Supplementary information

From Gene Discovery to Synthetic Biology: Recent Advances in the Biosynthesis of Oleanane -Type Pentacyclic Triterpenoids

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Table S1. Oleanane-type Pentacyclic Triterpenoid Biosynthetic Cytochrome P450 Genes

Gene Name	Substrate	Carbon Modification	Heterologous Host	Plant Name	Compound Name	Reference
CYP716A244	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Eleutherococcus senticosus*	Oleanolic acid	[1]
CYP716A48	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Olea europaea*	Oleanolic acid	[2]
CYP716C80	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Camptotheca acuminata*	Oleanolic acid	[3]
CYP716C81	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Camptotheca acuminata*	Oleanolic acid	[3]
CYP716C82	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Camptotheca acuminata*	Oleanolic acid	[3]
CYP716A254	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Anemone flaccida*	Oleanolic acid	[4]
CYP716A252	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Ocimum basilicum*	Oleanolic acid	[5]

CYP716A253	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Ocimum basilicum*	Oleanolic acid	[5]
CYP716A265	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Lagerstroemia speciose*	Oleanolic acid	[6]
CYP716A266	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Lagerstroemia speciose*	Oleanolic acid	[6]
CYP716A12	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Medicago truncatula*	Oleanolic acid	[7]
CYP716A52v2	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Panax ginseng*	Oleanolic acid	[8]
CYP716A75	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Maesa lanceolata*	Erythrodiol, Oleanolic acid	[9]
CYP716A233	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Terminalia arjuna*	Oleanolic acid	[10]
CYP716A432	β-amyrin	C-28 oxidation	*S. cerevisiae* *N. benthamiana*	*Terminalia arjuna*	Oleanolic acid	[10]
LmOAS1	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Lonicera macranthoides* *Lonicera japonica*	Oleanolic acid	[11, 12]
CYP716C49	Oleanolic acid	C-2α hydroxylation	*S. cerevisiae*	*Crataegus pinnatifida*	Maslinic acid	[13]

				Centella asiatica		
CYP716C55	Oleanolic acid	C-2α hydroxylation	*S. cerevisiae* *N. benthamiana*	*Lagerstroemia speciose*	Maslinic acid	6
CYP716C67	Oleanolic acid	C-2α hydroxylation	*N. benthamiana*	*Olea europaea*	Maslinic Acid	14
CYP716C88	Oleanolic acid, Hederagenin	C-2α hydroxylation	*S. cerevisiae* *N. benthamiana*	*Terminalia arjuna*	Maslinic Acid, Arjunolic Acid	10
CYP716C89	Oleanolic acid, Hederagenin, Erythrodiol, Oleanolic aldehyde	C-2α hydroxylation	*S. cerevisiae* *N. benthamiana*	*Terminalia arjuna*	Maslinic Acid, Arjunolic Acid, Hydroxy erythrodiol, Hydroxy oleanolic aldehyde	10
CYP716Y1	β-amyrin	C-16α hydroxylation	*S. cerevisiae*	*Bupleurum falcatum*	16α-hydroxy β-amyrin	7
CYP87D16	β-amyrin	C-16α oxidation	*S. cerevisiae*	*Maesa lanceolata*	16α-hydroxy β-amyrin	9
CYP714E107a	Oleanolic acid,	C-23 hydroxylation	*S. cerevisiae*	*Terminalia arjuna*	Hederagenin,	10

	Maslinic acid, Arjunic acid		*N. benthamiana*		Arjunolic acid, Arjungenin	
CYP714E107b	Oleanolic acid, Maslinic acid, Arjunic acid	C-23 hydroxylation	*S. cerevisiae* *N. benthamiana*	*Terminalia arjuna*	Hederagenin, Arjunolic acid, Arjungenin	[10]
CYP714E107a	Hederagenin	C-23 oxidation	*S. cerevisiae* *N. benthamiana*	*Terminalia arjuna*	Gypsogenic acid	[10]
CYP714E107b	Hederagenin	C-23 oxidation	*S. cerevisiae* *N. benthamiana*	*Terminalia arjuna*	Gypsogenic acid	[10]
CYP93E2	β-amyrin	C-24 hydroxylation	*S. cerevisiae*	*Medicago truncatula*	24-OH-β-amyrin	[15]
CYP72A61V2	24-OH-β-amyrin	Oxidation for Soyasapogenol B synthesis	*S. cerevisiae*	*Medicago truncatula*	Soyasapogenol B	[15]
CYP93E3	β-amyrin	C-24 hydroxylation	*S. cerevisiae*	*Glycyrrhiza uralensis*	24-OH-β-amyrin	[15]
CYP72A566	24-OH-β-amyrin	Oxidation for Soyasapogenol B synthesis	*S. cerevisiae*	*Glycyrrhiza uralensis*	Soyasapogenol B	[15]

CYP82AS1	β-amyrin	C-22 Hydroxylation	*S. cerevisiae*	*Tripterygium wilfordii*	22-Hydroxy-β-amyrin	16
CYP712K1	22-Hydroxy-β-amyrin	C-29 Carboxylation	*S. cerevisiae*	*Tripterygium wilfordii*	Abrusgenic acid	16
CYP712K2/K3	β-amyrin	C-29 Carboxylation	*S. cerevisiae*	*Tripterygium wilfordii*	3-Epikatonic acid	16
CYP716A12	β-amyrin	C-28 Oxidation	*N. benthamiana*	*Medicago truncatula*	Oleanolic acid	17
CYP716E60	β-amyrin	C6-β hydroxylation	*S. cerevisiae*	*Enkianthus chinensis*	6β -Hydroxy-β-amyrin	18
CYP716C80	β-amyrin	C2α-hydroxylation	*S. cerevisiae*	*Enkianthus chinensis*	2α-Hydroxy-β-amyrin	18
CYP716A862	β-amyrin	C28 oxidation	*S. cerevisiae*	*Enkianthus chinensis*	Oleanolic acid	18
CYP716A262	β-amyrin	C28 oxidation	*S. cerevisiae*	*Psammosilene tunicoides*	Oleanolic acid	19
CYP716A262	Oleanolic acid	C16α hydroxylation,	*S. cerevisiae*	*Psammosilene tunicoides*	Echinocystic Acid	19
CYP72A567	Oleanolic acid	C23 oxidation	*S. cerevisiae*	*Psammosilene tunicoides*	Hederagenin, Gypsogenin, Gypsogenic acid	19

CYP716A8	β-amyrin	C28 oxidation	*S. cerevisiae*	*Actinidia eriantha*	Erythrodiol, Oleanolic aldehyde, Oleanolic acid	[20]
CYP716A9	β-amyrin	C28 oxidation	*S. cerevisiae*	*Actinidia eriantha*	Erythrodiol, Oleanolic aldehyde, Oleanolic acid	[20]
CYP88A222 ***CYP88A164*** ***CYP88A108***	β-amyrin	C11 oxidation	*S. cerevisiae*	*Melia azedarach*	11-oxo-β-amyrin	[21]
CYP88D6	β-amyrin	C11 oxidation (two step)	*S. cerevisiae*	*Glycyrrhiza uralensis* *Glycyrrhiza glabra* *Glycyrrhiza inflate*	11-oxo-β-amyrin	[22]
CYP88D15	β-amyrin	C11 hydroxylation	*S. cerevisiae*	*Glycyrrhiza pallidiflora,* *Glycyrrhiza macedonica*	Oleana-9(11),12-dien-3β-ol, Oleana-11,13(18)-dien-3β-ol	[22]

CYP716A173 (SvC28)	β-amyrin	C28 oxidation	*N. benthamiana*	*Saponaria vaccaria*	Oleanolic acid	[23]
CYP716A379 (SvC16)	Oleanolic acid	C16α-hydroxylation	*N. benthamiana*, *S. cerevisiae*	*Saponaria vaccaria*	Echinocystic acid	[23]
CYP72A1130 (SvC23-1)	Oleanolic acid	C23 oxidation	*N. benthamiana*, *S. cerevisiae*	*Saponaria vaccaria*	Gypsogenic acid	[23]
CYP72A1131 (SvC23-2)	Oleanolic acid	C23 oxidation	*N. benthamiana*, *S. cerevisiae*	*Saponaria vaccaria*	Gypsogenic acid	[23]
CYP716A12	β-amyrin	C28 Oxidation	*Y. lipolytica*	*Medicago truncatula*	Oleanolic acid	[24]
CYP716C11	Oleanolic acid	C-2α hydroxylation	*Y. lipolytica*	*Centella asiatica*	Maslinic acid	[24]
CYP714E19p	Maslinic acid	C-23 hydroxylation	*Y. lipolytica*	*Centella asiatica*	Arjunolic acid	[24]
CYP714E19p	Oleanolic acid	C-23 hydroxylation	*Y. lipolytica*	*Centella asiatica*	Hederagenin	[24]
CYP716C11p	Hederagenin	C-2α hydroxylation	*Y. lipolytica*	*Centella asiatica*	Arjunolic acid	[24]
CYP716A249	β-amyrin	C-28 oxidation	*S. cerevisiae*	*Polygala tenuifolia*	Oleanolic acid	[25]
LmOAS1	β-amyrin	C28 Carboxylation	*N. benthamiana*	*Lonicera macranthoides*	Oleanolic acid, β-amyrin	[11]

CYP716A212	β-amyrin	C28 Hydroxylation	*S. cerevisiae*	*Ilex asprella*	Oleanolic acid	[26]
CYP714E88	Oleanolic acid	C23 Hydroxylation	*S. cerevisiae*	*Ilex asprella*	Hederagenin, Gypsogenic acid	[26]
CYP93A220	β-amyrin	C24 Hydroxylation	*S. cerevisiae*	*Ilex asprella*	α-Boswellic acid	[26]
CYP716A261	β-amyrin	C28 Hydroxylation	*S. cerevisiae*	*Conyza blinii*	Oleanolic acid	[27]

Table S2. Oleanane-type Pentacyclic Triterpenoid Biosynthetic UGT Genes

Gene Name	Substrate	Carbon Modification	Heterologous Host	Plant Name	Compound Name	Reference
UGT74AG5	Oleanolic acid, Hederagenin	C28 O-glucosylation	*E. coli*	*Ilex asprella*	Oleanolic acid 28-O-β-D-glucopyranoside, Hederagenin 28-O-β-D-glucopyranoside	28
UGT74AG11	Oleanolic acid, Hederagenin, Echinocystic acid, calenduloside E	C28-O-glucosylation	*E. coli*	*Hedera helix*	Oleanolic acid 28-O-glucopyranosyl ester, Hederagenin 28-O-glucopyranosyl ester, Echinocystic acid 28-O-glucopyranosyl ester, Chikusetsusaponin	29
UGT73C11	Oleanolic acid Hederagenin	C3 glucosylation	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 3-O-β-glucoside, Hederagenin 3-O-β-glucoside	30
UGT73C13	Oleanolic acid Hederagenin	C3 glucosylation	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 3-O-β-glucoside, Hederagenin 3-O-β-glucoside	30
UGT73C21	Oleanolic acid Hederagenin	C3 glucosylation	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 3-O-β-glucoside, Hederagenin 3-O-β-glucoside	30

UGT73C22	Oleanolic acid, Hederagenin	C28 glucosylation, C3 and C28 glucosylation	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 28-O-β-glucoside, Oleanolic acid 3,28-0-β-diglucoside, Hederagenin 28-O-β-glucoside, Hederagenin 3,28-0-β-diglucoside	30
UGT73C23	Oleanolic acid, Hederagenin	C3, C28 and 3,23 glucosylation, C3 and C28 diglucosylation	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 3,28-0-β-diglucoside, Hederagenin 3,28-0-β-diglucoside, Hederagenin 3,23-O-β-diglucoside	30
UGT73C25	Oleanolic acid, Hederagenin	C3, C28 and 3,23 glucosylation, C3 and C28 diglucosylation	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 3-O-β-glucoside, Oleanolic acid 28-O-β-glucoside, Oleanolic acid 3,28-0-β-diglucoside, Hederagenin 3-O-β-glucoside, hederagenin 28-O-β-glucoside, Hederagenin 3,28-0-β-diglucoside, Hederagenin 3,23-O-β-diglucoside	30
UGT73C26	Oleanolic acid, Hederagenin	C3 glucosylation	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 3-O-β-glucoside, Hederagenin 3-O-β-glucoside	30
UGT73C27	Oleanolic acid, Hederagenin	C3 glucosylation,	*N. benthamiana, E. coli*	*Barbarea vulgaris*	Oleanolic acid 3-O-β-glucoside, Hederagenin 3-	30

					O-β-glucoside	
UGT18	Calenduloside E, Chikusetsusapo-nin IVa	C3 glycosylation of glucuronic acid	*E. coli* *S. cerevisiae*	*Panax ginseng*	Zingibroside R1, Ginsenoside Ro	31
UGT8	Oleanolic acid, Calenduloside E, Zingibroside R1	C28 glucosylation	*E. coli* *S. cerevisiae*	*Panax ginseng*	Oleanolic acid 28-O-glucopyranosyl ester, Chikusetsusaponin IVa, Ginsenoside Ro	31
UGT1	Oleanolic acid 3-O-β-d-glucuronide, Zingibroside R1	C28 glycosylation	*E. coli*	*Panax japonicus var. major*	Chikusetsusaponin IVa, Ginsenoside Ro	32
UGT2	Oleanolic acid 3-O-β-d-glucuronide, Chikusetsusaponi n IVa	C3 glycosylation	*E. coli*	*Panax japonicus var. major*	Zingibroside R1, Ginsenoside Ro	32
PgCSyGT1	Oleanolic acid	C3 glucuronidation	*S. cerevisiae*	*Panax ginseng*	Calenduloside E	31
GAT1	Oleanolic acid	C3 glucuronidation	*E. coli*	*Panax zingiberensis*	Oleanolic acid 3-O-β-glucuronide	33
GAT2	Oleanolic acid	C3 glucuronidation	*E. coli*	*Panax zingiberensis*	Oleanolic acid 3-o-β-glucuronide	33

GAT3	Oleanolic acid	C3 glucuronidation	*E. coli*	*Panax zingiberensis*	Oleanolic acid 3-O-β-glucuronide	33
UGT73P1	Cauloside A	C3 rhamnose glycosylation	*N. benthamiana*, *E.coli*	*Lonicera macranthoides*	α-hederin	11
SvCslG	Quillaic acid	C3 glucuronidation	*N. benthamiana*, *S. cerevisiae*	*Saponaria vaccaria*	QA-C3-GICA	23
SvGalT	Quillaic acid	Transfers galactose onto QA-C3-GlcA	*N. benthamiana*, *S. cerevisiae*	*Saponaria vaccaria*	QA-C3-GICA-Gal	23
SvC3XyIT	Quillaic acid	Transfers xylose onto QA-C3-GlcA-Gal	*N. benthamiana*	*Saponaria vaccaria*	QA-C3-GICA-Gal-Xyl	23
Sv46DH	Quillaic acid	Converts UDP-glucose into UDP-4K6DG	*N. benthamiana*	*Saponaria vaccaria*	UDP-4K6DG	23
SvNMD	Quillaic acid	Reduces UDP-4K6DG to form UDP-D-Fucose	*N. benthamiana*	*Saponaria vaccaria*	UDP-D-Fucose	23
SvC28FucT	Quillaic acid	Transfers fucose onto the C28 of saponins	*N. benthamiana*, *S. cerevisiae*	*Saponaria vaccaria*	QA-C3-GICA-C28-Fuc	23

Table S3. Oleanane-type Pentacyclic Triterpenoid Biosynthesic Transcription Factors

TF Family	**TF Name(s)**	**Species**	**Metabolite**	**Target gene /promotor**	**Promotor-binding site**	**Reference**
WRKY	PgWRKY4	*Panax ginseng*	Gensenoside saponins	*PgSE*	W-box	[34]
R2R3-MYB	PnMYB4 PnMYB1	*Panax notoginseng*	Saponins	*PnSQS*, *PnSE*, *PnDS*	MYB	[35]
bHLH	PnbHLH	*Panax notoginseng*	Saponins	*PnSQS*, *PnSE*, *PnDS*	MYB	[35]
bHLH	TaMYC2	*Taraxacum antungense*	Taraxerol	*TaSQS*	E-box	[36]
bHLH	TSAR1, TSAR2	*Medicago truncatula*	Pentacyclic oleanane type soyasaponin	*MtHMGR1*	N-box	[37]
bHLH	GibHLH13	*Glycyrrhiza inflata*	Glycyrrhizin	*Giβ-AS*, GiCYP72A154 *GiCSyGT*	E-box	[38]
bHLH	GubHLH3	*Glycyrrhiza uralensis*	Soyasaponins / Soyasapogenol B	Guβ-AS, *GuCYP93E3*, *GuCYP72A566*	E-box N-box	[39]
MADS-box	PgMADS41, PgMADS44	*Panax ginseng*	Ginsenoside Ro	*PgSE-4*, *Pgβ-AS-13* Pg*CYP716A52v2-4*	CArG motif	[40]
WRKY	CoWRKY15	*Camellia oleifera*	Oleanane-type triterpenoid saponins	*CoSQS*	W-box	[41]

WRKY	ZjWRKY18	*Ziziphus jujuba*	Oleanolic acid	*ZjHMGR1*	W-box	[42]
WRKY	CbWRKY24	*Conyza bilinii*	Saponins	*CbβAS*	W-box	[43]
MYB	ZjMYB39, ZjMYB4	*Ziziphus jujube*	Ziziphine	*ZjFPS* *ZjSQS*	MYB	[44]
AP2/ERF	PjERF1	*Panax japonicas*	Chikusetsusaponin IV and IVa	*PjβAS*, *PjCAS*, and *PjSE*	GCC-box	[45]

Table S4. Advantage and limitation analysis of different host systems for OPTs heterologous production

Host Category	Key Examples	Major Advantages	Major Limitations / Liabilities

Native Plants	*Lotus japonicas*, *Olea europaea*	**Native Pathway Fidelity:** All endogenous enzymes, cofactors, organelles, and regulatory networks are present, enabling the synthesis of highly complex, modified OPTs.	**Low & Variable Yield:** OPT accumulation is often low, tissue-specific, and influenced by environment/stress. **Complex Extraction:**Mixtures of structural analogs complicate purification. **Slow Growth:**Long life cycles hinder rapid research and scaling.
Heterologous Plant Hosts	*Nicotiana benthamiana* (transient), *Medicago truncatula* (stable)	**Plant-Compatible Machinery:** Contains ER, redox partners (CPR), and lipid droplets that support functional expression of plant P450s and channeling. **Rapid Validation:** Transient agroinfiltration (e.g., Tsukuba system) allows ultra-fast (<2 week) pathway assembly and testing. **High-Performance Potential:** Can achieve very high yields per biomass unit (e.g., ~30 mg/g DW oleanolic acid).	**Agricultural Scaling:** Large-scale production requires significant land, is seasonal, and faces biocontainment/GMO regulations. **Quantitative Analysis Challenge:** Complex plant matrix complicates metabolite extraction and analysis compared to microbial systems.
Prokaryotic Host (Bacteria)	*Escherichia coli*	**Ultra-Rapid Growth:** Fast doubling (~20 min) enables quick genetic iterations. **High Genetic Tractability:** Vast toolkit for cloning, CRISPR, and library construction. **Defined Metabolism:** Simplifies metabolic modeling and omics analysis.	**Lacks Essential Eukaryotic Machinery:** No native sterol pathway, ER, or functional cytochrome P450 reductase (CPR) system. Requires extensive engineering to supply squalene/OS and functionalize P450s. **Product Toxicity:** Hydrophobic intermediates (e.g., β-amyrin) often accumulate intracellularly, inhibiting growth and limiting titers.
Eukaryotic Microbial Hosts (Yeast)	*Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Pichia pastoris*	**Balance of Fidelity & Tractability:** Native mevalonate pathway supplies precursors; contains ER and supports functional plant P450/CPR pairs. **Industrial Workhorse:** Excellent for	**Metabolic Burden:** Competition between heterologous pathway and native metabolism (e.g., for ergosterol synthesis) can limit yields. Requires extensive rewiring.

	scalable fermentation in bioreactors; GRAS status for pharmaceuticals. **Advanced Engineering:** Sophisticated tools for dynamic regulation, organelle engineering, and high-throughput screening.	**Limited "Drop-in" Success:** Functional expression of some plant P450s or UGTs may require N-terminal engineering or chaperone co-expression. **Precursor Sequestration:** Native metabolism may divert intermediates (like FPP) away from the OPT pathway.

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