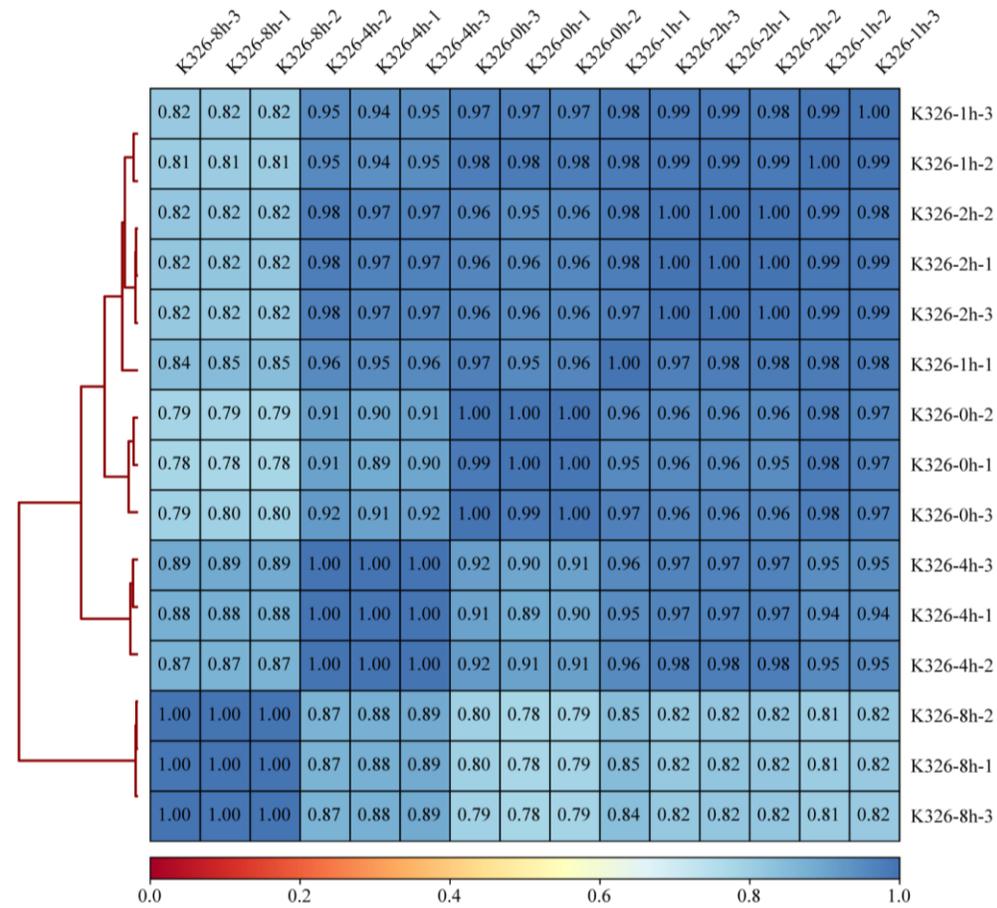
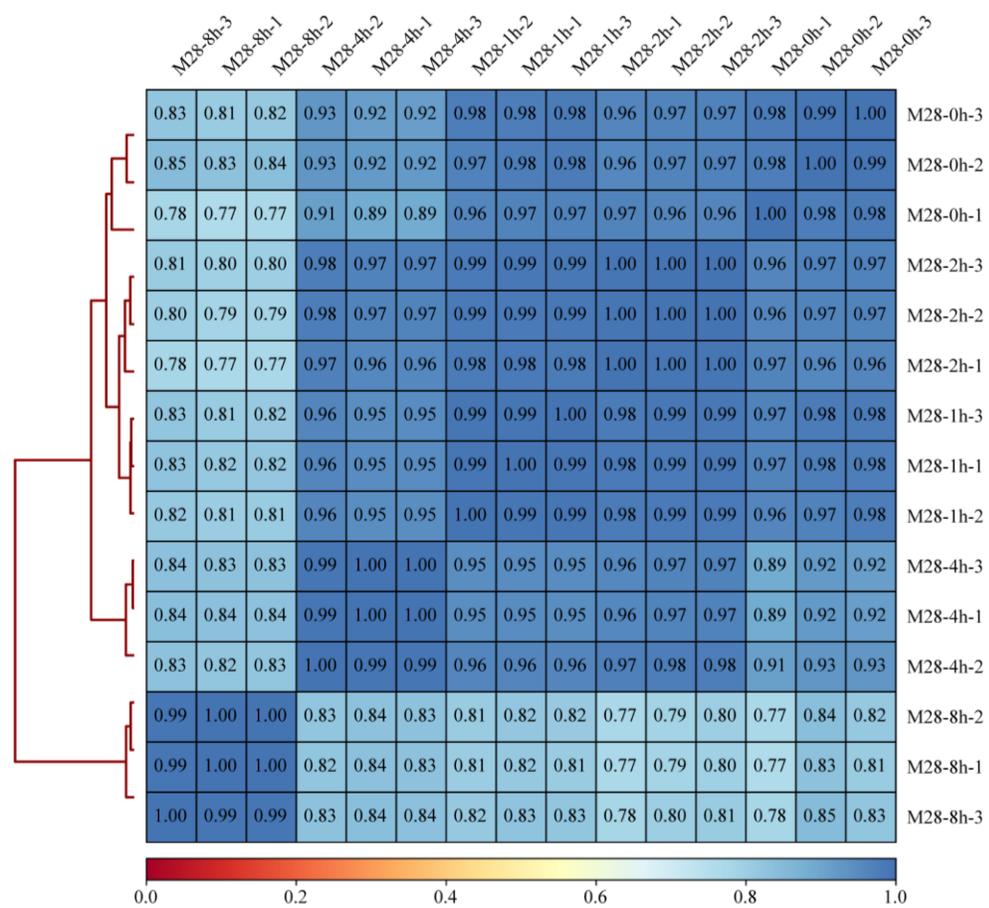


Supplementary Figure S1 Phenotypic comparisons between tobacco K326 and M28 at different time points after exposing to natural drought (ND) stress.

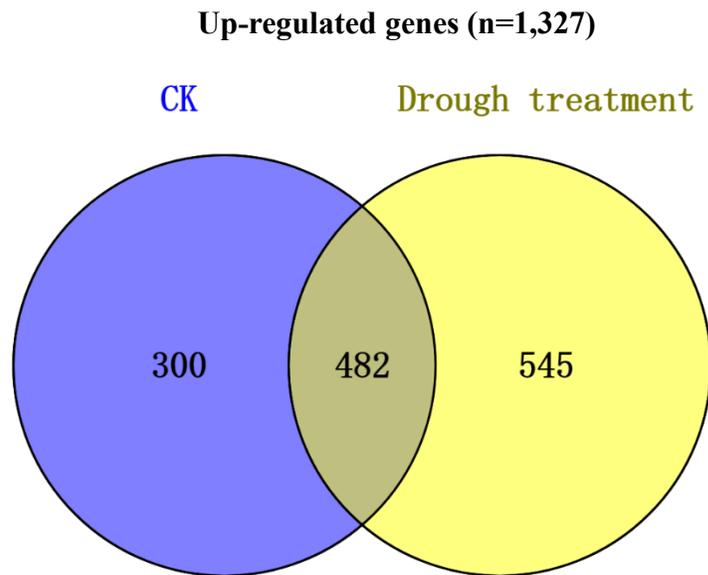


Supplementary Figure S2 Phenotypic comparisons between tobacco K326 and M28 at different time points after exposing to 200mM mannitol treatment (simulated drought, SD).

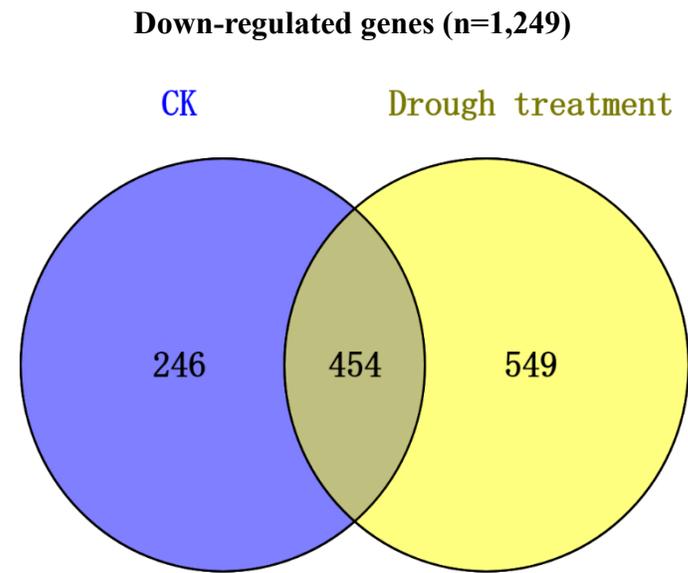


Supplementary Figure S3 Hierarchically clustered correlation matrix of RNA-seq data generated from M28 and K326 subjected to drought treatment at different time points. Three replicates for each time point. Pearson correlations were calculated and Hierarchically clustered by heatmap package in R.

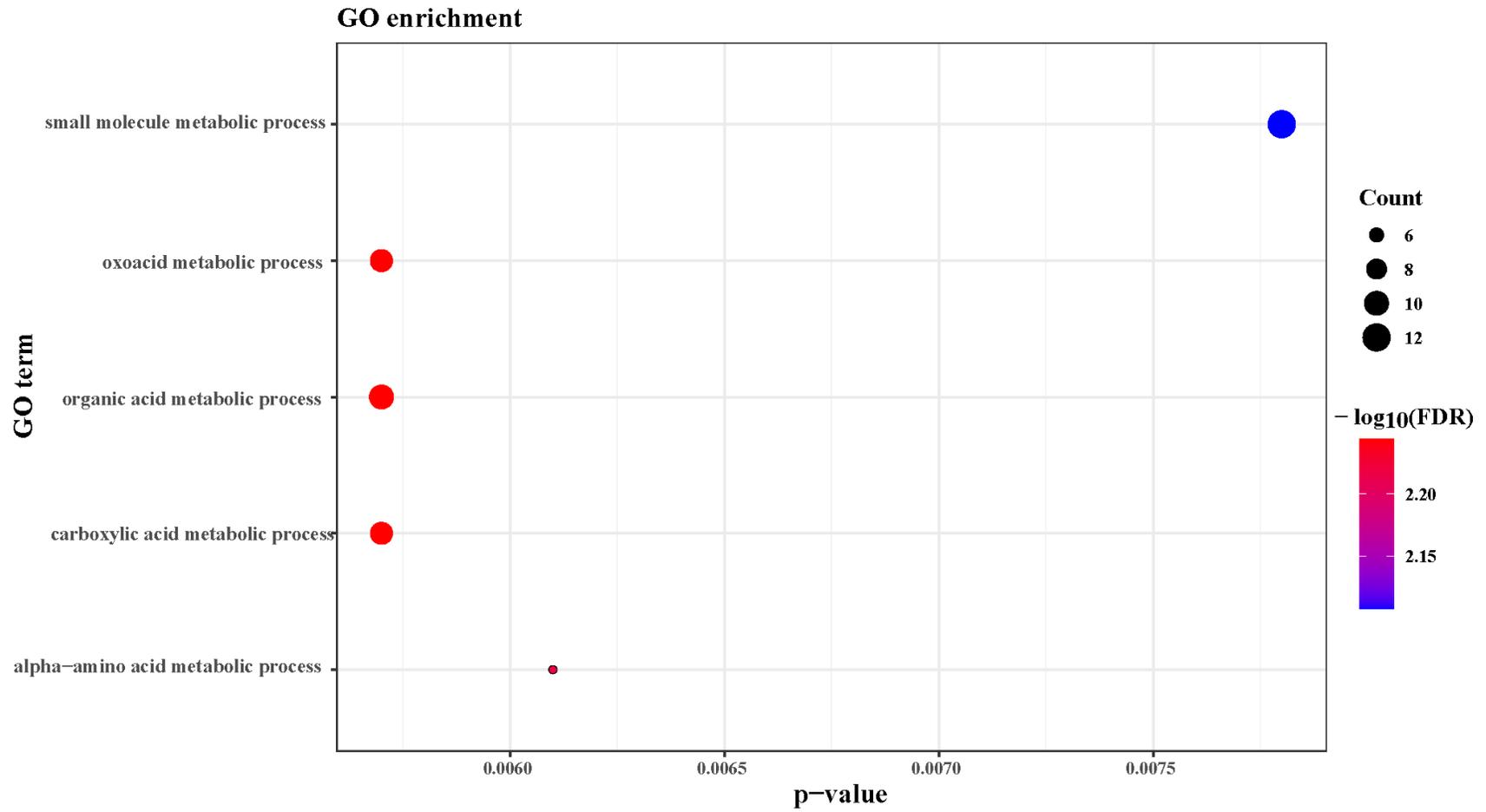
A



B

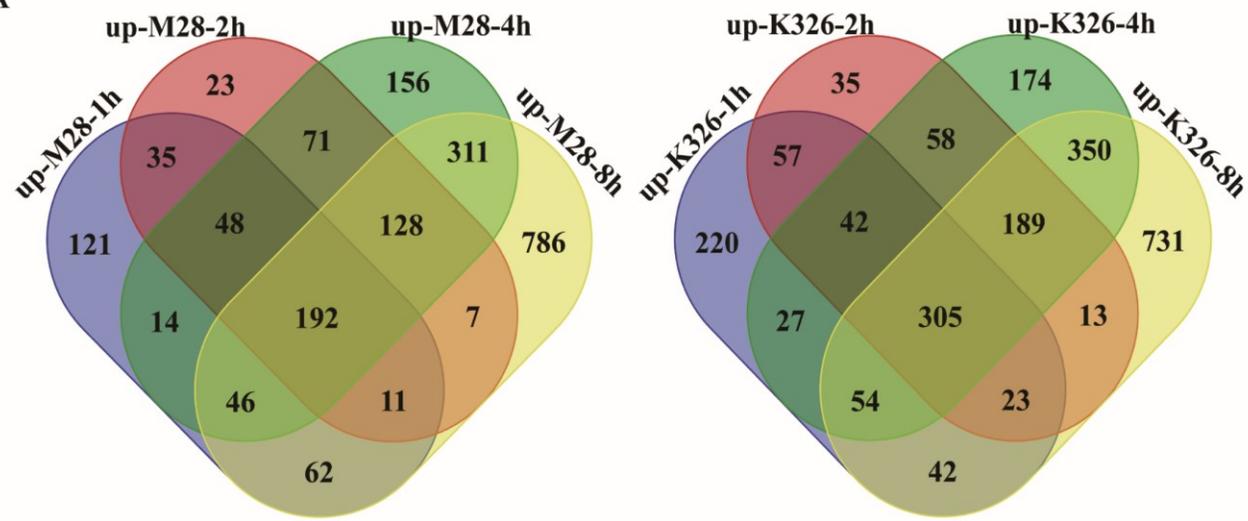


Supplementary Figure S4 Comparisons of genes that were up- or down-regulated before (CK) or after drought treatment.

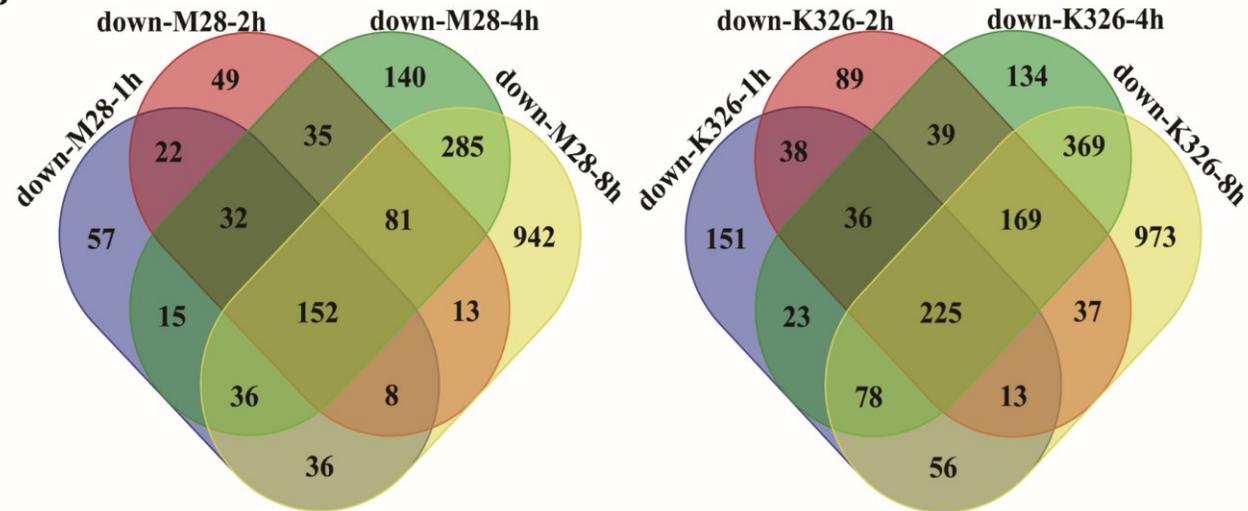


Supplementary Figure S5 GO term enrichment analyses of common down-regulated genes for all time points under drought treatment.

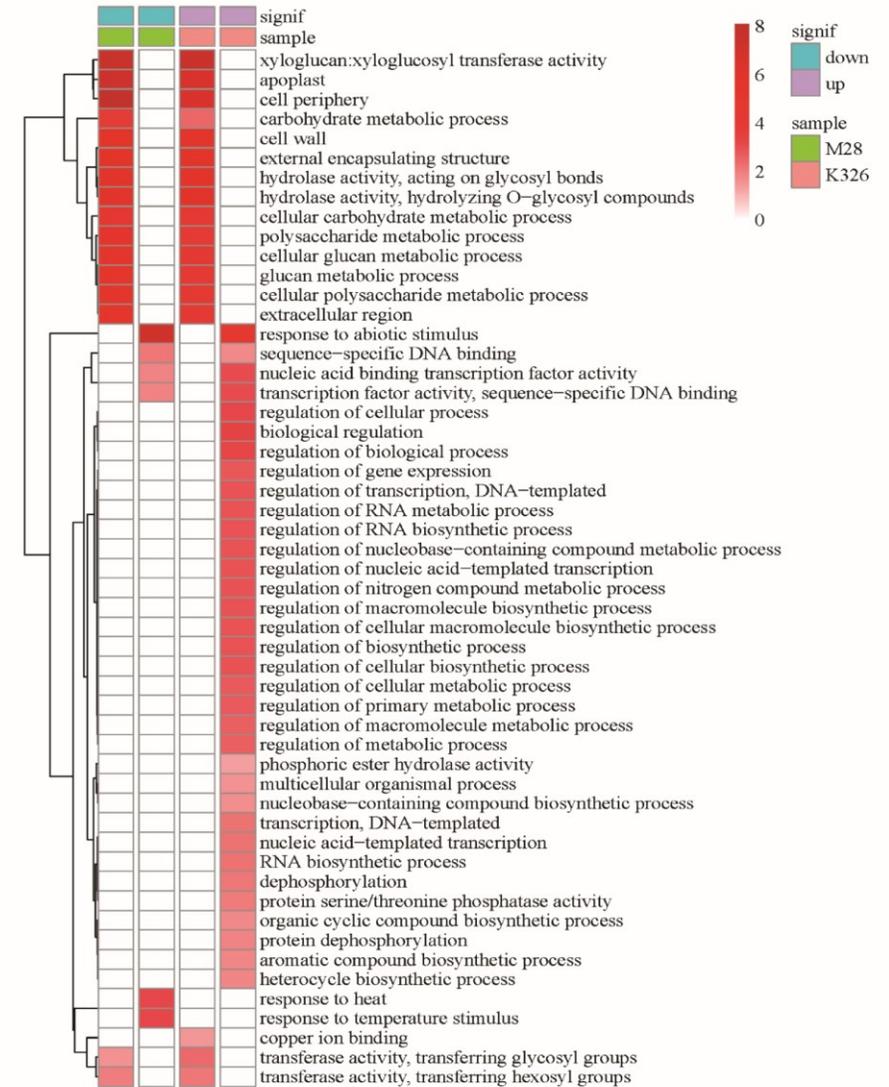
A



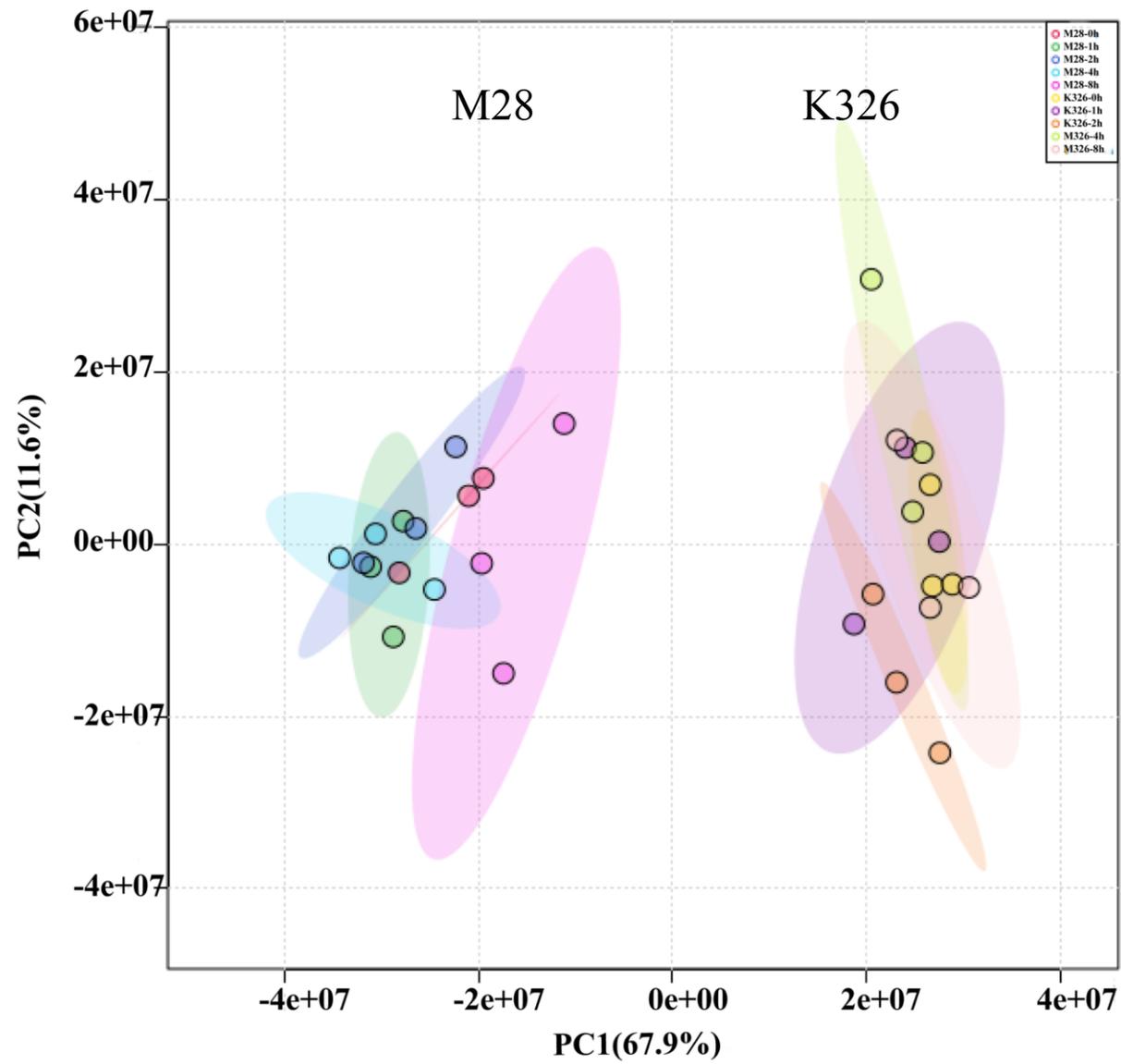
B



C



Supplementary Figure S6 Characterization of time-point-related up- or down-regulated genes in K326 or M28 under drought treatment. (A) Venn plots showing overlaps of time-point-related up-regulated genes in K326 (right) or M28 (left) under drought treatment. **(B)** Venn plots showing overlaps of time-point-related down-regulated genes in K326 (right) or M28 (left) under drought treatment. **(C)** GO term enrichment analyses of up- and down-regulated genes in K326 or M28 under drought treatment.



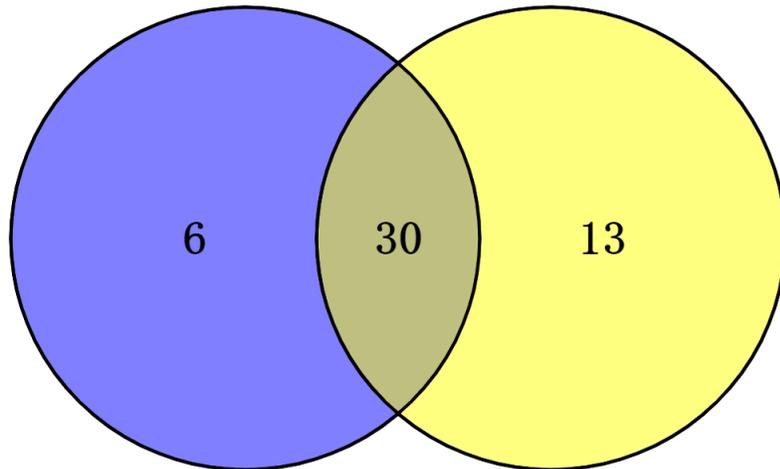
Supplementary Figure S7 PCA of metabolome generated from M28 and K326 subjected to drought treatment at different time points.

A

M28 vs K326 up-regulation

CK-up

Drought-up

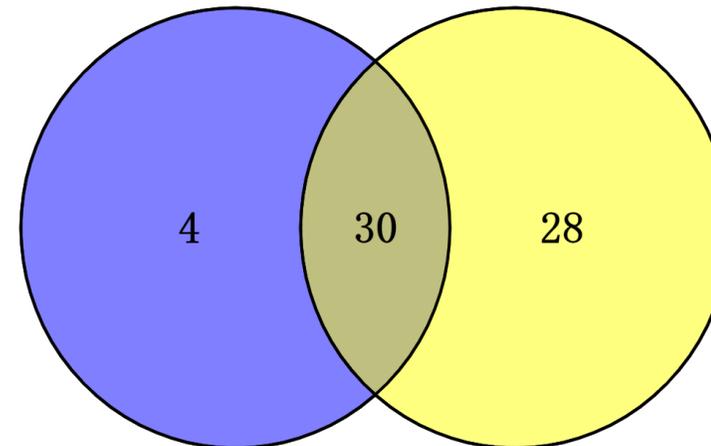


B

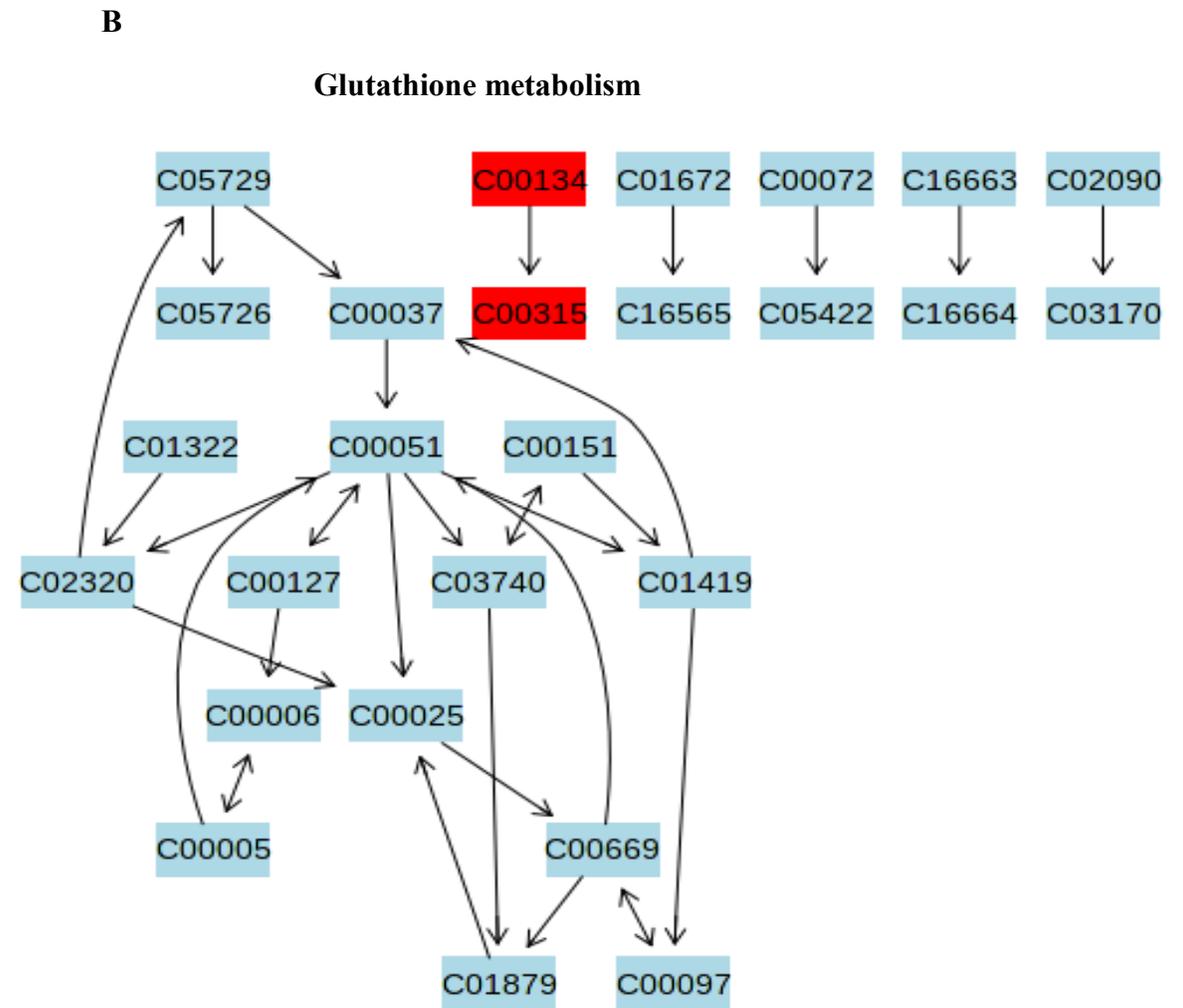
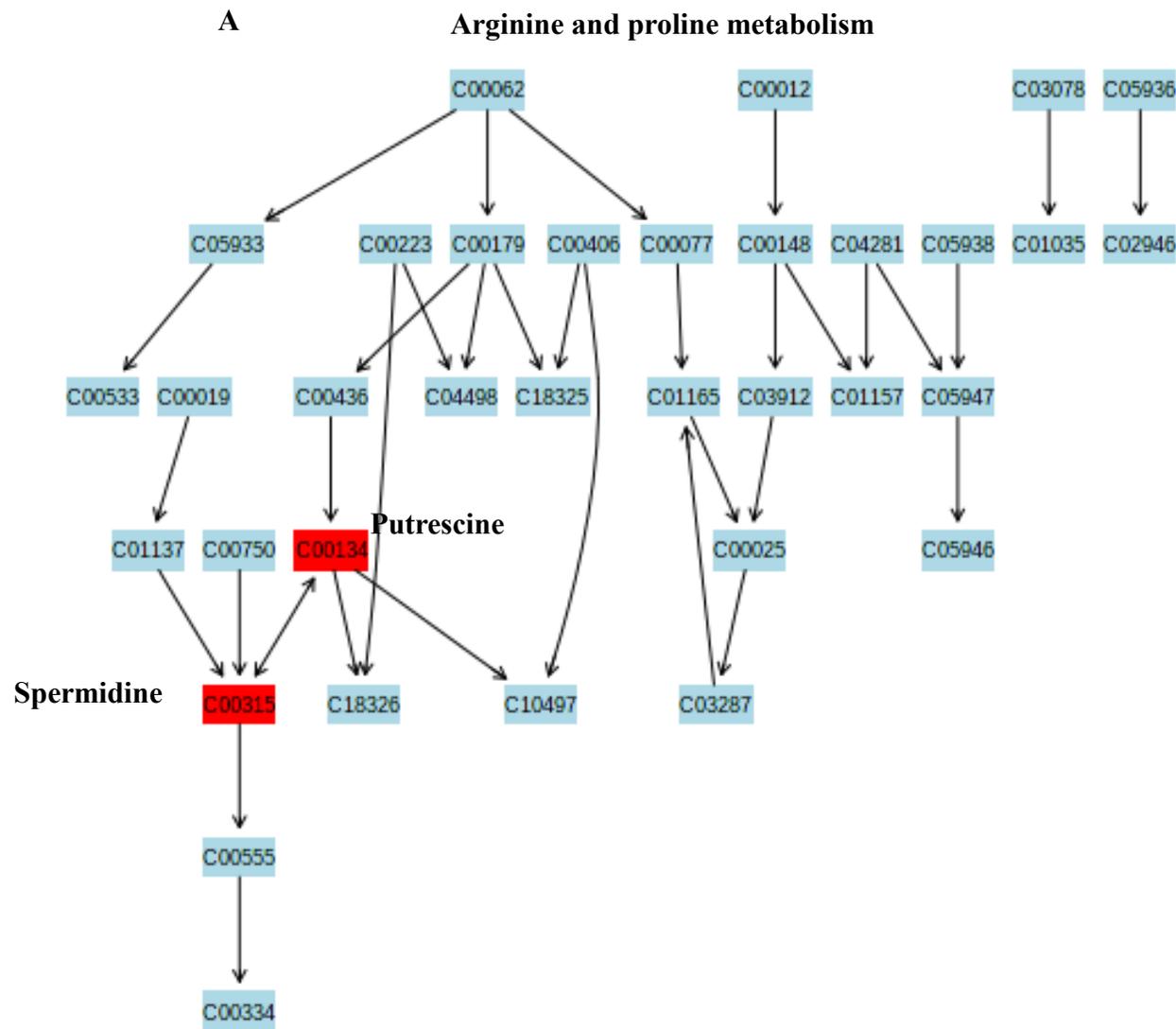
M28 vs K326 down-regulation

CK-down

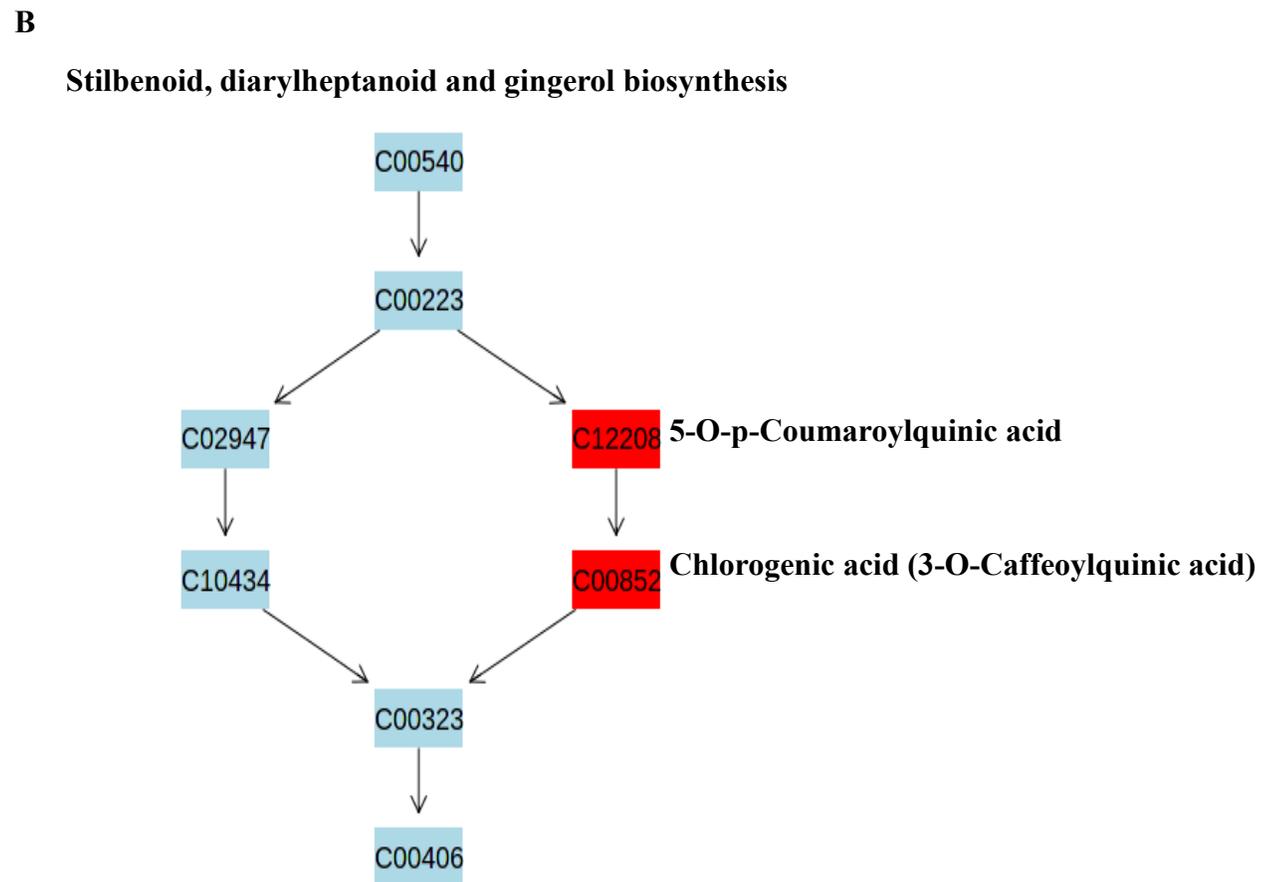
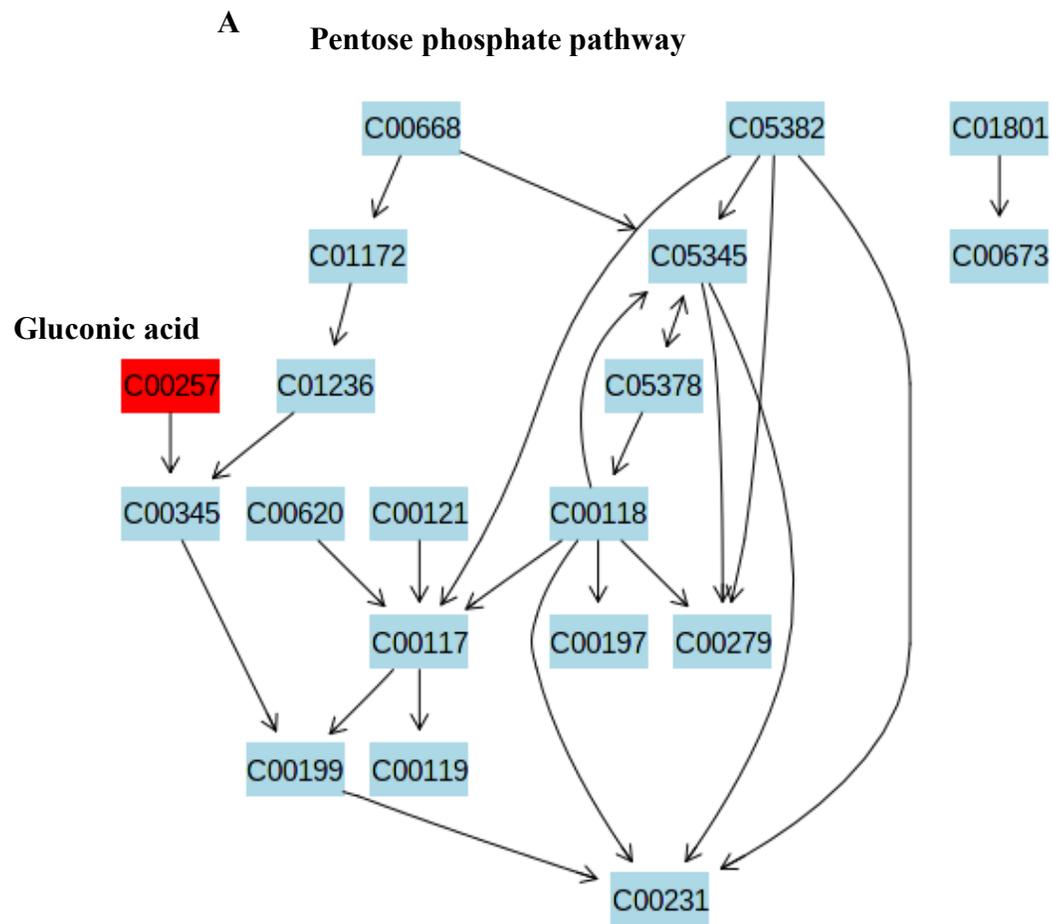
Drought-down



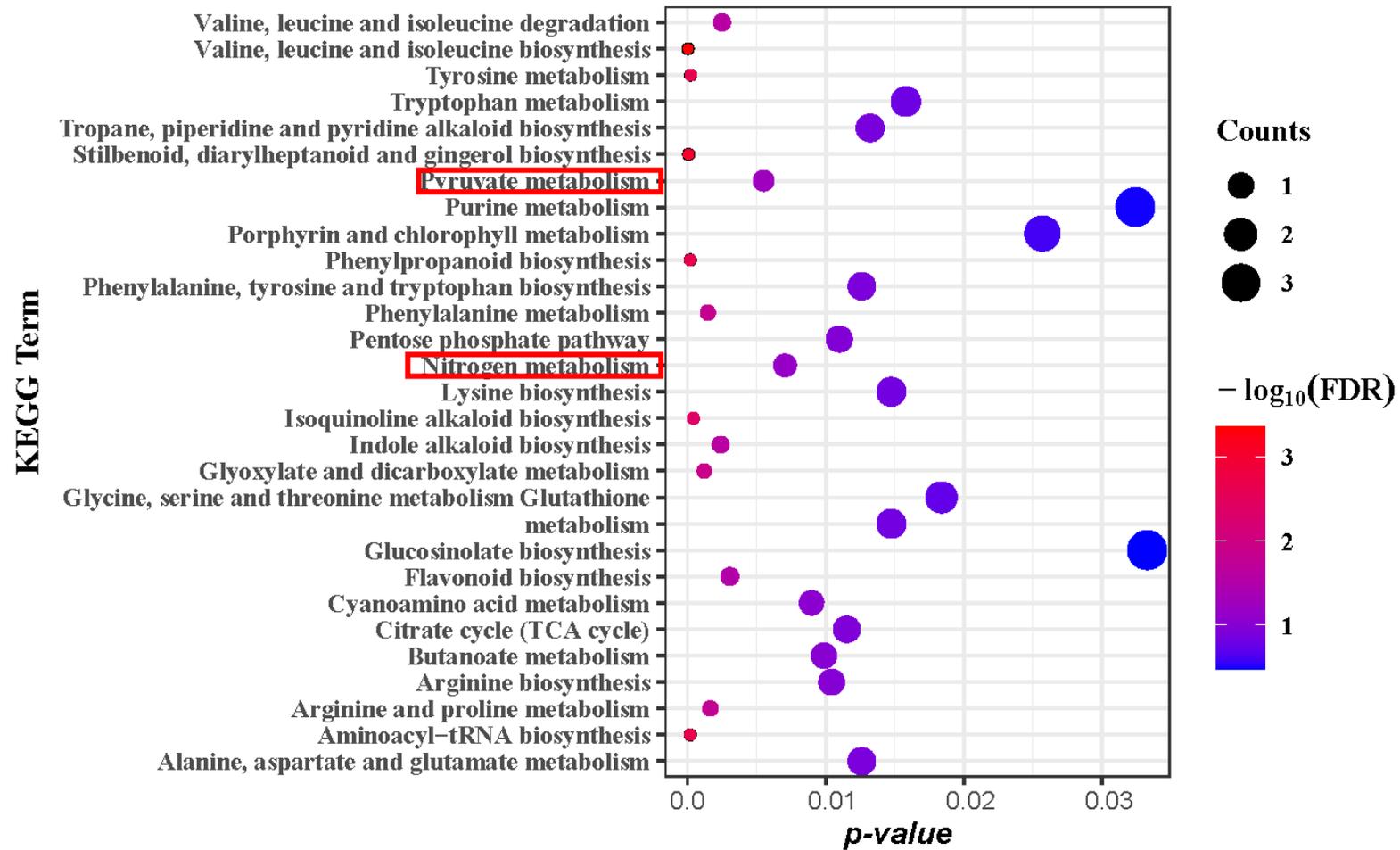
Supplementary Figure S8 Comparisons of differential metabolites between M28 and K326 before and after drought treatment.



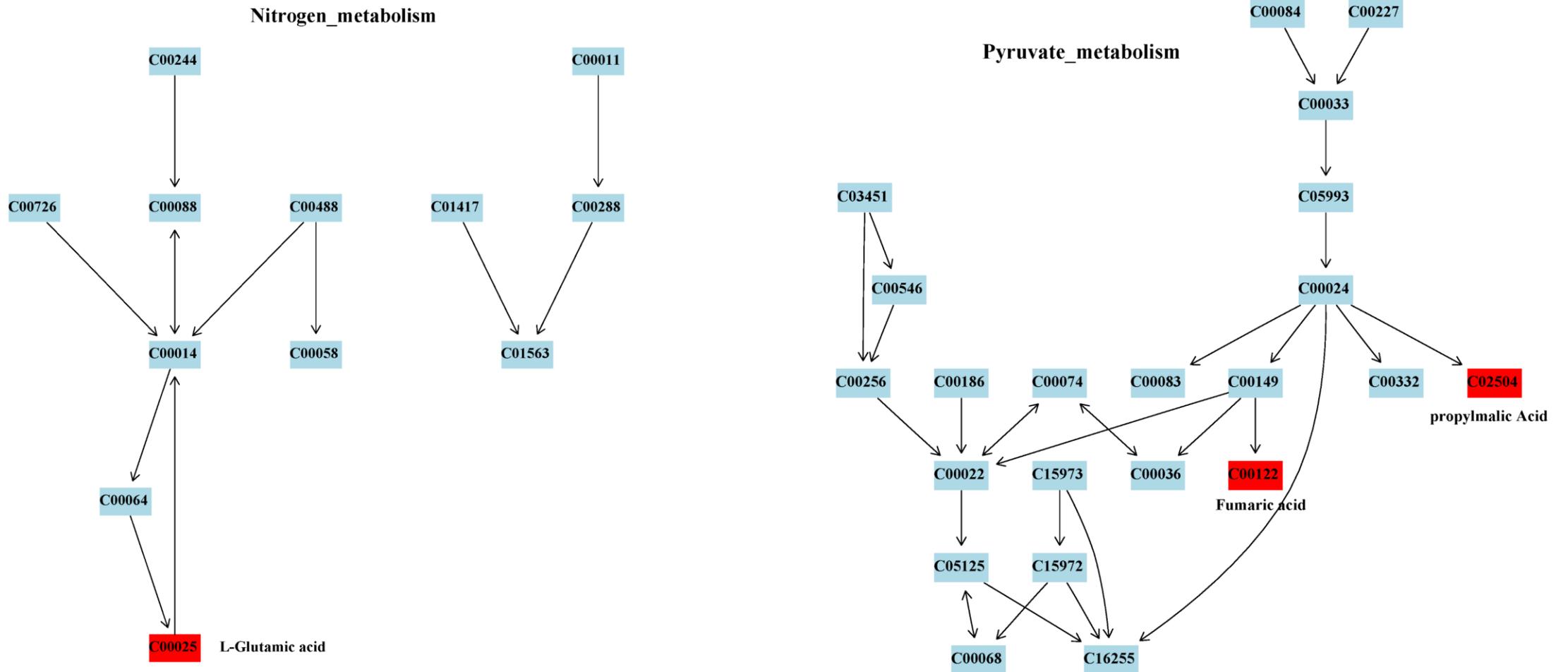
Supplementary Figure S9 KEGG analyses of common down-regulated metabolites between M28 and K326 after drought treatment



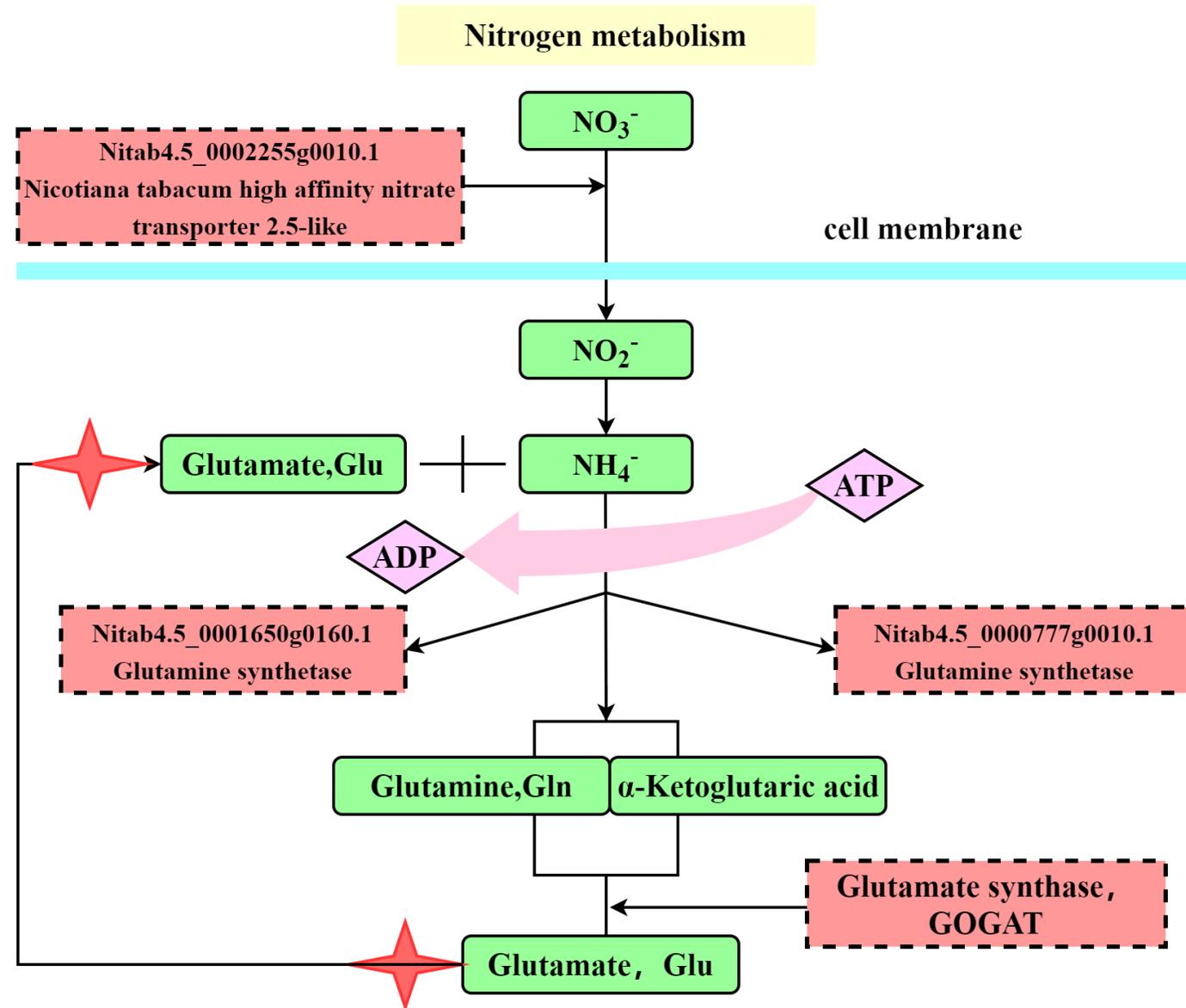
Supplementary Figure S10 KEGG analyses of common up-regulated metabolites between M28 and K326 after drought treatment



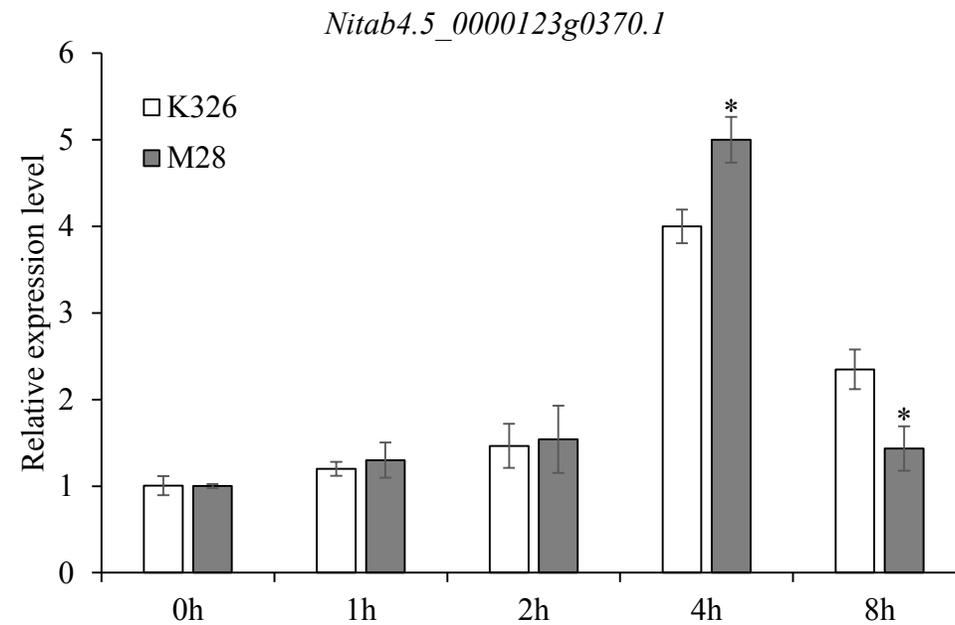
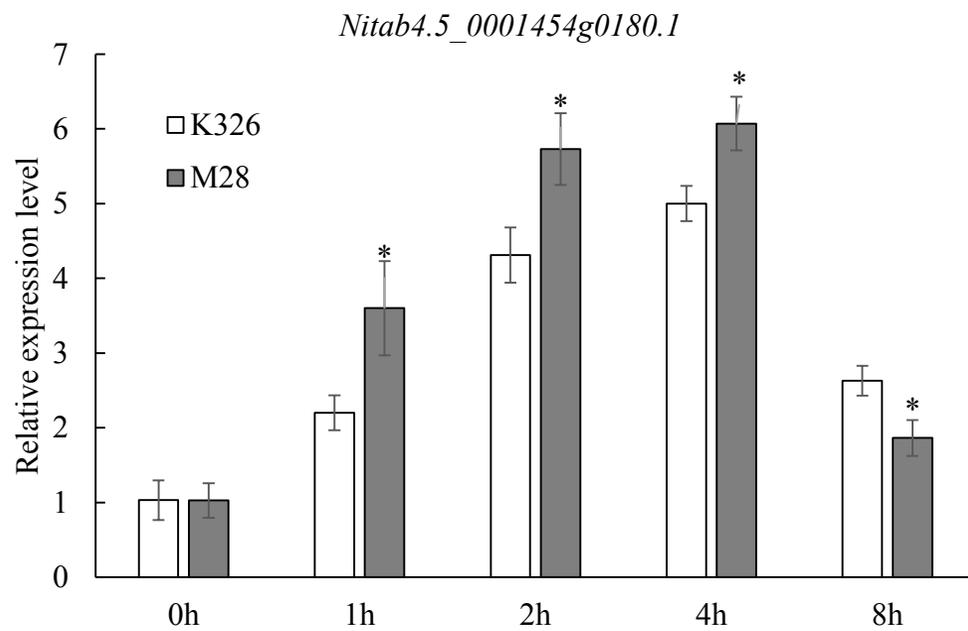
Supplementary Figure S11 KEGG analyses for all drought induced DEGs.



Supplementary Figure S12 Overview of the KEGG pathway of Nitrogen_metabolism (left) and Pyruvate_metabolism (right). The red indicates significantly up-regulated metabolites post drought treatment.

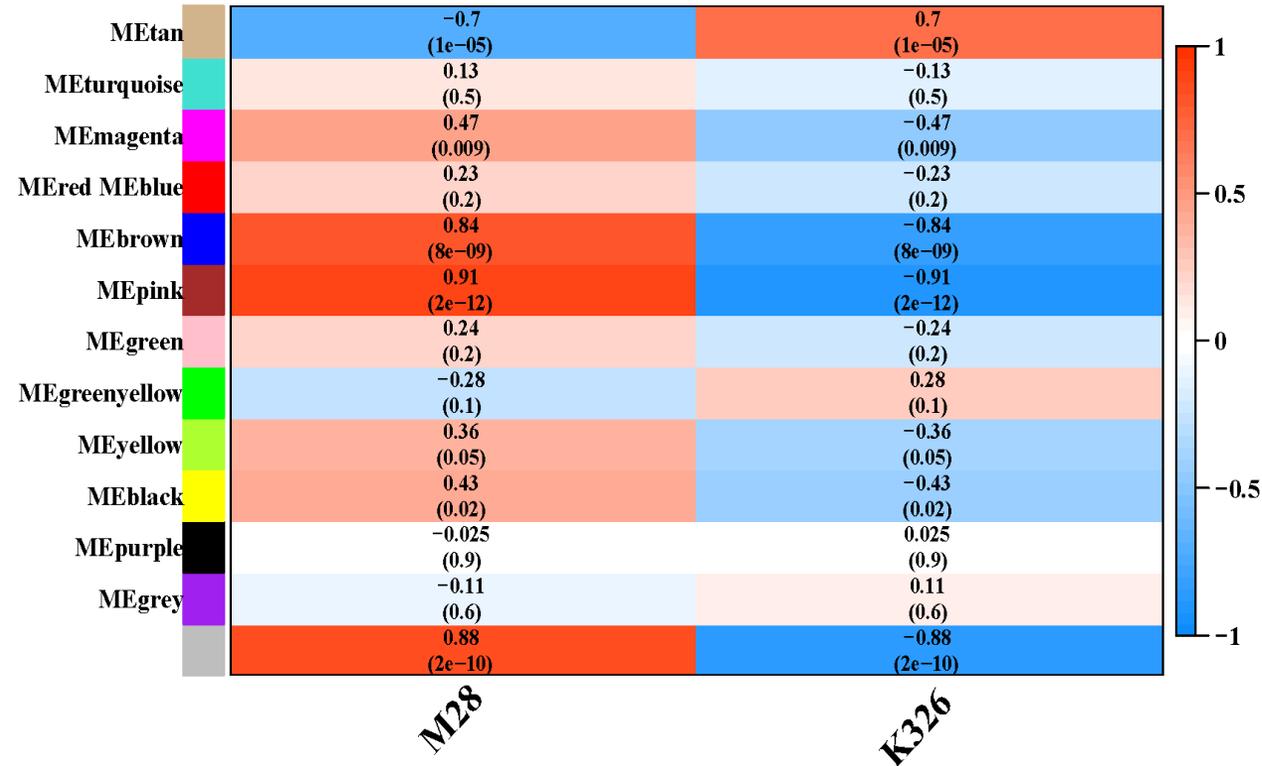


Supplementary Figure S13 Involvement of DEGs in combination with metabolites in Nitrogen_metabolism pathway for synthesis of glutamate in tobacco. The red representing DEGs and differential metabolites that were significantly enriched in the pathways of Nitrogen_metabolism .

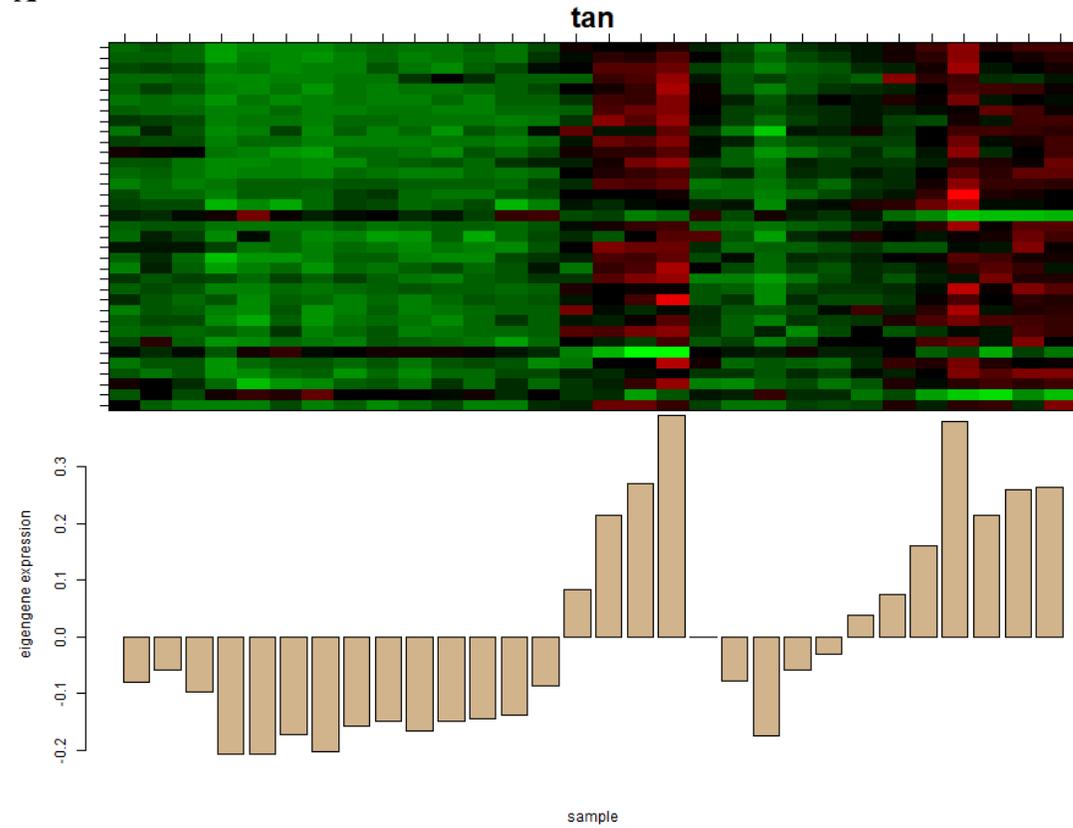
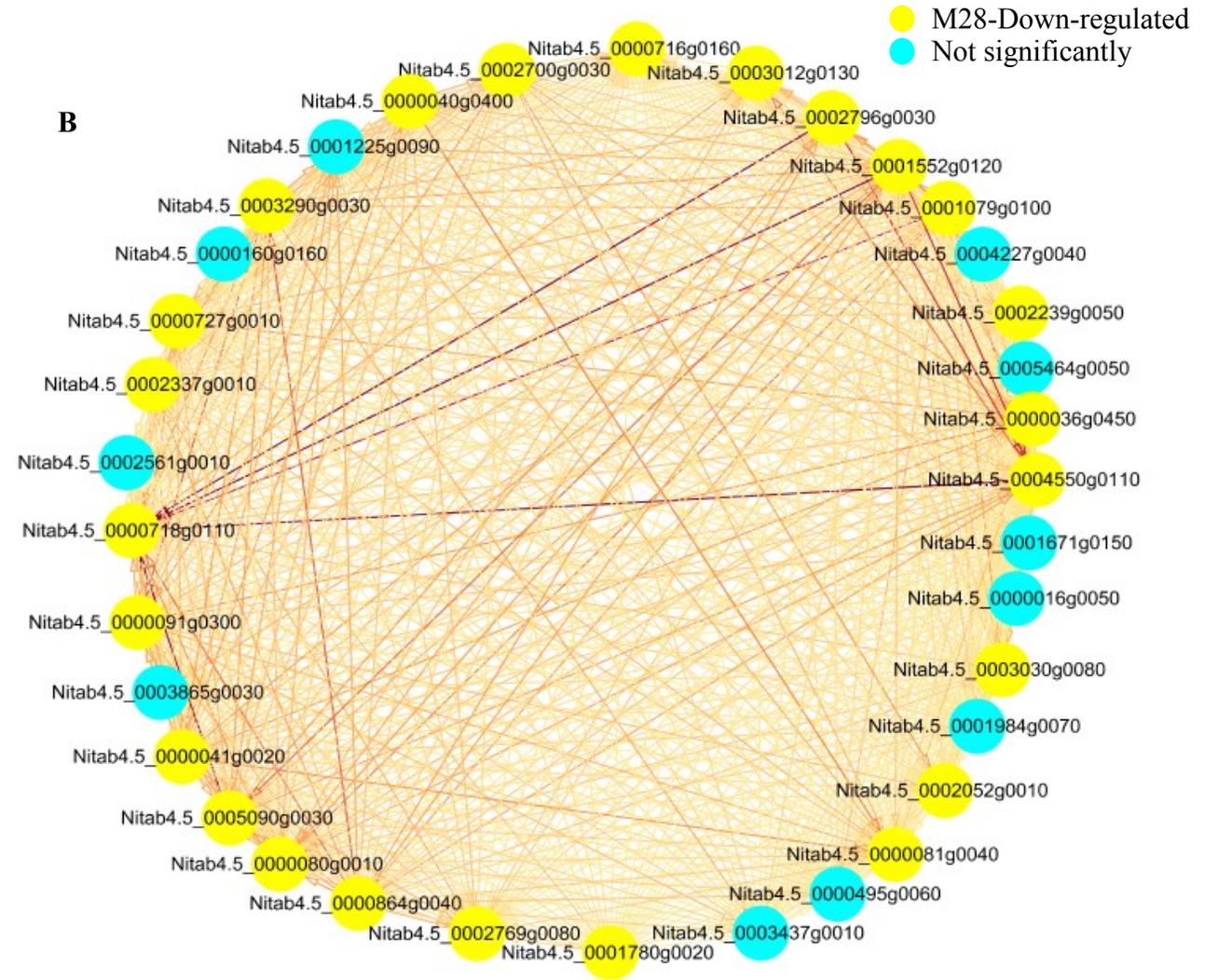


Supplementary Figure S14 Relative expression levels of *Nitab4.5_0001454g0180* and *Nitab4.5_0000123g0370* in overview of DEGs and metabolites involved in “Phenylpropanoid biosynthesis” and “Flavonoid biosynthesis”. Significance test was determined using t-test, *: $0.05 > p > 0.01$; **: $0.01 > p > 0.001$; ***: $p < 0.001$.

Module-pheno relationships



Supplementary Figure S15 Module-sample association. Each row represents a module. Each column corresponds to a specific sample. The color of each cell at the row-column intersection indicates the correlation coefficient between the module and sample. The red indicating a high degree of correlation between a specific module and sample.

A**B**

Supplementary Figure S16 WGCNA analyses. (A) Module Gene expression pattern in the tan module, the red representing up-regulated genes, the green representing down-regulated genes. (B) The correlation network of genes in the tan module. A gene network is constructed by WGCNA, in which each node represents a gene, and the connecting line between genes represents the co-expression correlation, the blue representing not significant genes and the yellow representing down-regulated genes between M28 and K326 before and after drought treatment. The genes with weights >0.1 are visualized by Cytoscape.

Supplementary Table S1. Summary of time-point-related drought-induced DEGs in M28 or K326 or between M28 and K326

| | DEGs | |
|------------------|----------------|--------------|
| | Down-regulated | Up-regulated |
| 28_0 vs K326_0 | 700 | 782 |
| 28_1 vs K326_1 | 503 | 502 |
| 28_2 vs K326_2 | 455 | 464 |
| 28_4 vs K326_4 | 521 | 557 |
| 28_8 vs K326_8 | 378 | 490 |
| 28_1 vs 28_0 | 358 | 529 |
| 28_2 vs 28_0 | 382 | 515 |
| 28_4 vs 28_0 | 776 | 966 |
| 28_8 vs 28_0 | 1,553 | 1,543 |
| 28_2 vs 28_1 | 41 | 58 |
| 28_4 vs 28_1 | 389 | 356 |
| 28_8 vs 28_1 | 1,224 | 1,135 |
| 28_4 vs 28_2 | 157 | 106 |
| 28_8 vs 28_2 | 1,072 | 918 |
| 28_8 vs 28_4 | 677 | 594 |
| K326_1 vs K326_0 | 620 | 770 |
| K326_2 vs K326_0 | 646 | 722 |
| K326_4 vs K326_0 | 1,073 | 1,199 |
| K326_8 vs K326_0 | 1,920 | 1,707 |
| K326_2 vs K326_1 | 298 | 175 |
| K326_4 vs K326_1 | 671 | 644 |
| K326_8 vs K326_1 | 1,519 | 1,384 |
| K326_4 vs K326_2 | 183 | 194 |
| K326_8 vs K326_2 | 1,223 | 996 |
| K326_8 vs K326_4 | 613 | 387 |

Supplementary Table S2. Summary of time-point-related drought-induced DiffExp metabolites in M28 or K326 or between M28 and K326

| | DiffExp metabolites statistics | |
|-------------------------|--------------------------------|--------------|
| | Down-regulated | Up-regulated |
| 28_0 vs K326_0 | 34 | 36 |
| 28_1 vs K326_1 | 16 | 26 |
| 28_2 vs K326_2 | 51 | 21 |
| 28_4 vs K326_4 | 9 | 36 |
| 28_8 vs K326_8 | 20 | 19 |
| 28_1 vs 28_0 | 0 | 21 |
| 28_2 vs 28_0 | 15 | 11 |
| 28_4 vs 28_0 | 7 | 22 |
| 28_8 vs 28_0 | 2 | 21 |
| 28_2 vs 28_1 | 18 | 2 |
| 28_4 vs 28_1 | 16 | 6 |
| 28_8 vs 28_1 | 11 | 10 |
| 28_4 vs 28_2 | 1 | 8 |
| 28_8 vs 28_2 | 1 | 23 |
| 28_8 vs 28_4 | 10 | 7 |
| K326_1 vs K326_0 | 4 | 9 |
| K326_2 vs K326_0 | 2 | 69 |
| K326_4 vs K326_0 | 10 | 11 |
| K326_8 vs K326_0 | 5 | 38 |
| K326_2 vs K326_1 | 1 | 17 |
| K326_4 vs K326_1 | 3 | 0 |
| K326_8 vs K326_1 | 4 | 23 |
| K326_4 vs K326_2 | 43 | 0 |
| K326_8 vs K326_2 | 20 | 13 |
| K326_8 vs K326_4 | 1 | 10 |

Supplementary Table S8. Up-regulated and Down-regulated genes in brown and Tan modules

| up-regulated genes in brown module | down-regulated genes in Tan module |
|---|---|
| Nitab4.5_0000040g0380 | Nitab4.5_0000036g0450 |
| Nitab4.5_0000050g0020 | Nitab4.5_0000040g0400 |
| Nitab4.5_0000062g0300 | Nitab4.5_0000041g0020 |
| Nitab4.5_0000092g0390 | Nitab4.5_0000080g0010 |
| Nitab4.5_0000110g0090 | Nitab4.5_0000081g0040 |
| Nitab4.5_0000117g0150 | Nitab4.5_0000091g0300 |
| Nitab4.5_0000173g0140 | Nitab4.5_0000716g0160 |
| Nitab4.5_0000178g0210 | Nitab4.5_0000718g0110 |
| Nitab4.5_0000195g0170 | Nitab4.5_0000727g0010 |
| Nitab4.5_0000303g0130 | Nitab4.5_0000864g0040 |
| Nitab4.5_0000458g0100 | Nitab4.5_0001079g0100 |
| Nitab4.5_0000500g0090 | Nitab4.5_0001552g0120 |
| Nitab4.5_0000525g0020 | Nitab4.5_0001780g0020 |
| Nitab4.5_0000856g0240 | Nitab4.5_0002052g0010 |
| Nitab4.5_0000978g0010 | Nitab4.5_0002239g0050 |
| Nitab4.5_0000993g0090 | Nitab4.5_0002337g0010 |
| Nitab4.5_0001315g0150 | Nitab4.5_0002700g0030 |
| Nitab4.5_0001315g0190 | Nitab4.5_0002769g0080 |
| Nitab4.5_0001925g0020 | Nitab4.5_0002796g0030 |
| Nitab4.5_0001996g0010 | Nitab4.5_0003012g0130 |
| Nitab4.5_0002367g0080 | Nitab4.5_0003030g0080 |
| Nitab4.5_0002682g0080 | Nitab4.5_0003290g0030 |
| Nitab4.5_0003286g0020 | Nitab4.5_0004550g0110 |
| Nitab4.5_0004274g0040 | Nitab4.5_0005090g0030 |
| Nitab4.5_0005820g0010 | |
| Nitab4.5_0005901g0010 | |

Supplementary Table S9. Blue module contained 15 up-regulated genes and 3 TFs

| up-regulated genes | TFs |
|---------------------------|--------------------|
| Nitab4.5_0000175g0070 | <i>bHLH</i> |
| Nitab4.5_0000540g0010 | <i>C3H</i> |
| Nitab4.5_0000082g0020 | <i>CCAAT</i> |
| Nitab4.5_0000986g0060 | up-regulated genes |
| Nitab4.5_0001788g0010 | up-regulated genes |
| Nitab4.5_0004624g0020 | up-regulated genes |
| Nitab4.5_0000312g0130 | up-regulated genes |
| Nitab4.5_0005745g0030 | up-regulated genes |
| Nitab4.5_0002887g0100 | up-regulated genes |
| Nitab4.5_0002079g0020 | up-regulated genes |
| Nitab4.5_0000142g0080 | up-regulated genes |
| Nitab4.5_0000832g0190 | up-regulated genes |
| Nitab4.5_0002093g0090 | up-regulated genes |
| Nitab4.5_0000471g0030 | up-regulated genes |
| Nitab4.5_0000130g0300 | up-regulated genes |
| Nitab4.5_0001239g0040 | up-regulated genes |
| Nitab4.5_0002716g0020 | up-regulated genes |
| Nitab4.5_0000463g0070 | up-regulated genes |

Supplementary Table S10. Primers used for gene expression analyses

| Gene name | Primer | Sequence |
|--------------------------------|--------|--------------------------|
| <i>NCED1</i> | F | GGCATTGCAGAACCATGGCCAAA |
| | R | GCTTTGCTGTTAGGGTCTCTTGG |
| <i>PP2C-37L</i> | F | ACCCGTCGTTTTGCAAAGGAG |
| | R | ACCTCTTCCACTGAGTTTCCC |
| <i>P450 84A1L</i> | F | GGGACAGCTGTAACTTAGG |
| | R | TAGACTCTGCTTACCAACCCAC |
| <i>18srDNA</i> | F | AGGATTGACAGACTGAGAGC |
| | R | CACAGACCTGTTATTGCCTC |
| <i>Nitab4.5_0001454g0180.1</i> | F | GCGAGTGTTCTTCACATCCTAAA |
| | R | GCAGAAGGAGTCTCGTAGAGAAGT |
| <i>Nitab4.5_0000123g0370.1</i> | F | TCCAATGTGTAGCCAAGGAAGG |
| | R | ACGGCTGGATCACGAGCAACA |