

1 **Insights into grapevine defense response against drought as revealed by biochemical,**  
2 **physiological and RNA-Seq analysis**

3 Muhammad Salman Haider, Cheng Zhang, Mahantesh M. Kurjogi Tariq Pervaiz, Ting Zheng,  
4 Chao bo Zhang, Chen Lide, Lingfie Shangguan, Jinggui Fang\*

5 College of Horticulture, Nanjing Agricultural University, Tongwei Road 6, Nanjing 210095, PR.  
6 China

7 \*Corresponding author

8 Jinggui Fang

9 Email: fanggg@njau.edu.cn

10 **Abstract**

11 Grapevine is economically important and widely cultivated fruit crop, which is seriously  
12 hampered by drought worldwide. It is necessary to understand the impact of glitches incurred by  
13 the drought on grapevine genetic resources. Therefore, in the present study RNA-sequencing  
14 analysis was performed using cDNA libraries constructed from both drought-stress and control  
15 plants. Results yielded, a total of 12,451 differentially expressed genes (DEGs) out of which  
16 8,022 genes were up-regulated and 4,430 were down-regulated. Further physiological and  
17 biochemical analyses were carried out to validate the various biological processes involved in the  
18 development of grapevine in response to drought stress. Results also showed that decrease in rate  
19 of stomatal conductance in-turn decrease the photosynthetic activity and CO<sub>2</sub> assimilation rate in  
20 the grapevine leaves and most ROS detoxification systems, including stress enzymes, stress-  
21 related proteins and secondary metabolites were strongly induced. Moreover, various hormones  
22 were known to be induced in the present study in response to drought. Overall the present study  
23 concludes that these DEGs play both positive and negative role in drought tolerance by  
24 regulating different biological pathways of grapevine. However our findings have provided  
25 valuable gene information for future studies of abiotic stress in grapevine and other fruit crops.

26 **Keywords:** Transcriptome, Drought-stress, Grapevine, Chlorophyll, Secondary metabolites,

27 **Introduction**

28 Grapevine (*Vitis vinifera* L.) is an economically important crop, having 7.8 million hectares of  
29 cultivated land with an annual production of 67.6 million tons worldwide <sup>1</sup>. The climate change  
30 pattern has influential effects on the survival and productivity of grapevine. Thus, growth of  
31 grapevine is consequently affected by abiotic stress, such as drought, salinity, etc. Drought has  
32 deleterious effects on grapevine cultivation worldwide <sup>2,3</sup>. Globally, 45% of the agricultural  
33 lands are under constant/ periodic water shortages <sup>4</sup>, finally resulting in nearly 50% of yield  
34 losses. Plants as being sessile organism are capable of making adaptive changes in physiology  
35 and morphology that allow them to tolerate environmental stress but these adaptations are  
36 inadequate to restore physiological water potential in the cell <sup>5</sup>. Plant response to these limited  
37 water conditions is mediated by expression of numerous genes encoding stress-related proteins,

38 enzymes and metabolites functioning in the various pathways of cell metabolism<sup>6</sup>. The genes  
39 induced under osmotic stress in plants are categorized into two groups such as, functional  
40 proteins and regulatory proteins<sup>7,8</sup>.

41 Water scarcity is not only threat for viticulture productivity, but also for wine quality<sup>9,10</sup>.  
42 Schultz proposed that an increase in environmental temperature due to rise in atmospheric CO<sub>2</sub>,  
43 is primary cause of water shortages for viticulture<sup>11</sup>. Grapevine possess the unique molecular  
44 machinery which adjusts the flow of water to leaf and then to the atmosphere by vessel anatomy  
45<sup>12</sup>, stomatal conductance<sup>13</sup> and aquaporin<sup>14</sup>. Consequently, the slow leaf and shoot growth,  
46 elongation of tendrils, inhibition of internodes extension, leaf enlargement, decline of an average  
47 diameter of xylem vessels and a minor stimulation in root growth under drought is observed in  
48 grapevine<sup>12</sup>.

49 RNA-seq is a novel technique that implicates deep-sequencing technology to achieve  
50 transcriptomic profiling of both model and non-model plants. This approach enables researchers  
51 to perceive novel genes in a single assay, allowing the detection of transcript information, allele-  
52 specific gene expression and single nucleotide variants without the availability of ESTs and gene  
53 annotations. Moreover, transcriptome data have also been used in characterizing large-scale  
54 genes governing the complex interaction and metabolic processes of plant under stress<sup>15</sup>. In  
55 addition, one step PCR enables researchers to predict the corresponding phenotype by detecting  
56 the expression of genes before recording the morphological changes in plant. Thus, advantage of  
57 this technique should be exploited in crop production, especially in countries with adverse  
58 environmental conditions. we have successfully implicated this technique in our previous study  
59 of different fertilization trials in grapevine<sup>16</sup>. However, the drought-regulated stress-response in  
60 grapevine has not been studied in detail so far. Therefore, the aim of this study is to elucidate the  
61 physiological responses of grapevine to drought stress, and further identify the DEGs in various  
62 biological pathways. These results also provide the defense-related gene information, which can  
63 be used for the development of drought-resistant grapevine cultivars.

## 64 **Results**

65 The sequence data obtained from the Illumina deep-sequencing was submitted to Short  
66 Read Archive (SRA) database at NCBI under accession number SAMN04914490. After  
67 filtering, raw data yielded 42.47 and 53.05 million clean reads in control and drought-stressed  
68 leaf samples, respectively. The sequence alignment (soap2/SOAPaligner;  
69 <http://soap.genomics.org.cn>) to the grapevine reference genome, allowed two base mismatches.  
70 The total mapped reads (73.44%) were corresponding to unique (72.01%) and multiple (1.44%)  
71 genomic positions (Supplementary: Table S1).

72 In current study, the sum of 12,451 DEGs was expressed under drought stress ( $|\log_2\text{Ratio}| \geq$   
73 1) and false discovery rate ( $\text{FDR} \leq 0.001$ ); whereas, 8,022 (64.43%) were up-regulated and 4430  
74 (35.57%) were down-regulated (Supplementary: Table S2).

## 75 **Gene ontology (GO) and KEGG analysis of differentially-expressed genes**

76 GO term mainly includes cellular components, molecular function and biological process.  
77 A sum of 12,451 (72.11%) transcripts were annotated and classified into 51 functional groups,  
78 including 21 in biological process, 16 in cellular component and 14 in molecular functions

79 (Supplementary: Table S3; Figure. S1). Under the biological process, out of 5994 transcripts,  
80 the predominant transcripts found to be in metabolic process which includes 4537 transcripts  
81 (75.7%; GO: 0008152), followed by cellular process consist of 3632 (60.6%; GO: 0009987) and  
82 single-organism process involves 3185 transcripts (53.1%; GO: 0044699). Whereas, in cellular  
83 component, out of 3940 reads the highest prevalence of transcripts were recorded in cell and cell  
84 part with 3247 (82.4%; GO: 0005623 and GO: 0044464, respectively) transcripts, followed by  
85 organelle” with 2358 (59.8% GO: 0043226) transcripts. Further 4291 and 3497 transcripts were  
86 observed with the catalytic (71.3% GO: 0003824) and binding (58.1% GO: 0005488) activity  
87 respectively in molecular function.

88 Several DEGs from the current study were subjected to KEGG annotation for further  
89 characterization of transcripts, where 12,451 transcripts were allotted to 306 KEGG pathways.  
90 The study revealed that the highest numbers of transcripts (2126) were involved in the metabolic  
91 pathway (1257 up-regulated, 167 down-regulated), followed by biosynthesis of secondary  
92 metabolites (out of 1160 transcripts; 681 were up-regulated, 479 were down-regulated), then 756  
93 transcripts were recorded in plant-pathogen interaction pathway (241 up-regulated, 241 down-  
94 regulated), while lowest transcripts (21) were recorded in sesquiterpenoid and triterpenoid  
95 biosynthesis pathway in which 14 transcripts were up-regulated and 7 transcripts were down-  
96 regulated (Supplementary: Table S4).

### 97 **Chlorophyll degradation and photosynthetic competences under drought stress**

98 The results of chlorophyll estimation unveil that 34.88% decrease of chl-a content in drought  
99 treated grapevine leaf ( $0.28 \pm 0.06 \text{ mg g}^{-1}$ ) when compared with that of control plant leaf ( $0.43 \pm$   
100  $0.11 \text{ mg g}^{-1}$ ). Similarly 21.92% decrease in chlb of leaf exposed to drought stress ( $0.57 \pm 0.04 \text{ mg}$   
101  $\text{g}^{-1}$ ) compared to control leaf ( $0.73 \pm 0.06 \text{ mg g}^{-1}$ ). In the same way, photosynthesis rate was also  
102 decreased by 32.20% in drought treatment ( $16.08 \pm 0.75 \mu\text{mole m}^{-2} \text{ sec}^{-1}$ ) when compared to  
103 control ( $23.67 \pm 0.81 \mu\text{mole m}^{-2} \text{ sec}^{-1}$ ). Moreover, stomatal conductance and  $\text{CO}_2$  assimilation  
104 rate also showed significant reduction by 40.00% ( $0.11 \pm 0.04$ ) and 44.44% ( $5 \pm 0.03$ ) in drought  
105 treated grapevine leaves compared to that of control (Table 1).

106 In the grapevine transcriptome, 29 DEGs involving in chlorophyll metabolic pathway responded  
107 differently to drought stress compared with control, of which 18 transcripts were up-regulated  
108 and 11 transcripts were down-regulated. However, out of 25 transcripts functioning in chl  
109 synthesis and degradation, 9 transcripts involved in chla synthesis (Glutamate tRNA Ligase;  
110 Radical S-adenosyl methionine domain-containing protein1; Protoporphyrinogen oxidase;  
111 Dehydrogenase/reductase SDR family member; Protochlorophyllide oxidoreductase, and four  
112 transcripts of Short chain dehydrogenase, TIC32) were significantly up-regulated; whereas, 7  
113 transcripts (2 transcripts of HemaA, Glutamate tRNA reductase 1; Proporphynogen oxidase 1; 2  
114 transcripts of CHLH, Magnesium chelatase H subunit; Protochlorophyllide oxidoreductase and  
115 Short chain dehydrogenase) were significantly down-regulated. Meanwhile, in the chl cycling  
116 process Chlorophyllide a oxygenase, (CAO) was significantly down-regulated, but Chlorophyll  
117 (ide) b reductase NYC1 (CBR) was up-regulated by the drought treatment. Whereas,  
118 Chlorophyllase-II, Pheophorbide a oxygenase, and Protochlorophyllide-dependent translocon  
119 component 52, were significantly up-regulated and 3 transcripts of Chlorophyllase-I, were  
120 down-regulated during the chl degradation process (Table 2, Figure, 1, ). The expression level of  
121 VIT\_08s0007g08540.t01 (307.93-106.65 RPKM) and VIT\_19s0014g03160.t01 (1360.37-307.58  
122 RPKM) revealed high profusion in chla synthesis pathway. Moreover, in the phytochromobilin

123 synthesis, the expression of Ferrochelatase-2 (VIT\_07s0031g03200.t01,  $|\log_2FC| = 3.032$ ), Heme  
124 oxygenase 1 (VIT\_11s0016g05300.t01,  $|\log_2FC| = 2.403$ ), Heme oxygenase 2  
125 (VIT\_18s0001g11040.t01,  $|\log_2FC| = 2.249$ ) and Phytychromobilin:ferredoxin oxidoreductase  
126 (VIT\_06s0009g03770.t01,  $|\log_2FC| = 1.705$ ) was also induced by the drought stress  
127 (Supplementary: Table S5).

128 In grapevine transcriptome, a sum of 23 DEGs related to photosynthesis pathway, including PSII  
129 (5), PSI (2), cytochrome b6-f complex (4), photosynthetic electron transport (4), F-type ATPase  
130 (4), photosynthesis-antenna proteins (4) were recorded sensitive to drought stress. In PSII (5  
131 DEGs), which includes psbB (2), psbCs (2) and psbW (1) and all 5 DEGs were found to be  
132 significantly down-regulated. psbC (VIT\_00s0396g00010.t01, 280.56 - 1.32 RPKM) possessed  
133 the high expression abundance. Moreover, two psaBs in PSI and two transcripts related to  
134 cytochrome b6-f complex (petA and petC) revealed significant reduction in their expression  
135 levels, perhaps two transcripts of petC were found to be increased with control group. Similarly,  
136 4 genes involved in the photosynthetic electron transport unveiled that, two transcripts of petF  
137 (VIT\_12s0035g00270.t01 and VIT\_06s0080g00410.t01) were down-regulated and two  
138 transcripts of petH (VIT\_04s0023g03510.t01 and VIT\_10s0003g04880.t01) were up-regulated  
139 when compared with control. In addition, the F-type ATPase-related genes (ATPF1B, ATPF1A,  
140 ATPF1G and ATPF0C) and photosynthesis-antenna proteins-related genes (LHCB1, LHCB2,  
141 LHCB3 and LHCB6) were found to be significantly down-regulated in drought treated leaves  
142 (Table 2, Supplementary: Table S6).

#### 143 **ROS system under drought stress**

144 The Malondialdehyde activity was increased significantly (60.93%) in drought treatment ( $8.61 \pm$   
145  $0.25 \text{ nmol g}^{-1}$ ) compared to control ( $5.35 \pm 0.21 \text{ nmol g}^{-1}$ ). A significant increase was observed in  
146 the activity of superoxide dismutase (75.16%), peroxidase (140.81%) and catalase (200.79%) in  
147 drought responsive grapevine leaves in comparison with control (Table 1). In transcriptomic  
148 analysis, one NADPH respiratory oxidase and five amine oxidases functioning in the ROS  
149 synthesis process were significantly up-regulated in drought treated grapevine leaf samples. In  
150 ROS scavenging system, 60 DEGs were identified that were categorized into Fe superoxide  
151 dismutase (2 transcripts), peroxidase (6 transcripts), catalase (3 transcripts), glutathione-  
152 ascarbate cycle (9 transcripts), glutathione peroxidase (1 transcript), glutathione S-transferase  
153 (26 transcripts), peroxiredoxin/thioredxin pathway (8 transcripts), alternative oxidases (3  
154 transcripts) and polyphenol oxidase (2 transcripts) (Figure 2; Supplementary: Table S7).

155 In our findings, two Fe-SODs were up-regulated, but both genes showed low expression  
156 abundance. In contrast, 2 Cu/Zn-SOD were significantly down-regulated ( $|\log_2FC| < 1$ ), CAT (3  
157 transcripts) and POD (2 transcripts) were significantly up-regulated. All three up-regulated CAT  
158 transcripts (VIT\_18s0122g01320.t01, from 2888.01 to 358.79 RPKM; VIT\_00s0698g00010.t01,  
159 from 428.21 to 106.90 RPKM; VIT\_04s0044g00020.t01, from 767.21 to 263.73 RPKM) showed  
160 high expression abundance; whereas, all up/down-regulated POD genes showed moderate to low  
161 expression abundance. Furthermore, 9 GSH-AsA (5 up-regulated, 4 down-regulated), 27 GPX-  
162 pathway (23 up-regulated, 4 down-regulated), eight Prx/Trx (5 up-regulated, 3 down-regulated),  
163 three AOX (2 up-regulated, 1 down-regulated) and two PPO (down-regulated) genes were  
164 identified in response to drought stress, (Table 3. Supplementary: Table S7).

#### 165 **Plant hormone signal transduction pathway under drought stress**

166 The hormonal level, including auxin was increased in drought treatment ( $1.626 \pm 0.03 \text{ ng g}^{-1}$   
167 FW) compared to control ( $1.373 \pm 0.02 \text{ ng g}^{-1}$  FW). Similar trend was observed in abscisic acid  
168 that is  $0.908 \pm 0.01$ , and  $0.257 \pm 0.01 \text{ ng g}^{-1}$  FW for drought and control treatments, respectively.  
169 In the same way jasmonic acid in drought treatment sample was  $1.67 \pm 0.05 \text{ ng g}^{-1}$  FW, whereas,  
170 in control it was found to be  $1.451 \pm 0.03 \text{ ng g}^{-1}$  FW. Further gibberellic acid (GA) in treated and  
171 control sample was recorded to be  $1.671 \pm 0.02$ , and  $1.53 \pm 0.02 \text{ ng g}^{-1}$  FW, respectively. Alike  
172 brassinosteroid also showed  $1.091 \pm 0.01$ , and  $1.073 \pm 0.01 \text{ ng g}^{-1}$  FW for drought and control  
173 treatment samples, respectively (figure 3). In grapevine transcriptome, several DEGs related to  
174 AUX, GA, ABA, JA, ET (ethylene), and BR were found in signal transduction pathways in  
175 drought stressed grapevine leaves. Under AUX signaling, three genes (down-regulated) related  
176 to auxin transport, eleven auxin response factors (7 up-regulated and 4 down-regulated) involved  
177 in the transcriptional repressors were detected. Moreover, fifteen genes in auxin induced and  
178 responsive proteins (2 up-regulated and 13 down-regulated), six IAA synthetase (GH3; 1 up-  
179 regulated and 5 down-regulated) and seventeen genes related to auxin and IAA induced proteins  
180 (SAUR; 5 up-regulated and 12 down-regulated) were perceived in grapevine under drought  
181 stress . Two natural receptors were up-regulated while four DELLA proteins were down-  
182 regulated in the GA under drought stress. Moreover, three ABA responsive proteins (down-  
183 regulated), two SNF1-related protein kinases 2 (SnRK2; up and down-regulated), three PP2C  
184 group (up-regulated) genes and six transcription factors (ABF, up-regulated) were involved in  
185 abscisic acid pathway. Six transcripts of jasmonate-ZIM-domain proteins (one up-regulated and  
186 5 down-regulated) and single jasmonoyl isoleucine conjugate synthase 1 (up-regulated) were  
187 found in JA hormonal signaling. Moreover, 12-oxophytodienoate reductase 2-like (up-regulated),  
188 linoleate 13S-lipoxygenase 2-1 (up-regulated) and allene oxide synthase (down-regulated) were  
189 identified in JA pathway under drought stress. Three ethylene-responsive transcriptional factors  
190 (3 up-regulated) being crucial to ET, five ethylene response factor (down-regulated) and three  
191 ACC oxidases (up-regulated) were perceived grapevine leaf tissue responding drought stress. In  
192 BRs, two transcripts related to BRASSINOSTEROID INSENSITIVE1 (up-regulated), ten  
193 (down-regulated) brassinosteroid-regulated proteins (BRU1) and 9 (down-regulated) D-type  
194 cyclins were functioning in plant hormone signal transduction pathway under drought stress  
195 conditions (Supplementary: Table S8).

## 196 **Proline metabolism under drought stress**

197 The proline level showed significant increase in grapevine leaves responding to drought stress  
198 ( $1.711 \pm 0.05 \text{ ng g}^{-1}$  FW) as compared with control plant leaves ( $1.624 \pm 0.04 \text{ ng g}^{-1}$  FW; Table  
199 1). In transcriptomic analysis, a total of 18 DEGs, including pyrroline-5-carboxylate synthetase ,  
200 proline dehydrogenase, Proline methyltransferase  $\alpha$ -Glutamyl kinase, Glutamic- $\alpha$ -semialdehyde  
201 dehydrogenase, Pyrroline-5-carboxylate dehydrogenase, Prolyl hydroxylase (4 transcripts),  
202 Acetyl-CoA: glutamate N-acetyl transferase 2 transcripts), N-Acetylglutamate kinase, Acetyl  
203 glutamic- $\alpha$ -semialdehyde dehydrogenase, Acetyl ornithine aminotransferase, Acetyl ornithine  
204 deacetylase (2 transcripts), Arginino succinate lyase (ASL) and Arginase were significantly up-  
205 regulated functioning in the proline synthesis and metabolism pathway in drought treatment  
206 compared to control (Figure, 4, Supplementary: Table S9).

## 207 **Biosynthesis of secondary metabolites under drought stress**

208 In transcriptomic study, 73 secondary metabolites related genes linked with shikimate acid (9),  
209 alkaloid (2), anthocyanin (33), lignin (21) and terpenoid (8) were recognized under drought  
210 treated grapevine leaves.

211 Shikimate acid (SA) pathway possessed one up-regulated 3-deoxy-D-arabino-heptulosonate-7-  
212 phosphate synthase 03 , two down-regulated 3-dehydroquinate dehydratase/shikimate  
213 dehydrogenase , one down-regulated shikimate kinase one up-regulated chorismate synthase 1 ,  
214 two down-regulated anthranilate phosphoribosyltransferase (AnPRT) and both up-regulated  
215 indole-3-glycerol phosphate synthase (IGPS) and tryptophan synthase beta chain 1 (TS1),  
216 respectively All SA genes have moderate transcript abundance.

217 In alkaloid biosynthetic pathway, genes related to strictosidine synthase 3 and D-amino-acid  
218 transaminase were down-regulated . Out of 33 genes in anthocyanin biosynthesis, 8 genes  
219 related phenylalanine ammonia-lyase (4-up-regulated and 4-down-regulated), one trans-  
220 cinnamate 4-monooxygenase (down-regulated), two 4-coumarate--CoA ligase-like 9 (up and  
221 down-regulated), 13 stilbene synthase (6 up-regulated and 7 down-regulated), 3 flavonol  
222 synthase/flavanone 3-hydroxylase (one up-regulated and 2 down-regulated), one 1-  
223 aminocyclopropane-1-carboxylate oxidase 5 (down-regulated), two dihydroflavonol-4-reductase  
224 (down-regulated), one anthocyanidin reductase (up-regulated) and one anthocyanidin 3-O-  
225 glucosyltransferase 2 (down-regulated) were observed, (Table 4, Figure 5, Supplementary: Table  
226 S10).

227 In grapevine transcriptome, 21 differentially expressed genes were identified in lignin  
228 biosynthesis, which were involved in the drought stress. It includes; 9 up-regulated genes related  
229 to shikimate O-hydroxycinnamoyltransferase, aldehyde 5-hydroxylase, two caffeoyl-CoA O-  
230 methyltransferase, cinnamoyl-CoA reductase 1, cinnamyl alcohol dehydrogenase 1, two  
231 peroxidase and laccase; whereas, 12 DEGs were down-regulated including, two caffeic acid 3 O-  
232 methyltransferase, one cinnamoyl-CoA reductase 1, five peroxidase, three laccase transcripts.

233 Further, eight genes were identified to be involved in terpenoid biosynthesis from which  
234 hydroxymethylglutaryl-CoA synthase, 1-deoxy-D-xylulose 5-phosphate reductoisomerase,  
235 isopentenyl diphosphate isomerase II and terpene synthase were up-regulated, while  
236 hydroxymethylglutaryl-CoA synthase, 1-deoxy-D-xylulose-5-phosphate synthase and two  
237 squalene epoxidase were down-regulated in drought-stressed grapevine leaves (Table 4, Figure  
238 5, Supplementary: Table S10).

### 239 **Heat shock protein (HSP) and pathogenesis-related protein (PR) in response to drought** 240 **stress**

241 The results revealed that 48 DEGs were identified in HSPs, including one HSP101 (down-  
242 regulated), three HSP90 (1 up-regulated and 2 down-regulated), two HSP70 (1 up-regulated, 1  
243 down-regulated), eighteen sHSPs (12 up-regulated and 6 down-regulated), twenty other HSP  
244 genes (17 up-regulated and 3 down-regulated) and heat-stress transcription factors (4 up-  
245 regulated and 1 down-regulated) . The high molecular weight HSPs (HMW HSPs), including  
246 HSP90s and HSP70s were also found to be up-regulated in our findings. One up-regulated  
247 transcript of HSP70s was expressed at higher abundance level (VIT\_17s0000g03310.t01; 650.81  
248 to 532.18 RPKM), compared with other HMW HSPs. The up-regulated,  
249 VIT\_16s0098g01060.t01 (from 706.59 to 1.98 RPKM) from sHSPs and

250 VIT\_14s0060g01490.t01 (from 363.93 to 355.88 RPKM) from other HSPs, expressed at  
251 moderate abundances, but remaining sHSPs, other HSPs and heat-stress transcription factors  
252 expressed at lower abundances (Table 5, Supplementary: Table S11).

253 In this study, 72 transcripts were identified as differentially-expressed genes, including ten  
254 pathogenesis-related protein PR-1 (4 up-regulated, 6 down-regulated), nine Beta-1,3-glucanase  
255 (PR2; 4 up-regulated, 5 down-regulated), nineteen chitinase (4 up-regulated, 15 down-regulated),  
256 fourteen thaumatin-like protein (PR5; 6 up-regulated, 8 down-regulated), four Pathogenesis-  
257 related protein 10 (2 up-regulated, 2 down-regulated), ten non-specific lipid-transfer protein  
258 (PR14; 5 up-regulated, 5 down-regulated), four Germin-like protein 2 (2 up-regulated, 2 down-  
259 regulated) and two pathogenesis-related transcription factors (2 down-regulated) to code disease  
260 resistance proteins. Conversely to HSPs, most of the PR showed down-regulation in grapevine  
261 leaves under drought stress. Moreover, 4 up-regulated transcripts, including PR1  
262 (VIT\_03s0088g00890.t01,  $|\log_2FC| = 8.75$ ), chitinase (VIT\_05s0094g00320.t01,  $|\log_2FC| =$   
263  $8.29$ ), thaumatin-like protein (VIT\_02s0025g04290.t01,  $|\log_2FC| = 3.84$ ) and Pathogenesis-  
264 related protein 10 (VIT\_05s0077g01600.t01,  $|\log_2FC| = 8.31$ ) were only expressed in treatment  
265 group. Additionally, ten dirigent proteins (3 up, 7 down-regulated) and thirteen proline related  
266 proteins (6 up, 6 down-regulated) were also recorded from this study (Table 5, Supplementary:  
267 Table S11).

#### 268 **qRT-PCR validation of DEGs from Illumina RNA-Seq**

269 In order to investigate the accuracy and reproducibility, 16 DEGs were selected from RNA-Seq  
270 results for quantitative real-time PCR, these transcripts represent all the major up/down-regulated  
271 functions that were identified in our transcriptome data including, metabolism, hormone  
272 signaling, disease resistance and regulatory proteins. The gene function, primer sequence,  
273 RPKM,  $\log_2$  values and qRT-PCR results are presented in Figure. 6; Supplementary: Table S12.  
274 The qRT-PCR findings of 16 (8 up-regulated and 8 down-regulated) selected genes were  
275 consistent with the RNA-seq results, revealing the accuracy and reliability of our RNA-seq  
276 results.

#### 277 **Discussion**

278 Drought stress suppresses the plant growth by inhibiting many physiological processes of plants.  
279 Chlorophylls (Chls) are the principal light-absorbing pigments and key components of  
280 photosynthesis in plants. The physiological and transcriptomic studies of grapevine leaves  
281 responding to drought stress have revealed that chl contents were remarkably decreased which  
282 in-turn inhibited the photosynthetic activity. Similarly decrease in chl content was reported in  
283 corn and chickpea in response to drought stress<sup>17,18</sup>. Moreover, transcriptomic data  
284 demonstrated that drought stress inhibited the chl biosynthesis process by suppressing the  
285 activity of key enzymes such as, HemA (Glutamyl-tRNA reductase 1) and CHLH (Magnesium  
286 chelatase H subunit), which play key role in chl<sub>a</sub> synthesis process<sup>19</sup>. Furthermore in chl cycle,  
287 the oxygenation reactions of chlorophyll(ide) a to chlorophyll(ide) b are catalyzed by  
288 chlorophyllide a oxygenase (CAO)<sup>20</sup>, whose activity was also decreased under drought stress,  
289 suggesting the obstructed process of chl cycle. In contrast, the chlorophyll(ide) b to a conversion  
290 is catalyzed by chlorophyll(ide) b reductase NYC1 (CBR) and its activity was up-regulated,  
291 suggesting that chl cycle process was also suppressed by the drought treatment<sup>21</sup>. Furthermore,  
292 PAO (pheophorbide a oxygenase) is regarded as an important chl catabolic enzyme<sup>22,23</sup> and

293 participated well in chl degradation process as its activity was increased under drought stress  
294 (Figure.1 Supplementary: Table S5).<sup>24</sup>Buchert and<sup>25</sup>Du have investigated the role of PAO as an  
295 important chl degradation enzyme during senescence of broccoli and banana, respectively.

296 Meantime, the photosynthetic activity, stomatal conductance and CO<sub>2</sub> assimilation rate was  
297 significantly decreased in grapevine leaves under drought stress as compared to control. Similar  
298 findings have also been reported in grapevine under Cu and drought stresses<sup>26,27</sup>. Moreover, the  
299 photosynthesis-related genes, involved in PSII, PSI, cytochrome b6-f complex, photosynthetic  
300 electron transport, F-type ATPase and photosynthesis-antenna proteins were significantly down-  
301 regulated in drought-induced grapevine leaves, but the extent of light-harvesting proteins (CP47,  
302 CP43), which binds the chl a molecules was down-regulated by the drought stress  
303 (Supplementary: Table S6). Perhaps, PsaB is regarded as the heart of PSI that binds P700 special  
304 chlorophyll pair<sup>28</sup> was also down-regulated under drought stress in our findings. Finally, drought  
305 stress gradually decreased the activities of PSII electron transport and light-harvesting complex  
306 (photosynthesis-antenna proteins). Available literature anticipated that stomatal closure reduced  
307 the CO<sub>2</sub> absorption which limits the photosynthetic activity in plants under drought stress  
308 environment<sup>29-31</sup>. Our findings on photosynthesis phenomenon at physiological and  
309 transcriptomic level suggested that drought stress definitely affected the primary photosynthesis  
310 metabolic process, and the decline in photosynthesis process was connected with the chlorophyll  
311 degradation.

312 ROS is the universal response of the plants against any type of environmental stress to prevent  
313 oxidative damage. Several studies have already been conducted on malondialdehyde under  
314 oxidative stress in different crops such as, wheat (*Triticum aestivum*) and oilseed rape (*Brassica  
315 napus*), proposed that MDA contents were induced by drought stress<sup>32-34</sup>. On the contrary, plants  
316 have the ability to accumulate the level of antioxidative enzymes to confer the severity of  
317 drought stress and similar investigations in olive<sup>35</sup> and wheat<sup>33</sup> support our findings of  
318 increased activity of ROS enzymes and MDA. The results of transcriptomic investigation  
319 showed that, one NADPH oxidase and five amine oxidases were significantly up-regulated,  
320 while both play key role in the ROS synthesis and accumulation under various kind of stress  
321 environments<sup>36</sup>. SODs are regarded as first line of defense against ROS which have two  
322 isozymes Fe-SOD and Cu/Zn-SOD in plant chloroplast<sup>36</sup>. It is worth mentioning that Fe-SODs  
323 was up-regulated, but Cu/Zn-SODs was down-regulated, which are in agreement with our  
324 previous findings in grapevine under Cu stress conditions<sup>26</sup>. Other enzymes, including CAT,  
325 POD, GSH-AsA cycle, PPO, GST, AO, MDHAR, DHAR and GR also possess the drought-  
326 responsive antioxidative defense system in grapevine<sup>37</sup>. Perhaps, non-enzymatic antioxidants  
327 such as, glutathione and proline also enhanced the ROS level in grapevine in response to  
328 drought-stress, which is consistent with the ROS scavenging system investigated in the *V.  
329 vinifera* and *S. lycopersicum* under drought stress<sup>38,39</sup>. Generally, ROS related analytical and  
330 transcriptomic findings present the broad spectra to understand their role at cellular level in  
331 response to drought stress.

332 Drought stress causes dehydration in plant cells. Plant hormones, such as abscisic acid, auxin,  
333 Gibberellin, ethylene, jasmonic acid and brassinostroid accumulate under dehydration condition  
334 and play important role of stress tolerance in plants<sup>40</sup>. In *Arabidopsis*, ABA activates the  
335 subclass III protein kinases of SnRK2 family, which further facilitate the regulation of stomatal  
336 conductance to regulate plant water status through guard cells<sup>41,42</sup>, favor our findings of

337 increased activity of SRK2I protein kinase under drought stress in grapevine leaves. The  
338 regulation of PP2C genes during the drought stress in grapevine leaves proposed that PP2C has  
339 its primary role in stress tolerance, especially in regulating ABA response<sup>43</sup>. The AUX gene  
340 family includes early response AUX genes, Aux/IAA, GH3 and SAUR and the regulators of  
341 AUX genes, ARF, while their activities were down-regulated in our findings. Wang et al.<sup>44</sup>  
342 investigated the AUX gene family in sorghum (*Sorghum bicolor*) and specified that most of  
343 these genes were induced by the exogenous application of IAA under drought stress conditions.  
344 Moreover, GA activity and the accumulation of DELLA proteins was up-regulated by the  
345 drought stress, while similar findings in *Arabidopsis* have suggested that DELLA proteins  
346 restrain the plant growth to promote survival of plant under drought stress<sup>44</sup>. JA biosynthesis and  
347 signaling together with ABA and other hormones have been extensively studied in many crops.  
348 In current investigations, JA amino acid conjugate (JAR1) was significantly up-regulated, while  
349 JAR1 are enduringly present in the plant leaves and together with ABA induce the stomatal  
350 closure under osmotic stress, have been extensively studied in *Arabidopsis*<sup>45</sup>. Interestingly,  
351 jasmonate-zim domain proteins (JAZ) were significantly down-regulated, which was observed  
352 up-regulated in another study in rice<sup>46</sup>, suggesting the severity of drought stress in grapevine  
353 leaves. Moreover, the activity of AOS and LOX were significantly increased, which is similar  
354 with the findings of Leng et al.<sup>36</sup> in *V. vinifera*. Ethylene is regarded as stress hormone because  
355 its synthesis is induced under different oxidative environments. Under drought stress, the  
356 synthesis of ethylene precursor 1-aminocyclopropane-1-carboxylate oxidase was up-regulated in  
357 grapevine, which stimulates plant development and functioning by inducing the diffusion  
358 possibility of ABA to its active site<sup>47,48</sup>. Furthermore, the expressions of the ethylene-related  
359 regulatory genes (ETR1 and CTR1) were intensely increased in our findings, suggesting their  
360 key role in ethylene biosynthesis as described by Schachtman and Goodger<sup>49</sup>. BRs are the only  
361 plant steroids, which induce the expression of many genes, especially during stress  
362 environments. Brassinosteroid Insensitive 1 (BRI1) was significantly up-regulated in our  
363 findings, which is known to play key role in plant growth, morphogenesis and response to  
364 drought stress. Feng, et al.<sup>50</sup> created RNAi mutants for bdBRI1 in *Brachypodium distachyon* and  
365 suggested that this gene produces a dwarf phenotype with enhanced tolerance against drought  
366 stress. BR signal transduction, from cell surface perception to activation of specific nuclear  
367 genes will be interesting to investigate in the future.

368 Plants cope with environmental stress by the accumulation of certain compatible osmolytes such  
369 as, proline, which is known to confer the drought tolerance in plants<sup>51</sup> and up-regulation of all  
370 the genes related to proline metabolism is the clear evidence of grapevine tolerance in our study.  
371 Proline biosynthesis commenced with the phosphorylation of glutamate, which then converted  
372 into glutamic- $\alpha$ -semialdehyde by Pyroline-5-carboxylate synthetase (up-regulated). Similarly,  
373 arginine is converted into ornithine by arginase (up-regulated) and then into GSA by the  
374 ornithine- $\delta$ -aminotransferase (not-detected). GSA is then converted into pyrroline 5-carboxylate  
375 (P5C) by impulsive cyclization. Finally, proline is synthesized from the P5C by P5C reductase  
376 (P5CR) enzyme<sup>51,52</sup>. In proline degradation pathway, proline is re-converted into P5C by Proline  
377 dehydrogenase (PDH; up-regulated) and then into glutamate by Pyrroline-5-carboxylate  
378 dehydrogenase (P5CDH; up-regulated). Thus PDH and P5CDH are believed to be most  
379 important enzymes in proline degradation to glutamate<sup>53,54</sup>. Hence, proline metabolism may  
380 regulate the gene expression during the drought stress.

381 In higher plants, accumulation of various secondary metabolites such as, amino acids,  
382 carbohydrates and lipids occur when plant is subjected to environmental stress<sup>55</sup>. Shikimate  
383 pathway not only act as bridge between central and secondary metabolism, but also serve as  
384 precursor for other secondary metabolites<sup>56</sup>. Additionally, Tyr is a precursor of IAA and initiate  
385 the synthesis of indole alkaloids and isoquinoline alkaloids, which prevent plants from oxidative  
386 stress<sup>57</sup>. Phe is considered as precursor of secondary metabolites family and PAL participates in  
387 phenylpropanoid biosynthesis; a key step towards biosynthesis of stilbenes, flavonoids, lignins  
388 and various other compounds<sup>58</sup>. STS (stilbene synthase) catalyzes the initial step of flavonoid  
389 biosynthesis pathway, which has the protective function during the drought stress<sup>59</sup>. Overall, 4  
390 PAL and 6 STS were significantly up-regulated in our findings, proposing the innate link with  
391 drought stress. The respective, up and down-regulation of 1-deoxy-D-xylulose 5-phosphate  
392 reductoisomerase and 1-deoxy-D-xylulose-5-phosphate synthase can act as rate limiting enzymes  
393 in MEP pathway, also found in cu-stressed grapevine leaves<sup>26</sup>. Dimethylallyl diphosphate and  
394 isopentenyl diphosphate are the universal 5 carbon precursors found in terpenoid synthesis. It has  
395 been reported that one isopentenyl-diphosphate isomerase II can catalyze isopentenyl  
396 diphosphate to form dimethylallyl diphosphate and one terpene synthase<sup>60,61</sup>, while both were  
397 up-regulated in our findings. The down-regulation of most of the genes related to anthocyanin,  
398 lignin and terpenoid biosynthesis have elucidated the negative role of drought stress on  
399 accumulation of secondary metabolites in grapevine leaves.

400 HSPs are ubiquitous stress-related proteins that act as molecular chaperone, HSP members  
401 participate in the protein synthesis, folding, aggregation and transportation from cytoplasm to  
402 different intracellular compartments<sup>62,63</sup>. In current study some high molecular weight HSPs  
403 (HSP101, HSP90 and HSP70) were down-regulated, but most of the genes related to small  
404 HSPs (sHSPs; 16-30kDa), other HSPs and heat stress transcription factors (HSFs) were up-  
405 regulated. In addition, pathogenesis-related (PR) proteins are derived from plant allergens  
406 and act as defense-responsive proteins by increasing their expression under pathogen attack  
407 and variable stress environments. Depending on the functions and properties, PR-proteins  
408 are classified into 17 families such as, beta-1,3-glucanases, chitinases, thaumatin-like  
409 proteins, peroxidases, small proteins (defensins and thionins) and lipid transfer proteins  
410 (LTPs)<sup>64,65</sup>. Most of the PR-proteins were down-regulated in our study, suggesting that  
411 drought stress posed negative effect on PR-proteins defense response. Contrarily, most of  
412 the genes related to dirigent-proteins (DIR), play role in lignin formation and proline-  
413 related proteins were up-regulated, suggesting their possible defensive-role in grapevine in  
414 response to drought stress.

## 415 **Conclusion**

416 Our results have provided substantial evidences to demonstrate that grapevine adaptation to  
417 drought stress is a multistep component system consisting of several genes that regulates various  
418 pathways. Out of 12,451 DEGs, 7987 DEGs were up-regulated and 4,464 DEGs were down-  
419 regulated. Nearly 2 fold up-regulations of DEGs clearly indicate their defense role in grapevine  
420 under various pathways in response to drought stress. The significant increase in the activity of  
421 ROS enzymes and hormones level revealed the defensive role of these enzymes and hormones  
422 during drought stress in grapevine leaves. Overall, study concludes that drought has severe  
423 effects on growth and physiology of the grapevine, but defense-related pathways assist grapevine  
424 to mitigate the drought severity.

## 425 **Materials and Methods**

### 426 **Plant material and drought treatments**

427 Two-year old grapevine (*V. vinifera* cv. ‘Summer Black’) pot grown plants were selected as  
428 experimental material which were grown in standard greenhouse condition ( $25 \pm 5^\circ\text{C}$ )  
429 under 16-h light/8-h dark photoperiod and 65% relative humidity (RH) at the Nanjing  
430 Agricultural University-Nanjing, China. Grapevine plants were subjected to drought with  
431 an interval of 20 days against control, each with three biological replicates. The fourth  
432 unfolded leaf from the shoot apex was collected from the each replicates of both control  
433 and drought treatment with the interval of 0 and 20th day, respectively, and the three  
434 samples were mixed to make one composite sample. After harvesting, the samples were  
435 immediately put in liquid nitrogen and then stored at  $-80^\circ\text{C}$  until analysis.

### 436 **Determination of important biochemistry and physiology-related traits**

437 The chlorophyll a and b contents was determined using spectrophotometer at 663 and 645  
438 nm, respectively as briefly explained by Leng et al. (2015). Photosynthesis activity,  
439 stomatal conductance and  $\text{CO}_2$  assimilation rate were carried out on mature leaf between 4<sup>th</sup>  
440 to 7<sup>th</sup> nodes from the shoot base for both control and drought treatment; between 9:00 -  
441 11:00 AM measured using LI-COR (LI-6400XT, Germany) meter as described by Tombesi  
442 et al. (2015). Malondialdehyde (MDA) contents were quantified by using thiobarbituric  
443 acid. The activities of antioxidant enzymes (SOD, POD and CAT) were measured using the  
444 method briefly described by Haider, et al. <sup>66</sup>. The activities of indole-acetic acid (IAA),  
445 abscisic acid (ABA), jasmonic acid (JA), gibberellic acid (GA) and brassinosteroid (BR)  
446 were measured following the method of Tombesi, et al. <sup>27</sup>. Three technical repeats were  
447 generated for all the quantifications. Data was subjected to one-way analysis of variance  
448 (ANOVA) at  $p < 0.05$ , using MINITAB (ver. 16) and represented as mean  $\pm$  standard  
449 deviation (SD).

### 450 **RNA extraction, cDNA library construction and Illumina deep sequencing**

451 Total RNA from leaf samples of both control and drought-stressed were extracted  
452 using Trizol reagent (Invitrogen, Carlsbad, CA, USA) (1% agarose gel buffered by Tris-  
453 acetate-EDTA was run to indicate the integrity of the RNA.) and subsequently used for  
454 mRNA purification and library construction with the Ultra<sup>TM</sup> RNA Library Prep Kit for  
455 Illumina (NEB, USA) following the manufacturer’s instructions. The samples were  
456 sequenced on an Illumina Hiseq<sup>TM</sup>2500 for 48h.

### 457 **Analysis of gene expression level, gene ontology (GO) and Kyoto encyclopedia of genes 458 and genomics (KEGG)**

459 After adaptor trimming and quality trimming, the clean reads were mapped to the *V.*  
460 *vinifera* transcriptome using Bowtie 1.1.2. Then, Sam tools and BamIndexStats.jar were  
461 used to calculate the gene expression level, and reads per kilobase per million (RPKM)  
462 value was computed from SAM files <sup>67</sup>. Gene expression differences between  $\log_2$  and  
463 early stationary phase were obtained by MARS (MA-plot-based method with Random  
464 Sampling model), a package from DEGseq 3.3 (Leng et al., 2015). We simply defined  
465 genes with at least 2-fold change between two samples and FDR (false discovery rate) less

466 than 0.001 as differential expressed genes. Transcripts with  $|\log_2FC| < 1$  were assumed to  
467 have no change in their expression levels. The gene ontology (GO) enrichment (p-value <  
468 0.05) was investigated by subjecting all DEGs to GO database  
469 (<http://www.geneontology.org/>) in order to further classify genes or their products into  
470 terms (molecular function, biological process and cellular component) helpful in  
471 understanding genes biological functions. Kyoto encyclopedia of genes and genomics  
472 (KEGG; the major public pathway-related database) was used to perform pathway  
473 enrichment analysis of DEGs<sup>68</sup>.

#### 474 **Illumina RNA-seq results validation by qRT-PCR**

475 In order to validate the Illumina RNA-seq results the drought-stressed grapevine leaf  
476 samples of each collection were applied to qRT-PCR analysis. Total RNA of the collected  
477 samples was extracted following the above mentioned method, and then was reverse-  
478 transcribed using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Dalian,  
479 China), following the manufacturers' protocol. Gene specific qRT-PCR primers were  
480 designed using Primer3 software (<http://primer3.ut.ee/>), for 20 selected genes with the  
481 sequence data in 3'UTR (Table S12). qRT-PCR was carried out using an ABI PRISM  
482 7500 real-time PCR system (Applied Biosystems, USA). Each reaction contains 10 $\mu$ l  
483 2 $\times$ SYBR Green Master Mix Reagent (Applied Biosystems, USA), 2.0 $\mu$ l cDNA sample,  
484 and 400 nM of gene-specific primer in a final volume of 20 $\mu$ l. PCR conditions were 2 min  
485 at 95°C, followed by 40 cycles of heating at 95°C for 10s and annealing at 60°C for 40s. A  
486 template-free control for each primer pair was set for each cycle. All PCR reactions were  
487 normalized using the Ct value corresponding to the Grapevine UBI gene. Three biological  
488 replicates were generated and three measurements were performed on each replicate.

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#### 494 **Conflict of Interest statement**

495 The authors declare that the research was conducted in the absence of any commercial or  
496 financial relationships that could be construed as a potential conflict of interest.

#### 497 **Authors' contributions**

498 Conceived and designed the experiments: MSH, JF. Perform the experiment: MSH, CZ.  
499 Analyzed the data: MSH, TP, LS. Contributed in reagents/ materials/ analysis tools: MSH,  
500 MMK, SJ, LA. Manuscript writing: MSH, MMK, JF. All the authors approved the final draft of  
501 manuscript.

#### 502 **Abbreviations**

503 ABA: Abscisic acid; ANOVA: Analysis of variance; BR: Brassinosteroid; CAT: Catalase;  
504 DEGs: Differentially-expressed genes; ESTs: Expressed sequence tags; FDR: False discovery  
505 rate; GA: Gibberellic acid; IAA: Indole-acetic acid; JA: Jasmonic acid; MARS: MA-plot-based  
506 method with Random Sampling model; MDA: Malondialdehyde; POD: peroxidase; qRT-PCR:

507 quantitative real-time PCR; RNA-seq: RNA-sequencing; ROS: Reactive oxygen species; SD:  
508 Standard deviation; SOD: Superoxide dismutase.

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681

682

683 **Table 1.** Comparison of some physiological and biochemical parameters in grapevine leaves  
684 under drought stress environment

<b>Physiological and biochemical parameters</b>	<b>Control</b>	<b>Drought treatment</b>	<b>Range of increasing %</b>
Chlorophyll contents (mg g <sup>-1</sup> )	1.16 ± 0.08	0.85 ± 0.09	-26.72
Chla contents (mg g <sup>-1</sup> )	0.43 ± 0.11	0.28 ± 0.06	-34.88
Chlb contents (mg g <sup>-1</sup> )	0.73 ± 0.06	0.57 ± 0.04	-21.92
Photosynthesis activity (μmole m <sup>-2</sup> sec <sup>-1</sup> )	23.67 ± 0.81	16.08 ± 0.75	-32.20
Stomatal conductance (μmole m <sup>-2</sup> sec <sup>-1</sup> )	0.15 ± 0.03	0.09 ± 0.02	40.00
Net CO <sub>2</sub> assimilation (μmole m <sup>-2</sup> sec <sup>-1</sup> )	9 ± 0.03	5 ± 0.03	44.44
MDA contents (nmol/g)	5.35 ± 0.21	8.61 ± 0.25	60.93
SOD activity (U/ g/ min)	371.56 ± 10.21	650.85 ± 15.7	75.16
POD activity (U/ g/ min)	18.23 ± 0.97	43.9 ± 1.01	140.81
CAT activity (U/ g/ min)	6.32 ± 1.21	19.01 ± 0.99	200.79
Proline (ng/g FW)	1.124 ± 0.04	1.711 ± 0.05	52.37

685

686

687 **Table 2.** List of differentially-expressed genes related to chlorophyll degradation and photosynthesis in  
688 grapevine perceived during drought stress.

Trait name	Description	No. of up-regulated	No. of down-regulated	sum
Chlorophyll synthesis	Chlorophyll a	9	6	15
Metabolism	Chlorophyll cycle	1	1	1
	Chlorophyll degradation	3	3	6
	psbB	0	2	2
Photosystem II	psbC	0	2	2
	psbW	0	1	1
Photosystem I	psaB	0	2	2
Cytochrome b6-f complex	petA	0	1	1
	petC	1	2	3
Photosynthetic electron transport	petF	0	2	2
	petH	2	0	2
	ATPF1B	0	1	1
F-type ATPase	ATPF1A	0	1	1
	ATPF1G	0	1	1
	ATPF0C	0	1	1
	LHCB1	0	1	1
Photosynthesis-antenna proteins	LHCB2	0	1	1
	LHCB3	0	1	1
	LHCB6	0	1	1

689 psbB, Photosystem II CP47 chlorophyll apoprotein gene; psbC, Photosystem II CP43 chlorophyll  
690 apoprotein gene; psbW, Photosystem II reaction center W protein; psaB, photosystem I P700 apoprotein  
691 A2 gene; petA, cytochrome f; petC, cytochrome b6-f complex iron-sulfur subunit 1; petF, ferredoxin-3;  
692 petH, ferredoxin--NADP reductase, leaf-type isozyme; ATPF1B, ATP synthase CF1 beta; ATPF1A, ATP  
693 synthase CF1 alpha; ATPF1G, ATP synthase gamma; LHCB1, chlorophyll a-b binding protein of LHCII;  
694 LHCB2, light harvesting chlorophyll A/B binding protein; LHCB3, light-harvesting chlorophyll binding  
695 protein 3 gene; LHCB6, chlorophyll a-b binding protein CP24 10A.

696 **Table 3.** List of differentially-expressed genes related to chlorophyll degradation and photosynthesis in  
697 grapevine perceived during drought stress.

Trait name	Description	No. of up-regulated	No. of down-regulated	sum
ROS synthesis	Rboh	1	1	2
	AO	5	0	5
	Fe-SOD	2	0	2
ROS scavenging	POD	2	4	6
	CAT	3	0	3
	MDAR	1	0	1
GSH-AsA cycle	DHAR	1	0	1
	GR	1	0	1
	Grx	2	4	6
GPX pathway	GPX	1	0	1
	GST	22	4	26
Prx/Trx	Prx	0	1	1
	Trx	5	2	7
Cyanide-resistant respiration	AOX	2	1	3
Copper-containing enzymes	PPO	0	2	2

698 Rboh, respiratory burst oxidase; AO, amine oxidase; Fe-SOD, Fe superoxide dismutase; POD,  
699 peroxidase; CAT, catalase, APX, ascorbate peroxidase; MDAR, monodehydroascorbate reductase;  
700 DHAR, dehydroascorbate reductase; GR, glutathione reductase; Grx, glutaredoxin; GPX, glutathione  
701 peroxidase; GST, glutathione S transferase; Prx, peroxiredoxin; Trx, thioredoxin; AOX, alternative  
702 oxidase, PPO, polyphenol oxidase.

703 **Table 4.** Elucidation on differential expression of genes related to secondary metabolites under drought  
704 stress.

Trait name	Description	No. of up-regulated	No. of down-regulated	sum
	DAHPS3	1	0	1
	B3D/SDH	0	2	2
	SHK	0	1	1
Shikimate acid pathway	CS1	1	0	1
	AnPRT	0	2	2
	IGPS	1	0	1
	TS	1	0	1
Alkaloids biosynthetic pathway	STR3	0	1	1
	DAT	0	1	1
	PAL	4	4	8
	TC4M	0	1	1
	STS	6	7	13
	4CL	1	1	2
Anthocyanin biosynthetic pathway	F3D	1	0	1
	FLSI	1	2	3
	DFR	0	2	2
	UFGT	0	1	1
	ANR	1	0	1
	SOH	1	0	1
	CA3M	0	2	2
	COM	2	0	0
Lignin biosynthetic pathway	CCR1	1	1	2
	CAD1	1	0	1
	POD	2	5	7
	LAC	1	3	4
	HMGS	1	1	2
	DXPS	1	1	2
Terpenoid biosynthetic pathway	IPI2	1	0	1
	TSE	1	0	1
	SED	0	2	2

705 DAHPS3, 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase 03; B3D/SDH, bifunctional 3-  
706 dehydroquininate dehydratase/shikimate dehydrogenase; SHK, shikimate kinase; CS1, chorismate synthase  
707 1; AnPRT, anthranilate phosphoribosyltransferase; IGPS, indole-3-glycerol phosphate synthase; TS,  
708 tryptophan synthase beta chain 1; STR3; strictosidine synthase 3; DAT, D-amino-acid transaminase;  
709 PAL, phenylalanine ammonia-lyase; TC4M, Trans-cinnamate 4-monooxygenase; STS, stilbene synthase;  
710 4CL, 4-coumarate--CoA ligase; F3D, flavanone 3-dioxygenase; FLS1, flavonol synthase/flavanone 3-  
711 hydroxylase; DFR, dihydroflavonol-4-reductase; UFGT; anthocyanidin 3-O-glucosyltransferase 2; ANR,  
712 anthocyanidin reductase; SOH, shikimate O-hydroxycinnamoyltransferase; CA3M, caffeic acid 3-O-  
713 methyltransferase; COM, caffeoyl-CoA O-methyltransferase; CCR1, cinnamoyl-CoA reductase 1; CAD1;  
714 cinnamyl alcohol dehydrogenase 1; POD; Peroxidase; LAC; laccase; HMGS, hydroxymethylglutaryl-  
715 CoA synthase; DXPS, 1-deoxy-D-xylulose-5-phosphate synthase; IPI2, isopentenyl diphosphate  
716 isomerase II; TSE, terpene synthase; SED, squalene epoxidase.

717

718

719 **Table 5.** List of differentially-expressed genes related to heat-shock proteins (HSPs) and pathogens  
 720 resistance (PRs) proteins in grapevine perceived during drought stress.

Trait name	Description	No. of up-regulated	No. of down-regulated	sum
	HSP101	0	1	1
	HSP90	1	2	3
	HSP70	1	1	2
Heat shock proteins	small HSP	12	6	18
	other HSP	17	3	20
	heat-stress transcription factor	4	1	5
PR-1	pathogenesis-related protein 2	4	6	10
PR-2	Beta-1,3-glucanase	4	5	9
PR-3,4,8,11	chitinase	4	15	19
PR-5	Thaumatin-like protein	6	8	14
PR-10	Pathogenesis-related protein 10	2	2	4
PR-14	lipid transfer protein	5	5	10
PR-15	germin-like protein 2	2	2	4
PTI	transcriptional activator		2	2
dirigent protein		3	7	10
proline related protein		6	6	12

721

## 722 **Figure legends**

723 **Figure 1. Chlorophyll metabolic pathway in drought-stress grapevine leaves.** GLTL, Glutamate  
724 tRNA ligase; HemaA, Glutamate tRNA reductase 1; GSA, Glutamate-1-semialdehyde; ALAD, Delta-  
725 aminolevulinic acid dehydrates; PBGD, porphobilinogen deaminase; UROS, Uroporphyrinogen III  
726 synthase; RMA1, Radical S-adenosyl methionine domain-containing protein 1; PPOX1; Proporphynogen  
727 oxidase 1; PPOX, Proporphynogen oxidase; UROD, Uroporphyrinogen III decarboxylase; CHLH,  
728 Magnesium chelatase H subunit; CHL1, Magnesium-chelatase I subunit; CHLD, Magnesium chelatase D  
729 subunit; CHLM, Mg-proto IX methyltransferase; CRD1, Mg-protophyrin IX monomethylester (oxidative)  
730 cyclase; POR, Protochlorophyllide oxidoreductase; DHR, Dehydrogenase/reductase SDR family member;  
731 SCD, Short chain dehydrogenase, TIC32; CHLG, CAO; Chlorophyllide a oxygenase; CBR,  
732 Chlorophyll(ide) b reductase NYC1; CLH1, Chlorophyllase-I; CLH2, Chlorophyllase-II; PAO,  
733 Pheophorbide a oxygenase; PDT, Protochlorophyllide-dependent translocon component 52.

734 **Figure 2. Reactive oxygen species (ROS) scavenging pathway in plants. (a) The ascorbate-**  
735 **glutathione (AsA-GSH) cycle, (b) The glutathione peroxidase (GPX) cycle. SOD (superoxide**  
736 **dismutase) initiate the line of defense by converting O<sub>2</sub><sup>-</sup> into H<sub>2</sub>O<sub>2</sub>, which is further detoxified by**  
737 **CAT (catalases), APX (ascorbate peroxidases (APX) and GPX (glutathione ascorbate).**

738 Abbreviations: DHA, dehydroascorbate; GSH, glutathione; GSSG, oxidized glutathione; GR, gkutathione  
739 reductase; MDAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase.

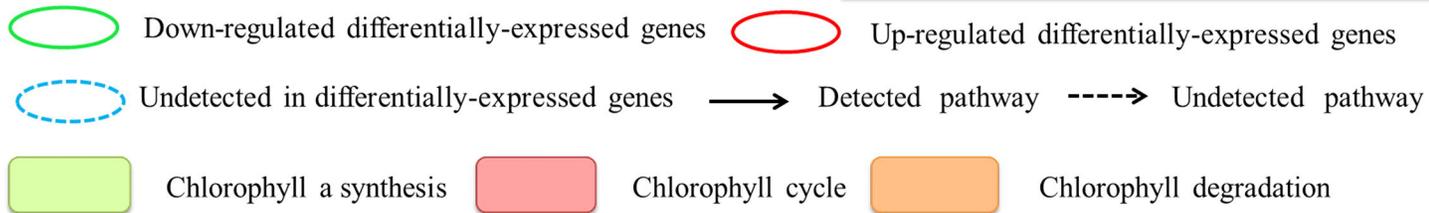
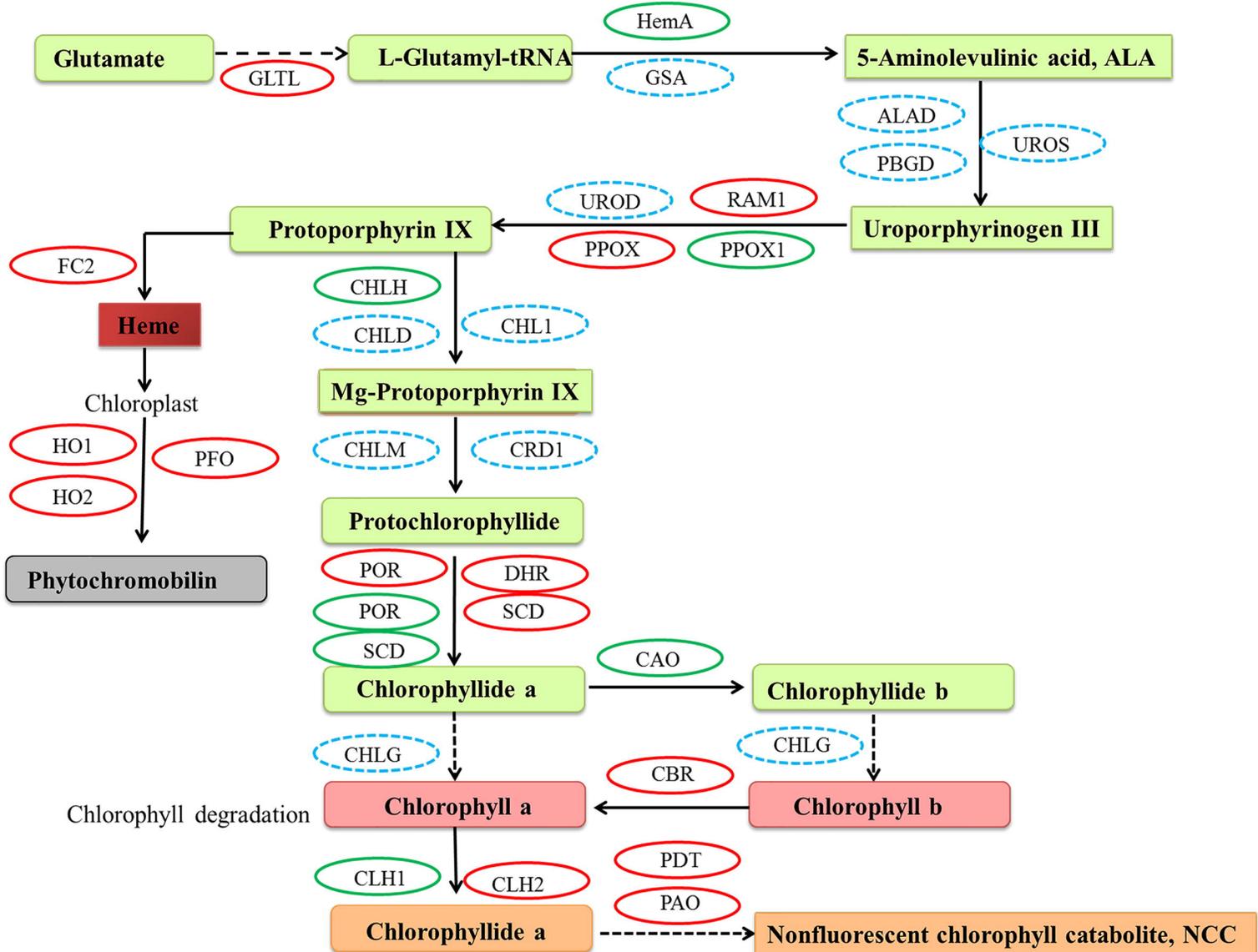
740 **Figure 3. The activities of different hormone, including IAA (indole-acetic acid), ABA (abscisic**  
741 **acid), JA (jasmonic acid), GA (gibberellic acid) and BR (brassinsteroid) in control and drought**  
742 **treatment.**

743 **Figure 4. Differential expressions of genes during biosynthesis and degradation of proline**  
744 **in response to drought stress. Given numbers represents the individual genes catalyzing**  
745 **specific reactions. P5CS, pyroline-5-carboxylate synthetase; ARG, arginase;  $\delta$ -AOT; ornithine-**  
746  **$\delta$ -aminotransferase; P5CR, pyrroline 5-carboxylate reductase; PDH, Proline dehydrogenase;**  
747 **P5CDH, Pyrroline-5-carboxyate dehydrogenase.**

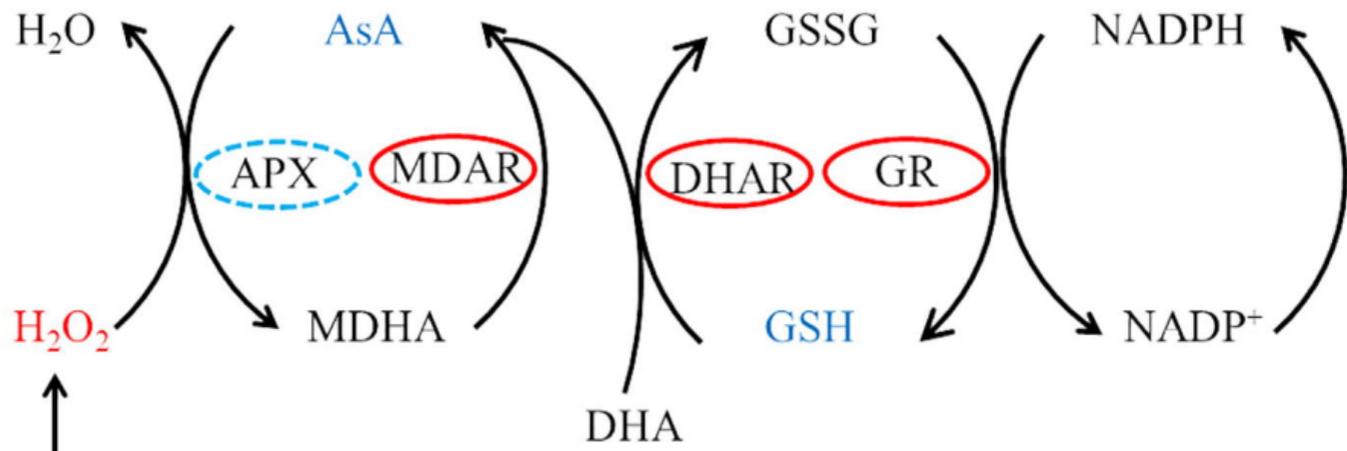
748 **Figure 5. Differential expression of genes related to secondary metabolites under drought stress.**  
749 DAHPS3, 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase 03; B3D/SDH, bifunctional 3-  
750 dehydroquininate dehydratase/shikimate dehydrogenase; SHK, shikimate kinase; CS1, chorismate synthase  
751 1; AnPRT, anthranilate phosphoribosyltransferase; IGPS, indole-3-glycerol phosphate synthase; TS,  
752 tryptophan synthase beta chain 1; STR3; strictosidine synthase 3; DAT, D-amino-acid transaminase;  
753 PAL, phenylalanine ammonia-lyase; TC4M, Trans-cinnamate 4-monooxygenase; STS, stilbene synthase;  
754 4CL, 4-coumarate--CoA ligase; F3D, flavanone 3-dioxygenase; FLS1, flavonol synthase/flavanone 3-  
755 hydroxylase; DFR, dihydroflavonol-4-reductase; UFGT; anthocyanidin 3-O-glucosyltransferase 2; ANR,  
756 anthocyanidin reductase; SOH, shikimate O-hydroxycinnamoyltransferase; CA3M, caffeic acid 3-O-  
757 methyltransferase; COM, caffeoyl-CoA O-methyltransferase; CCR1, cinnamoyl-CoA reductase 1; CAD1;  
758 cinnamyl alcohol dehydrogenase 1; POD; Peroxidase; LAC; laccase.

759 **Figure 6. Verification of relative expression levels of DEGs by qRT-PCR.** Error bars indicate standard  
760 deviation from 3 technical replicates of RT-qPCR. Expression patterns of 16 DEGs selected from  
761 different elucidated pathways by qRT-PCR (blue bar) and RNA-Seq (red dot). (1) Seq ID:  
762 VIT\_07s0151g00110.t01 (Chlorophyllase-1), (2) Seq ID: VIT\_00s0396g00010.t01 (psbC; Photosystem II  
763 CP43 chlorophyll apoprotein gene), (3) Seq ID: VIT\_00s2608g00020.t01 (psbB; Photosystem II CP47  
764 chlorophyll apoprotein gene), (4) Seq ID: VIT\_18s0001g08620.t01 (psaB; photosystem I P700 apoprotein  
765 A2 gene), (5) Seq ID: VIT\_19s0027g01930.t01 (peroxiredoxin (Prx)), (6) Seq ID:

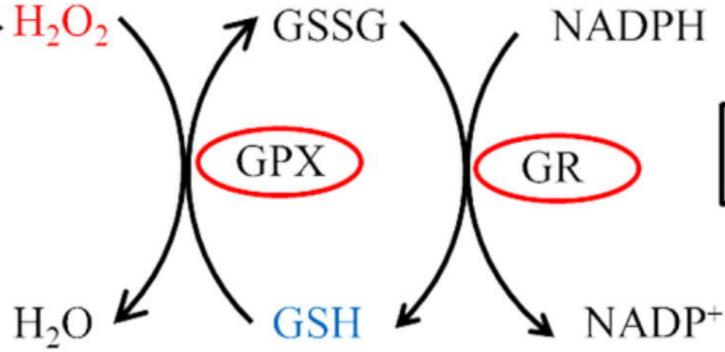
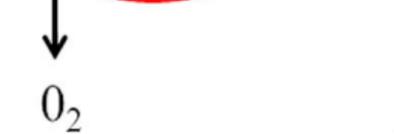
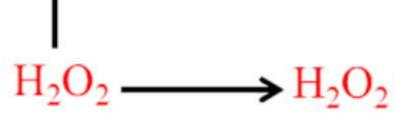
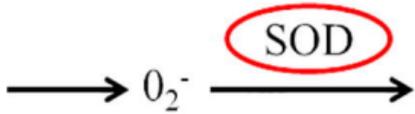
766 VIT\_11s0037g00940.t01 (S-adenosylmethionine decarboxylase proenzyme), (7) Seq ID:  
767 VIT\_12s0055g01020.t01 (peroxidase N1-like), (8) Seq ID: VIT\_18s0001g08550.t01 (squalene  
768 monooxygenase), (9) Seq ID: VIT\_06s0061g00790.t01 (Pheophorbide a oxygenase), (10) Seq ID:  
769 VIT\_17s0000g06130.t01 (glutathione S-transferase U9), (11) Seq ID: VIT\_04s0023g03230.t01 (auxin-  
770 induced protein 15A-like), (12) Seq ID: VIT\_01s0146g00350.t01 (BRASSINOSTEROID INSENSITIVE  
771 1-associated receptor kinase 1), (13) Seq ID: VIT\_01s0011g00480.t01 (glutamate 5-kinase), (14) Seq ID:  
772 VIT\_07s0129g00460.t01 (prolyl 4-hydroxylase 9), (15) Seq ID: VIT\_16s0039g01360.t01 (phenylalanine  
773 ammonia-lyase), (16) Seq ID: VIT\_03s0088g00710.t01 (pathogenesis-related protein PR-1).



a. AsA-GSH cycle

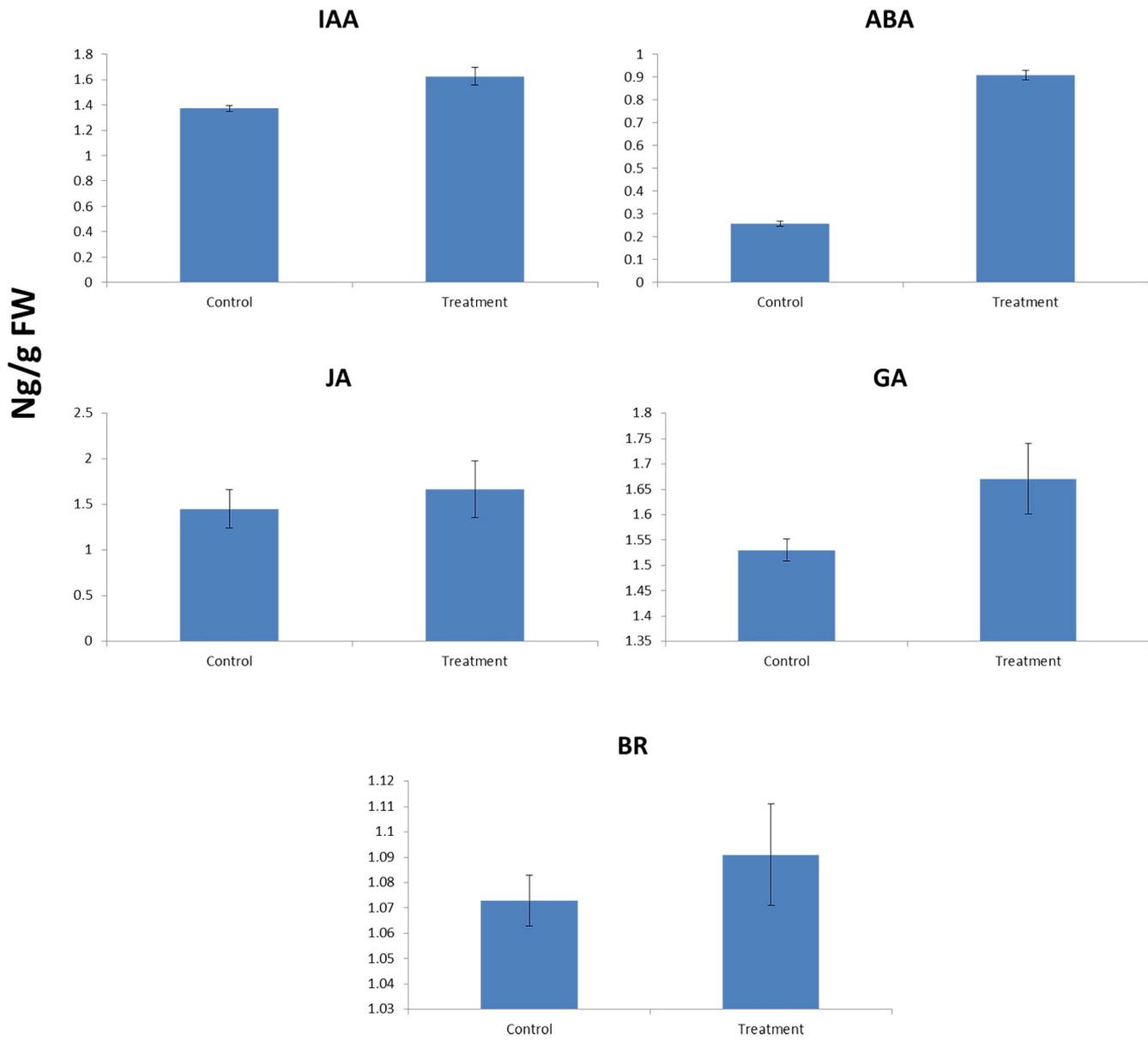


Drought stress



b. GPX cycle

○ Up-regulated differentially-expressed genes    ○ Not detected differentially-expressed genes



**IAA**

**ABA**

**JA**

**GA**

**BR**

**Ng/g FW**

Control

Treatment

Control

Treatment

Control

Treatment

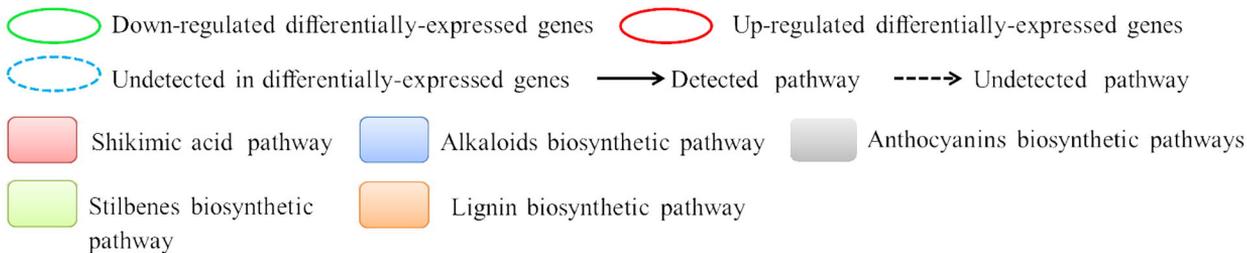
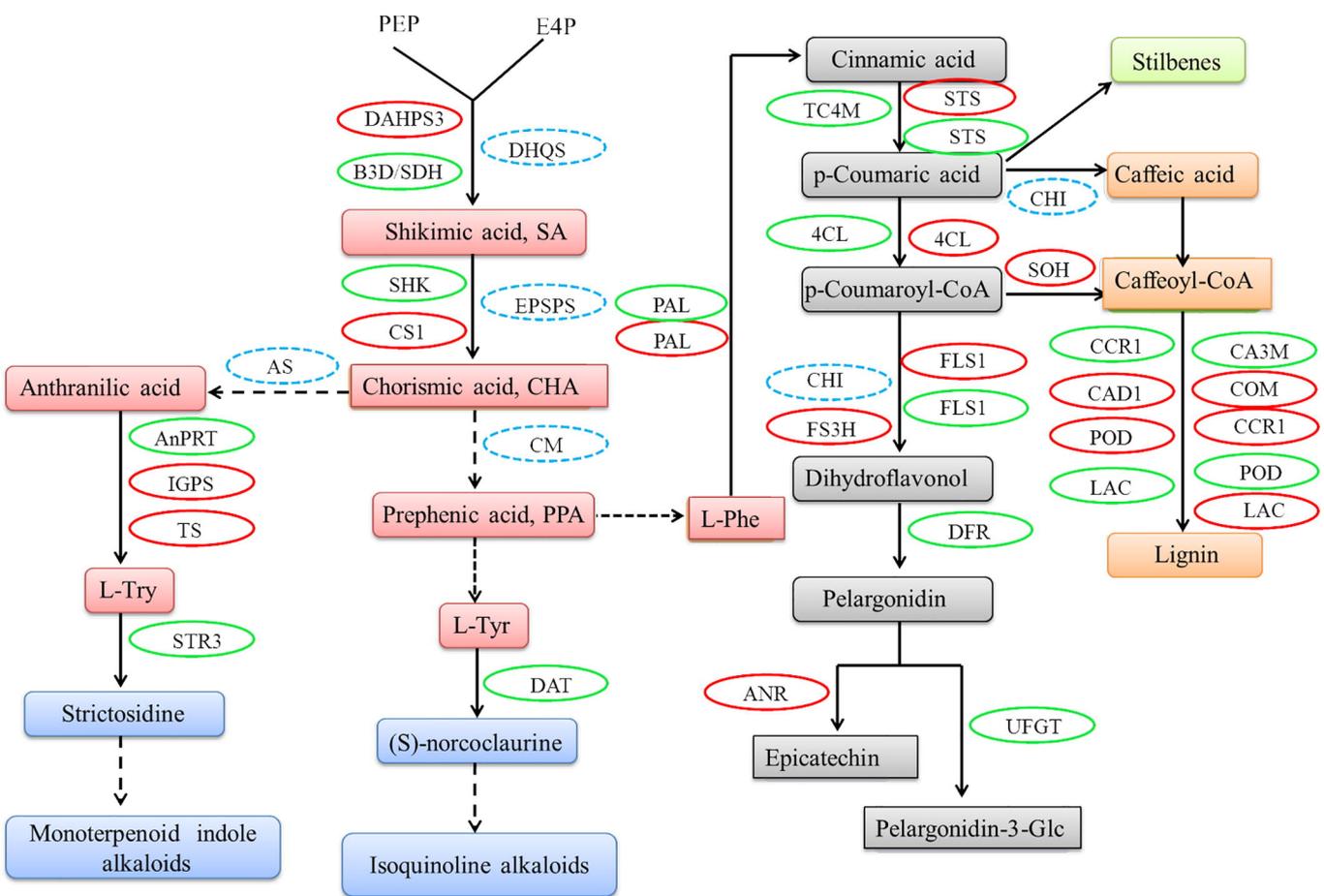
Control

Treatment

Control

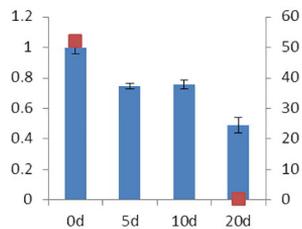
Treatment



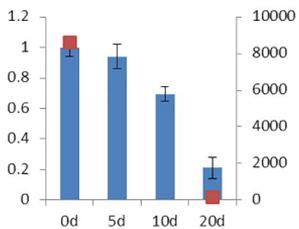


■ qRT-PCR

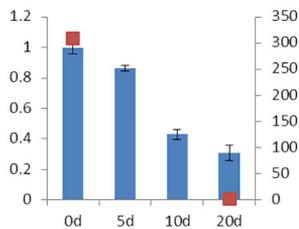
■ RNA-Seq



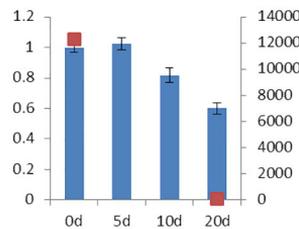
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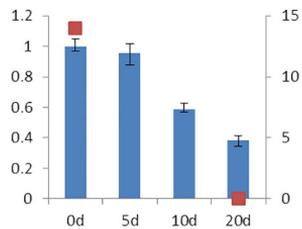
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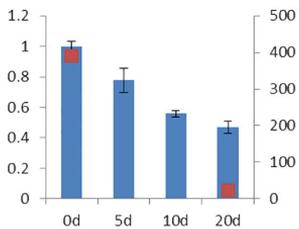
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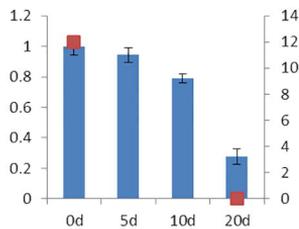
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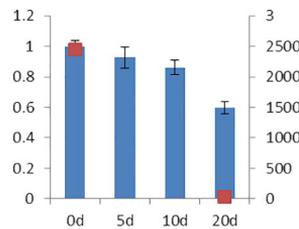
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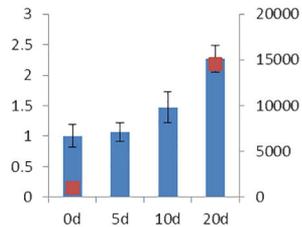
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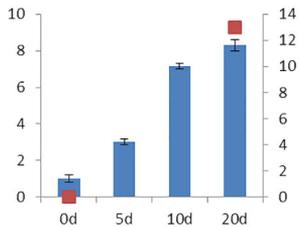
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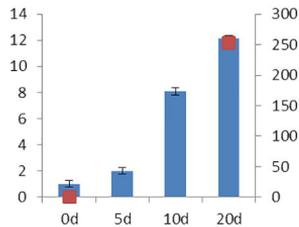
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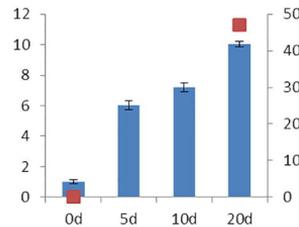
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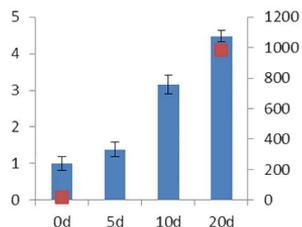
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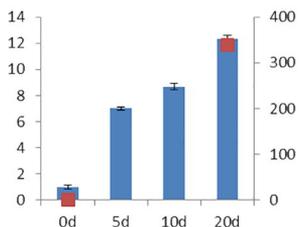
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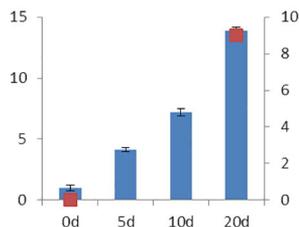
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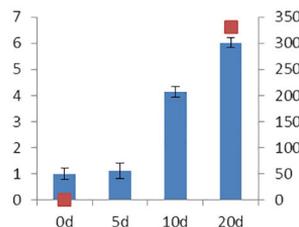
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Length of drought treatment

Relative Expression