**Supporting Information for**

**Metabolic and Transcriptional Analysis Reveals Flavonoid Involvement in the Drought Stress Response of Mulberry Leaves**

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**Figure S1.** Metabolic and transcriptional analysis of *M. Pendula* leaves under drought stress. (**A**) Principal component analysis (PCA) of the metabolome. (**B**) PCA of the transcriptome. (**C**) Volcano plot displaying differentially expressed genes (DEGs) identified through transcriptome analysis. (**D**) Venn Diagram showing annotation genes across four databases. (**E**) KEGG pathways of DEGs in mulberry leaves under drought conditions.

**Figure S2.** Non-rooted molecular phylogenetic tree of flavonoid related UDP-glycosyltransferase. The three candidate *MaUGTs* genes analyzed in this study are shown in red color. The GenBank accession numbers for the sequences are shown in parentheses: *Zm3GlcT* (X13501), *Vv3GlcT* (AF000371), *Ph5GlcT* (AB027455), *Vh5GlcT* (AB013598), *At7GlcT* (NM\_129234), *Sb7GlcT* (AB031274), *NtIS5* (AF346431), *At7RhaT* (NM\_100480), *MtUGT72L1* (EU434684), *OsCGT* (FM179712), *IpA3G2**˝GlcT* (AB192315), *PhA3G6˝RhaT* (Z25802), *GmF3G6˝RhaT* (NP\_001275524), *CsiF7G6˝RhaT* (DQ119035), and *MaF3G6˝RhaT*（KT324624).



**Figure S3.** Bioinformatic analysis of *MaFLS1*. (**A**) Phylogenetic analysis of flavonol synthase (FLS). The phylogenetic tree was constructed using the MEGA7 software with a neighbor-joining method. Sequence data from this article can be found in the NCBI database under the following accession numbers: *VcFLS* (AKJ87100), *EcFLS* (ALP48589), *PcFLS* (AY230249), *EgFLS* (AY230249), *MdFLS* (AAD26261), *PhFLS* (Q07512), *FaFLS* (AAZ78661), *RmFLS* (AJP36706), *GmFLS* (NP001237419), *GsFLS* (NP001237419), *AtFLS* (AED91333), *PpFLS* (AJO70134), *GbFLS* (GQ994432), *NiFLS* (AB078512), and *MaFLS1* (XM\_010098126). (**B**) Alignment of the amino acid sequences of the MaFLS1 and other FLS proteins. The typical of conserved 2-oxoglutarate-Fe (II) oxygenase domain is presented in the red box. The Fe2+-binding sites and oxoglutarate binding sites are indicated by red triangles and red ellipses, respectively.

**Table S1**. List of primer sequences used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gategory | Gene | Primer sequences | | |
| Forward (5' to 3') | Reverse (3' to 5') |
| Sequencing | *MaFLS1* | ATGGAGGTTGAGAGAGTTCAAG | TCACTGGG GAAGCTTGTTGAG |
| *MaF3GT5* | ATGGGCCACCTAGTTCCGAT | TCAGCCAGTGCT TTCTTTTAAGCT |
| *MaUGT76A2* | ATGCCGGACGGCTTATCC | TCATGCCAAGAAACCAAATTGGTC |
| *MaUGT90A1* | ATGGAATCGGCTATCGAGCC | TTAATAATTACTCGCATGATCTACT |
| qRT-PCR | *MaPAL1* | CCGGACCGACTTCTTCGAGC | GAATTCGGGCATCCCGAGCA |
| *MaPAL2* | GCAATGGAACAGAATCATGTCACAC | TCTAATGCCTGAGTAGCCTTGGAG |
| *MaPAL3* | TCACCACCGGCTTTGGTTCC | CCGGAGGCGGTTATGGTTCC |
| *MaC4H* | AACCTCCCTTGTAGCTCGTCCTG | CCATACCAGCAGTACCACCAATTCC |
| *Ma4CL1* | CCGAAGGGGGTGATGCTGAC | ATCACGATCGGCGGCACAAT |
| *Ma4CL2* | CATCTCGGAGCCGTCACCAC | TCGTGCCCGAAGAATACGGC |
| *MaCHS1* | TCACACCGATCGGCATCAGC | CCTGCTTCAGCCCCAGCTTT |
| *MaCHS2* | GGCAACCTCCGTCCAAGAAATC | TTGTGCTCGCTGTTGGTGATTC |
| *MaCHI* | GAGATAGCCACGTTCCCGCC | TCCACTTACCGGCAAGCCAC |
| *MaFLS1* | CCTCCCTCTCGCATCAACTACC | AACACCTTATCCGCCACCTCTC |
| *MaFLS2* | TGTCTTGGGCGGTGTTTTGTG | ACTTTGGCGGGTTTGTCTTGTC |
| *MaF3H* | ATCGCGGTGACGGAGGAGTA | GCGCTTCAGGCCAAGAGTGA |
| *MaF3'H* | CGTAATCGGGAACCTGCCCC | TGTGCTTGGCTCCGGAGTTG |
| *MaF3GT* | CATCGTCCGTCGCCTAGCTG | GAACAGCTCGATGCGCTCCT |
| *MaF3GT5* | CTGCCACGTCATCGACCTCC | TCCTCGGCCAGACACAGACA |
| *MaUGT76A2* | TGGAATTGGAGGAGTTGGAGAGAG | AGACGAACCACCTTCCCTTGTAG |
| *MaUGT90A1* | CACTCCTTCGCCCGATCCAC | GCCCATAGCGGATTGCGAGT |
| *MaF3G6''RT* | TAGTCCACTCCGGGTGGGTG | ACCTCCACCCCAGCCTTCAT |

**Table S4.** Statistical results of RNA-seq assembly data.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Genes number | GC percentage | N50 number | N50 length | Max length | Min length | Average length | Total assembled bases |
|
| 45642 | 40.4454 | 7996 | 2292 | 16569 | 201 | 1234 | 56354744 |

**Table S5.** Details of significantly changed genes involved in flavonoid [biosynthesis](https://fanyi.so.com/?src=onebox#biosynthesis) in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene ID | FPKMa | | Symbol | Putative annotationb |
| C | D |
| Unigene0023038 | 10.82 | 25.15 | *PAL1* | Phenylalanine ammonia lyase |
| Unigene0039394 | 101.63 | 235.33 | *PAL2* | Phenylalanine ammonium lyase |
| Unigene0022114 | 3.8 | 6.83 | *PAL3* | Phenylalanine ammonia lyase |
| Unigene0039210 | 18.87 | 30.42 | *4CL1* | 4-coumarate--CoA ligase 1 |
| Unigene0020203 | 4.97 | 7.23 | *4CL2* | 4-coumarate--CoA ligase 1 |
| Unigene0042667 | 20.98 | 164.28 | *CHS1* | Chalcone synthase |
| Unigene0042666 | 2.25 | 4.65 | *CHS2* | Chalcone synthase |
| Unigene0008170 | 1.25 | 0.41 | *CHS3* | Chalcone synthase |
| Unigene0029681 | 49.98 | 85.59 | *F3H* | Flavone 3-hydroxylase |
| Unigene0046320 | 47.38 | 114.49 | *F3'H1* | Flavone 3'-hydroxylase |
| Unigene0046319 | 5.28 | 16.23 | *F3'H2* | Flavone 3'-hydroxylase |
| Unigene0000347 | 31.78 | 99.27 | *FLS1* | Flavonol synthase/flavanone 3-hydroxylase |
| Unigene0014572 | 2.41 | 0.09 | *FLS2* | Flavonol synthase/flavanone 3-hydroxylase |
| Unigene0042252 | 11.02 | 5.49 | *FLS3* | Flavonol synthase/flavanone 3-hydroxylase |
| Unigene0046274 | 15.85 | 61.33 | *F3GT* | Anthocyanidin 3-O-glucosyltransferase |
| Unigene0033543 | 7.26 | 71.72 | *F3GT5* | Anthocyanidin 3-O-glucosyltransferase 5 |
| Unigene0015859 | 1.11 | 4.98 | *UGT76A2* | UDP-glucose glucosyltransferase 76A2 |
| Unigene0038284 | 5.5 | 13.72 | *UGT90A1* | UDP-glucose glucosyltransferase 90A1 |

aRepresents average of three replicates of normalized FPKM (reads per kb per million reads) values.

bPutative annotations according to BlastX queries against NCBI non-redundant (nr) protein database.