Supplementary Material

Table S1. The primers used in this study1

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| **Construct name** | **Primer name** | **Primer sequence (5＇-3＇)** |
| pYBA1132 | 1132-GhHSFA4a-F | TCTAGAACTAGT**GGATCC**ATGGATGATGTTCAGGGCAG |
| 1132-GhHSFA4a-R | TAAGCTTGATATC**GAATTC**CGTTCTCTCCGCAGGAGTAAG |
| pTRV2 | pTRV2-GhHSFA4a-F | TCTGTGAGTAAGGTTACC**GAATTC**CAGGAGCGTCAAGGATTTCAGTT |
| pTRV2-GhHSFA4a-R | ACGCGTGAGCTCGGTACC**GGATCC**CCCAGTTGTTGCAGGTGCAGTAG |
| pCAMBIA2300-35S-OCS | 2300-GhHSFA4a-F | GGACAGGGTACCCGG**GGATCC**ATGGATGATGTTCAGGGCAGC |
| 2300-GhHSFA4a-R | CACCATGGTACTAGT**GTCGAC**CGTTCTCTCCGCAGGAGTAAGA |
| RT-qPCR | qGhHSFA4a-F | TTGCCATAAATCTGACACCAC |
| qGhHSFA4a-R | TCATGTACCGCATTCTCCC |
| UBQ-F | AGCTCGGATACGATTGATAACG |
| UBQ-R | GAAGACGAAGAACAAGGGGAAG |
| Vd-ITS-F | TCCGTAGGTGAACCTGCGG |
| Vd-ITS-R | TCCTCCGCTTATTGATATGC |
| AtRubisco-F | GCAAGTGTTGGGTTCAAAGCTGGTG |
| AtRubisco-R | CCAGGTTGAGGAGTTACTCGGAATGCTG |
| qGhDFR-F | GCGACCCTGACAACTCGAAGAA |
| qGhDFR-R | GACTCGAAGTCCATAGGCGTGG |
| qGhANR-F | TGATCAAACCAGCGACCCAAGG |
| qGhANR-R | CCAAGCTGCCTTTTCAGCCAAC |
| qGhTPS-C2-F | TGCCGAGAACGACCTCTACACT |
| qGhTPS-C2-R | CCTCGAACATCGCTTATCACGGA |
| qGhTPS-XC14-F | ACTTTGAGCCCCAATATTCTCTTGGT |
| qGhTPS-XC14-R | TTGTCTCCCATGCTTAGCCACC |
| qGhJMT-F | GGACATAGTACGAGCGACGAGC |
| qGhJMT-R | GGAGGGTGCCAATCCAATACCC |
| qGhAOC-F | ATACGAAGGGCTCCGGATCGAA |
| qGhAOC-R | CATCTCCCCTGCTTTCACCTGG |
| qGhLOX6-F | AGGTCAGTGTCAGATCCAAGTGA |
| qGhLOX6-R | GCAATTGAGCACTGACATGTTGT |
| qGh9S-LOX4-F | GTCGGAAATTCGAAACGTCGGC |
| qGh9S-LOX4-R | CGGTTTGGAGGATAACCTGCGT |
| PCR | eGFP-F | CCTGAAGTTCATCTGCACCAC |
| eGFP-R | GACTGGGTGCTCAGGTAGTG |

1. The restriction recognition sequences of BamHI (GGATCC), EcoRⅠ(GAATTC) and SalⅠ (GTCGAC) within the primers are in bold and underlined. Genes included in qPCR assay are *GhHSFA4a* (XM\_016875437.1) *UBQ* (LOC107925174), *Vd-ITS* (MT899267.1), *AtRubisco* (ATCG00490), *GhDFR* (LOC107905441), *GhANR* (LOC107905961), *GhTPS-C2* (LOC107920690), *GhTPS-XC14* (LOC107920806), *GhJMT* (LOC107905447), *GhAOC* (LOC107910216), *GhLOX6* (LOC107961400), *Gh9S-LOX4* (LOC107954973).

Table S2 The significantly enriched pathway of GSEA analysis in GhHSFA4a-slienced cotton

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| --- | --- | --- |
| ID | NES | NOM p-val |
| LINOLEIC\_ACID\_METABOLISM (GHI00591) | 1.769 | 0 |
| FLAVONOID\_BIOSYNTHESIS (GHI00941) | 1.662 | 0 |
| PHENYLALANINE\_TYROSINE\_AND\_TRYPTOPHAN\_BIOSYNTHESIS (GHI00400) | 1.661 | 0 |
| GLYCOSYLTRANSFERASES (GHI01003) | 1.658 | 0 |
| PYRUVATE\_METABOLISM (GHI00620) | 1.639 | 0 |
| STRUCTURAL\_PROTEINS (GHI99992) | 1.628 | 0 |
| EXOSOME (GHI04147) | 1.538 | 0 |
| AMINO\_SUGAR\_AND\_NUCLEOTIDE\_SUGAR\_METABOLISM (GHI00520) | 1.471 | 0 |
| ASCORBATE\_AND\_ALDARATE\_METABOLISM (GHI00053) | 1.390 | 0 |
| NITROGEN\_METABOLISM (GHI00910) | 1.366 | 0 |
| PENTOSE\_AND\_GLUCURONATE\_INTERCONVERSIONS (GHI00040) | 1.323 | 0 |
| ENZYMES\_WITH\_EC\_NUMBERS (GHI99980) | 1.272 | 0 |



Figure S1 GhHSFA4a conserved domain2

1. Conserved domain is highlighted in orange.