## Supplementary Material

## UGT85A84 catalyzes the glycosylation of aromatic monoterpenes in *Osmanthus fragrans* Lour. flowers

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**Supplementary Figures and Tables** 

**Supplementary Figure 1**. GO classification of assembled unigenes

**Supplementary Figure 2.** KOG classification of assembled unigenes

Supplementary Figure 3. KEGG classification of assembled unigenes

**Supplementary Figure 4.** Analysis of differentially expressed genes (DEGs) between different blossoming stages

**Supplementary Figure 5.** Alignment of amino acid sequences of UGTs

Supplementary Figure 6. SDS-PAGE analysis of protein UGT85A84

**Supplementary Figure 7.** LC-MS analysis of sugar donors and aglycones

**Supplementary Figure 8.** Glycosylation of linalool and its oxides in *Osmanthus fragrans* flowers and its potential function.

Supplementary Table 1. Quality of transcriptome sequencing

**Supplementary Table 2**. Gene primers used for qRT-PCR analysis of four candidate *UGTs* 

**Supplementary Table 3**. Functional annotation of the transcriptome sequences

**Supplementary Table 4**. GenBank accession numbers used for construction of the phylogenetic tree in Figure 1

**Supplementary Table 5.** The correlation between transcript levels of MEP pathway genes and the contents of volatile, free and glycosylated aroma compounds

**Supplementary Table 6.**  $K_{\rm m}$  of UGTs from different plants

Table S1. Gene primers used for qRT-PCR analysis of four candidate UGTs

| Primer | Sequence |  |
|--------|----------|--|
|        |          |  |

| Actin-F       | 5'-ATTAGTCCTCTTCCAGCCTTCTTTG-3' |
|---------------|---------------------------------|
| Actin-R       | 5'-ATTATTTCCTTGCTCATACGGTCAG-3' |
| OfUGT85A82-F0 | 5'-AAGCCACCAAAGAAAGACG-3'       |
| OfUGT85A82-R0 | 5'-GCACTCCACTGCCAATACTC-3'      |
| OfUGT85A83-F0 | 5'-TTCGCAATGGAGGAGACTGA-3'      |
| OfUGT85A83-R0 | 5'-AGGACCAATGCCGTAAACTG-3'      |
| OfUGT85AF3-F0 | 5'-CGAGCGAAGACTCAAATCC-3'       |
| OfUGT85AF3-R0 | 5'-CAGGAGACAGTGGCGTGAT-3'       |
| OfUGT85A84-F0 | 5'-CTTGCTTGGCACCTTTCTG-3'       |
| OfUGT85A84-R0 | 5'-ACCATTTGTTACCTGGCTCAT-3'     |

Table S2. Quality of transcriptome sequencing

| Sample | Raw<br>reads | Clean reads | Clean<br>bases | Error(%) | Q20(%) | Q30(%) | GC(%) |
|--------|--------------|-------------|----------------|----------|--------|--------|-------|
| S1-1   | 55894680     | 54270372    | 8.14G          | 0.01     | 97.46  | 93.31  | 43.86 |
| S1-2   | 59656098     | 57908214    | 8.69G          | 0.01     | 97.38  | 93.13  | 44.36 |
| S1-3   | 50600770     | 49428240    | 7.41G          | 0.01     | 97.55  | 93.43  | 44.01 |
| S2-1   | 52328672     | 50804512    | 7.62G          | 0.02     | 97.3   | 92.96  | 43.56 |
| S2-2   | 45858390     | 44510034    | 6.68G          | 0.02     | 97.26  | 92.87  | 43.74 |
| S2-3   | 45792884     | 44666850    | 6.7G           | 0.01     | 97.41  | 93.16  | 44.16 |
| S3-1   | 57418394     | 55655706    | 8.35G          | 0.02     | 97.19  | 92.74  | 44.73 |
| S3-2   | 59510806     | 57524456    | 8.63G          | 0.01     | 97.73  | 94.11  | 45.02 |
| S3-3   | 55998928     | 46752178    | 7.01G          | 0.01     | 97.62  | 93.72  | 43.19 |
| S4-1   | 50486756     | 54124672    | 8.12G          | 0.01     | 97.88  | 94.41  | 44.34 |
| S4-2   | 50486756     | 49351558    | 7.4G           | 0.01     | 97.54  | 93.43  | 43.67 |
| S4-3   | 49707720     | 48051954    | 7.21G          | 0.01     | 97.5   | 93.48  | 45.3  |

<sup>&</sup>lt;sup>1</sup>1, 2 and 3 represent three independent biological replicates

**Table S3. Functional annotation of the transcriptome sequences** 

| Databases | Number of unigenes | Percentage(%) |  |
|-----------|--------------------|---------------|--|
|           |                    |               |  |

<sup>&</sup>lt;sup>2</sup>Q20: The percentage of bases with a Phred value >20

<sup>&</sup>lt;sup>3</sup>Q30: The percentage of bases with a Phred value >30

| Annotated in NR                    | 94927  | 65.83 |
|------------------------------------|--------|-------|
| Annotated in NT                    | 70565  | 48.94 |
| Annotated in KO                    | 38916  | 26.99 |
| Annotated in SwissProt             | 72330  | 50.16 |
| Annotated in PFAM                  | 65823  | 45.65 |
| Annotated in GO                    | 66202  | 45.91 |
| Annotated in KOG                   | 10056  | 27.78 |
| Annotated in all databases         | 20095  | 13.93 |
| Annotated in at least one database | 102789 | 71.28 |
| Total unigenes                     | 144186 | 100   |

Table S4. GenBank information and glycosylation substrates of plant UGTs used for construction of sequence alignment and phylogenetic tree

| Abbreviation | Plant species                 | Substrates   | Nucleotide ID      | Protein ID         |
|--------------|-------------------------------|--|--------------------|--------------------|
| UGT85A1      | Arabidopsis<br>thaliana       | Monoterpenes                                       |                    | AAF18537           |
| UGT85A2      | Arabidopsis<br>thaliana       | Citronellol, geraniol, perillyl alcohol            | AB016819           | BAA34687           |
| UGT85A5      | Arabidopsis<br>thaliana       | Citronellol, geraniol                              | AC068562           | AAF87255           |
| UGT85A7      | Arabidopsis<br>thaliana       | Terpineol, citronellol, geraniol, perillyl alcohol | AC068562           | AAF87257           |
| UGT85A23     | Catharanthus<br>roseus        | 7-Deoxyloganetin                                   | AB591741           | BAK55749           |
| UGT85A57     | Rubus suavissimus             | Diterpenoid  | MG592709           |                    |
| UGT85AF5     | Handroanthus<br>impetiginosus |  |                    | PIN05078.1         |
| UGT85AF6     | Sesamum indicum               | 7-Deoxyloganetin                                   | XM_01108960<br>0.2 | XP_0110879<br>02.1 |
| UGT85AF7     | Olea europaea                 | 7-Deoxyloganetin                                   | XM_02300545<br>5.1 | XP_0228612<br>23   |

Table S5. The correlation between transcript levels of MEP pathway genes and the contents of volatile, free and glycosylated aroma compounds

| Genes | Volatile | linalool | Free | linalool | and | Glycosylated linalool |
|-------|----------|----------|------|----------|-----|-----------------------|

|              | and its oxides | its oxides | and its oxides |
|--------------|----------------|------------|----------------|
| CMK-1        | -0.70          | 0.18       | 0.80           |
| CMK-2        | -0.66          | 0.27       | 0.75           |
| DXR          | -0.70          | 0.27       | 0.82           |
| DXS-1        | -0.74          | 0.12       | 0.72           |
| DXS-2        | -0.61          | 0.29       | 0.73           |
| <i>GPPS</i>  | -0.58          | 0.11       | 0.70           |
| HDS          | -0.61          | 0.31       | 0.73           |
| IDI          | -0.70          | 0.19       | 0.77           |
| IDS-1        | -0.52          | 0.37       | 0.67           |
| IDS-2        | -0.55          | 0.39       | 0.68           |
| IDS-3        | -0.57          | 0.21       | 0.68           |
| MCT-1        | -0.72          | 0.20       | 0.82           |
| MCT-2        | -0.64          | 0.29       | 0.75           |
| <i>MECPS</i> | -0.60          | 0.34       | 0.70           |
| LIS-1        | -0.60          | -0.11      | 0.59           |
| LIS-2        | -0.51          | -0.05      | -0.29          |
| LIS-3        | 0.32           | 0.31       | 0.36           |

The relative coefficient was analyzed according the circadian transcript levels and aroma compounds during the full blossoming period of *O. fragrans* flowers.

Table S6. K<sub>m</sub> of UGTs from different plants

| Plant  | Substrate   | $K_{\mathrm{m}}(\mu\mathrm{M})$ |
|--|-------------|---------------------------------|
|  | Nerol       | 417±4                           |
|  | Citronellol | 433±62                          |
| Vitis vinifera   | Geraniol    | 464±56                          |
| (Friedericke et al., 2014a; Friedericke et al., 2014b) | Nerol       | 204±27                          |
|  | Citronellol | 306±75                          |
|  | Geraniol    | 396±12                          |
|  | Nerol       | 211±16                          |
|  | Citronellol | 445±21                          |

|  | Geraniol                |      | 321±2      |  |
|--|-------------------------|------|------------|--|
|  | Nerol                   |      | 40±3.7     |  |
|  | S-Citronellol           |      | 55±1.3     |  |
|  | Geraniol                |      | 81±1.0     |  |
|  | 8-Hydroxylinalool       |      | 33±1.8     |  |
|  | Nerol                   |      | 118±4.7    |  |
|  | Citronellol             |      | 108±2.5    |  |
|  | Geraniol                |      | 355±14     |  |
| Camellia sinensis (Shoji et al., 2015)  Prunus persica (Wu et al., 2018)  Actinidia deliciosa (Yar-Khing et al., 2014) | Geraniol                |      | 44.2±3     |  |
|  | Geranyl glucopyranoside | β-D- | 78.1±19.6  |  |
|  | Linalool                |      | 463±80     |  |
|  | Geraniol                |      | 76.2±11.1  |  |
|  | Octan-3-ol              |      | 66.6±16.2  |  |
|  | Hexanol                 |      | 116.9±28.1 |  |