**Additional Material**

**A novel strategy to enhance terpenoids production using cambial meristematic cells of *Tripterygium wilfordii* Hook. f.**

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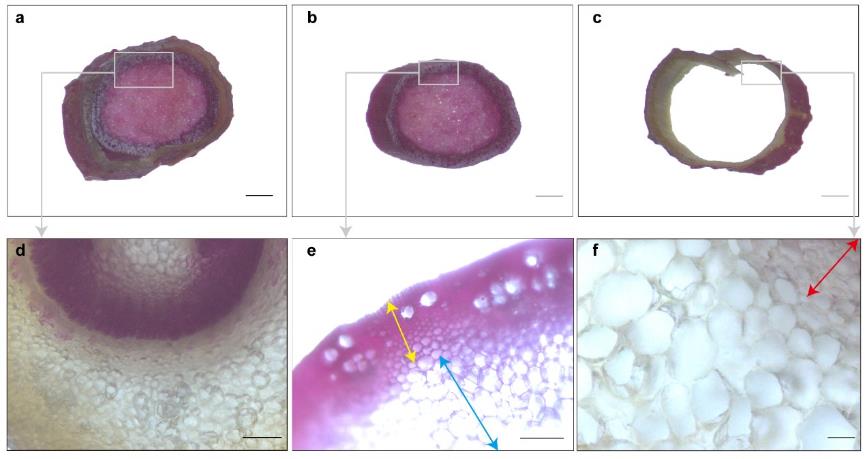
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**Figure S1** The isolation of cambium cell layer from xylem tissue of *T. wilfordii*. (a) The stem cross-sections of *T. wilfordii* were stained with phloroglucinol-HCl to detect lignin deposition (dyed in purplish-red). (b) Separated xylem and pith tissue from stem segment in panel a, stained with lignin-specific dye, phloroglucinol-HCl. (c) Cambium, phloem, cortex and epidermal tissue were peeled off from the xylem and pith tissue, from stem segment in panel a, stained with lignin-specific dye phloroglucinol-HCl. It was shown that phloroglucinol-HCl did not stain this tissue except epidermis. Xylem tissue was completely separated. (d) Optical microscopic observation of panel a. (e) Optical microscopic observation of panel b. The lignin-specific dye, phloroglucinol-HCl, stained these cells purplish-red. Yellow and blue arrow indicate xylem cell and pith cell layers, respectively. (f) Cross-section of cambium cell layer and phloem tissue from panel c. It was shown that phloroglucinol-HCl did not stain these cells. Red arrow bar indicates cambium cell layers. Scale bar is equivalent to 0.15 mm for a-c, 25 μm for d, 15 μm for e, and 10 μm for f.

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**Figure S2** Transcriptome data of CMCs and DDCs from *T. wilfordii*.(a) Transcript length distribution. (b) Success rate of gene annotation.

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**Figure S3** Amino acid sequence alignment between *T. wilfordii* cluster 96, cluster 95, cluster 71, cluster 04 and CMC marker genes. (a) Comparison of *T. wilfordii* cluster 96 and PXY. GenBank ID: PXY1 (NP\_200956), PXY2 (NP\_194594), PXY3 (AED97473), PXY4 (ACN59400). (b) Comparison of *T. wilfordii* cluster 95 and WUS. GenBank ID: WUS\_Arabidopsis (*Arabidopsis thaliana*; NP\_195280), WUS\_Medicago (*Medicago truncatula*; KEH44470), WUS\_Theobroma (*Theobroma cacao*; EOX90656), WUS\_Glycine (*Glycine soja*; KHN37332). (c) Comparison of *T. wilfordii* cluster 71 and CLV3 of CLE family. GenBank ID: CLE\_Medicago (*Medicago truncatula*; KEH44216), CLE\_Parasponia (*Parasponia andersonii*; PON78607), CLE\_Trema (*Trema orientalis*; PON95933). (d) Comparison of *T. wilfordii* cluster 04 and GA20-oxidase (GA20-ox). GenBank ID: GA20-ox\_Ricinus (*Ricinus communis*; EEF43925), GA20-ox\_Camellia (*Camellia sinensis*; AUD40403), GA20-ox\_Citrus (*Citrus unshiu*; BAU98538). Sequences were aligned by the multiple sequence alignment programme.

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**Figure S4** UPLC-QTOF-MS traces (total ion current) obtained with methanolic extracts of CMCs and DDCs. (a) UPLC-UV chromatogram of terpenoid standards. (b) UPLC-UV chromatogram of CMCs. (c) UPLC-UV chromatogram of DDCs. The characterization of the compound peaks 1 to 3 are given in Table S3.

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**Figure S5** MS spectra of compound 1 from the [M+H]+ ion at m/z 361.11. (a) MS spectrum of terpenoid standards. (b) MS spectrum of CMCs. (c) MS spectrum of DDCs.

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**Figure S6** MS spectra of compound 2 from the [M+H]+ ion at m/z 313.17. (a) MS spectrum of terpenoid standards. (b) MS spectrum of CMCs. (c) MS spectrum of DDCs.

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**Figure S7** MS spectra of compound 3 from the [M+H]+ ion at m/z 451.28. (a) MS spectrum of terpenoid standards. (b) MS spectrum of CMCs. (c) MS spectrum of DDCs.



**Figure S8** Terpenoids production of *T. wilfordii* DDCs following induction of 50 μmol L-1 MJ. (a) Total triptolide, celastrol and triptophenolide production in cells. (b) Total triptolide, celastrol and triptophenolide production in medium. The data represent the mean ± SD of five independent suspension cell cultures. DW, dry weight.

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**Figure S9** Effect of 50 μmol L-1-MJ on growth of *T. wilfordii* DDCs. Values represent means ± SD, n=5.

**Table S1** Length distribution of transcriptional data

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Min  length | Mean length | Median length | Max  length | N50 | N90 |
| Transcripts | 201 | 1033 | 704 | 17305 | 1611 | 453 |
| Unigenes | 201 | 1241 | 961 | 17305 | 1712 | 613 |

**Table S2** The primers used for qRT-PCR

|  |  |
| --- | --- |
| Primer name | Primer Sequence (5'-3') |
| Cluster 96-F | TTACAGTCAAGGGAGCGTCTC |
| Cluster 96-R | AGCCATACCGTTGGGAGTT |
| Cluster 95-F | AACCGATGGACTGGGACAC |
| Cluster 95-R | GGAGACAGAAACCTGCGAGA |
| Cluster 71-F | GTCTGCTGCGAGTGTAAGGC |
| Cluster 71-R | GGATCTGGACAACTGGGAACT |
| Cluster 04-F | ATTTGGGCTGACACCACATAC |
| Cluster 04-R | CTGCGAACCATTTACCATCTT |
| qEfα-F | CCAAGGGTGAAAGCAAGGAGAGC |
| qEfα-R | CACTGGTGGTTTTGAGGCTGGTATCT |
| qDXR-F | AATCTCCTTGTTCGGTTCCA |
| qDXR-R | GCTGGTTGAGGCTGCTGA |
| qDXS-F | GGCGACTACTGGGTCTTTCTT |
| qDXS-R | TGTCTTTGCGTATCATCATCCT |
| qGGPS-F | GGCAAGAGGGTTCGTCCAG |
| qGGPS-R | AAAGTAGTGCATCACCAGCAAG |
| qHMGS-F | CTGGAGGTAGGGAGCGAGAC |
| qHMGS-R | CCATAGCAGGCATTGGTTGA |
| qHMGR-F | GCCTTTGCTGCTGGACGACTA |
| qHMGR-R | CCGCTGCTCTTCTGGCTGAC |
| qFPS-F | CAGACCCTCACCTTCCATT |
| qFPS-R | AAGAGTAACCATAAGCAGCAGAC |
| qIDI-F | GTCCCTTCCACCCTAACC |
| qIDI-R | GCCCAACCACACGATCATTC |

**Table S3** Related data of target compounds detected by UPLC/Q-TOF MS

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Number | Retention time  (min) | [M+H]+ (m/z) | UV (nm) | Formula | Compound |
| 1 | 2.25 | 361.21 | 220 | C20H24O6 | Triptolide |
| 2 | 8.24 | 313.17 | 200 | C20H24O3 | Triptophenolide |
| 3 | 19.75 | 451.28 | 425 | C29H38O4 | Celastrol |