Transcriptomics analysis of field-droughted pear (*Pyrus spp.*) reveals potential drought stress genes and metabolic pathways

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Abstract

Drought acts as a major abiotic stress that hinders plant growth and crop productivity. It is critical, as such, to discern the molecular response of plants to drought in order to enhance agricultural yields under droughts as they occur with increasing frequency. Pear trees are among the most crucial deciduous fruit trees worldwide, and yet the molecular mechanisms of drought tolerance in field-grown pear remain unclear. In this study, we analyzed the differences in transcriptome profiles of pear leaves, branches, and young fruits in irrigation vs field-drought conditions over the growing seasons. In total, 819 differentially expressed genes (DEGs) controlling drought response were identified, among which 427 DEGs were upregulated and 392 DEGs were downregulated. Drought responsive genes were enriched significantly in monoterpenoid biosynthesis, flavonoid biosynthesis, and diterpenoid biosynthesis. Fourteen phenylpropanoid, 5 flavonoid, and 4 monoterpenoid structural genes were modulated by field drought stress, thereby indicating the transcriptional regulation of these metabolic pathways in fruit exposed to drought. 4438 transcription factors (TFs) belonging to 30 TF families were differentially expressed between drought and irrigation, and such findings signal valuable information on transcriptome changes in response to drought. Our study revealed that pear trees react to drought by modulating several secondary metabolic pathways, particularly by stimulating the production of phenylpropanoids as well as volatile organic compounds like monoterpenes. Our findings are of practical importance for agricultural breeding programs, while the resulting data is a resource for improving drought tolerance through genetic engineering of non-model, but economically important, perennial plants.

Key words: drought stress, transcriptomic, RNA sequencing, pear *(Pyrus spp)*

Introduction

Along with an increasing global population, drought is becoming one of the most persistent factors that limits agricultural production and food security around the world, especially in arid and semi-arid regions (Mittler, 2006). In turn, drought is responsible for losses in the multibillions annually (Fahad et al., 2017; Lesk and Rowhani, 2016). China is facing a perilous water crisis in which 50% of the national territory is located in arid and semi-arid regions (Hu and Zhang, 2001). The temporal-spatial distribution of annual precipitation causes 26.7% of the national land territorial area in Northwest China to have arid and semiarid climates, a region where drought is common. Predicting drought severity is difficult, and to do so requires consideration of several factors such as rainfall amount and distribution, evaporative demands, and the moisture storing ability of soils (Saud et al., 2017; Tadesse and Melkam, 2016). Globally, several management strategies have been implemented for improved crop production under drought environments (Bodner et al., 2015; Fahad et al., 2017). Among these, the development of crop varieties with an increased tolerance to drought functions as an important and effective strategy to combat drought.

Plants cope with water deficiency by complex mechanisms from molecular, biochemical and physiological processes at the cellular or whole plant level (Bray, 1997; Goufo et al., 2017; Huber and Bauerle, 2016; Ruggiero et al., 2017; Zandalinas et al., 2017). With the advent of new high throughput "-omics" technologies like proteome and transcriptome, notable strides have been made towards understanding the molecular mechanisms that regulate tolerance to drought. Previous studies have demonstrated signal transduction of drought stress perception to the nucleus via complex cellular signaling networks involving second messengers. These include reactive oxygen intermediates (ROIs) and calcium, calcium-associated proteins, and kinase cascade such as mitogen-activated protein (MAP) (Bray, 1997; Chen et al., 2002; Huber and Bauerle, 2016; Knight and Knight, 2001; Liu et al., 1998; Zandalinas et al., 2017). Drought stress signaling cascades are comprised of many stress-responsive genes. These include molecular chaperones such as late embryogenesis abundant (LEA) proteins and heat shock proteins (HSPs) that function as effector molecules. Other examples include transcription factors (TFs) like members of the APETALA2/ethylene-responsive element binding protein (AP2/EREPB), a basic leucine zipper (bZIP), WRKY, and MYB proteins that act as regulator molecules (Shinozaki and Yamaguchi-Shinozaki, 2007; Song et al., 2005; Wang et al., 2003). The physiological and molecular mechanisms of plant responses to drought have been extensively studied in model plants with dehydration treatments in controlled laboratory or greenhouse conditions (Li et al., 2016; Wang et al., 2018; Zarafshar et al., 2014). However, results from these studies most often translate poorly to field-grown plants. Clarifying the molecular mechanisms that regulate drought tolerance from crops grown under field conditions will facilitate a more thorough grasp of the complex interactions between drought response and environmental factors that crops encounter in the field during the growing season. As such, the task of developing an improved

80 understanding of molecular elements in responsiveness to field drought in non-model plants will
81 aid in both traditional and modern breeding applications towards improving stress tolerance.
82
83 Pear is one of the most vital fruit crops in the world and the second major crop among deciduous
84 fruits in China after apples (Silva et al., 2014). The crop has considerable value both
85 economically and in terms of personal health. In China, pear is primarily grown in the
86 Northwestern region, accounting for 60 percent of pear production in the country. YuluXiangli
87 (*Pyrus spp*) is an improved pear cultivar that is highly tolerant to drought, and it is an ideal
88 source for examining genomic responses to drought in order to explore valuable tolerance genes
89 (Okubo and Sakuratani, 2000; Zong et al., 2014). The full genome sequencing and resequencing
90 of multiple pear cultivars (Huang et al., 2015; Li et al., 2016; Wang et al., 2018; Wu et al., 2013)
91 have enabled several transcriptome studies of drought responses in pear, thereby revealing a
92 broad, multifaceted response to drought. Such a response features coordination between
93 phytohormone signaling pathways, the reduction of photosynthetic gene expression, and the
94 alteration in expression of genes involved in stress-induced leaf senescence. These studies,
95 however, have been restricted to greenhouses under certain durations of drought stimuli
96 treatment as opposed to field conditions that use early time points with samples exclusively from
97 leaves (Li et al., 2016; Wang et al., 2018).
98
99 The primary objectives of the present study were to identify differentially expressed genes
100 (DEGs) and to compare the gene expression patterns in leaf, branch, and fruit tissue of pear in
101 response to drought induced by withdrawal of irrigation in the field. The findings will provide an
102 unrivaled resource for understanding the mechanisms underlying drought resistance in pear.
103

Materials and Methods

105 *Plant growth conditions and drought treatment*
106 Field drought experiments were performed for 3 continuous years in a pear germplasm nursery at
107 the Institute of Fruit, Shanxi Academy of Agricultural Science, beginning on 21 October 2015
108 and concluding on 21 October 2018. The pear nursery is located in a semi-arid area of Taigu,
109 Shanxi Province, China (37°26′ N, 37°26′ E) with an altitude of 750 meters, and managed
110 according to common cultural practices in the region. In this region, the annual average
111 temperature is 9.8℃ with an annual accumulated temperature above 10℃ (AT10) of 3529℃.
112 The annual hours of sunshine range from 2,500 h to 2,600 h with an average frost-free period of
113 149 days. The annual rainfall is 450 mm, and the annual accumulative evaporation is 1800 mm,
114 which is approximately 4 times higher than the average total rainfall.
115
116 The pear cultivar YuluXiangli (*Pyrus spp*) was used in the experiment. YuluXiangli was derived
117 from a cross between *Pyrus bretschneiderie* and *Pyrus sinkiangensis,* and is resistant to drought.
118 The irrigation (control) and field-drought treatments were assigned via a randomized block
119 design with 3 replicates, where the fields were divided into 6 plots with 10 healthy and uniform

120   15-year-old pear trees per plot. Field drought plots were exposed to rainfall without additional
121   irrigation, whereas control plots were irrigated in November, May, and July, each of which
122   received 728.5 tons water/acre. The maximum water holding capacity was 30% in field-drought
123   treatment (severe drought) and 75% to 80% in control with irrigation. Fertilization and pest
124   controls were consistent among the field-drought and control plots.

125

126   In total, 100 young leaves, branches, and young fruits, including 10 from each tree, were
127   independently harvested on 5 May 2018, and were swiftly placed in liquid nitrogen and stored at
128   −80°C for RNA extraction.

129

130   At maturity, 10 fruits, each from a single tree, were independently harvested to determine fruit
131   soluble solids content with a handheld PAL-1 digital display sugar meter (Atago, Tokyo, Japan)
132   and single fruit weight.

133

134   ***Total RNA extraction, library preparation and sequencing***
135   Total RNA was extracted from young leaves, branches, and young fruits for each treatment using
136   RNApreo Pure Plant Kit (Tiangen, Beijing, China) in accordance with the manufacturer's
137   instructions. RNA purity and integrity were determined by Agilent 2100 Bioanalyzer (Agilent
138   Technology, USA) according to the manufacturer's instructions. The qualified RNA with an
139   RNA integrity number (RIN) of ≥7 and an 28S/18S ribosomal RNA ratio of ≥0.7 was applied
140   to construct 10 cDNA libraries (5 repeats for drought and irrigation, respectively). Equal
141   amounts of RNA from young leaves, branches, and young fruits for each treatment were mixed,
142   and then were diluted to 1 ng/μL for library construction. Briefly, RNA was enriched by
143   magnetic beads containing poly-T oligos and fragmented first to 200-300bp in length by ion
144   interruption, and reversed transcribed to the first strand of cDNA by 6-bp random primers. Then,
145   the first strand of cDNA was used as a template to synthesize the second strand of cDNA.
146   Library fragments were enriched by PCR amplification to select the fragment size of 300-400bp.
147   Equal amounts of libraries with different index sequences were pooled prior to sequencing and
148   diluted to 2 nM for paired-end sequencing on the Illumina HiSeq 2500 platform. All raw reads
149   were deposited in the NCBI repository with Bioproject: PRJNA655255
150   (https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA655255) under the accession number of
151   SRR12424088-SRR12424107.

152

153   ***Read mapping and transcript profiling***
154   The adapter and low-quality sequences were removed from the raw RNA-seq reads to generate
155   high-quality clean reads that were aligned to the pear genome reference
156   GCF_000315295.1_Pbr_v1.0_(https://ftp.ncbi.nlm.nih.gov/genomes/all/000/315/295/
157   GCF_000315295.1_Pbr_v1.0/) with HISAT2 (http://ccb.jhu.edu/software/hisat2/index.shtml).
158   Following the alignments, the raw counts for each pear gene were normalized as fragments per
159   kilobase of transcript per million mapped reads (FPKM) (Trapnell et al., 2010). Principal
160   component analysis (PCA) was performed to compare the log2-transformed FPKM values of the
161   expressed gene profiles among tissue-type and stages using the prcomp function in the R
162   program (https://www.rproject.org). The hierarchical clustering of samples was performed using

163   Pheatmap in R. Read coverage over gene body was calculated by RSeQC (Wang et al., 2012),
164   and the corresponding plot figure was made by using ggplot2 with R script.
165
166   ***Identification of Differentially Expressed Genes (DEGs)***
167   DEGs among tissue-types at different stages were located using the statistical package DEGseq
168   with the MA-plot-based method (Wang et al., 2010) in R version 3.0.3, where genes were
169   considered differentially expressed if |log2FoldChange| > 1, and an adjusted p value using
170   Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) (false discovery rate (FDR))
171   was <0.05.
172
173   ***Gene annotation (GO) and functional enrichment analysis***
174   The GO enrichment analysis for biological processes, molecular functions, and cellular
175   components was performed using TopGo (Alexa and Rahnenfuhrer, 2016) with P value < 0.05.
176   Pathway enrichment analysis was implemented on all DEGs in the KEGG (Kyoto Encyclopedia
177   of Genes and Genome) platform (http://www.genome.jp/kegg/) (Kanehisa et al., 2008). An
178   adjusted P value < 0.05 was considered statistically significant.
179
180   ***Statistical analysis***
181   Single fruit weight and soluble solid content were expressed as the mean ± standard error from
182   10 independent biological replicates by SPSS (V24.0, IBM Corporation, Armonk, NY, USA).
183   These were subjected to one-way analysis of variance (ANOVA), followed by Duncan's
184   Multiple Range post-hoc test, and the significance level was set to P < 0.01.
185
186   ***Validation of Transcripts by Quantitative Real-Time PCR (qRT-PCR)***
187   The expression levels of a set of randomly selected 13 DEGs were validated by a qRT-PCR
188   assay. Total RNA used for RNA-seq was treated with RNase-free DNase I (New England
189   Biolabs, Ipswich, MA, USA) to eradicate all contaminating DNA. 1000 ng total RNA was used
190   for the reverse transcription with PrimeScript^{TM} 1st stand cDNA Synthesis Kit. qRT-PCR was
191   performed with SYBR Premix Ex Taq (TaKaRa, Dalian, China) on ABI Step One RT-PCR
192   system, according to the manufacturer's instructions (20 μL reaction mix: 1 μL cDNA, 10μL
193   2×SYBR real-time PCR premixture, 0.4 μL each 10 μM primer, and 8.2 μL distilled water).
194   Three biological replicates with two technical replicates were performed for each sample. The
195   gene IDs and sequences of 13 primers are listed in *Table 1*. The PCR program was as follows:
196   95°C for 5 min, followed by 40 cycles of 95°C for 15s, and 60°C for 30s. Relative expression
197   was normalized to the internal control gene GAPDH gene with $2^{-\Delta\Delta CT}$ method (Livak &
198   Schmittgen, 2001). Pearson's correlation was performed using R software (ver. 3.2.4, R Core
199   Team, 2014) to determine the correlation of gene expression between qRT-PCR and
200   transcriptomic data.
201
202   **Results**

203 ***Effect of drought stress on physiological traits and antioxidant activities***
204 Two irrigation treatments were applied to pear trees over the course of 3 continuous years.
205 Irrigated pear trees were well irrigated, whereas pear trees subjected to deficit irrigation were not
206 irrigated over the same period of time. As shown in *Figure 1*, rainfalls during the 2018 season
207 were extremely scarce (*Figure 1a*), the consequence of which was a severe decrease in single
208 fruit weight and soluble solids content (*Figure 1b and c*).
209

210 ***RNA-seq and de novo assembly***
211 Paired-end RNA-Seq was performed on 10 cDNA libraries (5 repeats for drought and irrigation).
212 Each sample was independently aligned, processed for quality control, and then normalized. A
213 total of 400755040 clean reads *(Supplementary Table S1)* were generated, among which more
214 than 71.7% were mapped to the pear genome GCF_000315295.1_Pbr_v1.0_genomic.fna (*Table*
215 *2*). As indicated by FPKM, the expression values showed high correlations (Spearman
216 correlation coefficient (SCC) = 0.99) among biological replicates, which in turn demonstrated
217 that the sequencing quality was satisfactory for subsequent analyses. Principal component
218 analyses (PCA) revealed that the five replicates of each treatment were located nearest to each
219 other (*Figure 2*), thereby demonstrating the reliability of our datasets.
220

221 ***Identification of DEGs between field drought and irrigation treatment***
222 In total, 819 DEGs between drought and irrigation were identified by pairwise sample
223 comparisons (*Figure 3a*), among which 427 DEGs were upregulated and 392 DEGs were
224 downregulated in comparison to that of irrigation *(Supplementary Table S2)*. The expression
225 changes of genes in response to field drought are shown in *Figure 3b*. The highly expressed
226 (log2FoldChange < -3.5) drought specific genes (*Figure 3c*) included gene38569 encoding
227 Probable WRKY TF40, gene 1490 encoding WRKY TF 18, gene 30473 encoding ferritin-4,
228 gene 7768 and gene 6357 encoding 4-hydroxycoumarin synthase 1, gene 5151 encoding
229 histidine-containing phosphotransfer protein 4, gene 16914 encoding protein NIM1-
230 INTERACTING 1, and gene12366 encoding uncharacterized protein LOC103951864 (*Table 3*).
231 Genes that were highly expressed in irrigated samples but identified in drought samples included
232 gene 27148 encoding GDL79_ARATH GDSL esterase/lipase At5g33370, gene 5286 encoding
233 uncharacterized protein LOC103944059 isoform X1, gene 1170 encoding putative receptor
234 protein kinase ZmPK1, gene13865 encoding gibberellin-regulated protein 11, gene19880
235 encoding type I inositol 1,4,5-trisphosphate 5-phosphatase CVP2-like isoform X2, and three
236 genes (gene33465, gene39363, and gene34550) encoding palmitoyl-
237 monogalactosyldiacylglycerol delta-7 desaturase (*Figure 3d*, *Table 4*). The specific expression of
238 2 DEGs, WRKY TF 18 (gene 1490) and NIM1-INTERACTING 1 (gene 16914), was analyzed
239 by RT-qPCR. Consistent with our RNA-seq results, WRKY TF 18 (gene 1490) was highly
240 expressed in drought treatment at a relatively stable expression level, and the transcription of
241 NIM1-INTERACTING 1 *(*gene 16914*)* was consistent with the RNA-seq result only in the
242 irrigation (*Figure 4*).

243

Co-expression analysis of DEGs during field drought treatment

244 ***Co-expression analysis of DEGs during field drought treatment***
245 In order to investigate the co-expressed genes during field drought stress, all the genes that were
246 differentially expressed between drought and irrigation were statistically clustered into different
247 groups according to their expression profiles. The largest group (*Figure 5a*) included 539 genes
248 that predominantly annotated to RLP12_ARATH and increasingly expressed under field drought
249 conditions. Receptor-like protein 12 participated in the perception of CLV3 and CLV3-like
250 peptides to act as extracellular signals regulating meristems maintenance (149/539).
251 ZIFL1_ARATH Protein ZINC INDUCED FACILITATOR-LIKE 1 (120/539),
252 TMVRN_NICGU TMV resistance protein N (90/539), Y3475_ARATH Probable LRR receptor-
253 like serine/threonine-protein kinase At3g47570 (86/539), and WRK40_ARATH Probable
254 WRKY transcription factor 40 were responsible for the regulation of genes responsive to biotic
255 and abiotic stresses (79/539). The second largest group (*Figure 5b*) contained 293 genes whose
256 expression increased under field drought conditions. Genes in this cluster were mainly annotated
257 to BAMS_BETPL Beta-amyrin synthase, which catalyzes the formation of the most popular
258 triterpene among higher plants, HDAC6_HUMAN Histone deacetylase 6, HDAC6_HUMAN
259 Histone deacetylase 6, KAP1_ARATH Adenylyl-sulfate kinase 1, chloroplastic, and
260 RAP24_ARATH Ethylene-responsive transcription factor RAP2-4. The third largest group
261 (*Figure 5c*) contained 35 genes whose expression decreased under field drought conditions.
262

263 ***Functional analysis of DEGs between drought and irrigation.***
264 Functional analysis was performed to locate enriched GO terms and KEGG pathways involving
265 the DEGs. As shown in *Table 5*, DEGs were significantly assigned to microtubule
266 (GO:0005874), polymeric cytoskeletal fiber (GO:0099513), and supramolecular complex
267 (GO:0099080) in the cell component (CC) category. In the molecular function (MF) category,
268 DEGs were primarily assigned to microtubules motor activity (GO:0003777), motor activity
269 (GO:0003774), and microtubules binding (GO:0008017). In the biological process (BP)
270 category, DEGs were mainly assigned to microtubules-based movement (GO:0007018) and the
271 movement of cell or subcellular components (GO:0006928). These results demonstrate that
272 DEGs involved in binding, transport, and movement were critical during drought stress.
273

274 KEGG pathway enrichment analysis revealed that DEGs were notably enriched in plant
275 monoterpenoid biosynthesis, flavonoid biosynthesis, diterpenoid biosynthesis, cysteine and
276 methionine metabolism, phenylpropanoid biosynthesis, and carotenoid biosynthesis (*Figure 6,*
277 *Supplementary Table S3*), suggesting specific metabolic events during drought. DEGs were
278 identified using the log2 fold change of the transcript level in field drought compared to the
279 irrigation, and were mapped into the related metabolic pathways (*Table 6*), thereby revealing a
280 significant impact of field drought on secondary metabolism. Field drought modulated the
281 expression of many DEGs that codify for structural enzymes of the monoterpenoid biosynthesis,
282 flavonoid pathway, and phenylpropanoid biosynthesis (*Table 6*); the majority of these genes

283   were downregulated under field drought. Four DEGs including the salutaridine reductase-like
284   (SalR) gene family (gene18404, gene39888, gene39889, and gene10010) and nerolidol synthase
285   1-like (gene237) were involved in monoterpenoid biosynthesis, all of which were downregulated
286   (*Table 7*) in response to drought stress. Drought modulated the expression of the majority of the
287   structural flavonoid genes (*Table 7*), most notably three 3,5-dihydroxybiphenyl synthase-like
288   (gene7767, gene7762, and gene 6358), one leucoanthocyanidin reductase-like isoform X1
289   (gene3879), one BAHD acyltransferase *At5g47980*-like (gene7261), one salutaridinol 7-O-
290   acetyltransferase-like (gene10701), one vinorine synthase-like (gene34704), and 4-
291   hydroxycoumarin synthase 2 (gene7760). All the aforementioned genes were upregulated by
292   drought. The specific expression of 4 DEGs SalR (gene39889), 3,5-dihydroxybiphenyl synthase
293   (gene7767), BAHD acyltransferase (gene7261), and 4-hydroxycoumarin synthase 2 (gene7760)
294   was analyzed by RT-qPCR, and proved consistent with our RNA-seq results of high expression
295   in drought treatment at a relatively stable expression level (*Figure 4*).
296
297   ***Differentially expressed transcription factors under drought stress***
298   Transcription factors (TFs) play key regulatory roles in plant signaling responses, those which
299   activate or inhibit gene expression at the transcriptional level in response to stress. Field-drought
300   treatment led to a number of TFs being differentially expressed (*Figure 7*). In total, 4438
301   differentially expressed TFs were identified, belonging to 30 TF families such as bHLHs (basic
302   helix-loop-helix), NAC (NAM/ATAF/CUC), MYB (v-myb avian myeloblastosis viral oncogene
303   homolog), ERF (ethylene-responsive element binding factor), C2H2s and C3Hs (C2H2 and C3H
304   zinc-finger proteins), WRKYs (WRKY proteins), and bZIPs (basic region-leucine zipper).
305
306   ***Validation of DEG-based gene expression***
307   In order to validate the RNA-Seq gene expression results, qRT-PCR was performed to evaluate
308   the expression levels of the 13 randomly selected DEGs in irrigation vs field-drought conditions
309   *(Table 7)*. As shown in *Figure 4,* the expression of the 13 DEGs was largely identical between
310   RNA-Seq and qRT-PCR in spite of certain differences in the absolute fold change. The verified
311   results from the qRT-PCR demonstrated trends similar to the transcriptomic results, which
312   suggests that these DEGs could play significant roles in the regulation of production
313   performance under field-drought conditions.
314

Discussion
316   Drought is one of the vital factors limiting plant growth and distribution. Understanding the
317   complex mechanisms of drought responses in plants is essential for improving drought tolerance
318   through programmed selection with precise strategies of stress-testing, particularly in light of
319   ongoing global climate change. In the present study, we identified differentially expressed genes
320   under field-drought stress and irrigation control with RNA-Seq in the pear cultivar YuluXiangli.
321   A total of 819 DEGs were detected, and 4438 TFs were differentially expressed between drought
322   and irrigation control. Our findings represent valuable information on transcriptome changes in

323   response to drought. Drought responsive genes are mainly enriched in biosynthesis-related
324   pathways—monoterpenoid biosynthesis, flavonoid biosynthesis, and diterpenoid biosynthesis—
325   and they belong mainly to bHLHs, NAC, MYB, ERF, C2H2s, and C3Hs, as well as to WRKYs
326   transcription factor families. Our analysis provides a solid foundation for both the identification
327   and the functional analysis of potential candidate genes related to drought tolerance.
328
329   The prolonged and severe field drought imposed in this experiment modulated the accumulation
330   of phenylpropanoids, flavonoids, monoterpenoid biosynthesis, and several volatile organic
331   compounds in the pear. Previous studies demonstrated the drought-modulated accumulation of
332   phenylpropanoids, flavonoids, terpenoids, and carotenoids under drought (Li et al., 2018;
333   Murphy and Zerbe, 2020; Savoi et al., 2016; Sircelj et al., 2005). This accumulation acted as
334   antioxidants and protected plants from the adverse effects of drought conditions (Nichols et al.,
335   2015). Our study demonstrated modulation of the biosynthetic pathways of phenylpropanoids
336   and flavonoids by drought stress at the transcript level, leading to an enhanced accumulation of
337   derivatives of benzoic and cinnamic acids as well as several flavonoids. This was congruent with
338   previous results (Li et al., 2018; Murphy and Zerbe, 2020; Nichols et al., 2015; Savoi et al.,
339   2016; Sircelj et al., 2005). Five of 14 phenylpropanoid DEGs as well as all of the flavonoid
340   DEGs were upregulated under drought stress, the result of which enhanced the concentration of
341   accumulation within these compounds. Flavonoid aggregation in cytoplasm is capable of
342   effectively detoxifying drought-induced harmful H_2O_2 molecules. In the present study, the
343   elevated flavonoid aggregation was induced by drought stress condition, supporting previous
344   results in *Achillea pachycephala Rech.f.* (Gharibi et al., 2019), *Brassica napus* (Rezayian et al.,
345   2018), *Arabidopsis* (Nakabayashi et al., 2014), grape (Degu et al., 2015; Savoi et al., 2016), and
346   white clover (Ballizany et al., 2012). The physiological and molecular mechanisms underlying
347   the drought-induced accumulation of these compounds to modulate phenylpropanoid as well as
348   the flavonoid biosynthetic pathway need to be further elucidated by integrated transcriptome and
349   metabolite profiling.
350
351   The monoterpenoid biosynthesis was significantly modulated by the prolonged and severe field
352   drought conditions in the present experiment. Plant terpenes were synthesized in the plastids
353   through the 2C-methyl-D-erythritol-4-phosphate pathway (MEP), and in the cytosol through the
354   mevalonate (MVA) (Tholl, 2006). A number of terpenoid metabolites were involved in
355   adaptation to adverse environments (Pichersky and Raguso, 2018; Murphy and Zerbe, 2020),
356   including biotic and abiotic stresses; however, the knowledge of drought-modulated regulatory
357   mechanism of monoterpene biosynthesis is limited (Zhang et al., 2019). All of the four-terpene
358   synthase (TPS) genes encoding salutaridine reductase (SalR) and nerolidol synthase 1 involved
359   in monoterpene biosynthetic pathway were downregulated under drought conditions.
360   Salutaridine reductase catalyzes the stereo specific reduction of salutaridine to 7(S)-salutaridinol,
361   nerolidol synthase 1 converts geranyl diphosphate (GPP) into S-linalool, and farnesyl
362   diphosphate (FPP) into (3S)-E-nerolidol in the biosynthesis of morphin (Ziegler et al., 2009).
363   Morphine resides within the diverse class of metabolites called benzylisoquinoline alkaloids,

364 and drought stress, it has been noted, can increase alkaloids in opium poppy
365 (*Papaver* soniniferum) (Szabó et al., 2003). Our results were unlike previous findings in several
366 plants (Selmar and Kleinwächter, 2013), those such as *Chrysopogon zizanioides* (Ziegler et al.,
367 2009) and grapevine (Griesser et al., 2015; Savoi et al., 2016). Ziegler et al. (2009) reported
368 upregulation of the gene encoding Salutaridine reductase under drought stress specifically in leaf
369 tissue of *Chrysopogon zizanioides*. Drought-induced monoterpene production was observed in
370 several plants (Selmar and Kleinwächter, 2013) including grapevine leaves (Griesser et al., 2015;
371 Savoi et al., 2016). Six TPS genes, one of which included the nerolidol synthase 1-like gene,
372 were differentially expressed in response to abiotic stresses in *Santalum album* (Zhang et al.,
373 2019). Further biochemical and transcriptomic profiling is needed to address terpenoid
374 biosynthetic pathways and their spatiotemporal regulation in response to adverse drought stress.
375
376 Transcription factors (TFs) modulate diverse transcriptional regulation and play significant
377 regulatory roles in plant signaling responses to developmental and environmental changes (Ulker
378 and Somssich, 2004; Osakabe et al., 2014; Yu et al., 2015). In the present study, 4438
379 differentially expressed TFs were identified to promote or suppress abiotic stress responses,
380 including the bHLHs, NAC, MYB, ERF, C2H2s, C3Hs, and WRKY families. WRKY TFs have
381 been reported to be involved in drought stress responses through the ABA signaling pathway
382 (Ulker and Somssich, 2004; Osakabe et al., 2014). Overexpression of *ZmWRKY58* enhances the
383 drought and salt tolerance in transgenic rice (Cai et al., 2014). Drought-responsive WRKY
384 TFs *TaWRKY33* and *TaWRKY1* confer the transgenic *Arabidopsis* plants drought and/or heat
385 resistance (He et al., 2016). The cotton WRKY TF *GhWRKY33* reduces transgenic *Arabidopsis*
386 resistance to drought stress (Wang et al., 2019). In the present study, a total of 79 WRKY genes
387 induced by field drought treatment were grouped in cluster 1, the majority of which were
388 upregulated. Li et al. (2016) reported 637 transcription factors responsive to dehydration in pear,
389 among which 45 WRKY genes were differentially expressed. Huang et al. (2015) classified a
390 total of 103 WRKY TFs in the pear genome, demonstrating an improvement of tolerance to
391 drought by manipulating the PbWRKYs. Therefore, WRKY TFs may play significant roles in
392 regulating drought stress responses.
393

394 ## Conclusion
395 We utilized deep sequencing technology to investigate the transcriptome profiles in pear leaves,
396 branches, and young fruits in response to the prolonged field drought induced by irrigation
397 withdrawal. A total of 819 DEGs were detected, and 4438 TFs were differentially expressed
398 between drought and irrigation control, presenting valuable information on transcriptome
399 changes in response to drought. We illustrated the flavonoids and monoterpenoid biosynthesis-
400 related genes specifically expressed in drought and irrigation control during field-grown season
401 in pear. Validation of gene expression by 13 randomly selected genes was in correspondence
402 with transcriptomic results. Several candidate genes including flavonoid and terpenoid genes,
403 transcription factors, and drought-responsive elements, were involved in transcriptional

404 regulation of plant response to drought. Such information is important to germplasm
405 management and in endeavoring to improve pear productivity.
406

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414

Author contributions

416 Sheng Yang, Huangping Guo, and Baochun Fu conceived of the project. Sheng Yang and
417 Baochun Fu contributed to the original concept of the project. Mudan Bai and Guowei Hao
418 performed the genome sequencing. Sheng Yang and Mudan Bai performed the genome data
419 analyses. Sheng Yang and Baochun Fu collected samples and performed the phenotyping. Sheng
420 Yang, Huangping Guo, and Mudan Bai analyzed the data and wrote the paper.
421

Conflict of interest
423 The authors declare there are no competing interests.
424

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579
580

Figure 1

Weather conditions at the experimental site and impact of irrigation treatments on fruit weight and physiology

A.Daily rainfall and average temperature during the 2018 pear growth season. B.Single fruit weight. C. Soluble solids content.

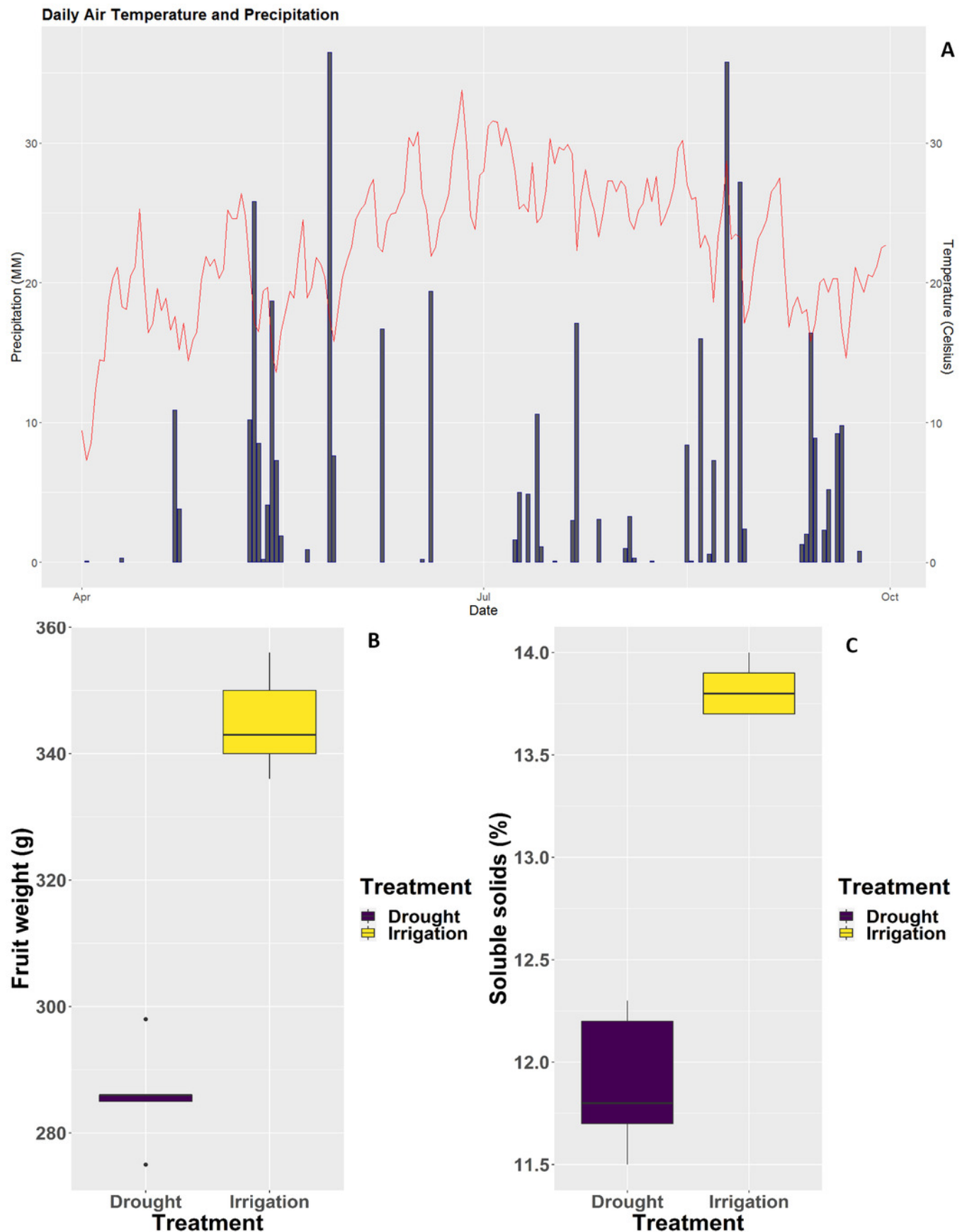



Figure 2

Principal component analysis (PCA) of the pear transcriptome of 10 samples collected from field drought and irrigation pear trees

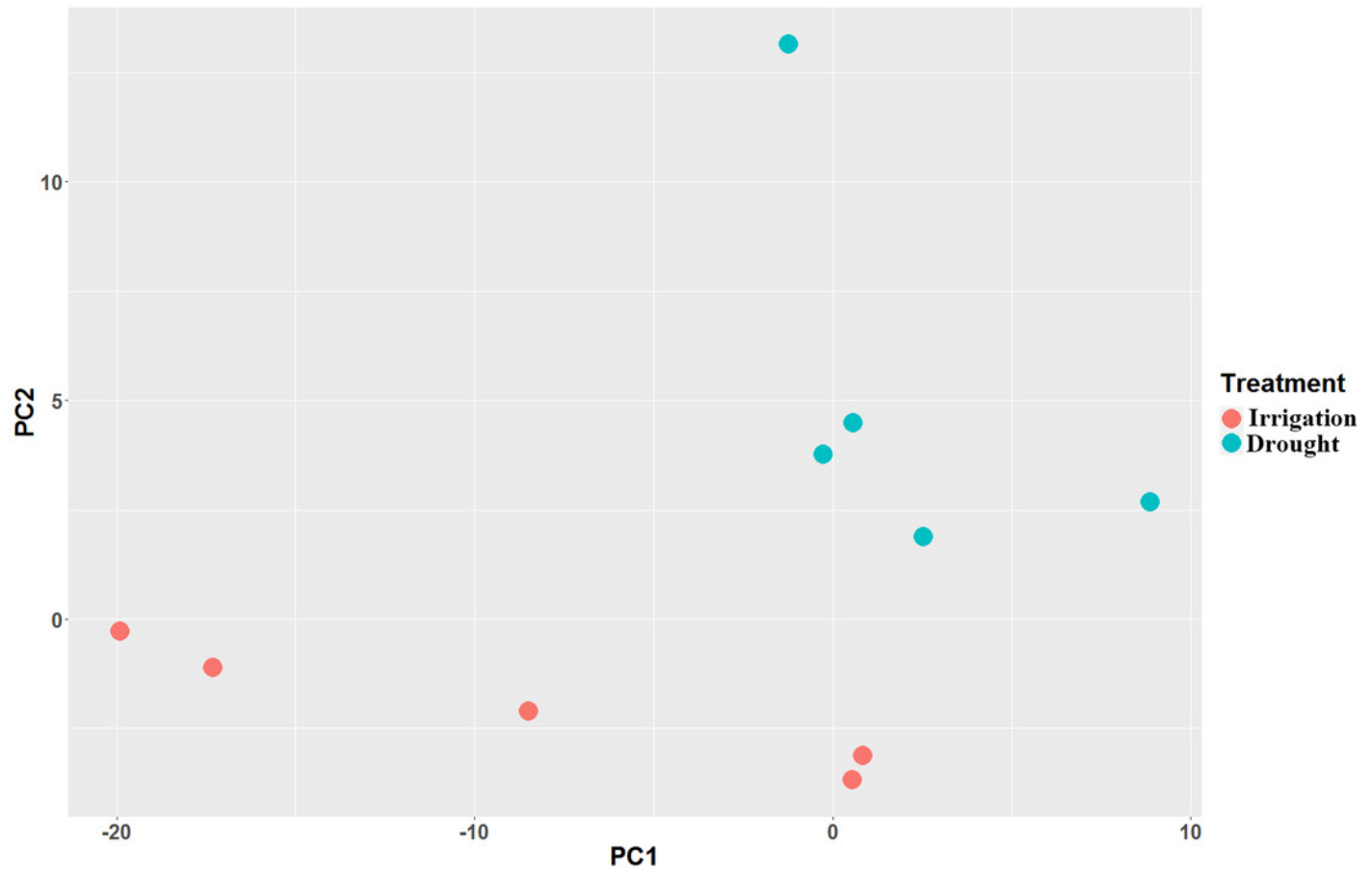



Figure 3

Differential expression analysis

A. Venn diagram of DEGs between drought and irrigation treatment. B. Heat map of the DEG expression levels. C. Highly expressed genes (log2FoldChange < -3.5) exclusively identified in field drought samples. D. Highly expressed genes identified exclusively in irrigation samples.

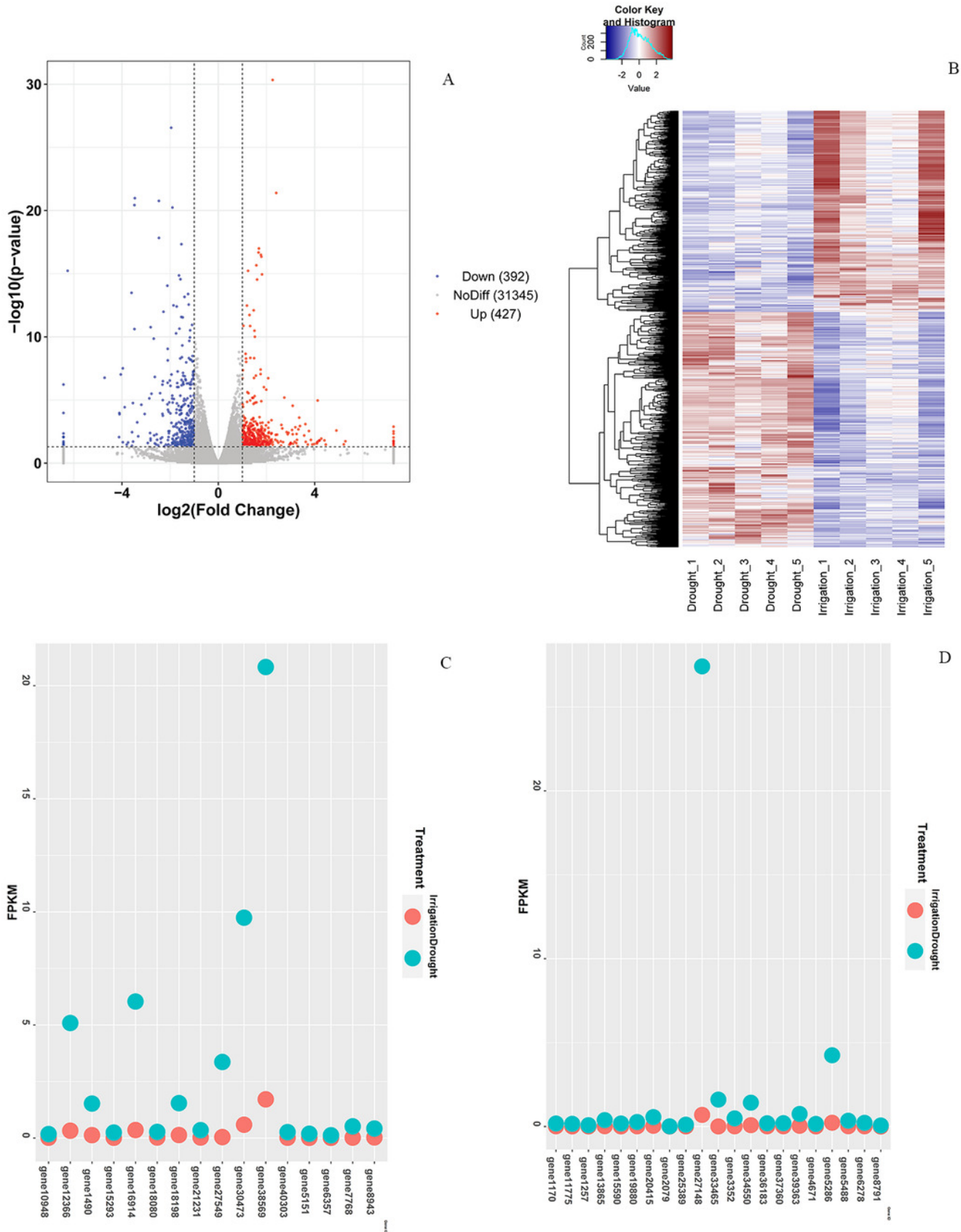



Figure 4

RNAseq and qRT-PCR validation results of differential gene expression under drought and irrigation. The left Y-axis indicates the gene expression levels calculated by the RPKM method. The right Y-axis indicates the relative gene expression levels.

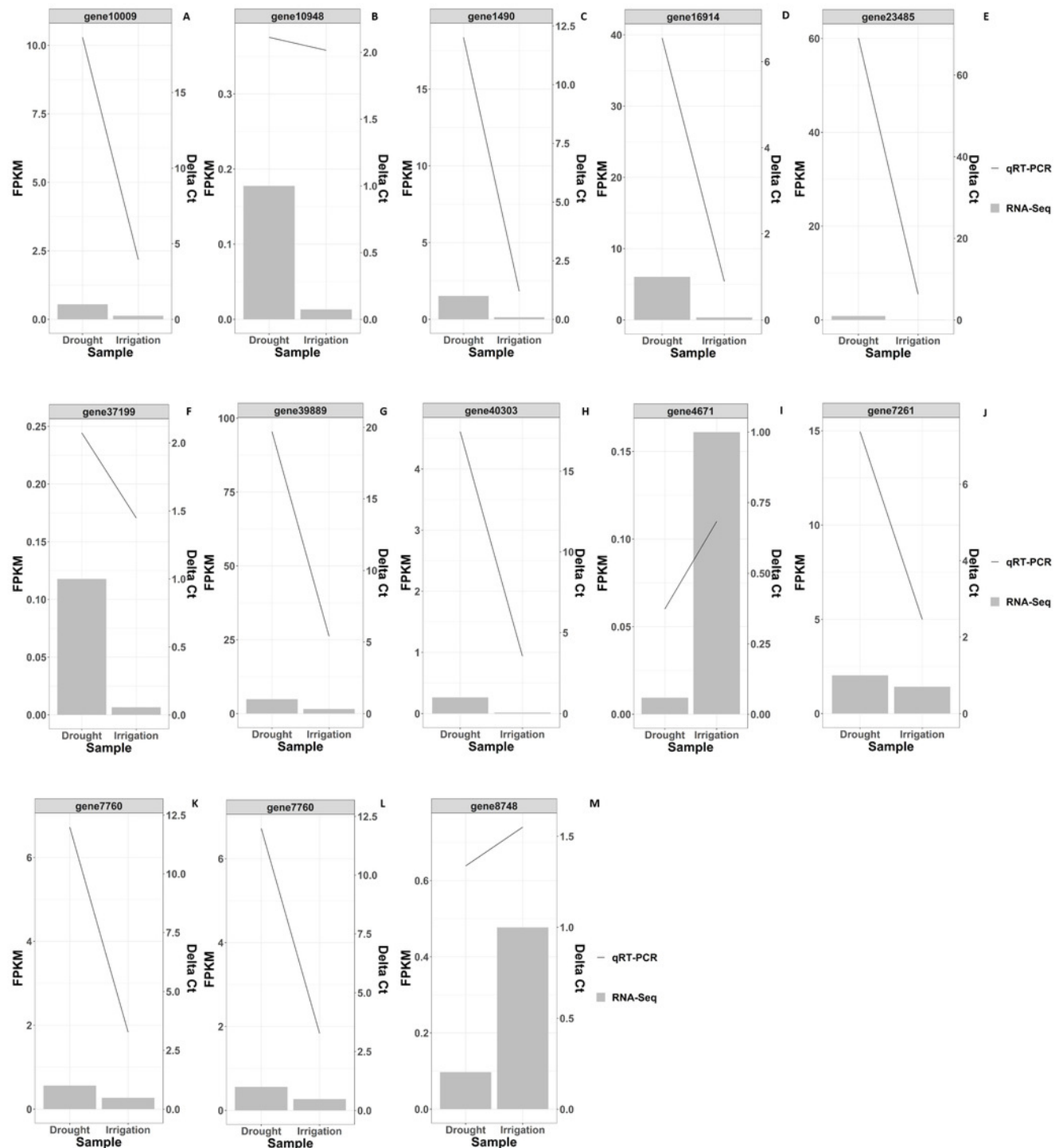
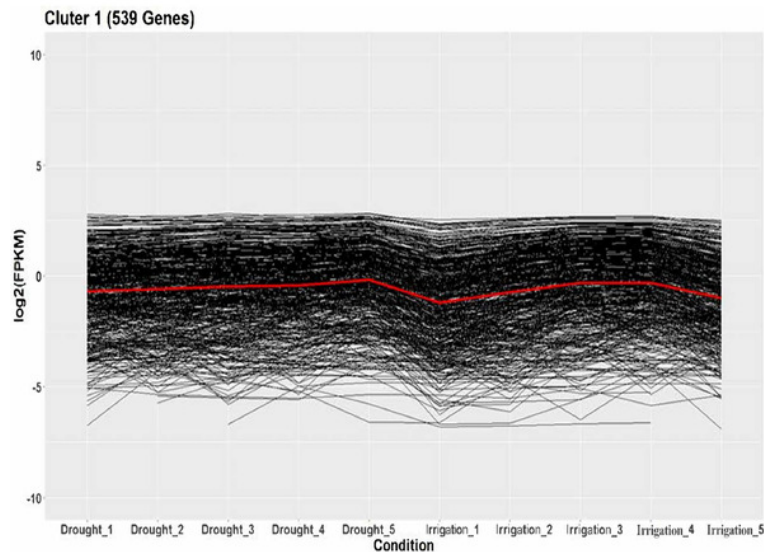
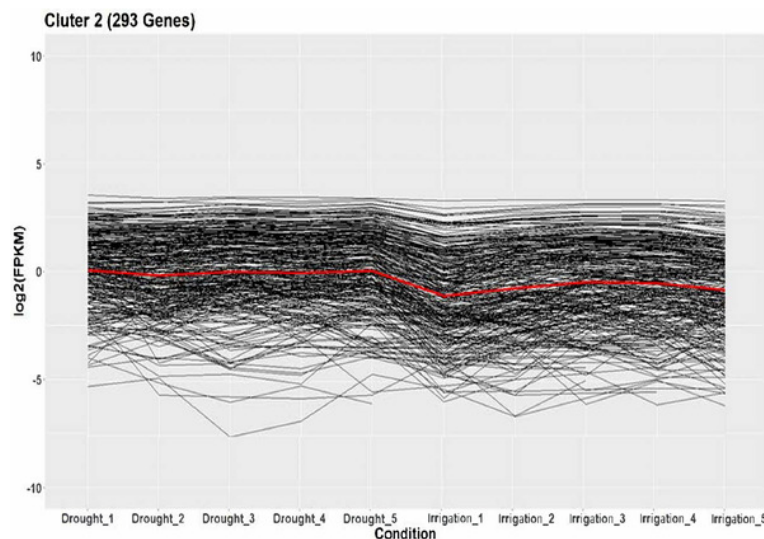



Figure 5

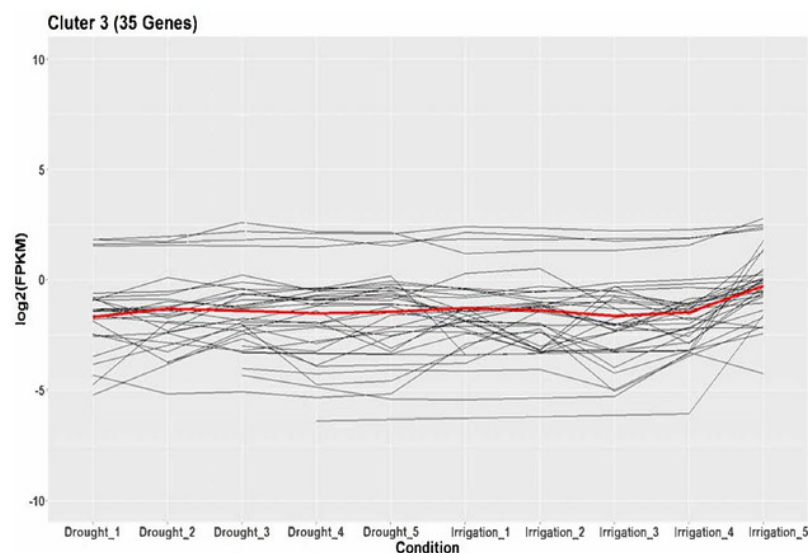
Clustering and gene ontology enrichment of DEGs between drought and irrigation treatment

A

Annotations of top 5 genes in cluster 1

Swissprot	Genes
RLP12_ARATH Receptor-like protein 12 OS=Arabidopsis thaliana GN=RLP12 PE=2 SV=2	149
ZIFL1_ARATH Protein ZINC INDUCED FACILITATOR-LIKE 1 OS=Arabidopsis thaliana GN=ZIFL1 PE=2 SV=1	120
TMVRN_NICGU TMV resistance protein N OS=Nicotiana glutinosa GN=N PE=1 SV=1	90
Y3475_ARATH Probable LRR receptor-like serine/threonine-protein kinase At3g47570 OS=Arabidopsis thaliana GN=At3g47570 PE=1 SV=1	86
WRK40_ARATH Probable WRKY transcription factor 40 OS=Arabidopsis thaliana GN=WRKY40 PE=1 SV=1	79

B

Annotations of top 5 genes in cluster 2

Swissprot	Genes
BAMS_BETPL Beta-amyrin synthase OS=Betula platyphylla GN=OSCBPY PE=1 SV=1	90
HDAC6_HUMAN Histone deacetylase 6 OS=Homo sapiens GN=HDAC6 PE=1 SV=2	55
KCS21_ARATH 3-ketoacyl-CoA synthase 21 OS=Arabidopsis thaliana GN=KCS21 PE=2 SV=1	50
KAP1_ARATH Adenylyl-sulfate kinase 1, chloroplastic OS=Arabidopsis thaliana GN=AKN1 PE=1 SV=1	40
RAP24_ARATH Ethylene-responsive transcription factor RAP2-4 OS=Arabidopsis thaliana GN=RAP2-4 PE=1 SV=1	40

C

Annotations of top 5 genes in cluster 3

Swissprot	Genes
SEC14_SCHPO Sec14 cytosolic factor OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) GN=sec14 PE=4 SV=1	27
AB3C_ARATH ABC transporter C family member 3 OS=Arabidopsis thaliana GN=ABCC3 PE=1 SV=1	20
BAHD1_ARATH BAHD acyltransferase At5g47980 OS=Arabidopsis thaliana GN=BAHD1 PE=2 SV=1	20
BH047_ARATH Transcription factor bHLH47 OS=Arabidopsis thaliana GN=BHLH47 PE=2 SV=1	20
CNBL7_ORYSJ Calcineurin B-like protein 7 OS=Oryza sativa subsp. japonica GN=CBL7 PE=2 SV=1	20

Figure 6

KEGG enrichment pathway analysis of differentially expressed genes between drought and irrigation pear trees

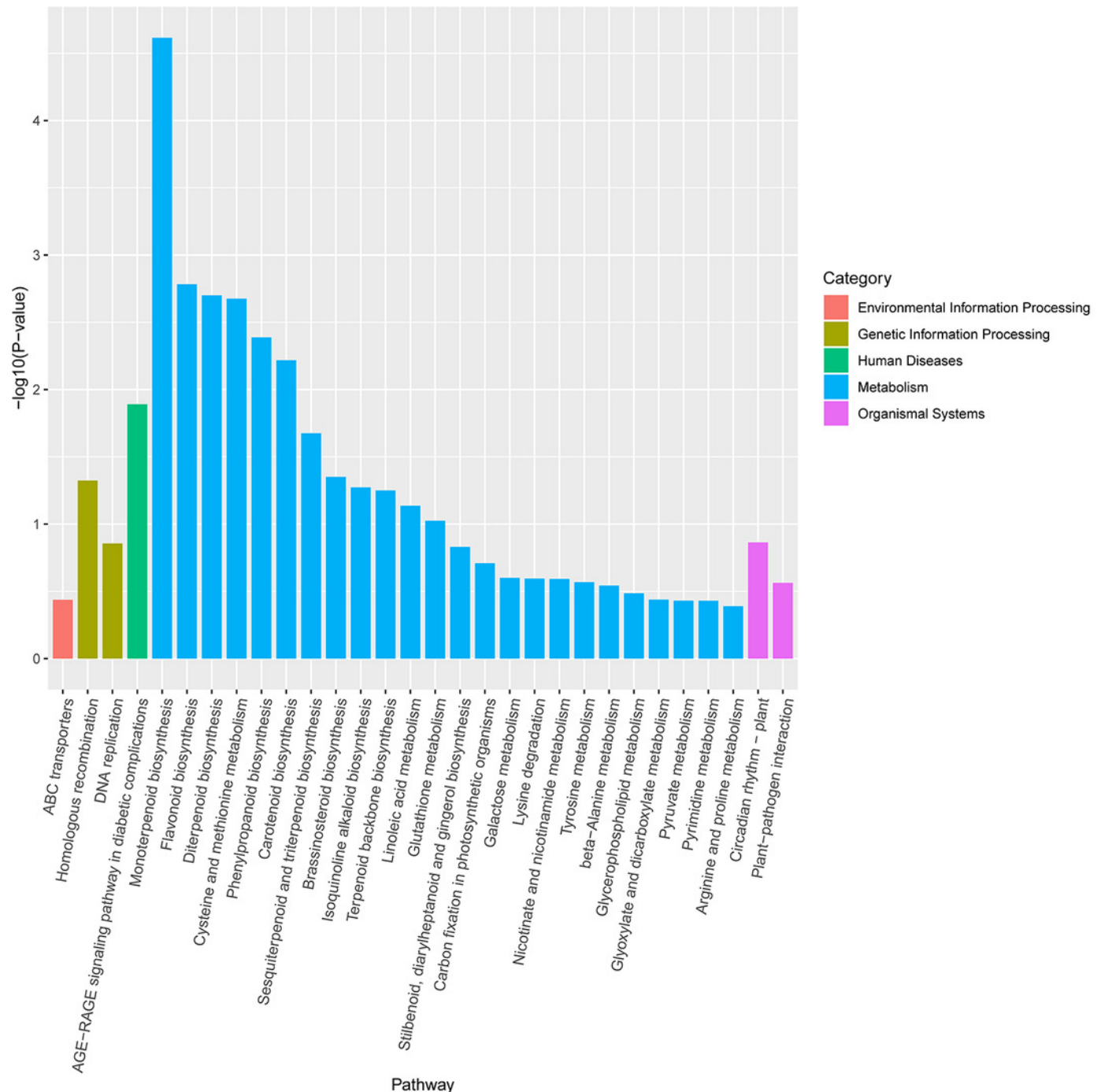
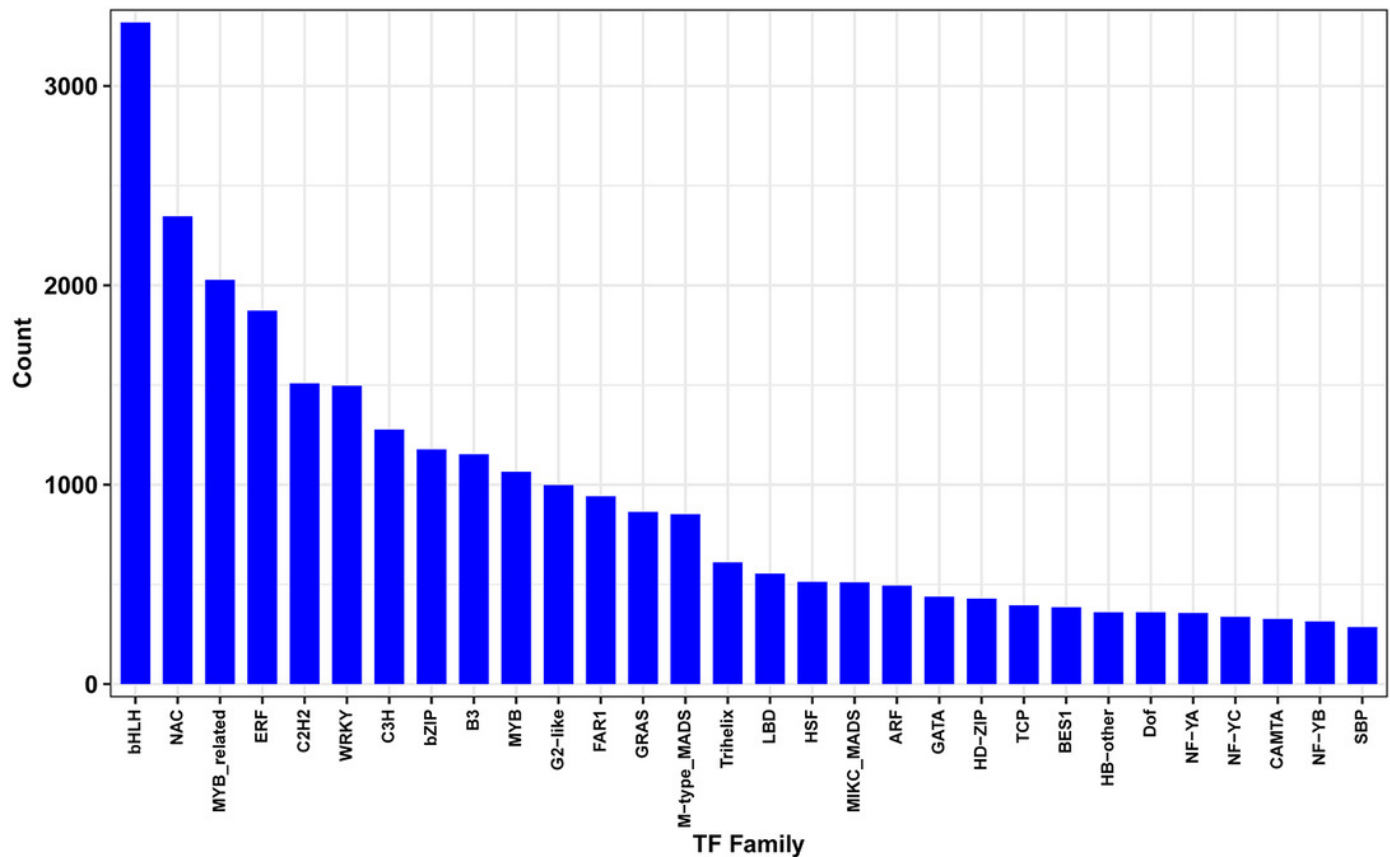



Figure 7

Differentially expressed transcription factors genes between drought and irrigation treatment

The gene IDs and primer sequences for qRT-PCR

1 Table 1. The gene IDs and primer sequences for qRT-PCR.

ID	Primer	5'to 3'
gene40303	gene40303-F	TGGAGGCAGATAGGGTGA
	gene40303-R	CCGTGTAGGAAGCAGTCG
gene10948	gene10948-F	AGCCTTGCTTCTTATTCGTC
	gene10948-R	ATTGCTTGAGTCCTTGCC
gene1490	gene1490-F	GTGCGATTACGAGCAAGAG
	gene1490-R	GAGGGGATGAAGGGTTGT
gene2348	gene2348-F	GAAACCTTCACTGCCAATCT
	gene2348-R	CTCATACCATCA ACCAACGA
gene37199	gene37199-F	GCTTGGGTGGCGTAGTAG
	gene37199-R	TCCTCCGTAATCAGGTTCTC
gene8748	gene8748-F	ATGCGGATGAGCTGTAATG
	gene8748-R	AGAACTTGGCGAGGAAAAC
gene4671	gene4671-F	TGGACAAGAAGAAGGCAAC
	gene4671-R	ATGGAAGTAAATGGCGTGA
gene10009	gene10009-F	GAGATGTGAGGAGGGGAAC
	gene10009-R	ATTCAGCCAGAGAGGCAA
gene7767	gene7767-F	GCTGGTTGCTATGCTGGT
	gene7767-R	TGTCAAGGTGGGTGTCAGT
gene39889	gene39889-F	GAGATGTGAGGAGGGGAAC
	gene39889-R	ATTCAGCCAGAGAGGCAA
gene7760	gene7760-F	TCGTTGGTGGAAATGTTGT
	gene7760-R	CAGTTGTGGTTTTGCCTTC
gene7261	gene7261-F	CGATACAAGAGATGGGGAAG
	gene7261-R	AGTCGGATTCACAGAAGCA
gene16914	gene16914-2F	TTATTCGTTGATTCGGAACTACCA
	gene16914-2R	TCTACCTCCTCCTCCTCCTT

Summary of read numbers based on the RNA-Seq data from field drought and irrigation samples

1   Table 2. Summary of read numbers based on the RNA-Seq data from field drought and irrigation
2   samples.

Sample	Clean_Reads	Total_Mapped	Multiple_Mapped	Uniquely_Mapped
Drought_1	44498956	32352950(72.70%)	3292204(10.18%)	29060746(89.82%)
Drought_2	40138692	29058326(72.39%)	2929579(10.08%)	26128747(89.92%)
Drought_3	40161076	27616611(68.76%)	3020158(10.94%)	24596453(89.06%)
Drought_4	44937140	32560917(72.46%)	3313185(10.18%)	29247732(89.82%)
Drought_5	41366108	29276201(70.77%)	3178099(10.86%)	26098102(89.14%)
Irrigation_1	40866274	29458607(72.09%)	3015133(10.24%)	26443474(89.76%)
Irrigation_2	38421602	28293159(73.64%)	2752309(9.73%)	25540850(90.27%)
Irrigation_3	36898382	26838597(72.74%)	2709184(10.09%)	24129413(89.91%)
Irrigation_4	37725898	26256731(69.60%)	2917913(11.11%)	23338818(88.89%)
Irrigation_5	35740912	25796677(72.18%)	2616670(10.14%)	23180007(89.86%)

Highly expressed genes identified in samples under field drought conditions
(log2FoldChange < -3.5)

1   Table 3. Highly expressed genes identified in samples under field drought conditions (log2FoldChange < -3.5).

Gene_ID	Irrigation_1.fpkm	Irrigation_2.fpkm	Irrigation_3.fpkm	Irrigation_4.fpkm	Irrigation_5.fpkm	Drought_1.fpkm	Drought_2.fpkm	Drought_3.fpkm	Drought_4.fpkm	Drought_5.fpkm	Swissprot	Description
gene10948	0.16	0	0.06	0.26	0.41	0	0	0	0.07	0	LOR6_ARATH Protein LURP-one-related 6 OS=Arabidopsis thaliana GN=At2g05910 PE=2 SV=1	protein LURP-one-related 6-like [Pyrus x bretschneideri]
gene12366	2.59	3.46	6.12	9.01	4.27	0.14	0.22	0.7	0.24	0.32		uncharacterized protein LOC103951864 [Pyrus x bretschneideri]
gene1490	0.52	1.22	1.03	2.73	2.14	0.33	0	0.05	0.16	0.11	WRK40_ARATH Probable WRKY transcription factor 40 OS=Arabidopsis thaliana GN=WRKY40 PE=1 SV=1	WRKY transcription factor 18 [Pyrus x bretschneideri]
gene15293	0.18	0.17	0.39	0.36	0.13	0	0.03	0	0.04	0	ACA12_ARATH Calcium-transporting ATPase 12, plasma membrane-type OS=Arabidopsis thaliana GN=ACA12 PE=2 SV=1	calcium-transporting ATPase 12, plasma membrane-type-like [Pyrus x bretschneideri]
gene16914	2.37	3.21	12.8	6.9	4.92	0.74	0.19	0	0.85	0		protein NIM1-INTERACTING 1 [Pyrus x bretschneideri]
gene18080	0.55	0.22	0.23	0.2	0.17	0.05	0	0.06	0	0	RKD4_ARATH Protein RKD4 OS=Arabidopsis thaliana GN=RKD4 PE=3 SV=1	uncharacterized protein LOC103948099 [Pyrus x bretschneideri]
gene18198	0.42	0.61	3.77	2.83	0.09	0	0	0.45	0.11	0.11	YE04_SCHPO Uncharacterized RNA-binding protein C17H9.04c OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) GN=SPAC17H9.04c PE=1 SV=1	uncharacterized RNA-binding protein C17H9.04c [Pyrus x bretschneideri]
gene21231	0	0.12	0.86	0.31	0.46	0	0	0.12	0	0		uncharacterized protein LOC103961606 [Pyrus x bretschneideri]
gene27549	3.17	3.81	2.73	1.63	5.48	0	0.14	0.04	0.04	0	REXO4_YEAST RNA exonuclease 4 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=REX4 PE=1 SV=1	RNA exonuclease 4 [Pyrus x bretschneideri]
gene30473	4.38	5.38	13.7	9.03	16.2	0	0.13	1.09	0.66	1.09	FRI3_SOYBN Ferritin-3, chloroplastic OS=Glycine max PE=2 SV=1	ferritin-4, chloroplastic-like [Pyrus x bretschneideri]
gene38569	12.6	14.1	22.1	27	28.3	2.01	1.2	3.31	1.66	0.4	WRK40_ARATH Probable WRKY transcription factor 40 OS=Arabidopsis thaliana GN=WRKY40 PE=1 SV=1	probable WRKY transcription factor 40 [Pyrus x bretschneideri]
gene40303	0.31	0.07	0.07	0.25	0.62	0	0	0	0	0.08		uncharacterized protein LOC103940893 [Pyrus x bretschneideri]
gene5151	0.49	0	0.13	0.22	0.12	0	0.06	0	0	0	AHP4_ARATH Histidine-containing phosphotransfer protein 4 OS=Arabidopsis thaliana GN=AHP4 PE=1 SV=2	histidine-containing phosphotransfer protein 4-like [Pyrus x bretschneideri]
gene6357	0.25	0	0.15	0.17	0.05	0	0	0.05	0	0	BIPS2_SORAU 4-hydroxycoumarin synthase 1 OS=Sorbus aucuparia GN=BIS2 PE=1 SV=1	4-hydroxycoumarin synthase 1-like [Pyrus x bretschneideri]
gene7768	0.49	0.45	0.53	0.64	0.5	0	0	0.1	0	0	BIPS2_SORAU 4-hydroxycoumarin synthase 1 OS=Sorbus aucuparia GN=BIS2 PE=1 SV=1	4-hydroxycoumarin synthase 1 [Pyrus x bretschneideri]
gene8943	0.56	0.53	0.25	0.34	0.43	0.05	0.1	0	0	0	RKD4_ARATH Protein RKD4 OS=Arabidopsis thaliana GN=RKD4 PE=3 SV=1	uncharacterized protein LOC103948099 [Pyrus x bretschneideri]

Highly expressed genes identified in samples under irrigation conditions
(log2FoldChange < -3.5)

1 Table 4. Highly expressed genes identified in samples under irrigation conditions (log2FoldChange < -3.5).

Gene_ID	Irrigation_1.fpkm	Irrigation_2.fpkm	Irrigation_3.fpkm	Irrigation_4.fpkm	Irrigation_5.fpkm	Drought_1.fpkm	Drought_2.fpkm	Drought_3.fpkm	Drought_4.fpkm	Drought_5.fpkm	Swissprot	Description
gene1170	0.03	0	0	0	0.04	0.11	0.22	0.24	0.37	0	KPRO_MAIZE Putative receptor protein kinase ZmPK1 OS=Zea mays GN=PK1 PE=2 SV=2	putative receptor protein kinase ZmPK1 [Pyrus x bretschneideri]
gene11775	0.02	0	0	0	0	0.17	0.08	0.03	0.03	0.53	GDL82_ARATH GDSL esterase/lipase At5g45670 OS=Arabidopsis thaliana GN=At5g45670 PE=2 SV=1	GDSL esterase/lipase At5g45670-like [Malus domestica]
gene1257	0.01	0	0	0.03	0	0.19	0.08	0.01	0.02	0.13	NACK1_TOBAC Kinesin-like protein NACK1 OS=Nicotiana tabacum GN=NACK1 PE=1 SV=1	uncharacterized protein LOC103955247 [Pyrus x bretschneideri]
gene13865	0	0	0	0.09	0	0.2	1.05	0.11	0.35	0.23	SNAK2_SOLTU Snakin-2 OS=Solanum tuberosum GN=SN2 PE=1 SV=1	gibberellin-regulated protein 11-like [Pyrus x bretschneideri]
gene15590	0	0	0	0.05	0	0.17	0.06	0.06	0.25	0.38	RADL1_ARATH Protein RADIALIS-like 1 OS=Arabidopsis thaliana GN=RL1 PE=2 SV=1	protein RADIALIS-like 3 [Malus domestica] gi|694378665|ref|XP_009365559.1| PR
gene19880	0	0.03	0	0.05	0	0.44	0.14	0.15	0.19	0.44	IP5P3_ARATH Type I inositol 1,4,5-trisphosphate 5-phosphatase CVP2 OS=Arabidopsis thaliana GN=CVP2 PE=1 SV=2	type I inositol 1,4,5-trisphosphate 5-phosphatase CVP2-like isoform X2 [Pyrus
gene19880	0	0.03	0	0.05	0	0.44	0.14	0.15	0.19	0.44	IP5P3_ARATH Type I inositol 1,4,5-trisphosphate 5-phosphatase CVP2 OS=Arabidopsis thaliana GN=CVP2 PE=1 SV=2	type I inositol 1,4,5-trisphosphate 5-phosphatase CVP2-like isoform X1 [Pyrus
gene20415	0	0	0	0.23	0	0.17	0.53	0.47	0.78	0.88		transcription repressor OFP8-like [Pyrus x bretschneideri]
gene2079	0	0	0	0	0	0.03	0.01	0.01	0	0.02		
gene25389	0.03	0	0	0	0	0.26	0.03	0.03	0	0.26	AB8G_ARATH ABC transporter G family member 8 OS=Arabidopsis thaliana GN=ABCG8 PE=2 SV=1	ABC transporter G family member 4-like [Pyrus x bretschneideri] gi|694405461|
gene27148	2.16	0.19	0.69	0.24	0.19	43.7	6.64	0.15	0.21	86.4	GDL79_ARATH GDSL esterase/lipase At5g33370 OS=Arabidopsis thaliana GN=At5g33370 PE=2 SV=1	GDSL esterase/lipase At5g33370-like [Pyrus x bretschneideri]
gene33465	0	0	0.05	0	0	1.89	0.57	0.41	0.42	4.75	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic OS=Arabidopsis thaliana GN=ADS3 PE=2 SV=2	palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic-like
gene3352	0.04	0	0	0	0.04	1.25	0.47	0.05	0.19	0.48	NAC98_ARATH Protein CUP-SHAPED COTYLEDON 2 OS=Arabidopsis thaliana GN=NAC098 PE=1 SV=1	protein CUP-SHAPED COTYLEDON 2 [Pyrus x bretschneideri]
gene34550	0	0.09	0.23	0	0.09	1.79	0.75	0	0.19	4.39	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic OS=Arabidopsis thaliana GN=ADS3 PE=2 SV=2	palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic-like
gene36183	0	0	0	0.08	0	0.43	0	0.1	0.1	0.35	IQD31_ARATH Protein IQ-DOMAIN 31 OS=Arabidopsis thaliana GN=IQD31 PE=1 SV=1	uncharacterized protein LOC103443739 [Malus domestica] gi|657977866|ref|XP_00
gene37360	0	0.08	0	0	0	0.46	0.32	0.17	0	0.09		uncharacterized protein LOC103937664 [Pyrus x bretschneideri]
gene39363	0.09	0.05	0	0	0.1	0.77	0.1	0.16	0.44	2.3	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic OS=Arabidopsis thaliana GN=ADS3 PE=2 SV=2	palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic-like
gene39363	0.09	0.05	0	0	0.1	0.77	0.1	0.16	0.44	2.3	ADS3_ARATH Palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic OS=Arabidopsis thaliana GN=ADS3 PE=2 SV=2	palmitoyl-monogalactosyldiacylglycerol delta-7 desaturase, chloroplastic-like
gene4671	0	0	0	0.05	0	0.1	0.05	0.11	0.12	0.42		uncharacterized protein LOC103943512 [Pyrus x bretschneideri]
gene5286	0.25	0.09	0.39	0.41	0	6.9	1.95	0.2	0.51	11.7		uncharacterized protein LOC103944059 isoform X1 [Pyrus x bretschneideri]
gene5286	0.25	0.09	0.39	0.41	0	6.9	1.95	0.2	0.51	11.7		uncharacterized protein LOC103944059 isoform X2 [Pyrus x bretschneideri]
gene5488	0.05	0.03	0	0.02	0	0.84	0.11	0.06	0.03	0.72	GRF4_ARATH Growth-regulating factor 4 OS=Arabidopsis thaliana GN=GRF4 PE=2 SV=1	growth-regulating factor 3-like isoform X1 [Pyrus x bretschneideri]
gene6278	0	0.04	0	0	0.04	0.22	0.08	0.12	0.08	0.59	C79D4_LOTJA Isoleucine N-monooxygenase 2 OS=Lotus japonicus GN=CYP79D4 PE=1 SV=1	isoleucine N-monooxygenase 2-like [Pyrus x bretschneideri]
gene8791	0	0	0	0.02	0	0.02	0.1	0.04	0.06	0.11	BGL12_ORYSJ Beta-glucosidase 12 OS=Oryza sativa subsp. japonica GN=BGLU12 PE=2 SV=2	beta-glucosidase 12-like [Pyrus x bretschneideri]

Top 10 GO terms of DEGs for each of the three GO categories in field drought samples compared to irrigation samples.

1   Table 5. Top 10 GO terms of DEGs for each of the three GO categories in field drought samples compared to

2   irrigation samples.

Category	GO.id	Term	Up	Down	DEG	Total	Pvalue	FDR
BP	GO:0007018	microtubule-based movement	14	0	14	44	7.10E-14	4.72E-11
BP	GO:0006928	movement of cell or subcellular component	14	0	14	45	1.00E-13	4.72E-11
BP	GO:0007017	microtubule-based process	14	1	15	118	1.20E-08	1.62E-06
BP	GO:0007349	cellularization	3	0	3	5	7.60E-05	0.003257636
BP	GO:0009558	embryo sac cellularization	3	0	3	5	7.60E-05	0.003257636
BP	GO:0008150	biological_process	111	95	206	9279	0.00012	0.004715
BP	GO:0019748	secondary metabolic process	1	5	6	41	0.00015	0.005440385
BP	GO:0055072	iron ion homeostasis	1	2	3	7	0.00026	0.008756429
BP	GO:0009698	phenylpropanoid metabolic process	1	4	5	31	0.00034	0.009926316
BP	GO:0019318	hexose metabolic process	0	5	5	32	0.00039	0.009926316
MF	GO:0003777	microtubule motor activity	14	0	14	44	3.30E-13	1.04E-10
MF	GO:0003774	motor activity	14	0	14	45	4.70E-13	1.11E-10
MF	GO:0008017	microtubule binding	14	0	14	60	3.60E-11	6.79E-09
MF	GO:0015631	tubulin binding	14	0	14	73	5.90E-10	9.27E-08
MF	GO:0008092	cytoskeletal protein binding	14	1	15	115	3.60E-08	2.68E-06
MF	GO:0019825	oxygen binding	1	2	3	4	4.30E-05	0.00202745
MF	GO:0033815	biphenyl synthase activity	0	3	3	4	4.30E-05	0.00202745
MF	GO:0003824	catalytic activity	93	67	160	5794	0.0001	0.0041
MF	GO:0003674	molecular_function	139	109	248	10119	0.00015	0.005440385
MF	GO:0016787	hydrolase activity	47	18	65	1920	0.00025	0.008731481
CC	GO:0005874	microtubule	11	0	11	70	2.70E-08	2.68E-06
CC	GO:0099513	polymeric cytoskeletal fiber	11	0	11	71	3.20E-08	2.68E-06
CC	GO:0099080	supramolecular complex	11	0	11	72	3.70E-08	2.68E-06
CC	GO:0099081	supramolecular polymer	11	0	11	72	3.70E-08	2.68E-06
CC	GO:0099512	supramolecular fiber	11	0	11	72	3.70E-08	2.68E-06
CC	GO:0015630	microtubule cytoskeleton	11	1	12	107	3.00E-07	1.95E-05
CC	GO:0005576	extracellular region	11	7	18	250	3.10E-07	1.95E-05
CC	GO:0044430	cytoskeletal part	11	1	12	124	1.50E-06	8.84E-05
CC	GO:0048046	apoplast	9	1	10	89	2.90E-06	0.000160865
CC	GO:0005856	cytoskeleton	11	1	12	139	4.90E-06	0.000256706

Top 10 pathways in metabolism related to DEGs in field drought samples compared to irrigation condition

1   Table 6. Top 10 pathways in metabolism related to DEGs in field drought samples compared to irrigation conditions.

Pathway	Level1	Level2	Up	Down	DEG	total_number	Pvalue	FDR
Fatty acid elongation	Metabolism	Lipid metabolism	3	4	7	60	0.000222794	0.015372772
Monoterpenoid biosynthesis	Metabolism	Metabolism of terpenoids and polyketides	0	4	4	21	0.000801546	0.018443153
Sesquiterpenoid and triterpenoid biosynthesis	Metabolism	Metabolism of terpenoids and polyketides	0	5	5	36	0.000801876	0.018443153
Phenylpropanoid biosynthesis	Metabolism	Biosynthesis of other secondary metabolites	5	9	14	303	0.004178352	0.057661261
Carbon fixation in photosynthetic organisms	Metabolism	Energy metabolism	1	6	7	104	0.005694659	0.065488575
Selenocompound metabolism	Metabolism	Metabolism of other amino acids	1	2	3	21	0.008750089	0.077724824
Cutin, suberine and wax biosynthesis	Metabolism	Lipid metabolism	3	1	4	40	0.009011574	0.077724824
Flavonoid biosynthesis	Metabolism	Biosynthesis of other secondary metabolites	0	5	5	75	0.01920126	0.132488694
Cysteine and methionine metabolism	Metabolism	Amino acid metabolism	5	2	7	154	0.040390415	0.232244887
Cyanoamino acid metabolism	Metabolism	Metabolism of other amino acids	4	1	5	118	0.09692841	0.514466178

Effects of drought on monoterpenoid pathway and flavonoid biosynthesis

1   Table 7. Effects of drought on monoterpenoid pathway and flavonoid biosynthesis.

Pathway	Gene id	foldChange	log2FoldChange	Gene Prediction
Monoterpenoid biosynthesis	gene18404	0.645958365	-0.630486914	Salutaridine reductase-like [Pyrus × bretschneideri]
Monoterpenoid biosynthesis	gene39888	0.423999841	-1.23786437	Salutaridine reductase-like [Pyrus × bretschneideri]
Monoterpenoid biosynthesis	gene39889	0.318598399	-1.650189078	Salutaridine reductase-like [Pyrus × bretschneideri]
Monoterpenoid biosynthesis	gene10009	0.233266788	-2.099947181	Salutaridine reductase-like isoform X1 [Pyrus × bretschneideri]
Monoterpenoid biosynthesis	gene10010	0.265228317	-1.914693282	Salutaridine reductase-like [Pyrus × bretschneideri]
Monoterpenoid biosynthesis	gene237	0.931868371	-0.101801911	(3S,6E)-nerolidol synthase 1-like [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene7767	-1.533981312	2.66172E-12	3,5-dihydroxybiphenyl synthase-like [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene7762	-0.49454945	0.307982886	3,5-dihydroxybiphenyl synthase-like [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene3879	-Inf	1	Leucoanthocyanidin reductase-like isoform X1 [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene7261	-0.510002646	0.062753961	BAHD acyltransferase At5g47980-like [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene10701	-0.035249252	0.880552832	Salutaridinol 7-O-acetyltransferase-like [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene34704	0.004359729	0.96585065	Vinorine synthase-like [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene6358	-Inf	0.000106527	3,5-dihydroxybiphenyl synthase-like [Pyrus × bretschneideri]
Flavonoid biosynthesis	gene7760	-1.047453808	0.04585677	4-hydroxycoumarin synthase 2 [Pyrus × bretschneideri]