

Supplementary information

Production of putative diterpene carboxylic acid intermediates of triptolide in yeast

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TABLE S1. Tobacco plant combinations.

Name	Genes expressed	Putative heterologous diterpene products
Control	<i>Cj</i> GGPPS, <i>Cj</i> DXS, p19	None
<i>Tw</i> TPS9+ <i>Tw</i> TPS27	<i>Cj</i> GGPPS, <i>Cj</i> DXS, p19, <i>Tw</i> TPS9, <i>Tw</i> TPS27	dehydroabietadiene, miltiradiene
DiTPS+ <i>Ps</i> CYP720B4	<i>Cj</i> GGPPS, <i>Cj</i> DXS, p19, <i>Tw</i> TPS9, <i>Tw</i> TPS27, <i>Ps</i> CYP720B4	Dehydroabietadiene, miltiradiene, dehydroabietic acid (DHA), “miltiradienic acid” (abieta-8,12-dien-18-oic acid)

TABLE S2. Yeast expression vectors.

Name	Promoter/Terminator	Yeast selection
pEVE1	P _{PGK1} /T _{CYC1} +P _{TEF1} /T _{ADH1}	<i>URA3</i>
pEVE2	P _{PGK1} /T _{CYC1} +P _{TEF1} /T _{ADH1}	<i>HIS3</i>
pEVE3	P _{CYC1} /T _{ADH2}	<i>LEU2</i>

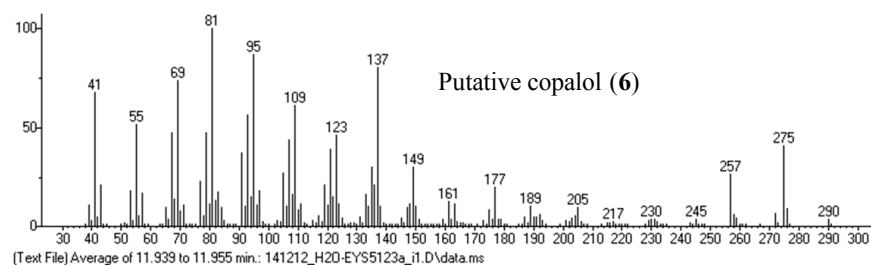
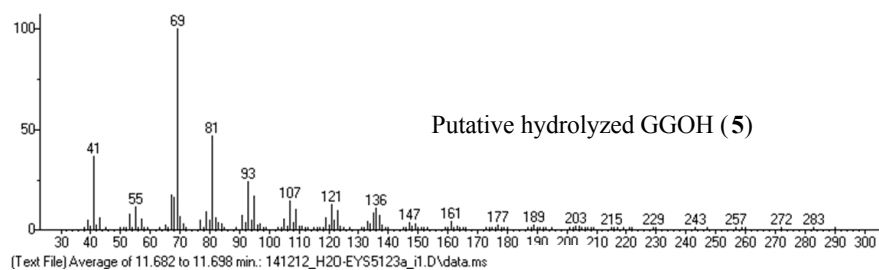
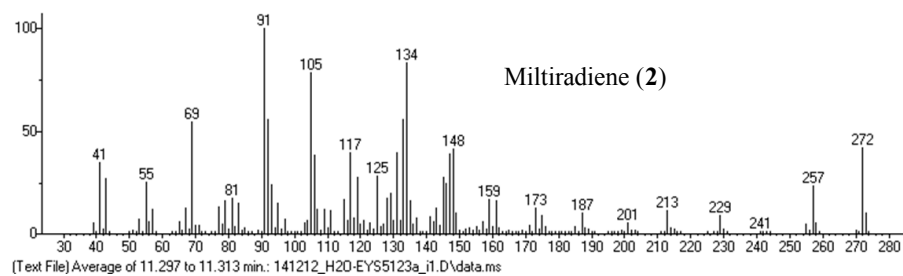
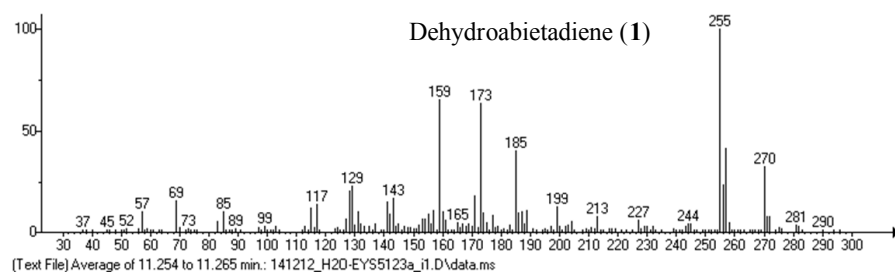
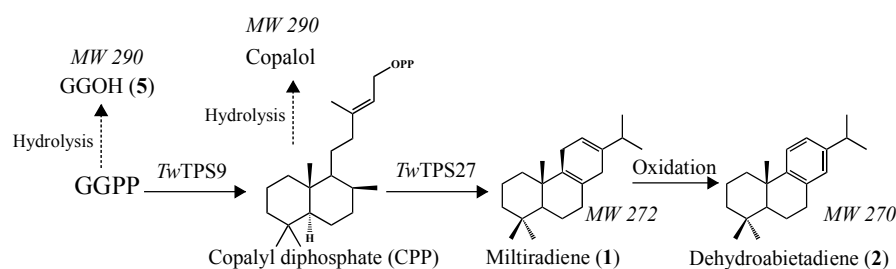


Figure S1. MS spectra and molecular weight (MW) of yeast produced products.

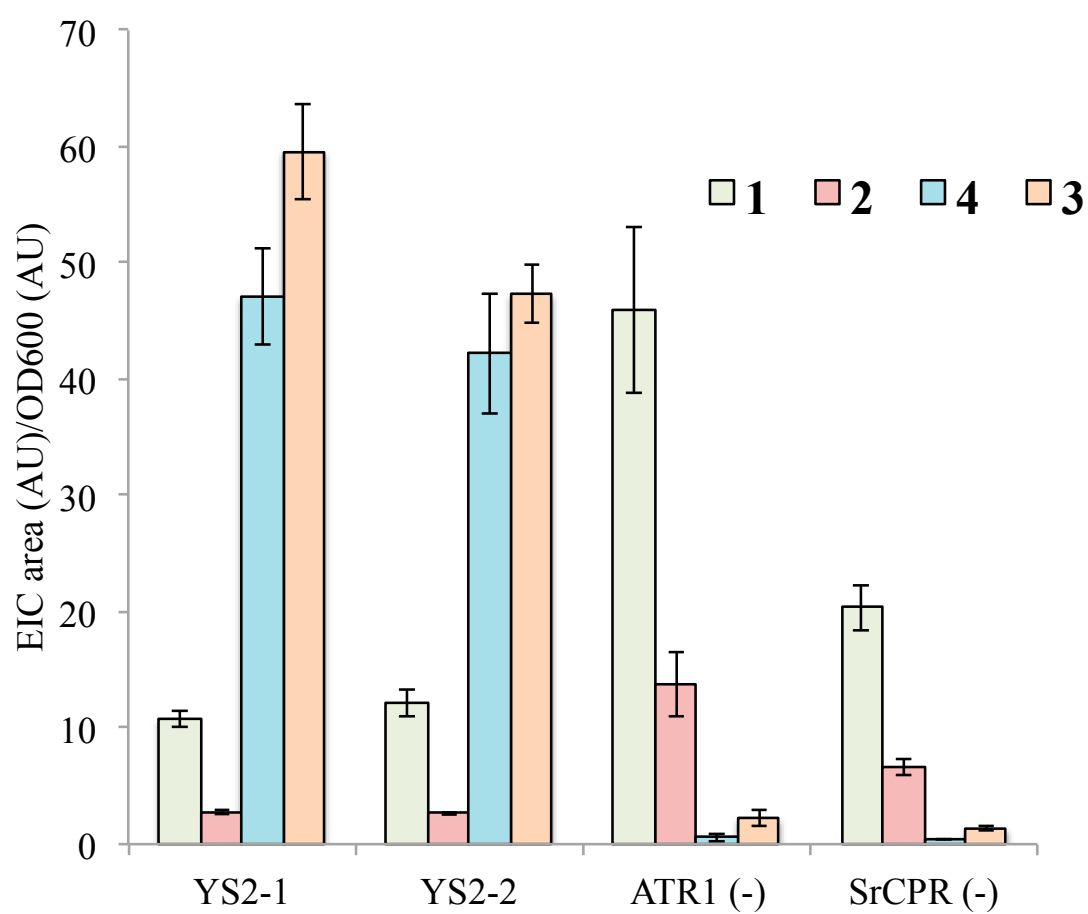


Figure S2. Diterpene production levels. Normalized levels (EIC area/OD₆₀₀) of miltiradiene (1), dehydroabietadiene (2), dehydroabietic acid (3) and miltiradienic acid (4) in yeast strains.

TABLE S3. Yeast codon optimized sequences.

Name	Sequence
CO <i>Tw</i> TPS9	ATGCACTCCTTGTGATGAAGAAAGTCATCATGTACTCCTCCCAAACCACTCATGTTTTTCCATCTCCATTGCACTGTACTAT CCCAAAGTCATCCTCATTTTTCTTGGATGCTCCAGTTGCTAGATTGCATTGCTTGTCTGGTCATGGTGCTAAAAAGAAGAGAT TGCACTTCGATATCCAACAAGGTAGAAATGCTGTTTCTAAGACTCATACTCCAGATGACTTGTACGCTAAGCAAGAATACTCT GTTCCAGAAATCGTTAAGGACGACGACAAAGAAGAAGAGTTCGTCAAGATCAAAGAACACGTTGACATCATCAAGTCCATGTT GTCATCTATGGAAGATGGTGAAATTTCCATTTCCGCTTATGATACTGCTTGGGTTGCTTTGATTCAAGACATTCTATAACAATG GTGCTCCACAATTCCCATCATCTTTGTTGTGGATTGCCGAAAATCAATTGCCAGATGGTTCTTGGGGTGATTCTAGAGTTTTT TTGGCTTTTCGACAGAATCATTAACACCTTGGCTTGTGTTGTTGCCTTGAAGTCTTGAAGTGTTCATCCAGATAAGTGCGAAAG AGGTATCTCATTTCTTGAAAGAAAACATCTCCATGTTGGAAAAGGACGACTCTGAACACATGTTGGTTGGTTTTGAATTTGGTT TCCCAGTCTTGTAGATATGGCTAGAAGATTGGGTATCGATGTTCCAGATGATTCTCCATTTCTTACAAGAAATCTACGTCCAA AGAGACTTGAAGTTGAAGAGAATCCCAAAGGATATCTGCATAACGTTCCAACCACTTTGTGTCATTCTTGGAAAGCTATTCC AGATTTGGATTGGACTAAGTTGTTGAAGTTGCAATGTCAAGACGGTTCCTTGTGTTTTTCTCCATCATCTACTGCTATGGCTT TCATCTCTCATTTCTGAAAGGACGAAAACGCTTGAGATACTTGATCTACGTTGTCCAAAGATTCAATGGGTGGCTCTACTGTTTTAT CCATACGATTTGTTTGAACATAACTGGGCCGTTGACAGATTGCAAAGATTAGGTATTTCCAGATTTCTCCAACCAGAAATCAG AGAATGTATGTCCTACGTTTACAGATACTGGACCAAGGATGGTATTTTCTGTACCAGAAACTCCAGAGTTCCAGATGTTGATG ATACAGCAATGGGTTTCAGATTATTGAGATTGCATGGTTACGAAGTTCACCCAGATGCTTTTAGACAATTTAAAAAGGGTTGC GAATTCATCTGCTACGAAGGTCAATCTCATCCAAGTGTACTGTCATGTATAACTTGTACAGGCCCTCCCAATTGATGTTTCC TGAAGAAAAGATTTTGGACGAAGCTAAGCAATTCACCGAAAAGTTTTTGGGTGAAAAGAGATCCGCTAACAGTTGTTGGACA AGTGGATTATTACCAAGGATTTGCCAGGTGAAGTTGGTTTTCGCTTTGGATGTTCCATGGTATGCTTCATTGCCAAGAGTTGAA GCTAGATTCTTCATCCAACATTACGGTGGTGAAGATGATGTTTGGTTGGATAAGGCATTATACAGAATGCCATACGTCACACAA CAACGCTCTATTTGGAATTGGCCAAGTTGGATTACAATTACTGTCAAGCCTTGCCATAGAACTGAATGGGGTAGAATTCAAAAGT GGTACGAAGAATGTAAGCCAAGAGATTTCCGTATCAGTAGAGAATGTTTGTGAGAGCTTACTTTATGGCTGCTGCCTCTATT TTTGAACTGAAAGATCAATGGAAAGATTGGCTTGGGCTAAGACTGCTATTTTGTGGAATCATCGTTTCTACTTCTCCGA AGTTGGTAACTCTACCGAACAAAGAATTGCTTTCACTACCGAATTTCTATTAGAGCTTCTCCAATGGGTGGTTACATTAAACG GTAGAAAATTGGACAAGATCGGTACTACCCAAGAATTGATCCAAATGTTTGGCTACCATCGACCAATTTTACACAAGATGCA TTTGTGCTTACGGTCATGATATTACTAGACACTTGCACAACCTCATGGAAAATGTGGTTGTTGAAATGGCAAGAAGAAGGTGA TAGATGGTTGGGTGAAGCCGAATTATTGATTCAAACCAATTAACTTGATGGCCGATCATAAGATCGTGAAAAGTTGTTTATGG GTCACACCAACTACGAACAATATTCTCTTTGACTAACAAGGTCTGCTACTCCTTGGGTGTCATGAATTGCAAAACAACAGA GAATTGGAACACGATATGCAAAGATTGGTCCAATTTGGTTTTGACCAACTCCTCTGATGGTATCGATTCCGATATTAAGAAAAC CTTCTTGGCCGTTGCTAAGAGATTTTACTACACTGCTTTTGTGATCCAGAAACCGTCAACGTTTCATATTGCTAAGGTTTTGT TCGAAGAGTTGATTAA
CO <i>Tw</i> TPS27	ATGGCTCCATTGGTTGTCTCCTTGACCATCTCCCATTTCGTTATTCAAACCTGGTTCTACTGCCTTGCAATTATTCTGCTTTGCC AGAAACCAGAACTAAGCACTGTCTATTCTTCTAGACCATTTCGCTCTATTAACTCCAACCTCCTTGCAAATGAATCAAAGACCAT TGACCGATTACAGACCAGCTATTTGGAATCCAGAATTGATCGATTCTTTGAACACCCCATACTCCTATCAATCTCATGGTACT CAATTGGACAAGTTGAGACAAGATGCCAAGAGATTATTGTCTCTACTTCTGATCCATGCTTGTGTTGAACCACGTTGAATC TATGCAAAGATTGGGTATTGCCTACCACTTCCAAGAAGAAATCGATTACTTGTGTAACACCAGAATCCAACCATACTCTCCAG ATGATCATGACTTGCCATACAACCTGCTTTGAGATTGAGAATCTTGAGAGACAACAACCTTCCAATCTCCTCTGATGTTTTCGGT AAGTTCATGTCCAGAGAAGGTAAATTTCTTGGAATCTTTGTCTAGAGATGTCAAGGGTTTGTGTCTCTGTATGAAGCTAGTTT CTTGGGTGTGATGGTGAAGTTATTTTGGACGAAGCCAAAGAATTTCTCCTCAAAGAATTTGAGAGCTTTGTTGGGTGAGATTGG AATCAACCTCTATTGATGTTGCCGAACAAGTCAAGCAATCATGCAAATTCCTTTGTTTTGGAGAATGCCCTAGAGTTGAAGCC AGAACTTCATTGACTTCTACCAAAAAGAAGGACGCCAAATCTTCTACCTTGTGGAATTGGCTAAGTTGGATTTTAACTTGGT CCAATCCACGTACCAACAAGAATTGAAAGAATTGTCTAAGTGGTGGGAAAACCTTGGGTTTCAAGCAAAAGTTGTCTTTTCAACCA GAGACAGATTGATGCAATCTTACTTCTCTACTACCGGTATTACCTTCAAGCCACAATTTTCCAAGCTAGAATTGCTGCTACC AAGTTTCATCAACATCGTTAACACCATCGATGATATCCACGATTACTACGGTTCTCAAGATGACTTGAAGTTGTTTGATTCCCG TGTTAAGAGATGGGATTTGGCTGCTATGGAAGAATTGCCAGATTACATGAAGATTTGCTACTTCGCCATGTACAACCTTGGTTA ACGAATTGGCTTACGACGTCTTGATCAATCAAGGTATAGATGTTTTGCCATGCTTGAGAGAAGCCTGGACTAAGTTTTGTGGT GCTGCATTTGTGAATCCCAATGGTGTTTACTGGTTACACTCCATCTATGGATGACTACTTGAAGAAGTGTGCGATTTCAT TGGTGTTCACGTTCTTTGAATTTTGTAGAGCAGATCAACAAGGTTCCAGATCTCCAATTGCTAATCTCATCTGATTGCT TGGAAGATCCTTTGTTGATTTGGTCTCTGTCTATCTGTAGATTGAACAACGATTTGGCTACCTTCCAACACGAATCTAAAACT GGTGAAGTCGTTTCTTCGTCAAGTCTACATGGTTGAAAAGGGTGTTCCTCAAGAACAAAGCCTGTGACGAAATTAGAGAATT GATTAAGCACGCCTGGAAGATGTTGAATACCGAAAAGAAGAAGATCTGACTTGCCACCATTGATGGTAGAAAATGTGTATGGATA CCCCAAGTTGTCAATGCTTGTATCAACATGGTGATGGTTTCGGTGTGCTATTGATTGACTAAGGATGTCATGTCCTCA TTGATCTTCAGACAAATCCCAATTTGA