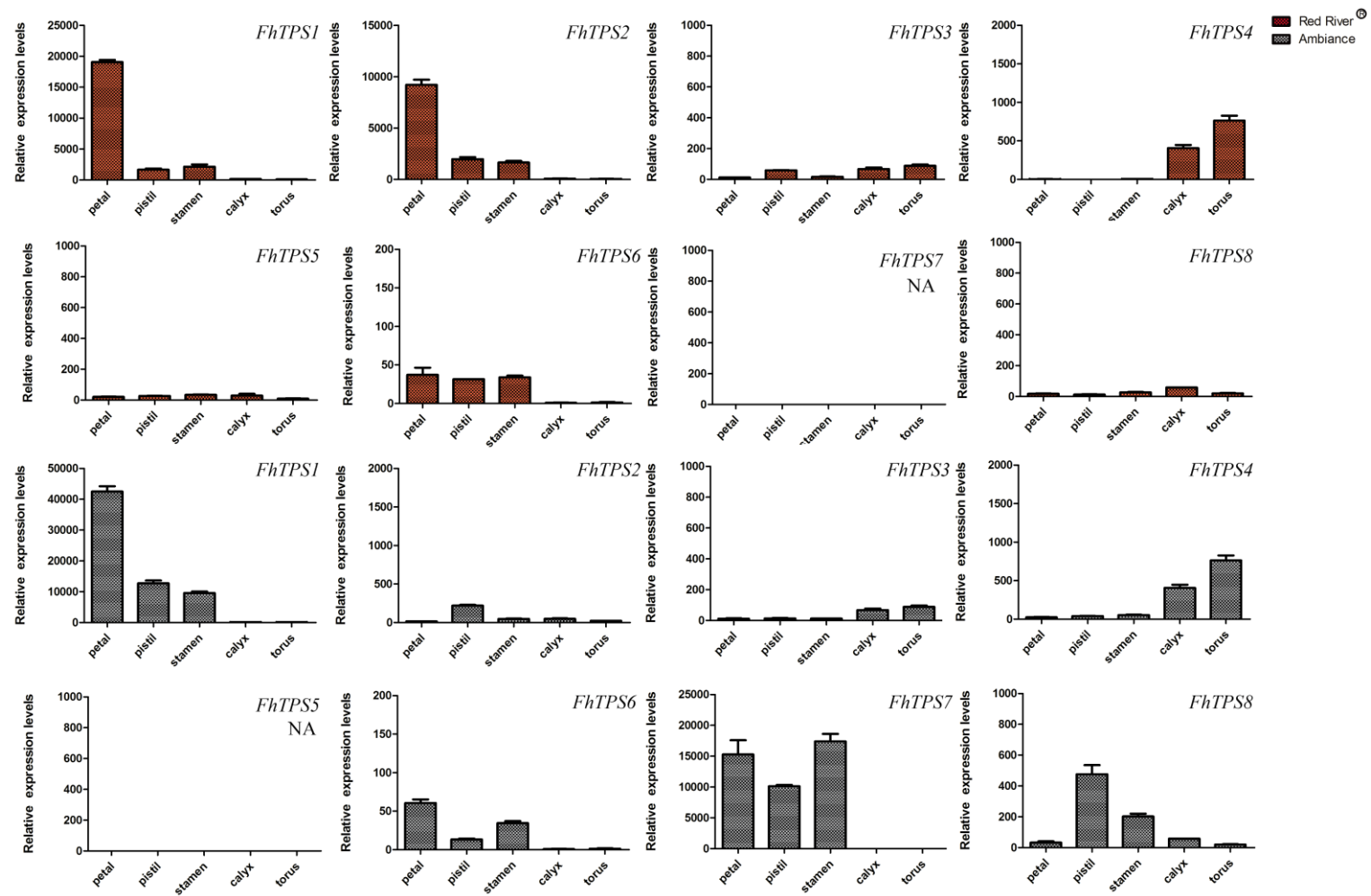


Fig. S13. Expression patterns of *FhTPS* genes in five flower tissues of Red River® and Ambiance

Real-time PCR gene expression analysis of *FhTPS* genes in five flower tissues of Red River[®] and Ambiance. Relative gene expression levels were calculated with formula $2^{-\Delta\Delta C_T}$. NA indicates no gene expression. Gene expression levels of *FhTPS* genes in Red River[®] and Ambiance are represented by red and gray frames, respectively. All results are presented as means \pm SD of triplicate experiments.

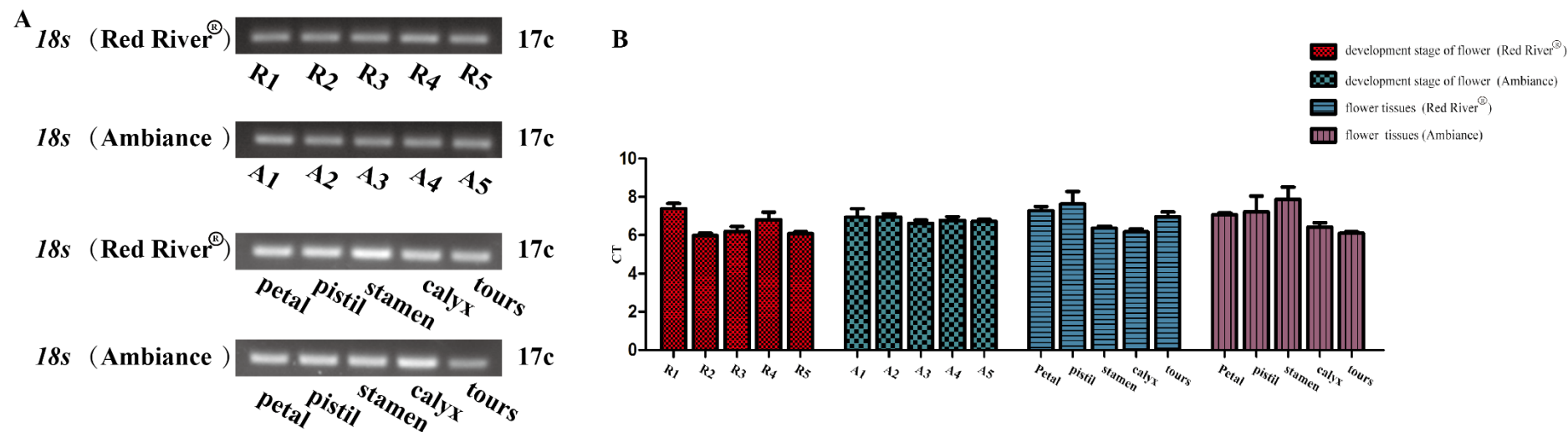


Fig. S14. Stability analysis of 18S rRNA in two cultivars of *Freesia hybrida*.

(A) The expression of 18S rRNA was analyzed at five developmental stages and in five flower tissues of two cultivars of *Freesia hybrida* by semi-quantitative PCR. 17C represented 17 PCR cycle. (B) The expression of 18S rRNA was analyzed at five developmental stages and in five flower tissues of two cultivars of *Freesia hybrida* by real-time PCR. The number in Y-axis represented the CT value of 18S rRNA.

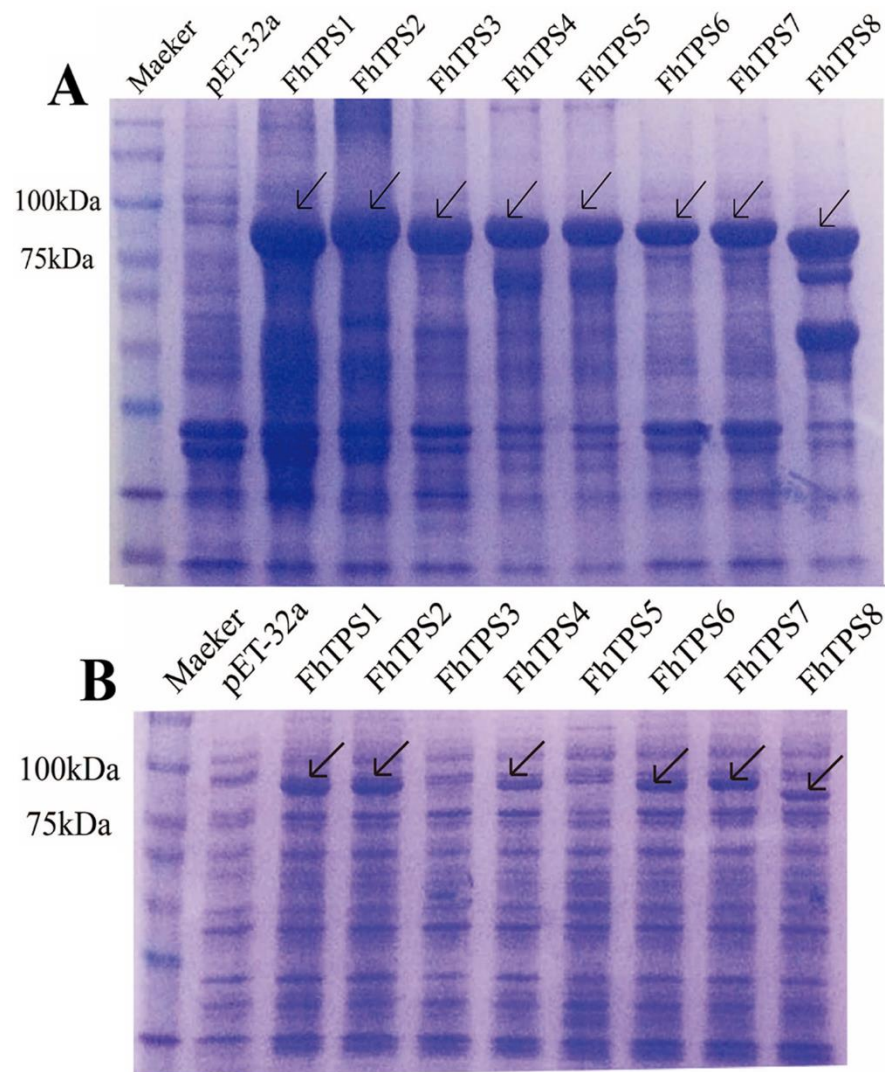


Fig. S15. Detection of FhTPS proteins in *E. coli* strain BL21 (DE3)

(A) SDS-PAGE gel showing recombinant 6His-tagged FhTPSs (FhTPS1-FhTPS8) proteins in the form of inclusion body protein. (A) SDS-PAGE gel showing recombinant 6His-tagged FhTPSs (FhTPS1-FhTPS8) proteins in the form of soluble protein. The position corresponding to the recombinant proteins are indicated by an arrow.

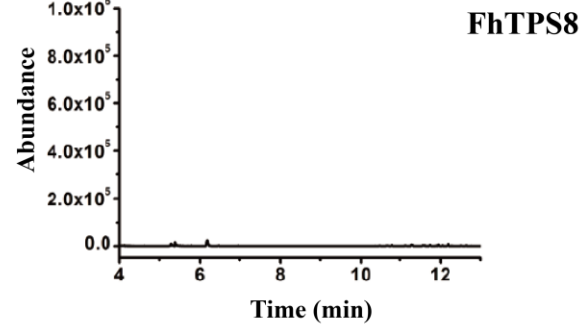
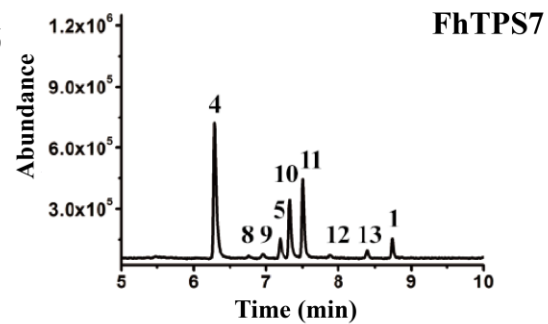
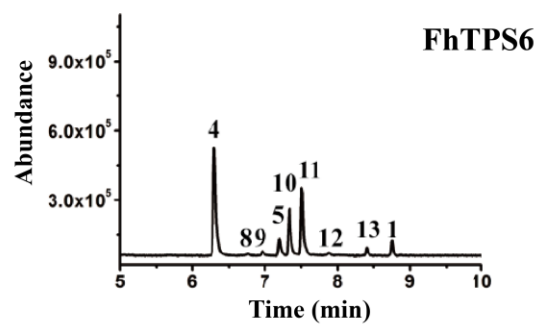
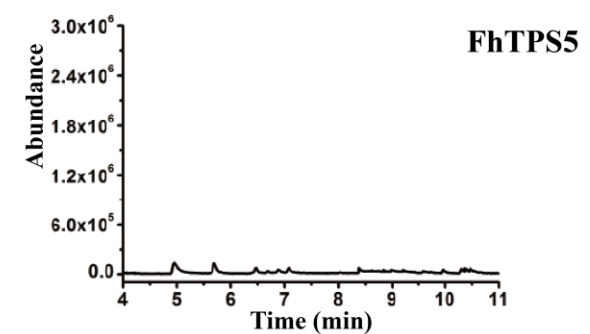
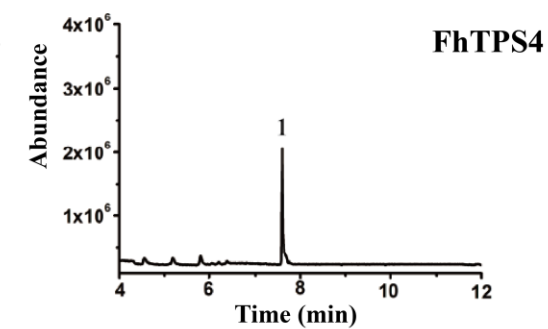
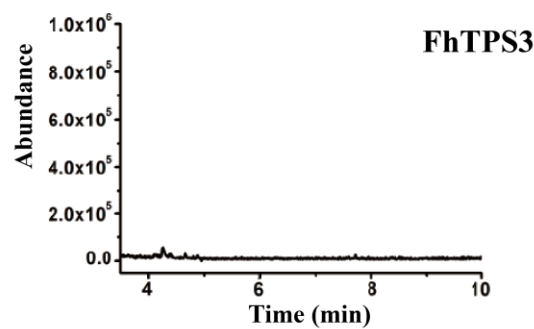
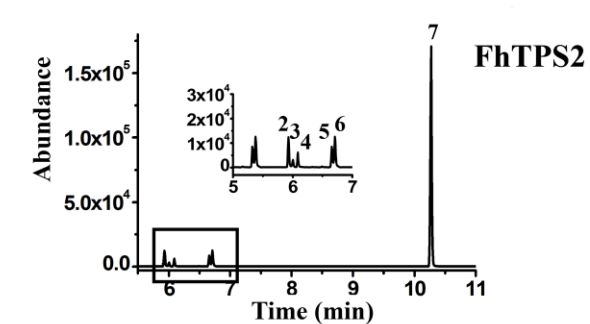
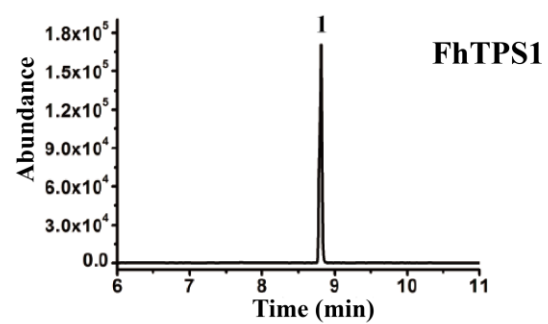
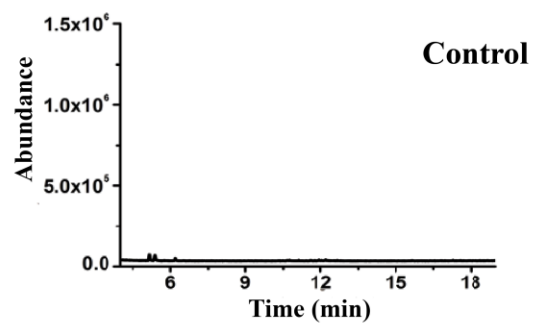


Fig. S16. *In vitro* enzymatic activity analysis of FhTPS proteins using GPP as substrate

Products catalyzed by FhTPS proteins (FhTPS1-FhTPS8) using GPP as substrate were subjected to GC-MS. Peaks marked with numbers were identified as monoterpenes by comparing mass spectra with the NIST 2008 mass spectra library. Each experiment was performed in three independent repeats to confirm the accuracy of the FhTPS products. The X axis represents the retention time of the peak outflow, and the Y axis represents the integral area of chromatographic peak. The enzymatic products are as follows: 1, Linalool; 2, Bicyclo [3.1.0] Thujene; 3, α -Pinene; 4, Myrcene; 5, D-Limonene; 6, 1,8 cineole; 7, α -Terpineol; 8, Thujene; 9, Isoterpinolene; 10, cis-Ocimene; 11, trans-Ocimene; 12, Terpinene; 13, Terpinolene.

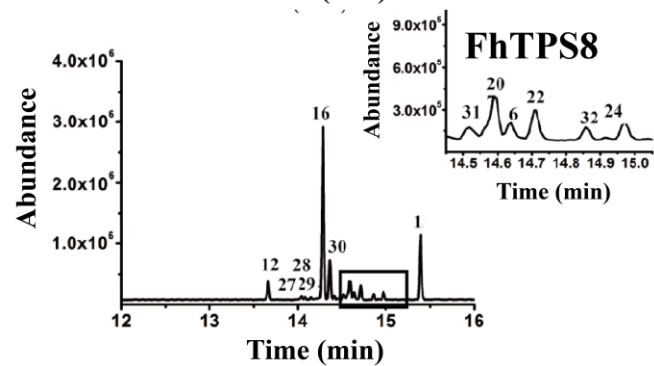
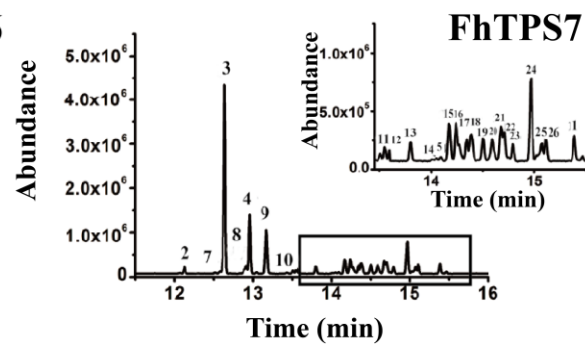
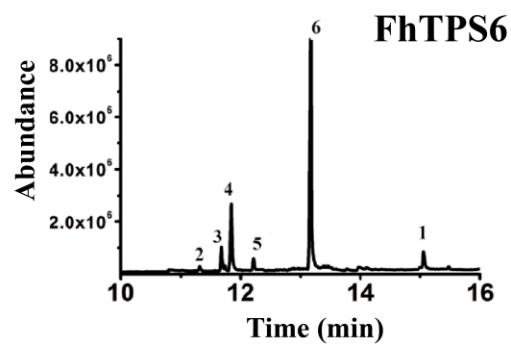
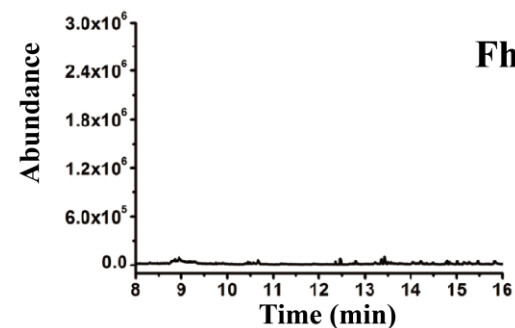
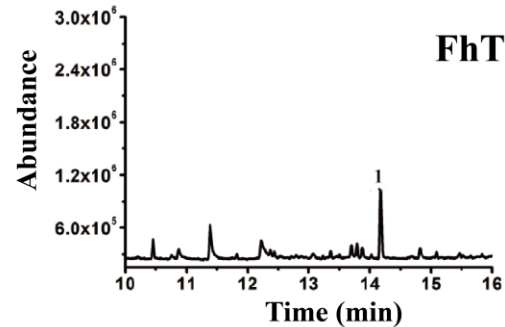
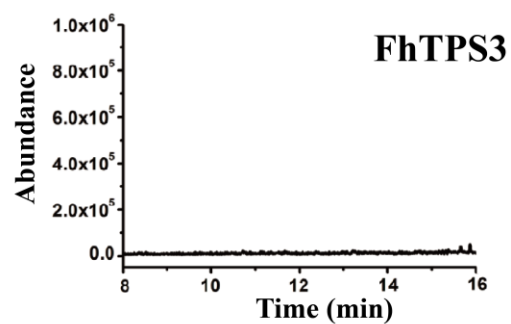
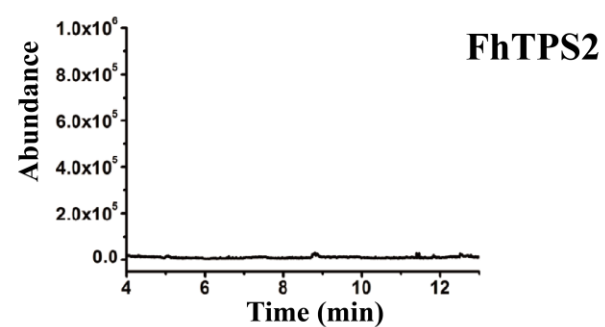
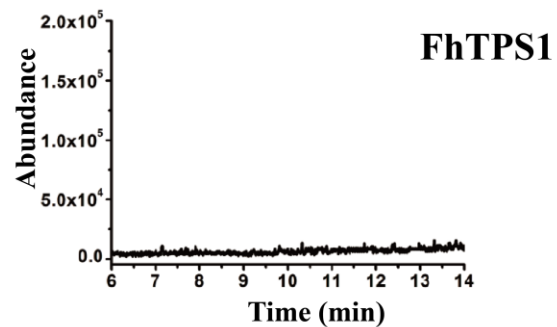
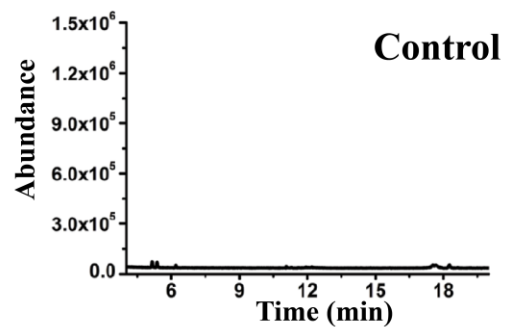


Fig. S17. *In vitro* enzymatic activity analysis of FhTPS proteins using FPP as substrate

Products catalyzed by FhTPS proteins (FhTPS1-FhTPS8) using FPP as substrate were subjected to GC-MS. Peaks marked with numbers were identified as sesquiterpenes by comparing mass spectra with the NIST 2008 mass spectra library. Each experiment was performed in three independent repeats to confirm the accuracy of the FhTPS products. The X axis represents the retention time of the peak outflow, and the Y axis represents the integrated area of the chromatographic peak. The enzymatic products are as follows: 1, Nerolidol; 2, α -Cubebene; 3, Copaene; 4, Elemene; 5, Caryophyllene; 6, Selinene; 7, Cycloisosativene; 8, Epi-bicyclosesquiphellandrene; 9, β -Maaliene; 10, β -Caryophyllene; 11, α -Guaiene; 12, Farnesene; 13, γ -Maaliene; 14, Himachalene; 15, Sativene; 16, α -Gurjunene; 17, γ -Cadinene; 18, γ -Muurolene; 19, γ -Gurjunene; 20, Germacrene; 21, α -Muurolene; 22, Guaia-1(10), 11-diene; 23, Eudesmene; 24, Cadina-3, 9-diene; 25, 1R, 3Z, 9S-2, 6, 10, 10-Tetramethylbicyclo [7.2.0] undeca-2, 6-diene; 26, Naphthalene 1, 2, 3, 4, 4a, 7-hexahydro-1, 6-dimethy-4-(1-methylethyl); 27, Isoledene; 28, unknown; 29, Acoradien; 30, Chamigrene; 31, Zingberene; 32, Sesquiphellandrene.

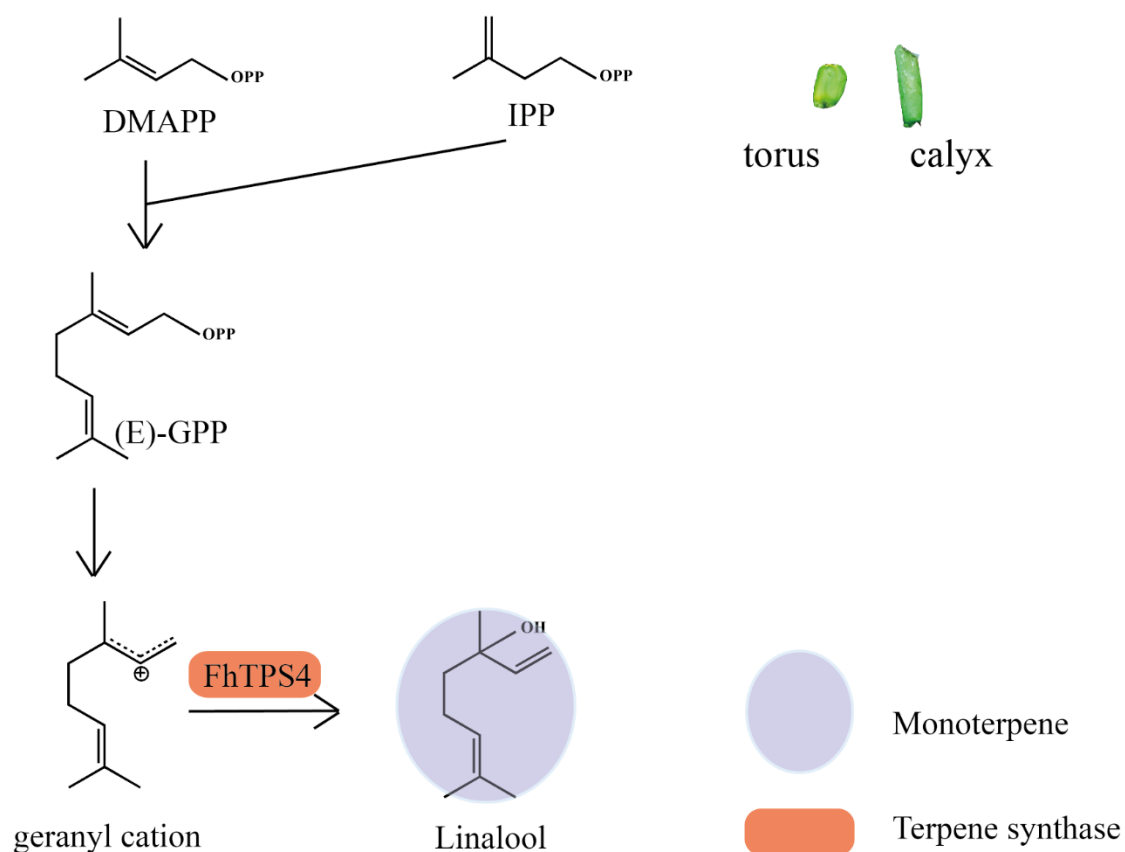


Fig. S18. Proposed model of terpene biosynthesis in torus and calyx of both *Freesia* cultivars.

FhTPS4 was specifically associated with linalool biosynthesis in these two flower tissues, and nerolidol, which was found in the *in vitro* enzymatic products catalyzed by FhTPS4, was not detected.

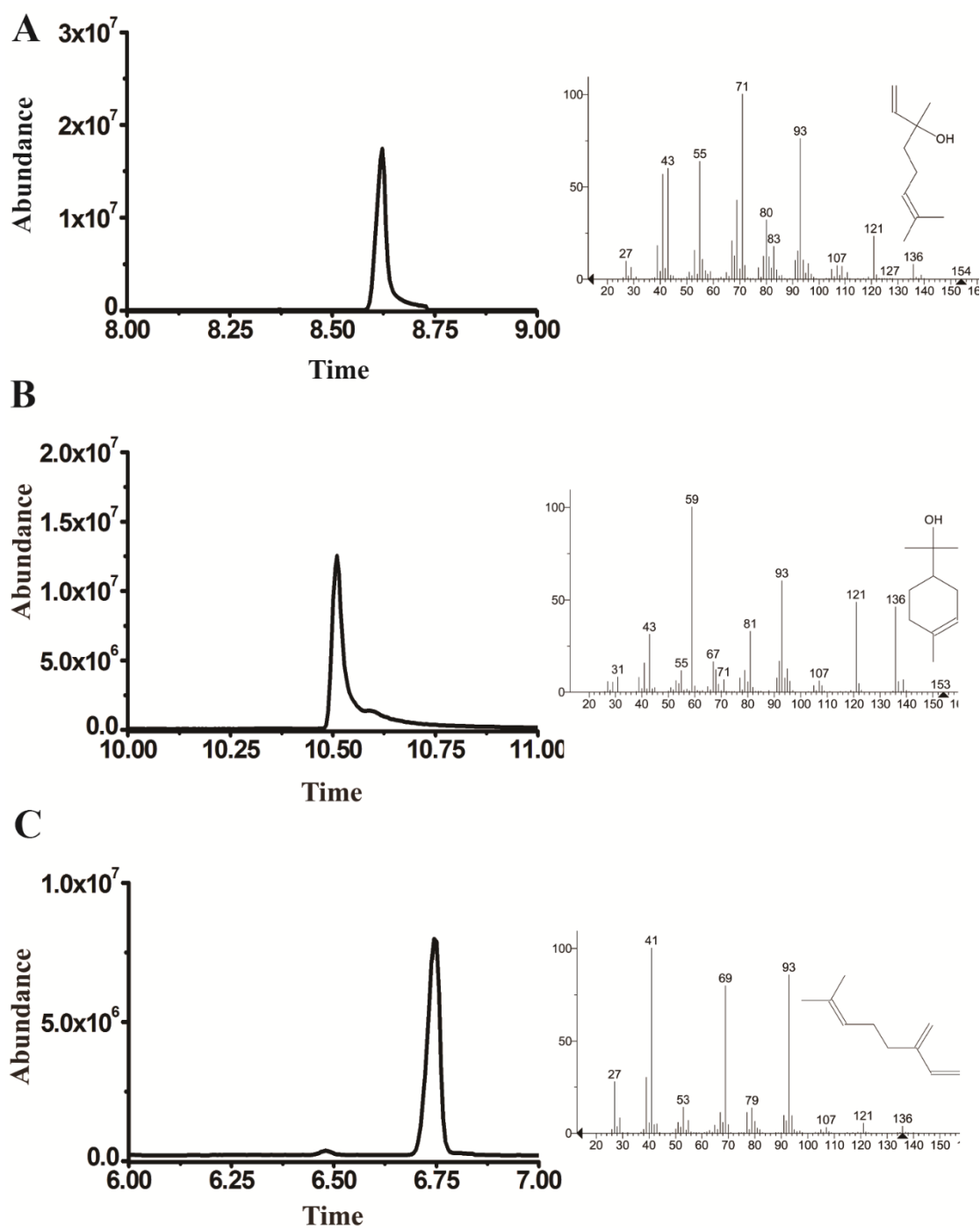


Fig. S19. *In vitro* assay of authentic standards by GC-MS

(A) Linalool. (B) α -Terpilenol. C. Myrcene. Mass spectra for each authentic standard are shown on the right.