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Supplementary Material

Combined transcriptome sequencing and prokaryotic expression to investigate the key enzyme in the 2-C-methylerythritol-4-phosphate pathway of *Osmanthus fragrans*

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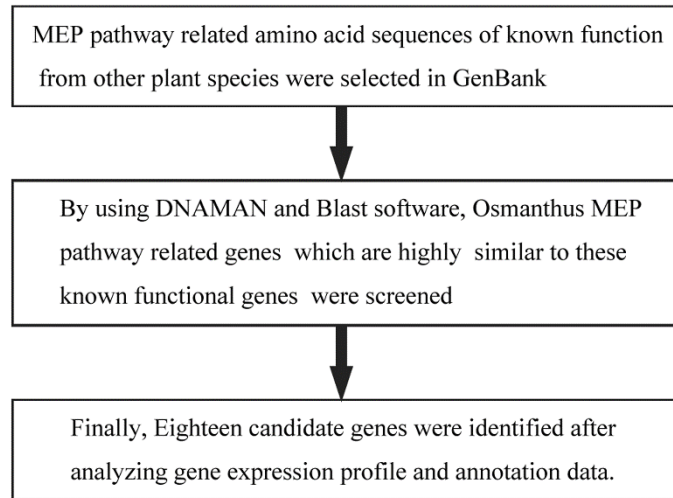


Fig. S1. Soluble expression of the fusion protein in *E. coli* BL21(DE3) detected by SDS-PAGE. M: protein maker; U: uninduced; I: induced; I1: 0.5mM IPTG; I2: 0.05mM IPTG; the arrows indicate the target protein.

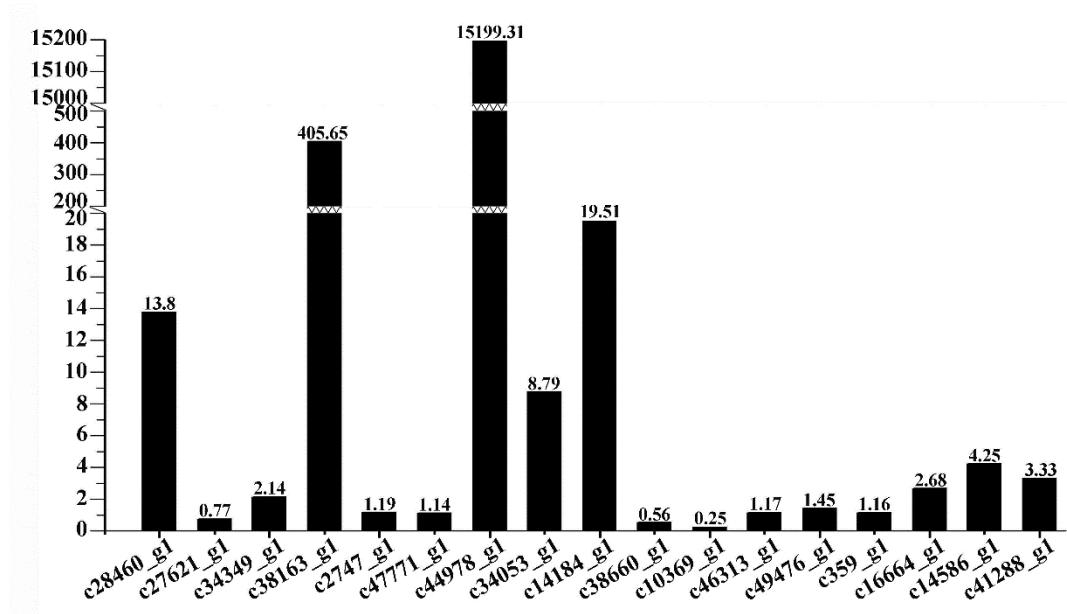


Fig. S2. Standard curve analysis of each primer pairs. A: *OfDXS1* B: *OfDXS2* C: *OfDXR1* D: *OfMCT1* E: *OfCMK1* F: *OfMDS1* G: *OfMDS2* H: *OfHDS1* I: *OfHDR1* J: *OfHDR2* K: *OfIDI1* L: *OfGGPPS1* M: *OfLSU1* N: *OfLSU2* O: *OfSSU* P: *OfTPS1* Q: *OfTPS2* R: *OfCYP450* S: *OfTPS1* T: *OfTPS2* U: *OfCYP450*.

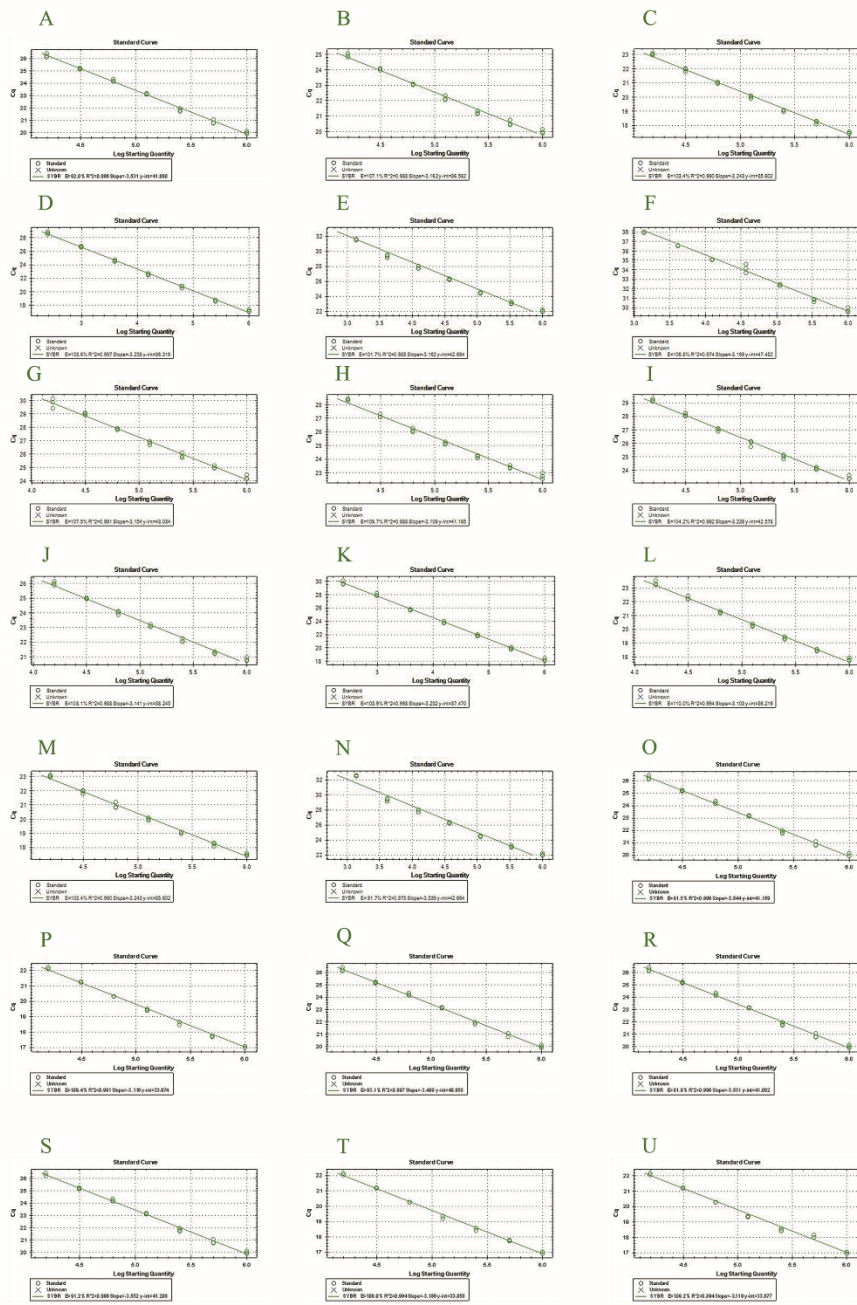


Fig. S3. Gene screening workflow.

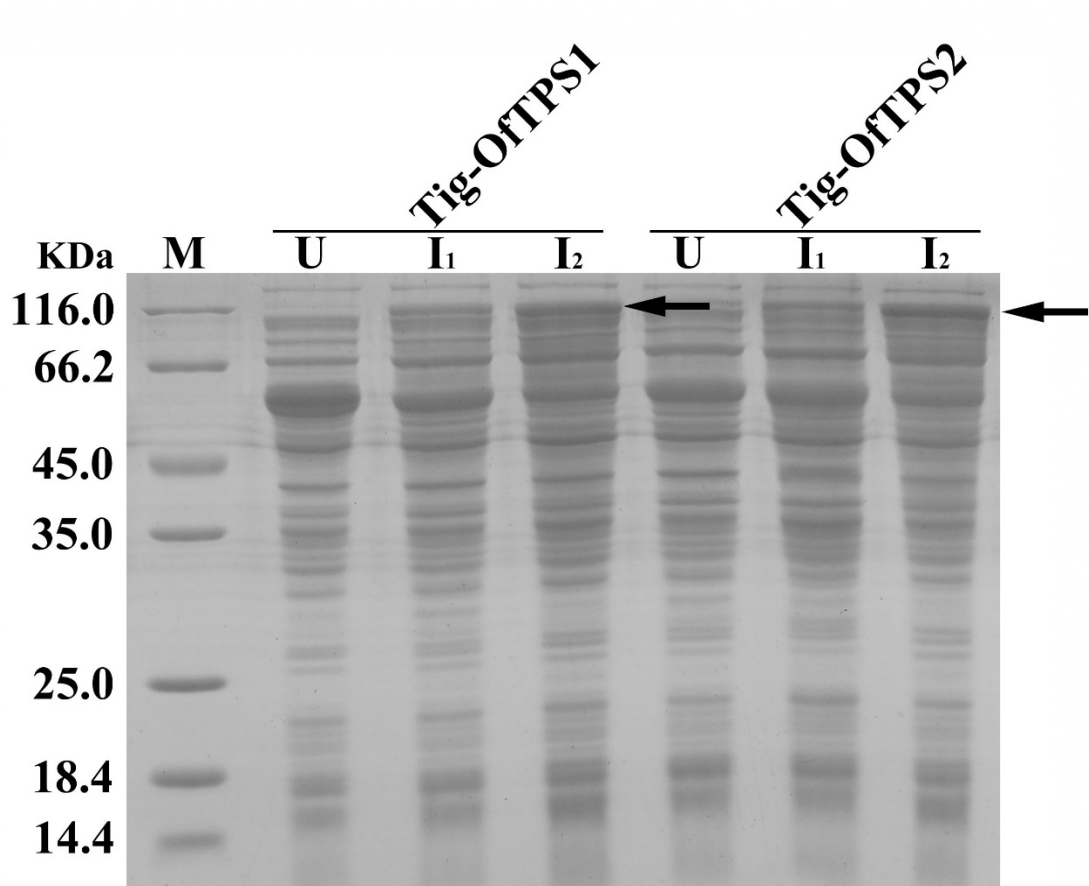


Fig. S4. Expression levels of 15 candidate TPS genes.

Table S1. List of primers used in this study

DXS: 1-deoxy-D-xylulose-5-phosphate synthase; DXR: 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MCT: 2-C-methyl-D-erythritol 4-phosphate cytidyl transferase; CMK: 4-(cytidine 59-diphospho)-2-C-methyl-D-erythritol kinase; MDS: mevalonate diphosphate decarboxylase; HDS: 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase; HDR: 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; IDI: isopentenyl diphosphate isomerase; GPPS: Homomeric Geranyl Diphosphate Synthases; LSU: Heteromeric Geranyl Diphosphate Synthases large subunit; SSU: Heteromeric Geranyl Diphosphate Synthases small subunit; TPS: Terpene synthase; CYP450: cytochrome P450

Primers used in qRT-PCR

Number	Primers	Sequences
1	18sRNA F	CCAGCACCACTGACCGAAGA
2	18sRNA R	TGAGCACGGCATAGACCCAAC
3	OfDXS1 F	GCAGAAGCGGAAGCAGACAAGG
4	OfDXS1 R	TTAATGCCTTCGCAGGACAAGCC
5	OfDXS2 F	AAGCCGTTCTGTGCCATCTATTCG
6	OfDXS2 R	CGGAGCCATCACCACCATGTTG
7	OfDXR1 F	AGCCACCTCCTCCAGCTTG
8	OfDXR1 R	TGGTCGGCAAGAAGAGTCACATTC
9	OfMCT1 F	GCCCGGAAAGGAGAGACAAG
10	OfMCT1 R	TGGCAGGAACACCTAGGACA
11	OfCMK1 F	GGTAGTAGTAATGCCGCCACAGC
12	OfCMK1 R	CGCCTGACCATTCTTGAGTTCC
13	OfMDS1 F	AGTGCAGTGGAAGCCGAGACTC
14	OfMDS1 R	GTGAGCTTCACAGCCTCGATCATG
15	OfMDS2 F	GTGGAAGCAGAGACGCAGAAGTC

16	OfMDS2 R	AGCAACACATCACCATCGGAGTG
17	OfHDS1 F	GAGCTTCAGCAAGGAGTGGAACC
18	OfHDS1 R	CAGAGCCATCACGATGAAGGACAC
19	OfHDR1 F	CTCCTCCTCGTCGGCTACTCTG
20	OfHDR1 R	ATGTCACCTTGTTACTCGCGGTTT
21	OfHDR2 F	CTCACGAAGAGACCGTAGCAACTG
22	OfHDR2 R	AACGTATGTCGCCTCTGCCATG
23	OfIDI1 F	GCCTCCTATTCGTCTGTCGCTTC
24	OfIDI1 R	CATAAGGCGTCTCTGGACAGCATC
25	OfGGPPS1 F	TGACTTCACTGGCACATCAACTTCC
26	OfGGPPS1 R	GATCGACAACAACCTCTCAGCTCAGG
27	OfLSU1 F	CTCGCCACAGAACATGGAGAAGAG
28	OfLSU1 R	ACCAGCAAGGATGGAATACCTCATTG
29	OfLSU2 F	AGATCCACGAAGCAATGCGGTATTC
30	OfLSU2 R	GCTCACAGGCGGCGATACATAAC
31	OfSSU1 F	ATCAAGTGGTGGACGATATGTTGGAAG
32	OfSSU1 R	CAACCTTCTCACCGTACTTCTCTAAGC
33	OfTPS1 F	CGCAGAATGCAGAGGCTAGA
34	OfTPS1 R	GCATGGATTGGACCATGTTG
35	OfTPS2 F	TGGCTCAAGACACCATAGGC
36	OfTPS2 R	AAGGCAGAACCAACTTGTCG
37	OfCYP4501 F	TGAATTCGGTTCACGGACAC
38	OfCYP4501 R	AGTCATTATCCGCCGCATCT

Primers used in Prokaryotic expression

Number	Primers	Sequences
1	OfTPS1 F	CCGGATCCGCCATGAACGAACTAACAATTTGAGTACATATCAACAG

2	OfTPS1 R	CGCTCGAGTTATGGCTGTGTCAATTGAATGGGCTCAATAATAACCGAC
3	OfTPS2 F	CCGGATCCTCGCCTAGTGTAACGCCATTGATTC
4	OfTPS2 R	CGCTCGAGTTAATTAATAGGAGTGAAGAACAAAGCCTGCACATAATTATAGACGCTGG

Primers used in Subcellular Localization

Number	Primers	Sequences
1	OfTPS1 F	GACTAGTATGGCACTTACCACCCTACTCCTAC
2	OfTPS1 R	CGGGATCCTGGCTGTGTCAATTGAATGG
3	OfTPS2 F	GACTAGTATGGAGTGTACAATGAGGAGTATTCCTTGTTTTCATCTAATG
4	OfTPS2 R	CGGGATCCATTAATAGGAGTGAAGAACAAAGCCTG

Table S2. The Pfam of OfTPS gene family member

The Pfam of OfTPS			
Number	Gene ID	Pfa	
1	c38163_g1	PF01397	PF03936
2	c44978_g1	PF01397	PF03936
3	c28460_g1	PF01397	
4	c27621_g1	PF01397	PF03936
5	c34349_g1	PF01397	PF03936
6	c2747_g1	PF01397	
7	c47771_g1	PF01397	PF03936
8	c34053_g1	PF01397	PF03936
9	c14184_g1		PF03936
10	c38660_g1	PF01397	
11	c10369_g1		PF03936
12	c46313_g1		PF03936
13	c359_g1		PF03936
14	c16664_g1	PF01397	
15	c41288_g1	PF01397	