Supplementary Files

MeJA elicitation on flavonoid biosynthesis and gene expression in the hairy roots of *Glycyrrhiza glabra* L.

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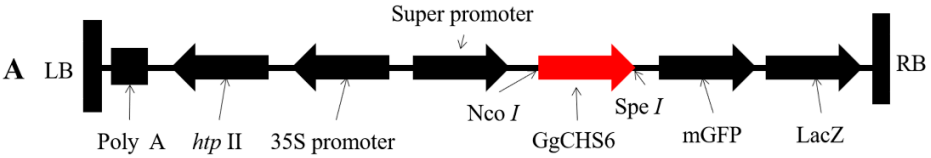
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1 Supplementary Figures and Tables

1.1 Supplementary Figures

A



B

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        10         20         30         40         50         60
ATGGTGAGTGTAGCTGAAATTCGCAAAGCTCAAAGGGCAGAAGGCCCTGCAAACATCTTG
 M  V  S  V  A  E  I  R  K  A  Q  R  A  E  G  P  A  N  I  L

        70         80         90        100        110        120
GCCATTGGTACTGCAAATCCACCAAATTGTGTTGATCAAAGTACTTATCCTGATTTTTAC
 A  I  G  T  A  N  P  P  N  C  V  D  Q  S  T  Y  P  D  F  Y

       130        140        150        160        170        180
TTTAAGATCACAAACAGTGAGCACAAGACCGAGCTTAAGGAAAAATTTCAGCGCATGTGT
 F  K  I  T  N  S  E  H  K  T  E  L  K  E  K  F  Q  R  M  C

       190        200        210        220        230        240
GATAAATCTATGATCAAGAAGCGATATATGTACCTAACGGAAGAGATTTTGAAAGAGAAT
 D  K  S  M  I  K  K  R  Y  M  Y  L  T  E  E  I  L  K  E  N

       250        260        270        280        290        300
CCTAACATTTGCGCTTATATGGCACCTTCTTTGGACGCTAGGCAAGACATGGTGGTCGTA
 P  N  I  C  A  Y  M  A  P  S  L  D  A  R  Q  D  M  V  V  V

       310        320        330        340        350        360
GAGGTGCCTAGACTAGGGAAGGAAGCTGCGGTCAAGCTATAAAAGAATGGGGCCAACCAA
 E  V  P  R  L  G  K  E  A  A  V  K  L  *  K  N  G  A  N  Q

       370        380        390        400        410        420
AGTCAAAGATCACCCACTTAATTTTTTGCACTACTAGGGTGTGGACATGCCGGCGCTGAT
 S  Q  R  S  P  T  *  F  F  A  L  L  G  C  G  H  A  G  A  D

       430        440        450        460        470        480
TACCAACTTACTAAACTCTTGGGTCTTCGCCCATATGTGAAAAGGTATATGATGTACCAG
 Y  Q  L  T  K  L  L  G  L  R  P  Y  V  K  R  Y  M  M  Y  Q

       490        500        510        520        530        540
CAAGGGTGTTTTGCAGGTGGCACGGTGCTTCGCTTGGCCAAAGACTTGGCGGAGAACAAC
 Q  G  C  F  A  G  G  T  V  L  R  L  A  K  D  L  A  E  N  N

       550        560        570        580        590        600
AAAGGTGCTCGTGTGCTAGTTGTTTGTTCTGAAGTTACTGCAGTCACATTCCGTGGCCCT
 K  G  A  R  V  L  V  V  C  S  E  V  T  A  V  T  F  R  G  P

       610        620        630        640        650        660
ACTGATACTCACCTAGATAGCCTTGTGGGACAAGCATTATTTGGAGATGGAGCAGCTGCA
 T  D  T  H  L  D  S  L  V  G  Q  A  L  F  G  D  G  A  A  A

       670        680        690        700        710        720
GTCATTGTTGGTTCTGACCCAATACCCGAAATTGAGAAGCCTATATTTGAGTTGGTTTGG
 V  I  V  G  S  D  P  I  P  E  I  E  K  P  I  F  E  L  V  W

       730        740        750        760        770        780
ACGGCACAAACAATAGCTCCAGATAGTGAAGGAGCCATTGATGGTCACCTTCGTGAAGTT
 T  A  Q  T  I  A  P  D  S  E  G  A  I  D  G  H  L  R  E  V

       790        800        810        820        830        840
GGGCTCACATTTCATCTTCTTAAAGATGTTCCCGGGATGTCTCAAAGAACATTGATAAAG
 G  L  T  F  H  L  L  K  D  V  P  G  M  S  Q  R  T  L  I  K

       850        860        870        880        890        900
CACTGACTGAGGCATTCCAACCATTAGGCATTCTGATTACAACTCAATCTTTTGGATTGC
 H  *  L  R  H  S  N  H  *  A  F  *  L  Q  L  N  L  L  D  C

       910        920        930        940        950        960
ACACCCAGGTGGACCGCAATTCTTGACCAAGTTGAGCAAAAGTTAGCTTTGAAACCTGAA
 T  P  R  W  T  A  I  L  D  Q  V  E  Q  K  L  A  L  K  P  E

       970        980        990       1000       1010       1020
AAGATGAAGGCCACTAGGGATGTGCTTAGTGATTATGGTAATATGTCAAGTGCATGTGTT
 K  M  K  A  T  R  D  V  L  S  D  Y  G  N  M  S  S  A  C  V

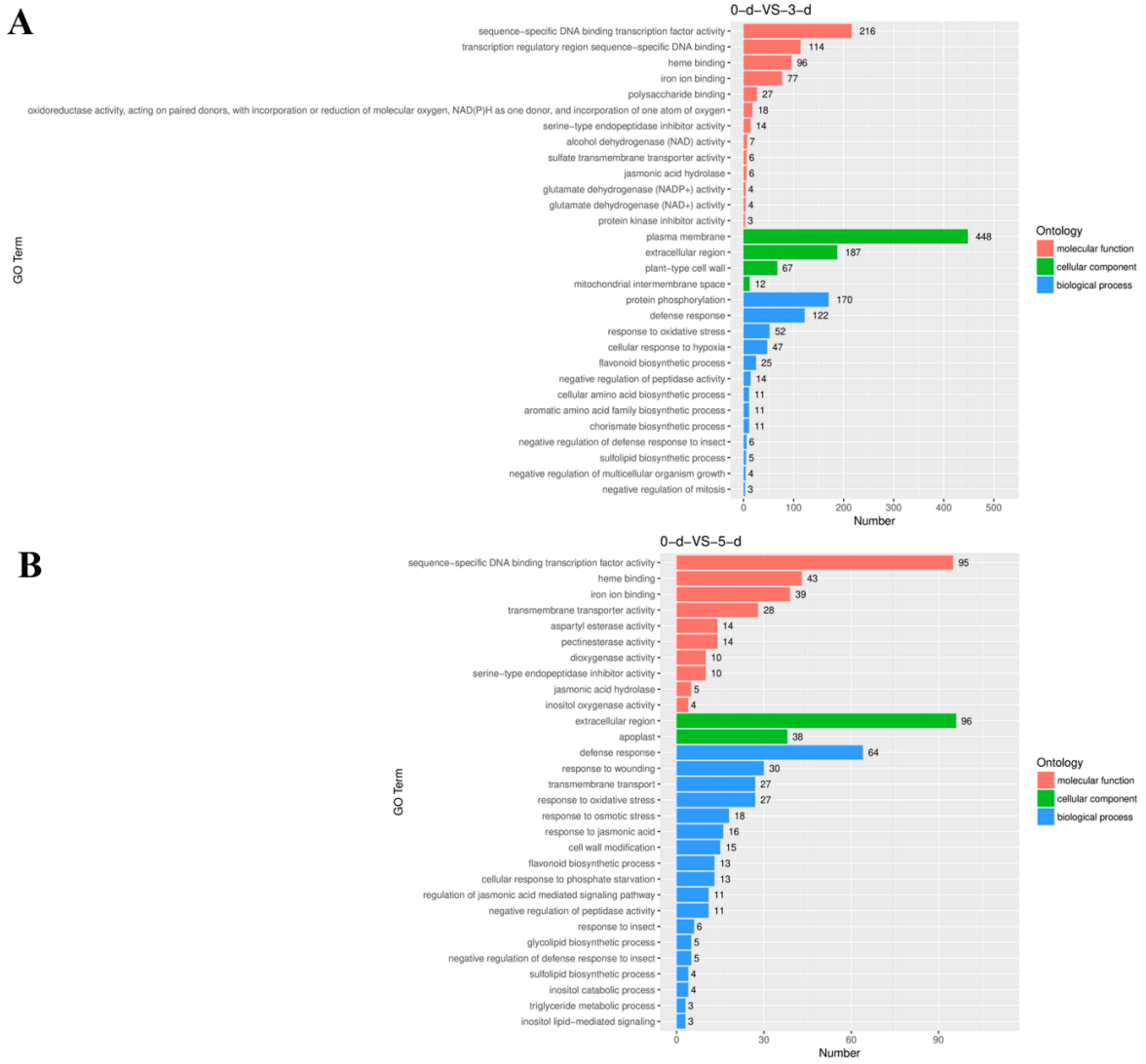
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 L  F  I  L  D  E  M  R  K  K  S  A  Q  N  G  L  K  T  T  G

      1090       1100       1110       1120       1130       1140
GAAGGACTCGAATGGGGTGTATTATTCGGCTTTGGACCTGGACTTACCATCGAAACTGTT
 E  G  L  E  W  G  V  L  F  G  F  G  P  G  L  T  I  E  T  V

      1150       1160       1170
GTTTTGCACAGTGTGGCTATATGA
 V  L  H  S  V  A  I  *
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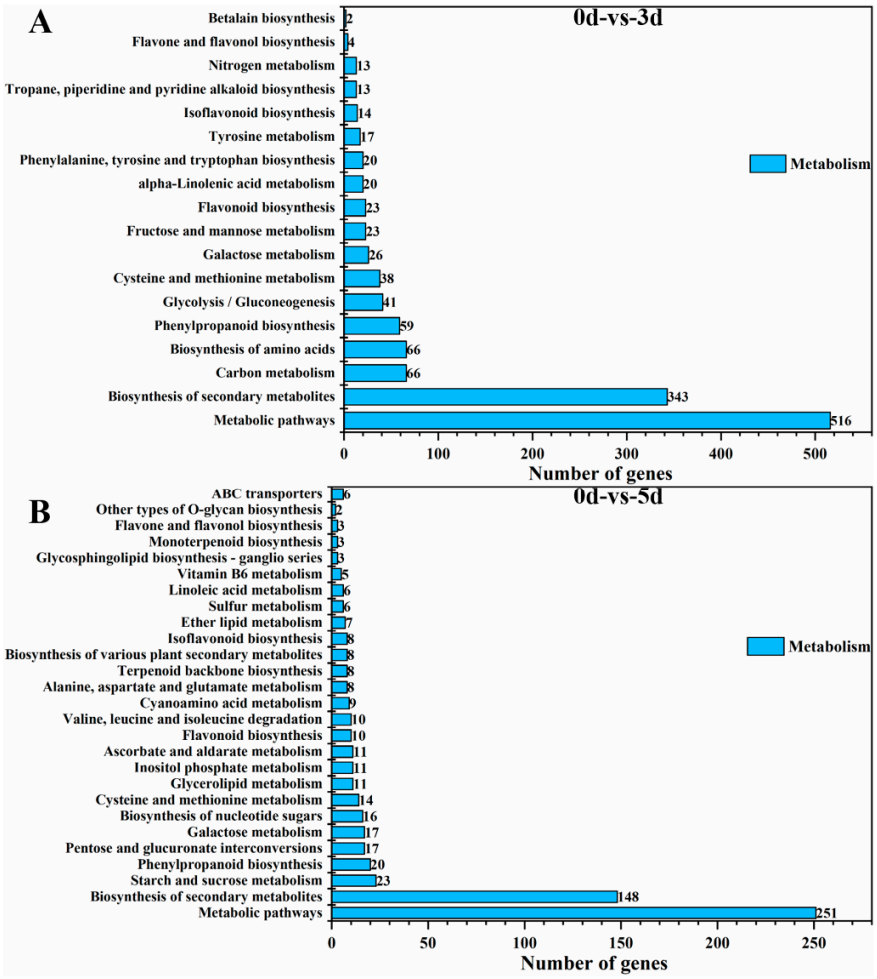
Supplementary Figure S1.

Construction of *GgCHS6* overexpression vector. **(A)** Schematic diagram of *GgCHS6* overexpression vector. **(B)** The DNA sequence of the *GgCHS6* and the sequence of the encoded protein.



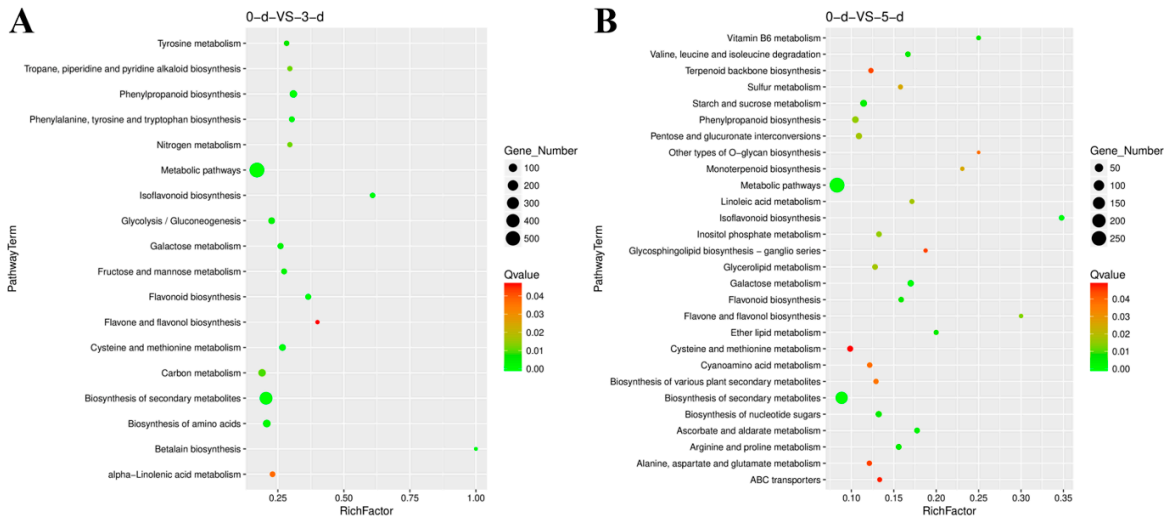
Supplementary Figure S2.

Gene Ontology (GO) functional classification analysis of DEGs in 0 vs. 3 d (A) and 0 vs. 5 d (B) based on RNA-Seq data. Based on sequence homology, all differentially expressed genes could be categorized into three main categories: molecular function, cellular component, and biological process.



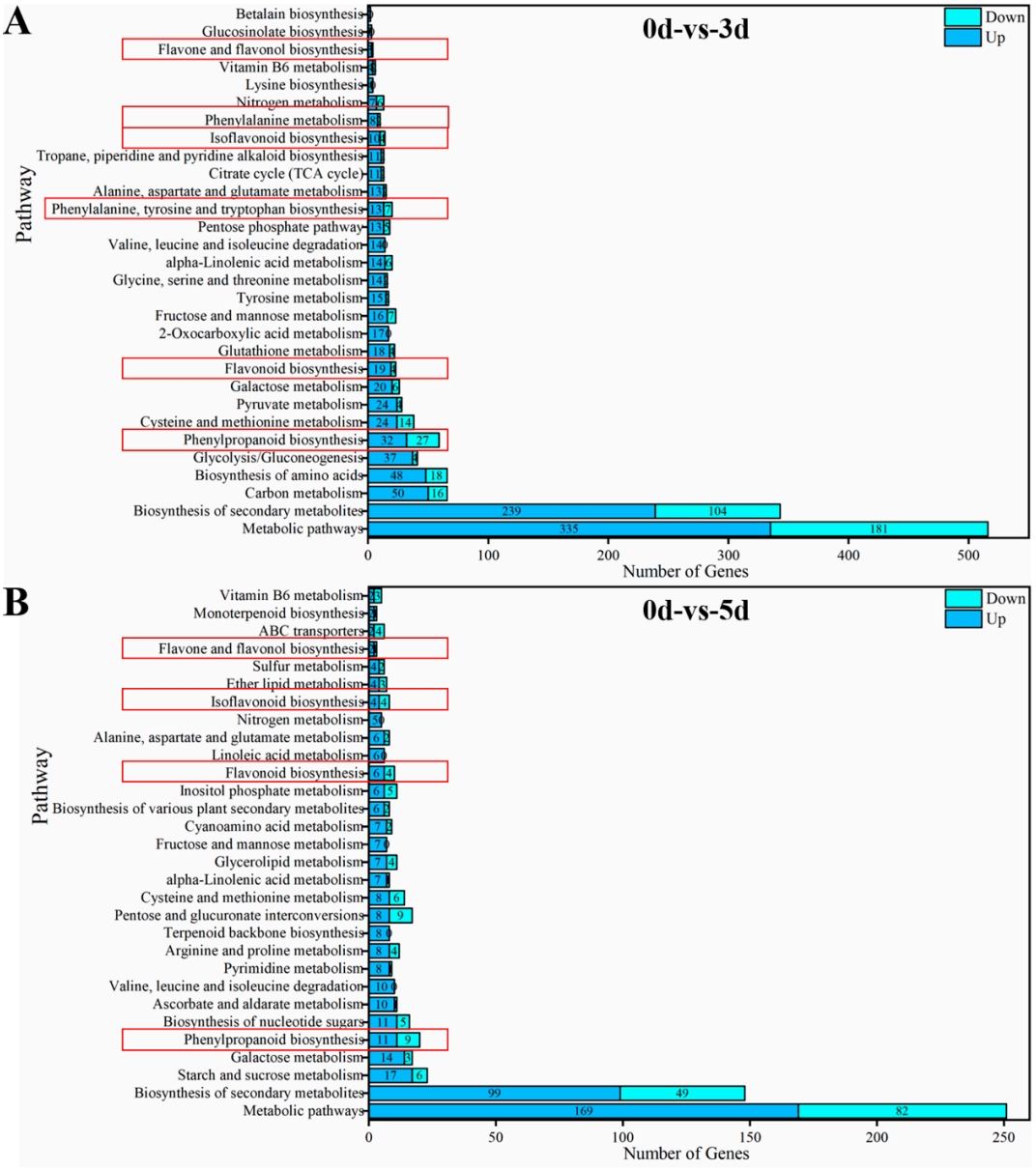
Supplementary Figure S3.

After 3 d and 5 d of MeJA treatment, the following KEGG pathways were mostly enriched in “metabolic pathways (ko01100)” (516 DEGs at 3 d and 251 DEGs at 5 d, respectively) and “biosynthesis of secondary metabolites (ko01110)” (343 DEGs at 3 d and 148 DEGs at 5 d, respectively).



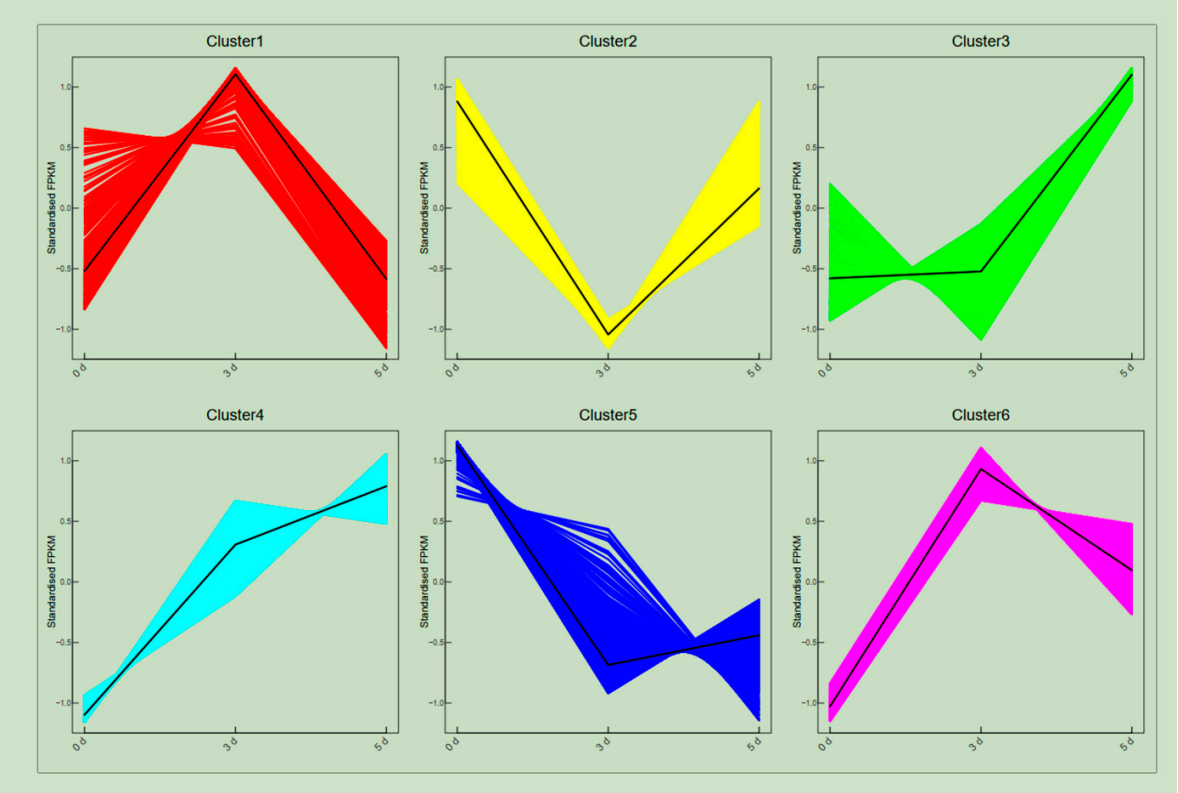
Supplementary Figure S4.

Enrichment analysis of transcriptomic data by KEGG. (A) The KEGG annotation of DEGs in HRs at 3 d post-MeJA treatment. (B) The KEGG annotation of DEGs in HRs at 5 d post-MeJA treatment.



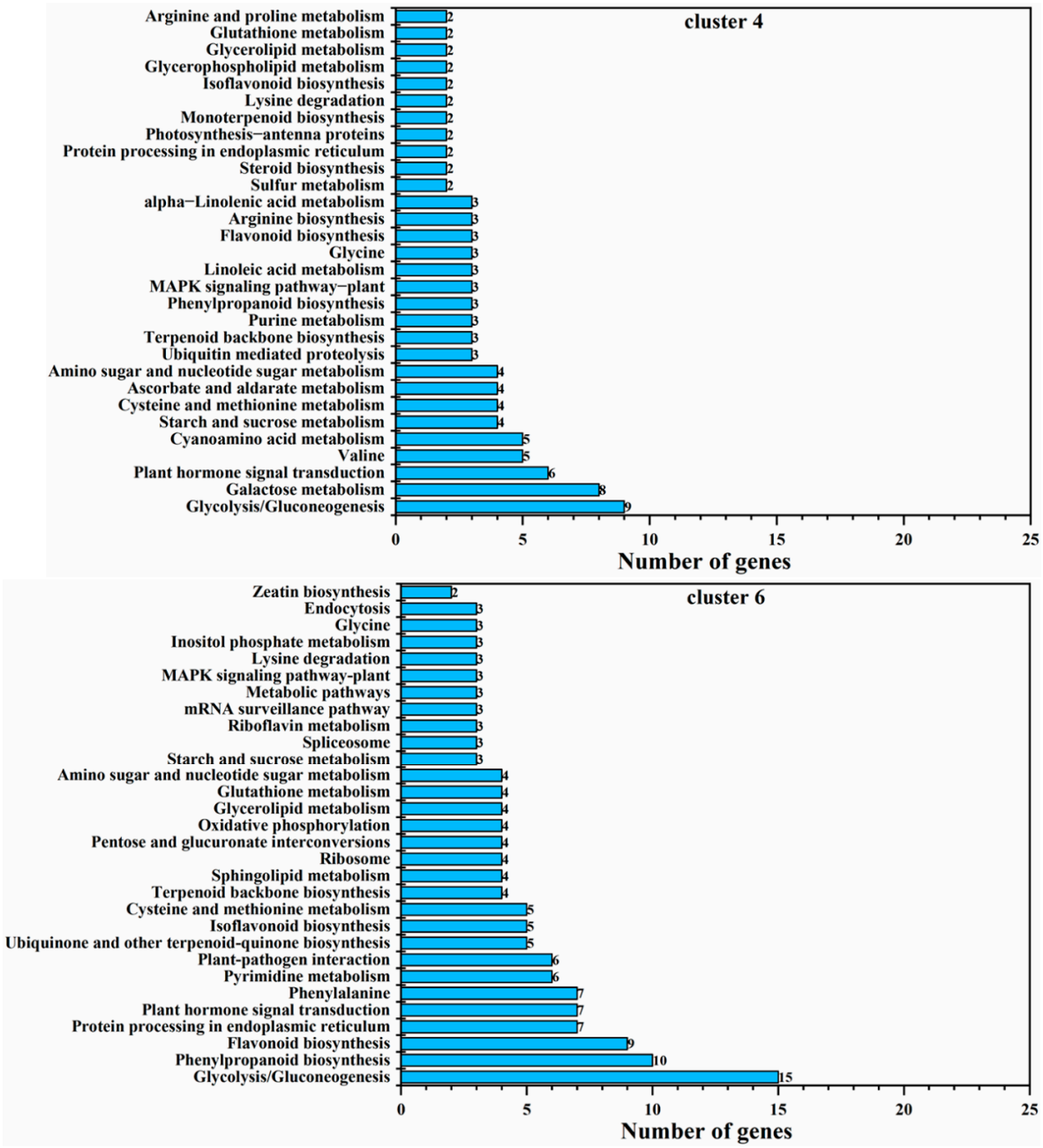
Supplementary Figure S5.

(A) After 3 days of treatment with MeJA, the metabolic pathways were enriched by up-regulated and down-regulated genes. **(B)** After 5 days of treatment with MeJA, the metabolic pathways were enriched by up-regulated and down-regulated genes. The pathways related to the synthesis of flavonoids are marked with red boxes.



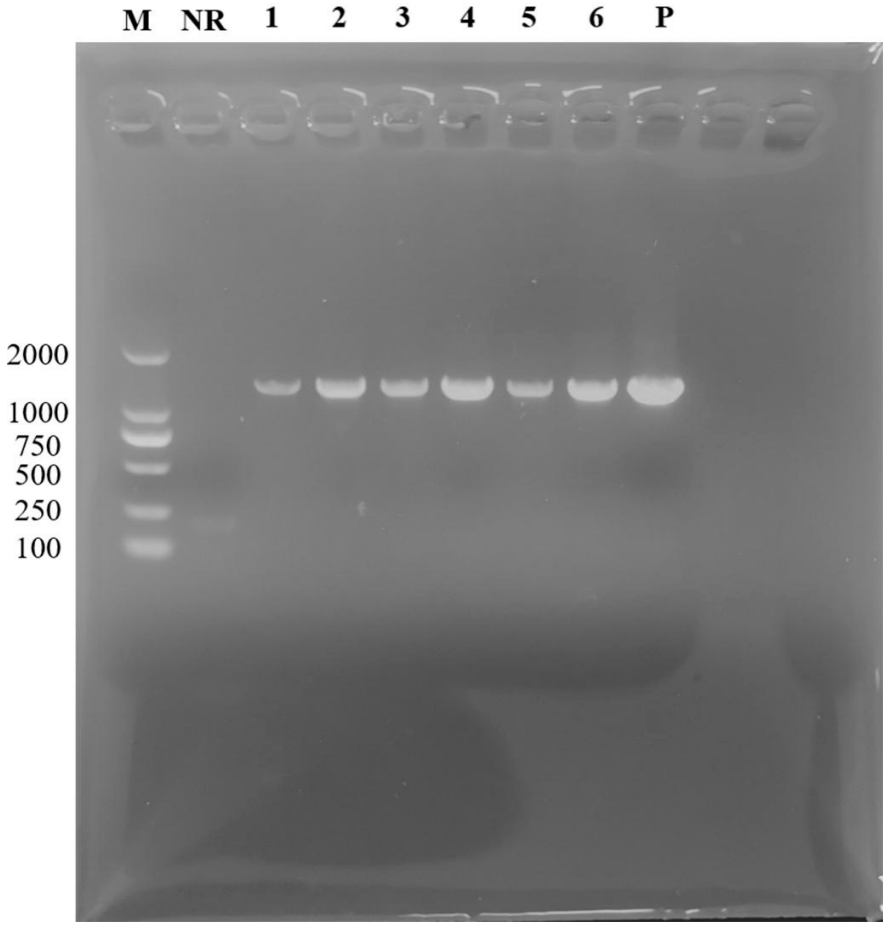
Supplementary Figure S6.

K-means clustering of gene expression profiles in all samples, resulting in six clusters with characteristic patterns.



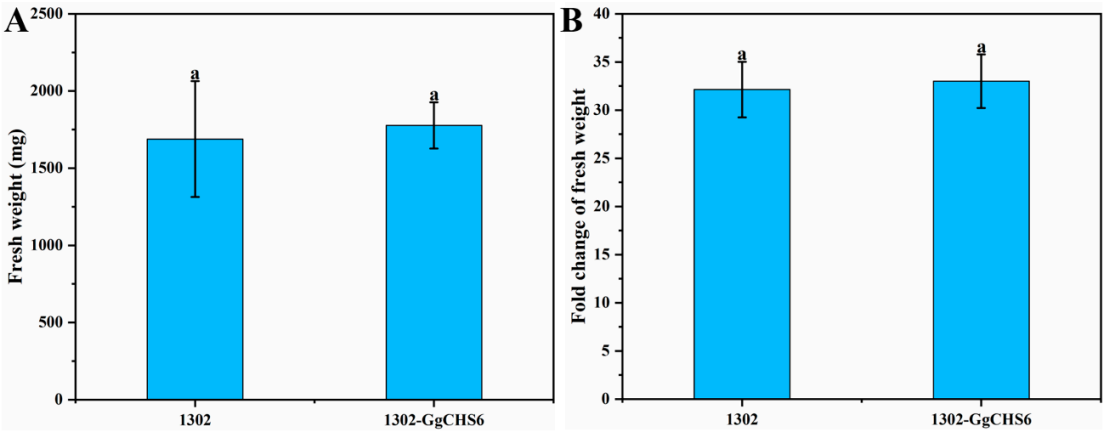
Supplementary Figure S7.

KEGG enrichment analysis of differentially expressed genes in cluster 4 and 6.



Supplementary Figure S8.

Identification of *GgCHS6*-overexpressing hairy roots by PCR. M: marker; NR: The normal root of licorice (negative control). 1-6: Different hairy root samples. P:1302-*GgCHS6* plasmid (positive control).



Supplementary Figure S9.

(A) The fresh weight of 1302 (empty vector) hairy roots and *GgCHS6*-overexpressing hairy roots at 28 days. **(B)** The fold increase in fresh weight of wild-type hairy roots and *GgCHS6*-overexpressing hairy roots at 28 days. Different small letters in the figure showed a significant difference (p<0.05).