

Supplementary Materials:

Table S1. Assembly statistics results of *Thymus* species.

Tools	Kmer Size	Species	Number of contigs	Total size (Mp)	Minimum length (bp)	Maximum length (bp)	Average length (bp)	L50 (bp)
Trinity	25	<i>T. daenensis</i>	91127	55.83	224	12126	614.35	819
		<i>T. vulgaris</i>	117835	68.35	224	14141	580.1	732
		<i>T. lancifolius</i>	123698	73.24	224	25032	592.09	767
		<i>T. persicus</i>	85084	58.47	224	13000	687.26	999
		<i>T. pubescens</i>	96976	59.99	224	15305	618.64	818
		<i>T. daenensis</i>	70626	56.14	201	14373	794.96	1350
Bridger	25	<i>T. vulgaris</i>	82450	68.78	201	17174	834.24	1414
		<i>T. lancifolius</i>	89526	72.02	201	26796	804.52	1416
		<i>T. persicus</i>	67166	59.58	201	21482	887.15	1500
		<i>T. pubescens</i>	67288	56.31	201	11592	836.99	1377
		<i>T. daenensis</i>	70235	54.72	201	13733	779.13	1316
		<i>T. vulgaris</i>	81814	67	201	15398	819.01	1396
BinPacker	27	<i>T. lancifolius</i>	89516	70.67	201	20523	789.51	1393
		<i>T. persicus</i>	66716	58.04	201	20248	869.98	1474
		<i>T. pubescens</i>	66565	54.54	201	14125	819.48	1345
		<i>T. daenensis</i>	66591	68.45	200	14373	1028.03	1553
		<i>T. vulgaris</i>	88953	94.52	200	17174	1062.68	1603
		<i>T. lancifolius</i>	94939	98.79	200	25544	1040.63	1634
BinPacker	25	<i>T. persicus</i>	67179	75.24	200	21482	1120.01	1665
		<i>T. pubescens</i>	67809	73.72	200	11592	1087.3	1574

Table S2. Velvet/Oases assembly statistics results of *Thymus* species.

Species/K-mers	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57	59	61	
<i>T. daenensis</i>	Number of contigs	64407	63611	61900	60130	56679	54699	53888	53499	52686	52284	51817	51422	51103	50413	50156	50024	49953	49739	49921	49370	48985
	Total size (Mp)	56.31	56.61	55.16	54.64	51.09	48.69	47.78	47.29	45.93	45.6	44.67	43.83	43.06	42.43	41.6	41.42	41.24	41.13	40.4	39.48	38.43
	Average length (bp)	874.36	890.06	891.18	908.85	901.47	890.32	886.68	884	871.8	872.3	862.11	852.39	842.64	841.81	829.46	828.05	825.73	827.11	809.42	799.68	784.71
	N50 length (bp)	1327	1343	1358	1384	1383	1375	1369	1364	1345	1341	1326	1310	1296	1305	1284	1285	1284	1295	1268	1257	1230
	Number of contigs	88534	86637	84537	80270	74483	71518	70248	69325	68744	68430	67662	66852	66980	66396	66031	65765	65972	65585	65698	64711	64646
<i>T. vulgaris</i>	Total size (Mp)	73.57	73.65	71.69	70.53	66.04	62.09	61.12	60.42	59.59	59.48	58.1	57.85	57.59	56.74	55.77	55.65	55.8	55.49	55.35	54.08	52.68
	Average length (bp)	831.04	852.79	848.05	878.72	886.71	868.26	870.08	871.59	866.91	869.31	858.76	858.76	859.87	854.59	844.68	846.2	845.88	846.13	842.61	835.75	814.93
	N50 length (bp)	1259	1289	1286	1338	1364	1333	1345	1346	1347	1348	1334	1334	1339	1331	1323	1332	1342	1362	1353	1352	1323
	Number of contigs	99808	98848	95024	91049	83022	80859	79882	78650	78013	77393	76437	76296	75789	74930	74324	74274	74090	74063	74088	74037	73396

<i>T. persicus</i>	Total size (Mp)	84.08	85.41	84.11	81.83	74.21	71.58	70.67	69.39	69.04	68.43	67.61	67.22	67	65.82	64.83	64.51	64.6	64.47	63.88	62.72	61.4	
	average length (bp)	842.5	864.1	885.17	898.8	893.91	885.26	884.73	882.35	885.08	884.21	884.54	881.14	884.08	878.49	872.38	868.61	871.99	870.59	862.3	850.64	836.63	
	N50 length (bp)	1303	1334	1369	1405	1420	1416	1416	1410	1415	1420	1420	1413	1427	1412	1409	1411	1421	1421	1429	1425	1414	1394
	Number of contigs	69437	64453	65231	61548	57545	57149	56241	55495	55238	55154	55206	54830	54804	54662	54427	54512	54655	54986	54966	54471	54769	
	Total size (Mp)	61.25	62.21	64.16	60.5	56.49	56.36	55.43	54.48	53.42	53.13	52.55	52.38	51.86	51.02	50.7	50.58	50.75	50.5	50.19	49.09	48.11	
	average length (bp)	882.15	965.3	983.72	983.03	981.78	986.33	985.72	981.86	967.25	963.46	952.04	955.43	946.38	933.45	931.57	928.05	928.69	919.98	913.14	901.23	878.49	
	N50 length (bp)	1357	1357	1498	1504	1502	1508	1502	1503	1479	1478	1457	1476	1460	1447	1443	1440	1449	1444	1434	1426	1401	1375
	Number of contigs	61109	58280	55685	54687	51719	50534	49772	49324	48909	48511	48245	48042	48199	47688	47783	47850	48078	48105	48491	48306	47938	
	Total size (Mp)	53.96	52.5	50.44	49.72	47.61	45.99	45.06	44.35	43.93	43.17	42.81	42.21	41.61	41.19	40.78	40.8	40.89	40.63	40.16	39.71	38.71	
	average length (bp)	883.03	900.82	905.86	909.58	920.61	910.12	905.52	899.28	898.3	890.02	887.44	878.68	863.43	863.82	853.59	852.75	850.69	844.61	828.27	822.05	807	
N50	130	133	135	135	138	137	136	135	135	134	134	133	130	130	129	129	130	129	127	126	125		

Table S3. The number of full length transcripts for three assemblers and five species.

Percent length coverage		100	90	80	70	60	50
<i>T. daenensis</i>	Trinity	3082	1114	883	866	905	1009
	Bridger	3374	1146	825	769	794	851
	BinPacker	3405	1156	842	777	791	826
<i>T. vulgaris</i>	Trinity	3234	1217	1034	976	1005	1114
	Bridger	3730	1258	901	812	841	840
	BinPacker	3806	1276	926	825	847	852
<i>T. lancifolius</i>	Trinity	3269	1212	1044	963	993	1096
	Bridger	3662	1239	910	819	866	861
	BinPacker	3722	1264	934	839	881	872
<i>T. persicus</i>	Trinity	3549	1211	928	817	837	873
	Bridger	3778	1204	872	729	728	712
	BinPacker	3815	1212	905	731	738	694
<i>T. pubescens</i>	Trinity	3445	1257	968	852	943	962
	Bridger	3544	1199	864	720	779	802
	BinPacker	3603	1198	877	752	791	807

Table S4. The contigs number of eleven genes involved in thymol and other monoterpene biosynthesis identified in *Thymus* transcriptomes.

TF families	<i>T. daenensis</i>	<i>T. vulgaris</i>	<i>T. lancifolius</i>	<i>T. persicus</i>	<i>T. pubescens</i>
bHLH	4630	6189	6533	4479	4631
NAC	2241	3234	3015	2316	2458
MYB_related	2232	3198	3099	2587	2649
ARF	2092	2018	2416	1796	1661
WRKY	2090	3011	2582	2246	2666
ERF	1672	2367	2313	1812	2108
C2H2	1583	2269	2449	1732	1768
C3H	1351	1855	1708	1458	1516
FAR1	1264	1752	1644	1315	1346
B3	1257	1773	1769	1494	1372
HD-ZIP	400	520	499	410	412
G2-like	78	86	103	99	76
GRAS	78	86	103	99	76
GATA	70	77	92	88	68
TALE	56	62	74	70	54
LBD	48	53	63	59	46
TCP	44	48	58	54	42
SBP	36	40	48	44	34
AP2	35	39	46	42	33
CO-like	31	34	41	37	29
HB-other	30	33	40	36	28
NF-YB	30	33	40	36	28
MIKC_MADS	26	29	34	30	24
Nin-like	26	29	34	30	24
YABBY	26	29	34	30	24

Dof	24	26	32	28	22
HSF	24	26	32	28	22
NF-YC	23	25	30	26	21
CPP	22	24	29	25	20
E2F/DP	20	22	26	22	18
SRS	20	22	26	22	18
WOX	20	22	26	22	18
ARR-B	19	21	25	21	17
GRF	19	21	25	21	17
BES1	17	19	22	18	15
BBR-BPC	16	18	21	17	14
CAMTA	15	17	20	16	13
NF-YA	14	15	18	14	12
GeBP	11	12	15	11	9
LSD	11	12	15	11	9
STAT	11	12	15	11	9
DBB	9	10	12	8	7
M-type_MADS	8	9	11	7	6
NF-X1	6	7	8	4	4
ZF-HD	5	6	7	3	3
RAV	4	4	5	1	2
S1Fa-like	4	4	5	1	2
VOZ	4	4	5	1	2
Whirly	4	4	5	1	2
EIL	3	3	4	0	1
HRT-like	3	3	4	0	1
HB-PHD	1	1	1	-	-
SAP	1	1	1	-	-

Table S5. The contigs number of eleven genes involved in thymol and other monoterpene biosynthesis identified in *Thymus* transcriptomes

Genes name	<i>T. daenensis</i>	<i>T. vulgaris</i>	<i>T. lancifolius</i>	<i>T. persicus</i>	<i>T. pubescence</i>
DXR	9	4	8	6	8
DXS	12	14	16	9	15
MCT	2	1	4	1	1
CMK	3	2	1	4	2
MDS	1	2	1	2	4
HDS	9	9	12	15	18
HDR	20	22	9	17	20
IDI	3	3	5	10	6
GGPS	6	7	4	9	9
GDS	1	2	2	3	2
TPS	15	25	9	21	9
Total	81	91	80	97	94

Table S6. Lengths of genes involved in thymol and other monoterpene biosynthesis identified in *Thymus* transcriptomes

Gene s name	<i>T.</i> <i>daenensi</i> s	<i>T.</i> <i>vulgari</i> s	<i>T.</i> <i>lancifoliu</i> s	<i>T.</i> <i>persicu</i> s	<i>T.</i> <i>pubescenc</i> e	<i>Salvia</i> <i>multiorrhiz</i> a	<i>T.</i> <i>vulgari</i> s	<i>T.</i> <i>caespititiu</i> s
DXR	1422	1422	1422	1422	1422	1425	-	-
DXS	2122	2122	2122	2122	2122	2145	-	-
MCT	915	915	910	915	915	915	-	-
CMK	1188	1188	1188	1188	1188	1191	-	-
MDS	705	705	705	705	705	705	-	-
HDS	2229	2229	2229	2229	2229	2229	-	-
HDR	1389	1389	1389	1389	1389	1392	-	-
IDI	862	906	731	862	862	918	-	-
GDS	1275	1275	1167	816	1275	1275	-	-
GGP S	1085	1085	1085	1085	1085	1095	-	-
TPS2	1791	1615	1231	1733	1197	-	1791	
TPS3	1154	1788	1788	1788	1618	-	1788	
TPS4	1654	1665	1639	1060	1665	-	-	1665
TPS5	1747	1747	1747	1747	1747	-	1747	-

Table S7. The unigenes subjected to positive selection

<i>T. daenensis</i>	<i>T. lancifolius</i>	<i>T. persicus</i>	<i>T. pubescens</i>
Histidine kinase 5	ABC transporter G family member 6	ABC transporter I family member 1	Probable anion transporter 4
AT-hook motif nuclear-localized protein 8	AT-hook motif nuclear-localized protein S10	E3 ubiquitin-protein ligase ATL31	L-ascorbate peroxidase
Probable dolichyl pyrophosphate Glc1Man9GlcNAc2 alpha-1,3-glucosyltransferase	Probable anion transporter 4	Berberine bridge enzyme-like 21	Actin-related protein 3
Probable RNA-binding protein ARP1	Anaphase-promoting complex subunit 1	Transcription factor bHLH106	RING-H2 finger protein ATL38
Histone-lysine N-methyltransferase ATXR6	Transcription factor bHLH110	Chaperone protein ClpB	E3 ubiquitin-protein ligase BRE1-like 2
Probable BOI-related E3 ubiquitin-protein ligase 3	Transcription factor BPE, (bHLH 31)	Cyclic nucleotide-gated ion channel 2	Cytochrome P450 71A8
Chaperone protein ClpB1	Serine/threonine-protein kinase BRI1-like 2	Furcadin hydrolase	Receptor protein-tyrosine kinase CEPR1
CLP protease regulatory subunit CLPX2	Histone-lysine N-methyltransferase CLF	LanC-like protein GCL2	Cyclic nucleotide-gated ion channel 2
Protein CLT1, chloroplastic	CLP protease regulatory subunit CLPX2	Lysophospholipid acyltransferase LPEAT2	Protein CONTINUOUS VASCULAR RING 1
Glucan endo-1,3-beta-glucosidase 5	Protein CLT1, chloroplastic	Probable mediator of RNA polymerase II transcription subunit 26b	Glucan endo-1,3-beta-glucosidase 6
F-box protein	Ubiquinone biosynthesis O-methyltransferase	Pentatricopeptide repeat-containing protein	F-box protein

Gibberellin 20 oxidase 2	Putative protease Do-like 14	Serine/threonine-protein phosphatase 7	Heat shock 70 kDa protein 8
ARF guanine-nucleotide exchange factor GNOM	Zinc finger BED domain-containing protein DAYSLEEPER	Protein NRT1/ PTR FAMILY 7.3	Probable serine/threonine protein kinase IRE
Copper-transporting ATPase HMA4	Elongator complex protein 1	Ribonuclease 3-like protein 2	Ent-kaurenoic acid oxidase 2
Kinesin-like protein KIN-12B	Embryogenesis-associated protein EMB8	NAD-dependent protein deacetylase SRT1	3-ketoacyl-CoA synthase 2
Transcription factor KUA1 (Myb-related protein H)	1-phosphatidylinositol-3-phosphate 5-kinase FAB1B	F-box protein SKIP23	Protein LAZ1 homolog 2
Transcription factor LHW (BHLH transcription factor delta)	F-box protein	Tubulin-folding cofactor D	L-type lectin-domain containing receptor kinase VII.1
Midasin (AtMDN1)	L-gulonolactone oxidase 3	Mitochondrial import inner membrane translocase subunit TIM8	RNA cytidine acetyltransferase 1
NADH dehydrogenase 1 alpha subcomplex subunit 13-B	Nuclear pore complex protein GP210	Probable LRR receptor-like serine/threonine-protein kinase	NADH-ubiquinone oxidoreductase chain 6
Protein phosphatase 2C 29	Type II inositol polyphosphate 5-phosphatase 15	Probable inactive receptor kinase	O-fucosyltransferase 2
GPI mannosyltransferase 1	Probable LRR receptor-like serine/threonine-protein kinase IRK	Protein YLS3 (Protein YELLOW-LEAF-SPECIFIC GENE 3)	OTU domain-containing protein
Pentatricopeptide repeat-containing protein	G-type lectin S-receptor-like serine/threonine-protein kinase LECRK1		Pectinesterase inhibitor 10
Respiratory burst oxidase homolog protein C	Probable 1-acyl-sn-glycerol-3-phosphate acyltransferase 4		Putative pentatricopeptide repeat-containing protein
G-type lectin S-receptor-like serine/threonine-protein kinase RLK1	MADS-box transcription factor 6		Pentatricopeptide repeat-containing protein
Receptor-like protein kinase 5	Mediator of RNA polymerase II transcription subunit 16		Pentatricopeptide repeat-containing protein
Disease resistance RPP8-like protein 3	Plastid division protein PDV1		Glucosidase 2 subunit beta (Glucosidase II subunit beta)
Subtilisin-like protease SBT5.4	Pentatricopeptide repeat-containing protein		Putative late blight resistance protein homolog R1A-6
Ethylene-responsive transcription factor SHINE 2	PRA1 family protein B4 (AtPRA1.B4)		DNA repair protein recA homolog 3
Single-stranded DNA-binding protein	Receptor-like cytosolic serine/threonine-protein kinase RBK2		Remorin 1.4
Thioredoxin-like 3-3 (Thioredoxin-like 1)	SCARECROW-LIKE protein 7		Probable disease resistance protein RXW24L

Beta-D-glucosyl crocetin beta-1,6-glucosyltransferase	Potassium channel SKOR (Stelar K(+) outward rectifying channel)	Tryptophan--tRNA ligase
Extra-large guanine nucleotide-binding protein 1 WEB family protein	SEC1 family transport protein SLY1 Protein trichome birefringence-like 16	Thylakoid luminal protein TL20.3, Ubiquitin-like domain-containing CTD phosphatase
Probable receptor-like serine/threonine-protein kinase	Ubiquitin carboxyl-terminal hydrolase 3 Vesicle-associated membrane protein 711 Vacuolar-processing enzyme (VPE) Probable inactive receptor kinase	Leucine-rich repeat receptor-like serine/threonine-protein kinase PHD finger protein

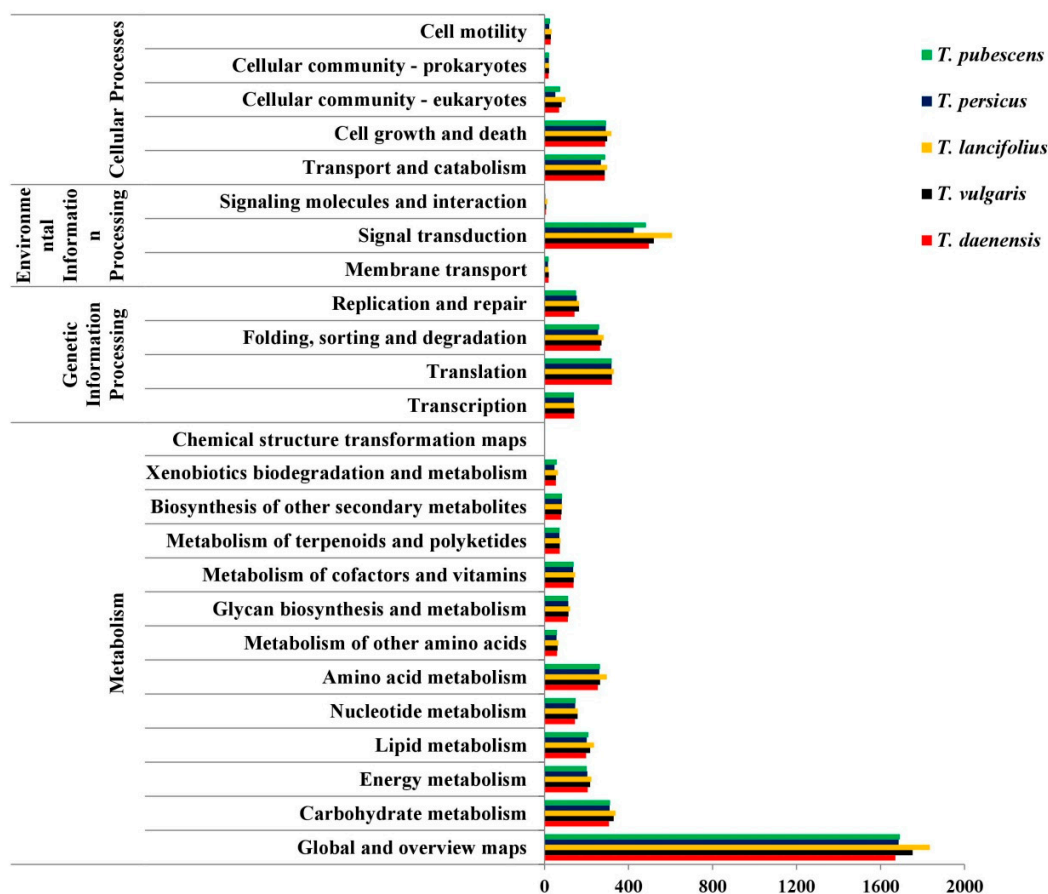


Figure S1. KEGG classification of unigenes for *Thymus* transcriptomes.

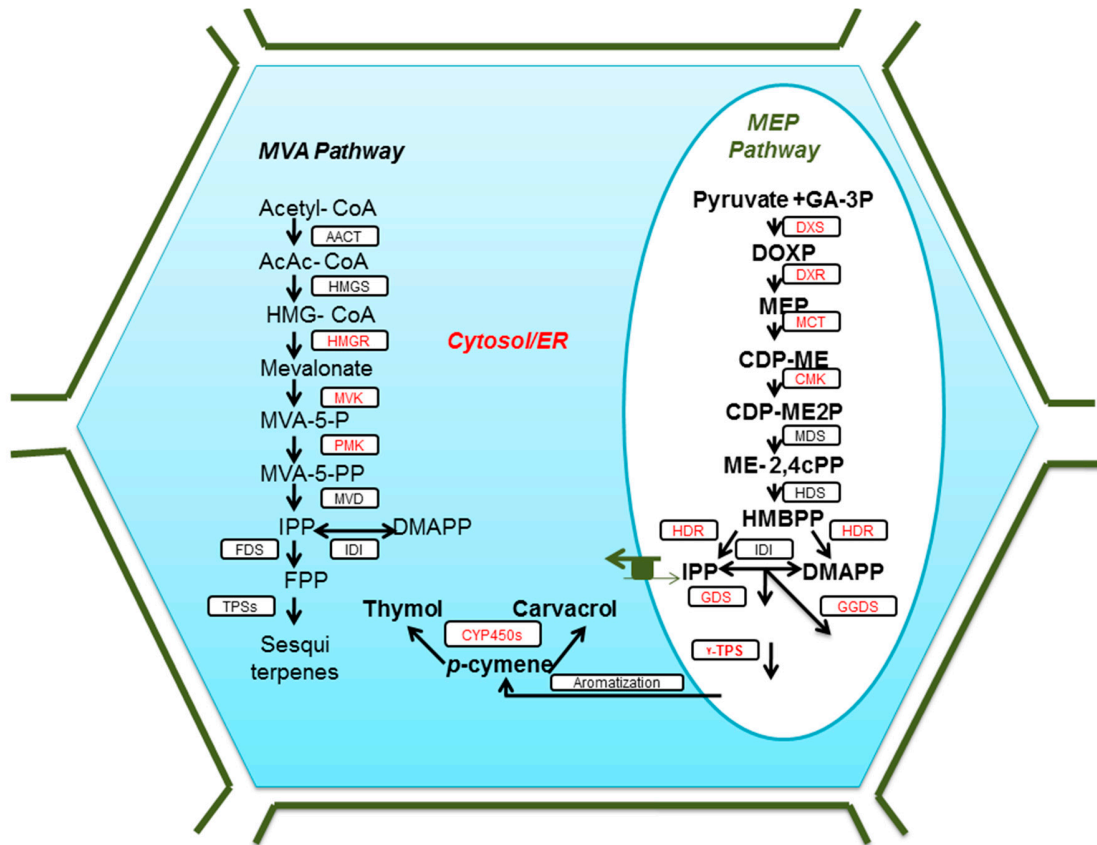


Figure S2. Schematic representation of thymol and carvacrol biosynthetic pathway.

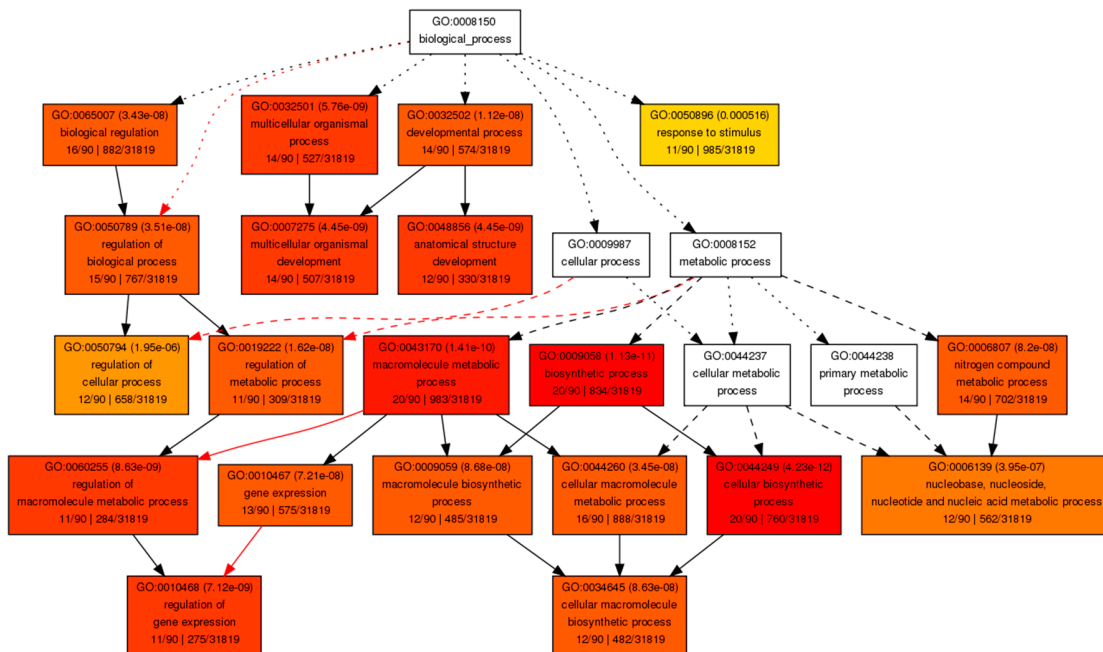


Figure S3. GO enrichment of PSGs related to ecological adaptation in *Thymus* species.

Tree scale: 0.1

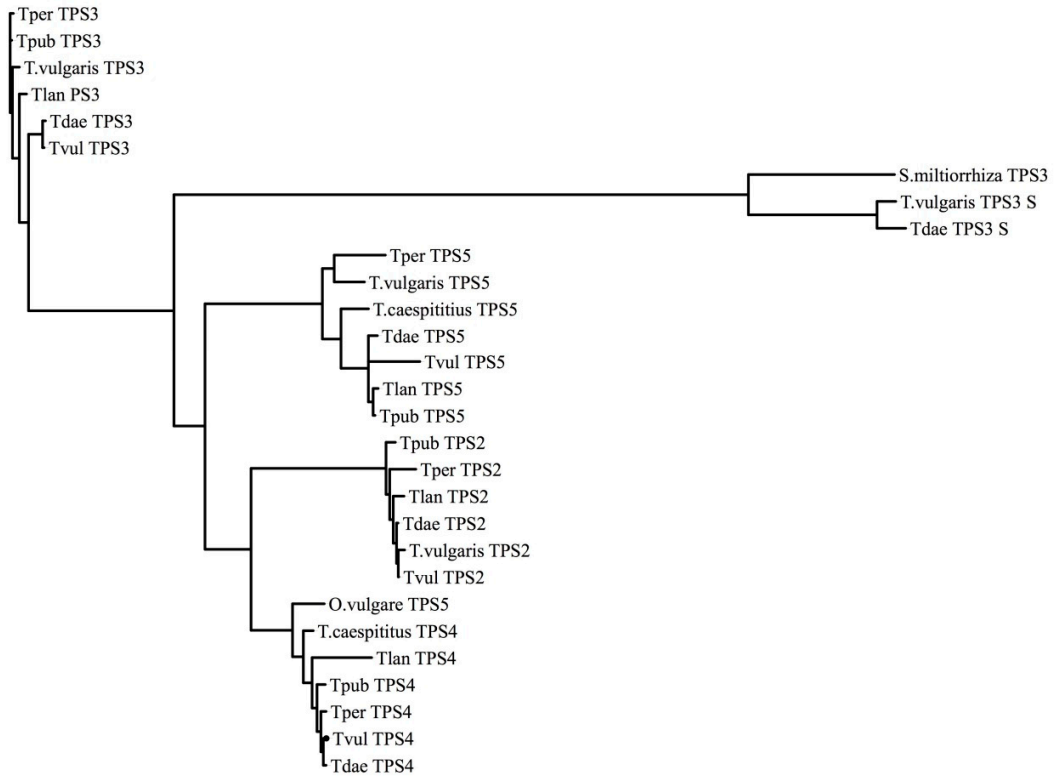


Figure S4. Maximum likelihood phylogeny of putative TPS genes in *Thymus* species.