

Supplementary material

Figure S1 Transformation of *O. pumila* explants for hairy roots lines. (A) Wild *O. pumila* seedlings; (B) Aseptic *O. pumila* seedlings; (C) Stem explants for transformation; (D) Hairy root derived from the wounding sites of stems; (E) Single-line hairy root cultured on plate; (F) Hairy root cultured in B5 liquid medium in flask

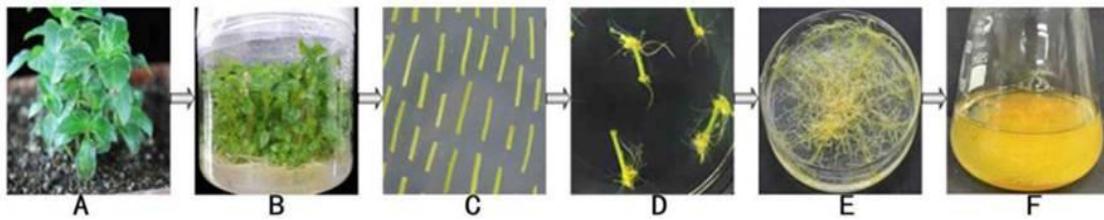


Figure S2 Expression pattern of *OpWRKY3* in transgenic hairy roots

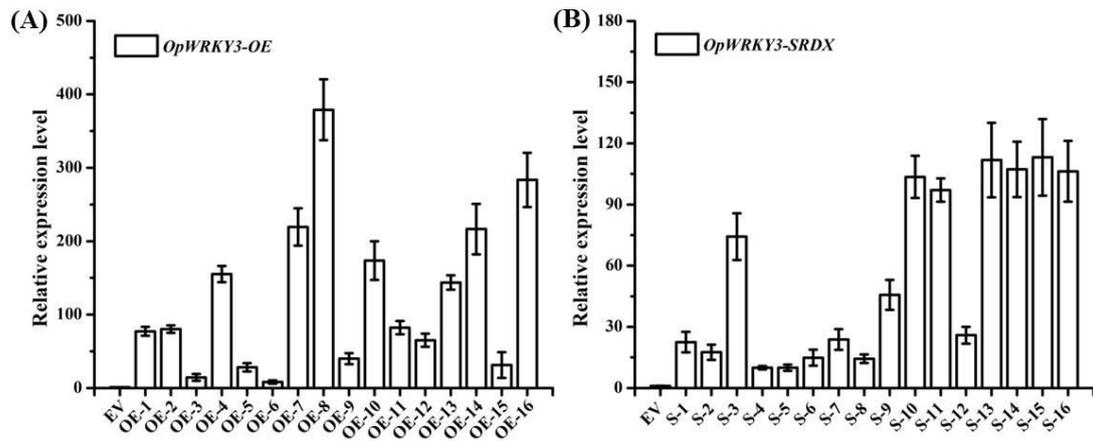


Figure S3 The fresh weight (FW) and dry weight (DW) of *OpWRKY3* transgenic hairy roots after shake-flask culture

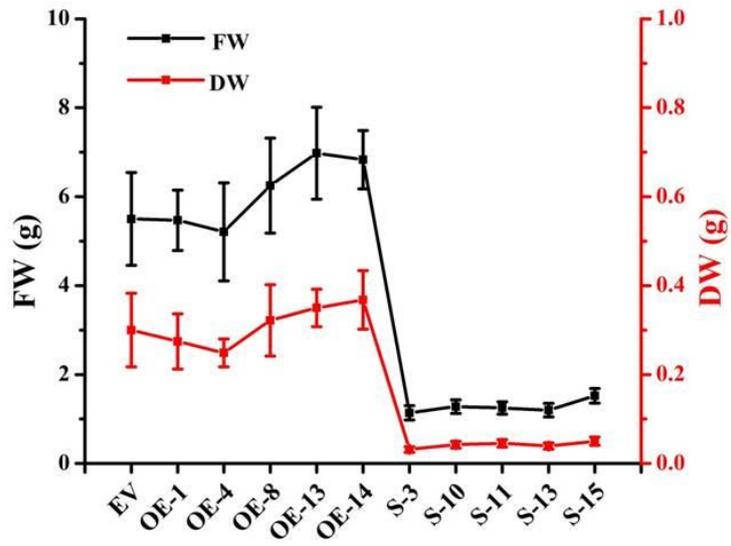


Figure S4 The content of CPT and its precursors in *OpWRKY3* transgenic hairy roots by HPLC. (A) The tryptamine content in *OpWRKY3* transgenic hairy roots by HPLC; (B) The loganin content in *OpWRKY3* transgenic hairy roots by HPLC; (C) The secologanin content in *OpWRKY3* transgenic hairy roots by HPLC; (D) The camptothecin content in *OpWRKY3* transgenic hairy roots by HPLC. Values are means \pm standard deviation of triplicate analyses. *, significant at $P < 0.05$; **, highly significant at $P < 0.01$.

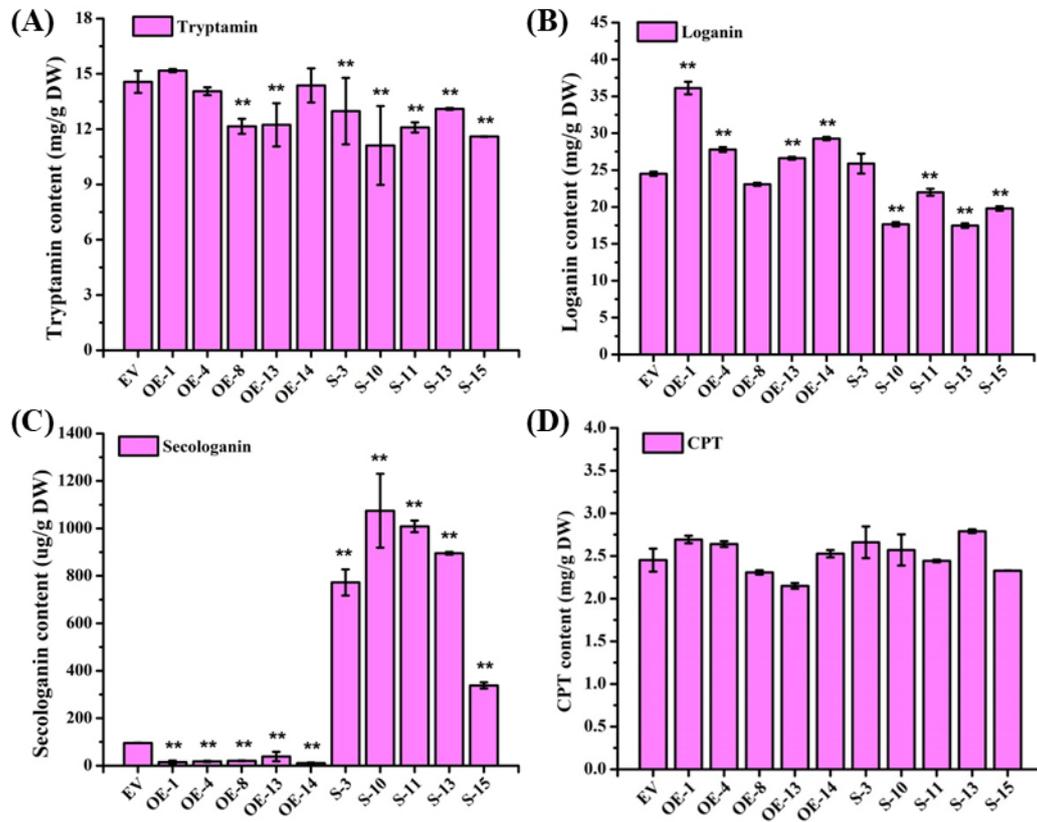


Figure S5 Camptothecin biosynthetic pathway in *O. pumila*. TSB, tryptophan synthase beta; TDC, tryptophan decarboxylase; G10H, geraniol-10-hydroxylase; 10-HGO, 10-hydroxy-geraniol oxidoreductase; SLS, secologanin synthase; CPR, NADPH-Cytochrome P450 reductase; STR, strictosidine synthase; SGD, strictosidine beta-glucosidase.

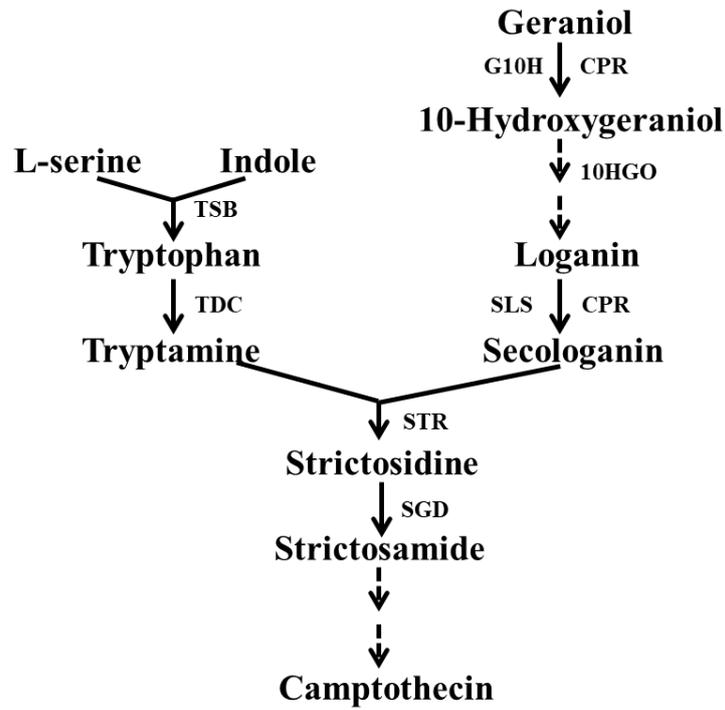


Figure S6 Expression vectors construction for transformation

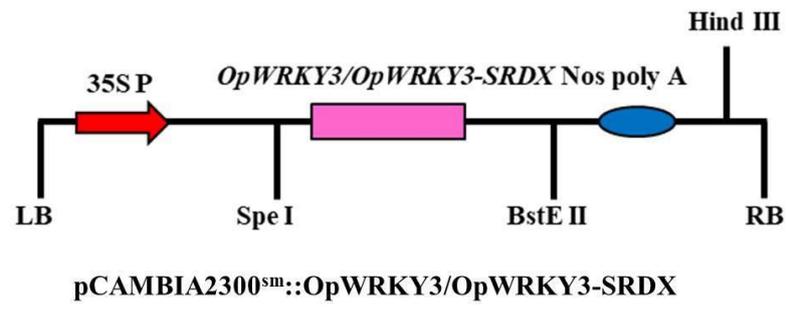


Figure S7 Analysis of tryptamine, loganin, secologanin and CPT standard by HPLC

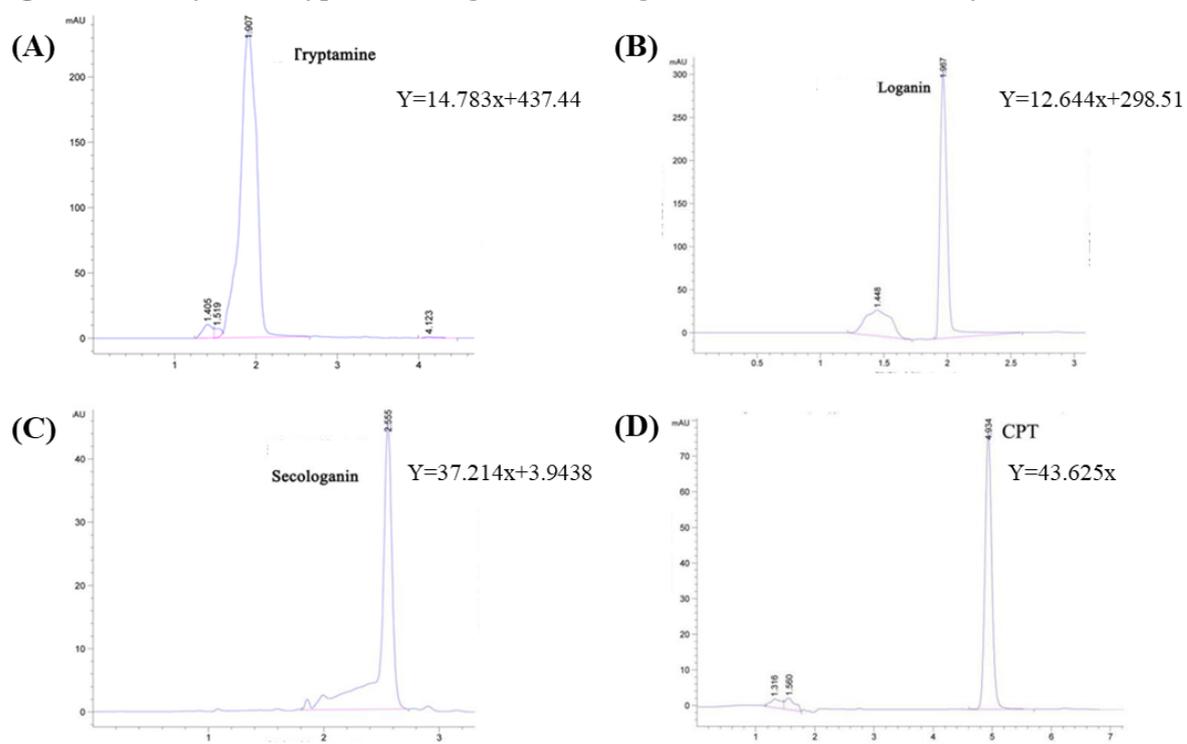


Table S1 Oligonucleotide primers used in this study

Primer name	Primer sequence (5→3)	Application
OpWRKY3-F1	CTTCAATTCTGCTCGTCTGTCGT	Cloning
OpWRKY3-R1	CAGGAGGTTTCCAGTTTCCACAA	Cloning
GFP-OpWRKY3-KF-BglII	GGAAGATCTATGGAGAATTCAGCAG	Subcellular localization
GFP-OpWRKY3-KR-KpnI	CGGGGTACCCCGAAAAGAATCCTGA	Subcellular localization
2300 sm -OpWRKY3-OE-KF-SpeI	ACTAGTATGGAGAATTCAGCAG	Overexpression construct assembly
2300 sm -OpWRKY3-OE-KR-BstEII	GGTCACCTCAGGAAAAGAATCC	Overexpression construct assembly
2300 sm -OpWRKY3-SRDX-KF-SpeI	ACTAGTATGGAGAATTCAGCAG	Repression construct assembly
2300 sm -OpWRKY3-SRDX-KR-BstEII	GGTCACCTCAAGCAAAACCTAATCTAAGTTCCAGATCC AAATCCAAGGAAAAGAATCCTGAATTATC	Repression construct assembly
RolB-F	GCTCTTGCAGTGCTAGATT	Positive identification
RolB-R	GAAGGTGCAAGCTACCTCTC	Positive identification
GusA-F	GTGAATCCGCACCTCTGG	Positive identification
GusA-R	ATCGCCGCTTTGGACATA	Positive identification
35S-F32	GAGGACCTAACAGAACTCGCC	Positive identification
OpWRKY3-R	CCCTCCCTAGTGTGCTGCTCGTTTA	Positive identification
OpACTIN-QF	AGCAGCATGAAGATTAAGGTTGTG	qRT-PCR
OpACTIN-QR	CACATCTGCTGGAAAGTGCTG	qRT-PCR
OpWRKY3-QF	CCGACGCAACAAAATCCACA	qRT-PCR
OpWRKY3-QR	TGTTATCGGCCACAGATGGG	qRT-PCR
OpSTR-QF	AGCCATGGTTGTGTCGATTCT	qRT-PCR
OpSTR-QR	TTCACCATCGGAGTCAAAAGC	qRT-PCR
OpTDC-QF	TAGGCTCAATCCAGGGAAAGG	qRT-PCR
OpTDC-QR	TCCCACAGCAAACCTCAACA	qRT-PCR
OpCPR-QF	CCGGCAATATGAGCATTCA	qRT-PCR
OpCPR-QR	GTTCTCGCCAAGCAGCAAA	qRT-PCR
OpG10H-QF	TGAGGAAGCCGATGTTCC	qRT-PCR
OpG10H-QR	TTTCATCACGCCCAATTGCC	qRT-PCR
Op10HGO-QF	TAATGGTTGGTGCACCGGAA	qRT-PCR
Op10HGO-QR	AGCTTTCAAGAGGCGTTCCA	qRT-PCR
OpSLS-QF	AAGCATCCCGAATGGCAAGA	qRT-PCR
OpSLS-QR	GAAGCATCACCGTTGGCATC	qRT-PCR
OpSGD-QF	ACCGGAAGGAGTGCCTATCT	qRT-PCR
OpSGD-QR	CCCCATTCCCATGTGTCCAA	qRT-PCR
OpTSB-QF	TGCAGGATGAAGATGGGCAG	qRT-PCR
OpTSB-QR	CAGAGTCGGGCACAGTTTCT	qRT-PCR
pB42AD-OpWRKY3-EcoR I-F	GATTATGCCTCTCCCGAATTCATGGAGAATTCAGCAGA TTGGGA	YIH
pB42AD-OpWRKY3-XhoI-R	TGGCGAAGAAGTCCACTCGAGTCAGGAAAAGAATCCT GAATTATCA	YIH
OpCPR-Pro EcoRI-W-boxII-F	aattcTTCTGGTCAAAACTTCTGGTCAAAACTTCTGGTC	YIH

OpCPR-Pro XhoI-W-boxII-R AAAACTc
 tegagAGTTTTGACCAGAAAGTTTTGACCAGAAAGTTTTG YIH
 ACCAGAAg
