

Supplementary Information: SREP-16-41203

Spatial transcriptome analysis provides insights of key gene(s) involved in steroidal saponin biosynthesis in medicinally important herb *Trillium govanianum*

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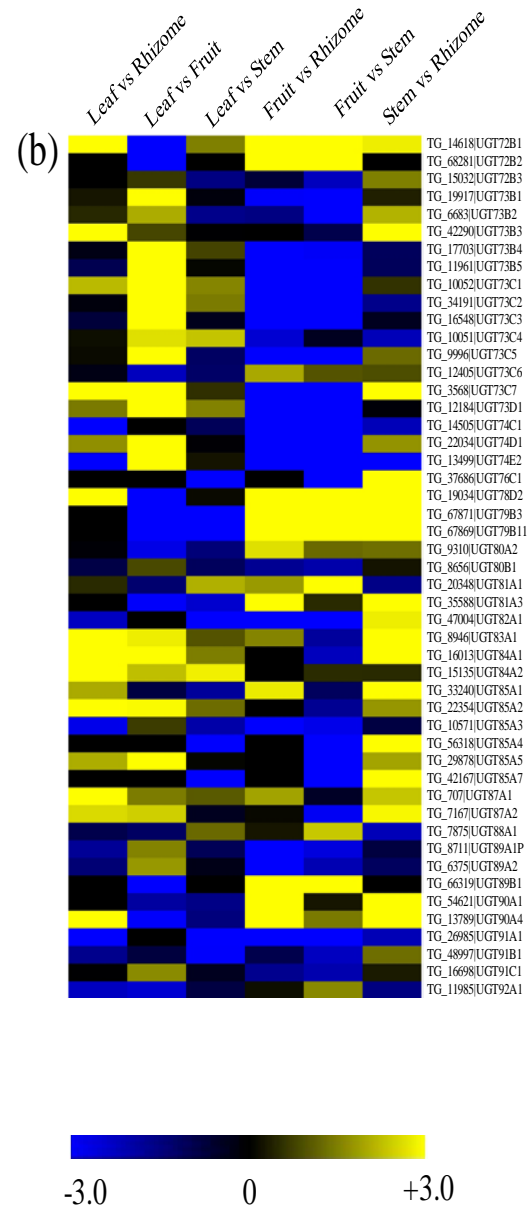
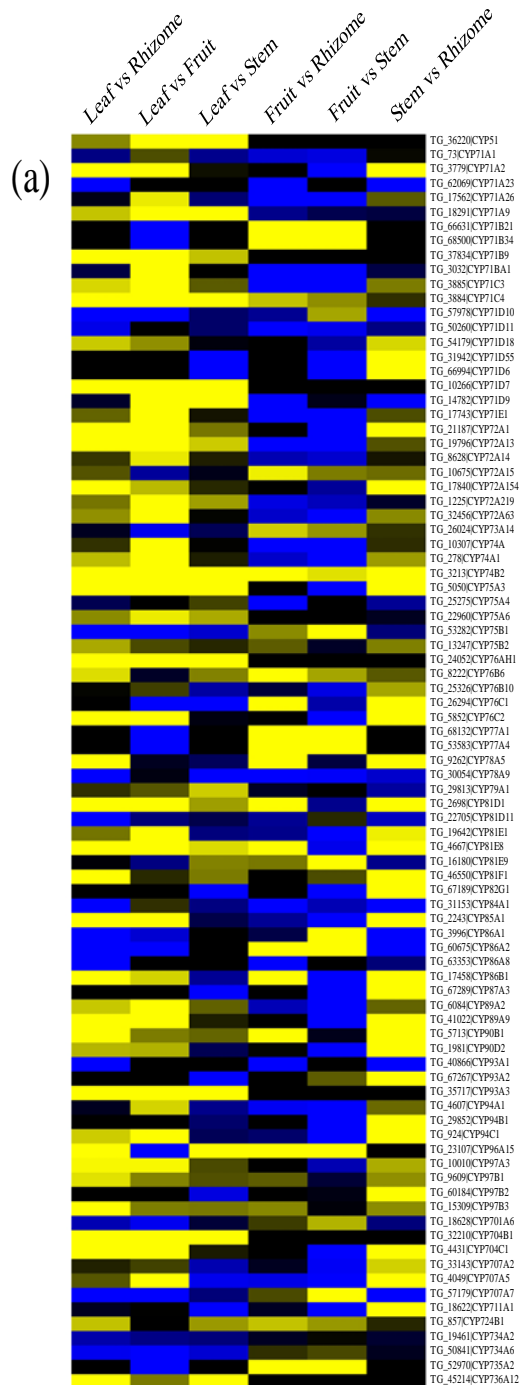


Figure S1

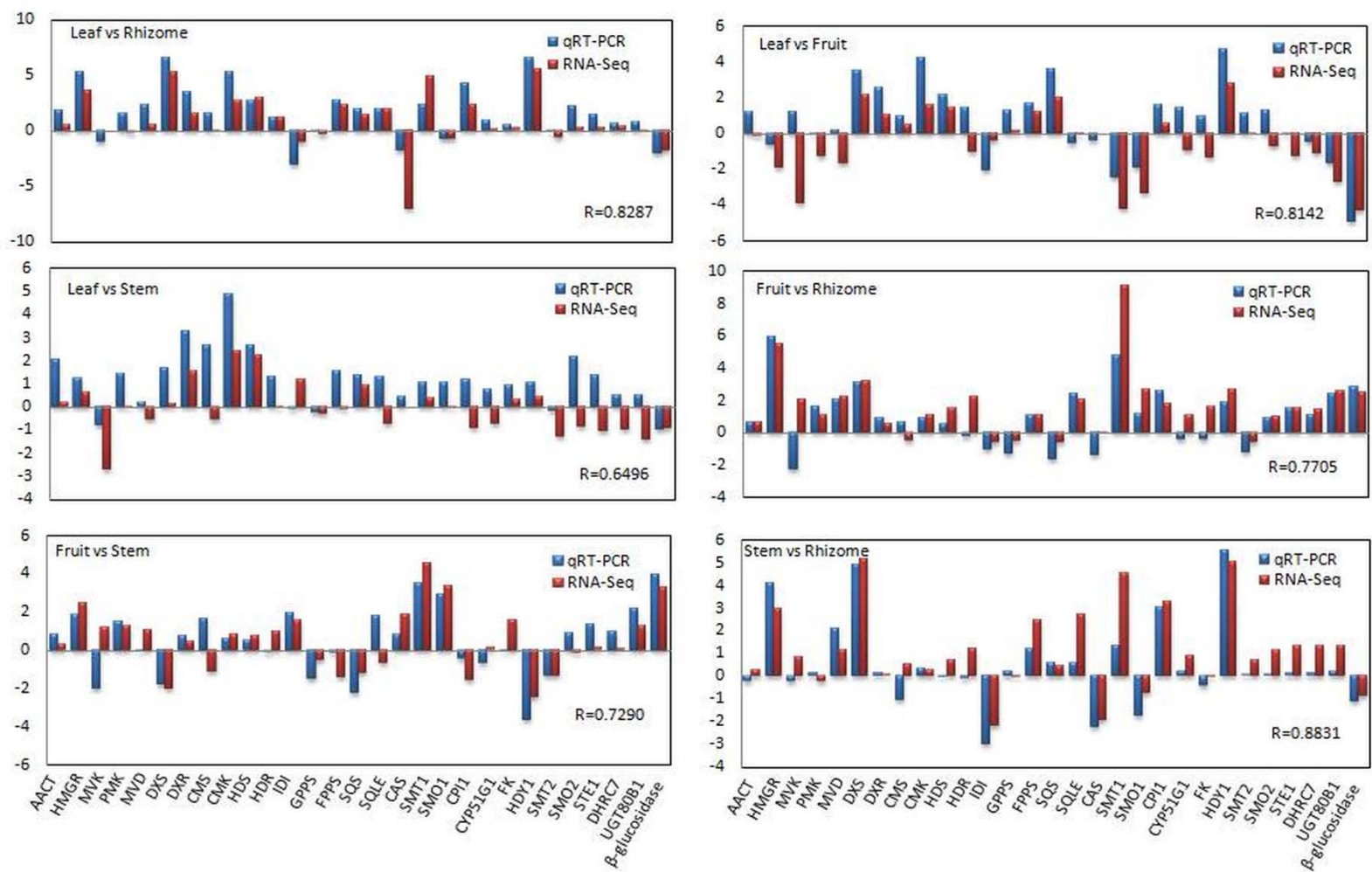


Figure S2

Supplementary Table S1: Summary of *de novo* assembly generated from *T. govianianum*.

Categories	Characteristics
Number of paired-end raw reads	173,974,146
N50 length	412
Minimum length (bp)	174
Maximum length (bp)	35,947
Average length (bp)	401
Total Contigs	69,174

Supplementary Table S3: Classification and number of transcripts related to transcription factors families.

S. No.	Family	Genes	Transcripts	S. No.	Family	Genes	Transcripts
1.	bHLH	250	1036	39.	CAMTA	24	46
2.	MYB_related	188	662	40.	NF-X1	9	45
3.	NAC	155	628	41.	CO-like	23	40
4.	FAR1	125	559	42.	CPP	16	40
5.	bZIP	147	516	43.	BBR-BPC	14	37
6.	B3	82	499	44.	SRS	8	37
7.	WRKY	128	484	45.	EIL	14	34
8.	C2H2	166	415	46.	ARR-B	25	33
9.	ERF	140	410	47.	DBB	15	33
10.	C3H	180	403	48.	ZF-HD	16	27
11.	MYB	128	386	49.	LSD	11	25
12.	G2-like	83	276	50.	VOZ	7	23
13.	GRAS	89	243	51.	HRT-like	6	19
14.	HD-ZIP	79	213	52.	GRF	14	18
15.	M-type	44	181	53.	RAV	7	16
16.	Trihelix	65	176	54.	Whirly	7	15
17.	GATA	52	173	55.	HB-PHD	3	7
18.	TCP	34	158	56.	LFY	1	1
19.	HB-other	45	156	57.	NZZ/SPL	1	1
20.	HSF	44	143	58.	SAP	1	1
21.	NF-YB	32	136				
22.	ARF	69	135				
23.	MIKC	34	134				
24.	TALE	41	107				
25.	LBD	37	106				
26.	E2F/DP	25	103				
27.	YABBY	13	97				
28.	SIFa-like	16	89				
29.	GeBP	17	88				
30.	SBP	35	87				
31.	Nin-like	22	78				
32.	Dof	34	72				
33.	STAT	5	65				
34.	NF-YA	18	63				
35.	BES1	23	62				
36.	NF-YC	26	62				
37.	WOX	13	60				
38.	AP2	22	48				

Supplementary Table S6. Pathways and number of transcripts related to secondary metabolite biosynthesis in *T. govanianum*

S. No.	Pathway ID	Secondary metabolites biosynthesis pathways	KO	Transcripts
1.	Ko00254	Aflatoxin biosynthesis	1	1
2.	Ko00942	Anthocyanin biosynthesis	1	1
3.	Ko00965	Betalain biosynthesis	1	1
4.	Ko00905	Brassinosteroid biosynthesis	4	4
5.	Ko00524	Butirosin and neomycin biosynthesis	2	3
6.	Ko00332	Carbapenem biosynthesis	2	2
7.	Ko00906	Carotenoid biosynthesis	14	18
8.	Ko00904	Diterpenoid biosynthesis	7	8
9.	Ko00944	Flavone and flavonol biosynthesis	3	4
10.	Ko00941	Flavonoid biosynthesis	12	13
11.	Ko00966	Glucosinolate biosynthesis	2	2
12.	Ko00901	Indole alkaloid biosynthesis	2	2
13.	Ko00950	Isoquinoline alkaloid biosynthesis	8	9
14.	Ko00261	Monobactam biosynthesis	6	9
15.	Ko00902	Monoterpenoid biosynthesis	3	3
16.	Ko00401	Novobiocin biosynthesis	3	4
17.	Ko00311	Penicillin and cephalosporin biosynthesis	1	1
18.	Ko00940	Phenylpropanoid biosynthesis	18	32
19.	Ko00523	Polyketide sugar unit biosynthesis	1	1
20.	Ko00909	Sesquiterpenoid and triterpenoid biosynthesis	2	2
21.	Ko00100	Steroid biosynthesis	18	23
22.	Ko00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	5	8
23.	Ko00521	Streptomycin biosynthesis	6	12
24.	Ko00900	Terpenoid backbone biosynthesis	33	47
25.	Ko00253	Tetracycline biosynthesis	3	3
26.	Ko00960	Tropane, piperidine and pyridine alkaloid biosynthesis	9	12
27.	Ko00908	Zeatin biosynthesis	4	7

Supplementary Table S7: List of expression primer sets of selected transcripts used for Real time-PCR analysis.

S. No.	Gene Symbol	Transcript ID	Forward 5'-3'	Reverse 5'-3'
1.	ACAT	TG_1384	GTGCCCAAATCGAGATCCT	CTTGCGGCACAGAGTATTCA
2.	HMGR	TG_7012	GGACGTGATTAGCATCTCAGG	TCACCACCTGCTCTTTGATG
3.	MVK	TG_27721	TCAGCTGGTGAATAAATGTGCT	GTCTCACCTGACCGGAACAT
4.	PMK	TG_12165	CTTTGCCTCCATTGATGACC	TGTCTCCATGGATCTCTGAGG
5.	MVD	TG_4239	CATTAGGCCCTGCATCAAAT	CAAGCCCGCCAATATTCTAC
6.	DXS	TG_13335	TTGGCAAAGGGAGGATACTG	CATCCCATGCTGCTCTACAA
7.	DXR	TG_1064	AGCCGCAATAGAGTCTGGAA	TCGGAATCAGCTGGCAGTAT
8.	CMS	TG_22136	CCCAAAGTGTTCCTGTCTG	AGAGATGGTTGGCTGAATGG
9.	CMK	TG_16822	TGCCTTGTGAGAAAGGAAGC	TCAGGAAGCGGAAGCACTAT
10.	HDS	TG_241	CCATTCAAGGATCTGGCAAC	GTCAGAACGCCCATGCTTAT
11.	HDR	TG_29033	AGCGGAGTCTTACGGGTTCT	GACGGTTGGGTTGTGGATAA
12.	IDI	TG_28659	GCTTTATGCGCCAGTTGAAT	TCTCACCGCAAACGGAAC
13.	GPSS	TG_15477	GTCAACATCGCTTGGGAAAG	AAGCCACGGTCAACAACCTC
14.	FPPS	TG_20699	GGGCAGATGTTGGATTTGAT	GCAACCGGAAGGTAGAAGG
15.	SQS	TG_2891	TTGATGCATGCTGAAGATTG	TGTAGCATTGCGCTAGTGTTC
16.	SQLE	TG_10901	GCCGGTTTCCTCTACGATAA	ACACCTCCGAGGCTCAGATA
17.	CAS	TG_61680	AGCCAATTGTGGGACACAG	GCCAGGACAGTTGTCAAGAA
18.	SMT1	TG_12295	CCAGTGGCAAGTACCATGAA	ACGGGCTTCCAGACATAAGA
19.	SMO1	TG_30966	CCCTTCTATGGAGGTGCAGA	GCCCTTGTCAGTTCGGTAAA
20.	CPI1	TG_15731	ATGAACAATGTGCCTCATGC	TGATTGTGGTAAGTCAGCAACA
21.	CYP51G1	TG_4589	AGGTGATGGAGCTCGTGTG	GACGACATGCTCCAATGCT
22.	FK	TG_15784	GCACCTCTCCTCGTCTCTTCT	CTATTGCTCGCGCTTTCATT
23.	HDY1	TG_1045	GCCGGTATGATAATCCACGA	TGGATGCGCTGTTTACTTTG
24.	SMT2	TG_2945	ACGATCACGGCAGACAAAGT	TGTAGAAGGTGTCGACGAAGG
25.	SMO2	TG_5104	GAACACTGTGGACGTGCTTG	TTGCCACACTGGACTGTCAT
26.	STE1	TG_5587	AGTTGCGATGAAGGCTATGC	TACATGGGCCAACCAACTTC
27.	DWF5	TG_27363	ATCCCGTAAATCTCGGTTCC	GGCATTTCCTTTGTTCTG
28.	UGT80B1	TG_9310	CGGCTCTAAGACCAGCAGAA	TTGGCAGAACCCAGAGATTC
29.	β -glucosidase	TG_4604	CAGCCAGAGGGTTCTCAAAG	GACGATCTGACTGCCCTTGT

The PCR amplification program was as follows: 95°C for 10min; 40 cycles of 95°C for 30s, 58°C for 30s and 72°C for 30s; followed by a melting-curve program of 95°C for 1min, 60°C for 30s and 95°C for 30s.

Supplementary Table S8: The number of transcripts related to Steroidal Saponin biosynthesis.

S. No.	Gene	Gene Symbol	KO	Transcripts
Terpenoid backbone biosynthesis	Acetyl-CoA C-acetyltransferase	ACAT	K00626	1
	Hydroxymethylglutaryl-CoA synthase	HMGS	K01641	2
	Hydroxymethylglutaryl-CoA reductase	HMGR	K00021	1
	Mevalonate kinase	MVK	K00869	1
	Phosphomevalonate kinase	PMK	K00938	1
	Diphosphomevalonate decarboxylase	MVD	K01597	1
	1-deoxy-D-xylulose-5-phosphate synthase	DXS	K01662	1
	1-deoxy-D-xylulose-5-phosphate reductoisomerase	DXR	K00099	1
	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	CMS	K00991	1
	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	CMK	K00919	1
	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	MCS	K01770	3
	(E)-4-hydroxy-3-methyl-2-butenyl-diphosphate synthase	HDS	K03526	5
	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	HDR	K03527	3
	Isopentenyl-diphosphate delta-isomerase	IDI	K01823	3
	Geranyl diphosphate synthase	GPPS	K14066	1
	Farnesyl diphosphate synthase	FPPS	K00787	1
Sesquiterpenoid and triterpenoid biosynthesis	Squalene synthase	SQS	K00801	1
	Squalene epoxidase	SQLE	K00511	1
Steroidal saponin biosynthesis	Cycloartenol synthase	CAS	K01853	1
	Sterol 24-C-methyltransferase	SMT1	K00559	1
	4,4-dimethyl-9beta,19-cyclopropylsterol-4alpha-methyl oxidase	SMO1	K14423	1
	Cycloeucalenolcycloisomerase	CPI1	K08246	1
	Sterol 14-demethylase	CYP51G1	K05917	1
	Delta14-sterol reductase	FK	K00222	1
	Cholesterol Delta-isomerase	HDY1	K01824	2
	24-methylenesterol C-methyltransferase	SMT2	K08242	1
	4-alpha-methyl-delta7-sterol-4alpha-methyl oxidase	SMO2	K14424	1
	Lanosterol Oxidase	STE1	K00227	1
	7-dehydrocholesterol reductase	DWF5	K00213	1
	Delta24-sterol reductase	DWF1	K09828	3
	Sterol 3-O-β-D-glucosyltransferase	UGT80B1	K05841	1
	β-glucosidase	β-glucosidase	K05349	3
	β-glucosidase	K01188	1	

Supplementary Figure legends

Supplementary Figure S1: Heatmap showing tissue-specific gene expression patterns in *T. govanianum*; (a) UGTs and (b) CYP450.

Supplementary Figure S2: Correlation analysis between qRT-PCR and RNA-seq data of steroidal saponin biosynthesis pathway genes using log₂ FC.

Supplementary Table Titles

Supplementary Table S1: Summary of *de novo* assembly generated from *T. govanianum*.

Supplementary Table S2: Functional annotation of *T. govanianum* transcripts with various public databases. **(Submitted as Supplementary dataset)**

Supplementary Table S3: Classification and number of transcripts related to transcription factors families.

Supplementary Table S4: CYPs Classification: Details of CYPs family, sub-family, genes and contig IDs. **(Submitted as Supplementary dataset)**

Supplementary Table S5: Global and tissue specific expression of cytochrome P450s (CYPs, s.no. 1), uridine diphosphate-glycosyltransferases (UGTs, s.no. 2), secondary metabolite biosynthesis pathways (s.no. 3) and steroidal saponin biosynthesis pathway (s.no. 4) in *T. govanianum*. Tissue wise expression of different categories was depicted with green colour (Leaf), Stem (Blue), Rhizome (yellow), Fruit (red). **(Submitted as Supplementary dataset)**

Supplementary Table S6. Pathways and number of transcripts related to secondary metabolite biosynthesis in *T. govanianum*.

Supplementary Table S7: List of expression primer sets of selected transcripts used for Real time-PCR analysis.

Supplementary Table S8: The number of transcripts related to Steroidal Saponin biosynthesis.