

Article

# Comparative analysis and identification of terpene synthase genes in *Convallaria keiskei* leaf, flower and root using RNA-sequencing profiling

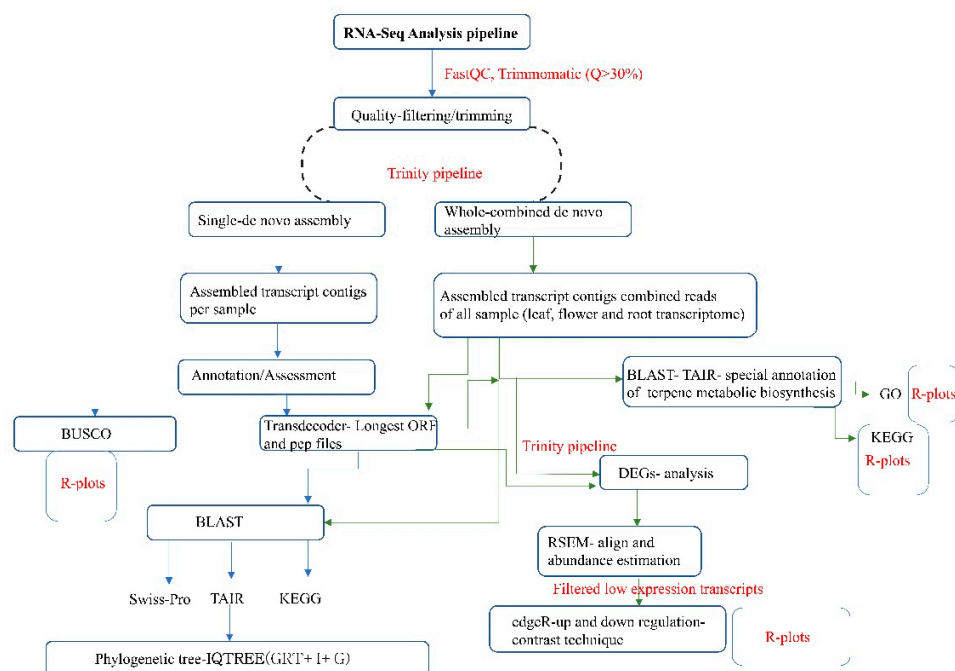
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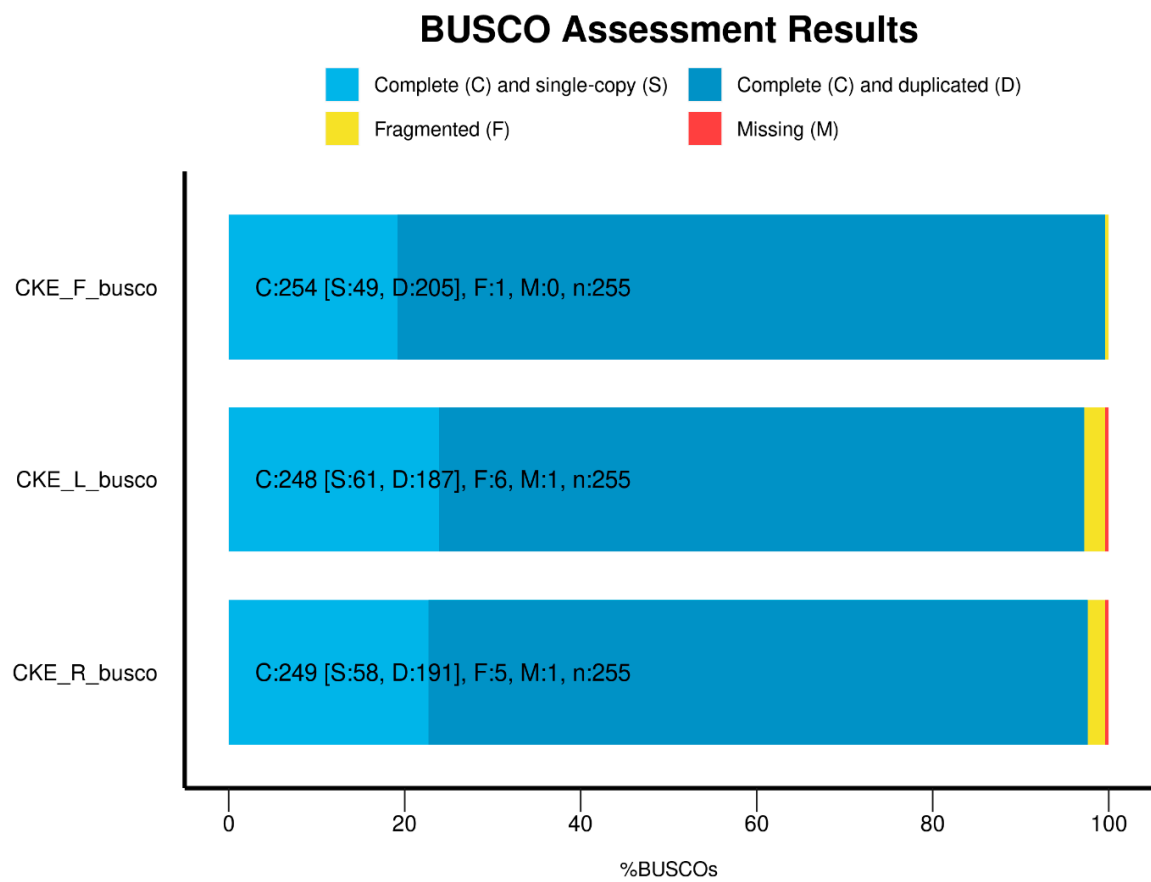
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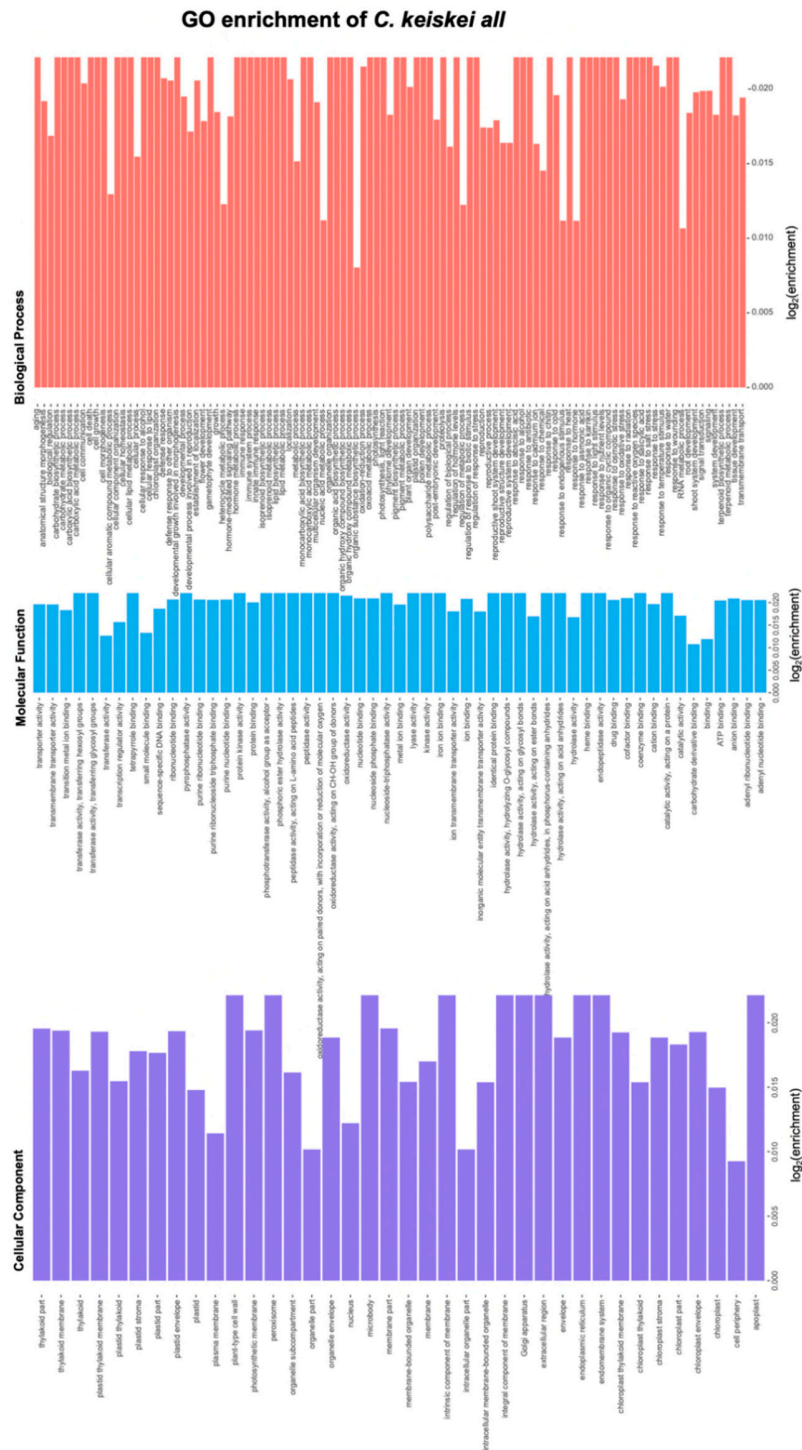
† Both authors contributed equally to this manuscript



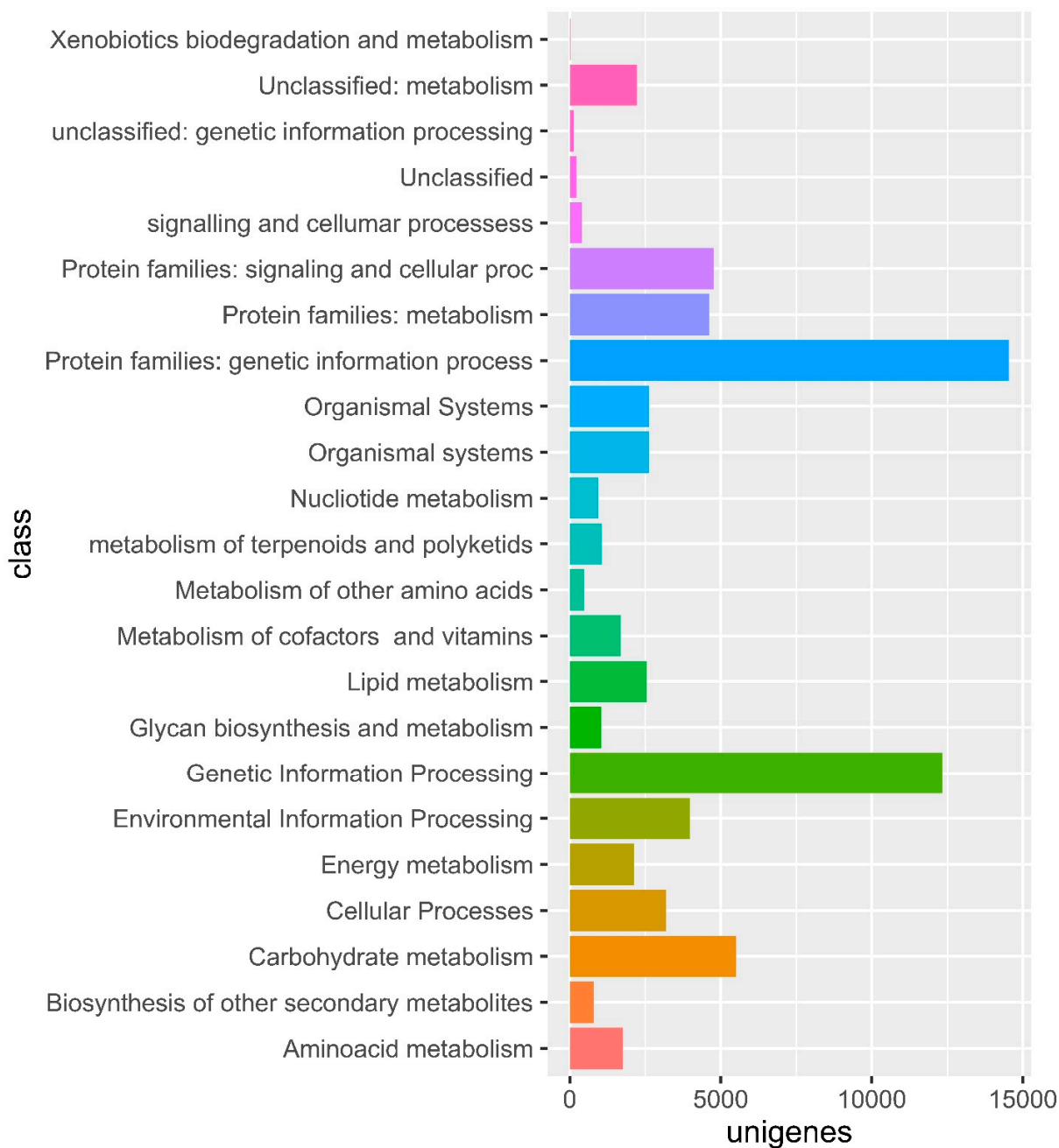
**Supplementary Fig S1:** Schematic diagram for a transcriptome assembly method



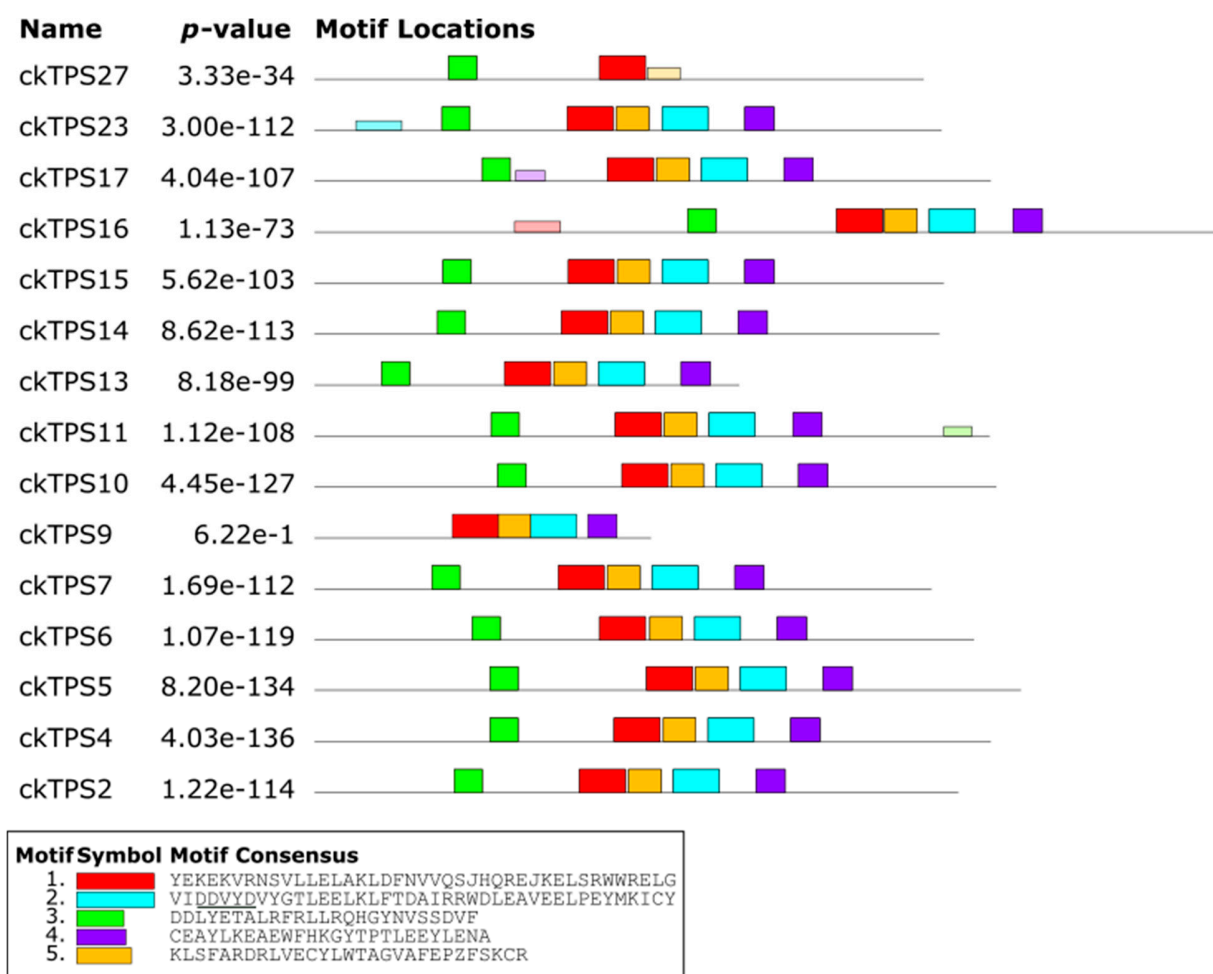
Supplementary Fig S2. BUSCO assessments of *Convallaria keiskei* transcriptome assembly



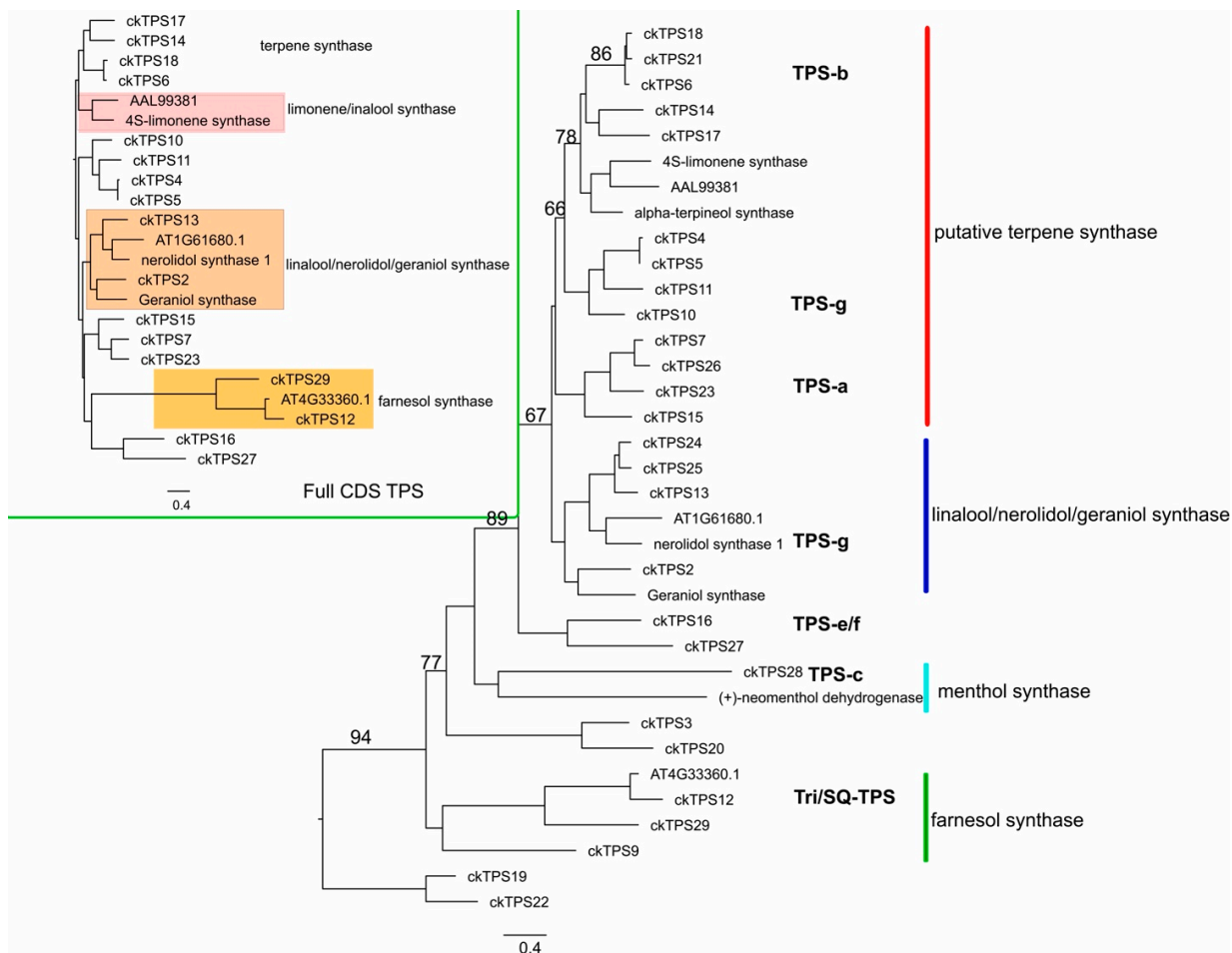
**Supplementary Fig S3.** Gene Ontology (GO) categories of combined transcriptome unigenes from the *C. keiskei* (TRAPID-enrichment analysis). (A) the distribution of biological process unigenes, (B) molecular function (C) cellular component in the *C. keiskei*



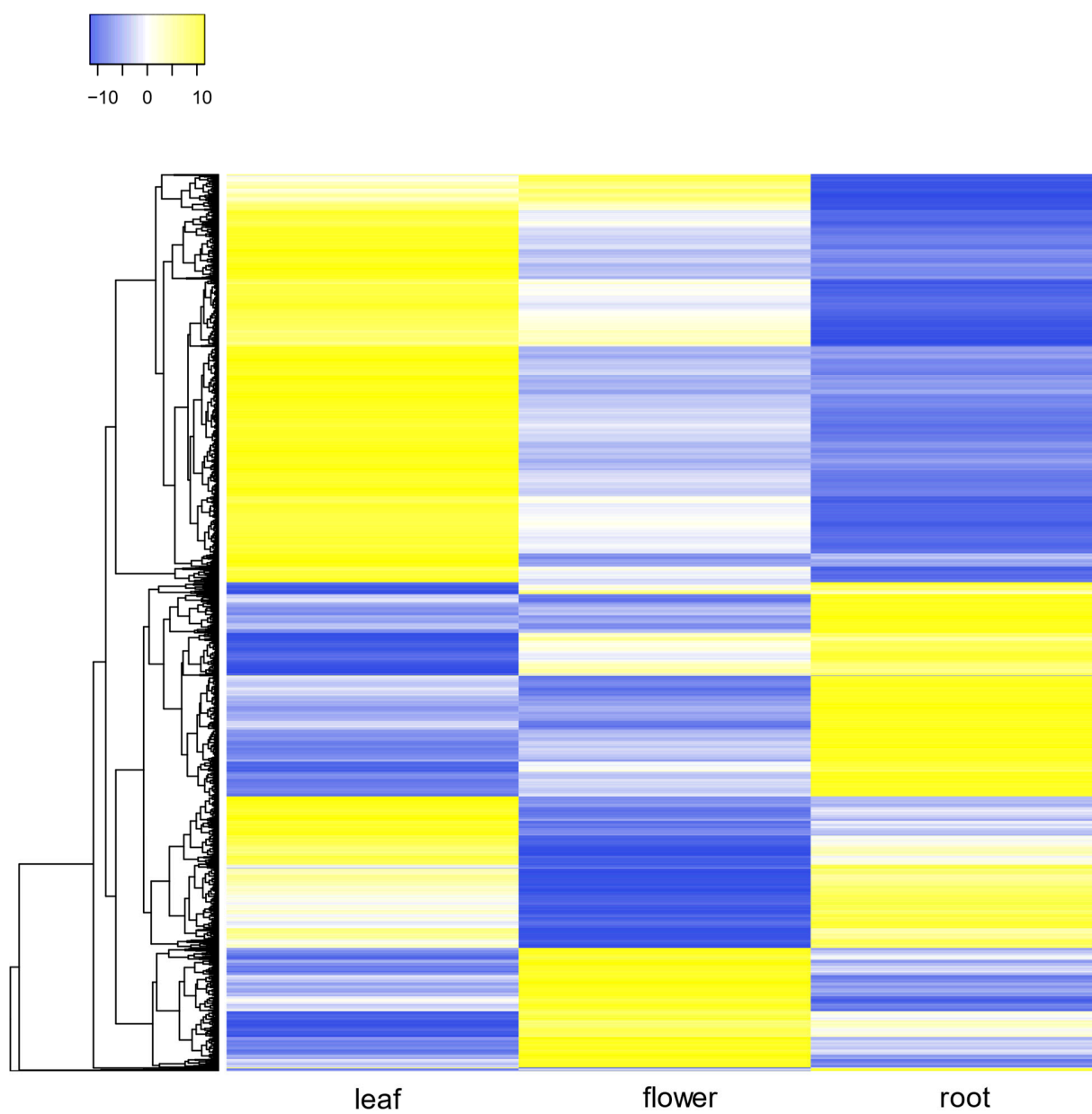
**Supplementary Fig S4.** The distribution of various classes of metabolic unigenes in the *C. keiskei* by KEGG analysis



**Supplementary Fig S5.** Phylogenetic tree analysis and identification of linalool, nerolidol, and farnesol gene in *C. keiskei*. The green color rectangular box indicates the phylogenetic tree of all functional terpene synthase genes in the *C. keiskei*



**Supplementary Fig S6.** MEME finding of the conserved domain (RRXXXD and DXXXD) of TPS in *Convallaria keiskei* transcripts

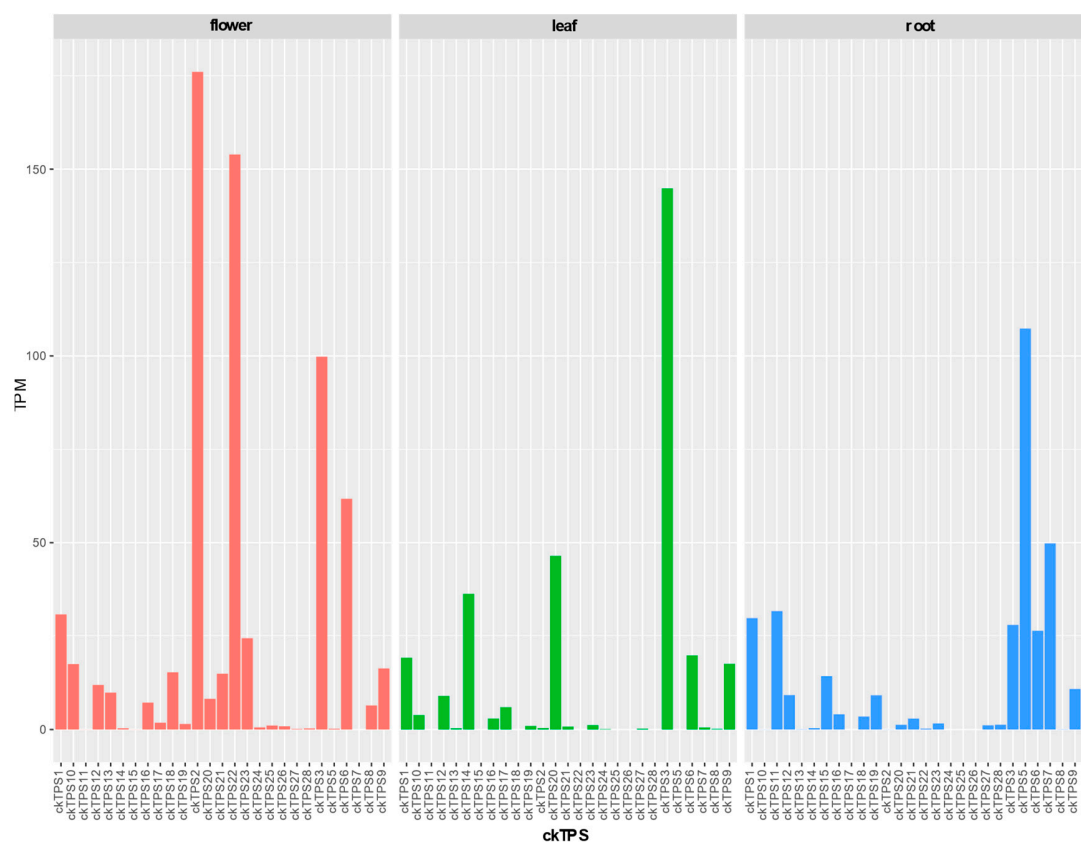


**Supplementary Fig S7.** Heatmap of differentially expressed 3,152 genes in *C. keiskei* from leaf, flower, and root tissue





**Supplementary Fig S8.** The top four ckTPS genes and their conserved domain of RRX8W and DDXXD domains are aligned sequences



**Supplementary Fig S9.** Bar plots of TPM value of RSEM assessment to the ckTPS genes align, and mapping of *Convallaria keiskei* reads quantification analysis.

**Supplementary Table S2:** List of highly expressed genes in the flower, leaf and root of *C. keiskei* and their role in the plants

Transcript ID gene name	Enzyme name	Metabolite name	Highly expression	Function
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<i>ckTPS2</i>	Geraniol synthase	Monoterpene	Flower	Floral scent, defense mechanism, stress response, medicinal properties
<i>ckTPS23</i>	Germacrene (Ger D) synthase	sesquiterpene	Flower	Floral scent, defense mechanism, stress response, medicinal properties
<i>ckTPS13</i>	Nerolidol synthase	Nerolidol (sesquiterpene)	Flower	Floral scent, defense mechanism, stress response, medicinal properties
<i>ckTPS7</i>	Valencene synthase	Valencene (sesquiterpene)	Flower and Root	Floral scent, citrus aroma, insect repellent, stress response, medicinal properties
<i>ckTPS4, 5, 10</i>	Trans-ocimene	Monoterpene	Flower and Root	Floral scent, plant communications, insect attraction and repellence, stress response
<i>ckTPS16, 27, 28</i>	Kaurene synthase	Diterpenoid	Flower, Leaf and Root	Precursor for plant hormone, gibberellins (GAS); plant growth and developmental process
<i>ckTPS11</i>	Trans-ocimene	Monoterpene	Leaf and Root	Floral scent, plant communications, insect attraction and repellence, stress response
<i>ckTPS6, 14, 17, and 18</i>	linalool synthase	Monoterpene	Flower	Floral scent, defense mechanism, stress response, medicinal properties

**Supplementary Table S4.** Top upregulated in leaf, flower, and root of *C. keiskei* using DEGs analysis

unigenes	leaf	flower	root	description
	leaf			
TRINITY_DN865_c1_g2	6.405638032	-1.26017827	-5.14545977	plastocyanin 1
TRINITY_DN5007_c0_g1	6.062331346	-2.22416415	-3.8381672	Aldolase superfamily protein
TRINITY_DN1598_c0_g2	5.989057744	-1.61804756	-4.37101018	galactinol synthase 4
TRINITY_DN21_c0_g1	5.787968911	-1.11100312	-4.67696579	sedoheptulose-bisphosphatase
TRINITY_DN1598_c0_g1	5.758316198	-2.11187826	-3.64643794	galactinol synthase 4
TRINITY_DN286_c0_g1	5.589524419	-1.40296613	-4.18655829	phosphoribulokinase
TRINITY_DN6998_c0_g1	5.236912939	0.337748707	-5.57466165	glyceraldehyde-3-phosphate dehydrogenase B subunit
TRINITY_DN5666_c0_g1	5.217190977	-1.43364094	-3.78355004	carbonic anhydrase 1
TRINITY_DN576_c0_g2	5.123788974	-0.615654	-4.50813498	Chalcone-flavanone isomerase

TRINITY_DN4758_c0_g1	5.087092031	-1.23966453	-3.8474275	hydroxypyruvate reductase
<b>flower</b>				
TRINITY_DN6014_c0_g1	-5.56887964	5.709013887	-0.14013425	lipid transfer protein 3
TRINITY_DN849_c1_g1	-2.18822494	4.806813116	-2.61858817	terpene synthase (ckTPS2)
TRINITY_DN2242_c0_g1	-0.97237219	4.599409052	-3.62703686	lipid transfer protein 3
TRINITY_DN1432_c0_g1	-1.10304837	4.14912112	-3.04607275	GDSL-like Lipase/Acylhydrolase
TRINITY_DN62992_c0_g1	-1.96567148	4.127189522	-2.16151804	glycosylphosphatidylinositol-anchored lipid protein transfer 1
TRINITY_DN9736_c0_g1	-1.8117389	4.033430645	-2.22169174	xyloglucan hydrolase 24
TRINITY_DN23_c0_g6	-3.47605855	3.93988504	-0.46382649	geranylgeranyl reductase
TRINITY_DN2504_c0_g1	-0.77319421	3.812773352	-3.03957915	expansin A8
TRINITY_DN1646_c0_g1	-2.69359083	3.676653456	-0.98306263	lipid transfer protein 6
TRINITY_DN2326_c0_g1	-1.23223387	3.393354818	-2.16112095	auxin response factor 8
<b>root</b>				
TRINITY_DN16033_c0_g1	-4.80268602	-2.23076249	7.033448508	Curculin-like lectin protein
TRINITY_DN95_c1_g1	-4.52951627	-1.91574356	6.445259824	magnesium transporter 4
TRINITY_DN16762_c1_g3	-4.18808015	-1.99380172	6.181881871	Lipase/lipoxygenase2C
TRINITY_DN11363_c0_g1	-4.48233334	-0.1852887	4.667622039	O-methyltransferase protein
TRINITY_DN2596_c0_g1	-0.39342283	-3.73521135	4.128634174	Glycosyl hydrolases 32 protein
TRINITY_DN7764_c0_g1	-1.71046321	-2.34420809	4.054671301	allene oxide synthase
TRINITY_DN5605_c1_g1	-0.69811789	-3.2526453	3.95076319	metallothionein 2A
TRINITY_DN38071_c0_g2	-2.11958887	-1.72672195	3.846310825	methylesterase PCR A
TRINITY_DN4058_c0_g1	-1.4682735	-2.09961065	3.567884145	terpene synthase (ckTPS7)
TRINITY_DN4084_c0_g1	-1.54330777	-1.94182255	3.485130324	ferulic acid 5-hydroxylase 1

**Supplementary Table S5.** Top down-regulated genes of leaf, flower, and root of *C. keiskei* using DEGs analysis

unigenes	leaf	flower	root	description
<b>leaf</b>				
TRINITY_DN6014_c0_g1	-5.56887964	5.709013887	-0.14013425	lipid transfer protein 3
TRINITY_DN16033_c0_g1	-4.80268602	-2.23076249	7.033448508	Curculin-like protein
TRINITY_DN95_c1_g1	-4.52951627	-1.91574356	6.445259824	magnesium transporter 4
TRINITY_DN11363_c0_g1	-4.48233334	-0.1852887	4.667622039	O-methyltransferase family protein
TRINITY_DN16762_c1_g3	-4.18808015	-1.99380172	6.181881871	Lipase/lipoxygenase2C PLAT/LH2 protein
TRINITY_DN976_c0_g3	-3.80086743	0.80665996	2.99420747	lipoxygenase 1
TRINITY_DN2025_c0_g1	-3.57718121	3.357453233	0.219727975	Plantinvertase/pectin methylesterase inhibitor
TRINITY_DN23_c0_g6	-3.47605855	3.93988504	-0.46382649	geranylgeranyl reductase
<b>flower</b>				
TRINITY_DN10_c0_g1	3.303408162	-4.68488032	1.381472157	O-methyltransferase family protein
TRINITY_DN4250_c0_g1	0.822584011	-4.04768682	3.225102806	O-methyltransferase family protein
TRINITY_DN2596_c0_g1	-0.39342283	-3.73521135	4.128634174	Glycosyl hydrolases family 32 protein
TRINITY_DN395_c0_g1	3.577352098	-3.68923201	0.111879912	O-methyltransferase family protein
TRINITY_DN6674_c0_g1	1.532701824	-3.36812021	1.835418385	beta glucosidase 16
TRINITY_DN5605_c1_g1	-0.69811789	-3.2526453	3.95076319	metallothionein 2A
TRINITY_DN1598_c1_g1	0.792285086	-3.21725581	2.424970728	galactinol synthase 1
TRINITY_DN7468_c0_g1	-0.43588815	-2.89105522	3.326943376	glutathione S-transferase TAU 20
TRINITY_DN3105_c0_g1	1.846122569	-2.86769847	1.0215759	Glycosyl hydrolases family 32 protein
<b>root</b>				
TRINITY_DN6674_c0_g2	4.525353113	1.442954261	-5.96830737	beta glucosidase 17
TRINITY_DN6998_c0_g1	5.236912939	0.337748707	-5.57466165	glyceraldehyde-3-phosphate dehydrogenase B subunit
TRINITY_DN865_c1_g2	6.405638032	-1.26017827	-5.14545977	plastocyanin 1
TRINITY_DN3898_c1_g1	4.989146557	-0.12373722	-4.86540933	glyceraldehyde 3-P dehydrogenase A
TRINITY_DN21_c0_g1	5.787968911	-1.11100312	-4.67696579	sedoheptulose-bisphosphatase
TRINITY_DN6873_c0_g1	2.71974644	1.929143933	-4.64889037	Glutathione S-transferase protein
TRINITY_DN499_c0_g1	4.051126712	0.47087128	-4.52199799	Glucose-6-phosphate/phosphate translocator-like protein
TRINITY_DN576_c0_g2	5.123788974	-0.615654	-4.50813498	Chalcone-flavanone isomerase protein
TRINITY_DN1598_c0_g2	5.989058	-1.61805	-4.37101	galactinol synthase 4