# Transcriptome Analysis of Genes Involved in Dendrobine Biosynthesis in *Dendrobium nobile* Lindl. Infected with Mycorrhizal Fungus MF23 (*Mycena* sp.)

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**Supplementary Figure S1. The effect of MF23 on growth of *D. nobile* tissue culture seedlings.**

**Supplementary Figure S2. Length distribution of assembled unigenes.**

**Supplementary Figure S3. Terpenoid backbone biosynthesis pathway.**

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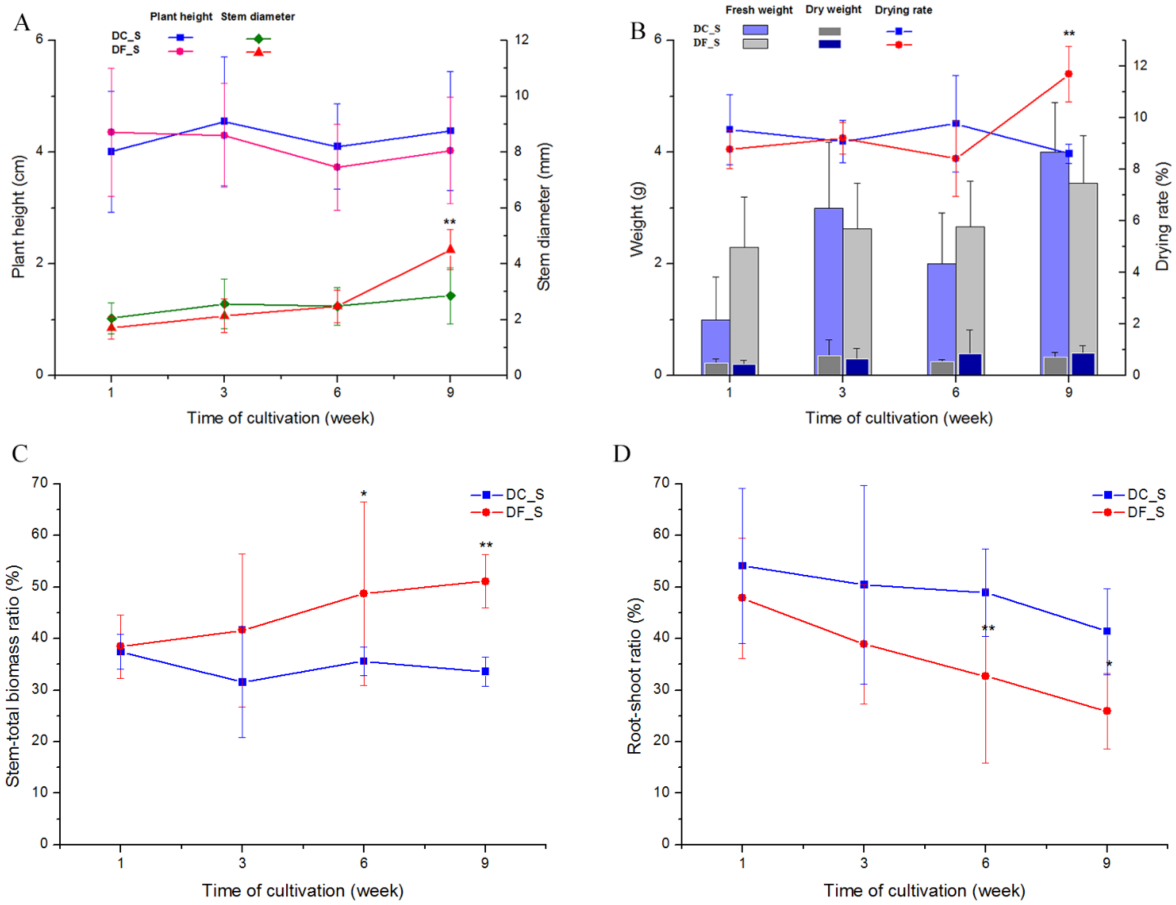
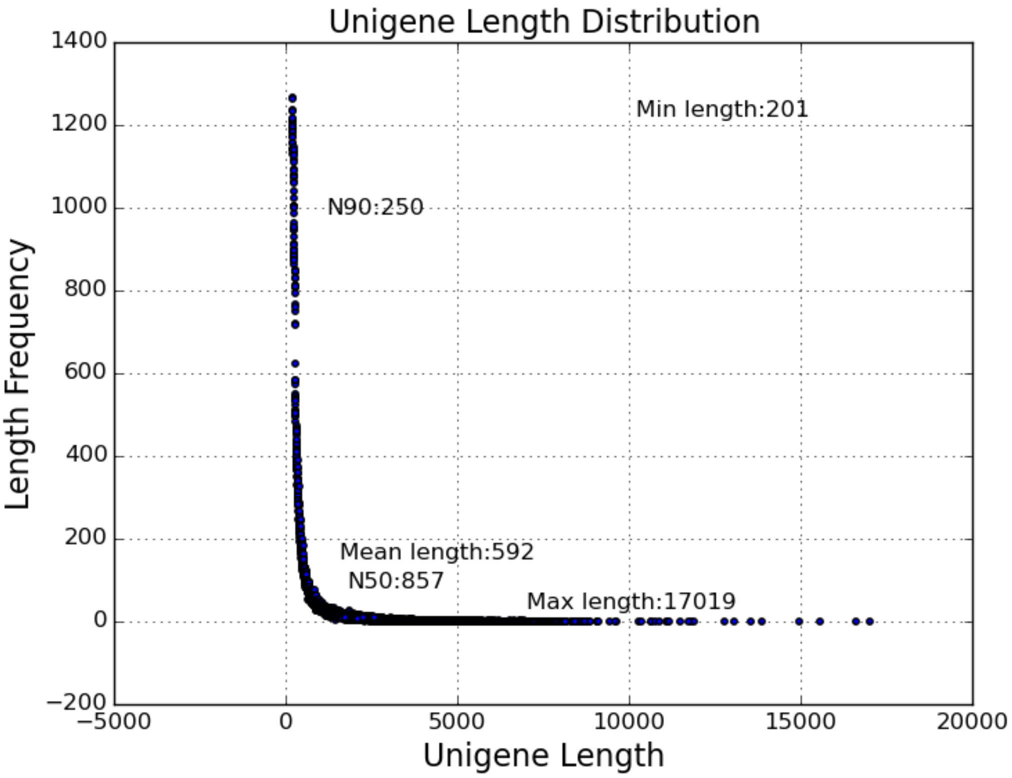
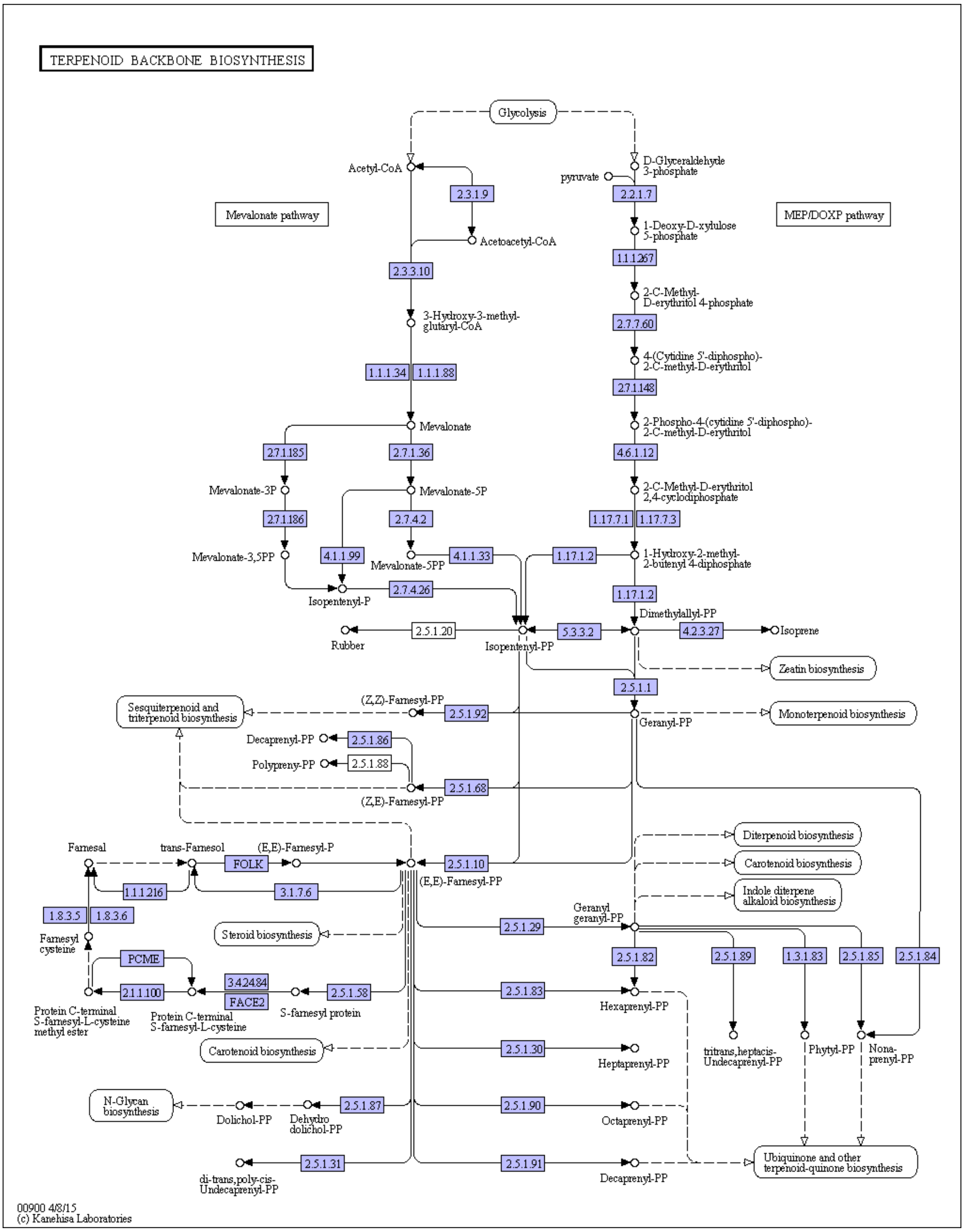


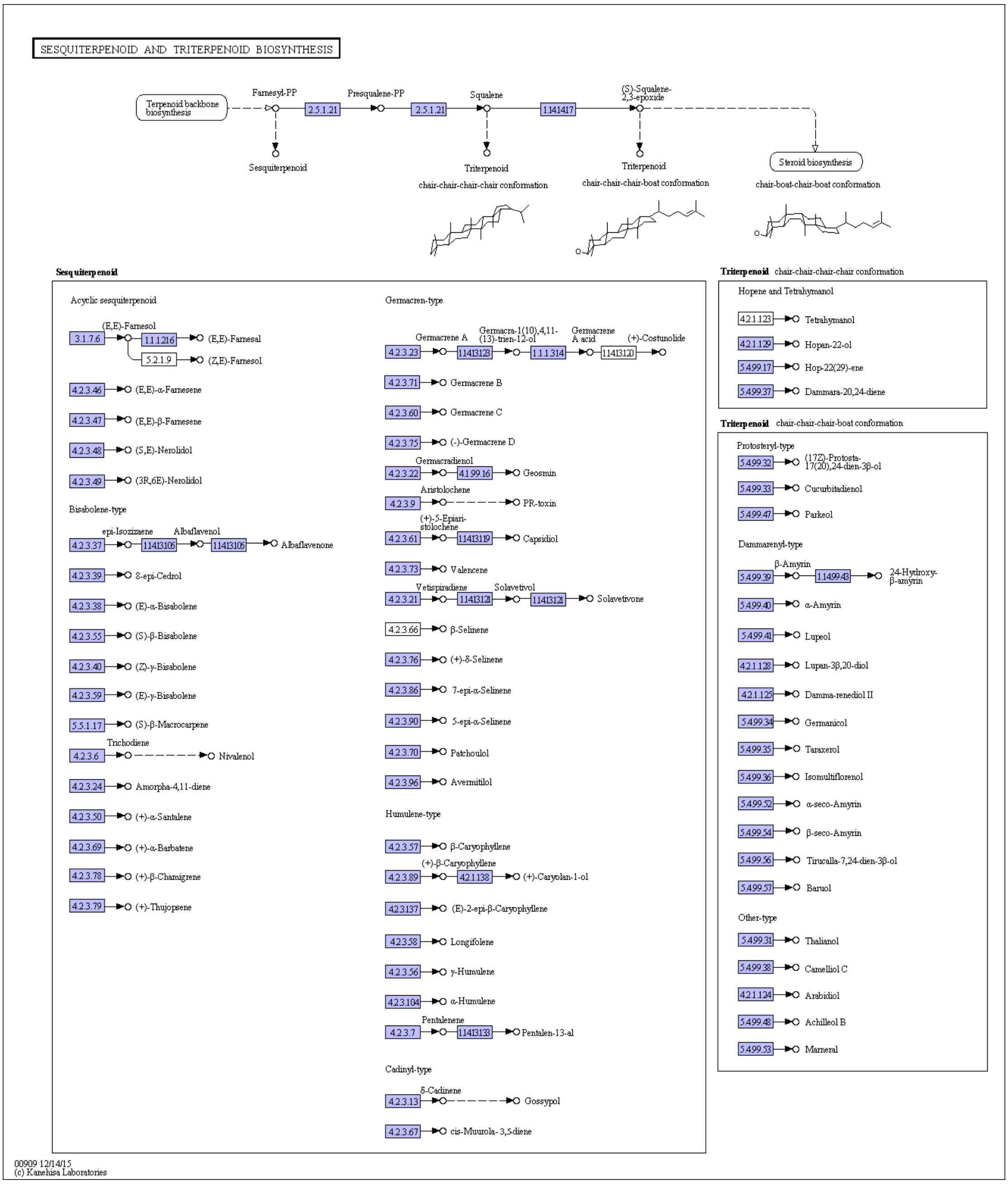
**Supplementary Figure S1. The effect of MF23 on the growth of *D. nobile* tissue culture seedlings. (A)** Plant height and stem diameter of *D. nobile* tissue culture seedlings. **(B)** Biomass of *D. nobile* tissue culture seedlings. **(C)** Stem-total biomass ratio of *D. nobile* tissue culture seedlings. **(D)** Root-shoot ratio of *D. nobile* tissue culture seedlings. DC_S represents for control group, DF_S represents for model group. Values are presented as the means ±SD (n=12); values with * are of statistical significance at $P < 0.05$, values with ** are of statistical significance at $P < 0.01$.



**Supplementary Figure S2. Length distribution of assembled unigenes.**



**Supplementary Figure S3. Terpenoid backbone biosynthesis pathway (derived from KEGG map00900).**



**Supplementary Figure S4. Sesquiterpenoid and triterpenoid biosynthesis pathway (derived from KEGG map00909).**

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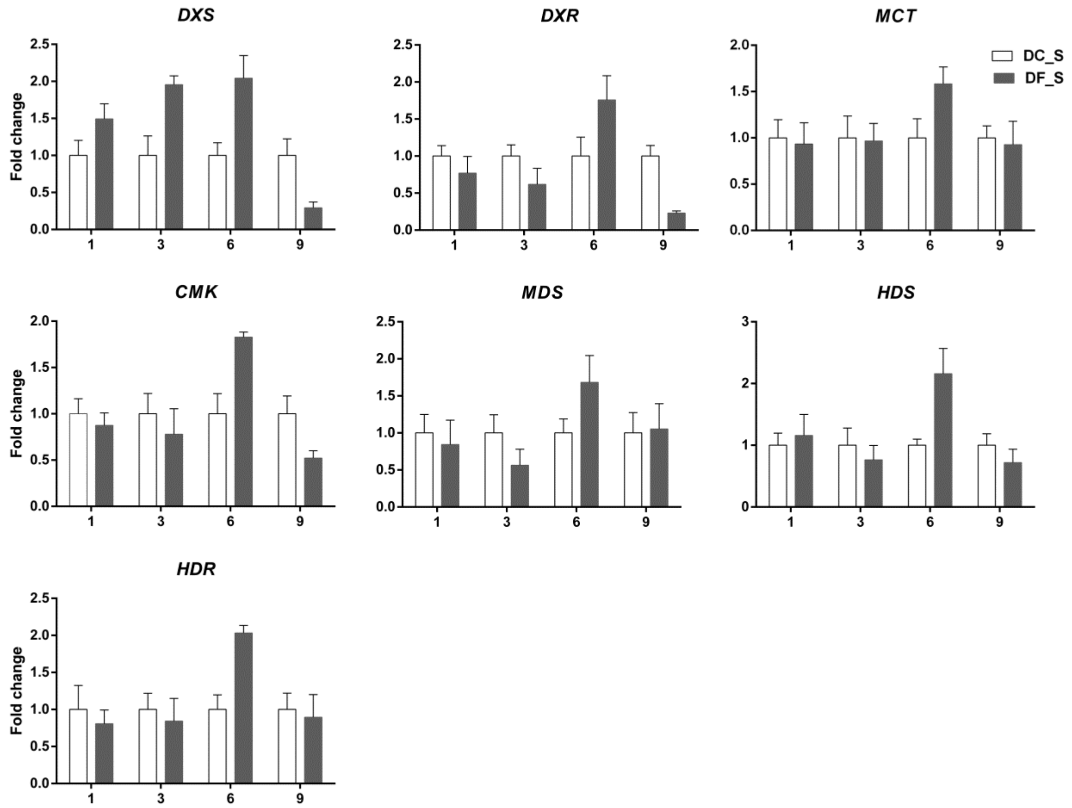
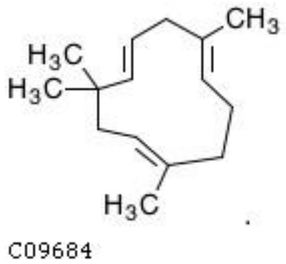


**Supplementary Figure S5. qRT-PCR analysis of key enzyme-coding genes involved in the MEP pathway in *D. nobile*.** The y-axes correspond to the mean fold changes in expression values, and the x-axes display the times of symbiotic culture (/weeks). White bars represent the control group (DC_S), and grey bars represent the model group (DF_S). For each qRT-PCR validation, three technical replicates were used, with a minimum of three biological replicates.



**Supplementary Figure S6. The structure of humulene.**

**Supplementary Table S1. Summary of sequences analysis.**

| Sample | Raw Reads | Clean Reads | Clean Bases | Error(%) | Q20(%) | Q30(%) | GC Content (%) |
|---|---|---|---|---|---|---|---|
| DC1_S1 | 57252114 | 55277910 | 8.29G | 0.01 | 97.53 | 94.05 | 46.16 |
| DC1_S2 | 44863528 | 43312840 | 6.5G | 0.01 | 97.56 | 94.09 | 45.75 |
| DF1_S1 | 58455694 | 56340260 | 8.45G | 0.01 | 97.37 | 93.7 | 46.2 |
| DF1_S2 | 52912596 | 50959096 | 7.64G | 0.01 | 97.69 | 94.33 | 47.63 |
| DC9_S1 | 47092876 | 45414294 | 6.81G | 0.01 | 97.25 | 93.47 | 46.48 |
| DC9_S2 | 46537374 | 44964612 | 6.74G | 0.01 | 97.49 | 93.96 | 46.59 |
| DF9_S1 | 53471134 | 51541182 | 7.73G | 0.01 | 97.54 | 94.09 | 47.3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DF9_S2 | 69564340 | 67080588 | 10.06G | 0.01 | 97.55 | 94.12 | 46.94 |

**Supplementary Table S2. BLAST analysis of non-redundant unigenes against public databases.**

| Database | Number of Unigenes | Percentage (%) |
|---|---|---|
| Annotated in NR | 56378 | 27.19 |
| Annotated in NT | 34493 | 16.64 |
| Annotated in KO | 18911 | 9.12 |
| Annotated in SwissProt | 48431 | 23.36 |
| Annotated in PFAM | 46843 | 22.59 |
| Annotated in GO | 47372 | 22.85 |
| Annotated in KOG | 27087 | 13.06 |
| Annotated in all Databases | 8015 | 3.86 |
| Annotated in at least one Database | 83778 | 40.41 |
| Total Unigenes | 207283 | 100 |

**Supplementary Table S3. Unigenes involved in backbone formation of sesquiterpene alkaloid dendrobine biosynthesis.**

| Pathway | Gene name | EC |
|---|---|---|
| MVA | Acetyl-CoA C-acetyltransferase (*AACT*) | 2.3.1.9 |
| | Hydroxymethylglutaryl-CoA synthase (*HMGS*) | 2.3.3.10 |
| | 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGR*) | 1.1.1.34 |
| | Mevalonate kinase (*MK*) | 2.7.1.36 |
| | Phosphomevalonate kinase (*PMK*) | 2.7.4.2 |
| | *Diphosphomevalonate* decarboxylase (*MVD*), | 4.1.1.33 |
| | Geranyl diphosphate synthase (*GPPS*) | 2.5.1.1 |
| | Farnesyl pyrophosphate synthetase (*FPPS*) | 2.5.1.10 |
| | Alpha-humulene synthase (*TPS21*) | 4.2.3.104 |
| MEP | 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*) | 2.2.1.7 |
| | 1-deoxy-D-xylulose-5-phosphate reductoisomerase (*DXR*) | 1.1.1.267 |
| | 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (*MCT*) | 2.7.7.60 |
| | 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (*CMK*) | 2.7.1.148 |
| | 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (*MDS*) | 4.6.1.12 |
| | 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase(*HDS*) | 1.17.7.3 |
| | 4-hydroxy-3-methylbut-2-enyl diphosphate reductase(*HDR*) | 1.17.7.4 |

**Supplementary Table S4. Recovery test of dendrobine.**

| Dendrobine (mg) | | | | Recovery (%) | Average recovery(%) | RSD(%) |
|---|---|---|---|---|---|---|
| Samples level | added | Total | Found | | | |
| 0.6089 | 0.5040 | 1.1129 | 1.1074 | 98.9 | 101.9 | 1.96 |
| 0.6089 | 0.5040 | 1.1129 | 1.1238 | 102.2 | | |
| 0.6089 | 0.5040 | 1.1129 | 1.1145 | 100.3 | | |
| 0.6089 | 0.5040 | 1.1129 | 1.1237 | 102.1 | | |

| | | | | |
|---|---|---|---|---|
| 0.6089 | 0.5040 | 1.1129 | 1.1328 | 104.0 |
| 0.6089 | 0.5040 | 1.1129 | 1.1327 | 104.0 |

## Supplementary Table S5. Primers designed for qRT-PCR.

| *Genes* | Unigene ID | forward primer sequence (5'-3') | reverse primer sequence (5'-3') |
|---|---|---|---|
| *AACT* | c90222_g1 | AGCCTTCCTTGGTTCATTGTCATCT | CTGTGCTGCTATCATTGTTGCCTTC |
| *HMGS* | c90508_g3 | GCGTTCAGCAGGAATCATTAGAGG | TCTCAAGCAAGGAAGTGACAACTGT |
| *HMGR* | c84175_g3 | GCCAGCAACATAGTATCAGCCATCT | CACCACCAACAGTTCCTACCTCAAT |
| *MK* | c86727_g1 | GCCTGCTCAATTCCTGCTATTAACA | GCGAGTTCTGACCTCCATTATTCCT |
| *PMK* | c89718_g2 | TGGAGGAAGCTATATGCCTGATGTG | CTGCCAGTCGTGTCCGTGTT |
| *MVD* | c89821_g1 | GGCTACGACGACTGTTGCTGTTAG | CTGAATGCCTCGCTTCTCATCCTC |
| *GPPS* | c84183_g4 | AACTGGCTCAAGCTGCTGAATACT | ACTGTCGGTTGTGGTATTGGCATAT |
| *FPPS* | c79611_g1 | TCACTAAGTTCAAGCGTGTCTTCAG | GCTCGTAGCTCGTCCTCTCGTA |
| *TPS21* | c91295_g3 | GTTGCGGCATCGGAAGGTCATTA | AGGGCGAGATGGAGTGATGGAAA |
| *DXS* | c89669_g1 | CGCAAAGAGCATCACAAAGCAGATT | GGACCAGGAGCAGGCATAGACT |
| *DXR* | c83260_g1 | CCAACCTACTCCAACGAACTCACTT | AACTCAATGGACATAGCGGACCTC |
| *MCT* | c87740_g2 | GCATCCATCGCCGCTATCAATCTA | TCCTTCACTCCAATCCCAACATCTG |
| *CMK* | c91297_g3 | GGCTGGCAGATGAAGTGGATAAGAA | CGACTGGAACTCCTGGCACATTAG |
| *MDS* | c89629_g3 | ACGGCGAGGTCACTGCTTCT | AGGCGTCGTTGTTGGCTTGG |
| *HDS* | c88411_g1 | TCACTGAGCCACCAGAAGAAGAGA | GCAGCAAGAGACCTGTAGAGAAGTT |
| *HDR* | c85735_g1 | CCGTTCTCTTCTCCGTCACCAT | GCTCCAGCGTCTCCTTCTTGT |
| *CYP71D55* | c76734_g1 | TGGATGAGGATCTCGTTGATGTTCT | CCGACTGAGCCTTACGCATTATCT |
| *CYP735A* | c76925_g1 | GAGTGGCAGAAGGCGTTAAGAGA | CGGCGAGTATAGACGAAGAACCT |
| *CYP71D10* | c73585_g1 | CGAAGCAGCGTGGCAGAGAA | TTGGCGTTGTAGAAGATGGCGTTA |
| *CYP94C1* | c88693_g2 | CATTCGCCGCAAGTCCTCGTA | GGCTTGACTACCTTCTTCCTCGTTA |
| *At3g26840* | c88964_g1 | CAACTTCAATGGCTCTCGCATCTG | GCGGCTCTACCACTGTCTCATC |
| *SALAT* | c84941_g1 | CGCAGTGAGAAGCCAAGTAGAGTT | TCTCCACCTCTTCCTGTCTTCCTTC |
| *METTL23* | c69161_g1 | AGCGGTCATCATCTGATTGAGTTCT | GCACTTCCTTGGTTCTTCATCTTCA |
| *ATX4* | c89731_g2 | CCCGAGCCAGTTTCTAAATCCCTTA | AGCCTCCTCTATTGCCAACCTTAAC |
| *OMT-3* | c88817_g1 | TGACTCTCGCTACCGCCATAGAA | TCGCAGTGGAGCAGGTGAAGA |
| *AAT2* | c88693_g5 | TCACAGCAATGCGGCGAGTC | CGGCAGAGGAGGAGGAGATGAAT |
| *DAT* | c83743_g5 | TGAATCGTGCTCCATGTTACCATTG | CCTGCTCGCCTTCTTCTCTTCTC |
| *BCAT2* | c82241_g1 | GCATCATAGTCGTCGTCATCATCAT | TCCACATCAGCATATTCATCCACAT |