

Supplementary Information

Identification of novel sesquiterpene synthase genes that mediate the biosynthesis of valerianol, which was an unknown ingredient of tea

Jun-ichiro Hattan¹, Kazutoshi Shindo², Tetsuya Sasaki³, Fumina Ohno¹, Harukuni Tokuda⁴,
Kazuhiko Ishikawa⁵, and Norihiko Misawa^{1*}

¹ Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, 1-308
Suematsu, Nonoichi-shi, Ishikawa 921-8836, Japan

² Department of Food and Nutrition, Japan Women's University, 2-8-1 Mejirodai, Bunkyo-ku, Tokyo
112-8681, Japan

³ Industrial Research Institute of Ishikawa, 2-1 Kuratsuki, Kanazawa-shi, Ishikawa 920-8203, Japan

⁴ Department of Complementary and Alternative Medicine, Clinical R&D, Graduate School of
Medical Science, Kanazawa University, 13-1, Takara-machi, Kanazawa-shi, Ishikawa 920-8640,
Japan

⁵ National Institute of Advanced Industrial Science and Technology, 1-8-31 Midorigaoka, Ikeda-shi,
Osaka 563-8577, Japan

*To whom correspondence should be addressed. E-mail: n-misawa@ishikawa-pu.ac.jp

Phone: 81 76 227 7525, Fax: 81 76 227 7557 (Ishikawa Prefectural University)

Supplementary Table S1 Terpenes and their ratio in the volatile compounds trapped by solid phase micro-extraction from the flowers of *Camellia hiemalis*

Component	content (%)
<i>cis</i> -Linalool oxide	1.37
Linalool	5.15
β -Elemene	0.08
α -Terpineol	0.11
Epoxylinool	0.79
Elemol	0.1
Hexahydrofarnesyl acetone	0.89
Eugenol	80.94
Guaiol	0.39
α -Muurolene	0.1
α -Eudesmol	0.07
β -Eudesmol	0.14
Total	90.13

Supplementary Table S2 Classification of amino acid substitutions observed in ChTPS1 clones

amino acid	location and interaction in the molecule	expected effects of substitutions on the enzyme activity
W42	surface	no effect
A68	surface, entrance of the cleft	unclear
E78	hydrogen bonding with H122, E121	E78Q has no effect
G139	loop*	G139C has no effect
V145	internal	unclear
G185	loop*	possibly changes the conformation
S290	surface	no effect
V295	internal	unclear
D317	surface	no effect
L319	internal	unclear
L321	surface	no effect
D365	surface	no effect
A395	surface	no effect
E399	surface	no effect
A443	internal	unclear
R477	surface, loop*, forming an ionic bond with E487	R477H possibly change the loop structure
M503	internal	unclear
K541	surface, loop*, hydrogen bonding with D542	K541N possibly change the peak1 productivity

*loop means β -turn

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Supplementary Fig. S1 Flowers of scented Camellia, *Camellia hiemalis*

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ZoTPS1 MELVDTPSLEVFEDVVVDRLQVAGFDFSFWDGYFITN---QKSQSEAWMNERAELKNEVRSMFQNV-TG 65
ZzZSS1 MERQ-SMALVGDKKEII-RKSFYHPTVWGDYFIQNSSLTHAKESTQRMKRVBELKVQVKSFMKDT-SD 65
ZzZSS2 MEKQ-SLTFDGDDEAKIDRKSSKYHPSIWGDYFIQNSSLTHAKESTQRMKRVBELKVQVKSFMKDT-SD 68
CbTPS1 MASS-QVGDMVNGNAEPTRHAKFPFSLWGDRT---SFTLDKQLWDKYGNEIEVLKEQVRSMVAVAGGRK 66
ChTPS1 MASS-QVGDMVNGNAEPTRHAKFPFSLWGDRT---SFTLDKQLWDKYGNEIEVLKEQVRSMVAVAGGRK 66

ZoTPS1 ILQTMNLIDTIQLLGLDYHFMEEIAKALDHLKD--VMSKYGLYEVALHFRLLRQKGFNISSDVFKKFKD 133
ZzZSS1 VLQIMILVDSIQLLGLDYHFEKEITAALRLIYE--ADVENYGLYEVSLFRLLRQHGYNLSPDVFNKFKD 133
ZzZSS2 LLQLMNLINSIQMLGLDYHFEKEIDEALRLIYE--VDDKSYGLYETSFRLLRQHGYNLSPDVFNKFKD 136
CbTPS1 AAQINLINVLERLGVSYHFEKEIEEQLEQLFAKFEDNEDYDLFTIALHFRIFRQHGKMSCDVFNKFRD 136
ChTPS1 AAQINLINVLERLGVSYHFEKEIEEQLEQLFAKFEDNEDYDLFTIALHFRIFRQHGKMSCDVFNKFRD 136

ZoTPS1 KECKFMEELKDDAKGLLSLYNAAYFGTKEETILDEAISFTKDNLTSLK--DLNPPFAKLVSLTKPIQ 201
ZzZSS1 DKGRFLPTLNGDAKGLLNLNAAYLGTHEETILDEAISFTKQLESLLG--ELEQPLAIBVSLFLETPLY 201
ZzZSS2 DNGSFISSLNGDAKGLLSLYNVSYLGTHGETILDEAKSFTKQPLVSLMS--ELEQSLAAQVSLFLEPLC 204
CbTPS1 SNCEFKETVSNDRGMLSLYEATYKIRGEGLDEAHAFHTIAQLESVGGPHLSSDLSEQVMHAKQSIH 206
ChTPS1 SNCEFKETVSNDRGMLSLYEATYKIRGEGLDEAHAFHTIAQLESVGGPHLSSDLSEQVMHAKQSIH 206

ZoTPS1 RSMKRIFTRSISYISIQDEPTLNETILELAKLDFNMLQCLHQKELKKICAWNNNLNLDIMHLNFIKRVVE 271
ZzZSS1 RRTFRLLVRKYIPIYQEKVMRNDTILELAKLDFNMLQSLHQEVEVKKITIWNNDLALTKS-LKFARDRVVE 270
ZzZSS2 RRNKILLARKYIILIYQEDAMRNNVILELAKLDFNMLQSLYQELKKISTIWNNDLAFKS-LSFTRDRVVE 273
CbTPS1 RGFPRLEAKHFISFYEKDAARNETTLRLAKLDFNMLQSLSHREELCHIFRWKKEIDLISK-VPYARDRAVE 275
ChTPS1 RGFPRLEAKHFISFYEKDAARNETTLRLAKLDFNMLQSLSHREELCHIFRWKKEIDLISK-VPYARDRAVE 275

ZoTPS1 CYCWSMVRHEPSCSRARLISTKLLMLITVDDTDYDSYSTLEESRLLTDAIQRWNPNEVDQLPEYLRDFF 341
ZzZSS1 CYWIVAVYFEPQYSRARVITSKAISLMSIMDDIYDNYSTLEESRLLTEAIERWEPQAVDCVPEYLRDFF 340
ZzZSS2 GYWWVLTIIYFEPQHSRARVICSKVFAFLSIMDDIYDNYGILEECTLLTEAIAKRWNPQADGLPEYLRDFF 343
CbTPS1 CFFWSTCAYYEPQHSVGRAVLTKIVLLSVTDDTDYDAYGTDELKLYTNAVQRWDASAMDELDPYMKLY 345
ChTPS1 CFFWSTCAYYEPQHSVGRAVLTKIMLLSVTDDTDYDAYGTDELKLYTNAVQRWDASAMDELDPYMKLY 345

ZoTPS1 LKMLNIFQEFENELA-PEEKFRILYLKEEWKIQSQSYFKECQWRDDNYVPKLEEHMRLSIISVGFVLFYC 410
ZzZSS1 LKLLKTYKDFEDEL-PNEKYRIPYLQEEIKVLSRAVFOEAKWGVERVYPALEEHLLVSLITAGYFVAVAC 409
ZzZSS2 LKLLKTFEDEFEL-LNEKYRMLYLQDEVKALAISSYLQEAKWGIERHVPSLDEHLHNSLISSGSSTVIC 412
CbTPS1 RALLNVYDEVERDLAKQGRAYGVHHSKEAFKEIVRSYEIEAEWFKEGYVASFEYMKNALVTSTGRLHTT 415
ChTPS1 RALLNVYDEVERDLAKQGRDYGVHHSKEAFKEIVRSYEIEAEWFKEGYVASFEYMKNALVTSTGRLHTT 415

ZoTPS1 GFLSGMEEAVATKDAFEWFASFPKIIEACATIRITNDITSMEREQKRAHVASTVDCYMKYGTSGDVAC 480
ZzZSS1 ASYVGLG-EDATKETFEWVASSPKILKSCSIHCRMLDDITSHQREQERDHFASSTVESYMKEHGTSKAVAC 478
ZzZSS2 ASFVGMG-EVATKEVFDWLSFSPKVVEACCVIGRLLNDIRSHLEQGRDHTASTVESYMKEHGDTNVDVAC 481
CbTPS1 SCFMGLEADVATTEAFEWILTTPKMVAASGAIGRLVDVMSNDEEQERGHVATGLDCYMKQHGVSQKEAI 485
ChTPS1 SCFMGLEADVATTEAFEWILTTPKMVAASGAIGRLVDVMSHDEEQERGHVATGLDCYMKQHGVSQKEAI 485

ZoTPS1 EKLGLGFVEDAWKTINEELLTETGLSREVIELSFHSAQTTEFVYKHVDAFTEPNTTMKENIFSLLVHPIPI 550
ZzZSS1 EKLQVMVEQKWKDLNEECLRPTQVARPLIEIILNLSRAMEDIYKHKDTYTNSNTRMKDNVSLIFVESFLI 548
ZzZSS2 EKLREIVEKAWKDLNNEELNPTKVPRLMIERIVNLSKSNEEIKYNDTYTNSDTTMKDNISLVLVESCDY 551
CbTPS1 VELYKMIENAWRDINEEMLKPTAISMKLLIHLNLSRISDVYKYVDGYTHP-EIKDHSVLSLFDPIPM 554
ChTPS1 VELYKMIENAWRDINEEMLKPTAISMKLLIHLNLSRISDVYKYVDGYTHP-EIKDHSVLSLFDPIPM 554

ZoTPS1 --- 550
ZzZSS1 --- 548
ZzZSS2 FNK 554
CbTPS1 --- 554
ChTPS1 --- 554

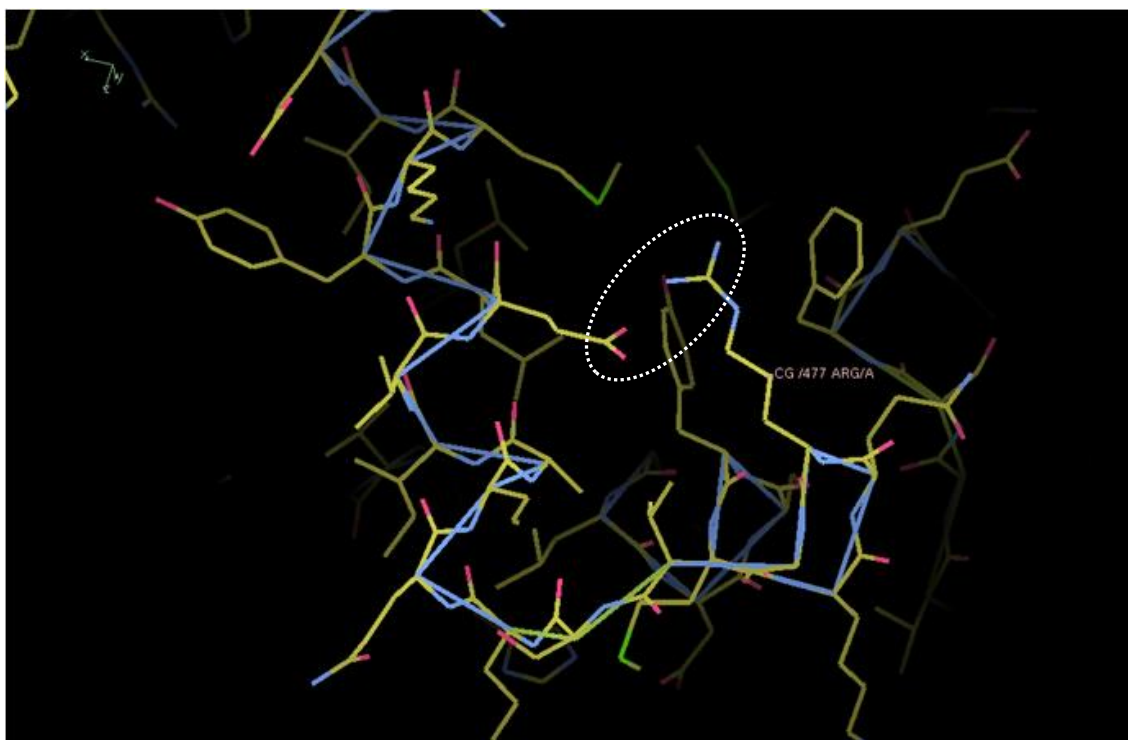
Supplementary Fig. S2 Amino acid alignment of sesquiterpene synthases derived from *Zingiber* plants and *Camellia* plants

The deduced amino acid sequences of the *Zingiber* terpene synthases (TPSs) together with *Camellia* TPSs, isolated by our group, were aligned. The TPS-conserved motifs RDR, DDxxD, and NSE/DTE [(N/D)Dxx(S/T)xxxE] are indicated by asterisks. Amino acids conserved in all TPSs are shown in black boxes, whereas amino acids that are not conserved between CbTPS1 and ChTPS1

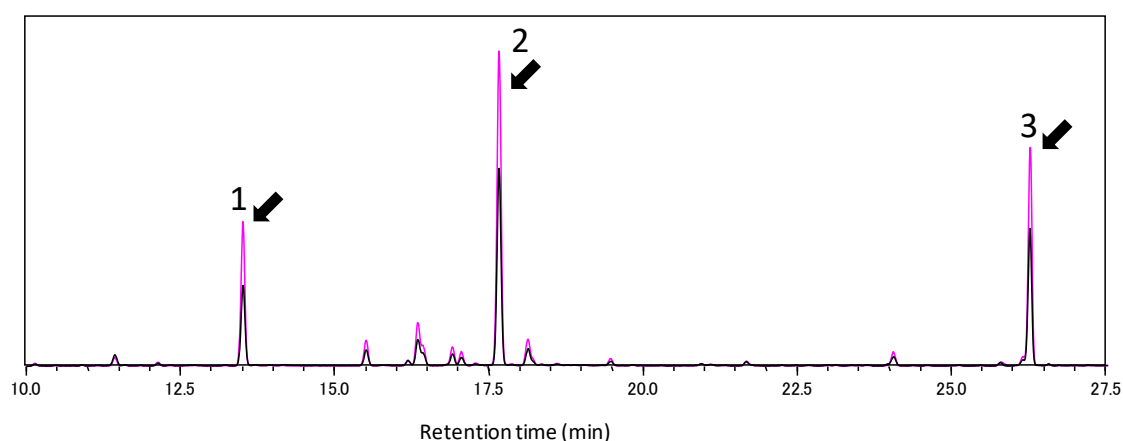
1 are indicated by +. Arrows indicate the sequences corresponding to the degenerate primers. Three
2 amino acids encircled by a simple square would correspond to E487 in ChTPS1. ZoTPS1, *Zingiber*
3 *officinale* β -bisabolene synthase³²; ZzZSS1, *Z. zerumbet* α -humulene synthase³⁴; ZzZSS2, *Z.*
4 *zerumbet* β -eudesmol synthase³³; CbTPS1, *Camellia brevistyla* hedycaryol synthase³⁰.
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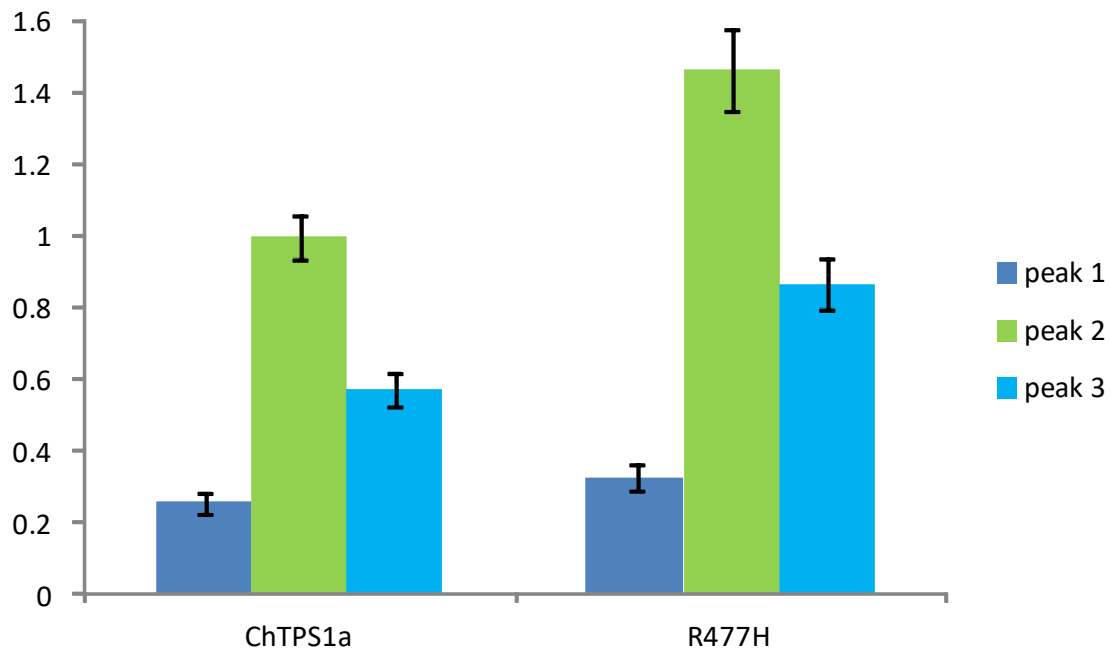
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11 **Supplementary Fig. S3** Expression analysis of the *Camellia hiemalis* *ChTps1* gene by reverse
12 transcription (RT)-polymerase chain reaction (PCR). Total RNAs were extracted from 3 independent
13 samples (1-3) of each tissue (flowers, leaves, and stems) and submitted for RT-PCR. Total RNA
14 without a treatment of reverse transcriptase was used as the template for the ‘w/o RT’ reaction. An
15 actin-like (ACT-like) gene of *C. hiemalis* was amplified as an internal control. Nine samples (three
16 replicates of each tissue) in each row (*ChTps1*, w/o RT, ACT-like) were simultaneously loaded into
17 one gel and electrophoresed.
18



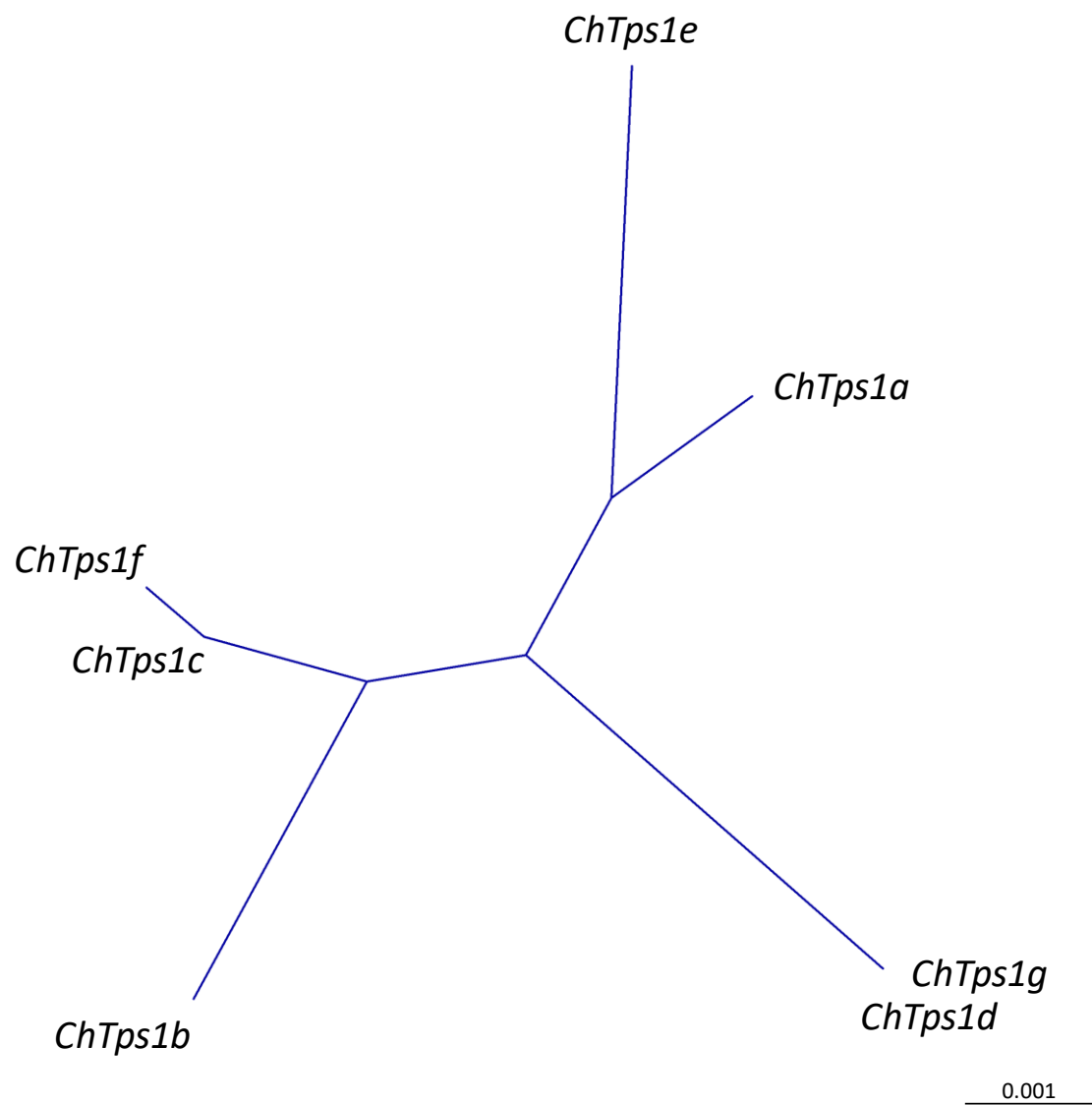
Supplementary Fig. S4 Schematic diagram of the loop structure around R477. An ionic bond between R477 and E487 was predicted in this model (dotted circle). The atoms were colored as C: yellow, N: blue, O: red, S: green. The backbone of polypeptide was shown in blue line.



Supplementary Fig. S5 Comparison of the *Camellia hiemalis* ChTPS1 and ChTPS1 (R477H) products. Volatile compounds biosynthesized by ChTPS1 and ChTPS1 (R477H) were extracted by decane and analyzed by GC-MS. The chromatograms were overlapped in the same figure (ChTPS1: black, ChTPS1(R477H): pink). Three peaks correspond to those in Fig. 2a.



Supplementary Fig. S6 Comparison of the peak areas obtained by GC-MS analysis of *ChTps1a* and *ChTps1*(R477H) clone's products. Three peak areas, shown in Fig. 2a (Peaks 1-3), of total ion chromatogram were obtained by the analysis. Relative abundance of each peak was depicted when the Peak 2 area of *ChTps1a* clone was set to 1. Bars represent means \pm standard deviation of three replicates.



Supplementary Fig. S7 Phylogenetic positions of the *ChTps1a-g* genes. The nucleotide sequences of these seven genes were compared phylogenetically.