Terpene profiling, transcriptome analysis and characterization of *cis*- β -terpineol synthase from *Ocimum*

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Abstract *Ocimum* species produces a varied mix of different metabolites that imparts immense medicinal properties. To explore this chemo-diversity, we initially carried out metabolite profiling of different tissues of five *Ocimum* species and identified the major terpenes. This analysis broadly classified these five *Ocimum* species into two distinct chemotypes namely, phenylpropanoid-rich and terpene-rich. In particular, β -caryophyllene, myrcene, limonene, camphor, borneol and selinene were major terpenes present in these *Ocimum* species. Subsequently, transcriptomic analysis of pooled RNA samples from different tissues of *Ocimum gratissimum*, *O. tenuiflorum* and *O. kilimandscharicum* identified 38 unique transcripts of

terpene synthase (TPS) gene family. Full-length gene cloning, followed by sequencing and phylogenetic analysis of three *TPS* transcripts were carried out along with their expression in various tissues. Terpenoid metabolite and expression profiling of candidate *TPS* genes in various tissues of *Ocimum* species revealed spatial variances. Further, putative *TPS* contig 19414 (*TPS1*) was selected to corroborate its role in terpene biosynthesis. Agrobacterium-mediated transient over-expression assay of *TPS1* in the leaves of *O. kilimandscharicum* and subsequent metabolic and gene expression analyses indicated it as a *cis*- β -terpineol synthase. Overall, present study provided deeper understanding of terpene diversity in *Ocimum* species and might help in the enhancement of their terpene content through advanced biotechnological approaches.

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Introduction

Plants have evolved specialized secondary metabolite biosynthetic pathways for the synthesis of structurally and functionally complex small molecules, which are essential for their survival. In plants, accumulation pattern of the secondary metabolites is complex as it changes in the tissue- and organ-specific manners. Secondary metabolites are crucial in the protection of plants against biotic and abiotic stresses. These molecules also play crucial role in attraction of pollinators and frugivores; adaptation under given environmental conditions and communications (Dixon 1999). Terpenes are major naturally occurring organic compounds present in all forms of living systems. Two simple five-carbon building blocks [isopentenyl

diphosphate (IPP) and dimethylallyl diphosphate (DMAPP)] are utilized in formation of isoprenoids. This isoprenoids synthesis is divided into four steps, which takes place either in cytosol (Mevalonic acid pathway) (McGarvey and Croteau 1995; Bach 1995) and/or plastids (Methyl erythritol pathway) (Rohmer et al. 1993; Sprenger et al. 1997; Eisenreich et al. 1998): (a) production of the five carbon (C5) monomers IPP and DMAPP; (b) condensation of C5-units (IPP and DMAPP) into geranyl diphosphate (GDP), farnesyl diphosphate (FDP) and geranylgeranyl diphosphate (GGDP); (c) cyclization and rearrangement of carbon backbone to form different forms of terpenes and (d) tailoring and downstream functional group modifications of the terpene skeleton. The compartmentalization of two biosynthetic pathways in plants that leads to the formation of DMAPP/IPP is not absolute, because in several cases one metabolite can exchange between these two pathways (Burke and Croteau 2002; Thiel and Adam 2002). Terpene synthases (TPS) catalyze biosynthesis with high regio- and stereo-chemical precision in a cascade of complex reactions involving highly reactive carbocationic intermediates that undergo a sequence of reactions such as cyclizations, alkylations, rearrangements, deprotonations and hydride shifts (Cane 1985; Croteau 1987; Cane 1990; Lesburg et al. 1998; Davis and Croteau 2000; Poulter 2006; Thulasiram and Poulter 2006; Tantillo 2011). Terpenes are classified on the basis of number of five carbon units present in the core structure viz. single isoprene unit (C5) are called hemiterpenes, two isoprene units (C10) are called monoterpenes and three isoprene unit (C15) called sesquiterpenes. Terpene derivatives are also used for the production of numerous pharmaceuticals including taxol (anti-cancer drug) (Jennewein and Croteau 2001) and artemisinin (anti-malarial drug) (Rodriguez-Concepcion 2004).

Monoterpenes consist of two isoprene units with the molecular formula $C_{10}H_{16}$ and are present in secretory tissues such as oil glands of higher plants, insects, fungi and marine organisms. These compounds are widely used in flavor and fragrance industry, due to their characteristic odor. Monoterpenes include three subtypes: acyclic (myrcene, geraniol, linalool), monocyclic (limonene, α -terpineol and terpinolene) and bicyclic (α -pinene, sabinene and camphor). Monoterpenes are biosynthesized from geranyl pyrophosphate (GPP) catalyzed by monoterpene synthase. Several monoterpenes have different medicinal properties including antibacterial, antifungal, antioxidant and anticancerous (Garcia et al. 2008). Generally, monoterpenes are synthesized in plastids through the MEP pathway in plants whereas in other higher organisms and in yeast, MVA pathway is used (Hampel et al. 2005). *Ocimum* is a genus of aromatic plants that belongs to the Lamiaceae family. Members belonging to *Ocimum* genus are known to

synthesize and accumulate various secondary metabolites including terpenes in the secretory and capitate peltate trichomes. Trichomes are located on the aerial parts of these plants and are important for interaction with surrounding by releasing volatiles. The metabolite fraction of *Ocimum* is mainly composed of mono- and sesquiterpenes, along with phenylpropanoids, which impart them medicinal properties, as well as flavor and taste. Here, we describe terpene profiling in six different tissues from five *Ocimum* species, which revealed species-specific distribution of mono- and sesqui- terpenes in different *Ocimum* species. These five species were selected on the basis of their distribution in phylogenetic analysis. Transcriptome profiling of leaf, inflorescence and stem tissue from *O. gratissimum*, *O. tenuiflorum* and *O. kilimandscharicum* revealed 38 putative TPS transcripts, which might be involved in biosynthesis of mono-, sesqui- and di-terpenes. Expression analysis of three putative TPSs (TPS1, TPS2 and TPS3) in different tissues of *Ocimum* species revealed significant expression variation in species-specific manner. Further, functional characterization of TPS1 along with *Agrobacterium*-mediated transient over-expression established its role as a *cis*- β -terpineol synthase.

Materials and methods

Chemicals and plant materials

All chemicals were purchased from Sigma-Aldrich (Sigma Chemical Co., USA), unless stated. *Ocimum* plants belonging to five species viz. *O. gratissimum* L. I and II (*Og* I and *Og* II), *O. tenuiflorum* I and II (*Ot* I and *Ot* II), *O. kilimandscharicum* Gürke (*Ok*), *O. americanum* (*Oa*) and *O. basilicum* I, II, III and IV (*Ob* I, *Ob* II, *Ob* III and *Ob* IV) were grown in a greenhouse at 25–28 °C with ~ 35–40% humidity and 16/8 h light and dark periods. All *Ocimum* plants species and types within species were phenotypically confirmed by taxonomist and voucher specimens of these the plants were submitted in the herbarium of Botanical Survey of India, Pune. All the samples were collected from 3 to 4 months old plants, immediately frozen in liquid nitrogen and stored at – 80 °C until further use. Fresh tissues were used for metabolite extraction and analysis.

Metabolite analysis from different *Ocimum* species

Metabolite analysis was carried out in six different tissues including young leaves (YL; top whorl), mature leaves (ML; third whorl), inflorescence (I), flower (F), stem (S) and root (R) from five *Ocimum* species. Extractions were performed by dichloromethane (DCM) extraction

method, as reported previously (Ramesha et al. 2016; Anand et al. 2016). Briefly, fresh tissues (5 g each) from different plant parts were harvested separately and immediately soaked in 50 mL DCM for 20 h at 28 °C. The combined organic phase was kept at – 20 °C for 2 h (for lipid precipitation) and filtered. The contents were dried, weighed, re-dissolved in 2 mL DCM and subjected to GC–FID and GC–MS analyses. Each sample consisted of 3 biological replicates with triplicates of each of them. GC–FID analyses were carried out on an Agilent 7890A instrument equipped with a hydrogen flame ionization detector and HP-5 capillary column (30 m × 0.32 mm × 0.25 µm, J and W Scientific, USA). Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The column temperature was raised from 70 °C to 110 °C at 2 °C min^{–1}, then raised to 180 °C at 3 °C min^{–1} and finally at 10 °C min^{–1} raised to 220 °C and held for 2 min. Injector and detector temperatures were 230 °C and 235 °C, respectively. GC–MS was carried out with an Agilent 5975C mass selective detector interfaced with an Agilent 7890A gas chromatograph using an HP-5 MS capillary column with helium as the carrier gas and using above-mentioned conditions. Compounds were identified by co-injection studies, comparing the retention time and mass fragmentation pattern with those of reference compounds and also compared acquired mass spectra and retention indices with those of NIST/NBS and the Wiley mass spectral library (software version 2.0, Dec. 2005).

RNA isolation and transcriptome sequencing

Total RNA was extracted from the different tissues using Spectrum™ Plant Total RNA Isolation Kit (Sigma Chemical Co., USA). For transcriptome analysis, total RNA of leaf, inflorescence and stem tissues from *Og*, *Ot* and *Ok* were mixed and preceded with library preparation. Transcriptome library was constructed according to the Illumina TruSeq RNA library protocol outlined in “TruSeq RNA Sample Preparation Guide” (Part # 15008136; Rev. A; Nov 2010) (Genotypic Pvt. Ltd., India) and as per the methodology of Krithika et al. (2015).

Sequence and phylogenetic analysis

The sequence analyses were carried out with Bioedit software (Ibis Biosciences, USA) and CLUSTALW2 program (www.ebi.ac.uk/Tools/msa/clustalw2/). ExPASy translate tool was used for Nucleotide sequences translation (<http://web.expasy.org/translate/>) and identical and similar amino acid residues were marked using BOX-SHADE 3.21 (http://www.ch.embnet.org/software/BOX_form.html). Phylogenetic analysis was performed using TPS1 (MH925775), TPS2 (MH925776) and TPS3

(MH925777), with other plant based TPS. Multiple sequence alignment was carried out with ClustalX 2.1 (<http://www.clustal.org/clustal2/>) and the phylogenetic tree was reconstructed with MEGA6 (Tamura et al. 2013) using the neighbour-joining method with 1000 bootstrap interactions.

Semi-quantitative and quantitative RT–PCR

The DNase-treated RNA (4 µg) was used for cDNA preparation in a 20 µL reaction using SuperScript™ III reverse-transcriptase system (Thermo Scientific). For semi-quantitative PCR, 2 µg DNase-treated RNA, each of young and mature leaves were mixed together and cDNA was synthesized as described. Semi-quantitative RT–PCR (sqRT–PCR) was performed for all three TPSs in 10 µL reaction consisting of 5 µL of 2X Jumpstart readymix, 1 µL each of 10 µM gene specific forward and reverse primers, 1 µL of cDNA optimized with endogenous control (18S rRNA) and nuclease free water was added to make up a volume of 10 µL. PCR was carried out for 30 cycles and the amplified products were run on 1% agarose gel. Quantitative RT–PCR (qRT–PCR) was performed by using SYBR Green chemistry. A typical reaction consisted of 5 µL of SYBR Green master-mix, 0.5 µL each of forward and reverse primer (10 µM) and 1 µL of diluted cDNA (1:2) with nuclease-free water added to make up a volume of 10 µL. For qRT–PCR reactions, elongation factor-1 (EF1) was used as an endogenous control and the reactions were carried out in triplicates for 3 biological replicates in the 7500 Fast Real Time PCR System (Thermo Scientific). Annealing temperature was kept at 58 °C and cycling conditions were kept as per the manufacture’s instruction. Primer sequences are as given in (Supplementary Table S1).

Cloning and in vivo expression analysis of TPS1

By comparing known TPS with de novo assembled RNA-Seq *Ocimum* transcriptome sequences, putative TPS encoding genes were identified using BLASTX. Coding sequence of TPS1 was obtained from *Ot* young leaf cDNA using full-length ORF primers (Supplementary Table S1). TPS1 full-length ORF was cloned into the pRI 101-AN vector for over-expression in plants, using *NdeI* and *BamHI* restriction sites in forward and reverse primer (Supplementary Table S1), respectively. After verifying the construct by sequencing, it was transformed into the *Agrobacterium tumefaciens* (GV3101) chemically competent cells and plated on Luria-agar plate containing 50 µg/mL kanamycin and 25 µg/mL rifampicin. *A. tumefaciens* (GV3101) cells carrying over-expression construct were grown in 2 mL LB media with the above-mentioned

Table 1 Major terpenes present in different *Ocimum* species

Class of metabolite	Name	Structure	% Composition in different species			
			OgI	OtI	Ok	ObI
Monoterpenes	Camphor		ND	0.11	47.33 ± 0.3	0.62
	Eucalyptol		ND	ND	19.65 ± 0.16	0.35
	β-trans-Ocimene		9.08	ND	ND	0.02
	Linalool		0.12	0.28	ND	30.78
	Germacrene D		9.52 ± 0.15	5.83 ± 0.35	5.19 ± 0.05	ND
	β-Caryophyllene		2.87 ± 0.03	14.5 ± 0.09	3.68 ± 0.02	ND
Sesquiterpenes	β-Cubebene		0.66 ± 0.05	2.33 ± 0.01	0.39 ± 0.004	ND
	β-Elemene		0.36 ± 0.05	0.89 ± 0.06	0.25 ± 0.002	ND
	δ-Cadinene		0.36 ± 0.001	3.11 ± 0.15	0.18 ± 0.01	0.04
	α-Copaene		1.54 ± 0.07	3.33 ± 0.12	0.56 ± 0.01	0.09

ND (not detected), OgI (*Ocimum gratissimum* I), OtI (*Ocimum tenuiflorum* I), Ok (*Ocimum kilimandscharicum*) and ObI (*Ocimum basilicum* I). Values are represented in relative area %. The results shown are an average of three biological replicates ± standard errors

antibiotics at 28 °C on rotary shaker set at 180 rpm for 2 days. This starter culture was used to inoculate 10 mL LB with the antibiotics and incubated overnight at 28 °C and 180 rpm. After incubation, cells were pelleted down by centrifugation at 10,000 rpm for 10 min at 4 °C. The cells were washed with half strength MS media, pH 5.4 and pelleted again. This cell pellet was re-suspended in half strength MS media, pH 5.4 to bring it to an O.D._{600nm} of 0.3–0.5. This suspension was used for syringe-assisted infection of the abaxial surface of the leaves. Three plants each were taken for over-expression, empty vector and uninfected control. *A. tumefaciens* (GV3101) cells harboring empty pRI 101-AN vector was used as control. The plants were kept at 22 °C, 18 h light and 6 h dark for acclimatization (3–4 days) before infection, and

maintained at the same conditions for the entire course of the experiment. The samples for volatile and real-time analysis were collected after 4 and 8 days after inoculation (DAI), and were processed as described in the earlier sections.

Statistical analysis

GC–MS analyses of volatiles and gene expression analysis were performed with three biological and three technical replicates each. Data sets were represented as mean $\pm$ standard deviation. Significant differences between control and *A. tumefaciens*-treated plants for metabolites and gene expression were determined using two-way ANOVA followed by Bonferroni's multiple comparisons test.

Fig. 1 Metabolite profiling of five species of *Ocimum*. **a**, **b** Venn diagram showing the unique and common metabolites from young leaves among five different species- **a** quantitative distribution and

b qualitative distribution; **c** Heat map showing the distribution of top 10 metabolites from different *Ocimum* species in young leaves

Fig. 2 Heat map showing relative abundance (%) of terpenoids from 10 *Ocimum* plants **a** Mature leaves, **b** Inflorescence, **c** Flower, **d** Stem and **e** Root. Different species and varieties are hierarchically clustered based on their abundance and diversity in mono- and sesquiterpenes

Results and discussion

Terpene diversity in different tissues across five *Ocimum* species

The terpene analysis indicated both quantitative (Fig. 1a, Supplementary Table S3) and qualitative (Fig. 1b, Table 1) differences in distribution of these compounds across different *Ocimum* species. We also analyzed the abundances of these metabolites in different tissues individually (Figs. 1c, 2a–e). Ten metabolites were common among different species, and were equally distributed between mono- and sesqui-terpenes. These ten common terpenes among *Ocimum* species could be represented as *Ocimum* specific chemical markers. In young leaves; linalool, ocimene, camphor and eucalyptol were the major monoterpenes in *Og*, *Ok* and *Oa*. Monoterpenes, ocimene and myrcene, were present in all the species studied. Young leaves were observed to be sesquiterpenes-rich in *Og* and *Ot*. Two species, *Ok* and *Oa* had comparable distribution of both mono- and sesqui-terpenes. Among sesquiterpenes, β-caryophyllene was detected in all the *Ocimum* species. A similar trend of metabolite distribution was observed in other tissues (Fig. 2a–e), where *Og* and *Ot* were found to be rich in sesquiterpenes, whereas in four different varieties of *Ob* species (*Ob* I–IV), monoterpenes dominated their metabolite profiles. An exception to this distribution was ocimene, which was found to be present in all the tissues of *Og*. This species-specific metabolite distribution was also evident from the cluster analysis, where species rich in

either types of metabolite, clustered together. This chemical diversity can be attributed to the various ways that the intermediates can fold and cyclize in the reaction, catalyzed by different terpene synthases. The product of these reactions can be further modified by other enzymes such as P450 dependent mono-oxygenases and various transferases. In all the different tissues analyzed, monoterpene-rich *Ob* species and sesquiterpene-rich species *Og* and *Ot* clustered together.

Transcriptome sequencing identifies 38 putative TPS transcripts in selected *Ocimum* species

Total RNA from leaf, inflorescence and stem tissue from *O. gratissimum*, *O. tenuiflorum* and *O. kilimandscharicum* were pooled and used for transcriptome analysis. With Illumina GA II platform, total of 4084829 (40.85 million) paired end reads (72 bp each), were created. In this, 38.25 million high quality reads (93.62%) were obtained with > 20 phred score and low-quality reads were trimmed. Velvet assembly generated a total of 220575 contigs with hash length of 55. These were used as input for Oases assembly to generate 253601 transcripts with average length of 255.9 bp. These transcripts were clustered to remove the redundancy using CD-HIT and generated 202081 unique transcripts. BLAST annotation of these transcripts was carried over with proteins of eudicotyledons family and the unannotated transcripts were further annotated with ESTs of Lamiids family. CAP3 analysis on these transcripts, gave 31034 contigs and 25855 singlets.

Fig. 3 Sequence alignment of TPSs with other reported monoterpene synthases. Conserved regions are marked in red. ObFS- *Ocimum basilicum* Fenchol synthase, ObMyS- *Ocimum basilicum* Myrcene

Synthase. TPS1, TPS2 and TPS3 from *Ocimum* species. RoCiS- *Rosmarinus officinalis* Cineole Synthase. Conserved regions are marked in red brackets and signal sequence indicated by red line

Pathway annotation was performed by KAAS (KEGG Automatic Annotation Server). Out of the 56889 transcripts, only 4294 were assigned 1888 unique KO numbers. A web-based server Virtual Ribosome identified 20302 transcripts to have an open reading frame (ORF) more than 100 amino acids (aa), whereas 88 were without any ORF. Pfam analysis was carried out on the peptide sequence of the transcripts > 100aa, where Pfam ID was assigned to 15737 transcripts. In particular, we identified 38 putative

TPSs, of which, 12 had similarities with monoterpene synthases, 13 were related to sesquiterpene synthases, 3 putative prenyl transferases; and 3 and 7 represented di- and triterpene synthases, respectively. In an attempt to understand the terpene accumulation in *Ocimum* species, three TPS contigs were selected and designated as TPS1 (Contig 19414), TPS2 (Contig 13517) and TPS3 (Contig 9641).

Fig. 4 Phylogenetic analysis of *TPS1*, *TPS2* and *TPS3* (marked in blue box) with other reported TPS sequences from different classes (a–f). Neighbor joining tree was constructed using MEGA 6 with 1000 bootstrap iterations

Three TPS shares sequence similarity with reported monoterpene synthases

Using amino acid sequence and protein function, TPSs have been organized into different sub-families, which includes, three angiosperm specific family (TPS-a, TPS-b and TPS-g), a gymnosperm specific subfamily (TPS-d), while TPS-c is most conserved among the land plants; TPS-e and f is conserved among the vascular plants (Bohlmann et al. 1998; Chen et al. 2011; Benabdelkader et al. 2015). Upon sequence analysis of these three contigs, presence of conserved domains and other characteristics of *TPS* gene family was evident (Fig. 3), including plastidial targeting sequence, characteristics of terpene synthases family TPS-b and TPS-g. TPS1 consists of 579 aa (63.7 kDa), TPS2 of 470 aa (51.7 kDa) and TPS3 of 580 aa (63.8 kDa), with TPS2 and 3 sharing 51% identity. TPS1

show 31% identity with TPS2 and 30% identity with TPS3. All three TPSs showed the presence of conserved features of plant TPSs like aspartate-rich region (DDxxD) which is involved in catalysis (Rynkiewicz et al. 2001) and xDx₆E' motif for metal cofactor binding (Degenhardt et al. 2009). A RR(X₈)W motif was present in the N-terminal region of TPS2 and 3, which participates in the substrate ionization and is a characteristic of TPS-a and b subfamilies (Williams et al. 1998; Martin and Bohlmann 2004). Upon phylogenetic analysis, TPS2 and 3 grouped together with other monoterpene synthases belonging to TPS-b family of TPSs, which is in accordance with their identity score. TPS1 grouped together with linalool/nerolidol synthase from *Solanum lycopersicum* and nerol synthase from *Glycine max*, both of which belonged to Tps-g family of TPS (Fig. 4). This family includes mono- and sesqui-terpene synthase from angiosperms.

Fig. 5 Expression analysis of *TPS1*, *TPS2* and *TPS3* by **a** sqRT-PCR using 18S rRNA as reference gene in leaves tissue of five *Ocimum* species and **b** qRT-PCR analysis with EF1 as endogenous reference

Tissue specific expression analysis of three TPSs among different *Ocimum* species

Transcript levels of three *TPSs* was analyzed in leaves of five *Ocimum* species using semi-quantitative RT-PCR, and significant tissue and species-specific variations were observed. Across different tissues, in general, inflorescence represented highest expression levels of these contigs, while stem having the least. *TPS1* transcript abundance was higher in leaf tissue of *Ot*, *ObII* and *Oa*, while *TPS2* expression was higher in all the five species analyzed (Fig. 5a). In qRT-PCR, except for *Ob* species, *TPS2* had very high expression levels in all tissues of the *Ocimum* species that were analyzed (Fig. 5b). In case of *OtI* and *II*, *TPS1* and 2 had similar expression profiles. Apart from *Ot*, *TPS1* was also found to be the most abundant contig in *ObII*, whereas in *Og* and *Ok*, it had low expression levels. On the other hand, *TPS3* was observed to be abundant only in *Ok*, in both semi-quantitative and qRT-PCR.

in different tissue of five species of *Ocimum*. *Og*- *Ocimum gratissimum*, *Ot*- *Ocimum tenuiflorum*, *Ok*- *Ocimum kilimandscharicum*, *Ob*- *Ocimum basilicum*, *Oa*- *Ocimum americanum*

Transient over- expression of *TPS1* indicated to be a *cis*- β -terpineol synthase

In order to analyze gene function of *TPS1*, transient over-expression analysis was carried on *Ok* plants. Accumulation of metabolites and transcript upon over-expression of *TPS1* was estimated by comparing them with that of control and empty vector infected plants (Fig. 5a, b). In qRT-PCR analysis, two-fold increase in *TPS1* was noted, in both local and systemic tissues, which was reflected in 7% increase in accumulation of *cis*- β -terpineol in 8 DAI (Fig. 6 and Supplementary Table S2). There was no significant difference in accumulation of any other metabolites upon over-expression of *TPS1* in *Ok* leaves. As compared to that of metabolite analysis, transcript abundance decreased at 8 DAI, an observation which was consistent with the reports suggesting instability of non-integrated T-DNA (Lacroix and Citovsky 2013).

Fig. 6 **a** *cis*-β-terpineol levels upon transient over-expression of *TPS1* in *O. kilimandscharicum*. Experiment was performed with 3 biological and 3 technical replicates (L-local tissue, S- systemic tissue). Metabolite level was checked in leaf tissues collected after 4 and 8 days of infection. Data expressed in terms of relative area % and bars represents the SD values. **b** *TPS1* expression levels determined by qRT-PCR. The values are expressed in comparison

to the transcript levels in empty vector. Two-way ANOVA followed by Bonferroni's multiple comparisons suggested significant difference between the data at $p < 0.001$ (indicated as ****), $p < 0.01$ (indicated as ***). **c** GC-chromatograms of metabolite analysis of control, empty vector infected and *TPS1* infected plants. 1- *cis*-β-terpineol and 2- Linalyl acetate (internal standard). *Refers to the hydrocarbon dodecane observed in the GC-MS runs

Conclusion

Detailed terpene analysis of five different *Ocimum* species indicated significant variations in their distribution across different tissues and species. Transcriptome sequencing led to detection of 38 unique *TPS* transcripts. Sequence and phylogenetic analysis of three full-length putative *TPS* transcripts identified them as monoterpene synthases. Their transcript abundance in five tissues across *Ocimum* species revealed higher abundance of *TPS2* in all the species, whereas *TPS1* and *TPS3* illustrated species-specific abundances. Further, Agro-infiltration based transient over-expression assay of *TPS1* indicated it to be a *cis*-β-terpineol synthase.

Note The nucleotide sequences reported in this paper has been submitted to the GenBank with accession number MH925775 (*TPS1*), MH925776 (*TPS2*) and MH925777 (*TPS3*).

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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