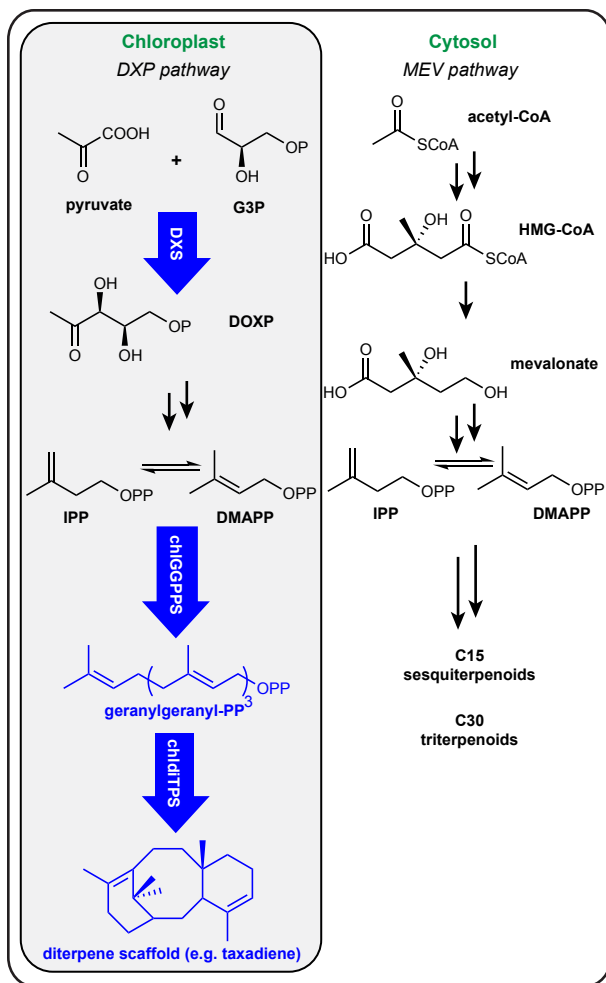
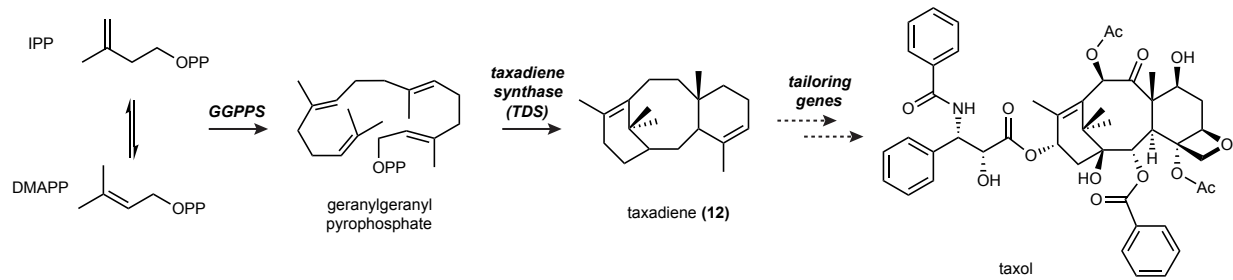


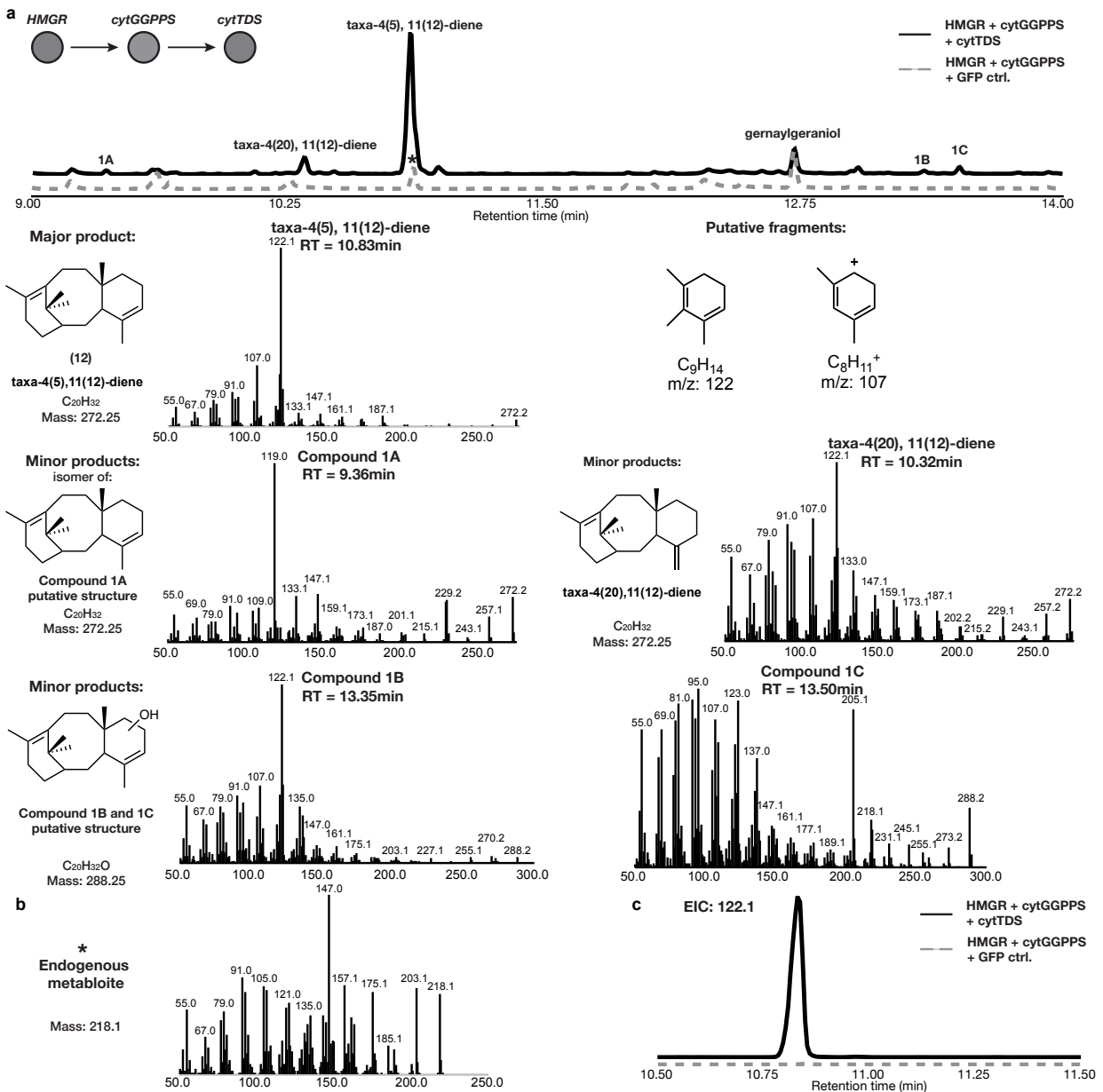
Supplementary Figure 1. Terpenoid biosynthesis in a plant cell can proceed via the chloroplast localized DXP pathway and the cytosol localized MEV pathway. G3P, glyceraldehyde 3-phosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate synthase; IPP, isopentyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.



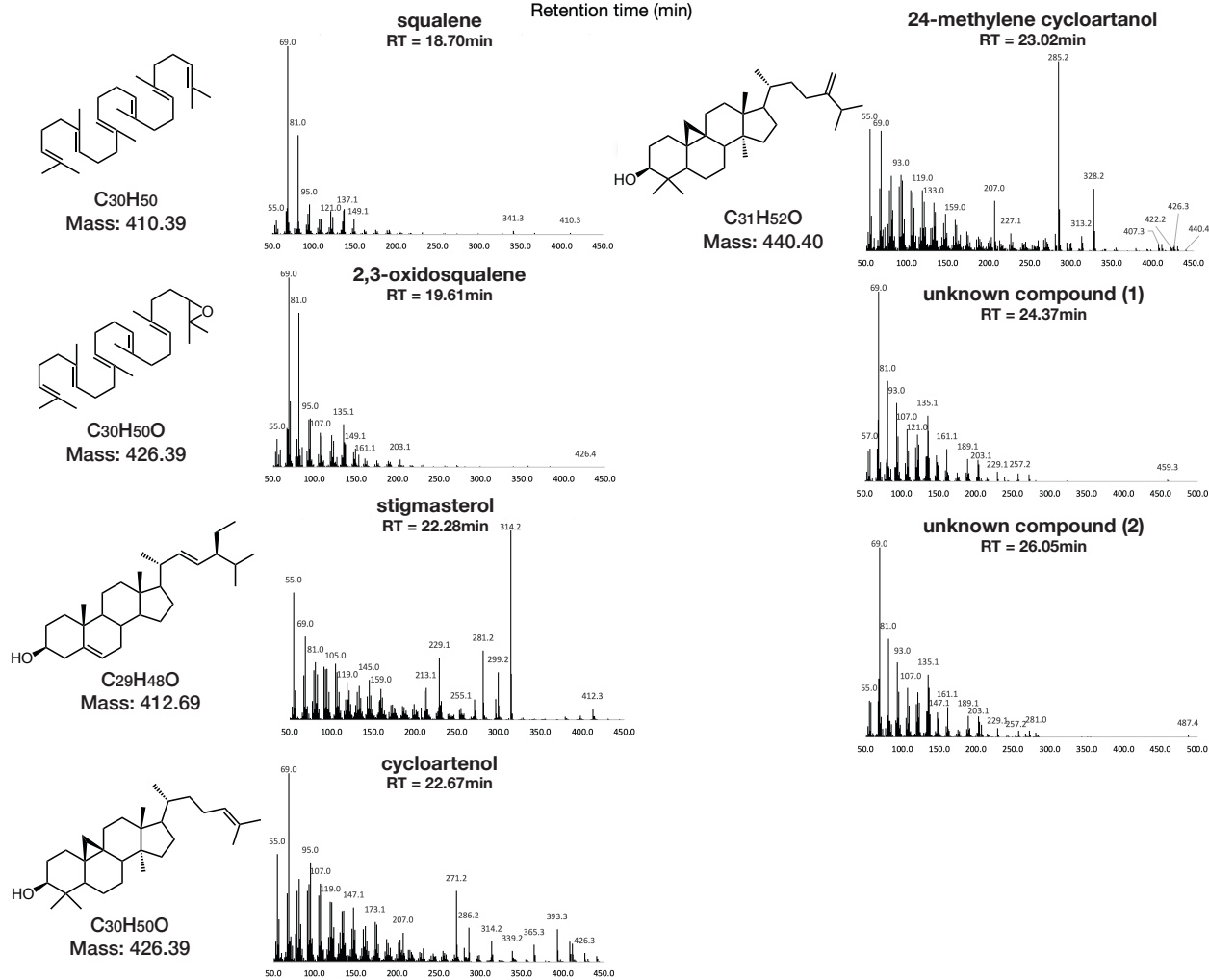
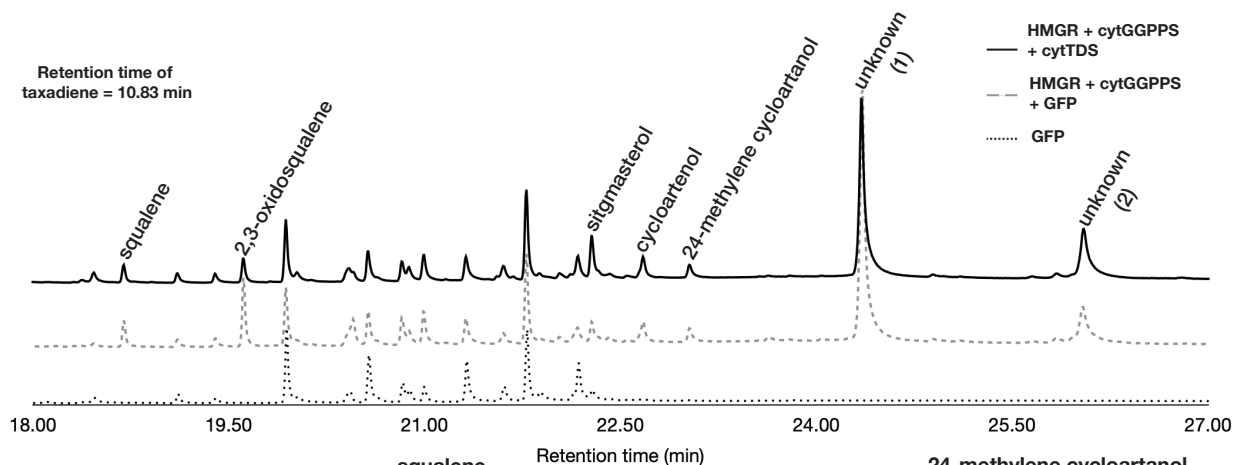
Supplementary Figure 2. Yield improvement of diterpenoids through the chloroplastic, DXP pathway requires overexpression of rate limiting gene DXS. Arrows in blue represent chloroplast localized and overexpressed genes.. G3P, glyceraldehyde 3-phosphate; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DOXP, 1-deoxy-D-xylulose-5-phosphate synthase; IPP, isopentyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; chIGGPPS, chloroplastic geranylgeranyl pyrophosphate synthase; chdiTPS, chloroplastic diterpene synthase(s); HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA;



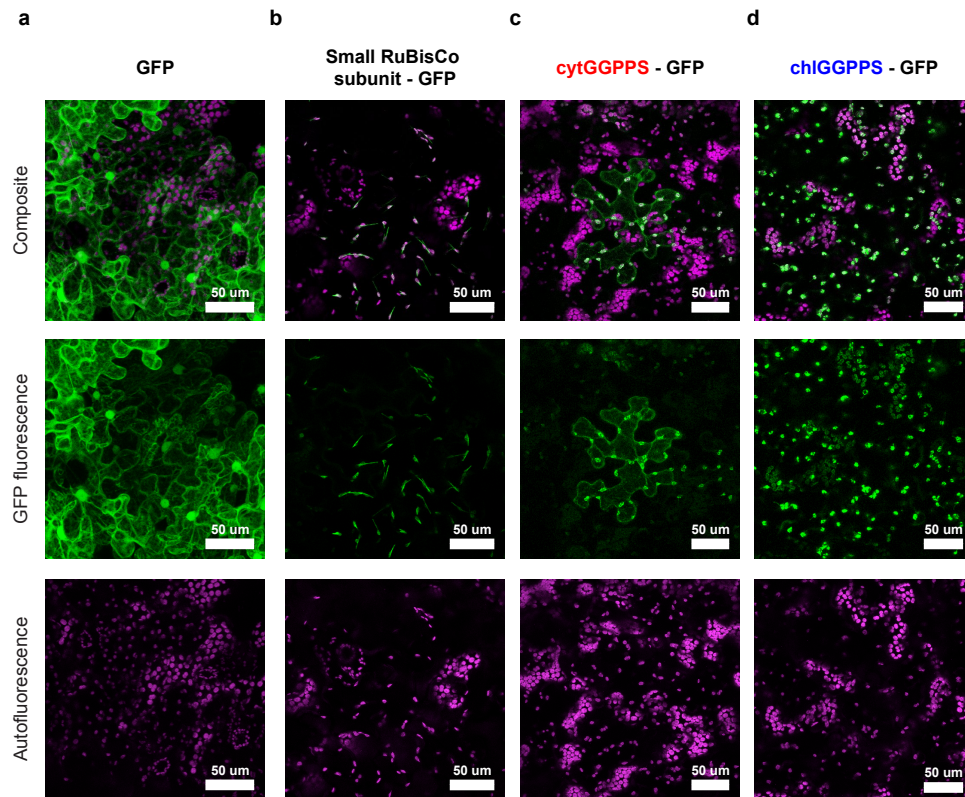
Supplementary Figure 3. Simplified taxol biosynthetic pathway



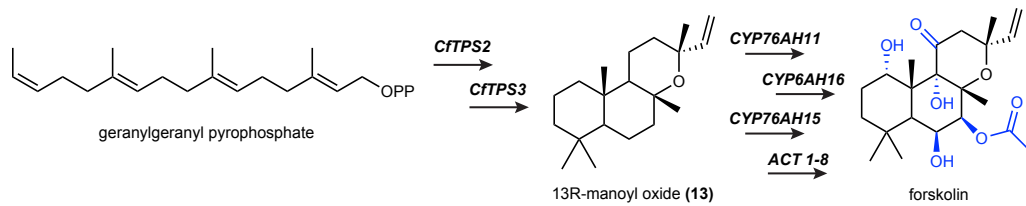
Supplementary Figure 4: Characterization of intermediates from overexpression of *cytTDS* via MEV engineering. (a) GC-MS total ion chromatogram (TIC) and MS spectra of products from overexpression of *HMGR*, *cytGGPPS* and *cytTDS* in *N. benthamiana*. Representative TICs are shown. Five products with m/z corresponding to diterpene derived scaffolds were observed upon expression of *cytTDS*. Identified products corresponded to **12**, isomer, taxadiene-4(20), 11(12)-diene, and three minor taxadiene-derived scaffolds⁵⁷. Putative ion structures for **12** were assigned as reference. Putative structures are preliminary and based on predicted formulas combined with analysis of fragment ions observed in MS spectra. An endogenous metabolite co-eluting with taxadiene was identified (* in the TIC). **(b)** MS spectra of endogenous metabolite. **(c)** GC-MS extracted ion chromatogram (EIC) using m/z = 122.1, corresponding an abundant fragment found in the MS of **12**, confirmed that **12** is only present upon *TDS* expression.



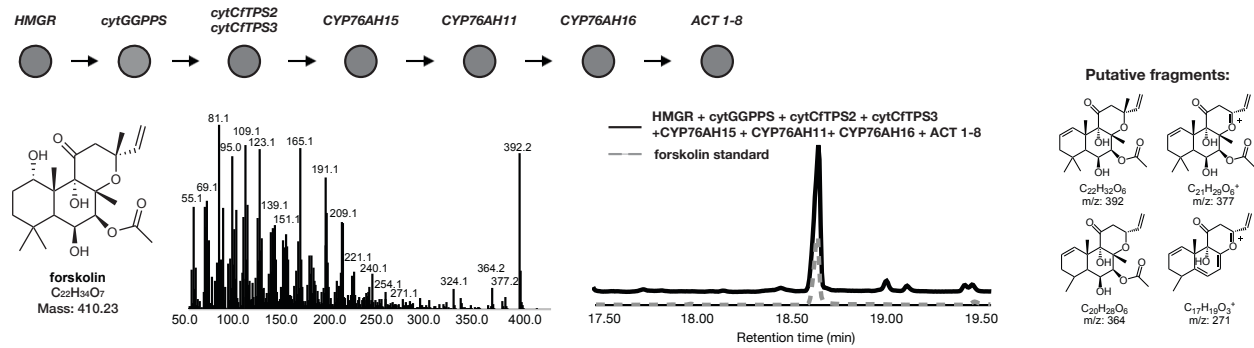
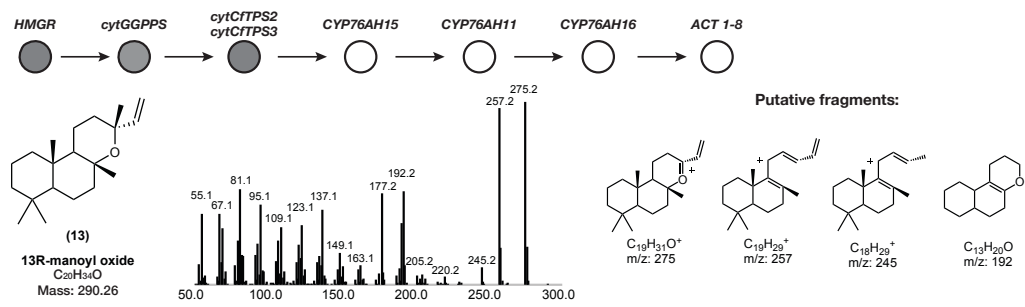
Supplementary Figure 5: Overexpression of *cytGGPPS* and *HMGR* alters endogenous triterpenoid metabolism. GC-MS total ion chromatogram (TIC) and MS spectra of triterpenoid derived products observed in *N. benthamiana*. Overexpression of *cytGGPPS* and *HMGR* results in the accumulation of endogenous phytosterols in leaf extracts independent of co-expression with *cytTDS*. Assignments for labeled peaks was done via comparison against MS spectra from previous reports⁵⁸.



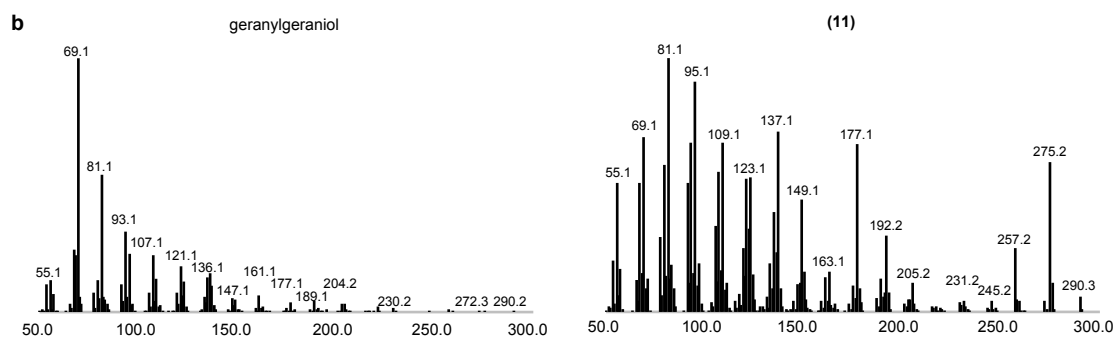
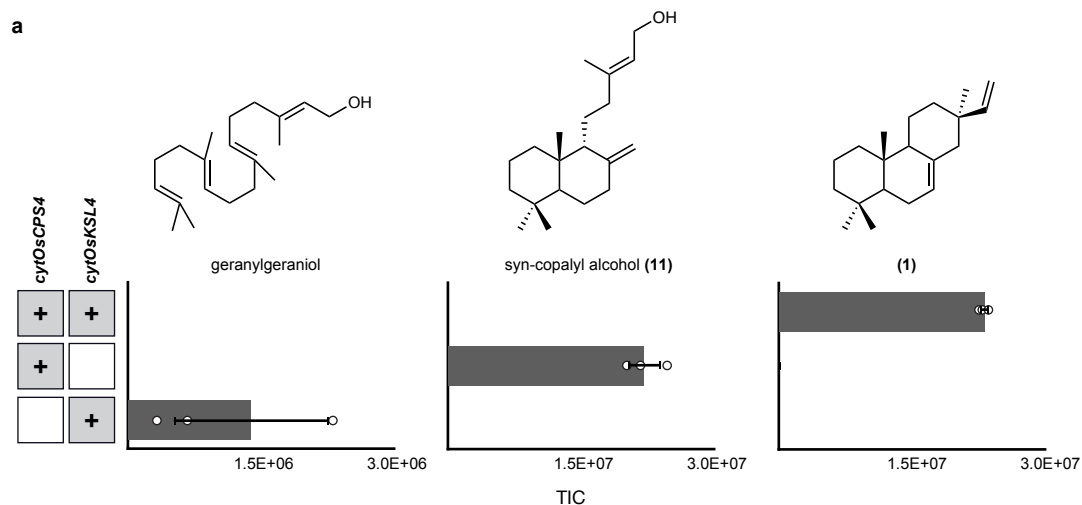
Supplementary Figure 6: Subcellular localization of overexpressed cytGGPPS-GFP and chlGGPPS-GFP fusion proteins and controls in *N. benthamiana*. (a) GFP localization and a (b) GFP fusion construct of the small subunit of RuBisCo indicate respective cytosolic and plastid localization. (c) Partial cytosolic localization of cytGGPPS-GFP was observed via the truncation of the predicted chloroplast transit peptide of GGPPS. (d) chlGGPPS-GFP overexpression resulted in plastid localization. Images of subcellular localization were chosen. All images show z-stacks of maximum intensity projections scaled to the same intensity range for GFP fluorescence (green; 488nm excitation, 500 - 550 emission), chlorophyll autofluorescence (magenta; 488nm excitation, 650nm excitation, 650 - 700 emission) and the two channel composite. Images are representative of three biological replicates repeated over two independent experiments.



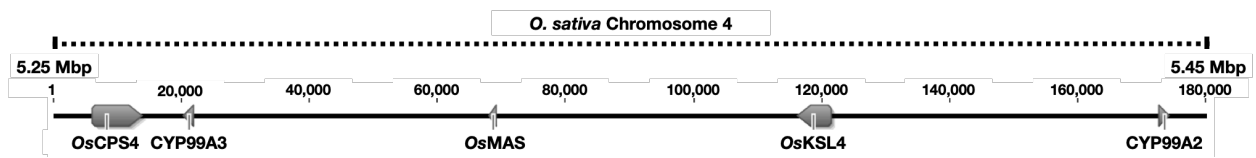
Supplementary Figure 7: Previously established forskolin biosynthetic pathway.



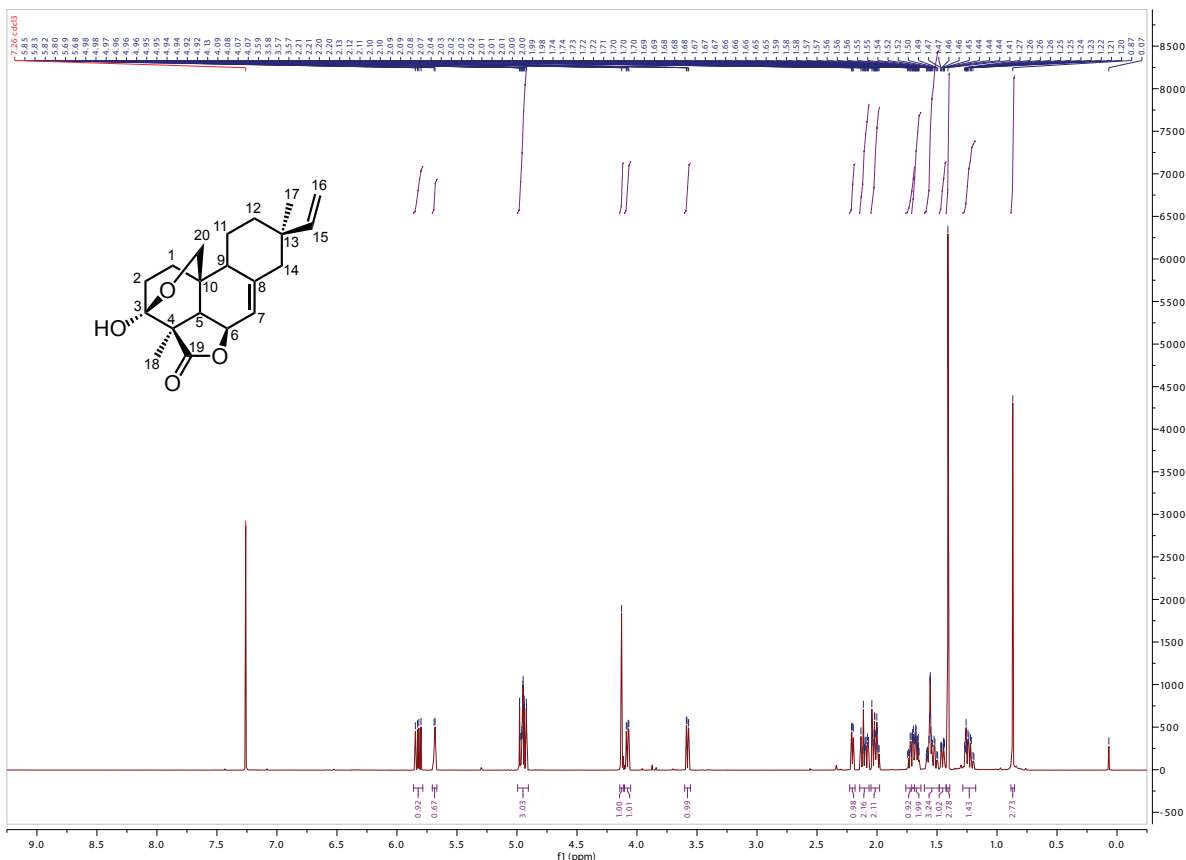
Supplementary Figure 8: MS spectra of 13R-manoyl oxide and forskolin. Putative ion structures for both products were assigned as reference.



Supplementary Figure 9: Characterization and yields of pathway intermediates upon overexpression of either *cytoCPS4* or *cytoKSL4* via cytosolic engineering. (a) Average GC-MS ion abundances of syn-pimaradiene precursors after overexpression of indicated genes via cytosolic engineering. Values and error bars represent the mean and the standard deviation of biological triplicates. (b) MS spectra of geranylgeraniol and **11.**



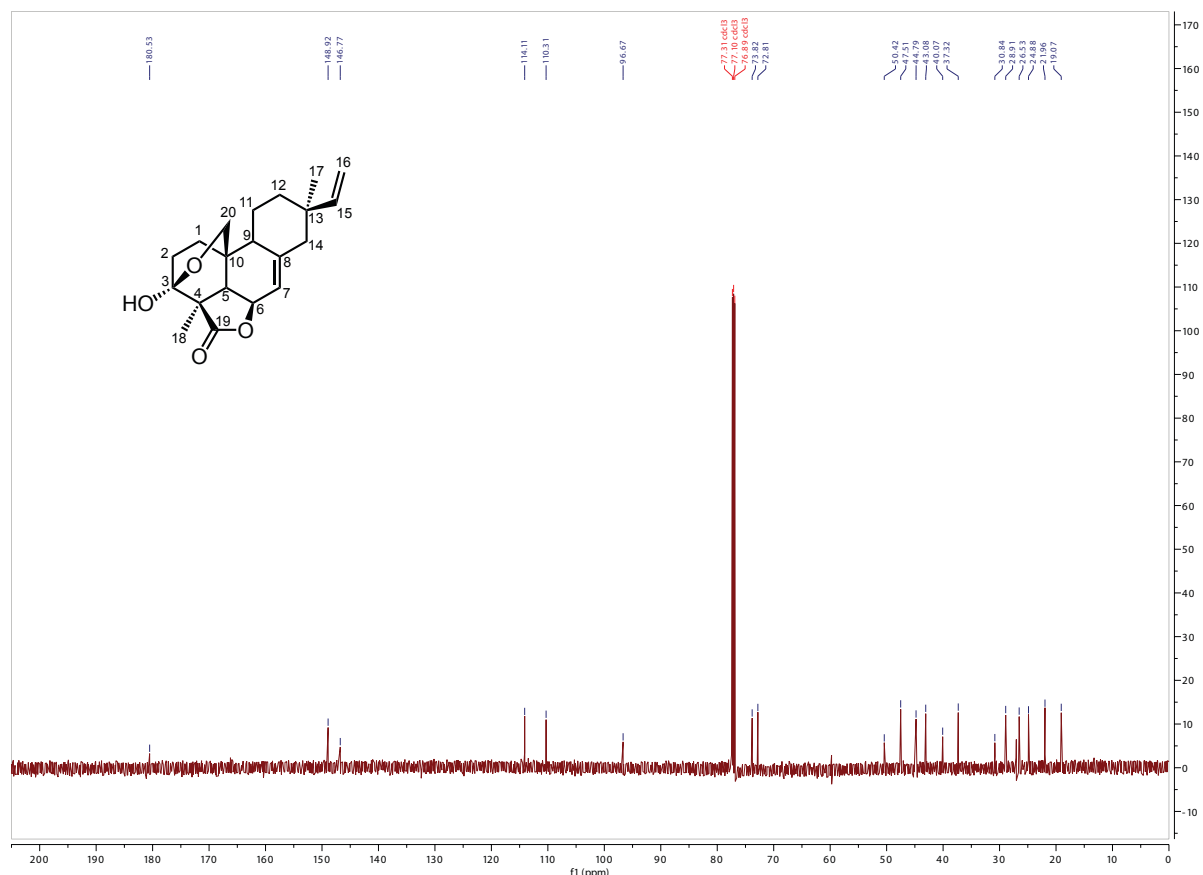
Supplementary Figure 10: Depiction of momilactone gene cluster on *Oryza sativa* chromosome 4. *OsCPS4*, *OsKSL4*, *CYP99A3* and *OsMAS* have previously been directly implicated in momilactone biosynthesis.



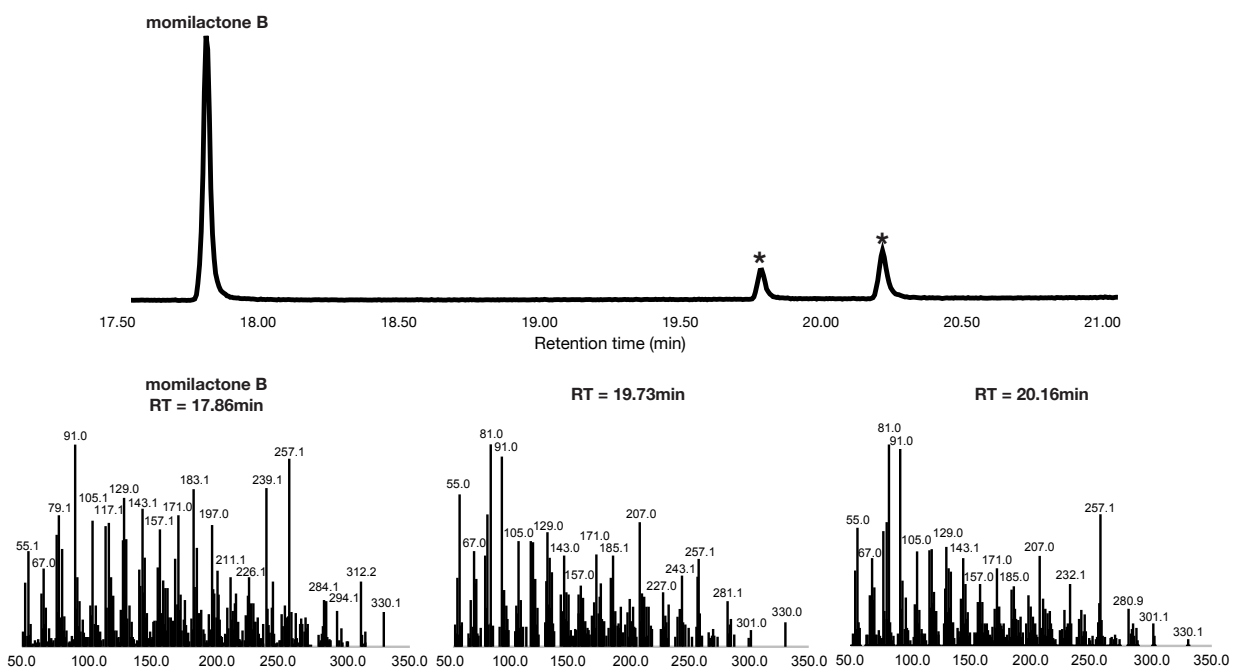
Proton	¹ H assignments (this study)			¹ H assignments ¹⁰		
	Shift (ppm)	Multiplicity	Coupling (Hz)	Shift (ppm)	Multiplicity	Coupling (Hz)
3-OH	4.13	bS			Not reported	
5-H	2.2	dd	6.8 and 2.1	2.2	dd	7.1 and 2.1
6-H	4.96	bd	4.3	4.94	dd	7.1 and 4.5
7-H	5.69	d	4.9	5.68	d	4.5
9-H	1.76 - 1.69	m			Not reported	
15-H	5.82	dd	17.4 and 10.8	5.83	dd	17.6 and 10.5
16-H _A	4.93	dd	10.7 and 1.0	4.92	dd	10.5 and 1.2
16-H _B	4.96	dd	17.4 and 1.0	4.95	dd	17.6 and 1.2
17-H ₃	0.87	S		0.87	S	
18-H ₃	1.41	S		1.4	S	
20-H _A	3.58	dd	9.2 and 2.1	3.55	dd	9.0 and 2.1
20-H _B	4.08	dd	9.2 and 3.4	4.07	bd	9.0

¹H NMR (600 MHz, Chloroform-*d*) δ 5.82 (dd, $J = 17.4, 10.8$ Hz, 1H), 5.69 (d, $J = 4.9$ Hz, 1H), 4.96 (dd, $J = 17.4, 1.0$ Hz, 1H), 4.96 (bd, 1H), 4.93 (dd, $J = 10.7, 1.0$ Hz, 1H), 4.13 (bs, 1H), 4.08 (dd, $J = 9.2, 3.4$ Hz, 1H), 3.58 (dd, $J = 9.2, 2.1$ Hz, 1H), 2.20 (dd, $J = 6.8, 2.1$ Hz, 1H), 2.14 – 2.07 (m, 2H), 2.05 – 1.98 (m, 2H), 1.76 – 1.69 (m, 1H), 1.71 – 1.63 (m, 2H), 1.60 – 1.48 (m, 2H), 1.48 – 1.42 (m, 1H), 1.41 (s, 3H), 1.28 – 1.18 (m, 1H), 0.87 (s, 3H).

Supplementary Figure 11: ¹H NMR spectra of momilactone B in CDCl₃ isolated from *N. benthamiana*.



Supplementary Figure 12: ^{13}C NMR spectra of momilactone B in CDCl_3 isolated from *N. benthamiana*.



Supplementary Figure 13: TIC from purified momilactone B from large scale isolation in *N. benthamiana*. Two impurities were not separable by silica-gel chromatography. Based on MS fragmentation patterns, these impurities possibly correspond to structural isomers of momilactone B or the ring-opened form of the lactol.

Supplementary Table 1: List of potential momilactone biosynthetic gene candidates.

	Name	Family	Gene ID	Chromosome	Rank				
					Bait: OsCPS4	Bait: CYP99A3	Bait: OsMAS	Bait: CYP99A2	Bait: OsKSL4
1	CYP99A3	P450	Os04g0178400	4*	3	N/A	76	26	271
2	CYP701A8	P450	Os06g0569500	6	4	6	89	7	154
3	CYP99A2	P450	Os04g0180400	4*	8	28	33	N/A	98
4	CYP701A9	P450	Os06g0568600	6	22	39	69	49	323
5	CYP71Z8	P450	Os10g0439800	10	31	84	7	18	230
6	CYP71Z5	P450	Os02g0529800	2	55	77	165	32	175
7	CYP94E3	P450	Os05g0382500	5	94	210	14	58	281
8	CYP76M14	P450	Os01g0561600	1	172	338	67	135	409
9	CYP76M8	P450	Os02g0569400	2^	620	581	882	273	4
10	CYP76M5	P450	Os02g0569000	2^	341	249	711	194	72
11	2-ODD candidate #1	2-ODD	Os09g0245500	9	48	12	232	139	503
12	2-ODD candidate #2	2-ODD	Os04g0662600	4	245	464	88	136	306
13	SDR candidate #1	SDR	Os07g0664000	7	16	27	42	89	558
14	OsMAS	SDR	Os04g0179200	4*	17	69	N/A	22	309
15	SDR candidate #2	SDR	Os07g0663700	7	66	158	20	43	24
16	OsCPS4	Terpene synthase	Os04g0178300	4*	N/A	3	19	8	243
17	OsKSL8	Terpene synthase	Os11g0474800	11	47	81	112	102	447
18	OsKSL7	Terpene synthase	Os02g0570400	2^	111	61	332	68	103
19	OsKSL4	Terpene synthase	Os04g0179700	4*	386	392	532	125	N/A

List is ranked via Pearson's correlation coefficient between genes present in the momilactone biosynthetic cluster and biosynthetic candidates. Potential biosynthetic genes shown correspond only to genes belonging to CYP, 2-ODD, SDR, and terpene synthase families. Gene names in bold correspond to genes involved in momilactone biosynthesis. A * or a ^ indicate genes present in either the momilactone gene cluster or the phytocassane gene cluster.

Supplementary Table 2: Yields of diterpenoid products engineered in this study.

Pathway	Compound	Compound use for standard	MEV Eng.		DXP Eng.		Plants	Plant weight (g)	Isolated (mg)	Isolated yield	
			mg/g FW	mg/g DW ¹	mg/g FW	mg/g DW ¹				ug/g FW	ug/g DW ¹
taxane biosynthesis	taxadiene (12)	5a-taxadiene-ol	0.13 ± 0.03	1.27 ± 0.31	0.02 ± 0.00	0.20 ± 0.03	-	-	-	-	-
momilactone biosynthesis	syn-pimaradiene (1)	GGOH	0.13 ± 0.06	1.32 ± 0.57	0.01 ± 0.01	0.13 ± 0.07	-	-	-	-	-
	(6)	momilactone B	0.06 ± 0.01	0.63 ± 0.10	-	-	-	-	-	-	-
	momilactone A (8)	momilactone B	0.03 ± 0.02	0.28 ± 0.15	-	-	-	-	-	-	-
	(7)	momilactone B	0.01 ± 0.01	0.12 ± 0.07	-	-	-	-	-	-	-
	momilactone B	momilactone B	0.02 ± 0.01	0.17 ± 0.08	-	-	80	216	3.6	16.7	167
forskolin biosynthesis	13-R-manoyl oxide (13)	GGOH	0.67 ± 0.07	6.67 ± 0.69	0.06 ± 0.01	0.57 ± 0.13	-	-	-	-	-
	forskolin	forskolin	0.13 ± 0.03	1.27 ± 0.26	0.02 ± 0.01	0.19 ± 0.09	-	-	-	-	-

¹Fresh to dry weight calculations correspond to typically observed ~90% weight loss after lyophilization of *N. benthamiana* leaves.

Supplementary Table 3: Germination efficiency of *A. thaliana* seeds (n=100).

Time days	DMSO mock	SA 1 mM	momilactone B		
			2.5 uM	12.5 uM	60 uM
0	0%	0%	0%	0%	0%
3	99%	0%	0%	0%	0%
5	99%	0%	97%	0%	0%
7	99%	0%	97%	0%	0%
10	99%	0%	97%	81%	0%

Supplementary Table 4: List of PCR primer pairs used in this study.

Purpose	Name	Primer Sequence (5' to 3')	
Metabolic engineering	HMGR	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGAAGAAAAAGCAAGCTGG
		Rev	GAAACCAGAGTTAAAGGCCTCGAGTCATGTTGTTGTTGTCGTTGTCCG
	chlGGPPS	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAGTCAGTTTTGCCTGAATGCAATG
	cytGGPPS	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGGCTTCTTATCAAGAATGC
		Rev	GAAACCAGAGTTAAAGGCCTCGAGTCAGTTTTGCCTGAATGCAATG
	DXS	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGGCTTCTTCTGCATTTC
		Rev	GAAACCAGAGTTAAAGGCCTCGAGTCAAAACAGAGCTCCCTTGG
Taxane biosynthesis	chlTDS	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAGTCATACTTGAATTGGATCAATATAAACTTTTC
	cytTDS	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGGTAATGATGAGCAGCAG
		Rev	GAAACCAGAGTTAAAGGCCTCGAGTCATACTTGAATTGGATCAATATAAACTTTTC
Forskolin biosynthesis	chlCfTPS2	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	(22-773)cytCfTPS2	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGAACAGCAATAAAAGGCAGTC
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	(46-773)cytCfTPS2	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGGTTGCAAGTCTGGATGCG
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	chlCfTPS3	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	(23-598)cytCfTPS3	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGCTGCAGCTGTTAAATGC
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	(38-598)cytCfTPS3	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGGGAATCTTGCCAGTCCACC
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	CYP76AH15	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	CYP76AH11	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	CYP76AH16	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
ACT 1-8	Fwd	ATTCTGCCCAAATTCGCGACCGGT	
	Rev	GAAACCAGAGTTAAAGGCCTCGAG	
Momilactone biosynthesis	chlOsCPS4	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	cytOsCPS4	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGCCGCCGTACCCGGCC
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	chlOsKSL4	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	cytOsKSL4	Fwd	ATTCTGCCCAAATTCGCGACCGGTATGGCGGTGATGTCGTCCTG
		Rev	GAAACCAGAGTTAAAGGCCTCGAG
	CYP99A3	Fwd	ATTCTGCCCAAATTCGCGACCGGT
		Rev	GAAACCAGAGTTAAAGGCCTCGAG

Purpose	Name	Primer Sequence
Momilactone biosynthesis	CYP76M8	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	OsMAS	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	CYP76M14	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	CYP701A8	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
Momilactone biosynthesis gene candidates	CYP99A2	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	CYP701A9	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	CYP71Z8	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	CYP71Z5	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	CYP94E3	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	CYP76M5	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	2-ODD candidate #1	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
	2-ODD candidate #2	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GAAACCAGAGTTAAAGGCCTCGAG
SDR candidate #1	Fwd ATTCTGCCCAAATTCGCGACCGGT	
	Rev GAAACCAGAGTTAAAGGCCTCGAG	
SDR candidate #2	Fwd ATTCTGCCCAAATTCGCGACCGGT	
	Rev GAAACCAGAGTTAAAGGCCTCGAG	
GFP fusion constructs	GFP	Fwd GGTTCCGGGAAGCATGACTAGCAAAGGAGAAGAAC
		Rev GAAACCAGAGTTAAAGGCCTCGAGTTATTTGTATAGTTCATCCATGCC
	Gibson overlaps chITDS-GFP	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GTTCTTCTCCTTTGCTAGTCATGCTTCCCGAACCTACTTGAATTGGATCAATATAAC
	Gibson overlaps cytTDS-GFP	Fwd ATTCTGCCCAAATTCGCGACCGGT ATGGTAATGATGAGCAGCAG
		Rev GTTCTTCTCCTTTGCTAGTCATGCTTCCCGAACCTACTTGAATTGGATCAATATAAC
	Gibson overlaps chlGGPPS-GFP	Fwd ATTCTGCCCAAATTCGCGACCGGT
		Rev GTTCTTCTCCTTTGCTAGTCATGCTTCCCGAACCGTTTTGCCTGAATGCAATGTAATC
	Gibson overlaps cytGGPPS-GFP	Fwd ATTCTGCCCAAATTCGCGACCGGT ATGGCTTCTTCAAGAATGC
		Rev GTTCTTCTCCTTTGCTAGTCATGCTTCCCGAACCGTTTTGCCTGAATGCAATGTAATC
Gibson overlaps Small RuBisCo subunit - GFP	Fwd ATTCTGCCCAAATTCGCGACCGGT ATGGCTTCTTCTATGCTCTCTTCC	
	Rev GTTCTTCTCCTTTGCTAGTCATGCTTCCCGAACCTTCGGAATCGGTAAGGTCAGG	

¹Nucleotides emphasized in **bold** represent those which hybridize to the gene of interest. Nucleotides emphasized in *italics* represent those which hybridize to the four peptide linker used to link fusion protein constructs. All other nucleotides consist of the 5' overlaps designed for Gibson assembly with pEAQ-HT vector. Gene accession numbers and IDs can be found in Supplementary Table 5.

Supplementary Table 5: Accession numbers and gene IDs used in this study.

Name	Organism	Gene ID / Accession	Reference
HMGR	<i>Nicotiana tabacum</i>	AT1G76490.1 / AY488113	51
GGPPS	<i>Taxus canadensis</i>	AF081514	60
DXS	<i>Arabidopsis thaliana</i>	AT4G15560.1 / BT002340	52
TDS	<i>Taxus brevifolia</i>	U48796	38
CFTPS2	<i>Plectranthus barbatus</i>	KF444507	42
CFTPS3	<i>Plectranthus barbatus</i>	KF444508	
CYP76AH15	<i>Plectranthus barbatus</i>	KT382358	20
CYP76AH11	<i>Plectranthus barbatus</i>	KT382349	
CYP76AH16	<i>Plectranthus barbatus</i>	KT382359	
ACT 1-8	<i>Plectranthus barbatus</i>	KT382363	
OsCPS4	<i>Oryza sativa</i>	Os04g0178300 / AB066270	25, 26
OsKSL4	<i>Oryza sativa</i>	Os04g0179700 / AB126934	27, 28
CYP99A3	<i>Oryza sativa</i>	Os04g0178400 / AK071864	29
CYP76M8	<i>Oryza sativa</i>	Os02g0569400 / XM_015768144.1	30
OsMAS	<i>Oryza sativa</i>	Os04g0179200 / XM_015778721.1	32
CYP76M14	<i>Oryza sativa</i>	Os01g0561600 / XM_015773010.1	This study
CYP701A8	<i>Oryza sativa</i>	Os06g0569500 / XM_015785419.1	43
CYP99A2	<i>Oryza sativa</i>	Os04g0180400 / XM_015778482.1	-
CYP701A9	<i>Oryza sativa</i>	Os06g0568600 / XM_015786143.1	-
CYP71Z8	<i>Oryza sativa</i>	Os10g0439800 / XM_015759387.1	-
CYP71Z5	<i>Oryza sativa</i>	Os02g0529800 / XM_015768673.1	-
CYP94E3	<i>Oryza sativa</i>	Os05g0382500 / XM_015782035.1	-
CYP76M5	<i>Oryza sativa</i>	Os02g0569000 / XM_015768639.1	30
2-ODD candidate #1	<i>Oryza sativa</i>	Os09g0245500 / XM_015755150.1	-
2-ODD candidate #2	<i>Oryza sativa</i>	Os04g0662600 / XM_015779149.1	59
SDR candidate #1	<i>Oryza sativa</i>	Os07g0664000 / XM_015790180.1	-
SDR candidate #2	<i>Oryza sativa</i>	Os07g0663700 / XM_015791272.1	-
Small RuBisCo subunit	<i>Arabidopsis thaliana</i>	RBCS1A / AT1G67090.1	-

Supplementary Table 6: Chloroplast transit peptide predictions using ChloroP and LOCALIZER.

Gene	Chloroplast transit peptide residues	
	ChloroP	LOCALIZER
GGPPS	(1 - 18)	(1 - 41)
TDS	(1 - 58)	(1 - 51)
<i>Cf</i>TPS2	(1 -45)	(1 - 21)
<i>Cf</i>TPS3	(1 - 37)	(1 - 22)
OsCPS4	(1 - 56)	(1 - 52)
OsKSL4	(1 - 81)	(1 - 73)

¹ Cells in gray indicate the truncated gene variant used in this study.

Supplementary Table 7: Table of Pfam numbers used to find momilactone biosynthetic genes

Family	Pfam #	ID
CYP450s	PF00067	p450
2-ODDs	PF03171	2OG-Fell_Oxy
	PF13532	2OG-Fell_Oxy 2
	PF13640	2OG-Fell_Oxy 3
	PF13661	2OG-Fell_Oxy 4
	PF13759	2OG-Fell_Oxy 5
	PF10014	2OG-Fe_Oxy_2
	PF16870	OxoGdeHyase_C
SDRs	PF00106	Adh_short
Terpene synthase	PF01397	Terpene_synth
	PF03936	Terpene_synth_C
Other PFAMs	PF01370	Epimerase
	PF01073	3Beta_HSD