Drought stress revealed physiological, biochemical and gene-expression variations in ‘Yoshihime’ peach (Prunus Persica L) cultivar

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ABSTRACT
It is indispensable to comprehend the mechanism that regulates plant responses to drought conditions to intensify the water use efficiency of stone fruits. The physiological, biochemical and molecular responses of drought-treated peach leaves were investigated. Results revealed that drought-treated plants manifested a significant attenuation in water potential as compared to control plants. Furthermore, sorbitol and proline contents were accumulated contrary to glucose, fructose, and sucrose that were dwindled significantly throughout the drought period. Similarly, the activities of antioxidant enzymes and expression pattern of related genes were hoisted to counter the lipid peroxidation in drought-treated plants. Moreover, reduced stomatal conductance has repressed the photosynthesis process and linked genes during drought stress. The expression level of regulatory genes (dehydration-responsive element-bindings and WRKYs) exhibited up-regulation in the drought-treated group. Overall, this study asserts that ‘Yoshihime’ peach cultivar possesses unique physiological, biochemical, and molecular responses under different spells of drought stress.

1. Introduction
Peach (Prunus persica L.) is one of the most valuable stone fruit crop belonging to Rosaceae family. The estimated production of peaches and nectarines has transcended by 22.5 million tons (FAO 2016). Apart from its ecological and economic significance, peach is also contemplated as the model plant for evolutionary biology and comparative genomic-related studies due to its smaller genome (300 Mb) size with shorter reproductive time (Zhebentsevaya et al. 2008; Tani et al. 2011). Recently, International Peach Genome Initiative has divulged the first draft of the peach genome (Peach v0.1, acquired from ‘Lovell’ haploid), which are publically accessible by the Genome Database for Rosaceae (http://www.rosaceae.org/peach/genome). Recent shifts in the climatic patterns have inflated the abiotic stresses, such as drought and salinity, which have adverse effects on peach growth and development (Sotiropoulos et al. 2010). Among these factors, drought has pernicious repercussions on plant distribution, productivity and genetic potential (Ashraf and Foolad 2007). Approximately 45% of the total agricultural terrains are under perpetual or intermittent water deficits conditions, letting almost 50% of yield losses every year globally (Abdelrahman et al. 2017). Therefore, screening the current and/or newly improved peach germplasm for their potential in mitigating the drought effects have turned out to be earnest (Morison et al. 2008; Sivritepe et al. 2008).

In the course of evolution, plants can streamline their morphological, physiological and metabolism-related responses at both organ and cellular levels to counter the drought severity (Haider, Kurjogi, et al. 2017). The abatement of water contents and water potential are among the primary effects in plants under drought stress, which ultimately affects the water movement to the new cell division sites (Escobar-Gutierrez et al. 1998). However, the extent of drought stress on physiological responses usually fluctuates in plants, though it constrains the photosynthesis rate, stomatal conductance, and carbon dioxide (CO2) assimilation (Jones 2007; Lovisolo et al. 2010). Reduced stomatal conductance prompts the decline in intracellular CO2 level which results in the over-production of reactive oxygen species (ROS) (Mahajan and Tuteja 2005; Chaves et al. 2009). Conflictingly, plant accrues certain osmolytes (proline) and sugars (raffinose and sorbitol) to prevent membrane disintegration and enzyme inactivation to diminish the turgor potential along with detoxification of ROS by re-establishing the cellular redox level (Mahajan and Tuteja 2005; Krasensky and Jonak 2012). Moreover, ROS enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) also play scavenging role in the degradation of free radicals (H2O2 and O2•−) (Bowler et al. 2003; Haider, Zhang, et al. 2017).

It is a well-known fact that plants have adaptive robustness at the physiological, molecular and cellular levels to tweak the water balance under drought stress. Numerous signaling-related networks play a crucial role to counter the effect of stress responses in plants. Under decreased relative water content (RWC), the signal transduction activates the hydrogen pump ATPase (H+ ATPase) proteins on the plasma membrane of root hairs, which incites the biosynthesis of unequivocal osmolytes to adjust water balance. Besides, various transcription factors (TFs) gene families, including
WRKY TF, ethylene-responsive factor (ERF), dehydration-responsive element-binding (DREB), myeloblastosis, myeloblastosis oncogene cellular (MYC), basic helix-loop-helix TF, and basic leucine zipper (bZIP) are implicated to trigger the specific genes to generate the requisite defense responses in plants (Abuzar et al. 2016; Zeng et al. 2017). Being largest families of TFs, WRKY proteins have been testified repeatedly against drought stress, for example, the over-expression of AtWRKY57 has hoisted the ABA level and promote the drought tolerance in Arabidopsis (Ren et al. 2010). Similarly, the AtWRKY63 curbed the antagonistic effects of drought by using ABA-signaling pathway, and also the higher activity of BdWRKY36 in transgenic tobacco assisted in drought stress tolerance (Li et al. 2013; Sun et al. 2015). However, the functional characterization of WRKY TFs has been carried out in many plant species, though regardless it still requires more consideration into its key defensive role in fruit plant species.

Yoshihime (21–18 × Akatsuki) is white fleshed, red pigmented and mid-season maturing table peach cultivar; recommended for commercial cultivation. However, this commercial cultivar has not yet been screened for its potential against oxidative stress. Therefore, the objectives of the current investigations were to assess the biochemical, physiological, and molecular responses of the drought-treated peach plants. Overall, this study will provide basic information related to the peach tolerance mechanism under moderate and severe drought stress, which can further be used for breeding of drought-tolerant peach cultivars.

2. Materials and methods

2.1. Plant material and drought stress

The experiment was carried at Nanjing Agricultural University, China using two-years-old peach ‘Yoshihime’ pot (2L) grown cultivar filled with peat-sand-soil 1:1:3 (v:v:v) in the standard greenhouse conditions (25 ± 5°C) supplemented with 65% relative humidity, and 16-h light/8-h dark photoperiod. Yoshihime was grafted on wild peach (Amygdalus persica L.) Batsch, which is still not characterized by its potential against oxidative stress. The experiment was laid out completely randomized design with three treatments and each provided with five single-plant replicates. The control treatment received 100 mL water per plant on daily basis, while drought stress treatments (mild and severe) were levied by withholding water. The severe stress-treated plants were maintained without water for 6 days, but mildly stressed plants were watered after 4 days (50 mL/day) for 2 days to modulate the drought intensity. All the biochemical, physiological and molecular investigations were carried at the final day (6th day) of the experiment. The third and fourth unfolded leaf from the shoot apex of control and both treatments were collected from each replicate 1 h before the end of photoperiod. The leaf samples were immediately put in liquid nitrogen and then stored at −80°C until further use.

2.2. Estimation of water potential, osmotic potential and relative-water contents (RWC)

The water potential (Ψ_w), osmotic potential (Ψ_p), turgor potential (Ψ_t) and RWC examinations were carried out at the third mature leaf. The Ψ_w was quantified by using Scholander Pressure bomb chamber (Mode, 3005; ICT, Australia). After removing the central veins of a leaf, lamina was used to measure Ψ_t and RWC. For Ψ_p estimation, the leaf sample was put in the syringe (2 mL) blocked with filter paper (Whatman, France) at the opening. The osmolality of leaf sap (25 µl) was measured using micro-osmometer (Roebling, Germany). The Ψ_p of leaf sap was then measured following the Van’t Hoff relation (Nobel 1983). The osmotic potential at full turgor (Ψ_sat) was estimated by the following formula:

\[ \Psi_{sat} = \frac{\Psi_p \times RWC}{100} \]

The turgor potential (Ψ_p) was estimated by the difference within Ψ_w and Ψ_p. For RWC estimation, fresh leaves were weighed to get fresh weight (FW), afterward, the petioles were submerged in water for overnight at 5 °C to reduce transpiration rate and avoid dry weight (DW) losses. Then the leaves (fully-rehydrated) were weighed again to get turgid weight and immediately put at 85°C for drying to get DW. RWC was estimated by following equation described by Morgan (1984):

\[ RWC = \frac{FW - DW}{TW - DW} \times 100 \]

2.3. Biochemical parameters estimation

Malondialdehyde (MDA) contents were quantified by using thiobarbituric acid method (Schmedes and Holmer 1989). The activity of SOD enzyme was estimated by determining the ability to inhibit photochemical reduction of nitroblue tetrazolium at 560 nm, while the POD activity was measured using guaiacol oxidation method at 470 nm, and finally the CAT activity was estimated by observing the degradation of H2O2 at 240 nm following the method briefly described by Haider, Zhang, et al. (2017). The quantification of most important sugars including glucose, fructose, sucrose, and sorbitol was carried out using high-performance liquid chromatography (HPLC; Shimadzu, Japan), which was connected with refractive index detector (RID-10 AL) as explained by Haider et al. (2014) and Abdelrahman et al. (2016). The proline contents were determined using sulfosalicylic acid (3%) and final calculations were made using proline standard calibration curve (Sigma-Aldrich) at 520 nm following the method briefly explained by Abrahám et al. (2010).

2.4. Physiological parameters estimation

The concentration of the chlorophyll (Chl) was estimated by using the SPAD meter (502 Minolta Co., Osaka, Japan) following the method of (Mielke et al. 2010). The measurements of net photosynthesis rate (AN), CO2 assimilation rate (C), stomatal conductance (gs), and transpiration rate (E) were conducted between 9:00 and 11:00 (GMT) at the fourth unfolded leaf using portable LI-COR meter (LI-6400XT, USA) following the method of Haider, Zhang, et al. (2017). The ratio between AN and gs defines the peach leaf water use efficiency (WUE).

2.5. RNA extraction and cDNA library construction

Total RNA was extracted from the leaf samples of both control and drought treatments (mild and severe) using Trizol-
reagent method (Invitrogen, Carlsbad, CA, USA). RNA purity and integrity were assessed by the A260/A280 absorbance ratio and 1.0% agarose gel. The concentration of total RNA was measured according to the A260 absorbance after genomic DNA had been digested by DNasel. cDNA was synthesized from 4 μg of DNA-free RNA using a Revert Aid™ First-Strand cDNA Synthesis Kit (Fermentas, Glen Burnie, MD, USA).

2.6. Quantitative real-time PCR

The Real-time quantitative PCR reaction was comprised of 10.0 μl of SYBR Premix Ex Taq™ (Takara, Japan), 0.4 μl of each primer (10 μM), 2 μl of cDNA, and 7.2 μl of RNase-free water in a total volume of 20 μl. The reaction was performed in a Light Cycler 1.5 instrument (Roche, Germany), started with a preliminary step of 95°C for 30 s followed by 40 cycles of 95°C for 5 s and 61°C for 20 s. A template-free control for each primer pair was set for each cycle. The details of primers used are shown in Table 1. All PCR reactions were normalized using the Ct value corresponding to the actin (KvActin1) gene. Three biological replicates were used and three measurements were performed on each replicate. For the drought-treated samples, expression levels produced by the qRT-PCR were expressed as a ratio relative to their corresponding controls, which were set to 1.

2.7. Statistical analysis

The estimated data from biochemical and physiological responses of drought-stressed peach leaves were subjected to the one-way analysis of variance (ANOVA) using SPSS. The estimated data from biochemical and physiological responses of drought-stressed peach leaves were subjected to the one-way analysis of variance (ANOVA) using SPSS (3. Results)

3. Results

3.1. Water relations and osmotic adjustment in response to drought stress

The water potential (Ψw), the osmotic potential at full turgor (ΨSAT), turgor potential (Ψp), and RWC showed significant differences relative to water relations between control and, mild and severe drought treatments (Table 2). The severe stress treatment induced significant reductions in water potential (−1.85 ± 0.22c MPa) followed by mild stress (−1.10 ± 0.23b MPa) when compared with control treatment (−0.70 ± 0.21a MPa) under prolonged drought stress (Table 2). The depletion of water contents from the pots of both (mild and severe) stress treatments have induced the drought level and decreased the transpiration rate of peach leaves. However, the mildly-stressed plants transpired 50% of control, while remaining water in pots was 15% of control by the end of the experiment. Meanwhile, the severely-stressed peach leaves transpired 20% of the control and remaining water contents in the pots were 5% of control. Furthermore, the values of ΨSAT, Ψp, and RWC were also significantly decreased in both mild and severe drought-stressed peach leaves by drought stress as compared to control treatment (Table 2).

3.2. Biochemical contents in peach leaves in response to drought stress

The contents of organic compounds were significantly affected by severe water deficit conditions in peach leaves (Table 3). Sorbitol was the most abundant sugar with 55%–70% accumulation out of the total soluble sugars. However, the amount of reducing sugars (glucose and fructose) and non-reducing sugar (sucrose) were significantly decreased throughout the drought period contrarily to the sorbitol contents as compared to control group. Overall, the sucrose contents at mild stress (23.2 ± 3.78 nmol C m⁻²) and severe stress (17.9 ± 2.53 nmol C m⁻²) were comparatively higher than the glucose (20.9 ± 1.25 – 17.3 ± 0.98 nmol C m⁻²) and fructose (8.5 ± 1.12 – 5.6 ± 0.78 nmol C m⁻²) in both mild and severe stressed drought treatments. Furthermore, the accumulation of proline contents occurred under stress conditions increased by 40.16% and 81.96% in mild and severe stress treatments, respectively, as compared to control (Table 3).

Table 1. Putative gene names and primer sequences for qRT-PCR analysis of gene expression in mild, and severe drought-responsive peach leaves.

| Putative gene function | Product length | Primer sequence (5′ -> 3′) |
|------------------------|---------------|--------------------------|
| Glutamyl-trNA reductase 1 (HemA)  | 248 | F: CCGGAAAAGCAATGGGAAGCTC | R: AGTGGCTGATGGCATGGA |
| Magnesium-chelatase subunit (ChlH) | 148 | F: GGGCAATCCAGTGGCTGGA | R: TCTTGGCAAGACCTAAAG |
| Photosystem I reaction center subunit (psaK) | 137 | F: GCGGTTGCTGGAGGCTGGA | R: TGGCTCAGCCAGCTACCA |
| Photosystem II core complex proteins (psb²) | 130 | F: AATGCTGCTTGTCGGCAA | R: ATGCTGCTTGTCGGCAA |
| Catalase (CATI) | 197 | F: GGCGTCACTCCTAAAGCAA | R: AGAACAACCCGGCAACAA |
| [SOD; Cu-Zn] | 162 | F: GGTGTCACCTTGCCAGGCTG | R: TCAACAGGGGCGCAAGACAG |
| L-APX | 182 | F: GGTCAACCTGTGAGGAATGTG | R: TCAATTCAGGGGCAAGACAG |
| DREB1A | 140 | F: CGAACGAAAACTTCTGGTC | R: TCTATCCAAGAAGGGGCA |
| DREB1B | 169 | F: GTGACCGAGGTGGTCAGGAG | R: ATGTCGAAAGAACGCAAGCA |
| Dehydration responsive element binding protein (DREB2A-like) | 188 | F: CGGAGGGAAAAACCCGCAAAG | R: GGGCCAGTTGAAGAAGTCCCT |
| DREB2B | 155 | F: GTGCCGGCCTGATGAGGC | R: TGTGAAGGAGTGTTGGC |
| WRKY11 | 173 | F: GGCAAGCGATTAGTGGC | R: TCTTCGTCGTTAGGCAAGCA |
| WRKY70 | 226 | F: TCCATTCGCGATGAGGCA | R: CATATCCGCTGTGAGCAGG |
| Actin (KvActin1) | 168 | F: GATTCGTGATGTTGACTGATG | R: GACAATCTCCCGTACAGG |

Table 2. Water potential (Ψw), the osmotic potential at full turgor (ΨSAT), turgor potential (Ψp) and RWC in control, mild stress and severe stress-responsive peach leaves.

| Treatment | Ψw (MPa) | ΨSAT (MPa) | Ψp (MPa) | RWC (%) |
|-----------|----------|------------|----------|---------|
| Control   | −0.70 ± 0.21a | −1.4 ± 0.8b | 1.5 ± 0.37a | 76 ± 5.6b |
| Mild stress* | −1.10 ± 0.23b | −1.7 ± 0.7a | 1.1 ± 0.29b | 72 ± 3.7b |
| Severe stress* | −1.85 ± 0.22c | −1.8 ± 0.9b | 0.6 ± 0.28a | 65 ± 2.5b |

*aSignifying significance level (α = 0.05). The significance level of 0.05 was used for indicators (a, b, c).
3.3. Chlorophyll degradation and photosynthesis incompetence in response to drought stress

Chlorophylls (Chls) are the most abundant pigments found in the biosphere, which actively participates in the photosynthetic process of green leaves by harvesting light energy and driving electron transport. The estimation of Chl contents unveiled that their accumulation was significantly reduced in mild stress (−16.66%) and severe stress (−40%) treatments as compared to control treatment (Figure 2). Moreover, net photosynthesis rate ($A_N$) was also decreased in mild stress from 16.18 ± 0.81 to 11.05 ± 0.75 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ and in severe stress from 16.18 ± 0.81 to 6.54 ± 0.11 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ as compared to control plants. Similarly, stomatal conductance ($g_s$), transpiration rate ($E$), and CO$_2$ assimilation rate ($C_i$) showed significantly marked reduction in their final values in both (mild and severe) drought treatments compared to control (Figure 1). Perversely, a significantly negative correlation was observed between $A_N$ and WUE, which was progressively increased by 26.19% and 59.52% during both mildly and severely affected peach leaves by drought stress compared to control (Figure 1).

For further verification of Chl biosynthesis and degradation process, and interrupted photosynthetic activity, the relative expression level of some genes involved in both pathways were investigated. Moreover, glutamyl-tRNA reductase 1 (HemA) and magnesium-chelatase subunit (ChlH) are the necessary genes to initiate the Chl biosynthesis which was also down-regulated by the drought stress; however, both (mild and severe) treatments did not show significant differences as compared to control (Figure 2). On the other hand, the activity of pheophorbide a oxygenase (PAO) was significantly up-regulated in both the drought treatments, indicating that degradation of chlorophyllide a into non-fluorescent Chl catabolites was enhanced by increasing the activity of this enzyme (Figure 2). Overall, these findings suggested that Chl synthesis process was severely repressed, but degradation process was induced by the drought stress in both mild and severe stress treatments as compared to control. Furthermore, the study of photosynthesis process revealed that the expression of genes-related to photosystem I reaction center subunit (psaK) and photosystem II core complex protein (psbY) were also significantly down-regulated in our findings (Figure 2).

3.4. ROS Synthesis and scavenging in response to drought stress

The enhanced activity of various antioxidant enzymes in the ROS system could occur in almost all type of environmental stress conditions. The MDA is an important marker to verify lipid peroxidation level, and its contents were significantly increased by 51.95% and 104.82% in mild and severe stress treatments, respectively, compared to control plants (Table 3). Similarly, the activities of ROS enzymes (SOD, POD, CAT, and APX) increased significantly in response to drought stress. The activity of SOD was higher (80.82%) all the times against drought stress. Meanwhile, the

| Parameters          | Control     | Mild stress* | Severe stress* |
|---------------------|-------------|--------------|---------------|
| MDA (nmol/g)        | 4.35 ± 1.21  | 6.61 ± 1.39   | 8.91 ± 1.27    |
| SOD activity (U g$^{-1}$ min$^{-1}$) | 371.56 ± 10.21 | 550.85 ± 15.78 | 671.88 ± 13.27 |
| POD activity (U g$^{-1}$ min$^{-1}$) | 18.23 ± 2.97   | 33.9 ± 2.01    | 52.01 ± 3.21   |
| CAT activity (U g$^{-1}$ min$^{-1}$) | 6.32 ± 1.21    | 15.01 ± 1.38   | 23.35 ± 1.56   |
| APX (U g$^{-1}$ min$^{-1}$) | 37.69 ± 1.52   | 47.95 ± 2.26   | 52.32 ± 1.67   |
| Proline (mg g$^{-1}$ FW) | 1.22 ± 0.94    | 1.71 ± 0.90    | 2.22 ± 0.02    |
| Glucose (nmol C m$^{-2}$) | 24.6 ± 1.11    | 20.9 ± 1.25    | 17.3 ± 0.98    |
| Fructose (nmol C m$^{-2}$) | 10.1 ± 1.05     | 8.5 ± 1.12     | 5.6 ± 0.78     |
| Sucrose (nmol C m$^{-2}$) | 28.3 ± 0.98     | 23.2 ± 3.78    | 17.9 ± 2.53    |
| Sorbitol (nmol C m$^{-2}$) | 85.2 ± 3.25    | 97.9 ± 2.89    | 108.3 ± 3.01   |
| Total sugars        | 148.2 ± 1.60   | 152.5 ± 2.26   | 149.1 ± 1.82   |

*Indicating significance level ($\alpha = 0.05$). The significance level of 0.05 was used for indicators (a, b, c).

Figure 1. Elucidation of the important physiological parameters, including Net photosynthesis rate ($A_N$), stomatal conductance ($g_s$), transpiration rate ($E$), WUE ($A_N$/$g_s$), net CO$_2$ assimilation ($C_i$), and chlorophyll content (CHL) in control, and mild and severe drought-stressed peach leaves.
activities of the POD, CAT, and APX were also increased with the prolonged stress duration in both mild and severe drought stress treatments as compared to control (Table 3).

The expression analysis of genes encoding ROS-scavenging system in peach leaves has demonstrated that POD, CAT, SOD, and APX were up-regulated in both mild and severe drought treatments as compared to control (Figure 2). Though the activity of APX was also significantly up-regulated in mild stress treatment as compared to control, there was no significant difference between both mild and severe stress treatments (Figure 2).

3.5. Role of transcription networks in response to drought stress
To undermine the regulatory mechanism of peach leaves against drought stress, the expression profiling of ABA-responsive genes (DREB1A, DREB1B, DREB2A, and DREB2B) and WRKY TFs (WRKY11 and WRKY70) were assessed in mild and severe treatments (Figure 3). The results depicted that all the ABA-responsive genes were up-regulated in response to drought in both mild and severely affected peach leaves. Moreover, DREB2A showed significantly 5.3-fold high expression level at mild stress and 9.4-fold at severe stress treatment as compared to control among all ABA-responsive genes (Figure 3). Among WRKY family, WRKY70 all the way revealed higher expression at both (mild and severe) stress treatments. Besides, WRKY11 also showed reduced expression at severe stress treatment, but there were no significant differences between mild stress treatment and control group, which indicates that in mild stress treatment the watering was applied after 4 days, may have controlled the expression level.

4. Discussion
The comprehensive study on versatile mechanism and responses to limited water conditions for developing resistant lines of fruit tree cultivars is gaining noticeable considerations. The choice of drought tolerant fruit cultivars is significantly imperative to avert future issues in the orchards with an aim to utilize water more efficiently.

Previously, some studies on Prunus rootstocks have shown a significant decrease in plant water status when subjected to limited water conditions (Rieger et al. 2003; Mellisho et al. 2011; Jiménez et al. 2013). In our findings, the values of $\Psi_{w}$, $\Psi_{p}$, and RWC in peach leaves were decreased in mildly and severely drought-treated peach cultivar. In plants, reduction in water contents and low $\Psi_{w}$ is a primary sign of water stress, which affects the movement of water into new growing regions (Kongsri et al. 2013). Our findings are in agreement with previously available reports on the $\Psi_{w}$, $\Psi_{p}$, and RWC of the peach plant, indicating decreased values with increased stress duration (Escobar-Gutierrez et al. 1998; Mellisho et al. 2011). The results of the current investigation proposed that peach plants showed an adjustment to dynamic drought spell probably because they have the ability to accumulate active osmolytes. Moreover, the current findings of potted plants appear to imitate the field conditions of stress in response to drought stress and allowed to unveil the drought responses induced by plants nevertheless to growth size orientation.

Plant growth mainly depends upon storage substance like carbohydrates, which are mobilized in the form of soluble sugars (Samii et al. 2016). P. Persica plants demonstrated variations in the composition of soluble sugar when subjected to different spells of drought (mild and severe) stress (Table 2). The reduction in glucose, fructose and sucrose concentration and an increased accumulation of sorbitol level accounts a common response of Rosaceae family in response to drought stress (Rieger et al. 2003; Cui et al. 2004; Jiménez et al. 2013). It has been well documented that instead of sucrose, sorbitol is preferentially synthesized even at low photosynthesis process in drought-affected peach leaves (Escobar-Gutierrez et al. 1998). The higher accumulation of leaf sorbitol could act as one of the real component required for osmotic adjustment during stress conditions (Krasensky and Jonak 2012). Moreover, the accumulation of particular osmolytes (proline) has also been well observed in Prunus species (Zrig et al. 2015; Khoyerdi et al. 2016). Similarly, in our findings, proline accumulation in the peach has depicted to play a tolerance-related role to confer drought stress and has been associated with stress tolerance (Garciasanchez et al. 2007; Bandurska et al. 2009). Krasensky and Jonak (2012) have described in their findings that proline may act as a molecular chaperone to stabilize structural proteins and also as ROS scavenger during stress conditions. Attributed to the alleged antioxidant role of sorbitol and proline, they can ameliorate malicious effects of oxidative stress by defending enzymes and membrane from damage (Ashraf et al. 2011; De Campos
et al. 2011). Regardless of whether both can likewise provide osmotic adjustment in peach leaves still need more considerations.

Chlorophylls (Chls) are the primary light-absorbing pigments and key components of the photosynthesis in plants. The physiological investigation of drought-responsive peach leaves has exhibited remarkably lower Chl contents as compared to control plants. Similarly, drought stress repressed the Chl accumulation has been reported in other species, including P. Persica (Jiménez et al. 2013), Z. mays (Mafakheri et al. 2010), and C. arietinum (Homayoun et al. 2011). The relative expression level of key Chl synthesis enzymes, such as \( \text{HemA} \) (glutamyl-tRNA reductase 1) and \( \text{ChlH} \) (magnesium chelatase H subunit) was down-regulated contrarily to \( \text{PAO} \) (PAO; up-regulated). Horsteneiner and Kräutler (2011) have regarded \( \text{HemA} \) and \( \text{ChlH} \) as the key enzymes of Chl biosynthesis, but their repressed activity has unveiled that drought stress inhibited the Chl synthesis process. Aberantly, \( \text{PAO} \) has been well demonstrated as Chl catabolic enzyme, and previous researchers have publicized that expression of \( \text{PAO} \) is persuaded by natural senescence and environmental stress in plants (Gray et al. 1997, 2002; Pružinska et al. 2005). Furthermore, the concomitant decrease in photosynthesis rate, stomatal conductance, net CO\(_2\) assimilation rate could specify that the obstructed stomatal conduc-
tance was the reason to limit photosynthesis under drought stress, which has already been reported in peach (Jiménez et al. 2013) and citrus (Garcíasanchez et al. 2007). Similarly, the photosynthesis-related genes involved in photosystem I (PSI; \( \text{psaK} \)) and photosystem II (PSII; \( \text{psbY} \)) were significantly down-regulated by the drought stress. Previous studies on Z. mays (Gao et al. 2015) and S. tuberosum (Thornton et al. 1996) have demonstrated that primary light-driven photosynthesis reaction occurs in the thylakoid membrane of PSII and PSI, which is very sensitive to the drought stress, support our findings.

MDA as an artifact of lipid peroxidation reflects the level of membrane lipid peroxidation in stressed plants. Previous studies depicted that accumulation of MDA significantly increased in response to drought stress (Silva et al. 2015; Khoyerdi et al. 2016). To counter the ROS synthesis, the activities of antioxidant enzymes (SOD, POD, CAT, and APX) were induced, which can adjust the balance by detoxification of excess ROS production (Khoyerdi et al. 2016). The SODs provide the first defense against ROS and resolve \( \text{H}_2\text{O}_2 \). Likewise, CAT and APX efficiently detoxify the \( \text{H}_2\text{O}_2 \) in plants as well. Haider, Zhang et al. (2017) investigated antioxidant enzymes (SOD, POD, and CAT) in drought-treated grapes and reported an increasing trend. Moreover, the oxidative stress-responsive enhanced activity of antioxidant enzymes have been reported in many crop plants (Sofo et al. 2005; Jin et al. 2015; Silva et al. 2015; Ahmad et al. 2018). Collectively, these findings suggested that peach may have up-regulated the activities of antioxidant enzymes to prevent ROS toxicity. Though, these activities were slightly declined in mild stress treatment after re-watering (after 4 days), but yet significantly higher than the control plants, has been previously validated by Bian and Jiang (2009). The expression level of antioxidant-related genes showed up-regulation to drought stress in our findings. Previous studies have specified that Cu-Zn SOD is an isoform of SOD, primarily responding to drought stress (Cao et al. 2017; Verslues et al. 2007). Our findings are consistent with that SOD activity was up-regulated during drought stress (Brossa et al. 2015). Ascorbate peroxidase (APX) utilizes Ascorbate-gluthionine (AsA–GSH) to alter the \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \), whereas, CAT is essentially requisite to assimilate \( \text{H}_2\text{O}_2 \) and locales in peroxisomes. Cao et al. (2017) investigated the expression level of antioxidant-related genes and demonstrated the similar trend of SOD, POD, and APX except for CAT which is inconsistent with our findings. Similar research in wild watermelon exhibited up-regulation of ROS scavenging enzymes both at early and late phases of drought stress (Yoshimura et al. 2008). The over-production of ROS is accompanied with oxidative stress, and reciprocal synthesis of ROS scavenging enzymes revealed that antioxidant system might be involved in the protection of peach plants from potential damages of drought stress.

The DREBs and WRKYs are important TFs that initiate the abiotic stress-related genes in plants to confer stress. The drought stress strongly induced the expression of ABA-responsive genes (DREB1A, DREB1B, DREB2A, and DREB2B) and WRKY TFs (WRKY11 and WRKY70) in our findings. The DREB TFs belong to the ABA-independent pathway, further categorized into DREB1 and DREB2, which belongs to ERF family of TFs and involved in two different signal transduction pathways (Agarwal et al. 2006). The expression of DREB1A and its homolog (DREB1B), and DREB2A and its homolog (DREB2B) is comprehensively investigated in many crops, for example, the expression of \( \text{AtDREB1A} \) was induced by the drought stress (Liu et al. 1998). Similarly, the expression of \( \text{DREB2A} \) and its homolog \( \text{DREB2B} \) was induced by salt and dehydration stresses (Nakashima et al. 2000). Not all the DREBs have the same responses to drought, for instance, \( \text{DREB2} \)-type TF (\( \text{TaDREB1} \)) isolated from wheat was induced by the low temperature and responded poorly to the drought stress, conflicting with our findings (Shen et al. 2003). Though, the over-expression (OE) of soybean \( \text{GmDREB1} \) and \( \text{GmDREB2} \) showed improved drought tolerance (Li et al. 2005). The ABA-independent and ABA-dependent pathways might regulate the transcriptional networks in response to osmotic stress, though the regulation information regarding both signaling pathways is still lacking. Previous reports depicted that \( \text{DREB2A} \) plays a key role in ABA-independent gene regulation under drought stress (Yoshida et al. 2014). Moreover, WRKY10 TF is the homolog of \( \text{AtWRKY65} \), relates to the group II of WRKY gene family and possesses transcriptional activation (Wang et al. 2013). The OE of \( \text{CmWRKY10} \) in chrysanthemum authenticated the involvement of this TF in drought stress tolerance, which further revealed the positive correlation between expression of \( \text{CmWRKY10} \) and survival rate of plants (Abuzar et al. 2016). The previous study on wheat \( \text{TaWRKY10} \) has elucidated that OE of this TF in this tobacco improves the proline and sugar contents by reducing ROS accumulation to mitigate drought stress (Wang et al. 2013). Besides, WRKY70 belongs to group III WRKY TF family, is a positive regulator of plant tolerance to osmotic stress. Li et al. (2013) explored the possible role of two WRKY double mutants (\( \text{wrky54wrky70} \)) in Arabidopsis and depicted that they play a significant role not only in plant defense but also in abiotic stress signaling. Based on our findings, it is obvious now that \( \text{DREB} \) and WRKY proteins are important TFs in playing a decisive role in imparting stress endurances in plants.
5. Conclusions

The findings of the current investigations have revealed that $\Psi_w$, $\Psi_p$, and RWC were reduced physiologically in drought-responsive ‘Yoshihime’ peach cultivar. Moreover, the Chl contents, $A_{NP}$, $g_o$, and $E$ were badly repressed by the mild and severe drought spells. Instead, the accumulation of sorbitol and proline can be implemented as drought tolerance markers for early selection of peach cultivar under controlled conditions. Moreover, the activity of important oxidative stress marker (MDA) was increased, but peach responded well by increasing the activities of antioxidant enzymes at both biochemical and molecular levels. The differential expression of TFs has revealed their role as positive regulator of stress-related genes to confer drought stress in peach, which could also be used as important osmotic stress marker. Overall, our findings have provided the basis for candidate gene selection to untangle the tolerance mechanism.

Disclosure statement

No potential conflict of interest was reported by the authors.

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