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Research article

Physiological and transcriptome analyses of the effects of exogenous dopamine on drought tolerance in apple

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ABSTRACT

Water shortage is one of the main limiting factors in apple (*Malus domestica* Borkh.) production. Although dopamine is produced in plants and has been linked with response to abiotic stress, the underlying mechanism remains unknown. In this study, physiological analyses revealed that pretreatment with 100 μM dopamine alleviated drought stress in apple seedlings. Dopamine inhibited the degradation of photosynthetic pigments and increased net photosynthetic rate under drought stress. Dopamine also reduced H_2O_2 content, possibly through direct scavenging and by mediating the antioxidant enzyme activity. Seedlings pretreated with dopamine had higher sucrose and malic acid contents but lower starch accumulation in their leaves. RNA-Seq analysis identified 1052 differentially expressed genes (DEGs) between non-treated and dopamine-pretreated plants under drought. An in-depth analysis of these DEGs revealed that dopamine regulated the expression of genes related to metabolism of nitrogen, secondary compounds, and amino acids under drought stress. In addition, dopamine may improve apple drought tolerance by activating Ca^{2+} signaling pathways through increased expression of CNGC and CAM/CML family genes. Moreover, analysis of transcription factor expression suggested that dopamine affected drought tolerance mainly through the regulation of WRKY, ERF, and NAC transcription factors.

1. Introduction

Plants under natural conditions often face multiple environmental constraints in terms of submergence, temperature extremes, salinity, and drought stress. Plant growth and productivity is negatively influenced by these abiotic stresses (Ashraf et al., 2018). Recently, a variety of approaches are being used to overcome abiotic stresses in plants (Savvides et al., 2016). Chemical priming is a promising field in plant stress physiology and crop stress management. Use of chemical compounds as priming agents has been found to improve plant tolerance significantly in various crop and non-crop species against a range of different individually applied abiotic stresses. For example, SA treatment increases the resistance of wheat (Triticum aestivum) seedlings to salinity and drought (Shakirova et al., 2003). Exogenous melatonin significantly increases the tolerance of both drought-tolerant *M. prunifolia* and drought sensitive *M. hupehensis* plants (Li et al., 2015b).

The catecholamine dopamine (3,4-dihydroxyphenethylamine) has best been characterized as a neurotransmitter in mammals; it is well known for its role in movement, motivation, reward, metabolism, and hormonal secretion (Acuna et al., 2016; Surmeier et al., 2014; Schultz, 1998; Wang et al., 2018). There have been few studies on dopamine in plants, but existing studies have found that it participates in several physiological and biochemical processes. In duckweed (*Lemna paucicostata*), 100 μM catecholamines induced more floral primordia, improved flower development, and sustained flowers for a longer time period (Khurana et al., 1987). Dopamine has also been shown to inhibit plant senescence and influence nutrient concentrations under drought conditions by regulating the uptake, transport, and resorption of nutrients (Liang et al., 2018a). Some reports suggest that it may interact with plant hormones (Dai et al., 1993; Protacio and Flores, 1992). Dopamine, as a cofactor in monovalent oxygen reduction, led to the production of ethylene in sugar beet leaves (Elstner et al., 1976). In addition, dopamine was identified as a strong water-soluble antioxidant that could suppress the oxygen uptake of linoleic acid and scavenge

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diphenylpicrylhydrazyl radicals (Kulma and Szopa, 2007). Some research suggests that catecholamines are involved in plant responses to environmental stresses by increasing resistance to pathogen infection, freezing damage, and salt stress (Dufeu et al., 2003; Li et al., 2015a; Skirycz et al., 2005).

Apple is one of the most important fruits in the world economy, and its production and consumption are highest in China. Apple production in China is focused in the Loess Plateau region of northwest China where there is abundant sunshine, large diurnal temperature variation, and a deep layer of loose soil. However, production in this area is threatened by water deficit (Yan et al., 2015). Drought stress causes accumulation of intracellular reactive oxygen species (ROS), leading to membrane lipid peroxidation, enzyme dysfunction, and protein oxidation and aggregation (Li et al., 2011; Tsugane et al., 1999). ROS can be scavenged through enzymatic pathways including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) (Hoffman et al., 2012; Sheikh-Mohamadi et al., 2018). In a previous study, application of exogenous dopamine significantly reduced the content of hydrogen peroxide (H_2O_2) and superoxide anion radicals (O^{2-}), consistent with decreased ROS levels (Elstner et al., 1976). In other research, dopamine protected plant against environmental stress by enhancing the antioxidant defense system and promoting ascorbate-glutathione cycle capacity (Guidotti et al., 2013; Li et al., 2015a).

During severe drought, components of the photosynthetic apparatus are easily perturbed by environmental stress. This damage may take the forms of disturbances in chloroplast homeostasis, decomposition of pigment complex, reduced photosynthetic rate, or restricted electron transport (Marian et al., 2004; Zhu, 2016). A previous study showed that dopamine significantly alleviated the decrease in chlorophyll content under salt stress (Li et al., 2015a). In addition, dopamine participates in photosynthetic oxygen reduction and the photophosphorylation of chloroplasts (Allen, 2003; Roshchina, 1990). When photosystem 2 (PS2) is blocked by DCMU (photosynthesis inhibitor), dopamine can reconstruct electron transport to $NADP^+$, whose donor capability plays a role in the mechanism of chloroplast adaptation under stress (Roshchina, 1990). Furthermore, exogenous dopamine significantly increases net photosynthetic rates and maximum photochemical efficiency (F_V/F_M), enabling plants to maintain their photosynthetic capacity (Li et al., 2015a).

Water deficit increases the content of soluble carbohydrates in plants; these may promote drought tolerance both as compatible solutes and antioxidants (And and Hitz, 1982; Smirnoff and Cumbes, 1989). Carbohydrates protect proteins and membranes against dehydration by inducing glass formation, and polyhydroxyl components can replace the structural water (Guy, 1990). A previous study showed that expression of the human dopamine receptor gene in transgenic plants resulted in changes in soluble sugar and starch content (Skirycz et al., 2005). Transgenic plants overexpressing the dopamine synthesis gene, tyrosine decarboxylase (TD), showed increased glucose and sucrose content and decreased starch content. These observations suggest that catecholamines may increase stress tolerance in plants by promoting accumulation of small, osmotically active carbohydrates.

However, the molecular mechanisms underlying dopamine-mediated drought resistance are still unclear. Quantifying the whole set of transcripts under specific physiological and ecological conditions is essential for understanding the regulatory mechanisms involved in adapting to these conditions (Davila et al., 2016). RNA-Seq method is a rapid technique employed profiling of genome-wide gene expression to identify key genes associated with specific situations such as abiotic and biotic stresses in plants (Lu et al., 2014; Wang et al., 2009). Hence, in addition to physiological experiments, RNA-Seq method was used to analyze the effects of exogenous dopamine on drought tolerance in apple.

2. Materials and methods

2.1. Plant material and experimental designs

Two-year-old 'Changfu 2' (*Malus domestica* Borkh. cv. *Naganofuji* No. 2) apple scions grafted onto *Malus hupehensis* rootstocks were grown in pots (size 30 × 26 × 22 cm). Grafted seedlings were cultured at a horticultural farm in Yangling, Shaanxi, China (34°20′N, 108°24′E).

At the initiation of the study (September 2014), 160 uniform trees approximately 1.0 m tall were chosen. Half were irrigated with nutrient solution containing 100 μM dopamine for 10 d. The concentration of dopamine used in this experiment was determined by a concentration gradient analysis performed in a previous trial that showed this level to be quite effective in alleviating the effects of stress on apple (Li et al., 2015a; Liang et al., 2017). Non-treated control and dopamine-treated plants were randomly assigned to normally watered plants and drought-stressed plants that were withheld for 10 d. Therefore, this was divided into four groups: (1) non-treated, normally watered control plants (CK); (2) non-treated, drought-stressed plants (DR); (3) dopamine-treated, normally watered plants (DA); and (4) dopamine-treated, drought-stressed plants (DADR). There were 40 plants in each group. Materials for testing were immediately collected and frozen in liquid nitrogen, then stored at −80 °C.

2.2. Physiological analysis

2.2.1. Relative water content (RWC) and relative electrolyte leakage (REL)

RWC was calculated according to Gaxiola et al. (2001). Fresh leaves were weighed, then soaked in deionized water overnight. After measuring the weight, the leaves were oven-dried overnight at 70 °C and the RWC was calculated. REL was measured according to Dionisio-Sese and Tobita (1998).

2.2.2. Measurement of net photosynthesis rate (P_n), intercellular CO_2 concentration (Ci), stomatal conductance (g_s) and instantaneous water-use efficiency (WUEi)

Photosynthetic parameters (Pn, Ci, g_s and WUEi) were measured using a portable infrared gas analysis system (Li-6400; LICOR, Lincoln, NE, USA) on sunny days during morning hours from 9 to 11 a.m. For each measurement, 10 fully mature leaves were measured from different seedlings per treatment.

2.2.3. Determination of photosynthetic pigments

For each measurement, photosynthetic pigments from fresh leaves were extracted with 80% acetone. The contents were measured by spectrophotometry using previously reported methods (Arnon, 1949).

2.2.4. H_2O_2 content and antioxidant enzyme activities

H_2O_2 in leaves was extracted and measured according to the method described by Patterson et al. (1984).

SOD activity in leaves was measured according to Giannopolitis and Ries (1977). POD activity was determined using guaiacol and hydrogen peroxide as substrates of the reaction and measured at 470 nm (Chance and Maehly, 1955). The activity of CAT was assayed at 240 nm by monitoring the decrease due to the decomposition of H_2O_2 (Chance and Maehly, 1955). The activity of APX was determined at 290 nm according to Nakano and Asada (1981).

2.2.5. Measurements of carbohydrate

Soluble carbohydrates (sorbitol, sucrose, glucose, galactose and malic acid) were extracted by the method previously described (Wang et al., 2010; Lisec et al., 2006). A Shimadzu GCMS-2010SE (Shimadzu Corporation, Kyoto, Japan) was then used to analyze carbohydrates after derivatization. Carbohydrates were identified by comparing the fragmentation patterns with the annotated quadrupole GC/MS spectral library downloaded from Golm Metabolome Database and the mass

spectral library generated from GC/MS system. Metabolite measurements were based on standard curves and internal standards for each metabolite. Starch was measured using colorimetric assay kits.

2.3. Transcriptome analysis

2.3.1. Total RNA extraction, library construction and illumina sequencing

Total RNA was extracted from leaves according to Chang et al. (1993). RNA quality and quantity were evaluated using a Nanodrop spectrophometer and gel electrophoresis. RNA purity was evaluated using NanoPhotometer®spectrophotometer (Implen, CA, USA). RNA concentration was determined in a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was evaluated using a 2100 Bioanalyzer (Agilent, CA, USA). The total RNA was subjected to mRNA purification using magnetic beads linked with oligo (dT). The mRNA molecules were fragmented into 400–600 bp fragments and used as templates for first cDNA strand with dNTP, RNaseH, a six-base random primer, and buffer. The second cDNA strand was synthesized using DNA polymerase I. The double-stranded cDNA was purified and repaired at the end. The A-tail was added and the sequencing adapters were connected to the end of cDNAs. Then 400–600 bp fragments were selected using agarose gel electrophoresis. After PCR amplification, the library was sequenced using Illumina HiSeq™ 2500.

2.3.2. Analyses of differentially expressed genes (DEGs)

Gene expression was measured as Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values. The DESeq R package was used to identify DEGs (fold changes ≥ 2 and false discovery rate (FDR) adjusted P-value < 0.05) (Anders and Huber, 2010). Gene Ontology (GO) terms were assigned from the Gene Ontology Database (http://www.geneontology.org/). Pathway enrichment analyses in plants were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) web server (http://www.genome.jp/kegg/) (Storey and Tibshirani, 2003).

2.3.3. Quantitative real-time PCR (qRT-PCR) analysis

The qRT-PCR was performed using SYBR Green qPCR kit (TaKaRa, Tokyo, Japan) and the iQ5 Multicolor Detection System (Bio-Rad Laboratories, Hercules, USA). Thermal cycling included an initial 60 s at 95 °C; then 40 cycles of 10 s at 95 °C, 30 s at 60 °C; followed by 10 s at 95 °C, 60 s at 65 °C, 1 s at 97 °C; and finally 30 s at 37 °C. The elongation factor 1 alpha gene (*EF-1α*; DQ341381) in *Malus* was used as a standard. We previously compared apple *EF-1α*, *actin*, and 18S rRNA as internal controls and found that *EF-1α* was more stable as a reference gene under saline conditions (Wang et al., 2012). All specific primer sequences for carbohydrate metabolic enzyme genes and selected DGE genes are listed in Supplementary Table S1. The melting curve was analyzed to test the primers' suitability to monitor the identity and specificity of the qRT-PCR products.

2.4. Statistical analysis

All data in this study were analyzed with SPSS software (version 17.0) and presented as means ± standard deviation (SD). Significant analysis was analyzed by one-way ANOVA and Tukey's tests, and $P < 0.05$ represented significant difference.

3. Results

3.1. Physiological analysis

3.1.1. Dopamine enhanced drought stress tolerance

100 μM dopamine pre-treatment alleviated apple leaf wilting under drought stress (Supplementary Fig. S1). RWC values for apple leaves were significantly reduced under drought stress. However, dopamine pre-treatment substantially alleviated this response over the stress period (Fig. 1A). Simultaneously, REL was significantly increased in untreated apple plants under drought stress but noticeably reduced in dopamine-pretreated plants under the same conditions (Fig. 1B). Thus, dopamine increased RWC and reduced REL under drought stress.

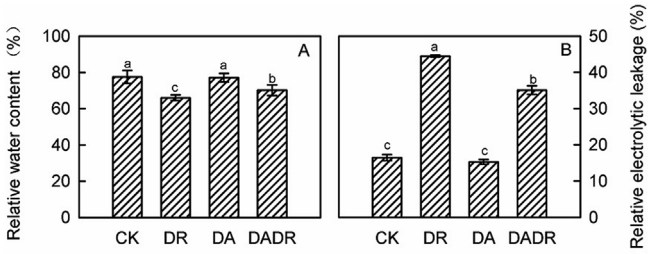

Fig. 1. Effects of dopamine on relative water content (A) and relative electrolytic leakage (B) under control and drought conditions (CK, control plants; DR, plants without dopamine pre-treatment under drought conditions; DA, plants pre-treated with dopamine in the absence of drought, and DADR, dopamine-pretreated plants exposed to drought). Values are means of five replicates ± SD. An ANOVA test and Tukey's test was performed. Different letters indicate significantly difference ($P < 0.05$).

3.1.2. Photosynthesis and photosynthetic pigments

Under drought conditions, net photosynthesis (P_n) decreased throughout the treatment period. However, at each time point, P_n was significantly higher in dopamine-pretreated plants than in non-treated plants (Fig. 2A). Stomatal conductance (g_s) declined rapidly after 4 days of drought in both drought-stressed groups, but values were significantly higher in dopamine-treated plants (Fig. 2B). The Ci values of dopamine-pre-treated plants were higher than those of untreated seedlings in early stages of drought, but lower in later stages (Fig .2C). In later stages of drought, WUEi values of dopamine-pretreated plants were higher than those of untreated plants (Fig. 2D).

Chlorophyll and other photosynthetic pigments decreased under drought conditions. As shown in Fig. 3, the content of chlorophyll *a* (chl a), chl a/b, total chlorophyll (chl t), and carotenoid (Car) decreased significantly in untreated plants under drought stress. However, 100 μM dopamine pretreatment significantly inhibited the degradation of photosynthetic pigments, and the contents of various chlorophyll forms did not differ significantly from those of unstressed control plants.

3.1.3. H_2O_2 content and antioxidant activities

Drought stress accelerated the accumulation of H_2O_2 in leaves

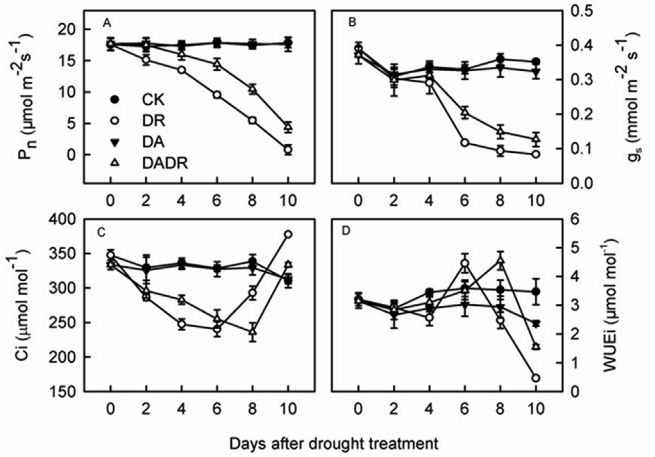

Fig. 2. Changes in net photosynthesis (P_n) (A); stomatal conductance (g_s) (B); intercellular CO_2 concentration (Ci) (C); and instantaneous water-use efficiency (WUEi) (D) under control and drought conditions. Values are means of five replicates ± SD. An ANOVA test and Tukey's test was performed.

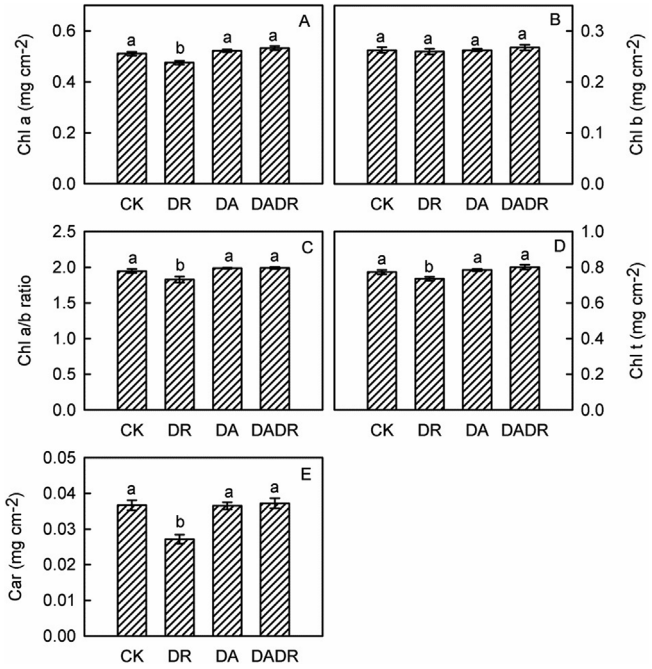

Fig. 3. Effects of 100 μM dopamine pretreatment on chlorophyll status under drought: Chl a (A), Chl b (B), Chl a/b ratio (C), total chlorophyll (Chl t; D), and carotenoid (Car; E). Values are means of five replicates ± SD. An ANOVA test and Tukey's test was performed. Different letters indicate significantly difference ($P < 0.05$).

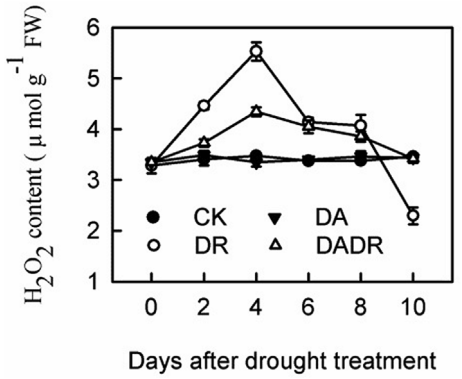

Fig. 4. Effects of dopamine on H_2O_2 contents in leaves under drought. Values are means of five replicates ± SD. An ANOVA test and Tukey's test was performed.

(Fig. 4). In non-treated control plants, H_2O_2 content reached a peak of ~160% of the initial levels on the fourth day after the onset of drought conditions, and then declined thereafter. Leaf H_2O_2 content in dopamine pre-treated plants showed a similar pattern during the drought, but H_2O_2 content was lower at all time points than in non-treated plants, with a peak on Day 4 of ~130% of initial levels, significantly lower than in non-treated plants. On the tenth day of drought treatment, H_2O_2 content in non-treated plants under drought was lower than that of unstressed control plants. Loss of physiological activity in untreated, drought-stressed plants may have reduced their ability to synthesize H_2O_2.

The activities of antioxidant enzymes were affected by drought but showed different patterns of activity in non-treated and dopamine-treated plants (Fig. 5). Under drought conditions, SOD activity in non-treated control plants initially decreased, and then increased. However, in dopamine-treated plants, SOD activity increased steadily (Fig. 5A). Drought caused a rapid increase in POD activity (Fig. 5B). In untreated

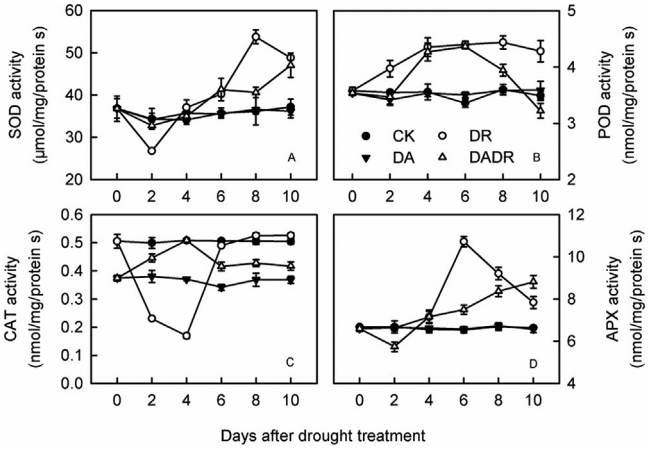

Fig. 5. Effects of dopamine on the main antioxidative enzymes activities in leaves under drought: (A) SOD, (B) POD, (C) CAT, (D) APX. Values are means of five replicates ± SD. An ANOVA test and Tukey's test was performed.

plants, POD activity remained relatively high; in contrast, dopamine pre-pretreated plants suppressed POD activity later in the drought period. CAT activity decreased under drought stress and then recovered to control level (Fig. 5C). In addition, CAT activity of dopamine-pretreated plants was higher than that of non-treated plants throughout the measurement period. APX activity first increased and then decreased under drought (Fig. 5D). After exogenous dopamine treatment, APX activity decreased slightly and then increased steadily.

3.1.4. Carbohydrate content and carbohydrate metabolism-related genes expression

The content of soluble carbohydrates in apple leaves was determined. As shown in Fig. 6A, sorbitol content showed no significant difference between dopamine-treated and nontreated control plants

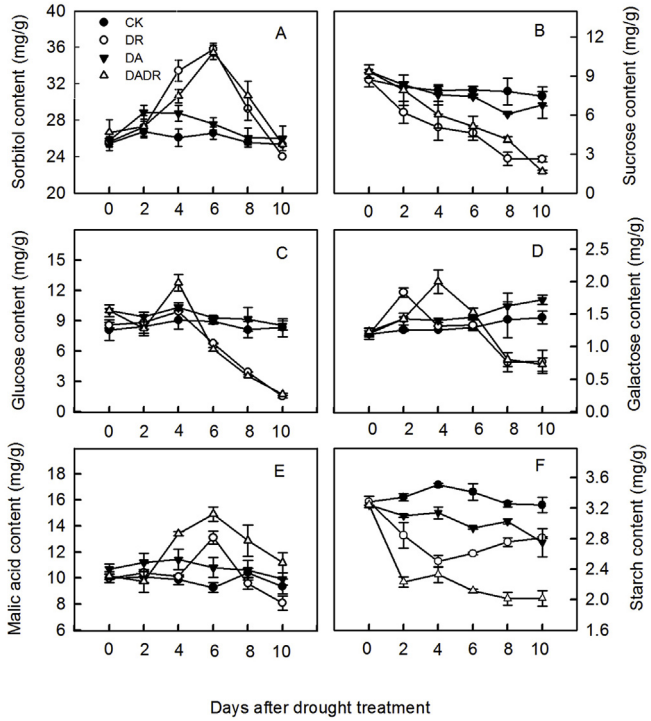

Fig. 6. Effects of dopamine on carbohydrates content in leaves under drought: sorbitol (A), sucrose (B), glucose (C), galactose (D), malic acid (E), starch (F). Values are means of five replicates ± SD. An ANOVA test and Tukey's test was performed.

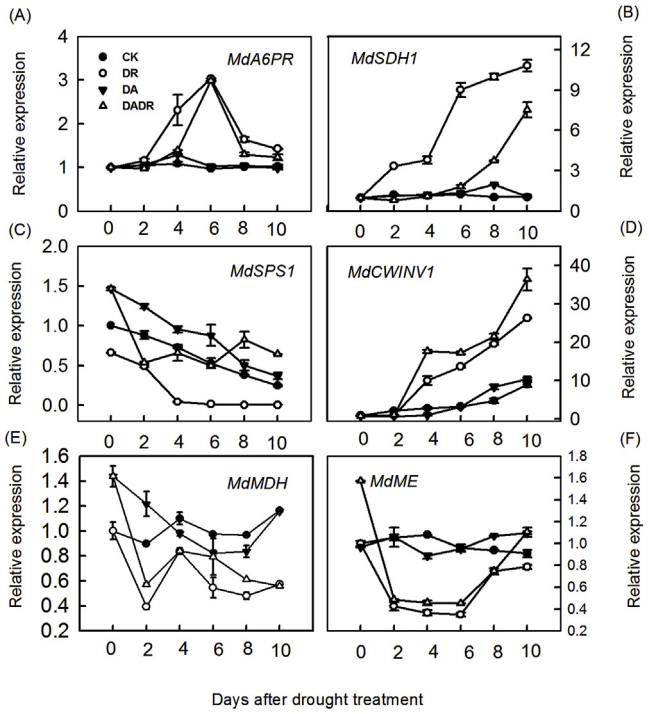

Fig. 7. Effects of dopamine on expression of carbohydrate metabolism-related genes: aldose-6-phosphate reductase (*MdA6PR*) (A), sorbitol dehydrogenase (*MdSDH1*) (B), sucrose phosphate synthase (*MdSPS1*) (C), cell wall invertase (*MdCWINV1*) (D), malate dehydrogenase (*MdMDH*) (E), malic enzyme (*MdME*) (F). Values are means of five replicates ± SD. An ANOVA test and Tukey's test was performed.

except on the fourth day. Dopamine increased sucrose content throughout the drought treatment (Fig. 6B). In Fig. 6C, the trend in glucose levels is similar to that of sorbitol. Glucose reached its peak on Day 4, and dopamine significantly increased glucose content. Dopamine-pre-treated plants showed increased galactose content in early drought, with the value reaching a peak on the fourth day, whereas non-treated plants reached a peak on the second day (Fig. 6D). Under drought, dopamine increased malic acid content but decreased starch content (Fig. 6 D and E). These results suggested that dopamine may promote sucrose and malic acid accumulation and starch degradation under drought stress.

The expression of genes encoding aldose-6-phosphate reductase (*MdA6PR*), sorbitol-dehydrogenase (*MdSDH1*), sucrose phosphate synthase (*MdSPS1*), cell wall invertase (*MdCWINV1*), malate dehydrogenase (*MdMDH*), and malic enzyme (*MdME*) was examined. In the early stages of drought, expression of *MdA6PR* was lower in dopamine-pretreated plants than in untreated plants (Fig. 7A). However, both groups of plants had similar *MdA6PR* expression levels in the later stages of drought. The expression of *MdSDH1* was lower and the expression of *MdSPS1* was higher in dopamine-pretreated plants under drought (Fig. 7B and C). Interestingly, *MdSPS1* expression showed a significant downward trend under unstressed conditions. Compared with untreated plants, dopamine-pretreated plants had higher expression of *MdCWINV1* under drought (Fig. 7D). Throughout the drought sampling period, dopamine increased the expression of *MdMDH and MdME* (Fig. 7E and F). These results suggest that dopamine can significantly influence the expression of some plant carbohydrate metabolism genes during drought stress.

3.2. Transcriptome analysis

3.2.1. Sequencing of mRNA and alignment to reference genome

To gain insight into the effects of exogenous dopamine on drought tolerance in apple, we performed transcriptional profiling of leaves from treatments described above, using plants exposed to drought or control conditions for three days. As shown in Table 1, four cDNA libraries (three replicates per library) were constructed. RNA-Seq of these libraries generated approximately 44–80 million clean reads under quality control and raw read filtering per sample. Most of the reads (> 70%) from the twelve libraries were further analyzed by comparison to the apple genome. Clean reads from every library showed a match rate of approximately 72% to the apple genome, indicating that the sequencing data could be used for subsequent transcriptome analysis.

3.2.2. Comprehensive profiling of transcript expression analysis

Analysis of DEGs was performed between every two groups (CK/DA, CK/DR, DA/DADR, and DR/DADR) based on FPKM with thresholds FDR < 0.05 and FC > 2. There were 414 DEGs (260 up- and 154 down-regulated) between CK and DA, 726 DEGs (539 up- and 187 down-regulated) between CK and DR, and 1195 DEGs (888 up- and 307 down-regulated) between DA and DADR. There were 1052 DEGs between DR and DADR, comprising 643 up-regulated and 409 down-regulated DEGs (Fig. 8A). There were 21 up-regulated DEGs, but there were no up-regulated DEGs in all groups (Fig. 8B). The number of up-regulated DEGs was higher than that of down-regulated DEGs across all four comparisons. Furthermore, 51 DEGs were up-regulated and no DEGs were down-regulated in both CK vs DR and DR vs DADR (Fig. 8B).

3.2.3. Gene ontology (GO) analyses under drought stresses

To explore how exogenous dopamine functions under drought stress, we performed GO analyses, focusing on differences between DR group and DADR group gene function. There were 617 DEGs, 638 DEGs and 372 DEGs were enriched into “biological process”, “cellular component” and “molecular function” respectively (Fig. 9, Supplementary Table S2). The main biological process categories were “metabolic process”, “cellular process”, “single-organism process”, “biological regulation”, and “response to stimulus”. DEGs in the molecular function

Table 1
Summary of RNA-Seq reads mapped to the apple genome.

Sample	Total Reads	Unmapped Reads	Unique Mapped Reads	Multiple Mapped reads	Mapping Ratio
CK-1	81714738	21871576 (26.77%)	53408732 (65.36%)	6434430 (7.87%)	73.23%
CK-2	44588916	12341425 (27.68%)	28658321 (64.27%)	3589170 (8.05%)	72.32%
CK-3	53227118	14741926 (27.70%)	34199274 (64.25%)	4285918 (8.05%)	72.30%
DA-1	44635202	12470451 (27.94%)	28592097 (64.06%)	3572654 (8.00%)	72.06%
DA-2	50345996	14174498 (28.15%)	32250152 (64.06%)	3921346 (7.79%)	71.85%
DA-3	47826248	13405500 (28.03%)	30628078 (64.04%)	3792670 (7.93%)	71.97%
DR-1	44440286	12318588 (27.72%)	28489838 (64.11%)	3631860 (8.17%)	72.28%
DR-2	51550440	14397765 (27.93%)	32980457 (63.98%)	4172218 (8.09%)	72.07%
DR-3	42883378	11977081 (27.93%)	27387349 (63.86%)	3518948 (8.21%)	72.07%
DADR-1	59102854	17241602 (29.17%)	37259460 (63.04%)	4601792 (7.79%)	70.83%
DADR-2	61992734	17892178 (28.86%)	39205918 (63.24%)	4894638 (7.90%)	71.14%
DADR-3	69440856	20049263 (28.87%)	43799705 (63.07%)	5591888 (8.05%)	71.13%

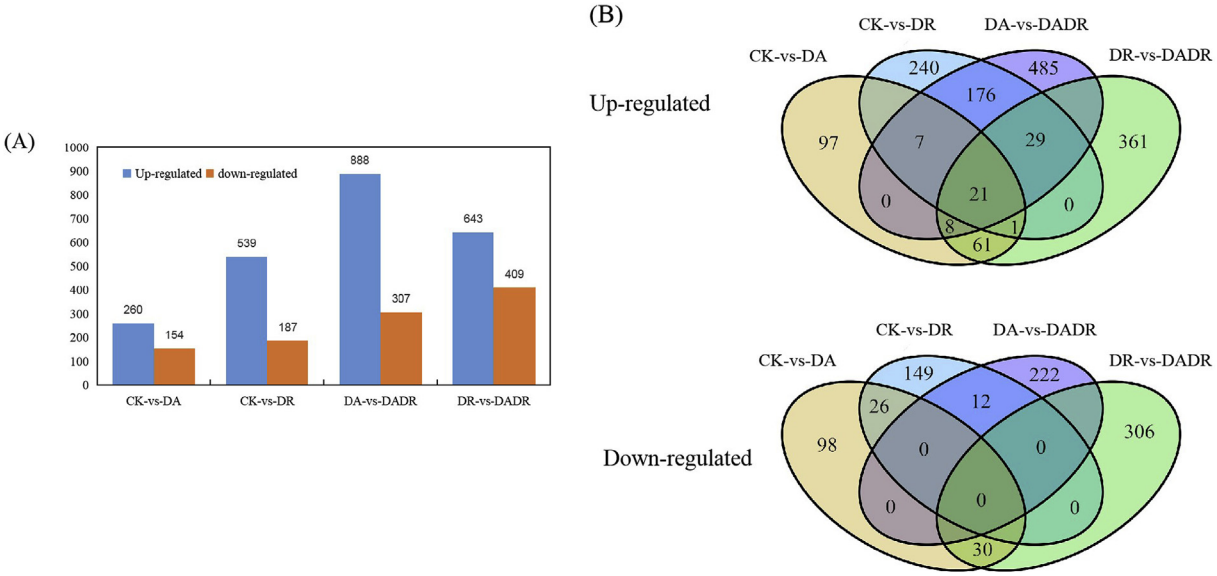

Fig. 8. Number of DEGs among the four comparison groups (A) and venn diagram showed overlap of up- and down-regulated DEGs in Pana (B).

category were related to “catalytic activity”, “binding”, “transcription factor activity”, and “transporter activity”. Most DEGs assigned to the “cell”, “cell part”, “organelle”, “membrane”. These results highlighted the involvement of dopamine in cellular metabolism, biological regulation, and response to stimulus under drought stress.

3.2.4. KEGG pathways enrichment analysis under drought stresses

To further analyze metabolic pathways and study gene functions, we identified 234 DEGs that were enriched into 95 KEGG pathways (Supplementary Table S3). The top 20 most obviously enriched pathways are shown in Fig. 10. The DEGs were enriched for “Nitrogen metabolism”, “Plant-pathogen interaction”, “Ribosome biogenesis in eukaryotes”, “Taurine and hypotaurine metabolism”, “Isoquinoline alkaloid biosynthesis”, “photosynthesis”, “Glyoxylate and dicarboxylate metabolism”, “Tyrosine metabolism”, “Glycosaminoglycan degradation”, “Thiamine metabolism”, “Lysine biosynthesis”, “Diterpenoid

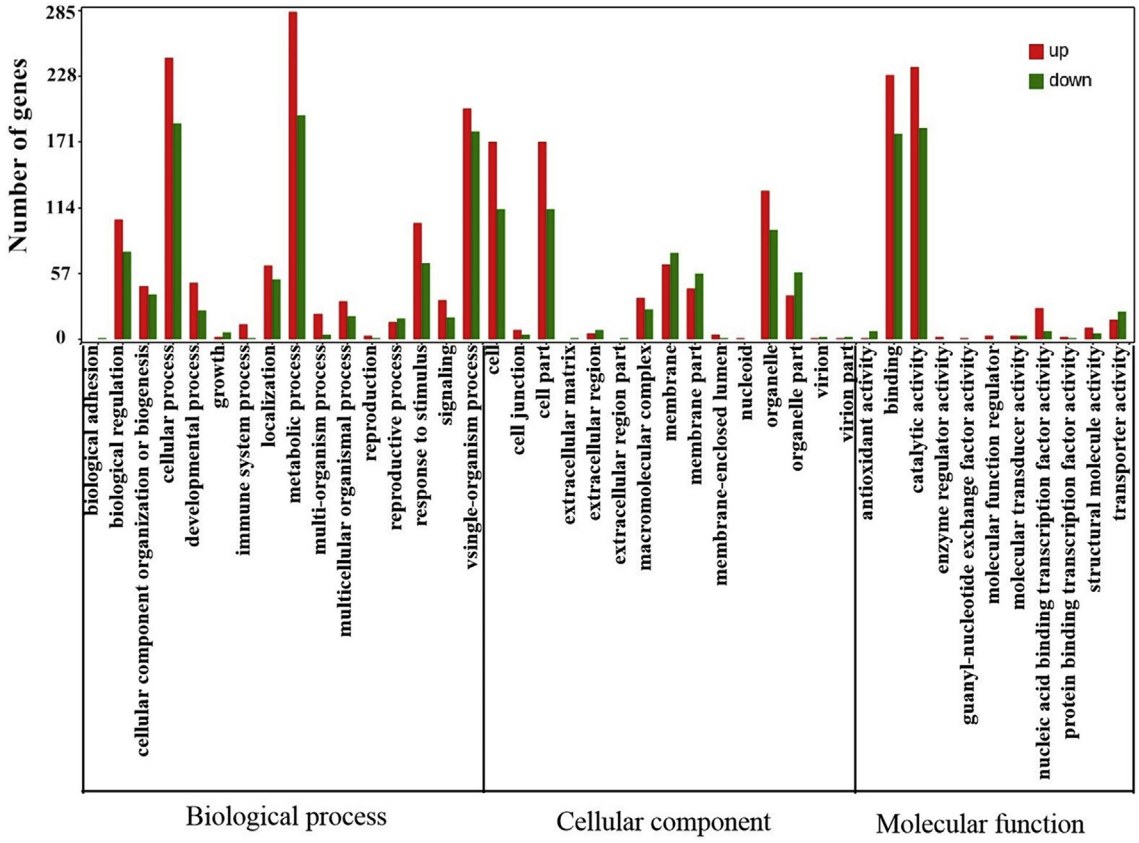

Fig. 9. GO annotation of DEGs between the DR and DADR. The x-axis indicates the GO classifications, and the Y-axis indicates the number of genes in each classification.

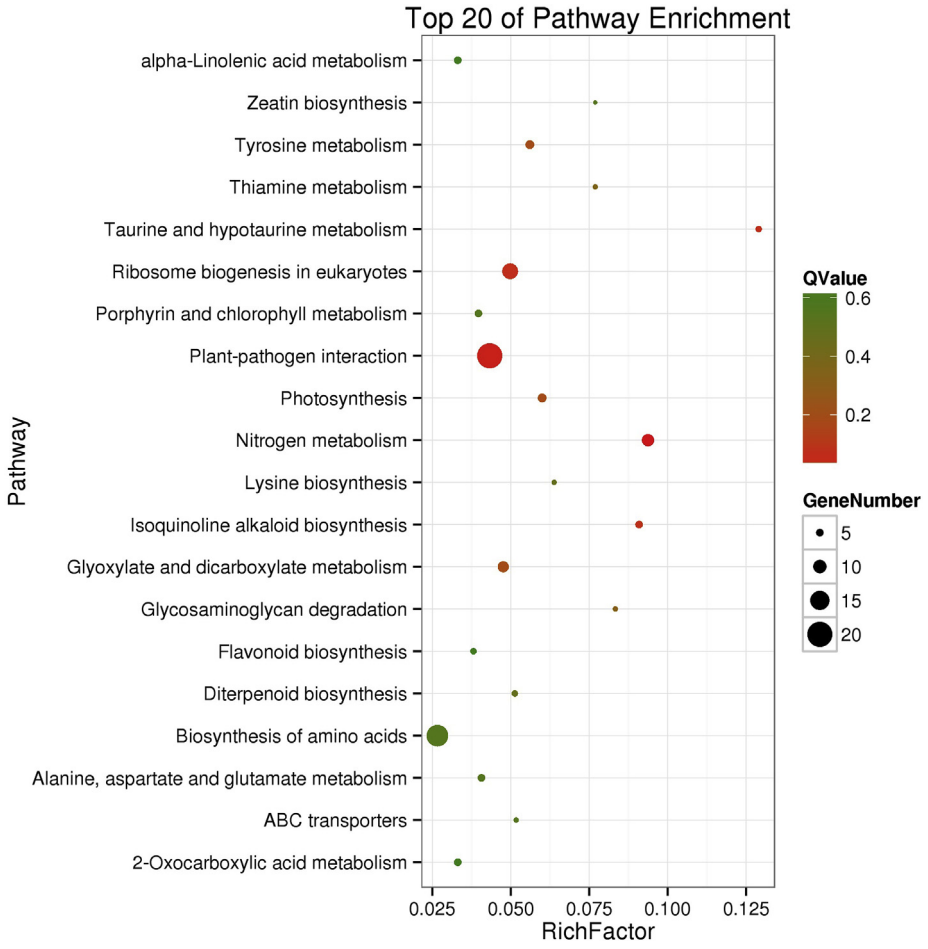

Fig. 10. KEGG enrichments of DEGs between the DR and DADR. The X-axis shows the rich factor. Green represents a high q value, and red represents a low q value. The Y-axis shows the top 20 KEGG pathways. The bigger size of spot, the more DEGs enriched. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

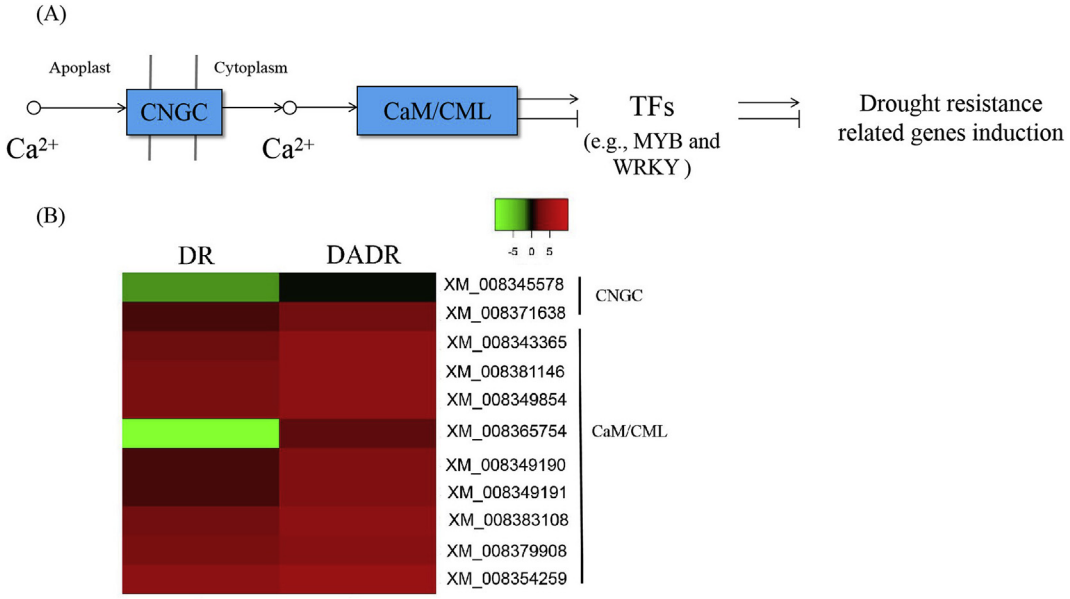

Fig. 11. Ca^{2+} signaling in response to osmotic stress (A) and a heat map of expression levels of CNGC and CAM/CML. The expression levels were assessed by log2-transformed FPKM values.

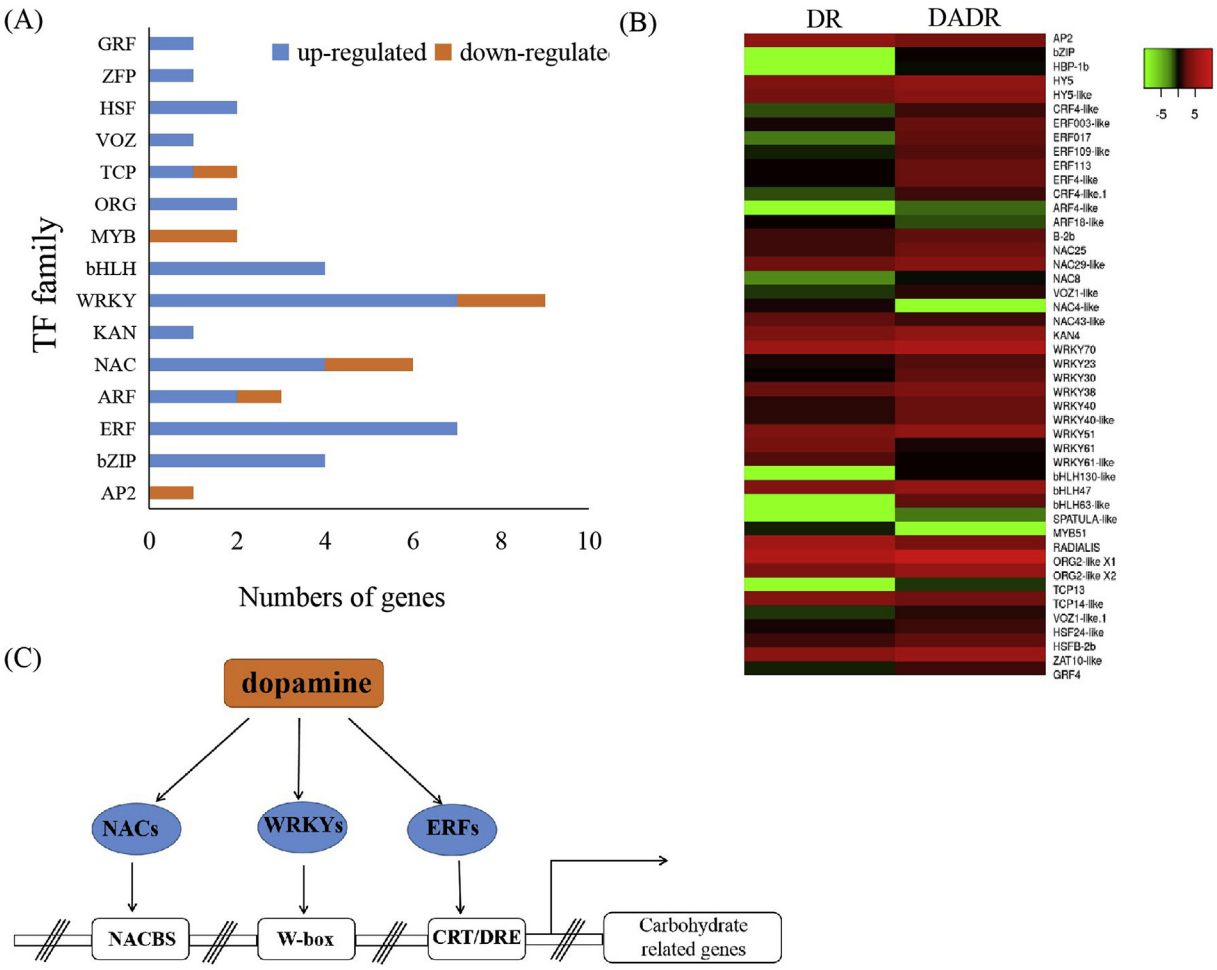

Fig. 12. The numbers of differentially expressed TFs between the DR and DADR (A), a heat map was constructed based on log2-transformed FPKM expression values of each TF (B), and regulation of dopamine on cis elements of carbohydrate related gene promoters under drought (C).

biosynthesis", "Alanine, aspartate and glutamate metabolism", "Zeatin biosynthesis", "porphyrin chlorophyll metabolism", "ABC transporters", "Biosynthesis of amino acids", "Flavonoid biosynthesis", "2-Oxocarboxylic acid metabolism" and "alpha-Linolenic acid metabolism". In addition, the most DEGs were enriched into "plant-pathogen interaction" pathway in this study, with a total of 20 DEGs. Specifically, 11 of these DEGs were mainly involved in Ca^{2+} signaling pathway, and all of them were significantly up-regulated (Supplementary Table S4, Fig. 11A and B). Two belonged to CNGC (cyclic nucleotide gated channel) gene family and nine belonged to CaM/CML (camlmodulin/calmodulin-like protein) gene family.

3.2.5. Differences in transcription factors (TFs) expression between DR and DADR

Transcription factors play an important role in abiotic stress via gene regulatory networks. Compared with DR group, a total of 46 TFs were differentially expressed in DADR group: 37 up-regulated and 9 down-regulated, from 15 different families (Fig. 12A and B, Supplementary Table S5). Most of the differentially-expressed TFs participate in drought stress response and the majority derived from the WRKY, ERF, and NAC families. All of these TFs have been previously characterized as regulating drought resistance. By analyzing the cis elements in carbohydrate related gene promoters region, there are many binding sites of WRKY, NAC and ERF (Fig. 12C, Supplementary Fig. S2). Dopamine may regulate the expression of carbohydrate related genes by regulating these TFs under drought.

3.2.6. Validation of the RNA-Seq results by RT-qPCR

To validate the accuracy of the RNA-Seq results, we manually selected 20 DEGs for RT-qPCR determination. As shown in Fig. 13, their expression profiles obtained via RT-qPCR were broadly consistent with those from the RNA-Seq data. These findings confirmed the reliability of the RNA-Seq results and reflected the actual transcriptome changes in this study.

4. Discussion

4.1. Photosynthesis and chlorophyll analysis

Plants acquire carbon and energy through photosynthesis, a process that is extremely sensitive to environmental stress. Stress damages pigment complexes, restricts electron transport, destroyed chloroplast structures, and reduces photosynthetic rates (Li et al., 2018; Marian et al., 2004; Shasha et al., 2015). Under drought stress, net photosynthesis decreases due to the lower in chlorophyll concentrations and reduced stomatal conductance (Bohnert and Jensen, 1996; Liang et al., 2018b). In the present study, dopamine increased net photosynthetic rate in apple seedlings under drought. In addition, exogenous dopamine increased g_s and Ci in drought-stressed plants. These results suggest that dopamine can significantly increase P_n by reducing stomatal limitation. WUEi is an important indicator of plant adaptation and resistance to drought (Shasha et al., 2015). In this study, higher WUEi was observed in dopamine-pretreatment plants and may have been a factor in their increased drought tolerance. The decrease of photosynthetic rate is

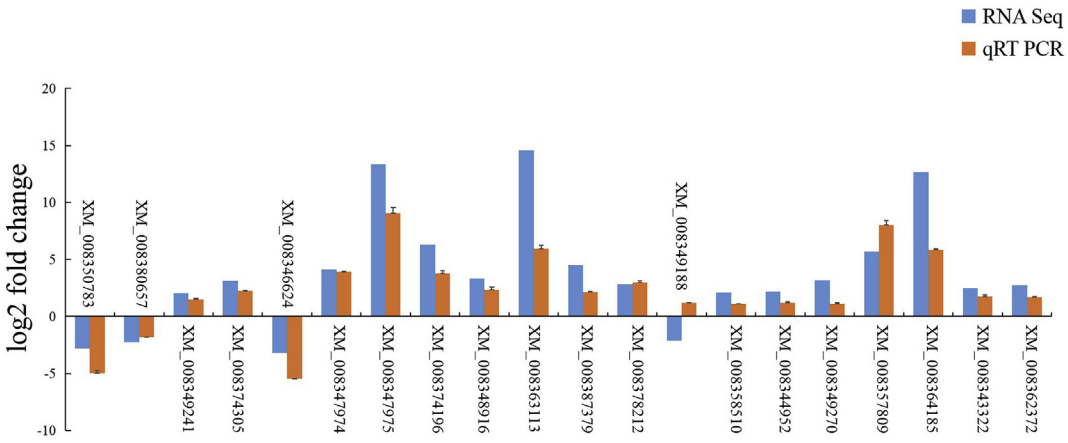

Fig. 13. Validation of the RNA-Seq results by qRT-PCR. Data are presented as the means of three replicates.

related to leaf photosynthetic pigments disturbance (Bai et al., 2013). Our experimental data showed that dopamine increased Car, Chl a, and Chl t content, keeping plants green and protecting plants from drought damage. Thus, dopamine improved plant drought resistance through multiple routes: reducing stomatal limitation, protecting chlorophyll from degradation, and increasing photosynthetic capacity.

4.2. H_2O_2 content and antioxidant activities analysis

Drought can also cause an imbalance in osmotic potential in plant tissues and increase the production of ROS (Jakab et al., 2005). Chloroplast is a major site of ROS production, including hydrogen peroxide, superoxide anion, hydroxyl radical, and singlet oxygen (Mignolet-Spruyt et al., 2016). ROS accumulation is mainly caused by gas exchange reduction, resulting in CO_2 limitation, O_2 accumulation and over-reduction of the photosynthetic electron transport chain during photorespiration (Miller et al., 2010). The generation of ROS also leads to the oxidation of amino acids and burst proteins structure (Kabiri et al., 2014). When exposed to drought stress, plants pretreated with the chemical agents have been shown to have lower ROS accumulation (Savvides et al., 2016). The mitigation of oxidative stress as a result of reduced ROS accumulation following chemical priming has been related either to the enhanced capacity of the antioxidant machinery or to the direct scavenging of ROS by the agents themselves (e.g., Melatonin) (Li et al., 2015b). Studies have shown that dopamine is also a similar antioxidant (Kulma and Szopa, 2007). Under salinity stress, it has been shown that exogenous dopamine may significantly reduce the content of H_2O_2 both by increasing the activities of antioxidative enzymes and by directly scavenging ROS (Li et al., 2015a). Similarly, our studies showed that dopamine inhibits the production of H_2O_2 under drought stress. To prevent oxidative damage, plants invoke the antioxidant defense system, including SOD, CAT, POD, APX and other enzymes (Hoffman et al., 2012; Sheikh-Mohamadi et al., 2018). As a very important antioxidant enzyme, APX controls the level of ROS via the ascorbate-glutathione cycle that transforms H_2O_2 to H_2O and O_2 (Mh, 2008; Sekmen et al., 2012). In the present study, APX activity of dopamine-pre-treated plants was remarkably higher than that of non-treated plants during the drought, compared with other antioxidant enzymes. Therefore, we speculate that the ability of dopamine to scavenge H_2O_2 in apple may be mainly related to its regulation of APX activity. Previous studies have shown that dopamine is oxidized to melanin, a powerful scavenger of ROS (Rosel et al., 1994). In addition, the activity of dopamine is similar to that of ascorbic acid, whose radical scavenging rate is faster than that of catechin, and similar to that of gallocatechin gallate (Kanazawa and Sakakibara, 2000). Therefore, dopamine can mitigate drought-induced ROS damage either directly or through antioxidant enzymes.

4.3. Carbohydrate metabolism analysis

Carbohydrates produced by photosynthesis not only promote plant growth, but also act as signals to regulate physiology and development (Meng et al., 2018a, 2018b). Accordingly, under low temperature and drought conditions, changes in carbohydrates can affect cell osmoregulation and plant environmental adaptation (Gibson, 2000; Jaspers and Kangasjarvi, 2010; Shulaev et al., 2008; Singh et al., 2018). Kulma and Szopa (2007) reported that catecholamines regulate the metabolism of some carbohydrates involved in many aspects of growth and development. Carbohydrate levels changed in plants that transformed with dopamine synthesis genes, suggesting that catecholamines are linked with sugar metabolism (Swiedrych et al., 2004). Previous research has shown that drought leads to starch degradation and improves osmotic regulation by accumulating soluble sugars (Tap, 2010; Duque and Setter, 2013). Sugars, especially sucrose, play a significant role in stress tolerance, serving as signaling molecules, osmolytes, and nutritional substances (Gong et al., 2014; Ruan et al., 2010; Zhang et al., 2017). In addition, drought stress leads to the accumulation of malic acid, which is positively correlated with drought resistance in plants (Hubac et al., 1986; Guicherd et al., 1997; Scott et al., 2019). In this study, exogenous dopamine was associated with higher amounts of sucrose and malic acid, but lower starch accumulation in leaves under drought conditions.

To further study how dopamine may promote drought resistance by regulating carbohydrate metabolism, the expression of genes related to carbohydrate metabolism was examined. Previous research has shown that sorbitol is synthesized in two sequential steps catalyzed by A6PR and Sor6P (sorbitol 6-phosphate) (Zhou and Cheng, 2008). It is then taken up into the cytosol of parenchyma cells and converted to fructose by SDH (Park et al., 2002). Fructose and glucose can be converted to sucrose by SPS. In the cell wall space, sucrose can also be converted to glucose and fructose by CWINV (Park et al., 2002). In addition, malic acid is synthesized through MDH and decarboxylated into pyruvate and CO_2 by the action of ME (Rustin and Claude, 1986). In our study, dopamine significantly influenced the expression of several carbohydrate metabolism genes in plants under drought stress. Dopamine-pretreatment increased the expression of *MdSPS1* and *MdMDH* under drought stress. These results suggest that dopamine improved apple drought tolerance by promoting the accumulation of sucrose and malic acid through increased the expression of *MdSPS1* and *MdMDH*. However, carbohydrate content in plants is dependent on multiple factors, including the expression of genes related to carbohydrate biosynthesis, metabolism, and transportation (Ma et al., 2017). Therefore, the specific mechanism by which dopamine affects carbohydrate content under drought requires further study.

4.4. Analyses of DEGs expression profiles between DR and DADR

The secondary effects of drought stress are complex, including oxidative stress; damage to cellular components such as proteins, nucleic acids, and membrane lipids; and metabolic dysfunction (Zhu, 2016). To understand better how dopamine functions in plants exposed to drought, we used RNA-Seq to characterize the influence of dopamine on gene expression under drought conditions. We identified 1052 DEGs between DR and DADR: 643 up-regulated and 409 down-regulated. GO analysis showed that most of the genes were involved in cellular metabolism, biological regulation, and response to stimulus. In this study, our results showed that dopamine affects carbohydrate metabolism under drought. In plants, carbohydrate levels are important regulators of nitrogen metabolism (Chen et al., 2015). Through the KEGG pathway annotation, "nitrogen metabolism" was one of the most enriched KEGG pathways in the present study (Supplementary Table S3). Nitrogen is an important mineral nutrient in plant growth and development, and nitrogen metabolism may be negatively affected by drought (He and Dijkstra, 2014; Marschnert et al., 2010). For example, nitrogen supply improved the drought resistance of *Abies fabri* (Guo et al., 2010). In addition, the accumulation of compatible solutes such as amino acids and secondary compounds can regulate osmotic regulation under drought stress (Gutbrodt et al., 2011; Liu et al., 2011; Zahoor et al., 2017). In this study, the top 20 most obviously enriched pathways suggested that dopamine regulates the expression of genes related to the metabolism of amino acids and secondary compounds under drought stress.

Furthermore, the largest number of DEGs were enriched into "plant-pathogen interaction" pathway, which mainly involved in Ca^{2+} signaling pathway. Previous studies have shown that Ca^{2+} signaling pathway can be activated by drought stress, and contribute to drought tolerance in plants (Batistic and Kudla, 2012; Jiang et al., 2013; Shou et al., 2004). In addition, several studies have found that the elevation of apoplastic Ca^{2+} induced by osmotic stress leads to activation of CNGC, causing further responses by CaM/CML (Leng et al., 2014; Reddy et al., 2011; Talke et al., 2003; Yang and Guo, 2018). In the present study, dopamine increased CNGC and CAM/CML family genes expression, which may further increase Ca^{2+} in the cytoplasm (Fig. 11A and B). CNGCs may be involved in Ca^{2+} signaling under abiotic stresses which lead to downstream responses such as drought, cold, growth, or development (Defalco et al., 2016; Nawaz et al., 2019; Zelman et al., 2012). An increasing number of CaMs/CML family members have been identified and shown to be involved in drought resistance and ABA responses (Perochon et al., 2011; Reddy et al., 2011). Besides, a number of CaM/CML-binding TFs, including MYBs (e.g. MYB14 and MYB70) and WRKYs (e.g. WRKY43, WRKY45), can induce drought resistance-related gene expression (Popescu et al., 2007; Kim et al., 2007). Therefore, dopamine may have increased apple drought resistance by activating the expression of CNGC and CAM/CML family genes.

Finally, dopamine promoted growth by mediating the antioxidant enzyme activity under drought. In plants, antioxidant systems and oxidative stress have been proved to be related to CaM, a Ca^{2+}-dependent activator (Gong et al., 1997). Previous research has found that SOD is a CaM-dependent enzyme, and GPX secretion and activation were regulated by CaM (Gong and Li, 1995; Xu and van Huystee, 1993). CaM may increase antioxidant enzyme activities and antioxidant gene expression by activating NADPH oxidase (Parvin et al., 2012). Therefore, dopamine may regulate antioxidant enzyme activity by activating Ca^{2+} signaling pathway through increased expression of CaM/CML genes.

4.5. Analyses of TFs responsive to dopamine under drought stress

Plant responses to drought are ultimately regulated by changes in gene expression, including that of dehydrins, transporters, and TFs (Kosová et al., 2014; Li et al., 2015c; Hu et al., 2006; Nakashima and Yamaguchi-Shinozaki, 2009). TFs are the main participants in water stress signaling. Some of them constitute the main hub in the signaling network, including WRKY, NAC bHLH, bZIP, ERF, and MYB (Tripathi et al., 2014). In this study, 46 transcription factor-encoding genes were differentially expressed between DR and DADR, mainly from the WRKY, NAC, and ERF families.

Of these, the most abundant TF members belonged to the WRKY transcription factor family. As one of the most important families of transcriptional regulators, WRKY genes play an important role in plant drought tolerance (Meng et al., 2016; Pnueli et al., 2002; Rushton et al., 2010). Compared to the DR group, the DADR group showed up-regulation of drought-related TFs such as WRKY 38, WRKY 40, WRKY 30, and WRKY 70 (Fig. 12B). Previous studies have shown that WRKY38 is involved in drought stress response and WRKY 40 is involved in ABA signaling (Albayrak et al., 2012; Chen et al., 2010). OsWRKY30 may be a substrate of MAPKs, activated by MAPKs to confer drought tolerance in rice (Shen et al., 2012). WRKY70 was positively involved in brassinosteroid-regulated growth but negatively involved in stomatal closure and drought response (Chen et al., 2017; Li et al., 2013). In addition, there are many binding sites of WRKY, NAC and ERF in carbohydrate related gene promoters region. The WRKY transcription factor HpWRKY3 promoted sugar accumulation in pitaya fruit by activating the transcription of sucrose metabolism genes (Wei et al., 2019). The TF SUSIBA2 participated in sucrose signaling in barley (Sun et al., 2003). Furthermore, they also found that SUSIBA2 is a regulatory TF in starch synthesis and demonstrated the involvement of a WRKY transcription factor in carbohydrate synthesis (Sun et al., 2003). Therefore, dopamine may affect the carbohydrate metabolism under drought conditions by regulating the expression of TFs.

NACs are plant-specific TFs, many of which play a positive role in drought resistance (Nakashima et al., 2012). Compared with DR group, NAC29-like and NAC25 were up-regulated in DADR (Fig. 12B). In previous studies, overexpression of NAC29 and NAC25 increased drought resistance in soybean during the reproductive stage (Xu et al., 2018). Thus, dopamine may affect drought tolerance in apples by regulating WRKY and NAC transcription factors to control complicated transcriptional networks. In addition, many TFs in ERF family were differentially expressed between DR and DADR (Fig. 12A and B), suggesting an important role of dopamine in ethylene signaling under drought stress.

5. Conclusions

In summary, physiological analyses revealed that dopamine can alleviate drought stress of apple seedlings through multiple mechanisms. First, dopamine may improve photosynthetic capacity by inhibiting the degradation of photosynthetic pigments and increasing net photosynthetic rate; second, dopamine may promote growth by suppressing the drought-associated boost in H_2O_2 production and mediating antioxidant enzyme activity; third, dopamine may promote starch breakdown and the accumulation of sucrose and malic acid. In addition, RNA-Seq was used to characterize the influence of dopamine on drought tolerance in apple. There were 1052 DEGs between DR and DADR, and GO analysis revealed that dopamine may affect responses to stimuli by regulating cellular metabolism and biological regulation under drought. KEGG pathway analysis further indicated that dopamine plays a major role in the metabolism of nitrogen, amino acids and secondary compounds under drought stress. Dopamine may improve drought tolerance through activating Ca^{2+} signaling pathway by activating the expression of CNGC and CAM/CML family genes. Furthermore, dopamine may affect apple drought tolerance by regulating the expression of WRKY, ERF, and NAC transcription factors. Our study provides valuable resources for further study of the molecular mechanisms by which exogenous dopamine influences drought tolerance and will contribute to improve apple production under drought stress.

Author contributions

C.L. and T.G. performed and analyzed most of the experiments. Z.Z. and X.L. helped obtain the experimental data. Q.W., Q.C. and Q.L performed the experiments. S.V.N. and C.E.W. revised the English composition. C.L. and F.M. provided all financial support and critical intellectual input in study design and manuscript preparation. All authors discussed the results and commented on the manuscript. The authors declare no competing financial interests.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2020.01.022.

References

Acuna, C., Liu, X., Südhof, T.C., 2016. How to make an active zone: unexpected universal functional redundancy between RIMs and RIM-BPs. Neuron 91, 792–807.
Albayrak, G., Yoruk, E., Diken, O., 2012. Quantitative gene expression analysis of WRKY38 and DREB2 transcription factors responsible for drought and salt tolerance in barley (*Hordeum vulgare* L.). N. Biotech. 29, S22.
Allen, J.F., 2003. Superoxide as an obligatory, catalytic intermediate in photosynthetic reduction of oxygen by adrenaline and dopamine. Antioxidants Redox Signal. 5, 7–14.
And, A.D.H., Hitz, W.D., 1982. Metabolic responses of mesophytes to plant water deficits. Annu. Rev. Plant Biol. 33, 163–203.
Anders, S., Huber, W., 2010. Differential expression analysis for sequence count data. Genome Biol. 11, R106.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Plant Physiol. 24, 1–15.
Ashraf, M.A., Akbar, A., Askari, S.H., Iqbal, M., Rasheed, R., Hussain, I., 2018. Recent advances in abiotic stress tolerance of plants through chemical priming: an overview. In: Rakshit, A., Singh, H. (Eds.), Advances in Seed Priming. Springer, Singapore.
Bai, T., Li, C., Li, C., Liang, D., Ma, F., 2013. Contrasting hypoxia tolerance and adaptation in *Malus* species is linked to differences in stomatal behavior and photosynthesis. Physiol. Plant. 147, 514.
Batistic, O., Kudla, J., 2012. Analysis of calcium signaling pathways in plants. Biochim. Biophys. Acta Biomembr. 1820, 1283–1293.
Bohnert, H.J., Jensen, R.G., 1996. Strategies for engineering water-stress tolerance in plants. Trends Biotechnol. 14, 89–97.
Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. Methods Enzymol. 2, 764–775.
Chang, S., Puryear, J., Cairney, J., 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Report. 11, 113–116.
Chen, H., Lai, Z., Shi, J., Xiao, Y., Chen, Z., Xu, X., 2010. Roles of *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. BMC Plant Biol. 10, 281.
Chen, D., Shiwen, W., Xiong, B., Cao, B., Deng, X.P., 2015. Carbon/nitrogen imbalance associated with drought-induced leaf senescence in sorghum bicolor. PLoS One 10, e0137026.
Chen, J., Nolan, T.M., Ye, H., Zhang, M., Tong, H., Xin, P., Chu, J., Chu, C., Li, Z., Yin, Y., 2017. *Arabidopsis* WRKY46, WRKY54, and WRKY70 transcription factors are involved in brassinosteroid-regulated plant growth and drought responses. Plant Cell 29, 1425–1439.
Dai, Y.R., Michaels, P.J., Flores, H.E., 1993. Stimulation of ethylene production by catecholamines and phenylethylamine in potato cell suspension cultures. Plant Growth Regul. 12, 219–222.
Davila, O.N., Coolen, S., Huang, P., Severing, E., van Verk, M.C., Hickman, R., Wittenberg, A., de Vos, M., Prins, M., van Loon, J., Aarts, M., van Wees, S., Pieterse, C., Dicke, M., 2016. Effect of prior drought and pathogen stress on *Arabidopsis* transcriptome changes to caterpillar herbivory. New Phytol. 210, 1344–1356.
Defalco, T.A., Moeder, W., Yoshioka, K., 2016. Opening the gates: insights into cyclic nucleotide-gated channel-mediated signaling. Trends Plant Sci. 21, 903–906.
Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9.
Dufeu, M., Martin-Tanguy, J., Hennion, F., 2003. Temperature-dependent changes of amine levels during early seedling development of the cold-adapted subantarctic crucifer Pringlea antiscorbutica. Physiol. Plant. 118, 164–172.
Duque, L., Setter, T., 2013. Cassava response to water deficit in deep pots: root and shoot growth, ABA, and carbohydrate reserves in stems, leaves and storage roots. Trop. Plant Biol. 6, 199–209.
Elstner, E.F., Konze, J.R.R., Selman, B.R., Stoffer, C., 1976. Ethylene formation in sugar beet leaves. Plant Physiol. 163–168.
Gaxiola, R.A., Li, J., Undurraga, S., Dang, L.M., Allen, G.J., Alper, S.L., Fink, G.R., 2001. Drought- and salt-tolerant plants result from overexpression of the AVP1 H+-pump. Proc. Natl. Acad. Sci. 98, 11444–11449.
Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases I. Occurrence in higher plants. Plant Physiol. 59, 309.
Gibson, S.I., 2000. Plant sugar-response pathways. Part of a complex regulatory web. Plant Physiol. 124, 1532–1539.
Gong, M., Li, Z.G., 1995. Calmodulin-binding proteins from Zea mays germs. Phytochemistry 40, 1335–1339.
Gong, M., Chen, S.N., Song, Y.Q., Li, Z.G., 1997. Effect of calcium and calmodulin on intrinsic heat tolerance in relation to antioxidant systems in maize seedlings. Funct. Plant Biol. 24.
Gong, X., Liu, M., Zhang, L., Ruan, Y., Ding, R., Ji, Y., Zhang, N., Zhang, S., Farmer, J., Wang, C., 2014. *Arabidopsis AtSUC2* and *AtSUC4*, encoding sucrose transporters, are required for abiotic stress tolerance in an ABA-dependent pathway. Physiol. Plant. 153, 119–136.
Guicherd, P., Peltier, J.P., Gout, E., Bligny, R., Marigo, G., 1997. Osmotic adjustment in *Fraxinus excelsior* L: malate and mannitol accumulation in leaves under drought conditions. Trees (Berl.) 11, 155–161.
Guidotti, B.B., Gomes, B.R., Siqueira-Soares, R.D., Soares, A.R., Ferrarese-Filho, O., 2013. The effects of dopamine on root growth and enzyme activity in soybean seedlings. Plant Signal. Behav. 8, e25477.
Guo, J., Yang, Y., Wang, G., Yang, L., Sun, X., 2010. Ecophysiological responses of *Abies fabri* seedlings to drought stress and nitrogen supply. Physiol. Plant. 139, 335–347.
Gutbrodt, B., Mody, K., Dorn, S., 2011. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. Oikos 120, 1732–1740.
Guy, C.L., 1990. Cold acclimation and freezing stress tolerance: role of protein metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41, 187–223.
He, M., Dijkstra, F.A., 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. New Phytol. 204, 924–931.
Hoffman, L., Dacosta, M., Ebdon, J.S., Zhao, J., 2012. Effects of drought preconditioning on freezing tolerance of perennial ryegrass. Environ. Exp. Bot. 79, 11–20.
Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., Xiong, L., 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc. Natl. Acad. Sci. 103, 12987–12992.
Hubac, C., Guerrier, D., Bousquet, U., 1986. Effect of far-red light on malate and potassium contents in cotton leaves: relation to drought resistance. Physiol. Plant. 66, 37–40.
Jakab, G., Ton, J., Flors, V., Zimmerli, L., Métraux, J.P., Mauchmani, B., 2005. Enhancing *Arabidopsis* salt and drought stress tolerance by chemical priming for its abscisic acid responses. Plant Physiol. 139, 267–274.
Jaspers, P., Kangasjarvi, J., 2010. Reactive oxygen species in abiotic stress signaling. Physiol. Plant. 138, 405–413.
Jiang, S., Zhang, D., Wang, L., Pan, J., Liu, Y., Kong, X., Zhou, Y., Li, D., 2013. A maize calcium-dependent protein kinase gene, ZmCPK4, positively regulated abscisic acid signaling and enhanced drought stress tolerance in transgenic *Arabidopsis*. Plant Physiol. Biochem. 71, 112–120.
Kabiri, R., Nasibi, F., Farahbakhsh, H., 2014. Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in nigella sativa plant under hydroponic culture. Plant Prot. Sci. 50, 43–51.
Kanazawa, K., Sakakibara, H., 2000. High content of dopamine, a strong antioxidant, in Cavendish banana. J. Agric. Food Chem. 48, 844–848.
Khurana, J.P., Tamot, B.K., Maheshwari, N., Maheshwari, S.C., 1987. Role of catecholamines in promotion of flowering in a short-day duckweed *Lemna paucicstata* 6746. Plant Physiol. 85, 10–12.
Kim, H.S., Park, B., Yoo, J., Jeong, M., Lee, S., Han, H., Kim, K., Kim, S., Yun, D.-J., Lee, S.Y., Chung, W., 2007. Identification of a calmodulin-binding NAC protein as a transcriptional repressor in Arabidopsis. J. Biol. Chem. 282, 36292–36302.
Kosová, K., Vítámvás, P., Prášil, I.T., 2014. Wheat and barley dehydrins under cold, drought, and salinity-what can LEA-II proteins tell us about plant stress response? Front. Plant Sci. 5.
Kulma, A., Szopa, J., 2007. Catecholamines are active compounds in plants. Plant Sci. 172, 433–440.
Leng, P., Yuan, B., Guo, Y., Chen, P., 2014. The role of abscisic acid in fruit ripening and esponses to abiotic stress. J. Exp. Bot. 65, 4577–4588.
Li, Y., Zhao, H., Duan, B., Korpelainen, H., Li, C., 2011. Effect of drought and ABA on growth, photosynthesis and antioxidant system of *Cotinus coggygria* seedlings under two different light conditions. Environ. Exp. Bot. 71, 107–113.
Li, J., Besseau, S., Törönen, P., Sipari, N., Kollist, H., Holm, L., Palva, E.T., 2013. Defense-related transcription factors WRKY70 and WRKY54 modulate osmotic stress tolerance by regulating stomatal aperture in *Arabidopsis*. New Phytol. 200, 457–472.
Li, C., Sun, X., Chang, C., Jia, D., Wei, Z., Li, C., Ma, F., 2015a. Dopamine alleviates salt-induced stress in *Malus hupehensis*. Physiol. Plant. 153, 584–602.
Li, C., Tan, D., Liang, D., Chang, C., Jia, D., Ma, F., 2015b. Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two Malus species under drought stress. J. Exp. Bot. 66, 669–680.
Li, J., Yu, G., Sun, X., Liu, Y., Liu, J., Zhang, X., Jia, C., Pan, H., 2015c. AcPIP2, a plasma membrane intrinsic protein from halophyte Atriplex canescens, enhances plant

growth rate and abiotic stress tolerance when overexpressed in *Arabidopsis thaliana*. Plant Cell Rep. 34, 1401–1415.
Li, C., Zhao, Q., Gao, T., Wang, H., Zhang, Z., Liang, B., Wei, Z., Liu, C., Ma, F., 2018. The mitigation effects of exogenous melatonin on replant disease in apple. J. Pineal Res. 65, e12523.
Liang, B., Li, C., Ma, C., Wei, Z., Wang, Q., Huang, D., Chen, Q., Li, C., Ma, F., 2017. Dopamine alleviates nutrient deficiency-induced stress in Malus hupehensis. Plant Physiol. Biochem. 119, 346–359.
Liang, B., Gao, T., Zhao, Q., Ma, C., Chen, Q., Wei, Z., Li, C., Li, C., Ma, F., 2018a. Effects of exogenous dopamine on the uptake, transport, and resorption of apple ionome under moderate drought. Front. Plant Sci. 9, 755.
Liang, B., Ma, C., Zhang, Z., Wei, Z., Gao, T., Zhao, Q., Ma, F., Li, C., 2018b. Long-term exogenous application of melatonin improves nutrient uptake fluxes in apple plants under moderate drought stress. Environ. Exp. Bot. 155, 650–661.
Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., Fernie, A.R., 2006. Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat. Protoc. 1, 387–396.
Liu, H., Wang, X., Wang, D., Zou, Z., Liang, Z., 2011. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* Bunge. Ind. Crops Prod. 33, 84–88.
Lu, X., Li, J., Yang, J., Liu, X., Ma, J., 2014. De novo transcriptome of the desert beetle Microdera punctipennis (Coleoptera: Tenebrionidae) using Illumina RNA-Seq technology. Mol. Biol. Rep. 41, 7293–7303.
Ma, Q.J., Sun, M.H., Lu, J., Liu, Y.J., Hu, D.G., Hao, Y.J., 2017. Transcription factor areb2 is involved in soluble sugar accumulation by activating sugar transporter and amylase genes. Plant Physiol. 174, 2348–2362.
Marian, C.O., Krebs, S.L., Arora, R., 2004. Dehydrin variability among rhododendron species: a 25-kDa dehydrin is conserved and associated with cold acclimation across diverse species. New Phytol. 161, 773–780.
Marschnert, H., Kirkby, E., Engels, C., 2010. Importance of cycling and recycling of mineral nutrients within plants for growth and development. Plant Biol. 110, 265–273.
Meng, D., Li, Y., Bai, Y., Li, M., Cheng, L., 2016. Genome-wide identification and characterization of WRKY transcriptional factor family in apple and analysis of their responses to waterlogging and drought stress. Plant Physiol. Biochem. 103, 71–83.
Meng, D., He, M., Bai, Y., Xu, H., Dandekar, A.M., Fei, Z., Cheng, L., 2018a. Decreased sorbitol synthesis leads to abnormal stamen development and reduced pollen tube growth via an MYB transcription factor, MdMYB39L, in apple (*Malus domestica*). New Phytol. 217, 641–656.
Meng, D., Li, C., Park, H.J., González, J., Wang, J., Dandekar, A.M., Turgeon, B.G., Cheng, L., 2018b. Sorbitol modulates resistance to Alternata alternate by regulating the expression of an NLR resistance gene in apple. Plant Cell 30, 1562–1581.
Mh, C.D.C., 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant Signal. Behav. 3, 156–165.
Mignolet-Spruyt, L., Xu, E., Idänheimo, N., Hoeberichts, F.A., Mü hlenbock, P., Brosché, M., Van Breusegem, F., Kangasjärvi, J., 2016. Spreading the news: subcellular and organellar reactive oxygen species production and signalling. J. Exp. Bot. 67, 3831–3844.
Miller, G.A.D., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R.O.N., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 33, 453–467.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Nakashima, K., Yamaguchi-Shinozaki, K., 2009. Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. Plant Physiol. 149, 88.
Nakashima, K., Takasaki, H., Mizoi, J., Shinozaki, K., Yamaguchi-Shinozaki, K., 2012. NAC transcription factors in plant abiotic stress responses. Biophys. Acta Gene Regul. Mech. 1819, 97–103.
Nawaz, Z., Kakar, K.U., Ullah, R., Yu, S., Zhang, J., Shu, Q.Y., Ren, X.l., 2019. Genome-wide identification, evolution and expression analysis of cyclic nucleotide-gated channels in tobacco (*Nicotiana tabacum* L.). Genomics 111, 142–158.
Park, S.W., Song, K.J., Kim, M.Y., Hwang, J.H., Shin, Y.U., Kim, W.C., Chung, W.I.I., 2002. Molecular cloning and characterization of four cDNAs encoding the isoforms of NADdependent sorbitol dehydrogenase from the Fuji apple. Plant Sci. 162, 513–519.
Parvin, S., Lee, O.R., Sathiyaraj, G., Khorolragchaa, A., Kim, Y.J., Devi, B.S.R., Yang, D.C., 2012. Interrelationship between calmodulin (CaM) and H_2O_2 in abscisic acid-induced antioxidant defense in the seedlings of Panax ginseng. Mol. Biol. Rep. 39, 7327–7338.
Patterson, B.D., Macrae, E.A., Ferguson, I.B., 1984. Estimation of hydrogen peroxide in plant extracts using titanium (IV). Anal. Biochem. 139, 487–492.
Perochon, A., Aldon, D., Galaud, J.P., Ranty, B., 2011. Calmodulin and calmodulin-like proteins in plant calcium signaling. Biochimie 93 0-2053.
Pnueli, L., Hallak-Herr, E., Rozenberg, M., Cohen, M., Goloubinoff, P., Kaplan, A., Mittler, R., 2002. Molecular and biochemical mechanisms associated with dormancy and drought tolerance in the desert legume Retama raetam. Plant J. 31, 319–330.
Popescu, S.C., Popescu, G.V., Bachan, S., Zhang, Z., Seay, M., Gerstein, M., Snyder, M., Dinesh-Kumar, S.P., 2007. Differential binding of calmodulin-related proteins to their targets revealed through high-density Arabidopsis protein microarrays. Proc. Natl. Acad. Sci. 104, 4730–4735.
Protacio, C.M., Flores, H.E., 1992. Growth stimulation by catecholamines in plant tissue/organ cultures. Plant Physiol. 98, 89.
Reddy, A., Ali, G., Celesnik, H., Day, I., 2011. Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. Plant Cell 23, 2010–2032.
Rosel, M.A., Mosca, L., Foppoli, C., Blarzino, C., Coccia, R., 1994. Lipoxygenase-catalyzed oxidation of 5-S-substituted catecholamines. Biochem. Biophys. Res. Commun. 200, 344–350.
Roshchina, V.V., 1990. Biomediators in chloroplasts of higher plants. 3. Effect of dopamine on photochemical activity. Photosynthetica 24, 117–121.
Ruan, Y.l., Jin, Y., Yang, Y.J., Li, G.J., Boyer, J.S., 2010. Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. Mol. Plant 3, 942–955.
Rushton, P.J., Somssich, I.E., Ringler, P., Shen, Q.J., 2010. WRKY transcription factors. Trends Plant Sci. 15, 247–258.
Rustin, P., Claude, L., 1986. Malate metabolism in leaf mitochondria from the crassulacean acid metabolism plant kalanchoë blossfeldiana poelln. Plant Physiol. 81, 1039–1043.
Savvides, A., Ali, S., Tester, M.A., Fotopoulos, V., 2016. Chemical priming of plants against multiple abiotic stresses: mission possible? Trends Plant Sci. 21, 329–340.
Schultz, W., 1998. Predictive reward signal of dopamine neurons. J. Neurophysiol. 80, 1–27.
Scott, E.R., Orians, C.M., Müller, C., Schweiger, R., Dukes, J.S., 2019. Combined impacts of prolonged drought and warming on plant size and foliar chemistry. Ann. Bot. https://doi.org/10.1093/aob/mcz004.
Sekmen, A.H., Turkan, I., Tanyolac, Z.O., Ozfidan, C., Dine, A., 2012. Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* Bark. Environ. Exp. Bot. 77, 63–76.
Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova, M.V., Fatkhutdinova, R.A., Fatkhutdinova, D.R., 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci. 164, 317–322.
Shasha, Z., Mingjun, L., Qingmei, G., Fengli, L., Sheng, Z., Wei, C., Lihua, Y., Yuan, Q., Fengwang, M., 2015. Physiological and proteome analysis suggest critical roles for the photosynthetic system for high water-use efficiency under drought stress in *Malus*. Plant Sci. 236, 44.
Sheikh-Mohamadi, M.H., Etemadi, N., Arab, M.M., Aalifar, M., Arab, M., 2018. Physiological and ascorbate-glutathione pathway-related genes responses under drought and heat stress in crested wheatgrass. Sci. Hortic. 242, 195–206.
Shen, H., Liu, C., Zhang, Y., Meng, X., Zhou, X., Chu, C., Wang, X., 2012. OsWRKY30 is activated by MAP kinases to confer drought tolerance in rice. Plant Mol. Biol. 80, 241–253.
Shou, H., Wang, K., Bordallo, P., 2004. Expression of the Nicotiana protein kinase (NPK1) enhanced drought tolerance in transgenic maize. J. Exp. Bot. 55, 1013–1019.
Shulaev, V., Cortes, D., Miller, G., Mittler, R., 2008. Metabolomics for plant stress response. Physiol. Plant. 132, 199–208.
Singh, R., Gupta, P., Khan, F., Singh, S.K., Sanchita, Mishra, T., Kumar, A., Dhawan, S.S., Shirke, P.A., 2018. Modulations in primary and secondary metabolic pathways and adjustment in physiological behaviour of Withania somnifera under drought stress. Plant Sci. 272, 42–54.
Skirycz, A., Swiedrych, A., Szopa, J., 2005. Expression of human dopamine receptor in potato (Solanum tuberosum) results in altered tuber carbon metabolism. BMC Plant Biol. 5, 1.
Smirnoff, N., Cumbes, Q.J., 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28, 1057–1060.
Storey, J.D., Tibshirani, R., 2003. Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. 100, 9440–9445.
Sun, C., Palmqvist, S., Olsson, H., Borén, Mats, Ahlandsberg, S., Jansson, C., 2003. A novel wrky transcription factor, susiba2, participates in sugar signaling in barley by binding to the sugar-responsive elements of the iso1 promoter. Plant Cell 15, 2076–2092.
Surmeier, D.J., Graves, S.M., Shen, W., 2014. Dopaminergic modulation of striatal networks in health and Parkinson's disease. Curr. Opin. Neurobiol. 29, 109–117.
Swiedrych, A., Stachowiak, J., Szopa, J., 2004. The catecholamine potentiates starch mobilization in transgenic potato tubers. Plant Physiol. Biochem. 42, 103–109.
Talke, I.N., Damien, B., Maathuis, F.J.M., Dale, S., 2003. CNGCs: prime targets of plant cyclic nucleotide signalling? Trends Plant Sci. 8, 286–293.
Tap, Z.S., 2010. Changes in carbohydrate metabolism and assimilate export in starch-excess mutants of *Arabidopsis*. Plant Cell Environ. 22, 1445–1453.
Tripathi, P., Rabara, R.C., Rushton, P.J., 2014. A systems biology perspective on the role of WRKY transcription factors in drought responses in plants. Planta 239, 255–266.
Tsugane, K., Kobayashi, K., Niwa, Y., Ohba, Y., Wada, K., Kobayashi, H., 1999. A recessive *Arabidopsis* mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification. Plant Cell 11, 1195–1206.
Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10, 57–63.
Wang, H.C., Ma, F.F., Cheng, L.L., 2010. Metabolism of organic acids, nitrogen and amino acids in chlorotic leaves of 'honeycrisp' apple (*Malus domestica* Borkh) with excessive accumulation of carbohydrates. Planta 232, 511–522.
Wang, S.C., Wang, R.C., Liang, D., Ma, F.W., Shu, H.R., 2012. Molecular characterization and expression analysis of a glycine-rich RNA-binding protein gene from *Malus hupehensis*. Rehd. Mol. Biol. Rep. 39, 4145–4153.
Wang, S., Che, T., Levit, A., Shoichet, B.K., Wacker, D., Roth, B.L., 2018. Structure of the D2 dopamine receptor bound to the atypical antipsychotic drug risperidone. Nature 555, 269.
Wei, W., Cheng, M.N., Ba, L.J., Zeng, R.X., Luo, D.L., Qin, Y.H., Liu, Z.L., Kuang, J.F., Lu, W.J., Chen, J.Y., Su, X.G., Shan, W., 2019. Pitaya hpwrky3 is associated with fruit sugar accumulation by transcriptionally modulating sucrose metabolic genes hpinv2 and hpsusy1. Int. J. Mol. Sci. 20.
Xu, Y., van Huystee, R., 1993. Association of calcium and calmodulin to peroxidase secretion and activation. J. Plant Physiol. 141, 141–146.
Xu, C., Xia, C., Xia, Z., Zhou, X., Huang, J., Huang, Z., Liu, Y., Jiang, Y., Casteel, S., Zhang, C., 2018. Physiological and transcriptomic responses of reproductive stage soybean to drought stress. Plant Cell Rep. 37, 1611–1624.
Yan, W., Deng, L., Zhong, Y., Shang, G.Z., 2015. The characters of dry soil layer on the loess plateau in China and their influencing factors. PLoS One 10, e0134902.
Yang, Y., Guo, Y., 2018. Elucidating the molecular mechanisms mediating plant salt-stress responses. New Phytol. 217, 523–539.
Zahoor, R., Wenqing, Z., Abid, M., Dong, H., Zhou, Z., 2017. Potassium application

regulates nitrogen metabolism and osmotic adjustment in cotton (*Gossypium hirsutum* L.) functional leaf under drought stress. J. Plant Physiol. 215, 30–38.
Zelman, A., Dawe, A., Gehring, C., Berkowitz, G., 2012. Evolutionary and structural perspectives of plant cyclic nucleotide-gated cation channels. Front. Plant Sci. 3, 95.
Zhang, C., Li, X., He, Y., Zhang, J., Yan, T., Xiaolong, L., 2017. Physiological investigation of C4-phosphoenolpyruvate-carboxylase-introduced rice line shows that sucrose metabolism is involved in the improved drought tolerance. Plant Physiol. Biochem. 115, 328–342.
Zhou, R., Cheng, L.L., 2008. Competitive inhibition of phosphoglucose isomerase of apple leaves by sorbitol 6-phosphate. J. Plant Physiol. 165, 903–910.
Zhu, J.K., 2016. Abiotic stress signaling and responses in plants. Cell 167, 313–324.

Glossary

APX: ascorbate peroxidase
CAT: catalase
Ci: intercellular CO2 concentration
DEGs: differentially expressed genes
FPKM: fragments per kb per million reads
GO: gene ontology
g_s: stomatal conductance
KEGG: Kyoto Encyclopedia of Genes and Genomes
Pn: net photosynthesis rate
POD: peroxidase
REL: relative electrolyte leakage
RNA-Seq: transcriptome sequencing
ROS: reactive oxygen species
RWC: relative water content
SOD: superoxide dismutase
TFs: transcription factors
WUEi: instantaneous water-use efficiency