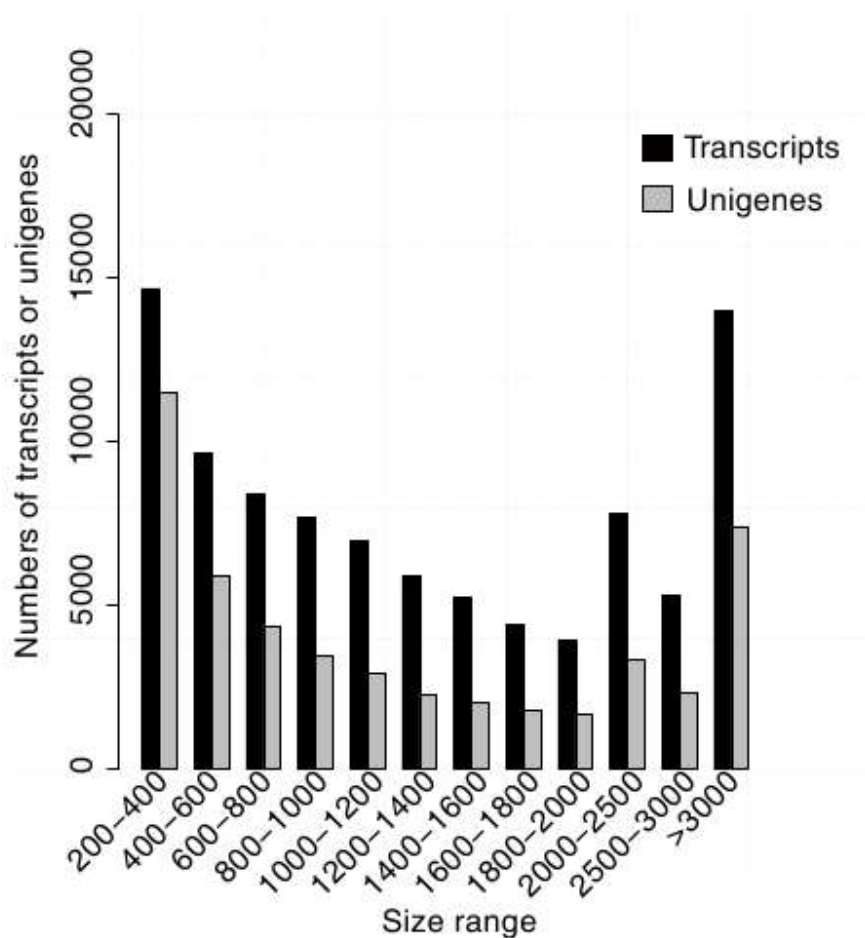




**Figure S3. Gene Ontology(GO) and kyoto encyclopedia of genes and genomes (KEGG) classification of assembled unigenes.**

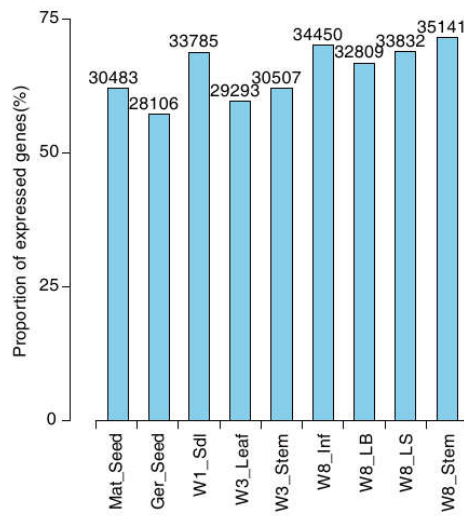
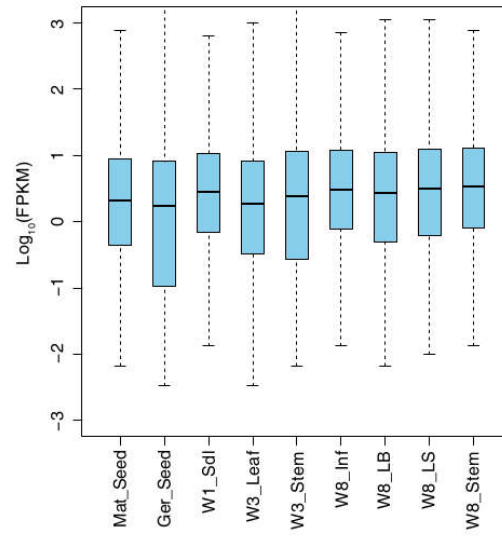
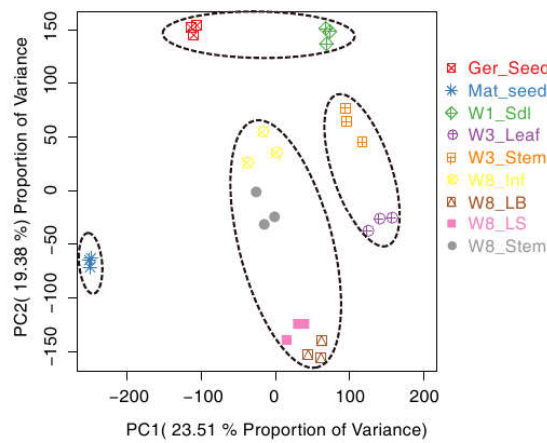
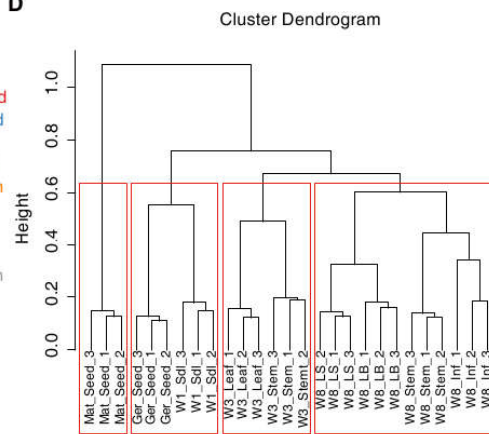
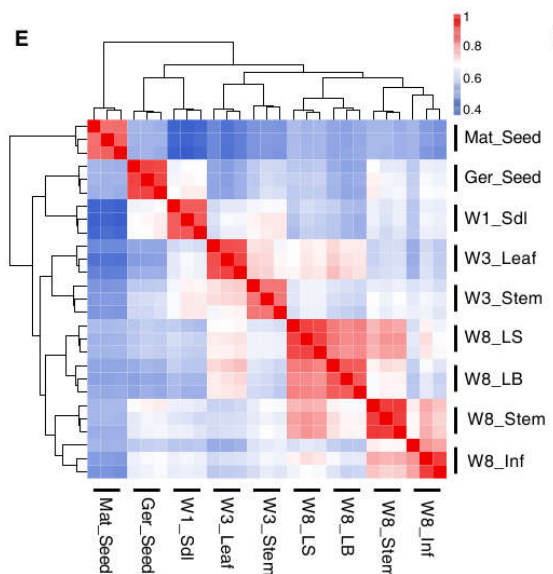
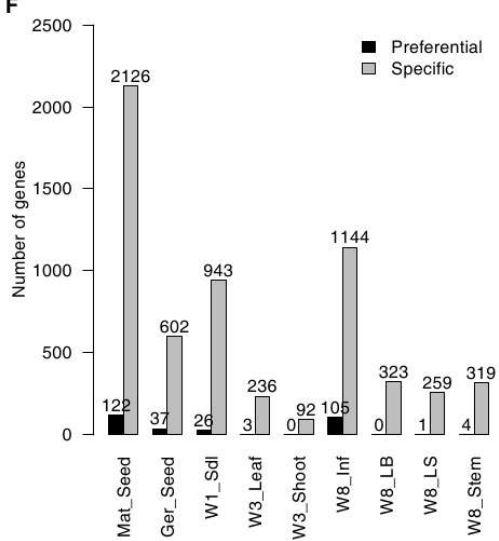
A. Each bar represents number of unigenes that were assigned to each level 2 GO term in the three categories. Red, blue, and green bars represent GO terms in biological process (BP), cellular component (CC), and molecular function (MF), respectively.

B. Each bar represents number of unigenes that were attributed to each KEGG category. A: Cellular processes; B: Environmental Information Processing; C: Genetic Information Processing; D: Metabolism; E: Organismal Systems.



**Figure S2. Length distribution of assembled unigenes and transcripts**

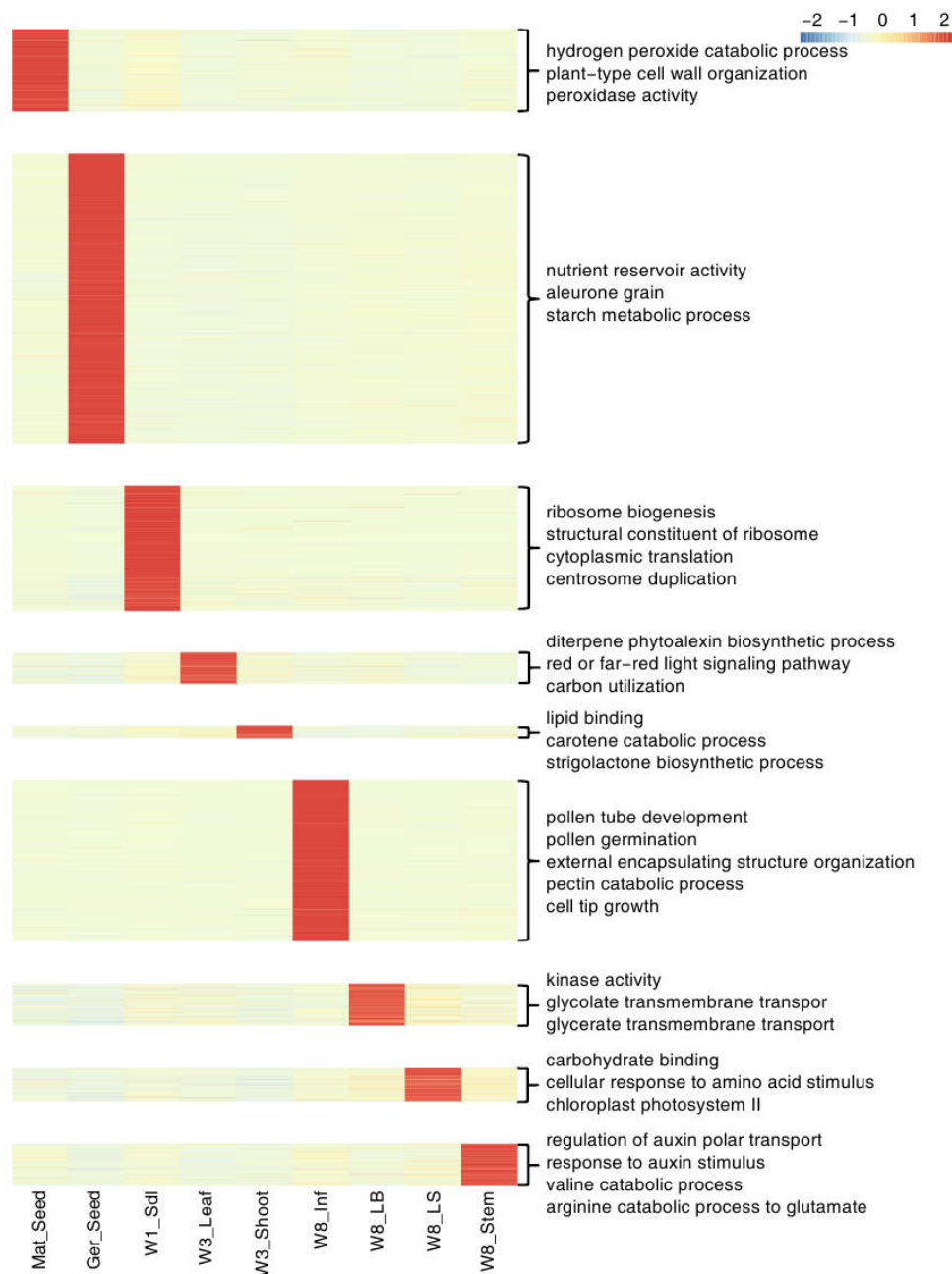
The black bars represent length distribution of transcripts and grey bars represent length distribution of unigenes.

**A****B****C****D****E****F**

**Figure S4. Global analysis of RNA-Seq data from different tissues.**

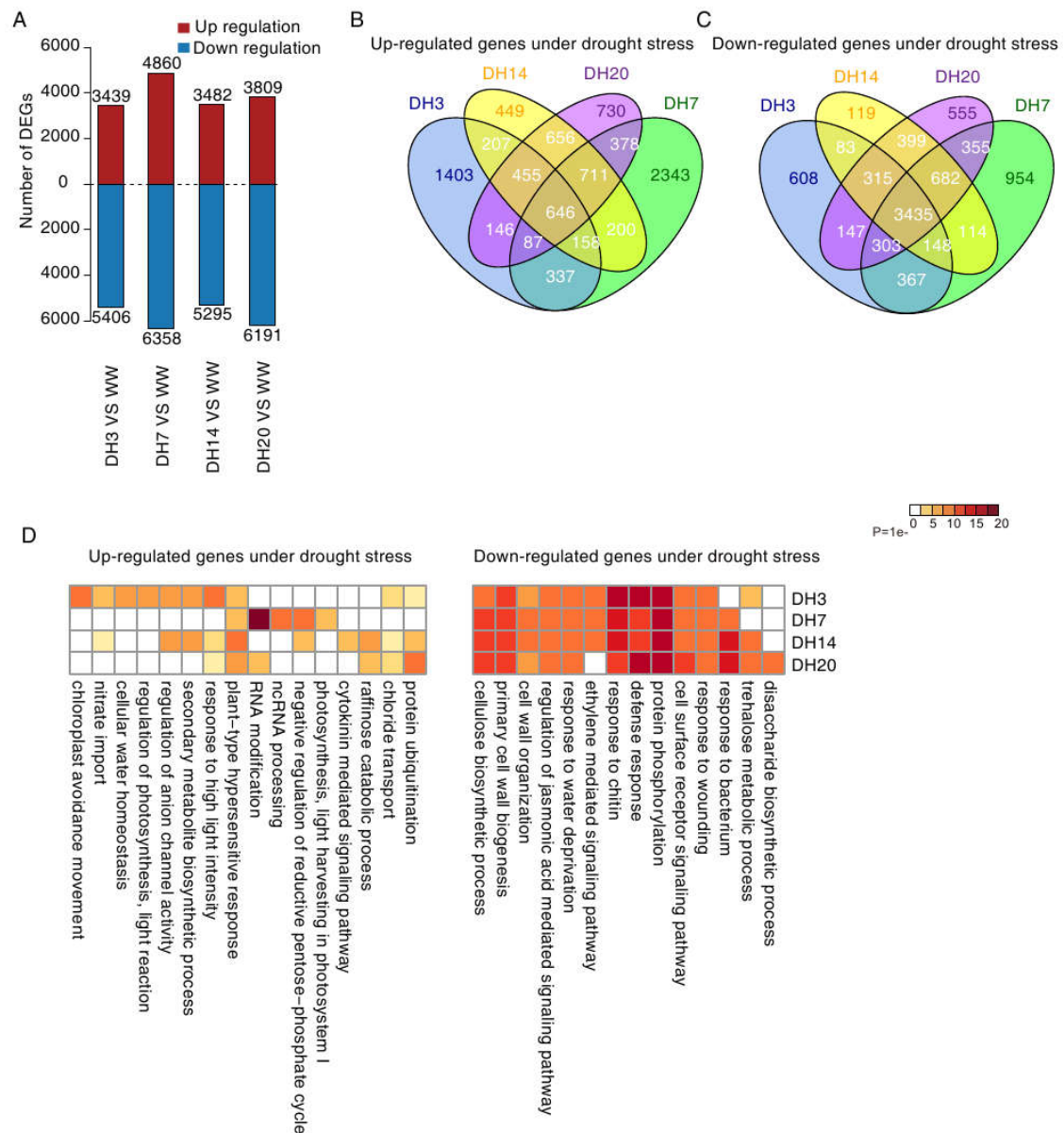
- A. Percentage of genes that were expressed in each sample. FPKM > 1 was used as the cutoff for identifying expressed genes. The number on the top of each bar represents the number of expressed genes in a particular sample.
- B. Box plot of gene expression levels in different samples.
- C. Principle component analysis (PCA) of gene expressions from different tissues
- D. Hierarchical clustering of overall gene expression among different samples. Samples from different tissues are clustered into four subgroups..
- E. Correlations of genes expressions from different tissues.
- F. Number of genes with tissue-specific or preferentially expressed expression. Grey bar indicated number of genes with tissue-specific expression. Black bar indicated number of genes with tissue-preferential expression.

"Mat\_Seed" denotes mature seeds at 1-3 days; "Ger\_Seed" denotes germinated seeds at 1-3 days, "W1\_Sdl" denotes seedlings at 1 week; "W3\_Leaf" denotes 3-week-old leaves; "W3\_Shoot" denotes 3-week-old shoot; "W8\_LB", "W3\_LS", "W8\_Stem", and "W8\_Inf" denote leaf blades, leaf sheaths, stems, and inflorescences at 8 weeks, respectively.



**Figure 4. Heatmap of tissue-specific and preferentially expressed genes in Broomcorn millet.**

Heatmap on the left show the expression patterns of tissue-specific and preferentially expressed genes. The gene expression level in each sample is colored according to the z-score that was calculated from the normalized expression level (FPKM). The most significantly enriched GO terms were shown on the right for each tissue-specific and preferentially expressed gene set.



**Figure 5. Up- and down-regulated genes under drought for 3, 7, 14, 20 days.**

A. Number of differentially expressed genes under drought for 3, 7, 14, 20 days. The numbers above and below the x axis represent the number of up and down-regulated DEGs, respectively.

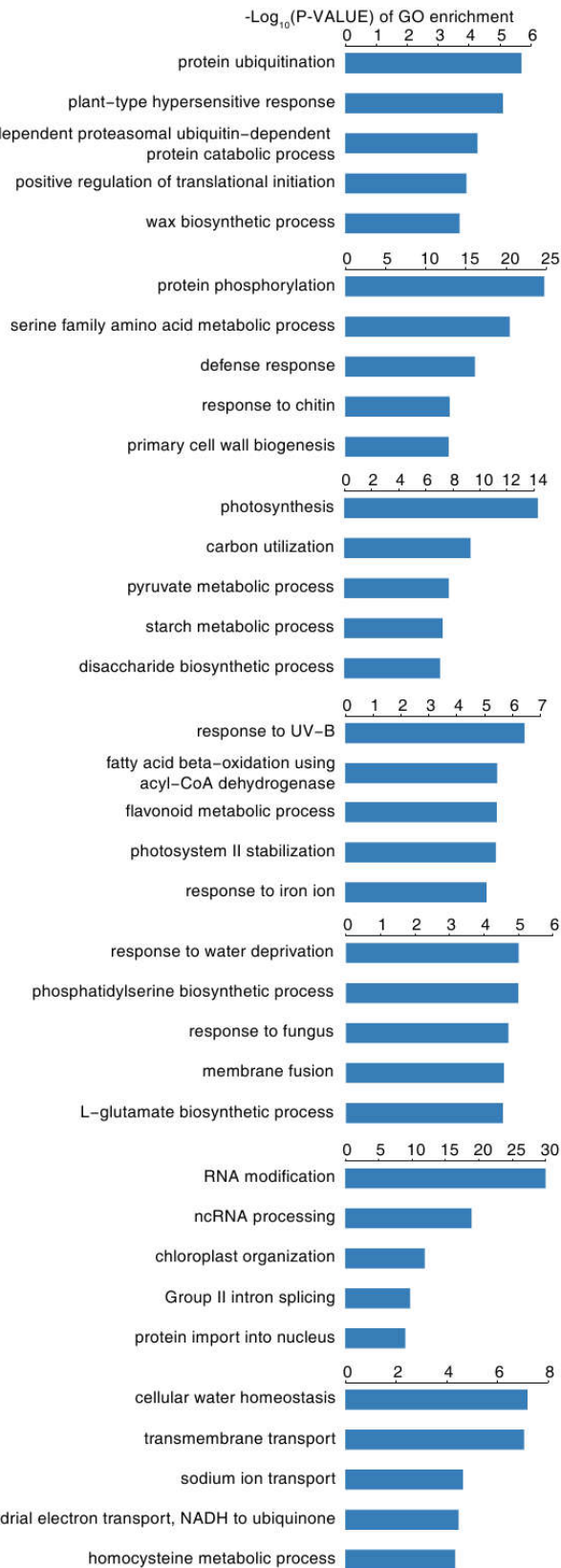
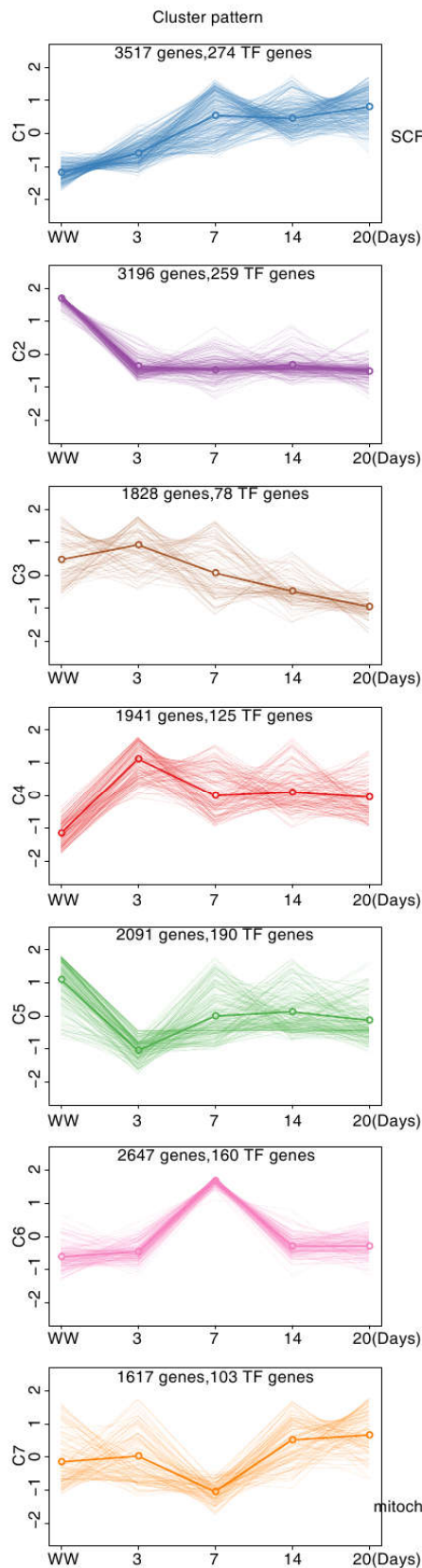
B. Venn diagram to show the overlaps of up-regulated genes under drought stress.

C. Venn diagram to show the overlaps of down-regulated under drought stress.

D. The most significantly enriched GO items in the biological process category of up-regulated or down-regulated genes under drought for 3, 7, 14, 20 days. Heatmap represents the  $-\log_{10}(p\text{-value})$  of overrepresented GO items.

WW represents well-watered sample. DH3, DH7, DH14, DH20 represents sample under drought stress for 3, 7, 14, 20 days, respectively.





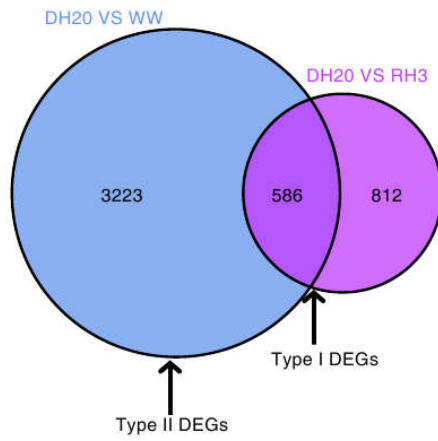
**Figure 6. Clusters of genes that displayed different expression trends along with the progression of drought treatment.**

For each cluster, z-scores of the normalized gene expression levels at different time points of drought treatment were plotted on the left. Each thin line represents an individual transcription factor gene in the cluster and the thick line represents the average gene expression level. The most enriched GO items in the biological process category were shown on the right. Lengths of the blue bar on the right represent  $-\log_{10}$  (p-value) of overrepresented GO items.

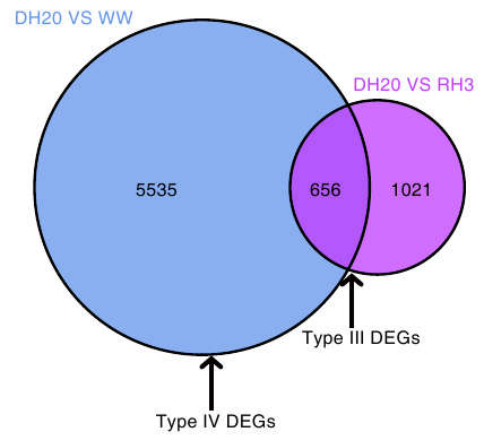
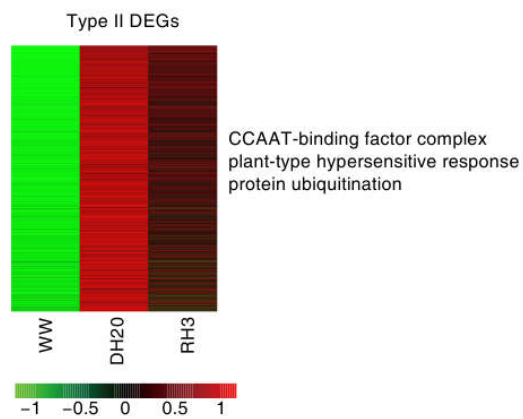
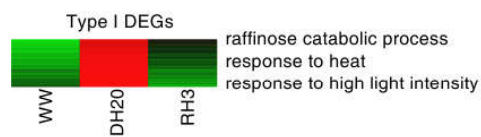
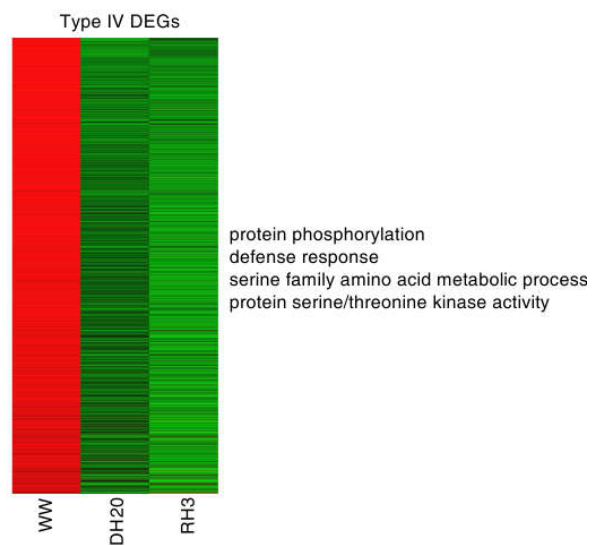
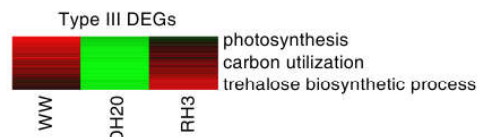


**A**

Up-regulated genes under drought stress

**B**

Down-regulated genes under drought stress

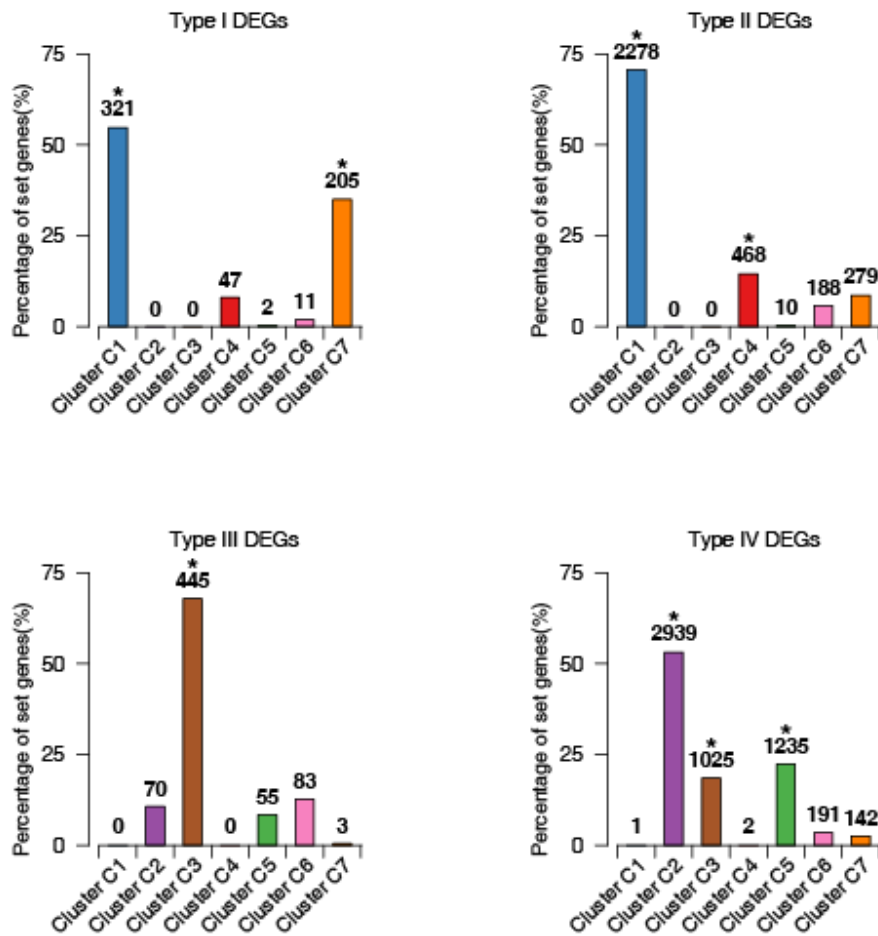
**C****D**

**Figure 7. DEGs under drought stress 20 days and rewatering for 3 days.**

A. Overlaps of up-regulated DEGs between DH20 *vs* WW and DH20 *vs* RH3. WW(well watered), DH20 (drought for 20 days), RH3 (re-watered for 3 days). Type I DEGs mean genes were up-regulated under drought stress and recovered their expression level after rehydration, type II DEGs mean genes were up-regulated under drought stress and not recovered their expression after rehydration.

B. Overlaps of down-regulated DEGs between DH20 *vs* WW and DH20 *vs* RH3. Type III DEGs mean genes were down-regulated under drought stress and recovered their expression after rehydration, type IV DEGs mean genes were down-regulated under drought stress and not recovered after rehydration

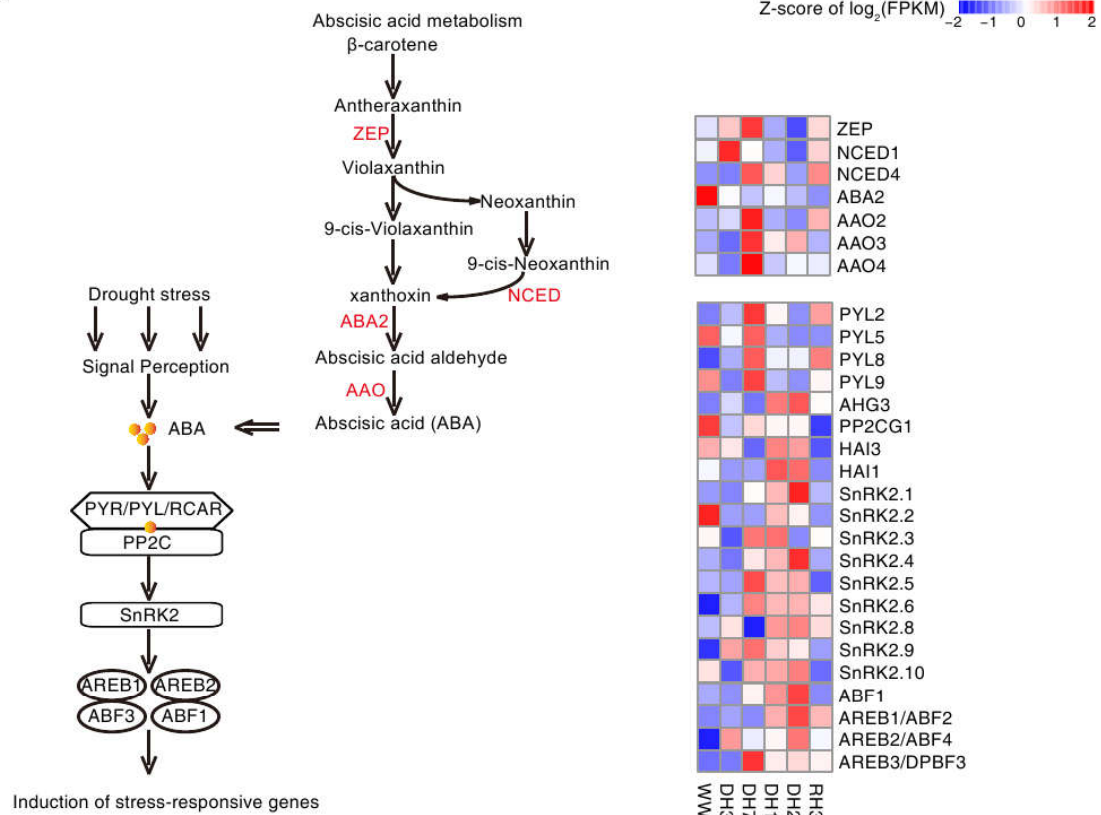
C. Gene expression and enriched GO terms of the four type DEGs indicated in (A) and (B). Left, heatmap show the z-scores of the normalized gene expression levels in WW, DH20, and RH3 samples. Right, Most significantly enriched GO terms.



**Figure S5. Number of DEGs in different clusters of different type .**

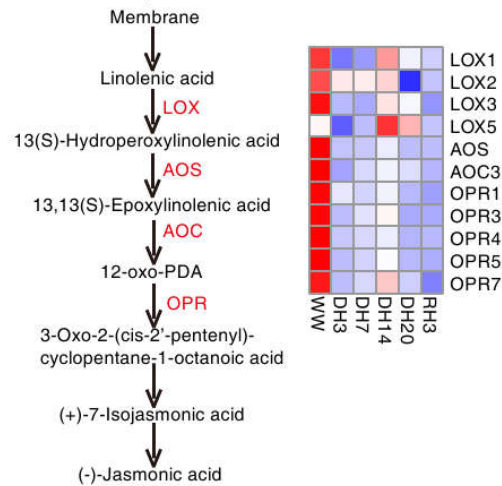
Number of DEGs in different gene clusters were indicated by the length of bar and shown on the top of the bar. Fisher's exact test were used to examine whether a type of DEGs were significantly overrepresented in k-means cluster. "\*" indicates p-value < 0.05

**A**

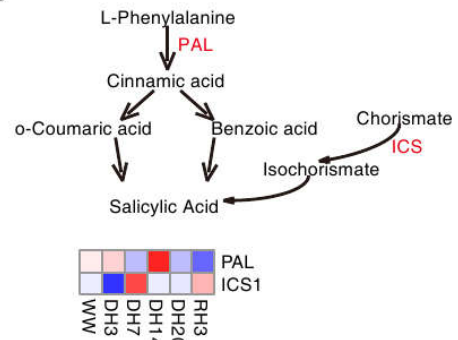


**B**

**Jasmonic acid synthesis**



**C**



**Figure S6. Expression level of genes involved in plant phytohormone under drought stress and rehydration**

A. Expression level of genes involved in abscisic acid metabolism and ABA-dependent pathways mediating to drought stress. Enzymes regulating key regulatory step and annotated in this millets transcriptome are shown in red.

A. Expression level of genes involved in jasmonic acid metabolism.

B. Expression level of genes involved in salicylic acid synthesis

Enzymes regulating key regulatory steps are shown in red. The gene expression level in each sample is colored according to the z-score calculated from the normalized expression level (FPKM).