



Improving photosynthetic characteristics and antioxidant enzyme activity of capsule wall and subtending leaves increases cotton biomass under limited irrigation system

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Abstract

The photosynthetic performance of the canopy boll-leaf system (BLS) reflects the material and energy exchange abilities between plant and external environment. A two-year field experiment determined the response to irrigation regimes [600 (W₁), 480 (W₂), and 360 (W₃) m³ ha⁻¹] of cotton BLS physio-biochemical traits. Decreasing irrigation, photochemical quenching coefficient, the electron transfer rate of PSII, chlorophyll, and stomata width of the BLS decreased; nonphotochemical quenching (NPQ), the activity of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), hydrogen peroxide (H₂O₂), and malondialdehyde (MDA) contents increased at 7–21 day interval after anthesis (TAA). Among them, W₂ increased SOD and POD by 3.5–42.1% and 1.4–57.8%, respectively, compared to W₁ treatment. NPQ and carotenoid contents of capsule wall and CAT of subtending leaves increased. Principal component analysis showed that NPQ, MDA, H₂O₂, POD, and CAT were positively correlated with the seed biomass. Therefore, cotton could protect photosynthetic apparatus by maintaining lower membrane lipid peroxidation and higher heat dissipation capacity of capsule wall and subtending leaves to ensure higher biomass accumulation under limited irrigation.

Keywords: antioxidative enzyme activity; boll-leaf system; drip irrigation; stomatal structure.

Introduction

Cotton (*Gossypium* spp.) is one of the most important fiber, edible oil, and animal feed crops worldwide (Tang

et al. 2005). According to the National Bureau of Statistics data for 2019, China's annual average cotton output accounted for 22.3% of the global output, while Xinjiang's cotton output accounted for 84.9% of the total national

Highlights

- Capsule wall increased carotenoid content and heat dissipation capacity in response to drought
- Low membrane lipid peroxidation in boll-leaf system ensures high biomass accumulation
- The stomatal density of capsule wall increased under limited irrigation

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Abbreviations: Car – carotenoids; CAT – catalase; Chl *a*(*b*) – chlorophyll *a*(*b*); BLS – boll-leaf system; ETR – apparent electron transfer rate; ETR_{II} – electron transfer rate of PSII; F₀ – the initial minimal fluorescence; FB₃ – the 3rd fruiting branch; F_m – maximum fluorescence; F_{m'} – the maximum fluorescence yield under light adaptation; F_t – the actual fluorescence yield, F_t/F_m – maximum quantum yield of PSII; MC – mepiquat chloride; MDA – malondialdehyde; NPQ – nonphotochemical quenching; POD – peroxidase; q_p – photochemical quenching coefficient; SE – standard error; SOD – superoxide dismutase; TAA – day interval after anthesis; Y_{II} – quantum efficiency of PSII.

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output, and 19.0% of the global output. Xinjiang is located in the hinterland of the Eurasian continent, with scarce precipitation, strong evaporation, an arid climate, and high dependence on irrigation for agricultural production. In this area, an insufficient water supply is the main obstacle to cotton production. Cotton is a drought-tolerant plant that adapts to dry conditions through significant responses in its internal physiochemical activities (Shareef *et al.* 2018). Therefore, cotton plant drought tolerance ability and tapping the potential of biological water saving can effectively promote the efficient and sustainable development of cotton in Xinjiang.

Photosynthesis is the prerequisite of cotton yield formation. Chlorophyll (Chl) fluorescence parameters can directly reflect the internal mechanism of plant photosynthesis (Krause and Weis 1991, Baker 2008). During leaf development, the minimal fluorescence yield of the dark-adapted state (F_0) and nonphotochemical quenching (NPQ) increased with the severity of drought stress (Tang *et al.* 2007, Yi *et al.* 2016), and the maximal quantum yield of PSII photochemistry (F_v/F_m), photochemical quenching coefficient (q_p), the quantum efficiency of PSII (Y_{II}), and apparent electron transfer rate (ETR) decreased with drought severity (Zhang *et al.* 2019). Stomata are the main channels and regulators of CO_2 and water vapor transport between higher plants and the outside world. It was found that with the increase of drought stress, the length, width, and opening of stomata decreased, while the density of stomata increased (Wang *et al.* 2019). Under a normal growth environment, plant antioxidant enzymes superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6), and other enzymes are relatively stable. When the plant is under water stress, the superoxide anion and redox substances in the plant increase. At the same time, endogenous antioxidative enzymes ensure an active defense strategy (Bange *et al.* 2004) to remove the excess active oxygen species in the plant (Miao *et al.* 2006, Miller *et al.* 2010, Gao *et al.* 2014, Subbarao *et al.* 2000) and enhance the antioxidant capacity of crops under drought stress (McKersie *et al.* 1993). In addition, drought stress significantly inhibited the production of plant biomass (Wang *et al.* 2016) and the accumulation of reproductive organ biomass (da Costa and Cothren 2011). Therefore, improving the photosynthetic performance of cotton under water deficit is crucial to improve cotton yield.

As a yield organ, nutrients needed for the growth and development of cotton bolls are mainly provided by photosynthesis of the 'boll-leaf system' (BLS: main stem leaves, boll subtending leaves, and capsule wall) (Mo and Tang 2013). In cotton, the canopy 'boll-leaf' is the main photosynthate 'sink' and 'source', and the 'boll-leaf' relationship reflects the coordination of the vegetative growth and reproductive growth of the cotton plant (Genty *et al.* 1989, Zhang *et al.* 2007, Gao *et al.* 2014). A change in water conditions will change the distribution of photosynthates among the plant components, and at the same time, it will lead to a change in the relationship between the sinks and sources and finally affect the economic yields of crop harvest. The increase in yield depends on the load capacity of leaves under different photosynthetic leaf areas (Luo

et al. 2011). However, in the later stage of cotton growth, the leaf area begins declining but the surface area of non-leaf green organs (bracts and bolls) increases. The photosynthetic oxygen-releasing capacity and photosynthetic enzyme activity of non-leaf green organs are relatively stable in the yield-formation stage; therefore, in the later stage of growth, the relative contribution of photosynthesis of non-leaf green organs to the whole cotton plant increases (Hu 2013).

There have been many studies on the response mechanism underlying photoinhibition of photosynthesis in cotton leaves under water stress (Bai *et al.* 2008, Shahenshah and Isoda 2010, Valladares and Pearcy 2010). However, due to the response of different organs to various water regimes (Zhang *et al.* 2011a), we hypothesized that drought stress would lead to different adaptability of different components of BLS to water deficit. Therefore, this experiment was conducted to study the effect of drought stress on the mechanism of light protection of cotton sinks and sources and its relationship with the biomass of each component to clarify the differences in the light protection ability of each component and consequently biological water-saving potential of cotton.

Materials and methods

Experimental site: The experiment was carried out in 2017–2018 at the Agriculture Test Station of Shihezi University (45°19'N, 86°03'E), Xinjiang, China. One cotton variety (*Gossypium hirsutum* L. cv. Xinluzao 45) was sown. The preplanting fertility of 0–20-cm topsoil layer was determined. The texture of the soil from the experimental site was medium loam, and the soil had pH 7.5, 12.5 g(organic matter) kg^{-1} , 1.45 g(total nitrogen) kg^{-1} , 54.9 mg(alkali nitrogen) kg^{-1} , 23.0 mg(available phosphorus) kg^{-1} , and 149 mg(available potassium) kg^{-1} . The mean temperatures during experimental period were in May–September 2017 and 2018, respectively, 21.0 and 18.2°C in May, 25.9 and 26.4°C in June, 31.3 and 28.3°C in July, 27.3 and 27.8°C in August, 19.5 and 16.1°C in September. The mean rainfall was 5.13 and 5.71 mm and the mean annual humidity was 31.1 and 30.0% in 2017 and 2018, respectively.

Experimental design: Generally, a total irrigation amount of 4,800–5,000 $\text{m}^3 \text{ha}^{-1}$ is required to achieve the seed cotton yield higher than 6,000 kg ha^{-1} in the northern Xinjiang region (Luo *et al.* 2016). A randomized complete block design with four replications was used in this study. The experiment included three drip-irrigation levels, named W_1 (conventional drip irrigation, 600 $\text{m}^3 \text{ha}^{-1}$ of water each time, control), W_2 (limited drip irrigation, 480 $\text{m}^3 \text{ha}^{-1}$ of water each time), and W_3 (deficit drip irrigation, 360 $\text{m}^3 \text{ha}^{-1}$ of water each time). From 45 d after emergence, eight irrigation treatments were conducted at 10-d intervals. The soil relative water in each plot was measured every after two to three days using a time-domain reflectometer (Trime T3, Germany). Before and after irrigation, the soil relative water content of W_1 fluctuated in 49.4–106.0%, with an average growth period of 75.5%; W_2 fluctuated within 48.3–96.6%, with an average growth period of

70.0%; W_3 fluctuated within 38.5–92.3%, with an average growth period of 65.2%. In the case of drip irrigation under film, the drip irrigation rates were controlled by a water meter (*DN32*, *Jiangxi Gamma Industry Co., Ltd.*, China) and switch ball valve. The irrigation was applied on the same dates for all the treatments, and the duration was approximately 10–14 h (07:30–21:30 h).

Field management: Sowing occurred on 23 April, 2018. Each plastic film was laid with two drip irrigation lines (*Beijing Luckrain Inc.*, China) to plant four rows of cotton with a spacing of 30 + 60 + 30 cm. The planting density was 18,000 plants ha^{-1} (the planting density commonly practiced in this region). Fertilization was performed according to recommended conventional practices. The basal fertilizer consisted of 1,500 kg(oil residue) ha^{-1} and 180 kg ha^{-1} of additional fertilizer applied with water (first *via* drip irrigation for 30 min, then *via* fertilizer), urea and potassium dihydrogen phosphate were applied at 975 kg ha^{-1} and 165 kg ha^{-1} , respectively. W_1 was treated with 6, 11, 26, 45, and 120 g ha^{-1} of mepiquat chloride (MC) at the cotyledon stage to the two-leaf stage, the 5–7-leaf stage, 2–3 d before the first irrigation, and 5–7 d after topping. No MC was applied before the first and second irrigations under W_2 and W_3 . Artificial topping was carried out on 10 July. Thiabendron (80% thaphthol, 450 g ha^{-1}) combined with ethephon (40% water; 1,350 ml) was used as a defoliant on 10 September. Other management practices, such as insect and weed control, were performed according to local agronomic practices.

Chlorophyll (Chl) fluorescence parameters: Four cotton plants from each treatment were randomly marked on the 7, 14, 21, 28, 35, 42, and 49 day interval after anthesis (TAA). The first main stem leaves, boll-subtending leaves, and capsule walls with good growth and no pests were marked at the first position of the 3rd fruiting branch (FB₃). After the gas-exchange parameters were measured, they were brought back to the laboratory under low temperature (in an insulated box). After dark adaptation for 30 min, the Chl fluorescence parameters were measured by a modulated fluorometer (*PAM 100*, *Walz*, Effeltrich, Germany). First, for determination of the F_0 and maximum fluorescence (F_m), actinic light with an intensity of 1,450 μ mol(photon) $m^{-2} s^{-1}$ was applied. After the fluorescence signal reached a steady state (4–5 min), saturated pulse light was applied for quenching analysis, and the actual fluorescence yield (F_t) and the maximal fluorescence yield of the light-adapted state (F_m') were measured at any time. The following Chl parameters were calculated:

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

$$Y_{II} = (F_m' - F_t)/F_m' \quad (2)$$

$$q_p = 1 - (F_t - F_0)/(F_m' - F_0) \quad (3)$$

$$NPQ = (F_m - F_m')/F_m' \quad (4)$$

$$ETR_{II} = (F_m' - F_t)/F_m' \times PAR \times 0.5 \times 0.84 \quad (5)$$

where 0.84 is a common leaf absorptance coefficient for C₃ plants and 0.5 assumes an equal distribution of excitation

between two photosystems (Björkman and Demmig 1987, Chen and Spreitzer 1992, Björkman and Demmig-Adams 1995, Zhang *et al.* 2010).

Photosynthetic pigment contents: After each Chl fluorescence test, the leaves and capsule walls were used for the determination of photosynthetic pigment contents, and attention was paid to the preservation of the leaves and capsule walls. Four discs were punched with a hole punch (6 mm in diameter) and placed into a 15-ml tube with a plug, and the pigment was extracted with 13 ml of 80% acetone. The tube was covered with a piece of black cloth, placed in the dark, and regularly shaken (extraction was performed for 4 d until the leaves were milky white) to mix the dipped matter for photosynthetic pigment extraction. A spectrophotometer (*UV-2401*, *Shimadzu*, Japan) was utilized to measure the optical density (OD) at wavelengths of 470, 645, and 663 nm. The control group was acetone (80%). The formulas were as follows:

$$\rho(\text{Chl } a) = 12.21 \text{ OD}_{663} - 2.81 \text{ OD}_{645} \quad (6)$$

$$\rho(\text{Chl } b) = 20.13 \text{ OD}_{645} - 5.03 \text{ OD}_{663} \quad (7)$$

$$\rho(\text{Car}) = [1,000 \text{ OD}_{470} - 3.27\rho(\text{Chl } a) - 104\rho(\text{Chl } b)]/229 \quad (8)$$

$$C = \rho V_t / (FV \times 1,000) \quad (9)$$

where $\rho(\text{Chl } a)$, $\rho(\text{Chl } b)$, and $\rho(\text{Car})$ were the chlorophyll *a*, chlorophyll *b*, and Car concentrations [$mg\text{ ml}^{-1}$]; OD_{470} , OD_{645} , and OD_{663} were the OD values under wavelengths of 470, 645, and 663 nm, respectively; C was the amount of the photosynthetic pigments [$mg\text{ cm}^{-2}$]; V_t was the total volume of the extracted solution, and FV was the area of the discs [cm^2].

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents and antioxidative enzyme activities: On 7, 14, 21, 28, 35, 42, and 49 TAA, the first main stem leaves, boll-subtending leaves, and bolls (capsule wall) were marked at the first position of FB₃. The activity of superoxide dismutase (SOD) and peroxidase (POD) was determined by the nitroblue tetrazolium method and the guaiacol method (Kochba *et al.* 1977), respectively. The activity of catalase (CAT), malondialdehyde (MDA), and the content of H_2O_2 were assessed by potassium permanganate titration (Kraus and Fletcher 1994), thiobarbituric acid colorimetry (Dhindsa *et al.* 1981), and redox titration (Nakano and Asada 1987, Baily *et al.* 2006), respectively.

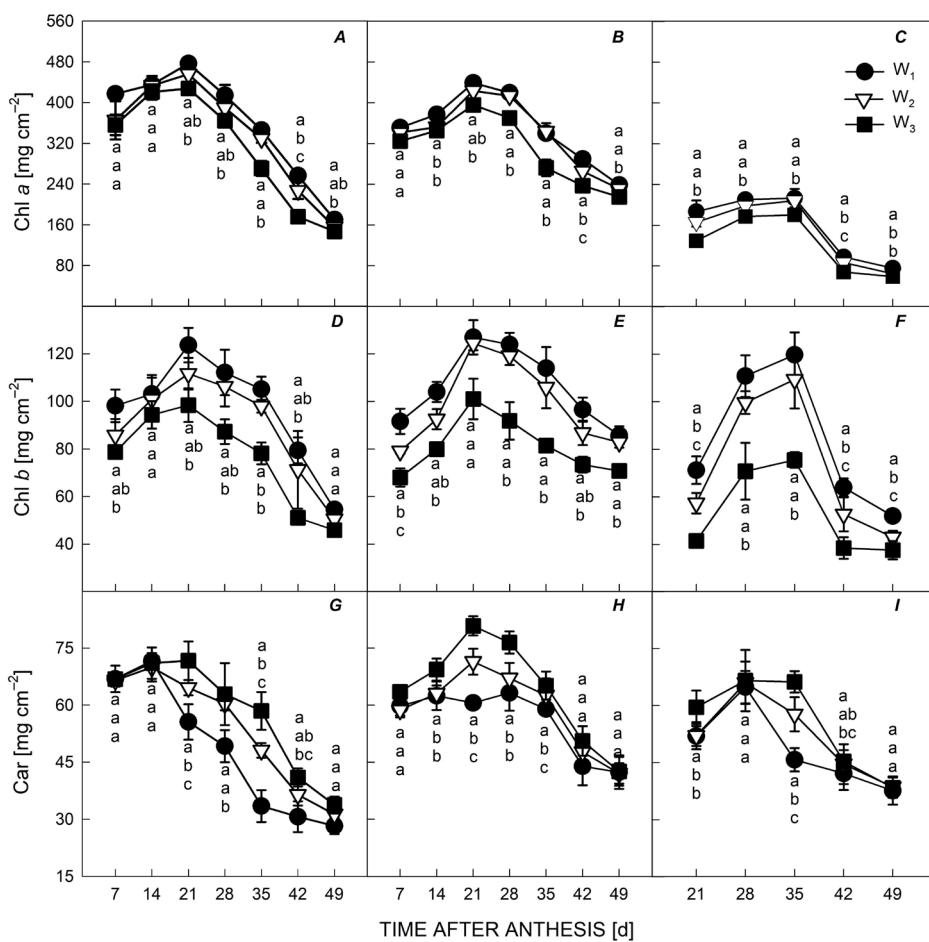
Stomatal structure: Three plants per plot were harvested on 21, 35, and 49 TAA and separated into main stem leaves, boll subtending leaves, and bolls (capsule wall) at the first position of FB₃. For stomatal density and size determination, two imprints of each sample were made using a transparent nail polish (avoiding veins), and the dried films were removed carefully with a tweezer. The film was mounted on a slide and observed under a microscope (*Phoenix 100-3B41L-IPL*, *Phoenix Optical Group Co., Ltd.*, China). Six measurements were made for each sample. Stomatal density was determined by counting the number of stomata in a fixed-size field of view.

Biomass accumulation: Six plants per plot were selected from four replications, bagged, labeled with plastic tags, and transported back to the lab on 7, 14, 21, 28, 35, 42, and 49 TAA. The main stem leaves, boll-subtending leaves, and bolls (capsule wall) at the first position of FB₃ were used to determine the green leaf area and biomass. The bolls were divided into capsule walls and seed cotton (fiber). The leaf and capsule walls area were measured using a *Licor-3000* instrument (*LI-COR Biosciences*, Lincoln, USA). Sample leaves, capsule walls, and seed cotton (only at 80°C) were oven dried at 105°C for 30 min and then at 80°C for 72 h to attain a constant mass.

Statistical analysis: The data were processed using *Microsoft Excel 2010*, and one-way analysis of variance (ANOVA) was performed using *SPSS 19.0*. The charts were drawn using *SigmaPlot 12.5* and *Origin 2019b*. The significance of differences between means was determined using *Duncan's* test at the $P<0.05$ level in the same period. The data are presented as the means \pm standard errors.

Results

Photosynthetic pigments: The proportions of Chl *a*, Chl *b*, and Car under different physiological conditions and in different cotton plant organs were not the same (Fig. 1). On 28–35 TAA, the contents of Chl *a* and Chl *b* in different



organs were as follows: boll subtending leaves > main stem leaves > capsule wall, while the contents of Car were as follows: boll subtending leaves > capsule wall > main stem leaves. With the decrease in drip irrigation, the contents of Chl *a* and Chl *b* in the main stem leaves, boll subtending leaves, and capsule walls decreased, and the content of Car increased gradually. On 35 TAA, the Chl *a* content of the main stem leaves, boll subtending leaves, and capsule wall of W₃ decreased by 21.8, 20.0, and 15.6%, respectively, compared with W₁, and by 17.8, 20.8, and 13.5% compared with W₂. The Chl *b* content of W₃ decreased by 25.7, 28.5, and 37.0% compared with W₁, and by 20.2, 23.1, and 31.0%, respectively, compared with those of W₂. The Car contents of W₃ increased by 42.7, 9.5, and 31.0% compared with those of W₁ and increased by 17.7, 3.9, and 12.9%, respectively, compared with those of W₂.

Chl fluorescence parameters: On 35 TAA, the F_v/F_m value showed a trend of capsule wall > boll-subtending leaves > main stem leaves (Fig. 2). At the early stage of boll development (7–21 TAA), there was no significant difference in F_v/F_m between the main stem leaves and the boll subtending leaves. On 35 TAA, W₂ decreased the F_v/F_m by 1.4% and increased by 0.3%, respectively, compared with boll-subtending leaves. The F_v/F_m values of W₃ decreased by 4.9 and 2.4%, respectively, compared to

Fig. 1. Changes in chlorophyll (Chl) *a*, Chl *b*, and carotenoid (Car) contents of main stem leaves (A, D, G), boll-subtending leaves (B, E, H), and capsule wall (C, F, I) under conventional drip irrigation (W₁), limited drip irrigation (W₂), and deficit drip irrigation (W₃) with the time after anthesis (TAA). Bars indicate SE ($n=4$). In *Duncan's* analysis, the difference between different treatments in the same period was significant ($P<0.05$).

W_1 and W_2 treatments. No significant differences between the values of the W_3 and the W_1 and W_2 treatments were observed.

It can be seen from Fig. 3 that q_p decreased in the BLS with the change in growth period. On 21–49 TAA, the main stem leaves of W_1 , W_2 , and W_3 showed decreases in q_p of 43.2, 35.5, and 46.7%, the boll-subtending leaves showed decreases of 43.2, 50.1, 58.0%, and the capsule wall showed decreases of 49.4, 60.6, and 73.0%, respectively. The trend of ETR_{II} among organs was different depending on the growth period; the main stem leaves and capsule wall decreased gradually, while that of the boll subtending leaves increased first and then decreased, and the peak value was reached on 21 TAA. With the decrease in irrigation water, ETR_{II} showed the following trend: $W_1 > W_2 > W_3$, which indicated that the electron transfer rate of the BLS was seriously affected by deficit irrigation.

Compared to W_1 , W_2 , and W_3 decreased the Y_{II} of each component but increased NPQ. On 21–49 TAA, the NPQ of each green organ changed with the growth period in the following order W_1 , W_2 , and W_3 treatments. The NPQ of the main stem leaves increased by 6.2, 8.4, 12.7%, respectively; the NPQ of boll-subtending leaves increased by 6.8, 8.9, 9.4%, and the NPQ of capsule wall increased by 42.9, 46.9, and 54.5%, respectively. This indicated that the components of the BLS could alleviate the effect of water stress on PSII and increase the light energy dissipated in the form of heat to protect against light inhibition damage to the photosynthetic apparatus.

Antioxidant enzyme system: H_2O_2 is the main product of lipid peroxidation, and the content of MDA reflects the level of lipid peroxidation and the degree of damage to the membrane structure to a certain extent. The H_2O_2 and MDA contents in the leaves were always higher than those in the capsule wall, which indicated that the degree of damage in the leaves was higher than that in the capsule wall under water deficit conditions (Fig. 4). On 21–49 TAA, the H_2O_2 content of the main stem leaves, boll subtending leaves, and capsule wall increased by 7.4–15.8,

1.4–21.4, and 4.5–17.8%, respectively, under W_2 , and by 19.6–72.3, 23.7–48.7, and 5.2–30.2%, respectively, under W_3 . The MDA content increased by 5.7–25.7, 1.2–20.4, and 3.0–13.8% under W_2 , and by 30.1–45.0, 9.6–38.4, and 7.5–27.0%, respectively, under W_3 .

The antioxidant enzyme system in plants, which includes SOD, POD, and CAT, can remove the active oxygen produced under adverse environments (Fig. 5). The SOD, POD, and CAT activities of the main stem leaves, boll-subtending leaves, and capsule wall were significantly affected by each treatment and the SOD, POD, and CAT activities of the capsule wall per unit mass were lower than those of the leaves. The SOD and POD activities of each component showed a trend of $W_1 < W_2 < W_3$. On 21–49 TAA, compared with W_1 , the SOD activity of the W_2 main stem leaves, boll-subtending leaves, and capsule wall increased by 10.7–23.2, 12.4–42.1, and 3.5–37.9%, and those of W_3 increased by 18.6–26.1, 22.1–70.1, and 13.8–66.6%, respectively. The POD activity increased by 1.4–9.9, 18.3–57.8, and 19.6–49.6%, respectively, under W_2 and by 10.2–55.7, 35.6–73.7, and 29.4–64.6%, respectively, under W_3 . The results showed that the SOD and POD activity of each component increased during water deficit, which protected the photosynthetic mechanism from drought damage. The activity of CAT varied among the different organs. The main stem leaves and capsule wall showed a tendency of first increasing and then decreasing CAT activity, while the boll-subtending leaves showed a trend of gradually increasing. On 35–49 TAA, the main stem leaves treated with W_2 had higher CAT activity.

Stomatal structure: Light micrographs showed significant variation in the stomatal density across the different organs on 35 TAA. For example, cotton leaves had significantly higher stomatal density, while capsule walls had much larger stomata than those of the other organs. Changes in stomatal density and dimensions (length and width) of the BLS components under the different treatments are shown in Table 1. Furthermore, stomatal density

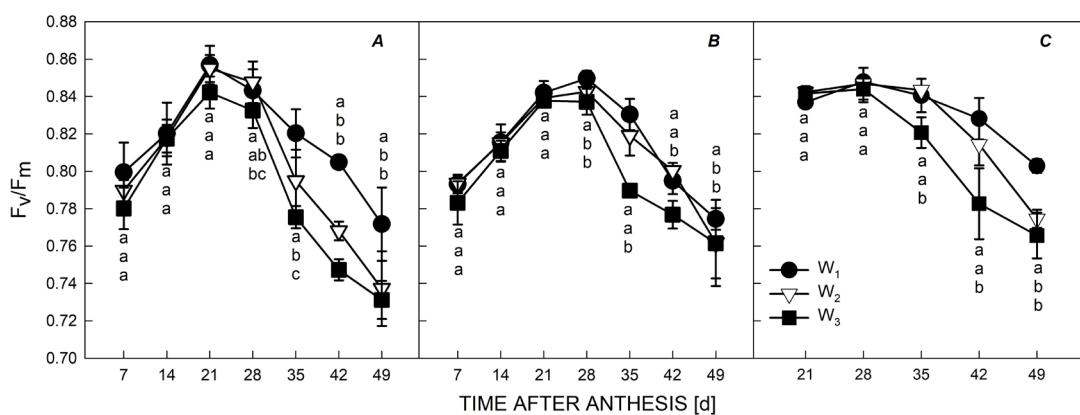


Fig. 2. Changes in the maximal quantum yield of PSII photochemistry (F_v/F_m) of main stem leaves (A), boll subtending leaves (B), and capsule wall (C) under conventional drip irrigation (W_1), limited drip irrigation (W_2), and deficit drip irrigation (W_3) with the time after anthesis (TAA). Bars indicate SE ($n = 3$). In *Duncan's* analysis, the difference between different treatments in the same period was significant ($P < 0.05$).

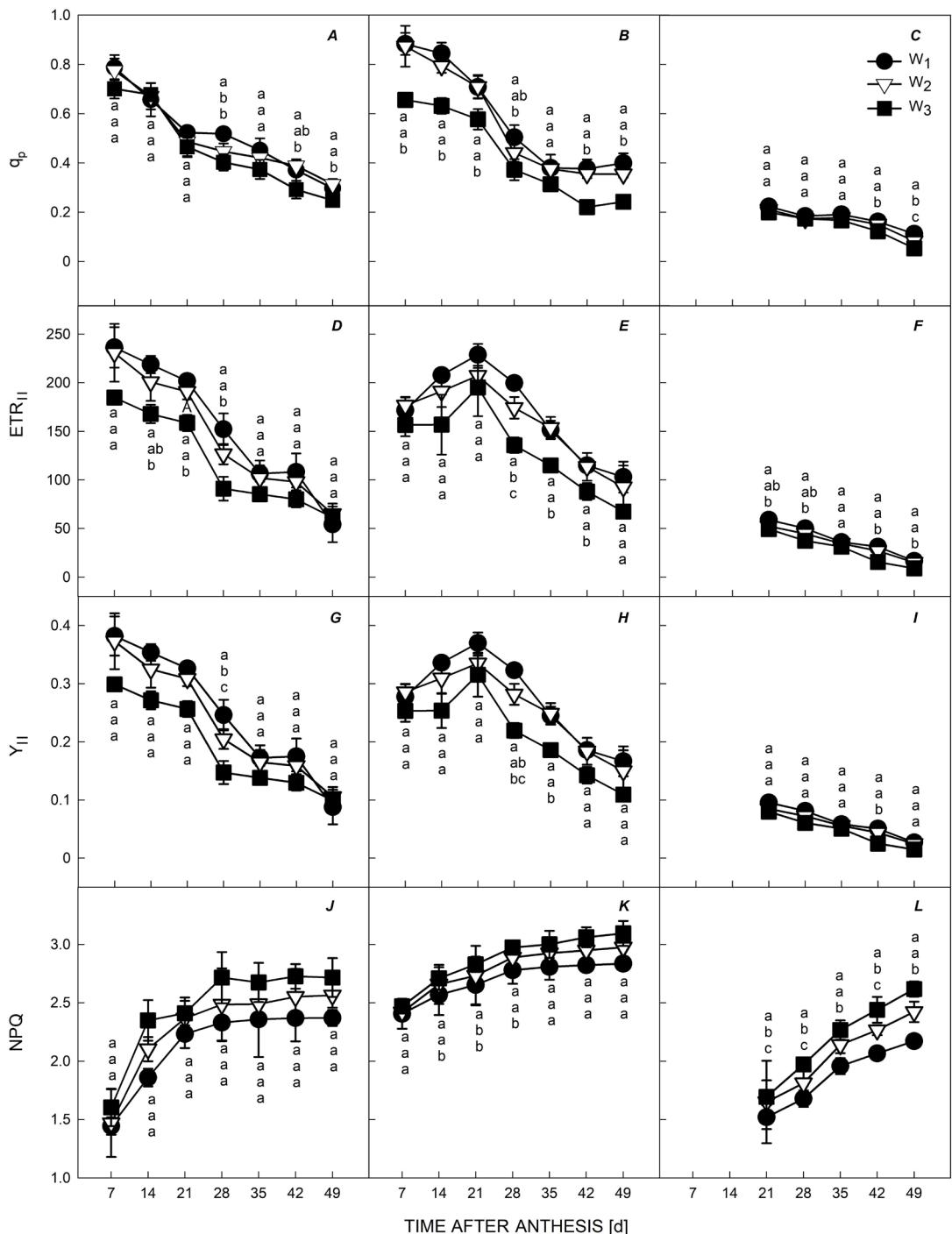


Fig. 3. Changes in the photochemical quenching coefficient (q_p), electron transfer rate of PSII (ETR_{II}), quantum efficiency of PSII photochemistry (Y_{II}), and the nonphotochemical quenching (NPQ) of main stem leaves (A,D,J,I), boll-subtending leaves (B,E,H,K), and capsule wall (C,F,I,L) under conventional drip irrigation (W₁), limited drip irrigation (W₂), and deficit drip irrigation (W₃) with the time after anthesis (TAA). Each value represents the mean \pm SE ($n = 3$). In Duncan's analysis, the difference between different treatments in the same period was significant ($P < 0.05$).

and dimensions (length and width) also decreased with decreasing irrigation intensities, with stomatal density showing the trend W₁ < W₂ < W₃, and the stomatal length and width showing the trend W₁ > W₂ > W₃. Compared to W₁, W₂ enhanced the stomatal density of the main stem

leaves, boll-subtending leaves, and capsule wall by 13.4, 23.5, and 7.5%, and those of W₃ were enhanced by 22.1, 32.7, and 8.9% on 35 TAA, respectively. Additionally, W₂ significantly reduced the stomatal length of the main stem leaves and boll subtending leaves by 7.2 and 8.9%,

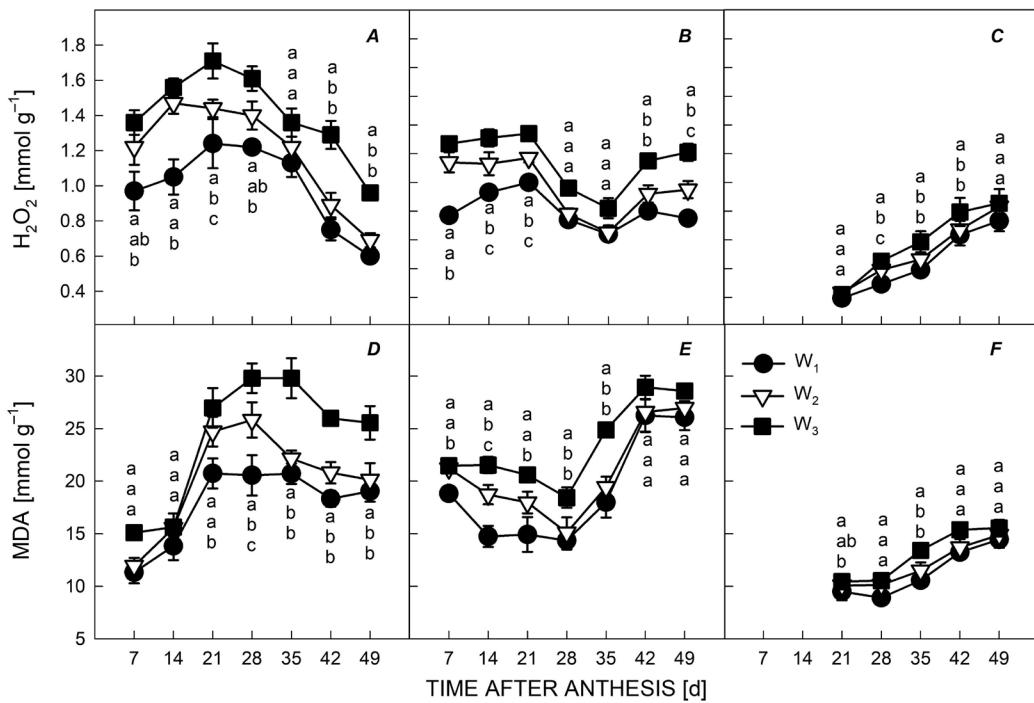


Fig. 4. Changes in hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) content of main stem leaves (A,D), boll-subtending leaves (B,E), and capsule wall (C,F) under conventional drip irrigation (W₁), limited drip irrigation (W₂), and deficit drip irrigation (W₃) with the time after anthesis (TAA). Each value represents the mean \pm SE ($n = 3$). In Duncan's analysis, the difference between different treatments in the same period was significant ($P < 0.05$).

respectively. The stomatal length of the capsule wall was slightly influenced by the treatments. Compared with W₁, the stomata width of the main stem leaves, boll-subtending leaves, and capsule wall decreased by 9.0, 10.7, and 18.1% in response to W₃, respectively. Moreover, drip irrigation slightly influenced the stomatal density and size of each organ on 49 TAA.

Biomass accumulation: The biomass of the main stem leaves, boll-subtending leaves, capsule wall, and seed cotton increased with increasing irrigation and peaked on 28, 35, 42, and 49 TAA, respectively (Fig. 6). W₂ had no significant difference on 21–49, 28–49, and 35–49 TAA on the biomass of the main stem leaves, boll-subtending leaves, and capsule wall of W₁, indicating that limited irrigation (W₂) mainly affected biomass accumulation by affecting the main stem leaves and boll-subtending leaves on 7–21 TAA but when the cotton bolls developed rapidly, from 21–28 TAA, the BLS mainly affected biomass accumulation by increasing the seed cotton mass. On 21–49 TAA, compared to those of W₃, the main stem leaves, boll-subtending leaves, capsule wall, and seed cotton increased by 10.5–15.9, 8.0–14.1, 4.3–7.6, and 0.9–24.6%, respectively, under W₂ and 14.5–19.3, 15.5–22.6, 6.8–14.8, and 9.2–35.3%, respectively, under W₁.

Principal component analysis: Through principal component analysis (PCA) and calculation (Fig. 7), we determined that 13 physiological indexes of cotton, such as Chl fluorescence parameters, antioxidant enzyme activities,

H₂O₂ and MDA contents, and seed cotton mass, were reflected by three principal components (characteristic values: $8.318 + 2.096 + 1.022 = 11.436$, $8.419 + 1.944 + 1.111 = 11.474$, $8.181 + 2.122 + 1.129 = 11.432$) at 88.0, 88.2, and 87.9%. The first three principal components provided a high degree of explanation for the total variation in the cotton BLS data. For the main stem leaves and boll subtending leaves of cotton, the first principal component clearly distinguished the water content and the second principal component clearly distinguished the period but for the capsule wall, these contributions were not obvious. For the main stem leaves, the contribution rate of the first principal component was 63.99%, which was characterized by factor variables, such as the contents of Chl *a*, Chl *b*, and Car, F_v/F_m, SOD and POD activities, and seed cotton mass, having a high load. The contribution rate of the second principal component was 16.1%, which was characterized by a high load of factor variables, such as the MDA and H₂O₂ contents, indicating that the Chl content and antioxidant enzyme system of cotton main stem leaves were greatly affected by water stress, resulting in serious damage to the cell membrane.

The contribution rate of the first principal component of boll-subtending leaves was 64.8%. Chl *a*, Chl *b*, F_v/F_m, and Y_{II} had higher positive loads, indicating that the change in water content had a major impact on the Chl fluorescence performance of boll-subtending leaves. The contribution of the second principal component was 15.0%, and the SOD and CAT activities and H₂O₂ content had higher positive loads, which indicated that the adverse stress caused great

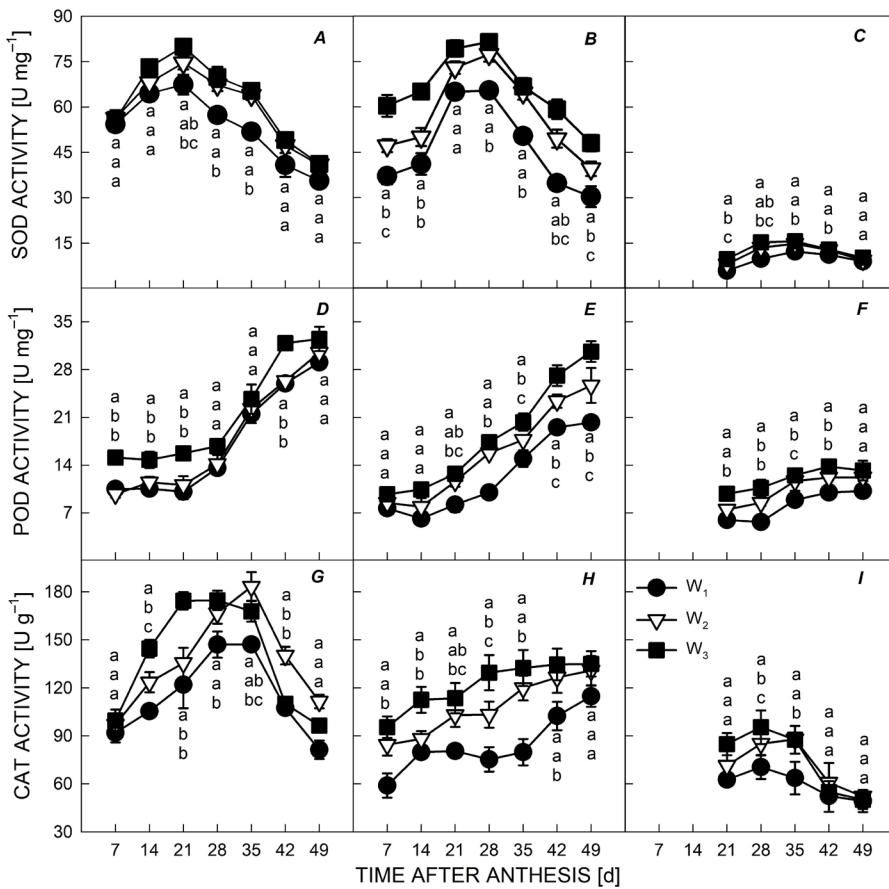


Fig. 5. Changes in the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activity of main stem leaves (A,D,G), boll-subtending leaves (B,E,H), and capsule wall (C,F,I) under conventional drip irrigation (W_1), limited drip irrigation (W_2), and deficit drip irrigation (W_3) with the time after anthesis (TAA). Each value represents the mean \pm SE ($n = 3$). In *Duncan's* analysis, the difference between different treatments in the same period was significant ($P < 0.05$).

damage to the cotton antioxidative enzyme system, so the active oxygen metabolism was out of balance. For the capsule wall, the contribution rate of the first principal component was 62.9%, which was characterized by the higher positive load of factor variables, such as the F_v/F_m , Y_{II} , q_p , and the contribution rate of the second principal component was 16.3%, which was characterized by the higher positive load of SOD, POD, and CAT activities, which shows that the Chl fluorescence parameters and cell antioxidative enzymes of the capsule wall of cotton were greatly affected by the different water treatments.

Discussion

Photoinhibition is the main nonstomatal limitation of plant photosynthesis (Murata *et al.* 2007). Chl fluorescence parameters are not only closely related to crop drought resistance but also directly reflect the degree of water stress damage to crops (Cao *et al.* 2017). Drought stress can affect the PSII in crops and thus affect the electron transfer rate and photochemical efficiency, finally leading to a reduction in CO_2 assimilation capacity (Zhang *et al.* 2011b). The F_v/F_m after dark adaptation of the plants is an important index to determine whether photoinhibition occurs; ETR_{II} reflects the electron transfer rate of PSII under any light intensity; NPQ reflects the photoprotection ability of PSII; and q_p represents the magnitude of photosynthetic activity (Li *et al.* 2014). Previous studies have found that

drought stress leads to a decrease in the F_v/F_m , ETR_{II} , and q_p values of crops and damage to the PSII active center, which inhibits the primary response of photosynthesis. To resist this damage, q_N and NPQ increase (Zhang *et al.* 2011b, Guo *et al.* 2016, Pilon *et al.* 2018). This study pinpointed that F_v/F_m of cotton BSL reached a high level on 21–28 TAA, while q_p decreased significantly from 7 TAA (capsule wall on 21 TAA) indicating that although cotton could maintain high photochemical efficiency and potential activity of PSII at flowering and boll stage (Guo *et al.* 2015), the proportion of light energy absorbed by PSII for photochemical reaction decreased (Tao *et al.* 2013). Under limited irrigation, the F_v/F_m of the main stem leaves, boll-subtending leaves, and capsule wall remained stable but the actual photochemical efficiency did not decrease significantly, while under deficit irrigation, the F_v/F_m of the leaves and capsule wall decreased, the NPQ increased correspondingly, and the NPQ of the capsule wall increased significantly. This indicated that there was no significant photoinhibition in the leaves and capsule wall of cotton under limited irrigation, but the electron transfer rate of the PSII reaction center was blocked under deficit irrigation, the share of light energy captured by antenna pigment used for photochemical reactions was reduced, the photochemical activity was reduced, and heat dissipation was enhanced (Gilmore 2001).

The capsule wall initiated the xanthophyll cycle to protect the photosynthetic mechanism from photoinhibition,

Table 1. Stomatal density, length, and width of main stem leaves, boll subtending leaves, and capsule wall on the adaxial side under conventional drip irrigation (W_1), limited drip irrigation (W_2), and deficit drip irrigation (W_3) at different time after anthesis (TAA). Values within columns followed by the same letter are statistically insignificant at the 0.05 level according to the *Duncan's* multiple range test. Values are means \pm SE, $n = 3$.

TAA	Parameters	Main stem leaves			Boll subtending leaves			Capsule wall		
		W_1	W_2	W_3	W_1	W_2	W_3	W_1	W_2	W_3
21	Density [mm^{-2}]	98.88 \pm 13.27 ^c	126.46 \pm 5.84 ^b	144.42 \pm 3.90 ^a	128.81 \pm 8.73 ^{bc}	174.34 \pm 13.27 ^{ab}	181.11 \pm 12.49 ^a	45.66 \pm 4.45 ^a	53.85 \pm 12.17 ^a	56.68 \pm 9.05 ^a
	Length [μm]	18.36 \pm 0.44 ^a	17.59 \pm 1.16 ^a	17.07 \pm 1.01 ^a	18.15 \pm 0.63 ^a	17.73 \pm 0.73 ^a	15.08 \pm 0.48 ^a	17.32 \pm 1.24 ^a	16.21 \pm 1.18 ^a	16.11 \pm 0.34 ^a
	Width [μm]	7.38 \pm 0.55 ^a	6.71 \pm 0.76 ^{ab}	5.97 \pm 0.44 ^{bc}	6.55 \pm 0.23 ^a	6.04 \pm 0.24 ^{ab}	5.52 \pm 0.78 ^{bc}	7.46 \pm 0.17 ^a	6.10 \pm 0.34 ^b	5.98 \pm 0.42 ^b
35	Density [mm^{-2}]	134.27 \pm 10.36 ^b	152.22 \pm 24.17 ^a	163.93 \pm 12.75 ^a	143.12 \pm 3.68 ^{bc}	176.81 \pm 14.03 ^{ab}	189.96 \pm 16.66 ^a	58.57 \pm 2.31 ^b	62.98 \pm 2.23 ^a	63.77 \pm 5.28 ^a
	Length [μm]	17.81 \pm 1.03 ^a	17.15 \pm 0.31 ^{ab}	16.52 \pm 0.39 ^{bc}	16.29 \pm 1.00 ^a	15.84 \pm 0.92 ^{ab}	14.84 \pm 0.54 ^{bc}	17.03 \pm 1.39 ^a	16.84 \pm 0.77 ^a	16.48 \pm 1.33 ^a
	Width [μm]	6.12 \pm 0.12 ^a	5.92 \pm 0.17 ^a	5.56 \pm 0.29 ^b	6.38 \pm 0.33 ^a	6.27 \pm 0.20 ^a	5.70 \pm 0.35 ^b	7.95 \pm 0.71 ^a	7.17 \pm 0.26 ^{ab}	6.51 \pm 0.89 ^{bc}
49	Density [mm^{-2}]	140.52 \pm 16.86 ^b	150.27 \pm 29.40 ^b	172.08 \pm 9.10 ^a	158.73 \pm 9.74 ^{bc}	171.74 \pm 6.37 ^{ab}	184.90 \pm 6.37 ^a	51.96 \pm 2.99 ^b	69.67 \pm 5.15 ^a	76.13 \pm 3.34 ^a
	Length [μm]	17.33 \pm 0.88 ^a	16.93 \pm 0.73 ^a	16.26 \pm 0.62 ^{ab}	14.76 \pm 0.80 ^a	14.65 \pm 1.14 ^a	14.49 \pm 0.63 ^a	16.78 \pm 1.50 ^a	16.61 \pm 0.55 ^a	15.56 \pm 1.54 ^a
	Width [μm]	5.95 \pm 0.56 ^a	5.74 \pm 0.33 ^a	5.22 \pm 0.25 ^a	5.51 \pm 0.80 ^a	5.21 \pm 0.12 ^{ab}	4.82 \pm 0.40 ^{bc}	7.32 \pm 0.59 ^a	6.39 \pm 0.20 ^a	5.81 \pm 0.18 ^b

which may be one of the mechanisms of maintaining stable photosynthetic activity. In addition, Car can quench singlet oxygen and protect photosynthetic organs under adversity. On 35–49 TAA, the change in Y_{II} in the capsule wall was more stable than that in the leaves and the content of Car increased under water deficit, which indicated that the capsule wall could also reduce the absorption of light energy and enhance the mechanism of light protection by accumulating Car to quench light energy (Hu 2013). The early adaptation of plants to water deficit conditions was mainly reflected in stomatal movement, which affects the photosynthetic carbon assimilation ability of leaves (Cornic 2000). Under long-term drought, stomatal density was positively correlated with stomatal conductance, the carbon assimilation rate, and water-use efficiency (Xu and Zhou 2008), which enabled plants to avoid adverse damage and reduce transpiration, thereby improving their water-use efficiency (Pan *et al.* 2011). With the decrease in irrigation, the stomatal density of the BLS increased, but the stomatal length decreased (except on the capsule wall), which was consistent with previous research results (Fraser *et al.* 2009, Yang *et al.* 2015). The stomatal density of the leaves and capsule wall increased and the stomatal aperture, especially the stomatal width, decreased by reducing the drip irrigation rate, which indicated that drought mainly caused stomatal aperture reduction.

Under adverse conditions, when excess light energy is not completely removed by photoprotection mechanisms such as heat dissipation, ROS accumulation, damage, and increased cell membrane permeability lead to damage from ROS (Yu *et al.* 2017), affecting plant metabolism. Enzymatic protection systems and antioxidant substances in plants are involved in the removal of ROS (Miao *et al.* 2006, Miller *et al.* 2010). Stress can induce plants to initiate their antioxidant enzyme system. SOD, POD, and CAT play roles in photoprotection, remove H_2O_2 and other ROS (O_2^-), reduce the degree of damage to the cell membrane, and thus maintain a high photosynthetic rate (Ullah *et al.* 2017a). Our experimental results showed that under limited irrigation and deficit irrigation treatments, BLS components with higher MDA and H_2O_2 contents and SOD, POD, and CAT activities also showed varying degrees of increases (Li *et al.* 2019). This shows that water deficit causes membrane lipid peroxidation, and each organ protects itself from harm, increases its antioxidative enzyme activity, and reduces adverse stress, which is consistent with previous research results (Yi *et al.* 2016, Ullah *et al.* 2017b).

On 7–21 TAA, the activity of SOD in the main stem leaves and boll-subtending leaves was high. On 35–49 TAA, the activity of POD in the main stem leaves and boll-subtending leaves was also high, indicating that SOD was sensitive to water and played a protective role against water deficit in the early stage of boll development, while POD played a major protective role in the late stage. After 35 TAA, the main stem leaves under limited irrigation showed higher CAT activity, which indicated that cotton leaves could further enhance their active oxygen-scavenging ability after moderate drought, resisting the oxidative damage caused by drought stress and improving

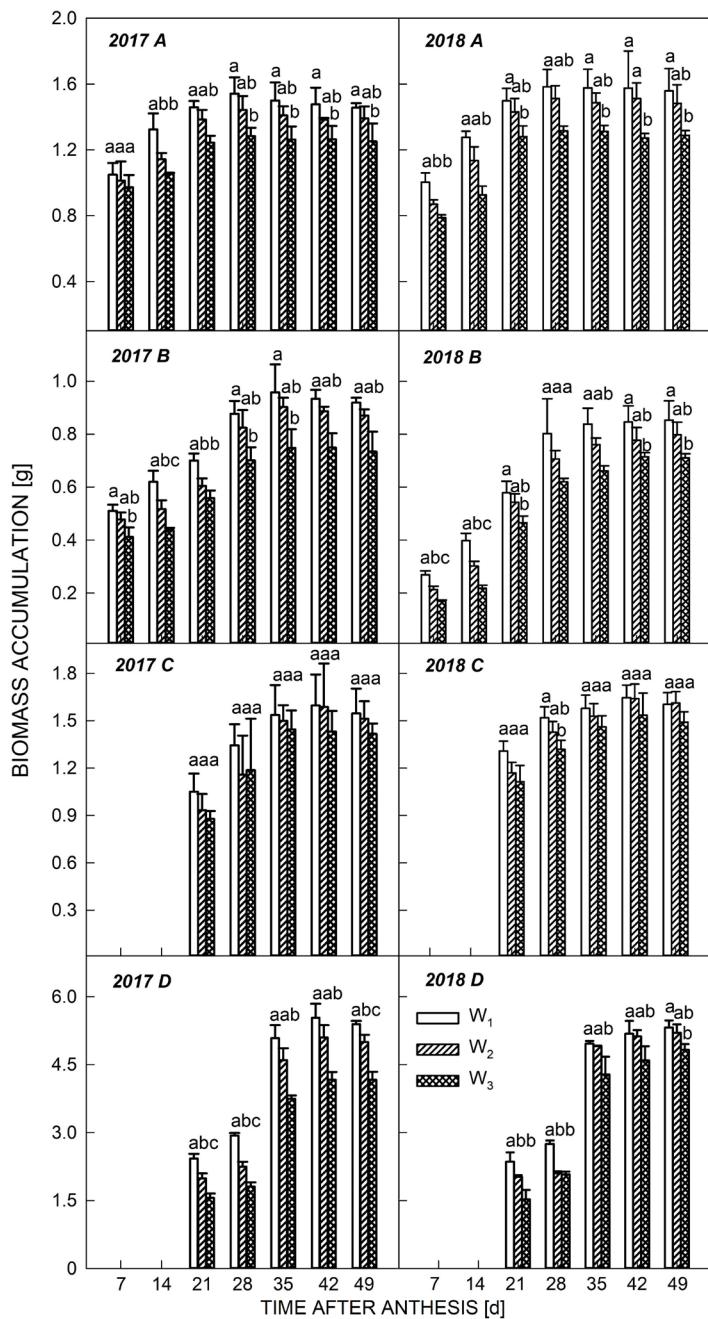


Fig. 6. Changes in biomass accumulation of main stem leaves (A), boll-subtending leaves (B), capsule wall (C), and seed cotton (D) under conventional drip irrigation (W₁), limited drip irrigation (W₂), and deficit drip irrigation (W₃) with the time after anthesis (TAA). Each value represents the mean \pm SE ($n = 3$). In Duncan's analysis, the difference between different treatments in the same period was significant ($P < 0.05$).

the drought tolerance of cotton (Liu *et al.* 2016). Compared to limited irrigation, deficit irrigation caused more serious damage to the antioxidant enzyme system. The threshold of resistance made it difficult to effectively remove ROS, and thus the drought tolerance of cotton leaves was low. In addition, compared to leaves, the activities of antioxidant enzymes and MDA and H₂O₂ contents in the capsule wall were smaller per unit mass; this indicated that the antioxidant system of the capsule wall was weaker, which was consistent with previous research results (Hu 2013).

Water is one of the main factors that affects the growth, development, physiological and biochemical processes of crops, and the water status is manifested in the accumulation

of biomass in various plant parts and the formation of yield (Meeks *et al.* 2019). A change in water conditions affects the dry matter distribution pattern between roots and crowns (Chen *et al.* 2004). For the cotton BLS, the early effect of irrigation on biomass accumulation was first manifested in the dry matter of leaves on 7–14 TAA but with the growth of the cotton bolls, the influence gradually shifted to the seed cotton mass. On 21–28 TAA, the decrease in irrigation significantly affected plants in the W₂ group, but with the growth and development of cotton bolls (Chen *et al.* 2019), there was no significant difference between the seed cotton biomass under W₁ and W₂. The possible reasons for this were the limited drip

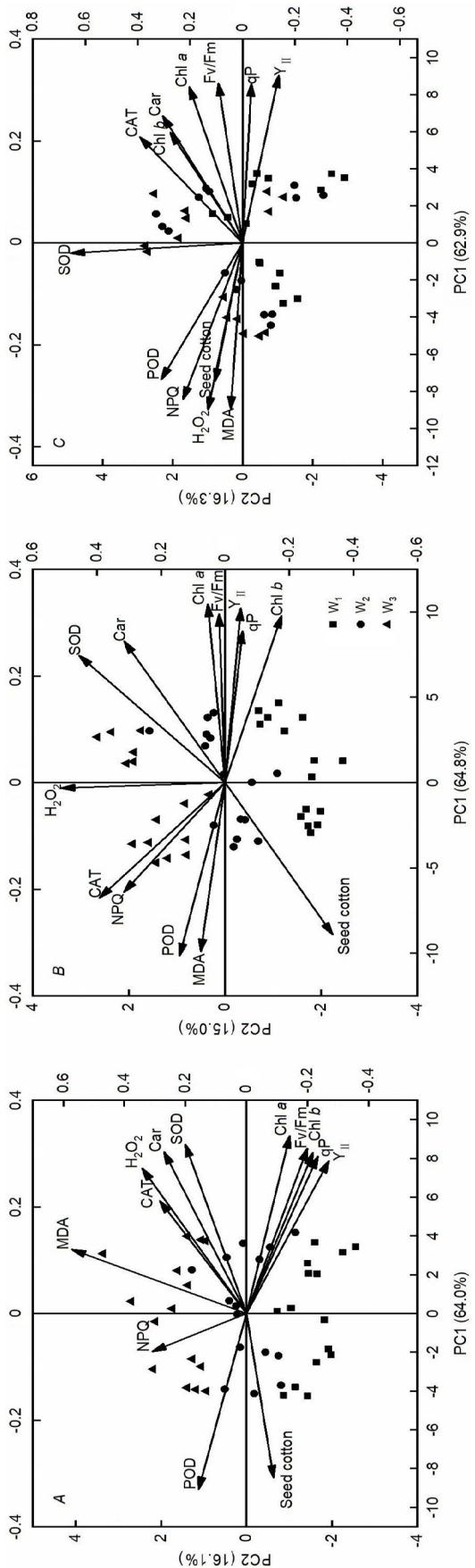


Fig. 7. Principal component analysis showing the relationship of the chlorophyll (Chl) *a*, Chl *b*, carotenoid (Car) content, maximum quantum efficiency of PSII photochemistry (F_v/F_m), photochemical quenching coefficient (q_p), nonphotochemical quenching (NPQ), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and seed cotton of main stem leaves (*A*), boll-subtending leaves (*B*), and capsule wall (*C*) under conventional drip irrigation (W_1), limited drip irrigation (W_2), and deficit drip irrigation (W_3).

irrigation and the possibility that the small amount of MC spray stimulated compensatory growth of components of the BLS (Kramer 1983).

Principal component analysis also showed that there was a significant correlation between the Chl fluorescence characteristics of each component of the BLS and the antioxidative enzyme system on the biomass accumulation of seed cotton. For W_3 , although the contribution of capsule wall biomass accumulation to BLS increased, the seed cotton mass significantly decreased. This was due to the different sensitivities of the different organs to water. When the amount of water was reduced excessively, the distribution of photosynthetic products was hindered, which led to differences in physiological changes (Chaves *et al.* 2002) and finally affected the development of cotton bolls.

Conclusion: In summary, in the early stage of boll development (on 35 TAA), the F_v/F_m of capsule wall under the limited irrigation increased by 2.9 and 5.8% compared to that of boll-subtending leaves and main stem leaves, and that of F_v/F_m under deficit irrigation increased by 3.8 and 5.5% compared to that of fruit branch and main stem leaf, respectively, and the NPQ and Car content increased with decreasing irrigation. On 35 TAA, the stomatal density of the capsule wall was higher over the main stem leaves and boll-subtending leaves and increased with the decrease in irrigation water. The activities of antioxidant enzymes and the MDA and H_2O_2 contents were lower than those of the main stem leaves and boll-subtending leaves. Therefore, the capsule wall maintained a high Car content and heat-dissipation ability to quench excess light energy, thus protecting the photosynthetic mechanism. Under water deficit, higher activities of SOD, POD, and CAT and the content of Car resulted in lower accumulation of active oxygen. This further protected the photosynthetic mechanism and led to increased biomass of the cotton BLS.

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