

Supplementary Table 1 Sequencing data statistics

| Sample Name | Sample ID | cDNA size | SMRT Cells | Reads of Insert | Read Bases of Insert | Mean Read Length of Insert | Mean Read Quality of Insert | Mean Number of Passes |
|-------------|-----------|-----------|------------|-----------------|----------------------|----------------------------|-----------------------------|-----------------------|
| Tree peony | F01 | 1-6k | 1 | 473,062 | 891,073,753 | 1,883 | 0.97 | 27 |

Supplementary Table 2 Full length sequence data

| cDNA | | Number | | | | | | | | | | |
|---------|------|-----------------|-----------------|-----------------------|------------------------|-----------------------|---------------------------------|-----------------------------|------------------------------|---------------------|-------------------------------------|----------------------------|
| Samples | Size | Reads of Insert | Number of reads | Number of prime reads | Number of poly-A reads | Number of short reads | Number of non-full-length reads | Number of full-length reads | Number of non-chimeric reads | Average read length | Full-Length non-chimeric Percentage | Artificial Concatemers (%) |
| F01 | 1-6k | 473,062 | 409,110 | 410,242 | 403,351 | 16,494 | 90,044 | 366,524 | 359,771 | 1,825 | 76.10% | 1.84% |

Supplementary Table 3 Clustering results by ICE

| Samples | cDNA Size | Number of consensus isoforms | Average consensus isoforms read length | Number of polished high-quality isoforms | Number of polished low-quality isoforms | Percent of polished high-quality isoforms (%) |
|---------|-----------|------------------------------|--|--|---|---|
| F01 | 1-6k | 158,245 | 2,007 | 117,680 | 40,102 | 74.37% |

Supplementary Table 4 Data output statistics using the SGS sequencing platform

| Samples | Read Number | Base Number | GC Content | %≥Q30 |
|--------------|-------------|---------------|------------|-------|
| High Noon'-1 | 23,743,135 | 7,083,754,008 | 45.18 | 95.29 |
| High Noon'-2 | 27,363,068 | 8,171,858,244 | 45.71 | 94.73 |

| | | | | |
|--------------|------------|---------------|-------|-------|
| High Noon'-3 | 26,151,034 | 7,817,595,230 | 45.35 | 95.15 |
| Roufurong'-1 | 23,849,725 | 7,102,437,096 | 45.54 | 94.91 |
| Roufurong'-2 | 24,209,670 | 7,231,381,174 | 44.80 | 95.28 |
| Roufurong'-3 | 28,420,112 | 8,471,070,046 | 45.12 | 95.14 |

Supplementary Table 5 Annotation of transcript datasets to public databases

| Annotated databases | Annotated number | 300<=Length<1000 | Length>1000 |
|---------------------|------------------|------------------|-------------|
| COG | 24,465 | 3,299 | 21,166 |
| GO | 30,995 | 5,428 | 25,566 |
| KEGG | 26,108 | 4,714 | 21,392 |
| KOG | 36,590 | 5,586 | 31,002 |
| Pfam | 47,713 | 6,969 | 40,743 |
| Swiss-Prot | 45,865 | 7,282 | 38,581 |
| eggNOG | 55,743 | 9,525 | 46,216 |
| Nr | 56,674 | 9,853 | 46,819 |
| All annotated | 56,974 | 9,972 | 46,999 |

Supplementary Table 6 Genes mapped to 125 KEGG biochemical pathways

| #pathway | pathway_id | Gene_number |
|--|------------|-------------|
| Glycolysis / Gluconeogenesis | ko00010 | 598 |
| Citrate cycle (TCA cycle) | ko00020 | 335 |
| Pentose phosphate pathway | ko00030 | 255 |
| Pentose and glucuronate interconversions | ko00040 | 282 |
| Fructose and mannose metabolism | ko00051 | 222 |

| | | |
|---|---------|-----|
| Galactose metabolism | ko00052 | 270 |
| Ascorbate and aldarate metabolism | ko00053 | 185 |
| Fatty acid biosynthesis | ko00061 | 207 |
| Fatty acid elongation | ko00062 | 91 |
| Fatty acid degradation | ko00071 | 238 |
| Synthesis and degradation of ketone bodies | ko00072 | 26 |
| Cutin, suberine and wax biosynthesis | ko00073 | 249 |
| Steroid biosynthesis | ko00100 | 93 |
| Ubiquinone and other terpenoid-quinone biosynthesis | ko00130 | 102 |
| Oxidative phosphorylation | ko00190 | 583 |
| Photosynthesis | ko00195 | 135 |
| Photosynthesis - antenna proteins | ko00196 | 49 |
| Purine metabolism | ko00230 | 479 |
| Caffeine metabolism | ko00232 | 10 |
| Pyrimidine metabolism | ko00240 | 340 |
| Alanine, aspartate and glutamate metabolism | ko00250 | 212 |
| Glycine, serine and threonine metabolism | ko00260 | 283 |
| Cysteine and methionine metabolism | ko00270 | 415 |
| Valine, leucine and isoleucine degradation | ko00280 | 215 |
| Valine, leucine and isoleucine biosynthesis | ko00290 | 88 |
| Lysine biosynthesis | ko00300 | 51 |
| Lysine degradation | ko00310 | 126 |
| Arginine and proline metabolism | ko00330 | 281 |
| Histidine metabolism | ko00340 | 58 |
| Tyrosine metabolism | ko00350 | 148 |
| Phenylalanine metabolism | ko00360 | 180 |

| | | |
|---|---------|-----|
| Tryptophan metabolism | ko00380 | 99 |
| Phenylalanine, tyrosine and tryptophan biosynthesis | ko00400 | 181 |
| beta-Alanine metabolism | ko00410 | 181 |
| Taurine and hypotaurine metabolism | ko00430 | 50 |
| Selenocompound metabolism | ko00450 | 127 |
| Cyanoamino acid metabolism | ko00460 | 221 |
| Glutathione metabolism | ko00480 | 313 |
| Starch and sucrose metabolism | ko00500 | 776 |
| N-Glycan biosynthesis | ko00510 | 130 |
| Other glycan degradation | ko00511 | 136 |
| Other types of O-glycan biosynthesis | ko00514 | 23 |
| Amino sugar and nucleotide sugar metabolism | ko00520 | 458 |
| Glycosaminoglycan degradation | ko00531 | 36 |
| Glycerolipid metabolism | ko00561 | 204 |
| Inositol phosphate metabolism | ko00562 | 191 |
| Glycosylphosphatidylinositol(GPI)-anchor biosynthesis | ko00563 | 75 |
| Glycerophospholipid metabolism | ko00564 | 249 |
| Ether lipid metabolism | ko00565 | 83 |
| Arachidonic acid metabolism | ko00590 | 51 |
| Linoleic acid metabolism | ko00591 | 54 |
| alpha-Linolenic acid metabolism | ko00592 | 188 |
| Sphingolipid metabolism | ko00600 | 112 |
| Glycosphingolipid biosynthesis - globo series | ko00603 | 22 |
| Glycosphingolipid biosynthesis - ganglio series | ko00604 | 23 |
| Pyruvate metabolism | ko00620 | 436 |
| Glyoxylate and dicarboxylate metabolism | ko00630 | 276 |

| | | |
|---|---------|-----|
| Propanoate metabolism | ko00640 | 127 |
| Butanoate metabolism | ko00650 | 84 |
| C5-Branched dibasic acid metabolism | ko00660 | 40 |
| One carbon pool by folate | ko00670 | 101 |
| Carbon fixation in photosynthetic organisms | ko00710 | 376 |
| Thiamine metabolism | ko00730 | 91 |
| Riboflavin metabolism | ko00740 | 24 |
| Vitamin B6 metabolism | ko00750 | 63 |
| Nicotinate and nicotinamide metabolism | ko00760 | 47 |
| Pantothenate and CoA biosynthesis | ko00770 | 119 |
| Biotin metabolism | ko00780 | 61 |
| Lipoic acid metabolism | ko00785 | 9 |
| Folate biosynthesis | ko00790 | 47 |
| Porphyrin and chlorophyll metabolism | ko00860 | 134 |
| Terpenoid backbone biosynthesis | ko00900 | 260 |
| Monoterpene biosynthesis | ko00902 | 59 |
| Limonene and pinene degradation | ko00903 | 31 |
| Diterpenoid biosynthesis | ko00904 | 9 |
| Brassinosteroid biosynthesis | ko00905 | 42 |
| Carotenoid biosynthesis | ko00906 | 101 |
| Zeatin biosynthesis | ko00908 | 26 |
| Sesquiterpenoid and triterpenoid biosynthesis | ko00909 | 67 |
| Nitrogen metabolism | ko00910 | 90 |
| Sulfur metabolism | ko00920 | 102 |
| Phenylpropanoid biosynthesis | ko00940 | 349 |
| Flavonoid biosynthesis | ko00941 | 115 |

| | | |
|--|---------|------|
| Anthocyanin biosynthesis | ko00942 | 12 |
| Flavone and flavonol biosynthesis | ko00944 | 30 |
| Stilbenoid, diarylheptanoid and gingerol biosynthesis | ko00945 | 33 |
| Isoquinoline alkaloid biosynthesis | ko00950 | 78 |
| Tropane, piperidine and pyridine alkaloid biosynthesis | ko00960 | 87 |
| Aminoacyl-tRNA biosynthesis | ko00970 | 268 |
| Biosynthesis of unsaturated fatty acids | ko01040 | 140 |
| Carbon metabolism | ko01200 | 1225 |
| 2-Oxocarboxylic acid metabolism | ko01210 | 254 |
| Fatty acid metabolism | ko01212 | 367 |
| Degradation of aromatic compounds | ko01220 | 23 |
| Biosynthesis of amino acids | ko01230 | 1114 |
| Vancomycin resistance | ko01502 | 3 |
| ABC transporters | ko02010 | 68 |
| Ribosome biogenesis in eukaryotes | ko03008 | 303 |
| Ribosome | ko03010 | 1062 |
| RNA transport | ko03013 | 746 |
| mRNA surveillance pathway | ko03015 | 544 |
| RNA degradation | ko03018 | 418 |
| RNA polymerase | ko03020 | 132 |
| Basal transcription factors | ko03022 | 142 |
| DNA replication | ko03030 | 116 |
| Spliceosome | ko03040 | 826 |
| Proteasome | ko03050 | 255 |
| Protein export | ko03060 | 144 |
| Base excision repair | ko03410 | 90 |

| | | |
|---|---------|-----|
| Nucleotide excision repair | ko03420 | 161 |
| Mismatch repair | ko03430 | 88 |
| Homologous recombination | ko03440 | 97 |
| Non-homologous end-joining | ko03450 | 24 |
| Phosphatidylinositol signaling system | ko04070 | 185 |
| Plant hormone signal transduction | ko04075 | 631 |
| Ubiquitin mediated proteolysis | ko04120 | 474 |
| Sulfur relay system | ko04122 | 31 |
| SNARE interactions in vesicular transport | ko04130 | 92 |
| Regulation of autophagy | ko04140 | 115 |
| Protein processing in endoplasmic reticulum | ko04141 | 961 |
| Endocytosis | ko04144 | 498 |
| Phagosome | ko04145 | 292 |
| Peroxisome | ko04146 | 324 |
| Plant-pathogen interaction | ko04626 | 484 |
| Circadian rhythm - plant | ko04712 | 253 |

Supplementary Table 7 Primers used for in-fusion cloning and qRT-PCR in tree peony.

| Primers | Primer Sequences (5'-3') | Description |
|--------------------|--|-------------------|
| <i>PsMYB4</i> -F | ACACGGGGACTCTTGACATGGGAAGGTCTCCTTGTGTTGT | In-fusion cloning |
| <i>PsMYB4</i> -R | AAGTTCTTCTCCTTTACTCTTCATCTCCAATCTTCTGTA | In-fusion cloning |
| <i>PsMYB111</i> -F | ACACGGGGACTCTTGACATGGGGAGGGCACCCGTGTTGT | In-fusion cloning |
| <i>PsMYB111</i> -R | AAGTTCTTCTCCTTTACTAGAAAGAAGCCAAGCAACCAT | In-fusion cloning |
| <i>PsTT8</i> -F | ACACGGGGACTCTTGACATGGCTGCGCCGCCGTAAAC | In-fusion cloning |
| <i>PsTT8</i> -R | AAGTTCTTCTCCTTTACTAGGAATTATTGGTGTATTGC | In-fusion cloning |
| <i>PsEGL3</i> -F | ACACGGGGACTCTTGACATGGCAACTGGGCTCCAAATT | In-fusion cloning |

| | | |
|-----------------------|---------------------------------------|------------------------|
| <i>PsEGL3</i> -R | AAGTTCTTCTCCTTACTACACTTGTGAATAACTCTTG | In-fusion cloning |
| <i>PsSPL9</i> -F | ACACGGGGACTCTGACATGGAAATGGGTCGAGCGTT | In-fusion cloning |
| <i>PsSPL9</i> -R | AAGTTCTTCTCCTTACTAAGTGACCAGTCATCTGCTG | In-fusion cloning |
| <i>PsUbiquitin</i> -F | GACCTATACCAAGCCGAAG | qRT-PCR for tree peony |
| <i>PsUbiquitin</i> -R | CGTCCAGCACCAAC | qRT-PCR for tree peony |
| F01_cb7425_c19-F | GTAATCTGGTGGTGGTTTCGT | qRT-PCR for tree peony |
| F01_cb7425_c19-R | TCGAAAGAAAGGAACCGTCA | qRT-PCR for tree peony |
| F01_cb9709_c126-F | ACTACCAACTCACCAAAC | qRT-PCR for tree peony |
| F01_cb9709_c126-R | CGACCAAAGAACATCAAATGAG | qRT-PCR for tree peony |
| F01_cb13354_c0-F | CGCAGGAGTAAGAGGTTGGA | qRT-PCR for tree peony |
| F01_cb13354_c0-R | CTCTGAATATTGTTGCCCGT | qRT-PCR for tree peony |
| F01_cb4550_c42-F | GAGCATGGAGGTAGAACAGAT | qRT-PCR for tree peony |
| F01_cb4550_c42-R | TCCCCATTCTTGCTTGC | qRT-PCR for tree peony |
| F01_cb4550_c24-F | CACTCTCAACCAAAACAATCC | qRT-PCR for tree peony |
| F01_cb4550_c24-R | CACTCTCAACCAAAACAATCC | qRT-PCR for tree peony |
| F01_cb4853_c44-F | AAAACCCCTCAATCCAGCTTC | qRT-PCR for tree peony |
| F01_cb4853_c44-R | AAATCCCCAGTCTCACATG | qRT-PCR for tree peony |
| F01_cb6443_c9-F | TTTTGTGATACTGCCCTCT | qRT-PCR for tree peony |
| F01_cb6443_c9-R | TATTAGTGAAAGATGGCCC | qRT-PCR for tree peony |
| F01_cb12671_c6-F | GTTAATGGACTCTGGGTTGA | qRT-PCR for tree peony |
| F01_cb12671_c6-R | ATCCCTTCCTCGGCATGTT | qRT-PCR for tree peony |
| F01_cb7851_c6-F | CGTCTTGGTTCTGCTGGGTT | qRT-PCR for tree peony |
| F01_cb7851_c6-R | ACTGCTACTTCCACCACCATT | qRT-PCR for tree peony |
| F01_cb8732_c11-F | AGAACAAAACCTACAACCGCC | qRT-PCR for tree peony |
| F01_cb8732_c11-R | ATTCCTGCTCTCTCTCCTC | qRT-PCR for tree peony |
| F01_cb8475_c68614-F | GTTCAGACAGTAGTGCCAAAG | qRT-PCR for tree peony |

| | | |
|---------------------|------------------------|------------------------|
| F01_cb8475_c68614-R | CTTCCTTATTCTCCGATCTCC | qRT-PCR for tree peony |
| F01_cb3984_c5-F | AAGTTCGGGTCATGGTTATT | qRT-PCR for tree peony |
| F01_cb3984_c5-R | ACGTTGTGTGCATCTGTT | qRT-PCR for tree peony |
| F01_cb8475_c28561-F | AAAAGAATTGACGAGGGACCAG | qRT-PCR for tree peony |
| F01_cb8475_c28561-R | AGCTCCACCATTATGCCATC | qRT-PCR for tree peony |
| F01_cb3692_c19-F | TCCACTCACCATCACTTCCAC | qRT-PCR for tree peony |
| F01_cb3692_c19-R | CTCAGACATCTCAGTTGCAT | qRT-PCR for tree peony |
| F01_cb13401_c3-F | TCTTGTACATATGCACTGCC | qRT-PCR for tree peony |
| F01_cb13401_c3-R | ACCCGCAGTATTGAAGCTTA | qRT-PCR for tree peony |
| F01_cb9156_c0-F | CTTCATCTGCTCACTTCAAC | qRT-PCR for tree peony |
| F01_cb9156_c0-R | CGGGAAAATGAAGTTGTGTT | qRT-PCR for tree peony |

Supplementary Table 8 Primers used for qRT-PCR in transgenic tobacco.

| Primers | Primer Sequences (5'-3') | Description |
|-----------|--------------------------|--------------------------------|
| NtTubA1-F | CTCCTATGCTCCTGTCATTTC | qRT-PCR for transgenic tobacco |
| NtTubA1-R | GGCGAGGATCACACTTAAC | qRT-PCR for transgenic tobacco |
| NtbHLH-F | GCAGCAGTGTAGCTGGATATG | qRT-PCR for transgenic tobacco |
| NtbHLH-R | AGCAGAACTCATTGAGGAAGACC | qRT-PCR for transgenic tobacco |
| NtSPL9-F | CTCCTTTGGCTTCTGCTT | qRT-PCR for transgenic tobacco |
| NtSPL9-R | CTCGCTCTGGTGGTTCTAA | qRT-PCR for transgenic tobacco |
| NtC4H-F | ACAACAGAGAAAGGCCGGCAA | qRT-PCR for transgenic tobacco |
| NtC4H-R | AGGGGCTTACATGACACCAA | qRT-PCR for transgenic tobacco |
| NtCHS-F | TGACACCCACTGGATAGTTAG | qRT-PCR for transgenic tobacco |
| NtCHS-R | CGACCTCTGGAATTGGATCAG | qRT-PCR for transgenic tobacco |
| NtCHI-F | CTTTCTGCCCGCTAAATG | qRT-PCR for transgenic tobacco |
| NtCHI-R | TTTCTGCCACCTCTCTG | qRT-PCR for transgenic tobacco |

| | | |
|------------------|-------------------------|--------------------------------|
| <i>NtF3H</i> -F | CAAGGCATGTGATGGATATGG | qRT-PCR for transgenic tobacco |
| <i>NtF3H</i> -R | TGTGTCGTTCAGTCCAAGG | qRT-PCR for transgenic tobacco |
| <i>NtF3'H</i> -F | AGGCTCAACACTTCTCGT | qRT-PCR for transgenic tobacco |
| <i>NtF3'H</i> -R | CATCAACTTGGGCTTCT | qRT-PCR for transgenic tobacco |
| <i>NtFLS</i> -F | GAACTTGAAGGGAAAAGGGGTTG | qRT-PCR for transgenic tobacco |
| <i>NtFLS</i> -R | GTAGGAGGGAGGATTTAGGCC | qRT-PCR for transgenic tobacco |
| <i>NtDFR</i> -F | AACCAACAGTCAGGGGAATG | qRT-PCR for transgenic tobacco |
| <i>NtDFR</i> -R | TTGGACATCGACAGTTCCAG | qRT-PCR for transgenic tobacco |

Supplementary Table 9 Primers used for genome walking and Dual-luciferase assay.

| Primers | Primer Sequences (5'-3') | Description |
|--------------------|---|-----------------------|
| <i>PsCHS</i> -SP1 | GGTGGGTGATTTAGATTGGG | Genome walking |
| <i>PsCHS</i> -SP2 | CACCATGTCCTGACGTGCATCC | Genome walking |
| <i>PsCHS</i> -SP3 | CTTCGGTCAGGTACATATAGCGTT | Genome walking |
| <i>PsFLS</i> -SP1 | CTCTCTTCCAACACTCTGCAAC | Genome walking |
| <i>PsFLS</i> -SP2 | CCCATTCTTGCTTGCACTGTC | Genome walking |
| <i>PsFLS</i> -SP3 | TCTTTGCTTGCACTGTCGATGG | Genome walking |
| <i>PsCHS</i> -F | GTACCGGGCCCCCTCGAGGTCGACAGAGGAGAACGATAGGATCGT | Dual-luciferase assay |
| <i>PsCHS</i> -R | GGCTGCAGGAATTGATATCAAGCTTTGTGTGCTGGGCTCGGTT | Dual-luciferase assay |
| <i>PsFLS</i> -F | GTACCGGGCCCCCTCGAGGTCGACAGTGGAGTAGCAAAGGTTG | Dual-luciferase assay |
| <i>PsFLS</i> -R | GGCTGCAGGAATTGATATCAAGCTGCTGTTTGAAAGCAGA | Dual-luciferase assay |
| <i>PsMYB111</i> -F | GGCCGCTCTAGAACTAGTGGATCCATGGGAGGGCACCCGTGTTG | Dual-luciferase assay |
| <i>PsMYB111</i> -R | ATCGATAAGCTTGTATCGAATTCTCAAGAAAGCAAGCAACCA | Dual-luciferase assay |