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RESEARCH ARTICLE

The Relative Contribution of Non-Foliar Organs of Cotton to Yield and Related Physiological Characteristics Under Water Deficit

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Abstract

Water deficit is one of the most important causes of decreased yield in cultivated plants. Non-foliar green organs in cotton play an important role in yield formation at the late growth stage. Although better photosynthetic performance was observed in a non-foliar organ (bract) compared with leaves under water deficit. However, the physiological response of each organ in cotton to water deficit has not been comprehensively studied in relation to the water status and photosynthesis characteristics. We studied the maintenance of water status of each organ in cotton by measuring their relative water content, proline content and stomatal characteristics. Water deficit significantly decreased the surface area of each organ, but to a lesser extent in non-foliar organs. Our results showed that the relative contribution of biomass accumulation of non-foliar organs increased under water deficit. Non-foliar organs (bracts and capsule wall) showed less ontogenetic decrease in O_2 evolution capacity and in RuBPC activity (per dry weight) as well as better antioxidant systems than leaves at various days after anthesis. We conclude that the photosynthesis from non-foliar organs is important for increasing cotton yield especially under water deficit conditions.

Key words: non-foliar organ, water deficit, water status, antioxidant systems, biomass accumulation, cotton

INTRODUCTION

Drought is considered to be the main environmental factor limiting plant growth and yield worldwide, especially in semi-arid areas (Boyer 1982). It is well known that one of the primary physiological targets of water deficit is photosynthesis (Chaves 1991; Cornic 1994; Lawlor 1995). In cotton, drought significantly decreased leaf photosynthetic rate, but the green non-foliar organs (bracts) were not affected to the same extent as leaves (Wullschleger *et al*. 1990). Under water deficit, when photosynthesis in leaves is largely depressed, ear photosynthesis may be the main photosynthetic contributor to grain filling in wheat (Evans *et al*. 1972; Bort *et al*. 1994; Sánchez-Díaz *et al*. 2002).

Compared to the flag leaf, the photosynthetic parts of the ear in wheat have physiological and morphological traits that may confer resistance to water deficit (Morgan 1980; Blum 1985; Knoppik *et al*. 1986; Xu and Ishii 1990; Araus *et al*. 1993; Bort *et al*. 1994). Under water deficit conditions, the ability to maintain cell water status is essential for continued growth. Compared with that of the subtending leaf and bracts, the water status in cotton fruit is less sensitive to drought (van Iersel and Oosterhuis 1996). Drought also causes a series of alterations in biochemical processes related to photosynthesis.

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In chloroplasts, for example, water deficit induces generation of active oxygen species (AOS) (Asada 1999). The AOS trigger deleterious reactions that involve degradation of proteins, enzyme inactivation, pigment bleaching and membrane injury (Asada and Takahashi 1987; Virgin *et al*. 1991; Irigoyen *et al*. 1992; Metha *et al*. 1992). To prevent oxidative damage, plants have evolved a complex antioxidant defence system, such as superoxide dismutase (SOD), asocrbate peroxidase (APX), glutathione reductase (GR), glutathione, ascorbic acid, and carotenoids (Car) (Liebler *et al*. 1986; Elstner 1987; Larson 1988). However, there have been few studies of the photosynthetic characteristics of non-foliar organs in response to water deficit. Compared with leaves, studies examining the effects of water deficit on antioxidant systems in non-foliar organs are few, despite the importance of antioxidant defence systems in helping chloroplasts to ameliorate oxidative stress and the fact that oxidative stress strongly affects photosynthetic capacity.

The objective of the present study was to investigate different physiological processes of leaf and non-foliar organs in response to water deficit. In this context, relative water content (RWC), proline content and stomatal characteristics were determined in various green organs in cotton under drought. In order to reveal their different capacities to resist drought, we also compared the photosynthetic rate, lipid peroxidation, RuBPC activity, soluble protein as well as antioxidant systems of leaves and non-foliar organs of cotton grown under both well-watered conditions and water-deficit stress. Also, the photosynthetic role of non-foliar organs to contribution to yield formation under drought was examined. Understanding the physiological photosynthetic responses of each green organ to drought is essential for a holistic elucidation of the resistance of cotton to drought.

RESULTS

Relative water content and stomatal charactertics in response to water deficit

The RWC is the most commonly used water status parameter to assess the degree of water deficit. Drought induced a larger decrease in RWC of the main stem compared with that of the other organs during the growth stages. Under water deficit, the reduction of RWC in leaf and main stem, at 20 day after anthesis (DAA), was 16.7 and 18.2%, respectively. The RWC of the bract was significantly lower than those in the other organs both under well-watered and water-deficit stress conditions. However, there was less decrease (10.04%) in RWC of bracts than in leaves. The RWC in capsule wall showed the least reduction (only 3.37%) among the green organs under water-deficit stress compared with well-watered conditions throughout growth stages (Fig. 1).

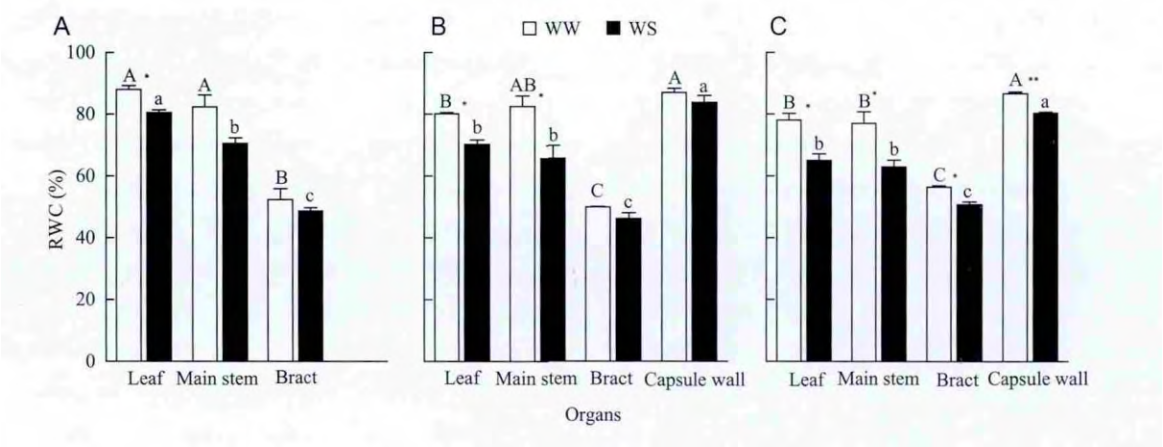

Fig. 1 Relative water content (RWC) of leaf, main stem, bract, and capsule wall of well-watered (WW) and water-deficit stress (WS) plants of cotton grown in field at 5 day after anthesis (DAA) (A), 15 DAA (B) and 20 DAA (C). Each value represents the mean±SD of three measurements. The same as below. Different letters denote significant differences among four green organs under WW (uppercase letters) and WS conditions (lowercase letters). Significant differences between two different water treatments: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. The same as below.

The stomatal density of the main stem and capsule wall was significantly lower than that of the leaf and bract. The stomatal density on the abaxial side of leaf and bract significantly increased under water deficit (Table 1). However, a decrease in stomatal size on the abaxial side in leaf and bract was observed under water deficit.

Table 1 Stomatal density (number of stomata mm^{-2}) and stomatal aperture (μm) in leaf, bract, main stem, and capsule wall of cotton, *Gossypium hirsutum* L., grown in the field at 20 day after anthesis

	Leaf		Bract		Main stem		Capsule wall	
Parameters	WW	WS	WW	WS	WW	WS	WW	WS
Stomatal density								
Adaxial side	176±14.8	178±16.5	68.8±6.41	64.5±8.78	23.0±3.21	27.3±7.85	47.1±15.4	54.5±14.0
Abaxial side	224±16.9	280±39.3***	136±20.9	175±16.5***				
Stomatal aperture size								
Breath on adaxial side	4.00±1.11	2.76±0.53**	5.05±0.49	7.93±0.96***	3.96±0.92	3.02±0.24	4.10±1.38	4.05±0.72
Length on adaxial side	18.6±1.16	19.1±0.78	16.4±1.44	17.0±1.79	15.1±1.39	17.8±1.32	15.8±4.36	15.1±1.74
Breath on abaxial side	4.09±0.95	2.55±0.98**	4.74±1.94	3.04±0.36*				
Length on abaxial side	14.2±1.19	10.5±1.36***	12.6±3.60	12.1± 0.93				

WW, well-watered; WS, water-deficit. All data are presented as the mean±SD. Significant differences in each organ between two different water treatments: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Surface area, biomass weight and the biomass accumulation of each organ and relative contribution of each organ to the whole plant during the late growth stage

Drought may initially inhibit leaf growth and development, significantly reducing leaf area (Chaves *et al*. 2003). From Jul. 30th (98 d after sowing) to Aug. 22nd (120 d after sowing), the surface area of leaf and stalks decreased about 4.2 and 5.1% under well-watered conditions, while the surface area of bracts and bolls increased about 3.3 and 135%, respectively (Fig. 2-A). During the period from Jul. 30th to Aug. 22nd, the surface area of leaf, stalks and bracts decreased about 33.77, 15.09 and 8.31% under drought, respectively (Fig. 2-B).

The inhibitory effect of drought upon total biomass production is well documented in the literature (Bajji *et al*. 2001; Lizana *et al*. 2006). Consistent with this, the total dry matter weight per cotton plant significantly decreased under water deficit; from Jul. 30th to Aug. 22nd, the total above-ground biomass per plant increased by about 39.2 and 11.6 g under well-watered and drought conditions, respectively (Fig. 2-C, D).

More than 90% of crop biomass is derived from photosynthetic products. Under well-watered conditions, the dry weight of bolls increased by 217% from Jul. 30th to Aug. 22nd. Thus, yield formation was

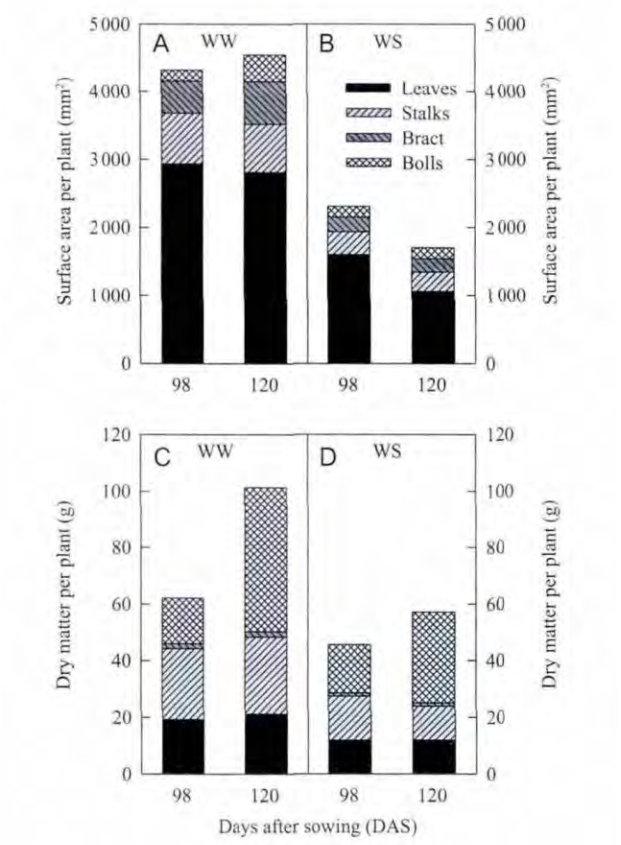

Fig. 2 Changes in surface area of leaves, stalks, bracts and bolls of a single cotton plant (A, B) and the plant dry weight (C, D) under two water treatments at 98 and 120 days after sowing (DAS).

achieved during this stage. The biomass accumulation of each organ was estimated by multiplying the O_2 evolution rate per area by the area and duration of the respective organ. As shown in Table 2, from Jul. 30th to Aug. 22nd, the biomass accumulation of the whole

Table 2 Biomass accumulation of each organ, estimated with surface area and photosynthesis rate of each organ during Jul. 30th to Aug. 22nd[1)]

Organs	Surface area as the same as Jul. 30th		Surface area as the same as Aug. 22nd	
	WW (g)	WS (g)	WW (g)	WS (g)
Leaves	33.6	9.98	32.2	2.97
	(74.2%)	(72.1%)	(67.8%)	(65.3%)
Stalks	6.39	1.70	6.06	0.65
	(14.1%)	(12.3%)	(12.8%)	(14.3%)
Bracts	3.18	0.97	4.22	0.39
	(7.0%)	(7.0%)	(8.9%)	(8.5%)
Bolls	2.12	1.19	5.00	0.54
	(4.7%)	(8.6%)	(10.5%)	(11.9%)
Total	45.29	13.84	47.5	4.66

[1)] The percent contribution of each organ to the photosynthesis rate of the whole plant is shown in parentheses. We assumed the the organs were exposed to the saturating 1 200 μmol photon m^{-2} s^{-1} for 8.5 h d^{-1}. WW and WS plants of cotton grown in field.

plant was estimated to be about 45.33-47.50 and 4.55-13.84 g under well-watered and water-deficit stress conditions, respectively. Water deficit decreased the percent contribution of photosynthesis of leaves to the yield formation. By contrast, the percent contribution of photosynthesis of non-foliar organs to the yield formation either increased or decreased only marginally under water deficit.

Response of O_2 evolution capacity, soluble protein content and photosynthetic enzyme to water deficit

The O_2 evolution rate, on both a DW (dry weight) basis or leaf area basis, decreased significantly in each organ of drought stressed plants in comparison with that of well-watered plants during each growth stage (Fig. 3). At 5 DAA, there was less reduction of the O_2 evolution rate in the main stem and bract as compared with the leaf in response to drought. At 20 DAA, the O_2 evolution rate on a DW basis in leaf, main stem, bract, and capsule wall exhibited different degrees of decrease in response to water deficit, being 43.2, 30.4, 13.7, and 36.3%, respectively.

Decreased leaf RWC has been correlated positively with reduced photosynthetic rate (Pugnaire *et al*. 1996). Similarly, we found that RWC was positively correlated with O_2 evolution rate on a DW basis in

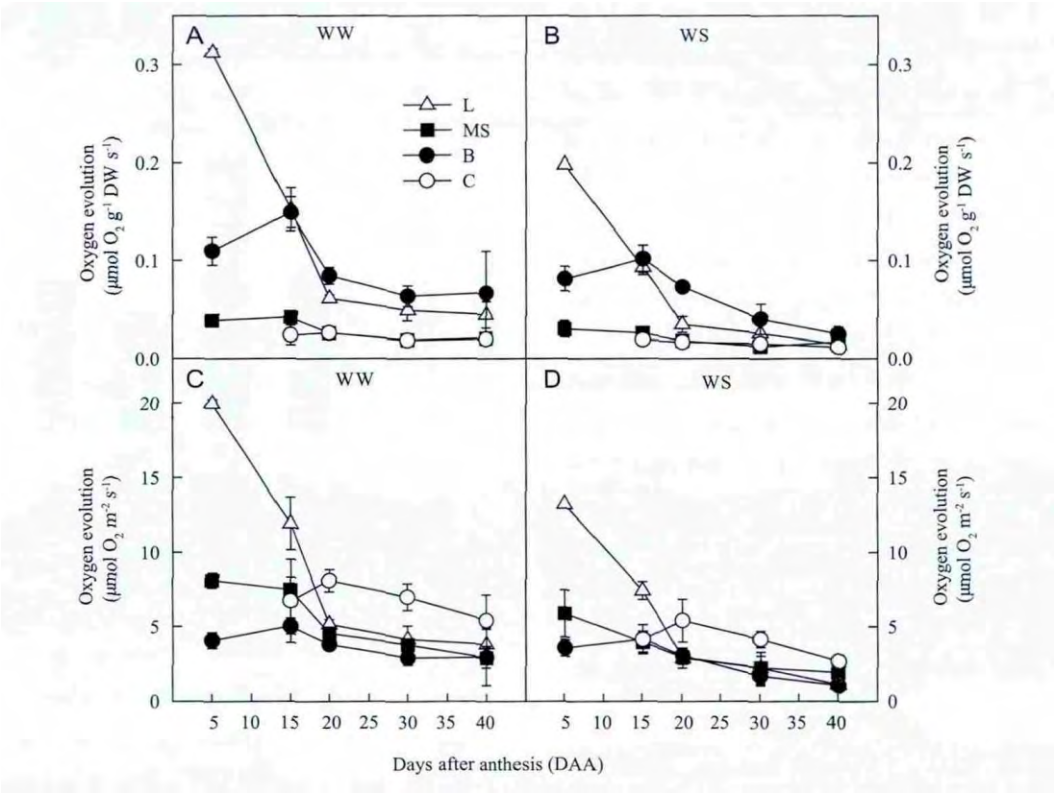

Fig. 3 The oxygen evolution rates of leaf, main stem, bract and capsule wall expressed on a dry weight basis (A, B) and an area basis (C, D) of WW and WS plants of cotton grown in field at various DAA. L, leaf; MS, main stem; B, bract; C, capsule wall. The same as below. Each value represents the mean±SD of six measurements.

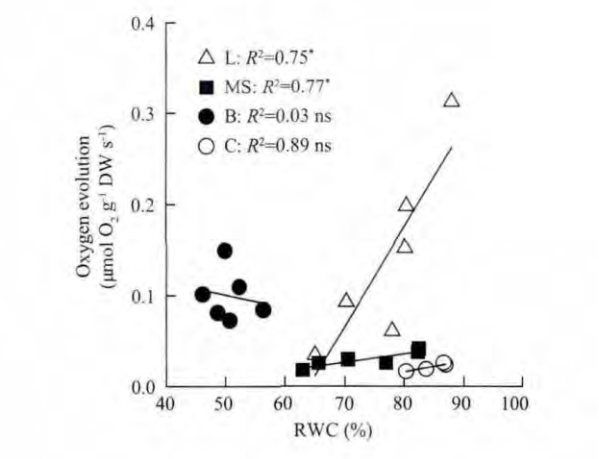

Fig. 4 Relation between RWC and oxygen evolution rate in each organ of cotton. Values are average means of six replicates each organ under two water treatments at 5, 15 and 20 DAA. The solid lines represent the best-fit linear regressions for each organ: *, $P<0.05$; ns, not significant.

leaves (P=0.025), main stem (P=0.022) and capsule wall (P=0.056) (Fig. 4). The result suggests that the photosynthesis of leaf, main stem, capsule wall is related to their water status.

In each treatment, soluble protein content decreased in leaf, main stem and bract during the period between 5 and 20 DAA (Fig. 5-A). Further, there was a significant decrease in soluble protein content of leaf, main stem and bract of drought-stressed cotton in comparison with well-watered plants on a given day after anthesis. At 5 DAA, the decrease of soluble protein in the main stem was significantly less than in other organs in response to water deficit. At 20 DAA, there was relatively greater reduction of the soluble protein content in leaf under water deficit than other organs, then followed by the main stem, capsule wall and bract in that order.

Under water deficit, the RuBPC activity in leaf, main stem and bract at 5 DAA decreased by about 35.9, 16.0 and 21.0%, respectively (Fig. 5-B). At 20 DAA, the RuBPC activity in the leaf, main stem and bract from drought-stressed cotton decreased significantly in comparison to well-watered cotton, with a reduction of 43.0, 35.7 and 22.8%, respectively. In contrast, there was no significant difference in RuBPC of capsule wall of well-watered bolls compared with water-deficit stress bolls.

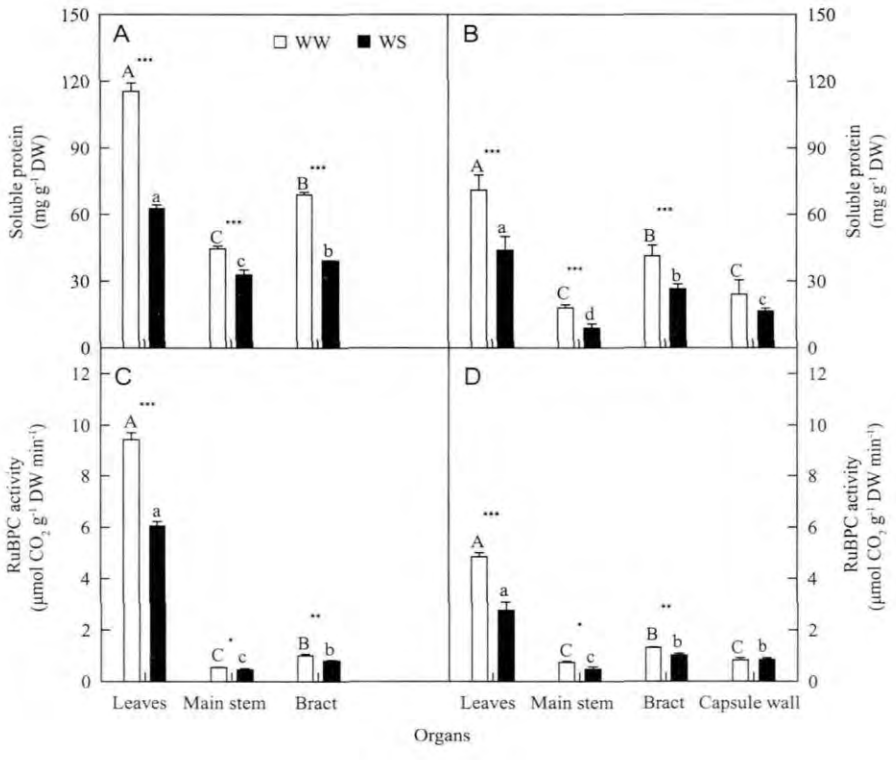

Fig. 5 Soluble protein content and RuBPC activity expressed on the basis of dry weight of leaf, main stem, bract and capsule wall of WW and WS plants of cotton grown in field during at 5 DAA (A, C) and 20 DAA (B, D).

Proline content, cellular damages, antioxidant systems and relationships between them

Proline plays a major role in osmotic adjustment (Pérez-Alfocea 1993). Water deficit increased proline content in each organ both at 5 and 20 DAA. At 5 DAA, the highest proline accumulation occurred in the main stem, among the three organs, in response to water deficit (Fig. 6). Under water deficit at 20 DAA, there was about 131 and 141% increase in proline accumulation in the main stem, and in the capsule wall, respectively, both increases being higher than in the leaf (42.0%) (Fig. 6). However, there was no significant increase in the already-high proline content in the bract in response to water deficit at either stage after anthesis. The proline content in the four green organs was significantly correlated with their RWC in a quadratic manner (P=0.002; Fig. 7).

Under water deficit, MDA content and ion leakage in the leaf and other non-leaf organs gradually increased (Fig. 8-A and B). At 5 DAA, the increase in MDA due to drought stress was the largest in the main stem; in the other extreme, the capsule wall exhibited a lower increase in MDA content than the other organs. Ion leakage in the leaf, main stem, bract and capsule wall in response to water deficit showed the same trend as did the MDA content.

SOD, the first enzyme in the detoxifying process, converts $O_2^{\cdot-}$ radicals to H_2O_2. Drought resulted in a significant increase of SOD activity in main stem at 5 DAA, while SOD activity decreased at 20 DAA (Fig. 8-C). At 20 DAA, there was also a significant

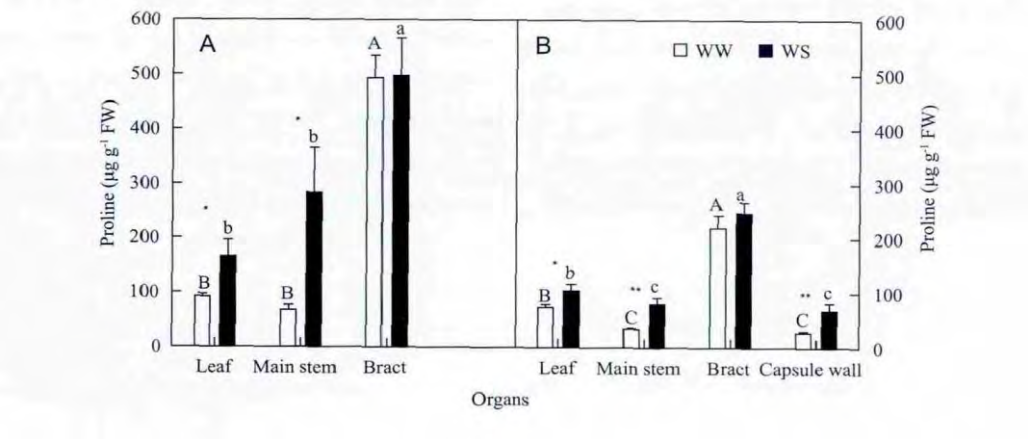

Fig. 6 Proline content expressed on the basis of dry weight of leaf, main stem, bract and capsule wall of WW and WS plants of cotton grown in field at 5 DAA (A) and 20 DAA (B).

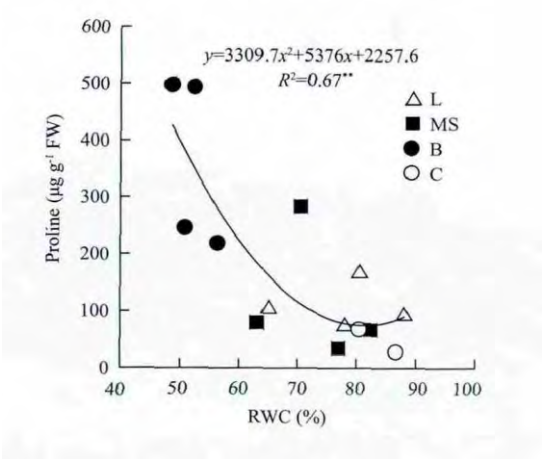

Fig. 7 Relation between RWC and proline concentration in the four green organs of cotton. Values are average means of three replicates each organ under two water treatments at 5 and 20 DAA. The same as below.

decrease in SOD activity in leaf and capsule wall after the water-deficit stress treatment. Water deficit caused a decrease in APX activity in each organ at 5 DAA. At 20 DAA, the APX activity in leaf significantly decreased under water deficit, but increased significantly in the main stem and capsule wall (Fig. 8-D).

DISCUSSION

Differences in maintenance of water status among four green organs of cotton in response to drought stress

Plant metabolism is dependent on leaf water status, as

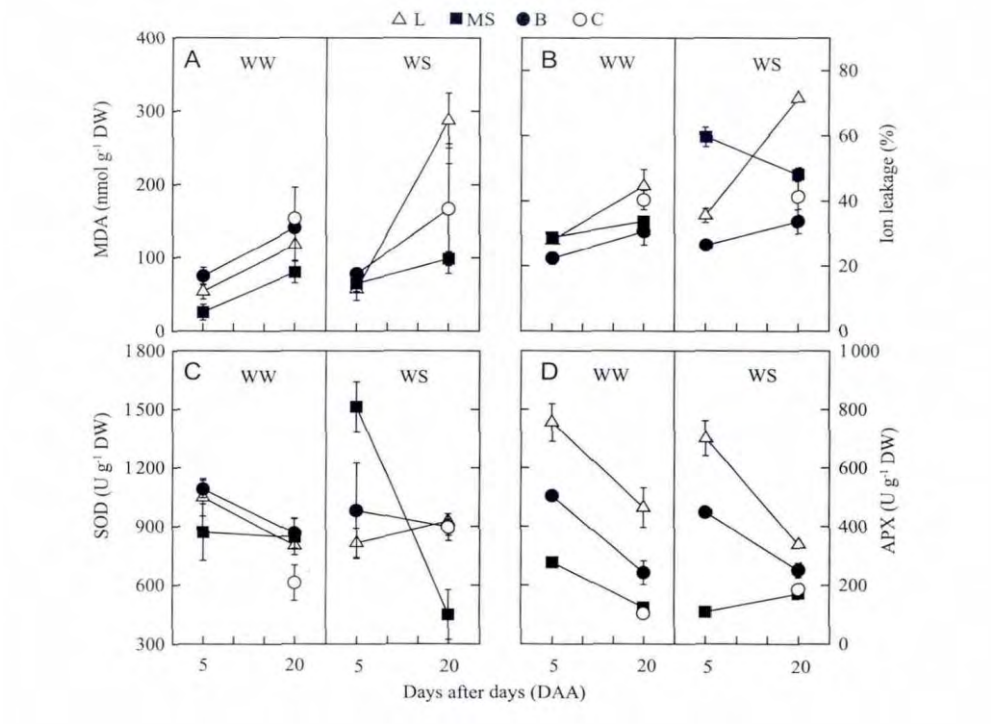

Fig. 8 Malondialdehyde (MDA) content (A), ion leakage (B), SOD (C) and APX (D) of leaf, main stem, bract and capsule wall of WW and WS plants of cotton grown in field both at 5 and 20 DAA.

measured, for example by RWC (Sinclair and Ludlow 1985). The RWC in different jute species decreased under drought stress (Chowdhury and Choudhuri 1985). Similarly, the RWC in all green cotton organs showed a decreasing trend under water deficit (Fig. 1). The foliar photosynthesis rate of higher plants is known to decrease as the RWC also decreases (Cornic 2000; Lawlor and Cornic 2002). The RWC in leaves and main stem were correlated positively with photosynthetic rate (Fig. 4), indicating that the photosynthesis of leaves and main stem was depended on their water status. Under water deficit, the ability to maintain cell water status is essential for continual growth.

Maintenance of cell water status can be achieved by the accumulation of compatible solutes such as proline (Sofo *et al*. 2004; Ben Ahmed *et al*. 2006; Ashraf and Foolad 2007) and stomatal regulation (Athar and Ashraf 2005; Ben Ahmed *et al*. 2007). The capacity for osmotic adjustment in terms of accumulating compatible solutes (e.g., proline) has been considered an important physiological adaptation of plants to resist drought (Morgan 1984). The proline content of tomato leaves, significantly correlated with RWC of leaves in a quadratic function, indicating that proline is a reliable indicator of the environmental stress imposed on plants (Claussen 2005). Similarly, in this study, the proline content in each cotton organ indicated its water status (Fig. 7).

Many reports have suggested that proline accumulation is significantly higher in drought-tolerant cultivars than drought-sensitive cultivars of wheat (*Triticum aestivum*, Nayyar 2003), mulberry (*Morus alba* L., Reddy *et al*. 2004) and olive (*O. europaea* L., Ben Ahmed *et al*. 2009). Therefore, we suggest that the significantly greater proline accumulation in the main stem than in leaf (Fig. 6) may help to maintain a relatively stable photosynthetic rate in response of the main stem to drought. Tambussi *et al*. (2005) reported that the better performance of the ear under water deficit (compared to the flag leaf) is related to its higher RWC. In our study, the capsule wall maintained significantly higher RWC than other organs both under well-watered and water-deficit stress conditions (Fig. 1). It was consistent with less effect of water deficit on O_2 evolution rate per DW in capsule wall compared with other organs under water deficit (Fig. 3). It is interesting that the bract maintained a relatively

stable RWC (Fig. 1) while its proline content, already high, increased little under water deficit conditions (Fig. 6). Possibly, the water status of the bract was maintained by its proline content. Indeed, as RWC decreased, there was an increase in proline content of the four organs (Figs. 1 and 6), presumably in a response that regulated the water status.

In addition, stomata regulation could also maintain RWC. It is well known that leaf water status always interacts with stomatal conductance, and under drought stress, a good correlation was often observed between leaf water potential and stomatal conductance (Medrano *et al*. 2002). Further, water deficit leads to an increase in stomatal density (Zhang *et al*. 2006; Xu and Zhou 2008), but a decrease in stomatal size (Spence *et al*. 1986). Under water deficit conditions, we indeed observed an increase in stomatal density and a reduction in stomatal size on the abaxial side both of leaf and bract (Table 1). For the leaf, although a reduction in stomatal aperture would limit CO_2 uptake, this limitation may have been counteracted by an increased stomata density on the abaxial side. By contrast, no increase in stomatal density or stomatal size was observed on the adaxial side of leaves: the close packing of the palisade cells on the adaxial side of the tissue (picture not shown) may have restricted CO_2 diffusion even if stomatal density and/or size had been increased. We are not sure why the stomatal density on the abaxial side of the bract, which lacks palisade tissue (Bondada *et al*. 1994) and which does not face the boll, was increased (Table 1). Possibly, the increase in stomatal density was related to the observation that the bract is adapted to a high CO_2 environment (Hu *et al*. 2013). Elevated CO_2 benefits sunflower growing under water deficit (Tezara *et al*. 2002), because a smaller Rubisco carboxylation capacity and greater light capture with greater capacity for synthesis of NADPH and ATP would predispose plants to photo-oxidative damage (Scarascia-Mugnozza *et al*. 1996). Therefore, the absence of significant changes in stomatal density and stomatal size on the adaxial side of bract which faces the cotton fruit (boll) would help CO_2 diffusion from the high respiration rate tissue (boll) to the bract under water deficit. On the other hand, the smaller stomatal size on the abaxial side, which faces air, would help to maintain a high CO_2 concentration inside the bract and minimize water loss from the bract. Indeed, stomatal conductance is reduced in bracts (14.3%) to a lesser extent than in leaves (28.9%) during peak reproductive development under moderate water deficit (Wullschleger *et al*. 1990). Thus, the relatively stable photosynthetic rate in the bract may be attributable to its growth in a high CO_2 environment.

Another important factor that would help bracts maintain a stable water status under drought would be the abundance of intercellular air spaces (bract lacked palisade tissue, Bondada *et al*. 1994). Under certain conditions, the air spaces may be filled with water (Willmer 1983). Accordingly, we suggest that the abundant intercellular air space in the bract may be filled with water under drought. To test this hypothesis, further research is necessary to clarify the water transport mechanism between bract and leaf or boll.

Optimal active oxygen-scavenging systems of bracts and capsule wall result in stable photosynthetic rate under drought

A more stable photosynthetic rate under drought is an indicator of better drought tolerance. In cotton, Wullschleger *et al*. (1990) reported that water deficit significantly decreased leaf photosynthetic rate (by 17.7-44.5%), while bracts showed less reduction in photosynthesis (10.0-11.1%). Their finding is consistent with our result (Fig. 3). The photosynthetic components (Chl., Rubisco and the light-harvesting complex of photosystem II) in awn and ear bracts were much less affected by water deficit than those in the leaves (Martinez *et al*. 2003). Variation in photosynthetic rate has been attributed to the amounts and activities of Rubisco (Lawlor *et al*. 1989). Rubisco is usually the major soluble protein of photosynthetic tissue, comprising about 50% (Makino *et al*. 1983). Decreases in soluble protein were observed in leaf, main stem, capsule wall and bract under drought (Fig. 5-A and B), indicating that Rubisco content decreased under water deficit. Compared with the leaf, less reduction of RuBPC activity in the bract in response to water deficit, may relate to its growth in a high CO_2 environment (less decrease in CO_2 concentration under drought stress). At 5 DAA, less reduction of soluble protein and RuBPC activity in the main stem may

result in less reduction of photosynthetic rate (Figs. 3-A and 5-A). At 20 DAA, significant decrease in soluble protein and stable RuBPC activity in capsule wall suggests that the decrease in photosynthetic rate was related to the reduction of soluble protein (Figs. 3-B and 5-B).

Under water deficit, closure of stomata decreases intercellular CO_2 concentration, resulting in an accumulation of NADPH at the expense of NADP; consequently, oxygen acts as an alternate acceptor of electrons leading to the formation of the superoxide radical, and from it H_2O_2 and hydroxyl radicals are produced. A decrease in membrane stability is reflected in the extent of lipid peroxidation caused by active oxygen species (Smirnoff 1993). Ion leakage is an indicator of cell membrane stability and integrity, which is commonly considered as one of the best physiological components of drought tolerance in plants (Kocheva *et al*. 2004; Xu *et al*. 2008). In the present study the increase in MDA content was accompanied by a marked increase in ion leakage in the main stem under water deficit at 5 DAA (Fig. 8-A and B). It might have been caused by a significant increase in SOD and a decrease in APX, resulting in an increase of H_2O_2 which was potentially toxic for cells (Elstner 1987). SOD plays a key role in quenching active oxygen (Fu and Huang 2001), working as a catalyst to dismutate $O_2^{\cdot-}$ into H_2O_2. APX and SOD are major enzymes of the water-water cycle, which maintains electron flow through the photosynthetic apparatus, especially when CO_2 fixation is limited or inhibited (Asada 1999). A number of studies have found that Cu/Zn SOD or the thylakoid-bound APX enhances the abiotic stress tolerance of transgenic plants (Gupta *et al*. 1993; Yabuta *et al*. 2002). The enhancement of active oxygen-scavenging system in transgenic tobacco plants (over-expressing SOD in chloroplasts) not only increased SOD level but also APX activity (Gupta *et al*. 1993). The observed positive correlations between SOD and APX in bract and capsule wall suggest that the increase of SOD activity (Fig. 9-A), combined by increase of APX, was better able to scavenge active oxygen species under water deficit. Therefore, we suggest that the better active oxygen-scavenging system of bracts and capsule walls may have resulted in less MDA and ion leakage under water deficit.

There was a positive relationship between proline content and SOD (Fig. 9-B) and APX (Fig. 9-C) in bracts. Possibly, the bract could maintain its relatively stable RWC under water deficit through stomatal regulation, and less change in RWC may result in little change in proline content. On the other hand, proline could stabilize the structures and activities of enzymes (Chaves *et al*. 2003). Under water deficit, a significant proline accumulation was observed in the leaf, main stem and capsule wall. However, a positive correlation between proline content and SOD or APX was only observed in the capsule wall. Possibly, the high Car/Chl. ratio in the capsule wall might have preserved its oxygen-scavenging system (data not shown), since Car plays an important role in photoprotection (Demming-Adams and Adams 1996; Adams *et al*. 1999). By contrast, perhaps the

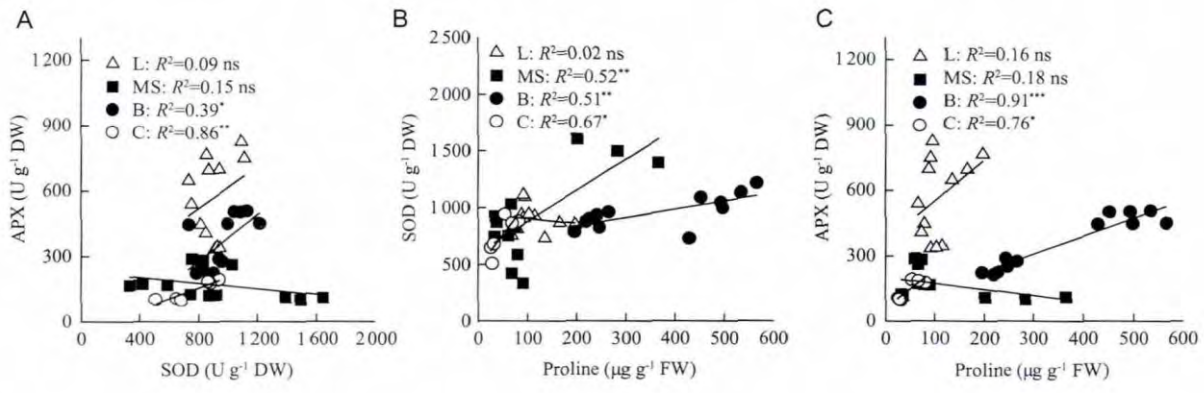

Fig. 9 Correlations between SOD activity and APX activity (A), between proline content and SOD activity (B), and proline and APX activity (C) in each organ. The solid lines represent the best-fit linear regressions for each organ: *, P<0.05; **, P>0.01; ***, P>0.001; ns, not significant.

oxygen-scavenging systems in leaves and main stem had been damaged under water deficit.

An increase in the relative contribution of biomass accumulation from non-foliar organs to the whole cotton plant

The total contribution of non-foliar green organs, including ears and peduncles, accounts for about 40-50% of grain mass per ear (Araus *et al*. 1993; Wang *et al*. 2001). An important photosynthetic contribution was from the non-foliar green organs in cotton, especially at the later growth stage (Hu *et al*. 2012). Under water deficit, the surface area of leaves decreased by 33.8% at the later growth stage, from 98 to 120 days after sowing, while the surface area of non-foliar organs decreased by 9.4% (Fig. 2-B). However, a decrease in leaf area could also be beneficial to plants under drought because it allows a reduction of leaf transpiration. Inhibition of photosynthesis under water deficit determines plant growth, survival and yield. Compared with leaves, the O_2 evolution rate per area in non-foliar organs was less affected by drought from 20 to 40 DAA (Fig. 3-C and D). The decrease in total biomass production of cotton plants was mainly due to a reduction of photosynthesis under water-deficit stress (Fig. 2-C and D). Since a non-foliar organ (bract) could maintain a relatively stable photosynthetic rate, even under drought stress, Wullschleger *et al*. (1990) suggested that the relative contribution of assimilates from the bracts may increase during adverse environmental conditions such as water deficit, or during canopy senescence when leaf photosynthesis is severely reduced. Thus, because of less decrease in surface area and photosynthetic rate of non-foliar organs under drought, we suggest that the photosynthetic contribution from non-foliar organs to the whole cotton plant might increase under water deficit.

In order to check the relative contribution of each organ changes in response to water deficit, the biomass accumulation was estimated based on an 8.5-h photosynthetically active duration per day and optimal irradiance of 1 200 μmol photons m^{-2} s^{-1} (Fig. 6 in Hu *et al*. 2012). The average value of the photosynthetic capacity was taken from the 15 to 40 DAA as the average photosynthetic rate of each organ during the period Jul. 30th to Aug. 22nd (except two rainy days, Fig. 10). If we assume the surface area of each organ was taken on Aug. 22nd as the photosynthetic area from the Jul. 30th to Aug. 22nd, it was found that the total biomass accumulation of the whole plant was about 45.3 and 13.8 g under well-watered and water-stressed conditions, respectively (Table 2). From the Jul. 30th to Aug. 22nd, the total dry matter per plant increased by about 39.2 and 11.6 g under well-watered and water-deficient conditions, respectively (Fig. 1-C and D). These results indicate that the calculation of biomass accumulation of each organ during the later growth stage (from 98 to 120 days after sowing) was fairly accurate.

Table 2 shows that the relative contribution of non-leaf organs to the whole plant increased under water deficit, though leaves still made the major contribution to the yield of cotton. Thus, further research should be conducted to improve not only the drought resistance of cotton leaves, but also the drought tolerance of the non-foliar organs.

CONCLUSION

The relative photosynthetic contribution of green non-foliar organs to the whole cotton plant increased under water deficit, especially at the late growth stage. Under water deficit, there was a smaller decrease in surface area of non-foliar organs than in leaves (Fig. 2-A and B). Moreover, non-foliar organs (bract and capsule wall) showed less ontogenetic decrease in O_2 evolution capacity, photosynthetic enzyme activity and better antioxidant systems than leaves in response to drought stress. Thus, the relative photosynthetic contribution of the non-foliar organs to the whole-plant was expected to increase under water deficit. The estimated relative contribution of biomass accumulation of leaves to the whole plant was 65.3-72.1% under water deficit (Table 2). These results suggested that the yield formation is largely affected by leaf photosynthesis under water deficit, because the majority of cotton biomass is produced by leaves. However, the relative photosynthetic contribution of non-foliar organs increased under water deficit was about 27.9-34.7%, thereby providing a substantial contribution to yield formation at the late growth

stage. Therefore, it is also important to consider the photosynthesis of non-foliar organs in cotton when breeding for drought-tolerant cotton.

MATERIALS AND METHODS

The study was carried out in an experimental field of Agricultureal College, Shihezi University, Xinjiang, China (45°19´N, 86°03´E) in 2010. Cotton (*Gossypium hirsutum* L. *cv*. Xinluzao 13) seeds were sown on the 24th April and allowed to grow under field conditions with under-mulch drip irrigation. Fig. 10 shows the meteorological conditions in terms of maximum and minimum temperature and precipitation during growth stages (from Jun. to Oct.). There were two levels of irrigation treatment, namely, well-watered (WW) treatment and water-deficit stress (WS). The well-watered plots were irrigated according to standard local practice whereas the drought-stressed plots were irrigated to the extent of 20% (moderate water deficit) of the well-watered plots after sowing. Weeds and pests were controlled in the field using standard management practices.

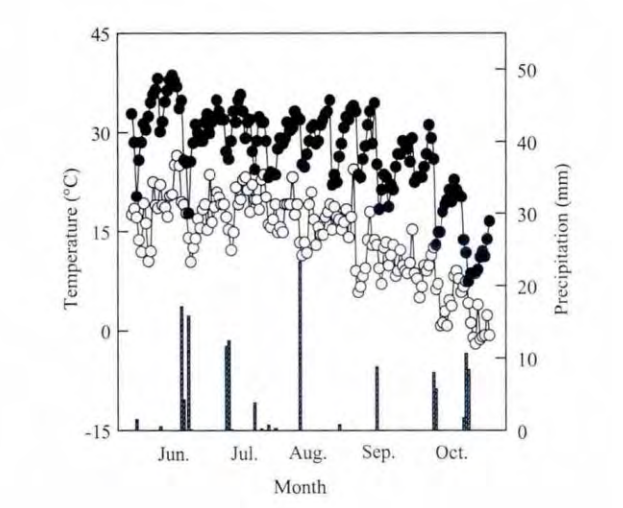

Fig. 10 Maximum and minimum temperature (circles) and precipitation (bars) at the study site.

Relative water content determination

Leaves and associated main stem, bracts and capsule wall were collected from both well-watered and drought-stressed cotton at 5, 15 and 20 DAA to determine relative water content (RWC), which was calculated as RWC (%)=(FW-DW)/(TW-DW)×100, where FW is the fresh weight of these tissues, TW is the turgid weight after rehydrating samples for 24 h and DW is the dry weight after oven-drying samples at 85°C for 48 h.

Stomatal characteristics

Stomatal density was measured on each organ at 20 DAA. The organs were coated with a thick layer of nail polish. The dried replicas were carefully peeled from the organs and placed on microscope slides. Three dried replicas from each organ, and three subsamples per replica were used to determine stomatal sizes and densities.

Surface area and biomass weight

The surface area of leaves, subtending leaves and bracts was measured using a leaf area meter (LI-300, Li-Cor, Lincoln, NE, USA). The surface area of stalks (including carpopodium and petiole) could be calculated from the length and diameter of stem section (taken as cylinders). The surface area of the bolls was measured according to Hu *et al*. (2012), disregarding ones which were younger than 15 DAA. After drying to constant dry weight at 85°C (≥24 h) dry weights were determined. These measurements were taken for both well-watered plants and drought-stressed plants on Jul. 30th (98 days after sowing) and Aug. 22nd (120 days after sowing). We did not include the surface area or biomass of the fallen leaves or other organs.

The biomass accumulation of each organ and relative contribution to the whole plant during the late growth stage

In order to investigate the relative contribution of each organ to the whole plant during the yield formation stage, we estimated the biomass accumulation of each organ from Jul. 30th to Aug. 22nd (the late full bolling stage) as following. Considering that the cotton plants were exposed to 1 200 μmol photons m^{-2} s^{-1} or more for 8.5 h in Xinjiang on a sunny day in summer (data not shown), the biomass accumulation of each organ was estimated by multiplying the O_2 evolution rate per area by total area of the respective organ and photosynthetic duration:

Biomass=P×10^{-6} mol O_2 (μmol O_2)$^{-1}$×Area×10^{-4} m^2 cm^{-2}× 8.5 h d^{-1}×20 d×3 600 s h^{-1}×(1/6) mol $C_6H_{12}O_6$×180 g $C_6H_{12}O_6$ (mol $C_6H_{12}O_6$)$^{-1}$

Where, P is the O_2 evolution rate (μmol m^{-2} s^{-1}) and area (cm^2) is the surface area of each organ. Here, we estimated the biomass accumulation of each organ from Jul. 30th to Aug. 22nd under two conditions. In one case, we assumed that the area of each organ was maintained the same as on Jul. 30th during this period; in the other case, we assumed that the area of each organ was maintained the same as on Aug. 22nd. It would take about 26, 40 and 28 d to achieve the maximum dry-weight accumulation of the first three bolls at node 10 (Wullschleger and Oosterhuis 1990). The dry weight of bolls would increase by about 200% from 10-26, 10-40 and 17-28 DAA (Fig. 1-B in Wullschleger

and Oosterhuis 1990). The dry matter per plant increased about 63.2% on the Aug. 22nd compared to the Jul. 30th, and the total dry weight of bolls on the Aug. 22nd increased about 217% compared to that on the Jul. 30th (Fig. 2-A and B). Thus, we assumed that the major biomass of cotton was formed during the Jul. 30th to Aug. 22nd (the later full bolling stage). In our study, we did not measure the oxygen evolution rate of the capsule wall after 10 DAA. Here, the average value from 15 to 40 DAA was taken as the average photosynthetic capacity of each organ during this period (from Jul. 30th to Aug. 22nd except two rainy days, Fig. 10). The percent contribution of photosynthesis of each green organ to the yield formation was estimated by the biomass accumulation of each organ divided by the total biomass accumulation of the whole plant (sum of all green organs).

Oxygen evolution rate

The leaves, bracts, bolls and the main stem under the main leaf were removed from the plant, placed on moistened cloth, and then speedily taken to the lab. Samples of leaves, bracts, main stem and capsule wall were cut into small sections (1×2 mm^2). Oxygen evolution capacity of the sectioned samples was measured at 25°C using a ChloroLab2 liquid-phase O_2 electrode system (Hansatech Instruments, Norfolk, UK), in which the CO_2 was saturating for photosynthesis. The reaction mixture (2.0 mL) consisted of 20 mmol L^{-1} $NaHCO_3$ and 60 mmol L^{-1} Tris-HCl (pH 7.5). Illumination (1 200 μmol photons m^{-2} s^{-1}) was provided by red light-emitting diodes. The area of leaves, main stem, bracts and capsule wall were also recorded at each measurement time point. All the O_2 evolution capacities were calculated on an area basis (μmol O_2 m^{-2} s^{-1}) and dry weight basis (μmol O_2 g^{-1} DW s^{-1}). Data were averaged from six replicates.

Extraction and assay of key enzyme in the Calvin cycle and soluble protein

The RuBPC activity of leaves, bolls, bracts, bolls and main stems was investigated at 5 and 20 DAA. The extraction of enzymes was carried out according to Sayre *et al*. (1979) with slight modifications. Green tissue (0.2 g) was ground with a mortar and pestle (4°C) containing a small amount of sand and 1.0 mL of grinding media consisting of 0.1 mol L^{-1} Tris-HCl (pH 7.8), 100 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} EDTA, 20 mmol L^{-1} mercaptoethanol, 100 kg m^{-3} glycerin, and 10 kg m^{-3} polyvinylpyrrolidone. Following centrifugation at 15 000×g for 10 min at 4°C, the supernatant was used for enzyme assays. RuBPC activity was assayed by the method of Camp *et al*. (1982). An enzyme extract was added to a reaction mixture that contained 50 mmol L^{-1} Tricine-NaOH (pH 7.9), 10 mmol L^{-1} KCl, 1 mmol L^{-1} EDTA, 2 mmol L^{-1} dithiothreitol (DTT), 0.2 mmol L^{-1} NADH, 5 mmol L^{-1} ATP, 15 mmol L^{-1} $MgCl_2$, 10 mmol L^{-1} $NaHCO_3$, 5 mmol L^{-1} phosphocreatine, 2 U mL^{-1} creatine phosphokinase, 4 U mL^{-1} each of NAD-dependent glyceraldehyde-3-P-dehydrogenase and 3-P-glycerate kinase in a final volume of 1 mL. The mixture was incubated at 25°C for 5 min. Reactions were initiated by the addition of 0.5 mmol L^{-1} RuBP.

The soluble protein content of leaves was measured according to the Coomassie brilliant blue G_{250} method described by Read and Northcote (1981). The soluble protein content was measured by a spectrophotometer (U-3900, Hitachi, Tokyo, Japan) at 595 nm.

Proline content

Proline content was determined by the ninhydrin method (Bates *et al*. 1973). Three replicates of each organ were obtained from cotton with different water treatments at 5 and 20 DAA.

Cellular damage and antioxidant enzymes

Lipid peroxidation was measured in terms of malondialdehyde (MDA) (Hodges *et al*. 1999). To determine cell membrane stability and integrity, ion leakage was measured according to a simplified method (Nayyar 2003). Tissues were thoroughly washed, cut into small discs and placed in vials filled with 10 mL deionized water. After incubation at 25°C for 12 h in dark condition, the electrical conductivity (initial *EC*) in the bathing solution was determined by a conductivity meter (DDSJ-308A, Leici, Shanghai, China). Then the samples were heated at 100°C for 20 min and conductivity (final *EC*) in the bathing solution was read again:

$$\text{Ion leadage was defined as } EC\,(\%)=\frac{\text{Initial } EC}{\text{Final } EC}\times 100$$

Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) method (Fu and Huang 2001). One unit of SOD activity was defined as the amount of enzyme required to produce a 50% inhibition of reduction of NBT at 560 nm. APX activity was assayed according Ushimaru *et al*. (1997), following the decrease in $OD_{290\ nm}$ owing to H_2O_2-dependent ascorbate oxidation. One unit of APX activity was defined as an absorbance change 0.1 min^{-1}.

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