

Supplementary Materials

Functional characterization of a *Dendrobium officinale* geraniol synthase DoGES1 involved in floral scent formation

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Supplementary Table legends

Table S1. Three candidate GES sequences from the *D. officinale* genome.

Table S2. The reported TPS proteins from other plant species used in the phylogenetic analysis.

Table S3. Gene-specific primers used in the experiments.

Supplementary Figure legends

Figure S1. Transcription levels of *DoGES1*, *DoGES2*, and *DoGES3* in different *D. officinale* tissues.

Figure S2. Agarose gel electrophoresis of cDNA amplification of the *DoGES1* gene.

Figure S3. SDS-PAGE analysis of DoGES1 recombinant protein expressed in *Escherichia coli* BL21.

Figure S4. Content of geraniol in semi-open *D. officinale* flowers at 8:00, 11:00, 14:00, and 17:00.

Figure S5. Putative regulatory *cis*-elements in the *DoGES1* promoter.

Figure S6. Different developmental stages of *D. officinale* 'Zhongke 5' flowers.

Table S1. Three candidate GES sequences from the *D. officinale* genome.

Name	Sequences
DoGES1	MEEELRPCRFSLLLAEQTMQRLADGAAFSTPVSRRSANYQPSLWDDNYIQSL PDGSLDATQVNLWEKLKEEVRHLIDQNKQNDTIELLEYVNTLCQLGISYHFESIKNVLT FIASSMESLSNILKNSLHGSALLFRLREYGIKALNTREDFLVRSFKNENGSGF KVHIVNDVKGMLSLYEASYLSVEGEDDLDEAMEFTTKHLSNYLKEPSLIHPSLVEQ ISHALHLPLHWRMPTLHTMWFIDTYEKQENTNYSLFEFAKLDFNMVQSIYKKEV KEMSSWRSIGLAGDEFSFARDRLMENYFWAMGCALEPHFWRCRKEITKLVSIIIT TIDDIYDTYGSIEELVFTNAVDEWKIIEIQSLPNCMRKALLTLINTMNEIAFAFSKE KGLDILPQLKRPWGYQCKAYLVEAIWYNTRYIPTLNEYMENAWLSIGTALVLT VAYLLEDLTKEALNSLELYFDVTRYSCMITRLYDDLGTSTDELQRGDIPKSIQCYM NETNVSEVVARDHIRKLIKKYWKLFNGEYFSNFNLEESFKRYALNLPMAQCIYQ YGDGYGKPDRETRDRIISLIKPIPL
DoGES2	MEGDLQSSPPSRRSANYQPTLWDDSYIQSLPDSSLDAAQVNLWEKLNEEVRHLID HTKQKDIIELLELEYVNTLCQLGISYHFESIKNVLTFIASSMESLSNILKNSLHGSALLF RLLREYGIKALNTREDFLVRSFKNENGSGFKVHIVNDVKGMLSLYEASYLSVEGED DLDEAMEFTTKHLSNYLKEPLLIHPSLVEQISHALELPLHWRMPRLHTRWFIDAY ERQENMNHSLLEFAKLDFNMVQSICKKEVKEMSSWRSIGLAGDEFSFARDRLM ENYFWTMGCTFEPHFWRRCRKEITKLASLITIDDIYDIYGSVEELMLFTNAVNEWK ITEIQSLPNCMRKALLAIINTMNDTACAFSKEKGLDILPQLKQAWGDQCKAYLVE AIWYNTRYIPTLNEYMENAWLSVAISLVINAAYLLEDLTKEALNSLELYFDVTRY SSMVTRLYDDLGTSTDELQRGDVPKSIQCYMNETNVSEFVARDHIRQLIKKYWRL FNGEYFSNFNLEESFKRYALNLPMAQCIYQYGDGYGKPDRETRDKIISLIKPIPL
DoGES3	MEGDLPSPPSRRSANYQPSLWDDSYIQSLPDSSLDATQINLWEKLNKEARHLIEH NKQKDIIELLE YINTLCQLGISYHFESIKNVLTLIASSIENLSNILKNSLHGSALLFRL LREYGIKALNTSEDFLVRSFKNENGSGFKVHIVNDVKGMLSLYEASYLSVEGEDDL EAMEFTIKHLSNYLKEPLLIHPSLVKQISHAIELPLHWRSRPLHTRWFIDAYERQEN MNPSLLEFAKLDFNMVQSICKKEVKEMSSWRRSIGLASDEFSFARDRLMENYFWI MGCTFEPHFWRRCRKEITKFASLISTIDDIYDIYGSVEELVFTNAVDEWKIIEIQSLP NCMKTTLLALINTMNDIACAFLKEKGLDILPQLKRAWGDQCKAYLVEAIWYNT RYTPTLNEYMDNAWLSAAVPLVLTAAYLLEDLTQALNSLQFYFDVTRYSSMV ARLYDDLETSTDELQRGDVPKSIQCYMNETNVLESVARDHIRQLIKKYWKLNGE YFSNFNLEESFKRYALNLPMAQCIYQYGDGYGKPDRETKDRIISLIKPIPL

Table S2. The reported TPS proteins from other plant species used in the phylogenetic analysis.

Species	TPS	Accession ID in NCBI
<i>Santalum album</i>	Monoterpene synthase	ACF24767
<i>S. album</i>	β -bisabolene synthase	AIV42941
<i>S. austrocaledonicum</i>	β -bisabolene synthase	ADO87003
<i>S. spicatum</i>	α -bisabolol synthase	E3W206
<i>Malus domestica</i>	α -farnesene synthase	AAX19772
<i>Prunus dulcis</i>	Geraniol synthase	QEE82241
<i>P. dulcis</i>	(<i>E,E</i>)- α -farnesene synthase	QEE82244
<i>Ocimum basilicum</i>	Geraniol synthase	AY362553
<i>O. basilicum</i>	<i>R</i> -linalool synthase	AAV63789
<i>Vitis vinifera</i>	(-)- α -terpineol synthase	AAS79352
<i>Arabidopsis thaliana</i>	β -caryophyllene/ α -humulene synthase	AAO85539
<i>Cucumis sativus</i>	(<i>E,E</i>)- α -farnesene synthase	Q66PX9
<i>Fragaria vesca</i>	Germacrene D synthase	LOC101314066
<i>S. album</i>	Sesquiterpene synthase	ACF24768
<i>S. spicatum</i>	Sesquiterpene synthase	E3W208
<i>A. thaliana</i>	<i>S</i> -(+)-linalool synthase	Q84UV0
<i>Antirrhinum majus</i>	nerolidol/linalool synthase 1	ABR24417
<i>V. vinifera</i>	(3 <i>S</i>)-linalool/(<i>E</i>)-nerolidol synthase	ADR74212
<i>Camellia sinensis</i>	(<i>E</i>)-nerolidol synthase	KY033151
<i>Pinus abies</i>	(<i>E</i>)- α -bisabolene synthase	Q675L6
<i>Abies grandis</i>	(<i>E</i>)- α -bisabolene synthase	AAC24192
<i>P. taeda</i>	α -farnesene synthase	Q84KL5
<i>P. abies</i>	(-)-linalool synthase	Q675L2
<i>P. taeda</i>	(-)- α -terpineol synthase	Q84KL4
<i>Solanum lycopersicum</i>	Copalyl diphosphate synthase	BAA84918
<i>Oryza sativa</i>	<i>ent</i> -copalyl diphosphatesynthase1	Q6ET36
<i>S. lycopersicum</i>	<i>ent</i> -kaurene synthase	AEP82778
<i>O. sativa</i>	Kaurene synthase 1	Q0JA82
<i>S. habrochaites</i>	Santalene and bergamotene synthase	ACJ38409
<i>Clarkia breweri</i>	Linalool synthase 2	AAD19840
<i>V. vinifera</i>	P(<i>E</i>)-nerolidol/(<i>E,E</i>)-geranyl linalool synthase	NP001268004
<i>Actinidia deliciosa</i>	Terpene synthase	ACO40485

Table S3. Gene-specific primers used in the experiments

Name	Sequences (5'→3')	Description
DoGES1-F	ATGGAGGAAGAACTTCGCCCCG	Gene cloning
DoGES1-R	TAGAGGAATGGGCTTAATTA	
DoGES1-32a-F	GGCTGATATCGGATCCTATGGAGGAAGAACTTCGCCCCG	pET32a vector construction
DoGES1-32a-R	GTGCGGCCGCAAGCTTTAGAGGAATGGGCTTAATTA	
DoGES1-3300-F	CTCGGTACCCGGGGATCCTATGGAGGAAGAACTTCGCCCCG	pCAMBIA 3300 vector construction
DoGES1-3300-R	GGCCAGTGCCAAGCTTTAGAGGAATGGGCTTAATTA	
DoGES1-YFP-F	ATTTACGAACGATAGCCATGGCTATGGAGGAAGAACTTCGCCCCG	YFP vector construction
DoGES1-YFP-R	AGATCTGAGTCCGGACCATGGTTAGAGGAATGGGCTTAATTA	
DoGES1-qRT-PCR-F	GCCTTTACGAGGCTTCTTATCT	qRT-PCR analysis
DoGES1-qRT-PCR-R	TTGTTCCACTAACGAGGGATG	
DoGES2-qRT-PCR-F	GCTTATGAGAGGCAAGAGAACA	qRT-PCR analysis
DoGES2-qRT-PCR-R	CCACCAACTAGACATCTCCTTAAC	
DoGES3-qRT-PCR-F	TGATGCTTATGAGAGGCAAGAG	qRT-PCR analysis
DoGES3-qRT-PCR-R	TCATCGCTAGCAAGACCAATAC	
DoEF-1 α -F	GCTTGAGAAGGAGCCCAAGT	qRT-PCR analysis
DoEF-1 α -R	CCAACAGCCACAGTTTGTCC	

Gene-specific primers for real-time quantitative reverse transcription PCR (qRT-PCR) were designed by the PrimerQuest online tool (<http://www.idtdna.com/Primerquest/Home/Index>). The *D. officinale* actin gene (DoEF-1 α) was obtained from NCBI (JF825419). F, forward; R, reverse.

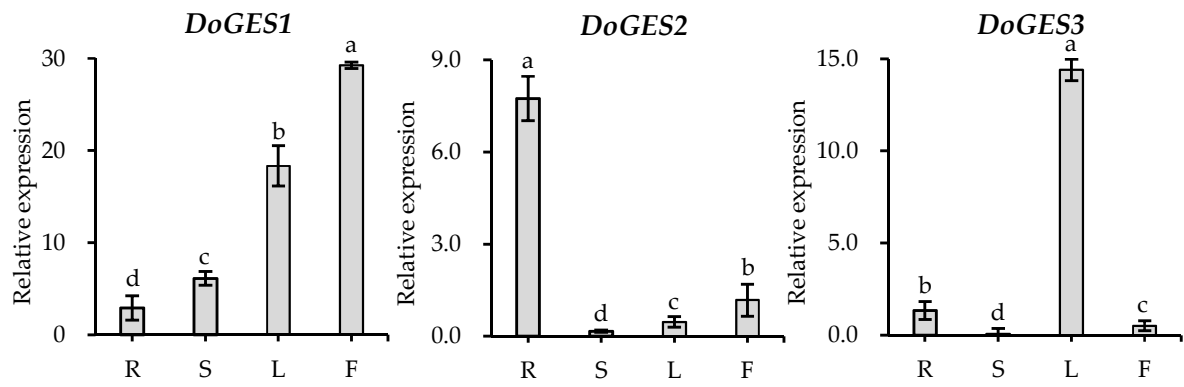


Figure S1. Transcription levels of *DoGES1*, *DoGES2*, and *DoGES3* in different *D. officinale* tissues. Different letters above error bars (standard deviation) ($n = 10$) indicate significant differences between treatments ($p < 0.05$, Duncan's multiple range test). R, roots; S, stems; L, leaves; F, flowers.

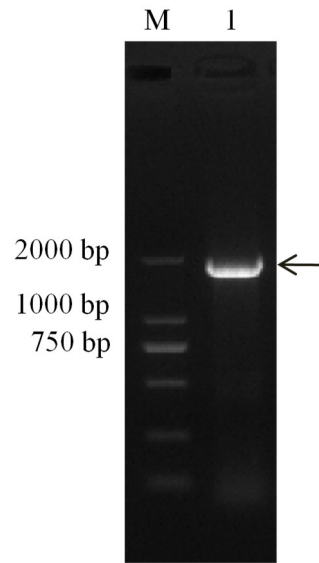


Figure S2. Agarose gel electrophoresis of cDNA amplification of the *DoGES1* gene. M, marker D2000 (M122, GenStar Inc., Beijing, China).

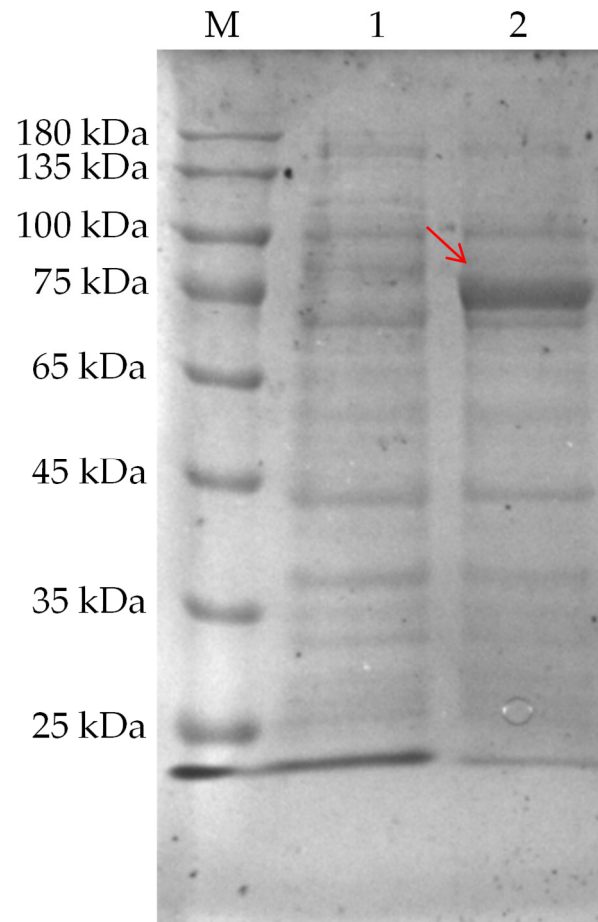


Figure S3. SDS-PAGE analysis of DoGES1 recombinant protein expressed in *Escherichia coli* BL21. Lane 1 indicates empty vector pET32a. Lane 2 indicates the elute of pET32a-DoGES1 protein. M indicates protein marker 10-180 kDa (M221, GenStar Inc., Beijing, China).

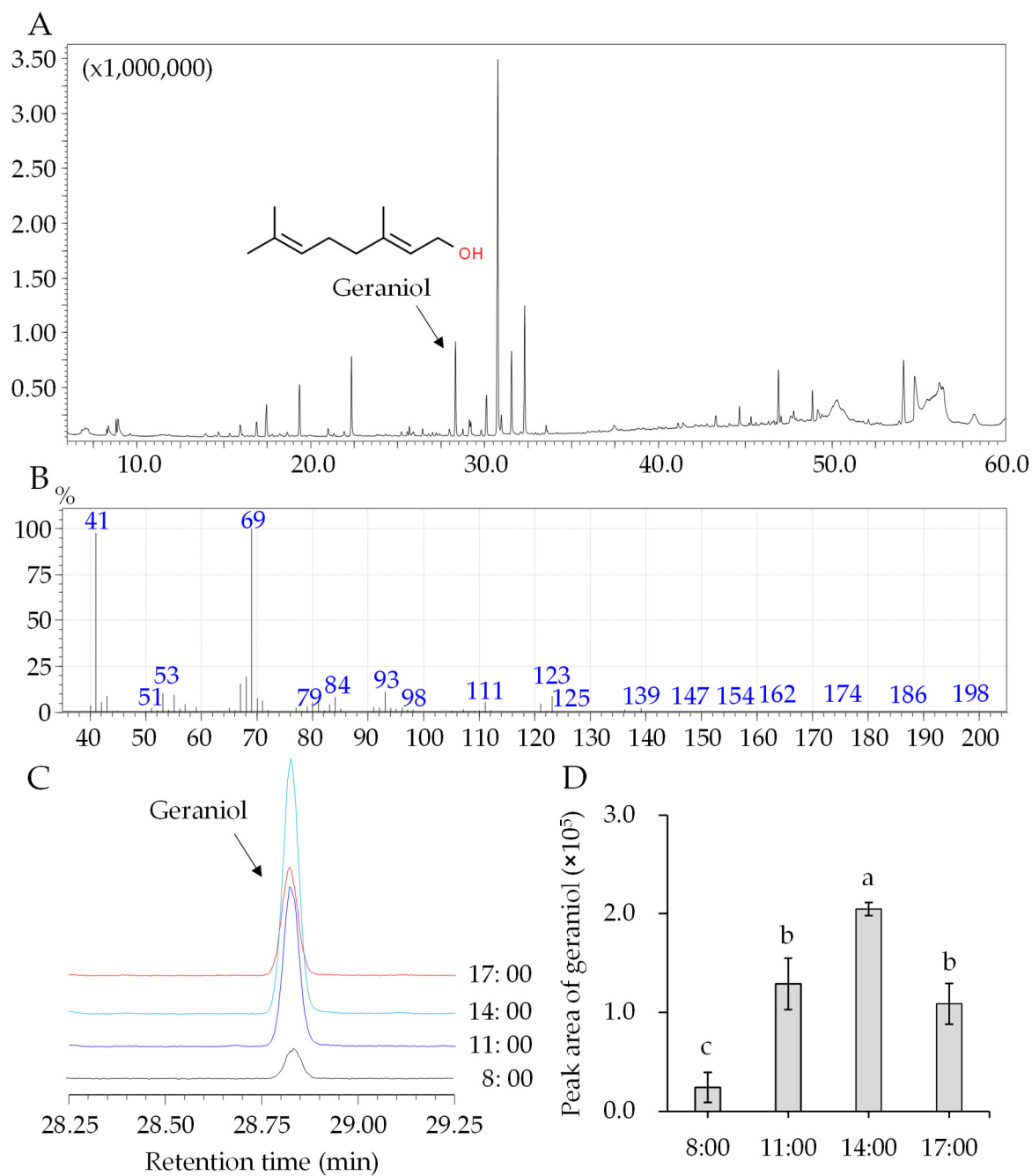


Figure S4. Content of geraniol in *D. officinale* semi-open flowers at 8:00, 11:00, 14:00, and 17:00. Each bar represents the mean (\pm standard error, $n = 10$) of three independent biological replicates. Different letters above the bars indicate significant differences ($p < 0.05$, Duncan's multiple range test).

TTGGAGGATTGTAGTCGTCGACCAACGGAGATTGAAGAATCATAATTGAGCGATGATGGTTGACTATCGACGAAGGTT
AGATTTATCATAGTCGGGCAACTAAGGTTGAAATATCATATTTGGTAGTTGATGGAGGTCGTTAGTATGAAAAGCTTTATAA
ATGTTTAAATTTAAATAAGATAATATAGTCTTTTGATATTTGTTAATGGAACGTGAATATTTTAAAGACATGTCCTTGAC
TTTTATGCGCTTTGTTAGTCATTGAATACTATTGTCAAAAATAATTGATATTATAATCTCTTTAAACAATCAAATTTTAGGCAT
AATTATATTCATCCATATAGATTTATATAATAGTTTAAAAAGTGACAACATAATAAAATTTTAAATTAATTTTACAAA
ATTTATTTTAAATAAAAATTTAATAATGAAGTCATCTAATTCACCACATATAATTGAAGAGCTAAGAGAAAATAAAAAAAA
GGAGGGAGATTCAAGAGCTAAGAGAAAATAAAGAATTCTCTGTATTTTCCAAGCTATCATCGTCGCTGACTGCGCCAGCA
GCAATCGCCGCCAGCATTTGTCTGACTTCTATCAATATTTAACTCAGTTATTGAATTTTAAATCATCTCTTATAGATATCAA
TTTTGTAATTCCTACAAGAAAACCTTGGCAATTGTTCTTAATTCTAAATAATCCTATCTCAGCAAATGTGAAGAAGGGA
AGATATATTATTGTATCTCATATTAATGCAAAAAATAGGATGATGAAAAGGATACCATAAAAAATTCATACAATCGGCACA
AATCTCGATATATTTGAATTGAAAACCTTCAAAATGAAGTTTTTGATCATCCAACTTTGAGTTTAAACAACTTATATT
CGAAGTGAAGTGTTGATGAAAACGAATCTACCGATGCTACAAAATGATTCAATCCAATTCAAAATGAGCTCAAATCAAG
CTCTTAAGTTATGTTTGGAAAACCTTTTAAATATAAATTAATTGATCGAACAACCTCCAATCAAAGCATGCTCATATTCACT
CGAGCAACTAACGAGCCAAATCTAACCAAGCACAAAGTCACTTGATTGAAACATGATTAAGACATTCAAATATTGATAA
ACAATCCACGGTCAACAAGATCAATATGGACTGTACGATGAAATGCCTTGACTTATGGCCAAATTAACCAAAATGTTCA
TCTCAAAAAGCTTATAAATAGTGCCTATGGAGGTTCAATAGTTGAGAAAATTAATAATTCTCTACTTCTTAACTGT
GTCATAGCCAACTAACACCACTAACAATTATCATACCACCGCCAAGCTTTTTTCAACATATAGACATATTGTTAGGCTT
TTTTAATCATCTTTTATGAACATTAAGTTTCTATAATTCCTACAAGAACTCCGATAGTTATTCTCAATCAATAAATAATC
TCATATTTTCCAACTATGACGAAGGGAAGATTCTTATATCTTATATTATAATGGATTATTATAATTAGGCTAAGCTTCT
TGGATTTTTTTTTCAATAGACAGGATATTAACAACAATTCTACCGACACAAATTTTTTAGCAAGTAACAGTAGTAATTG
ATAAAAATTAATAAATAAATGAAATAAAAATTAATAATCACTAAATATTATTGAATTATTGAGAAAATAAATAAAGTCAATA
CAAAAAGGTTTTTCCAAGTCTTCTTAACTAATCAAGCACTAAAATGAAAATTAGTCTCATTCTGACGGCCATGCAT
GTAATAATGCTTTTACATTCACGAAATAACATATTAATCGAGTTGGGTGAAAGCAACTTTTGGAAAGGTAAGTCAAATCC
CAAATTTCTTAATAAATAAACAAGAGCTACAAACAAGTGGGTATAAATTATGATTGAAACAACACTACGTTACAGAGCA
GAGATATTGCTTCATTAGGCTTTTCCAGCTTTCTTCTTCAATTTCCCTTCTGTATTCTTAAATAAGAAGAAATG (position 0)

Figure S5. Putative regulatory *cis*-elements in the DoGES1 promoter. The CGTCA-motif is indicated in red at the -1440 position. The MYC motif is indicated in blue at +675, -731, and -1473 positions. PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) was used for the promoter analysis. The initiation codon of DoGES1 gene (ATG) is set as position 0.



Figure S6. Different developmental stages of *D. officinale* 'Zhongke 5' flowers. Three developmental stages of *D. officinale* 'Zhongke 5' flowers, includes budding, semi-open flower, and fully-open flower.