

Research Article



Drought-Induced Oxidative Damage and Antioxidant Responses in Blackberry Cultivar 'Hull Thornless'

Haiyan Yang¹, Hongxia Liu¹, Wenlong Wu^{1*}, Weilin Li² and Lianfei Lyu¹

¹Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, People's Republic of China; ²Nanjing Forestry University, Nanjing 210037, People's Republic of China.

Abstract | Drought stress is a limiting factor for plant cultivation in many areas of China. In present study, the blackberry cultivar 'Hull Thornless' was chosen for drought stress analysis. All plants were grown in pots under greenhouse conditions, and were subjected to 20-day drought stress by withholding irrigation, followed by re-watering for 5 days. The changes in leaf water content (LWC), electrolyte leakage (EL), concentrations of photosynthetic pigments, protein, soluble sugar, hydrogen peroxide (H₂O₂), malondialdehyde (MDA), ascorbate (AsA) and reduced glutathione (GSH), activities of superoxide dismutase (SOD) and peroxidase (POD) in leaves of 'Hull Thornless' were investigated. In the treatment group, water stress significantly increased EL, and the accumulation of photosynthetic pigments, protein, soluble sugar, H₂O₂ and MDA. After re-watering, all of those parameters showed decreases of different degree. LWC was significantly decreased in response to drought treatment. However, after 5 days of re-watering, the LWC restored. Moreover, SOD activity increased gradually under drought stress, except for the day 15. After re-watering, SOD activity was still at higher level. Under drought treatment, POD activity and levels of AsA and GSH first ascended and then followed by a decline. After re-watering, they all decreased, but AsA and GSH levels were remain higher than the controls. The results indicated that the tolerance of blackberry cultivar 'Hull Thornless' to drought stress was enhanced by activating the antioxidant system, and altering the levels of photosynthetic pigments.

Received | December 07, 2018; **Accepted** | April 20, 2019; **Published** | August 01, 2020

***Correspondence** | Wenlong Wu, Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, People's Republic of China; **Email:** 1964wwl@163.com; haiyanyang_025@126.com

Citation | Yang, H., H. Liu, W. Wu, W. Li and L. Lyu. 2019. Drought-induced oxidative damage and antioxidant responses in blackberry cultivar 'Hull Thornless'. *Pakistan Journal of Agricultural Research*, 33(3): 643-651.

DOI | <http://dx.doi.org/10.17582/journal.pjar/2020/33.3.643.651>

Keywords | Blackberry (*Rubus* spp.), Drought stress, Re-watering, Oxidative stress, Antioxidants

Introduction

Water deficiency is a severe environmental stress, and has a negative impact on crop yields worldwide (Pennisi, 2008). Approximately one-third of the world's arable land suffers from chronically inadequate supplies of water for agriculture. Plants have evolved complex mechanism to perceive, respond and adapt to water deficit. For instance, they can avoid drought by accumulating osmolytes, reducing evaporative water loss, scavenging reactive oxygen

species (ROS) and synthesizing new substances such as proteins and phytohormones. To date, the effects of drought stress on plants have been widely studied at physiological, molecular and cellular levels (Pan et al., 2006; Pyngrope et al., 2013; Sapeta et al., 2013; Yue et al., 2012), but the responses to re-watering after drought stress and related mechanism are still lacking. The capacity for rapid recovery after re-watering may be adaptive to plants (Galmés et al., 2007). Previous studies reported that several species of stressed plants can recover after re-watering (Ortño et al., 2005;

Blackberries (*Rubus* spp.) are widely distributed around the world and are extremely tolerant of site and soil conditions (Strik, et al., 2007). 'Hull Thornless' was selected in 1968 at Carbondale, Il, by Jack Hull as a result of the cross SIUS 47 × Thornfree (Galletta et al., 1981). It is the predominant erect blackberry grown worldwide and is also one of the main cultivars in China. However, in China, the blackberry plants were mainly cultivated in the hilly areas where water irrigation is required, especially in the summer time. Followed with global warming, the hot weather occurred in many areas and indicated that drought is becoming a common environmental stress that severely limits plant growth and development. Thus, to elucidate drought related traits of this species is not only important to understand how drought tolerant plants are functioning in their natural environments but also improve its agronomic characteristics.

Up to date, the detailed and comprehensive studies on physiological adaptation of blackberry to drought stress are still lacking. In the present study, the potential role of antioxidant defense system in enhancing its protecting 'Hull Thornless' from oxidative stress of drought was examined by analyzing enzyme activities and some low-molecular antioxidants content such as SOD, POD, AsA and GSH. We also investigated the basic parameters such as leaf water content, the membrane electrolyte leakage, photosynthetic pigments, protein, soluble sugar, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), in leaves of 'Hull Thornless'. The present research inferred that a comprehensive adaption was employed for blackberry to cope with drought stress.

Materials and Methods

Plant materials

This experiment was carried out in the Institute of Botany, Jiangsu Province and Chinese Academy Sciences, Nanjing, China. One-year-old seedlings of 'Hull Thornless', which were obtained by layering, were used in the tests. Plants were grown in plastic pots (26 cm upper diameter, 18 cm lower diameter, and 24 cm high) containing a mixture of loamy garden soil and peat soil (3:2, v/v) and maintained in a greenhouse where temperature ranged 19-26 °C, relative humidity 65-70%, and natural light/dark cycle. These plants were normally irrigated until the

beginning of the treatments.

Plants were divided in two groups. The first group was maintained under well-watered conditions throughout the period of the experiment, while the second group was subjected to 20-day drought stress by withholding irrigation, and then plants were re-watered to field capacity and followed by a recovery for 5 days. Samples of fully expanded leaves at the fourth or fifth positions from the apex of the shoot were harvested at five treatment periods (0, 10, 15 and 20 days drought and a 5 day recovery period from drought stress). This experiment was set up as a randomized complete design with six replications.

Assay of leaf water content

Leaf water content (LWC) was determined using the equation: $LWC (\%) = [(FW - DW)/FW] \times 100\%$. Fully developed leaves were harvested and weighed immediately to determine the fresh weight (FW) and were then oven dried at 60 °C till constant weight for dry weight (DW) (Turner, 1981).

Assay of electrolyte leakage (EL)

Electrolyte leakage (EL) was determined according to Yan et al. (1996). Six fresh leaf discs with the diameter of 5 mm were put in a 10 ml volume of vials containing 2 ml deionized water. Then they were vacuumized via a vacuum pump for 3h and then electrical conductivity of the solution (L_t) was determined at 25°C. After this, the leaves were treated in boiling water for ten minutes. The final electrical conductivity (L_0) of the samples was measured after equilibration at 25°C. EL (%) data was obtained according to the calculation formula $(L_t/L_0) \times 100$.

Assay of photosynthetic pigment

Chlorophylls contents were determined according to Lichtenthaler (1987). Leaf samples (~0.2g fresh sample) were taken and the chlorophylls were extracted in 80 % chilled acetone. Absorbance of centrifuged extracts was measured at 470, 647 and 663 nm.

Assay of protein and soluble sugar contents

The content of soluble protein was estimated by the method of Bradford (1976) using bovine serum albumin as a standard. Soluble sugar content was measured using the method of Ji et al. (2008).

Assay of leaf lipid peroxidation and H_2O_2 contents

The lipid peroxidation status was expressed as malondialdehyde (MDA) content and was determined as the 2-thiobarbituric acid-reactive metabolites (Heath and Packer, 1968). The concentration of MDA was calculated by using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as nmol g^{-1} fresh weight. The content of H_2O_2 was measured by the reagents purchased from Nanjing Jiancheng Bioengineering Institute, China.

Assay of antioxidant enzyme activities

Plant material (0.5 g) was homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.8), and then centrifuged at $10,000 \times g$ for 20 min at 4°C . Supernatant was used to measure the activities of enzymes. The activity of SOD was assayed by the method of Stewart and Bewley (1980) by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The activity of POD was measured by using the guaiacol method (Maehly, 1955).

Assay of ascorbate (AsA) and reduced glutathione (GSH) contents

The plant leaves (0.2 g) were homogenized in ice-cold 5% (w/v) trichloroacetic acid and then centrifuged at $10,000 \times g$ for 20 min at 4°C . AsA was determined following the method of Law et al. (1983). To measure total AsA, 0.2 mL of treated-supernatant was added to 0.5 mL of 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA and 0.1 mL of 0.5 mM N-ethylmaleimide, 0.4 mL of 10% (w/v) trichloroacetic acid, 0.4 mL of 44% (v/v) H_3PO_4 , 0.4 mL of 4% (w/v) 2,2-dipyridine (dissolved with 70% ethanol) and 0.2 mL of 3% (w/v) $FeCl_3$. Then the mixture was incubated at 40°C for 40 min. The absorbance of the mixture was read at 525 nm and the result was expressed as AsA content in the tissue (mg g^{-1} fresh weight). GSH was measured using the method of Anderson (1985). The supernatant (0.25 mL) was mixed with 2.6 mL of 150 mM phosphate buffer (pH 7.7) and 0.18 mL 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB). Then the mixture was incubated at 30°C for 5 min. The absorbance of the mixture was read at 412 nm and the level of GSH was expressed as $\mu\text{g g}^{-1}$ fresh weight.

Statistical analysis: The experiments were repeated at least three times. The mean values with standard deviation (SD) are shown in the table and figures in the next section. The results were derived from statistical

analysis using analysis of variance (ANOVA). Levels of significance were indicated by Duncan's multiple range test at $p < 0.05$.

Results and Discussion

Leaf water content

To assess the ability of this cultivar to retain water under drought, LWC was determined. The results of this experiment showed a significant reduction of the LWC in 'Hull Thornless' (Figure 1). At 20 days after drought stress, LWC was reduced to 49.21%, however, after 5 days of re-watering, the LWC restored, and was recorded as 81.15% in leaves of the plant.

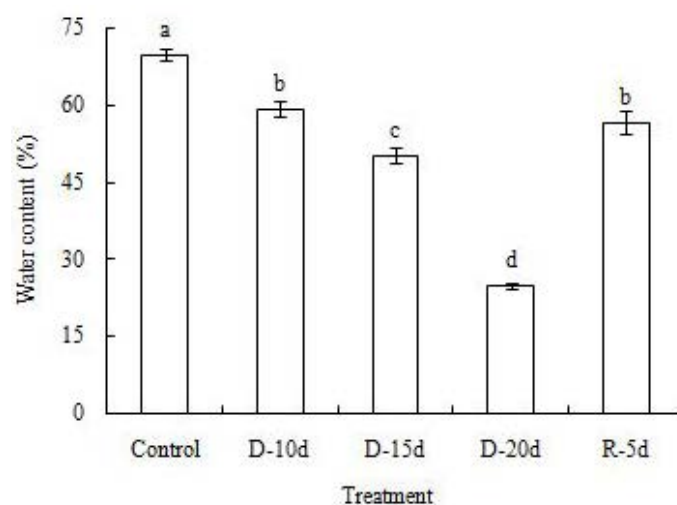


Figure 1: Leaf water content in 'Hull Thornless' subjected to drought stress and re-watering. Control: well-watered; D: drought stress treatments of 10, 15, and 20 days, and R: 5 days after re-watering. Data are means \pm S.D. ($n = 3$). Values designated over the bars in different letters are significantly different at $p < 0.05$.

Membrane permeability

Drought stress resulted in a significant increase in membrane electrolyte leakage in the leaves of 'Hull Thornless' (Figure 2). Membrane electrolyte leakage raised 2.7 fold on day 20 of drought stress compared to the control, which was recorded as 82.42% in leaves of the plant. After re-watering, the electrolyte leakage decreased to approximately the control level.

Protein, MDA and H_2O_2 and soluble sugar contents

As shown in Table 1, a significant alteration in protein content was found in the leaves of 'Hull Thornless'. Compared with the control, the maximal increase was recorded as 140.7% in leaves of the plant on the day 10. After re-watering, the protein content was still remained higher than the control.

Table 1: Protein, MDA, H₂O₂ and soluble sugar contents in 'Hull Thornless' subjected to drought stress and re-watering.

Control: well-watered; **D:** drought stress treatments of 10, 15 and 20 days and **R:** 5 days after re-watering. Data are means±S.D. (n = 3). Different letters in the same column indicate that means are significantly different at p < 0.05.

Treatment	Protein content (mg g ⁻¹ FW)	MDA content (nmol g ⁻¹ FW)	H ₂ O ₂ content (μmol g ⁻¹ FW)	soluble sugar content (nmol g ⁻¹ FW)
Control	2.28±0.09d	11.74±2.55e	824.92±24.82d	82.63±1.95d
D-10d	3.21±0.03a	15.26±1.07d	1151.60±59.71c	104.73±8.60d
D-15d	2.79±0.09c	42.51±0.65b	1984.06±132.50b	279.35±9.15b
D-20d	3.09±0.03b	62.10±2.18a	3463.07±46.92a	393.33±11.27a
R-5d	3.07±0.05b	18.56±0.89c	858.68±23.46d	221.09±26.46c

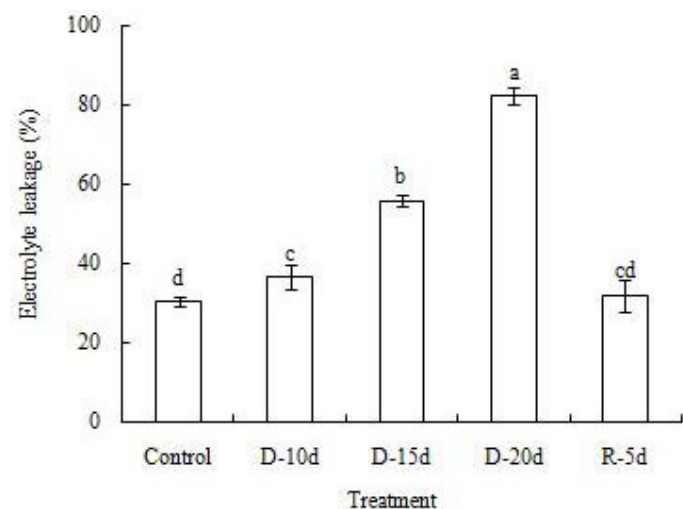


Figure 2: Electrolyte leakage in 'Hull Thornless' subjected to drought stress and re-watering. Control: well-watered; D: drought stress treatments of 10, 15, and 20 days, and R: 5 days after re-watering. Data are means±S.D. (n = 3). Values designated over the bars in different letters are significantly different at p < 0.05.

Under drought stress, the contents of MDA, H₂O₂ and soluble sugar in the leaves of 'Hull Thornless' increased in a linear fashion with the progression of stress. On the day 20, they all reached the peak values, to 5.3, 4.2 and 4.8 times of the control levels, respectively. Moreover, H₂O₂ content was restored to control level by re-watering, whereas MDA and soluble sugar contents remained significantly higher than those of controls.

SOD and POD activities

The activities of SOD and POD showed different changes in leaves of 'Hull Thornless' during the drought stress period (Figure 3). SOD activity increased gradually under drought stress, except for the day 15. After 5 days of re-watering, SOD activity was still at higher level compared with control. Under drought treatment, POD activity first ascended and then followed by a decline. It reached the peak on the day 10 and showed an increase of up to 311.27%

in comparison to the control. After 5 days of water recovery, POD activity was significantly lower than the control.

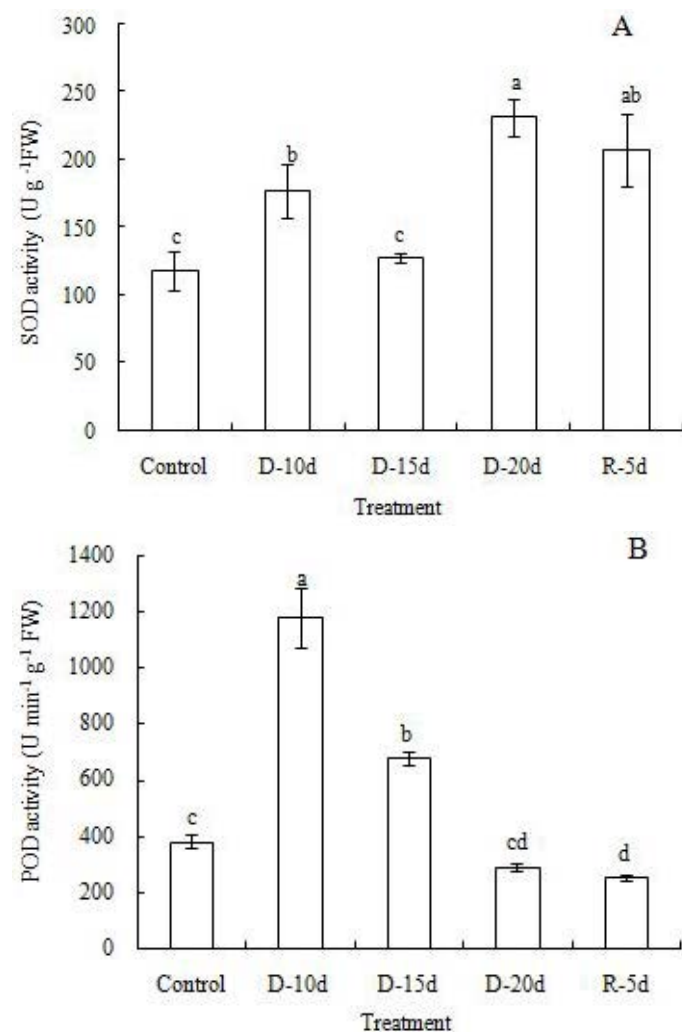


Figure 3: SOD (A) and POD (B) activities in 'Hull Thornless' subjected to drought stress and re-watering. Control: well-watered; D: drought stress treatments of 10, 15, and 20 days, and R: 5 days after re-watering. Data are means±S.D. (n = 3). Values designated over the bars in different letters are significantly different at p < 0.05.

AsA and GSH contents

Compared to controls, the contents of AsA and GSH increased rapidly under drought stress (Figure 4). The

maximum increases were 80.19% and 128.56%, on the day 10 when compared to the controls. After re-watering, AsA and GSH levels were still 1.2 and 1.1 times higher than the controls, respectively.

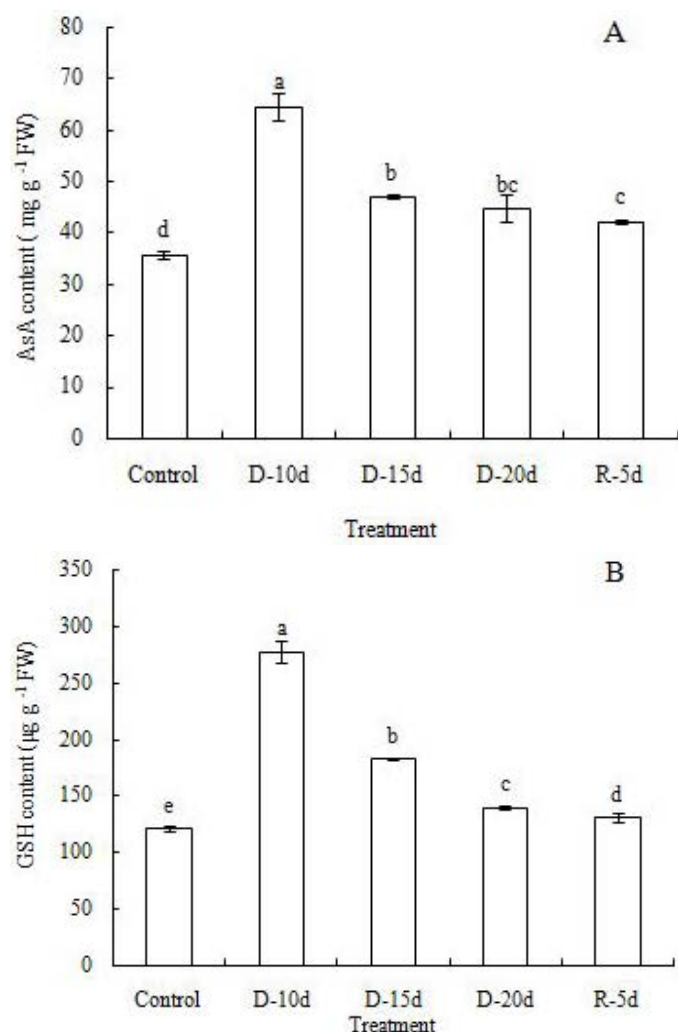


Figure 4: AsA (A) and GSH (B) contents in 'Hull Thornless' subjected to drought stress and re-watering. Control: well-watered; D: drought stress treatments of 10, 15, and 20 days, and R: 5 days after re-watering. Data are means±S.D. (n = 3). Values designated over the bars in different letters are significantly different at $p < 0.05$.

Chlorophyll content

As show in Table 2, drought stress significantly increased the contents of chlorophyll *b* (Chl *b*) and chlorophyll (*a+b*) compared to the controls. The maximal increase in Chl *b* and chl (*a+b*) contents were recorded as 128.07% and 61.92% in leaves of 'Hull Thornless' on the day 15 and 20, respectively. After 5 days of water recovery, the contents of Chl *b* and chl (*a+b*) decreased, which were 54.39% and 55.52% of the control levels. Compared with the control, the content of chlorophyll *a* (Chl *a*) first slightly decreased and then increased. After 5 days of re-watering, Chl *a* content decreased to 56.29% of the background level.

Drought is one of the most important environmental extremes that constraints to plant survival and to crop productivity in natural and agricultural habitats. Under drought stress, plant water status acts as a regulatory switch for the myriad of physiological, biochemical and morphological responses of the plant, and LWC is proposed as a selection criterion for drought tolerance (Banik et al., 2016). In 'Hull Thornless', LWC was significantly decreased under drought stress, however, during the recovery period, the LWC returned to its starting levels rapidly (Figure 1), indicating that plants might have evolved complex mechanisms to sense water availability and re-program their metabolism.

It is well known that drought stress causes a marked increase in oxidative damage to plants (Pan et al., 2006; Pyngrope et al., 2013; Sapeta et al., 2013; Yue et al., 2012). MDA is the decomposed product of polyunsaturated fatty acids in bio-membranes and usually used as the criterion of stress-induced oxidative damage to membrane (Yue et al., 2012). In the present study, a time-dependent increase in the levels of MDA was observed in the leaves of 'Hull Thornless' (Table 1), which is in agreement with the results of other studies (Pan et al., 2006; Yue et al., 2012). However, after 5 days of re-watering, this parameter was significantly decreased. Per-oxidation of membrane lipids may result in enhanced membrane fluidity, which may also led to enhanced electrolyte leakage (Li et al., 2013). This effect was also confirmed by the greatly increased membrane electrolyte leakage when 'Hull Thornless' suffered soil drought stress (Figure 2). Generally, plants have evolved an efficient system for decomposing ROS, comprising of enzymatic and non-enzymatic mechanisms (Miller et al., 2010). In the present study, the activities of SOD and POD enzymes were increased in 'Hull Thornless' leaves when compared with the control groups seems to be the physiological adaptive mechanisms to regulate its redox status under drought stress conditions (Figure 3).

SOD is the first line of defense against ROS by dismutating $O_2^{\cdot-}$ into H_2O_2 (Pan et al., 2006; Ahmed et al., 2009; Singh et al., 2010). It exists in the cytoplasm, mitochondria, chloroplasts and peroxisomes (Vyas and Kumar, 2005). In higher plants, SOD exists in multiple isoforms that are developmentally regulated and highly reactive to abiotic stress (Pan et al., 2006). In previous studies, different responses of SOD have been shown depending on drought severity, duration

and species. For example, high levels of SOD activity were reported in several studies including *Glycyrrhiza uralensis Fisch* (Pan et al., 2006), olive (Ahmed et al., 2009) and cabbage (Singh et al., 2010) under drought treatments. Confirming these studies, in this study, SOD activity increased gradually under drought stress, except for the day 15. After 5 days of re-watering, SOD activity was still higher than the control plants (Figure 3A). A higher sensitivity of SOD to drought stress played a key role in protective mechanism in 'Hull Thornless' leaves. While SOD is the first line of defense against oxidation stress, POD is the second. POD is considered as a stress marker enzyme having a higher affinity for removal of H_2O_2 (Foyer and Noctor, 2005; Pan et al., 2006). In the present study, POD activity first ascended and then followed by a decline on day 15, suggesting that the protective function of POD to harmonize and maintain the stability of the membrane system had reached an upper limit (Figure 3B). Confirming this, H_2O_2 levels were markedly increased during drought treatments. However, after 5 days of re-watering, H_2O_2 levels were rapidly decreased, faster catabolism of H_2O_2 must be helpful for better recovery from drought stress.

Apart from these antioxidant enzymes mentioned above, the levels of antioxidants like AsA and GSH during drought stress were all higher than those in control, which would increase the tolerance of plants to free radical damage (Pyngrope et al., 2013). Besides, previous studies on plants with altered contents of AsA and GSH also proved that these two antioxidants play important roles in the tolerance of plants to environmental stresses (Noctor and Foyer, 1998; Mittova et al., 2003; Pyngrope et al., 2013). AsA is considered as a powerful antioxidant because of its ability to donate electrons in a number of enzymatic and non-enzymatic reactions. AsA has a key role in removal of H_2O_2 via AsA-GSH cycle (Noctor and Foyer, 1998; Pyngrope et al., 2013). Significant increase in AsA content under drought stress (Figure 4A) could suggest that ascorbate synthesis was stimulated and it might be also resulted from a concurrent enhancement in GSH content. In 'Hull Thornless', less remarkable enhancement in AsA content observed after drought periods of 10 and 15 days was probably due to a greater formation of H_2O_2 and its poisonous derivatives.

GSH functions as an antioxidant in many ways. It can

react chemically with $O_2^{\cdot-}$, $\cdot OH$, H_2O_2 and, thus, can function directly as a free radical scavenger (Mittova et al., 2003; Pyngrope et al., 2013). In present study, GSH content was greatly increased under drought stress (Figure 4B). The accumulation of GSH might be due to the stimulation of enzymes which were responsible for its synthesis. However, when the stress increased, the GSH content dropped and redox state became more oxidized, which marked the degradation of the system. Besides, GSH is a crucial low molecular weight non-protein thiol that plays an important role in intracellular defense against ROS-induced oxidative damage (Pyngrope et al., 2013). During drought stress, it may also protect the thiol status of proteins in the leaves of 'Hull Thornless'.

In this experiment, it could be seen from Table.1 that drought stress enhanced the content of soluble protein in 'Hull Thornless' leaves; then, after re-watering, the plants still maintained higher protein level. It was suggested that protein accumulation under drought stress could increase the bound water content, cell water holding capacity, and help the plants to avoid the drought induced damages (Jiang and Huang, 2002). Similarly, soluble sugar content was also increased in plant leaves during the drought stress period (Table 1). Bolouri-Moghaddam et al. (2010) suggested that sugars are important regulatory molecules with both signalling and putative ROS scavenging functions in plants. Soluble sugars and the enzymes associated with their metabolic pathways are widely believed to be connected to oxidative stress and ROS signaling. Besides, Li et al. (2006) found that glucose changes the expression of 6.6% of all genes in *Arabidopsis thaliana*, suggesting changes in soluble sugars in plants may cause changes in activities of many enzymes. Thus, in 'Hull Thornless', soluble sugars might act as signaling molecules and influence the enzymes in the antioxidant system.

Chlorophyll degradation is a common response in plants under drought stress (Guerfel et al., 2009; Liu et al., 2011). However, in the present study, chlorophyll contents were greatly increased in the leaves of 'Hull Thornless' (Table 2). Compared with the control, the content of Chl *a* first slightly decreased and then increased, while the content of Chl *b* gradually increased during the drought stress period. In our experiments, the increment of chlorophyll contents was mainly due to the increase in Chl *b* content. This has also been reported for *Jatropha curcas*,

Table 2: Pigment contents in 'Hull Thorn less' subjected to drought stress and re-watering. **Control:** well-watered; **D:** drought stress treatments of 10, 15 and 20 days and **R:** 5 days after re-watering. Data are means±S.D. (n = 3). Different letters in the same column indicate that means are significantly different at $p < 0.05$.

Treatment	Control	D-10d	D-15d	D-20d	R-5d
Chla (mg g ⁻¹ FW)	1.67±0.01b	1.65±0.01b	1.48±0.08c	2.78±0.15a	0.94±0.03d
Chlb (mg g ⁻¹ FW)	1.14±0.06c	1.38±0.28c	2.6±0.07a	1.76±0.19b	0.62±0.04d
Chl(a+b) (mg g ⁻¹ FW)	2.81±0.06c	3.04±0.28c	4.08±0.1b	4.55±0.35a	1.56±0.06d

which suggested that the high chlorophyll contents under drought stress was due to the increase in Chl *b* content (Sapeta et al., 2013). Besides, future studies are needed to clarify the role of chlorophyll changes in drought tolerance of blackberry.

Conclusions and Recommendations

In summary, drought stress induced pronounced oxidative damage in leaves of 'Hull Thornless'. Drought stress accelerated the generation of reactive oxygen species (ROS), provoked membrane lipid peroxidation, and disrupted the balance of Chlorophyll metabolism. It is suggested that drought stress tolerance of blackberry was associated with its ability to maintain higher constitutive activity of SOD and POD, and induced levels of anti-oxidative compounds (AsA and GSH). Increased protein and soluble sugar also play roles in reducing ROS directly and decreasing lipid peroxidation in blackberry. However, after re-watering, SOD activity, AsA and GSH levels, protein and soluble sugar contents were still higher than controls, respectively. Besides, LWC was almost restored after re-watering. All of these suggested that 'Hull Thornless' has a good recovery capacity after re-watering. Our results support the idea that blackberry is a suitable variety in areas with limited water availability.

Author's Contribution

Haiyan Yang conducted the experiment, collected and analyzed the data, and prepared the draft. Wenlong Wu and Weilin Li participated in the design of the study and revised the manuscript. Hongxia Liu and Lianfei Lyu helped measurements of antioxidant enzymes. All authors read and approved the final manuscript.

Acknowledgement

This research was supported by National Natural Science Foundation of China (No.31700606)

and Jiangsu Agricultural Science and Technology Innovation Fund Project No CX(19)2013.

References

- Ahmed, C.B., B.B. Rouina, S. Sensoy, M. Boukhris and F.B. Abdallah. 2009. Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. *Environ. Exp. Bot.* 67: 345–352. <https://doi.org/10.1016/j.envexpbot.2009.07.006>
- Anderson, M.E. 1985. Determination of glutathione and glutathione disulfide in biological samples. *Method Enzymol.* 113: 545–548. [https://doi.org/10.1016/S0076-6879\(85\)13073-9](https://doi.org/10.1016/S0076-6879(85)13073-9)
- Banik, P., W.P. Zeng, H.L. Tai, B. Bizimungu and K. Karen, Tanino. 2016. Effects of drought acclimation on drought stress resistance in potato (*Solanum tuberosum* L.) genotypes. *Environ. Exp. Bot.* 126: 76–89. <https://doi.org/10.1016/j.envexpbot.2016.01.008>
- Bolouri-Moghaddam, M.R., K. Le Roy, L. Xiang, F. Rolland and W. Van den Ende. 2010. Sugar signalling and antioxidant network connections in plant cells. *FEBS. J.* 277: 2022–2037. <https://doi.org/10.1111/j.1742-4658.2010.07633.x>
- Bradford, M.M. 1976. A rapid and sensitive method for the quantity of protein dyebinding. *Anal. Biochem.* 72: 248–254. <https://doi.org/10.1006/abio.1976.9999>
- Foyer, C.H. and G. Noctor. 2005. Oxidant and antioxidant signalling in plants, a reevaluation of the concept of oxidative stress in a physiological context. *Plant Cell, Environ.* 28: 1056–1071. <https://doi.org/10.1111/j.1365-3040.2005.01327.x>
- Galletta, G.J., A.D. Draper, R.G. Hill Jr., R.C. Blake and R.M. Skirvin. 1981. 'Hull Thornless' blackberry. *Hort. Sci.* 16: 796–797.
- Galmés, J., J. Flexas, R. Savé and H. Medrano. 2007. Water relations and stomatal characteristics of

- Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant Soil*. 290: 139–155. <https://doi.org/10.1007/s11104-006-9148-6>
- Guerfel, M., O. Baccouri, D. Boujnah, W. Chaïbi and M. Zarrouk. 2009. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars. *Sci. Hort.* 119: 257–263. <https://doi.org/10.1016/j.scienta.2008.08.006>
- Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts I. Kinetic and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125: 189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Ji, W.D., G.X. Shi, Q.S. Xu, Y. Xu, H.Y. Yang and K.H. Du. 2008. Effects of Ni²⁺ on physiological characteristics and submicroscopic structure of *Salvinia natans* leaves. *Environ. Sci.* 29: 2308–2313.
- Jiang, Y. and B. Huang. 2002. Protein alterations in tall fescue in response to drought stress and abscisic acid. *Crop Sci.* 42: 202. <https://doi.org/10.2135/cropsci2002.0202>
- Law, M.Y., S.A. Charles and B. Halliwell. 1983. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and of paraquat. *Biochem. J.* 210: 899–903. <https://doi.org/10.1042/bj2100899>
- Li, F., X.Y. Qin, Y.H. Xie, X.S. Chen, J.Y. Hu, Y.Y. Liu and Z.Y. Hou. 2013. Physiological mechanisms for plant distribution pattern: responses to flooding and drought in three wetland plants from Dongting Lake, China. *Limnol.* 14: 71–76. <https://doi.org/10.1007/s10201-012-0386-4>
- Li, Y., K.K. Lee, S. Walsh, C. Smith, S. Hadingham, K. Sorefan, G. Cawley and M.W. Bevan. 2006. Establishing glucose and ABA-regulated transcription networks in *Arabidopsis* by microarray analysis and promoter classification using a Relevance Vector Machine. *Genome Res.* 16: 414–427. <https://doi.org/10.1101/gr.4237406>
- Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic membranes. *Method Enzymol.* 148: 350–382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Liu, C., Y. Liu, K. Guo, D. Fan, G. Li, Y. Zheng, L. Yu and R. Yang. 2011. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ. Exp. Bot.* 71: 174–183. <https://doi.org/10.1016/j.envexpbot.2010.11.012>
- Liu, D., X. Liu, Y. Meng, C. Sun, H. Tang, Y. Jiang, M.A. Khan, J. Xue, N. Ma and J. Gao. 2013. An organ-specific role for ethylene in rose petal expansion during dehydration and rehydration. *J. Exp. Bot.* 64: 2333–2344. <https://doi.org/10.1093/jxb/ert092>
- Maehly, A.C. 1955. Plant peroxidase. *Methods Enzymol.* 2: 801–813. [https://doi.org/10.1016/S0076-6879\(55\)02307-0](https://doi.org/10.1016/S0076-6879(55)02307-0)
- Miller, G., N. Suzuki, S. Yilmaz and R. Mittler. 2010. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ.* 33: 453–467. <https://doi.org/10.1111/j.1365-3040.2009.02041.x>
- Mittova, V., F.L. Theodoulou, G. Kiddle, L. Gómez, M. Volokita, M. Tal, C.H. Foyer and M. Guy. 2003. Coordinate induction of glutathione biosynthesis and glutathione-metabolizing enzymes is correlated with salt tolerance in tomato. *FEBS Lett.* 554: 417–421. [https://doi.org/10.1016/S0014-5793\(03\)01214-6](https://doi.org/10.1016/S0014-5793(03)01214-6)
- Noctor, G. and C.H. Foyer. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49: 249–279. <https://doi.org/10.1146/annurev.arplant.49.1.249>
- Ortuño, M.F., J.J. Alarcón, E. Nicolás and A. Torrecillas. 2005. Sap flow and trunk diameter fluctuations of young lemon trees under water stress and rewatering. *Environ. Exp. Bot.* 54: 155–162. <https://doi.org/10.1016/j.envexpbot.2004.06.009>
- Pan, Y., L.J. Wu and Z.L. Yu. 2006. Effect of salt and drought stress on antioxidant enzyme activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis* Fisch). *Plant Growth Regul.* 49: 157–165. <https://doi.org/10.1007/s10725-006-9101-y>
- Pyngrope, S., K. Bhoomika and R.S. Dubey. 2013. Reactive oxygen species, ascorbate-glutathione pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (*Oryza sativa* L.) seedlings subjected to progressing levels of water deficit.

- Protoplasma. 250: 585–600. <https://doi.org/10.1007/s00709-012-0444-0>
- Pennisi, E. 2008. Plant genetics: the blue revolution, drop by drop, gene by gene. *Sci.* 320: 171–173. <https://doi.org/10.1126/science.320.5873.171>
- Stewart, R.R.C. and J.D. Bewley. 1980. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.* 65: 245–248. <https://doi.org/10.1104/pp.65.2.245>
- Singh, B.K., S.R. Sharma and B. Singh. 2010. Antioxidant enzymes in cabbage: variability and inheritance of superoxide dismutase, peroxidase and catalase. *Sci. Hortic.* 124: 9–13. <https://doi.org/10.1016/j.scienta.2009.12.011>
- Sapeta, H., J.M. Costa, T. Lourenco, J. Maroco, P. van der Linde and M.M. Oliveira. 2013. Drought stress response in *Jatropha curcas*: growth and physiology. *Environ. Exp. Bot.* 85: 76–84. <https://doi.org/10.1016/j.envexpbot.2012.08.012>
- Strik, B.C., J.R. Clark, C.E. Finn and M.P. Bañados. 2007. Worldwide blackberry production, 1995 to 2005 and predictions for growth. *Hort. Technol.* 17: 205–213. <https://doi.org/10.21273/HORTTECH.17.2.205>
- Turner, N.C. 1981. Techniques and experimental approaches for the measurement of plant water status, *Plant Soil.* 58: 339–366. <https://doi.org/10.1007/BF02180062>
- Vyas, D. and Kumar, S. 2005. Tea (*Camellia sinensis* (L.) O. Kuntze) clone with lower period of winter dormancy exhibits lesser cellular damage in response to low temperature. *Plant Physiol. Bioch.* 43: 383–388. <https://doi.org/10.1016/j.plaphy.2005.02.016>
- Yan, B., Q. Dai, X. Liu, S. Huang and Z. Wang. 1996. Flooding-induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant Soil.* 179: 261–268. <https://doi.org/10.1007/BF00009336>
- Yue, L.J., S.X. Li, Q. Ma, X.R. Zhou, G.Q. Wu, A.K. Bao, J.L. Zhang and S.M. Wang. 2012. NaCl stimulates growth and alleviates water stress in the xerophyte *Zygophyllum xanthoxylum*. *J. Arid Environ.* 87: 153–160. <https://doi.org/10.1016/j.jaridenv.2012.06.002>