PHOTOSYNTHETICA
International Journal for Photosynthesis Research

DOI 10.32615/ps.2023.036 PHOTOSYNTHETICA 62 (1): 6-15, 2024

Regulation of photosynthesis and water-use efficiency in pima and upland cotton species subjected to drought and recovery

J.M. HAN*, J. FLEXAS**, D.L. XIONG***, J. GALMÉS**, and Y.L. ZHANG*,+

*Key Laboratory of Oasis Eco-agriculture, Xinjiang Production and Construction Corps, Shihezi University, 832003 Shihezi, China**
*Research Group on Plant Biology under Mediterranean Conditions, Universitat de les Illes Balears/Instituto de Investigaciones Agroambientales y de Economía del Agua (INAGEA), Carretera de Valldemossa Km 7.5, 07121 Palma de Mallorca, Illes Balears, Spain***
*National Key Laboratory of Crop Genetic Improvement, MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, 430070 Wuhan, Hubei, China****

Abstract

Photosynthesis is extremely sensitive to drought and decreases due to co-occurring stomatal, mesophyll, and biochemical limitations. Few studies have shown the effects of a drought cycle (drought–rewatering) on photosynthesis and water-use efficiency (WUE) in cotton. Here, the dynamics of photosynthesis in upland (*Gossypium hirsutum*) and pima (*G. barbadense*) cotton were analyzed during a drought cycle. While both species decreased net CO_2 assimilation rate (P_N) to a similar extent under drought, in pima cotton rewatering rapidly restored P_N, the stomatal conductance (g_s), the maximum carboxylation rate (V_{cmax}), and the maximum electron transport rate above the control level. Meanwhile, the response of leaf intrinsic WUE (WUE_i) to drought and recovery was different between the two cotton species, which was mainly co-regulated by V_{cmax} and g_s. In conclusion, pima and upland cotton exhibit different response patterns of P_N and WUE_i during the whole process of drought and recovery.

Keywords: drought; mesophyll conductance; photosynthesis; recovery; stomatal conductance; water-use efficiency.

Introduction

Photosynthesis is the main determinant of plant carbon uptake and final photosynthetic productivity but, at the same time, it is also one of the most sensitive and critical processes to be influenced by drought (Flexas and Medrano 2002, Chaves *et al.* 2009). Consequently, improving the responses of leaf photosynthesis and intrinsic water-use efficiency [WUE_i, *i.e.*, the ratio of net CO_2 assimilation (P_N) to stomatal conductance (g_s)] to drought has been a scientific goal for decades (Costa *et al.* 2007, Morison *et al.* 2008, Flexas *et al.* 2010, Singh and Reddy 2011,

Highlights

- Upland and pima cotton differ in response of water-use efficiency to drought
- After rewatering, photosynthesis recovers faster in pima than in upland cotton
- The maximum carboxylation rate and stomatal conductance coordinate in cotton

Received 4 July 2023
Accepted 26 September 2023

+Corresponding author
phone: +86-993-2058005
e-mail: zhangyali_shzu@163.com

Abbreviations: BL – biochemical limitation; C_c – chloroplastic CO_2 concentration; C_i – substomatal CO_2 concentration; g_m – mesophyll conductance; g_s – stomatal conductance; J_{max} – maximum electron transport rate; LMA – leaf mass per area; MCL – mesophyll limitation; P_N – net photosynthetic rate; R_D – dark respiration; R_d – day respiration; SL – stomatal limitation; V_{cmax} – maximum carboxylation rate; WUE_i – intrinsic water-use efficiency.

Acknowledgments: This research was supported by the National Natural Science Foundation of China (grant no. U1903302); the 111 Project (grant no. D20018). Y.L. Zhang acknowledges China Scholarship Council (CSC) for funding his research. J. Flexas acknowledges support by the project PGC2018-093824-B-C41 [Ministerio de Economía y Competitividad (MINECO), Spain] and the ERDF (FEDER). Y.L. Zhang is especially grateful to Antònia Romero Munar for supporting and helping his life during his visit to Spain, also very grateful for the company from his son (Shixun Zhang) when he was performing his research.

Conflict of interest: The authors declare that they have no conflict of interest.

Cano *et al.* 2014). However, since photosynthesis and WUE_i are two complex traits involving many plant leaf characteristics and processes (Tomás *et al.* 2013, Gago *et al.* 2014), it might be difficult to improve them simultaneously only through the modification of single trait (Flexas *et al.* 2016). Under drought conditions, WUE_i increases due to decreased g_s, but low g_s also restricts CO_2 uptake by limiting CO_2 influx and thus P_N, therefore improvements in WUE_i may be realized at the expense of photosynthetic productivity (Lawson and Blatt 2014, Han *et al.* 2016, McAusland *et al.* 2016). In addition, numerous studies have shown that drought decreased photosynthesis *via* decreased CO_2 diffusion including not only g_s but also mesophyll conductance to CO_2 (g_m) in C_3 plants (Grassi and Magnani 2005, Flexas *et al.* 2012a, 2018; Galmés *et al.* 2013, Zhou *et al.* 2013, 2014) and, sometimes, the biochemical capacity of leaves, the latter reflected by the maximum carboxylation rate (V_{cmax}) and the maximum electron transport rate (J_{max}) (Thimmanaik *et al.* 2002, Zhou *et al.* 2013, 2014; Flexas *et al.* 2016). Meanwhile, g_s, g_m, V_{cmax}, and J_{max} are also important factors indirectly influencing WUE_i (Flexas *et al.* 2013, Gago *et al.* 2014, Nadal and Flexas 2019), and high WUE_i is still an important trait to minimize the loss of water and maximize the photosynthetic carbon assimilation in many plant species (Blum 2009, Sinclair and Rufty 2012, Vadez *et al.* 2014, Long *et al.* 2015).

Several studies have analyzed instantaneous physiological limitations to photosynthesis under short- and/or long-term drought (Grassi and Magnani 2005, Han *et al.* 2018, Nadal and Flexas 2019, Zhu *et al.* 2021). However, analyses of photosynthetic recovery after drought are still scarce. The recovery of photosynthesis caused by drought and rewatering could partially offset the effects of drought on final plant productivity (Flexas *et al.* 2009, Gallé *et al.* 2009, 2011). Moreover, as the frequency of droughts is increasing, determining the recovery time is critical since, if a second drought period arrives before plants have recovered from the previous, the plant function might be permanently affected with enormous consequences for plant yield and even survival (Schwalm *et al.* 2017). Therefore, studying plant drought acclimation and quantifying how long it takes for plant photosynthetic CO_2 assimilation to return to pre-stress levels upon rewatering is necessary to determine the final effects of drought on plants.

Cotton (*Gossypium*) is considered one of the most drought-tolerant crops in the world and plays an important role in the global economy, with upland (*G. hirsutum*) and pima cotton (*G. barbadense*) being the most widely cultivated. Upland cotton is known for its high yield potential while pima cotton has superior fiber quality. Thus, increasing yield and enhancing fiber quality by interspecific hybridization and introgression has been an important breeding goal for cotton improvement. As upland cotton has wide adaptation and pima cotton is only grown in semi-arid and arid areas in the world, their responses to drought are distinct. In previous works, it has been shown that upland and pima cotton have different physiological strategies, for example, of photochemical acclimation to drought, for which upland cotton dissipates excess energy by enhanced photorespiration and Mehler reaction (water–water cycle) while pima cotton does it by photorespiration and heat dissipation, depending on the level of drought (Yi *et al.* 2016a). Additionally, the photosystems of upland cotton are relatively stable under drought and, after rewatering, decreased photosynthesis can be completely restored to the control values (Yi *et al.* 2016b). However, up until now, no studies analyzed the overall effects of a drought cycle (drought–rewatering) on photosynthesis and water-use efficiency in pima and upland cotton, which is crucial for the development of water-saving irrigation schedules in cotton. Therefore, the aim of the present study was to (*1*) assess the photosynthetic resilience of these two cotton species (*G. hirsutum* and *G. barbadense*) to drought; (*2*) analyze the differences in photosynthetic traits and water-use efficiency between two cotton species in response to drought and rewatering.

Materials and methods

Plant materials and water treatments: Two *Gossypium* species *G. hirsutum* L. (upland cotton, cv. Xinluzao 45) and *G. barbadense* L. (pima cotton, cv. Xinhai 28) were germinated and grown in a natural light source greenhouse at the Universitat de les Illes Balears, Palma, Mallorca, Spain, on April and May 2016. Seeds were planted into 3.0-L pots filled with a mixture of soil and organic substrate, and ten pots were prepared for each species. After germinating, plants were thinned to a single plant per pot. Greenhouse air temperatures were set to 28°C for the 14-h photoperiod and 25°C at night; the relative humidity was kept at ~50%; supplemental light intensity above ~350 μmol(photon) m^{-2} s^{-1}. Plants were watered daily at full substrate saturation until the drought treatment was performed, and the 50% Hoagland's solution was applied once per week (Hoagland and Arnon 1950). The cotton plants were subjected to progressive water withholding followed by sustained water stress at a constant level before rewatering. Water stress (WS) was applied to half of the plants for each species at 60 d after planting by stopping water, and the pots were weighted at nightfall every day. Relative substrate water content reached the expected drought level (50%) on the 11th day of drought treatment. Then drought state was maintained by weighting pots every day to keep relative substrate water content constant at 50% until rewatering to 100% on the 19th day (detailed in Fig. 1). The light-saturated stomatal conductance (g_s) of all drought cotton plants on the first day of drought acclimation was within the range of 0.05–0.12 mol(H_2O) m^{-2} s^{-1}, which proved that the plants were suffering from moderate drought as we expected when relative substrate water content was kept at 50% (Flexas *et al.* 2012b). The topmost full-expanded youngest leaves on the main stems were marked and used to perform the measurements over the whole experiment.

Gas exchange and chlorophyll fluorescence: Concomitant measurements of gas exchange and chlorophyll

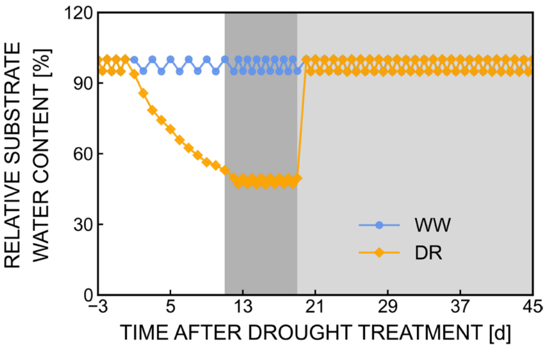

Fig. 1. The relative substrate water content over the whole experiment. The period of drought acclimation (from day eleven to nineteen) is indicated by *the dark grey area*, and the days under rewatering (from day nineteen to forty five) by *the pale grey area*.

fluorescence were performed 12 times during the study period, specifically on the 0 (drought imposition, shortly named as DI), 4 (DI), 7 (DI), 10 (DI), 14 (drought acclimation, shortly named as DA), 17 (DA), 21 (rewatering, shortly named as RW), 23 (RW), 25 (RW), 33 (RW), 37 (RW), and 42th (RW) day, using an open gas-exchange system (*Li-6400*, *Li-Cor*, *Inc*., Lincoln, NE, USA) connected to a leaf fluorometer chamber (*Li-6400-40*, *Li-Cor*, *Inc*.). Leaf temperature during the photosynthesis measurements was set to 25°C, the leaf vapor pressure deficit (VPD) was kept in the range of 1.3 to 2 and the ratio of red to blue light was set to 90:10% PPFD to maximize stomatal aperture. Illumination was provided by an LED source and CO_2 concentration in the *Li-6400* leaf chamber was provided by a CO_2 cylinder.

Photosynthesis was induced with a PPFD of 1,500 µmol(photon) m^{-2} s^{-1} and ambient CO_2 concentrations (C_a) of 400 µmol mol^{-1}. Once a steady state was reached (usually 20 min after clamping the leaf), data were recorded, and then a CO_2-response curve (P_N/C_i curve – P_N, net assimilation rate; C_i, substomatal CO_2 concentration) was performed. Gas exchange and chlorophyll fluorescence were first measured at 400 µmol mol^{-1}, then C_a was decreased stepwise until 50 µmol mol^{-1}, upon completion of measurements at low C_a, this was returned to 400 µmol mol^{-1} to restore the original P_N. Then, C_a was increased stepwise to complete the curve. The number of different C_a values used for the curves was 11, and the time lag between two consecutive measurements at different C_a was restricted to 2–4 min, so that each curve was completed in 30–50 min. Simultaneous quantum yield of PSII (Φ_{PSII}) was determined by measuring steady-state fluorescence (F_s) and maximum fluorescence using the multiphase flash method [a light-saturating pulse at the end of phase 1 was *ca*. 8,000 µmol(photon) m^{-2} s^{-1}, Loriaux *et al*. 2013]:

$$\Phi_{PSII} = F_m' - F_s/F_m' \quad (1)$$

The electron transport rate (ETR) was then calculated as

$$ETR = \Phi_{PSII} \times PPFD \times \alpha \times \beta \quad (2)$$

where α is leaf absorptance and β reflects the partitioning of absorbed quanta between PSI and PSII. Since no significant differences in α × β were observed between well-watered and drought plants (Flexas *et al.* 2009), as routinely assumed, α and β were set to be 0.84 (Björkman and Demmig 1987, Schreiber 2004) and 0.5 (von Caemmerer 2000), respectively. In addition, leakage of CO_2 into and out the leaf cuvette was determined with photosynthetically inactive leaves of each species enclosed in the leaf chamber (obtained by heating the leaves until no variable chlorophyll fluorescence was observed), and used to correct measured leaf fluxes (Flexas *et al*. 2007).

Leaf dark respiration (R_D) was determined by gas exchange (*Li-6400*) after plants were dark-adapted for more than half an hour in the evening.

Photosynthetic capacity parameters (V_{cmax} and J_{max}) were obtained by fitting P_N–C_c curves (C_c, chloroplastic CO_2 concentration) based upon the FvCB model (Farquhar *et al.* 1980, Sharkey 1985) modified by Harley *et al.* (1992) and Sharkey (2016). The key input constants in the model (*i.e.*, Michaelis–Menten constants, K_c and K_o; the CO_2-compensation point in the absence of mitochondrial respiration, Γ*) may vary across species. Given that it is challenging to directly measure or derive their actual values, we used the values from Bernacchi *et al.* (2002), which have been widely adopted in many studies (*e.g.*, Sharkey *et al*. 2007, Sharkey 2016, Wu *et al*. 2023, Xiong 2023).

Estimation of g_m by the variable *J* method: Mesophyll conductance (g_m) was estimated by the variable *J* method (Harley *et al*. 1992) as

$$g_m = \frac{P_N}{C_i - \dfrac{\Gamma^*\left[J_{flu} + 8\left(P_N + R_d\right)\right]}{J_{flu} - 4\left(P_N + R_d\right)}} \quad (3)$$

where P_N and C_i were taken from gas-exchange measurements at saturating light. The value of Γ* from Bernacchi *et al.* (2002) was used for estimating g_m from the variable *J* method. R_d is day respiration and was assumed to be 0.5 times the measured dark respiration (R_d = $R_D/2$) (Piel *et al.* 2002, Niinemets *et al.* 2005). To show the reliability of g_m estimation, we checked whether it met the criterion 10 > dC_c/dP_N > 50 for each data point generated, as suggested by Harley *et al.* (1992).

Note up to now, it is challenging to obtain accurate g_m, especially under water deficit conditions, using the current few approaches (*i.e.*, the variable *J* method, carbon isotope discrimination method, P_N–C_i curve fitting method). With the wide availability of advanced instruments for measuring gas exchange and chlorophyll fluorescence, the majority of studies of g_m adopted the variable *J* method (Gilbert *et al.* 2012). Since this study focused on the dynamics of photosynthetic characteristics during the progressive drought and subsequent recovery process instead of their respective absolute values, potential estimation bias (or error) induced by approaches should not affect our data analysis and conclusions. In addition, our study did not consider the potential effect of the alternative electron sink (*i.e.*, photorespiration, nitrate reduction, and the Mehler reaction) on the estimation of

g_m with the variable J method, which deserves further comprehensive investigation in cotton plants.

Quantitative limitation analyses on P_N: Quantitative stomatal limitation (SL), mesophyll limitation (MCL), and biochemical limitation (BL) were investigated respectively in the cotton leaves according to the method of Grassi and Magnani (2005) as modified by Flexas *et al.* (2009). The quantitative changes in light-saturated assimilation can be expressed in terms of parallel changes in stomatal and mesophyll conductance and in biochemical capacity as follows:

$$\frac{\mathrm{d}P_N}{P_N} = \mathrm{SL} + \mathrm{MCL} + \mathrm{BL} = l_{st} \times \frac{\mathrm{d}g_s}{g_s} + l_{mc} \times \frac{\mathrm{d}g_m}{g_m} + l_b \times \frac{\mathrm{d}V_{cmax}}{V_{cmax}} \tag{4}$$

$$l_{st} = \frac{\frac{g_{tot}}{g_s}\frac{\partial P_N}{\partial C_c}}{g_{tot} + \frac{\partial P_N}{\partial C_c}} \tag{5}$$

$$l_{mc} = \frac{\frac{g_{tot}}{g_m}\frac{\partial P_N}{\partial C_c}}{g_{tot} + \frac{\partial P_N}{\partial C_c}} \tag{6}$$

$$l_b = \frac{g_{tot}}{g_{tot} + \frac{\partial P_N}{\partial C_c}} \tag{7}$$

where g_{tot} is total conductance to CO_2 between the leaf surface and the sites of carboxylation ($1/g_{tot} = 1/g_s + 1/g_m$); l_{st}, l_{mc}, and l_b are the corresponding relative limitation ($0 < l_i < 1$, i = st, mc, b). Here, V_{cmax} can be replaced by ETR, which is tightly coupled with V_{cmax} (Galmés *et al.* 2007, Gallé *et al.* 2009). $\partial P_N/\partial C_c$ was calculated as the slope of P_N–C_c response curves over a C_c range of 50–100 µmol mol^{-1} (Tomás *et al.* 2013).

$$\frac{\mathrm{d}g_s}{g_s} = \left(g_s^{ref} - g_s\right) / g_s^{ref} \tag{8}$$

$$\frac{\mathrm{d}g_m}{g_m} = \left(g_m^{ref} - g_m\right) / g_m^{ref} \tag{9}$$

$$\frac{\mathrm{d}j}{\mathrm{ETR}} = \left(j_m^{ref} - \mathrm{ETR}\right) / j_m^{ref} \tag{10}$$

where g_s^{ref}, g_m^{ref} and j_m^{ref} are the reference values of stomatal and mesophyll conductance and of electron transport rate. For well-watered cotton, in order to assess the effects of leaf aging on photosynthesis, the first values of g_s, g_m, and ETR (before leaf aging) were used as a reference. Values for well-watered plants for each day were considered as the reference for the drought plants determined on the same day since P_N in well-watered cottons gradually decreased as cotton leaves aging. Therefore, the photosynthesis limitations calculated in drought cottons only reflect drought limitations and did not include the limitation from leaf aging. Whenever drought cotton plants had higher g_s, g_m, and J_{max} than well-watered plants, its corresponding limitation was set to zero (Flexas *et al.* 2009).

Statistical analysis: Data in figures are presented as the means ± standard error of at least three plants per treatment and species. A mixed analysis of variance (*ANOVA*) was used to compare how the photosynthetic parameters change across time (measurement day) between well-watered (WW) and drought and recovery (DR) treatments in upland and pima cottons (Table 1S, *supplement*). Interrelations between variables were investigated by nonlinear regression analysis. All analyses and figures were performed in *Python version 3.8*.

Results

Variation of gas-exchange parameters: The net CO_2 assimilation rate (P_N) of well-watered cotton plants progressively declined during the experiment, from 20.5 µmol(CO_2) m^{-2} s^{-1} at the beginning of the experiment (day 0) to 10.1 µmol(CO_2) m^{-2} s^{-1} by the end of the experiment (day 42) in upland cotton (Fig. 2*A*), and from 21.4 to 8.7 µmol(CO_2) m^{-2} s^{-1} in pima cotton (Fig. 2*B*), which were caused by leaf aging (more details in 'Discussion'). P_N declined with prolonged drought, reaching minimum values of around 10.5 µmol(CO_2) m^{-2} s^{-1} and 9.0 µmol(CO_2) m^{-2} s^{-1} by day 14 in upland and pima cotton, respectively. Upon rewatering, P_N almost completely returned to the well-watered level in about 3 d in pima cotton (Fig. 2*B*), but it tooked up to 7 d in upland cotton (Fig. 2*A*).

The observed variations in P_N were accompanied by similar variations in g_s, which, in drought upland and pima cotton plants, declined to 0.11 and to 0.06 mol(H_2O) m^{-2} s^{-1}, respectively (Table. 1, Fig. 2*C,D*). Mesophyll conductance to CO_2 (g_m) followed a somewhat different pattern (Fig. 2*E,F*). In upland cotton, g_m was similar to the value of well-watered treatment in the drought period, but significantly lower than that in the first 7 d after rewatering. In pima cotton, no significant differences of g_m were observed between well-watered and drought and recovery treatments during the whole experimental process.

The maximum carboxylation rate (V_{cmax}) and maximum electron transport rate (J_{max}) decreased in the process of drought imposition in upland and pima cotton (Fig. 3). The decline ranges of V_{cmax} and J_{max} during the drought acclimation relative to the well-watered treatment were smaller in pima cotton than those in upland cotton. V_{cmax} and J_{max} increased on the 3rd day after rewatering (the 21th of the whole experiment) in pima cotton but on the 5th day after rewatering (the 23rd of the whole experiment) in upland cotton. Relative to well-watered treatment, upland cotton had lower or similar V_{cmax} and J_{max} under drought and recovery treatment over the whole experiment, but in pima cotton, rewatering resulted in similar or higher V_{cmax} and J_{max} (Fig. 3).

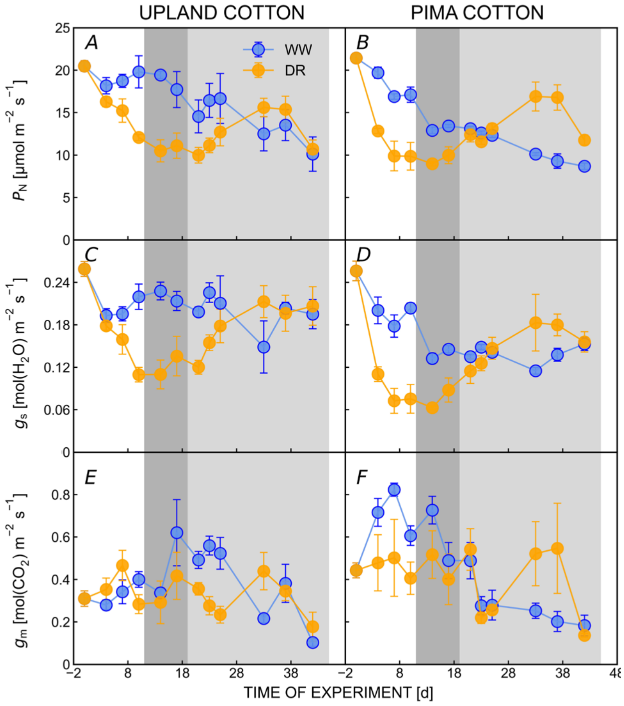

Fig. 2. Variation during the experiment of net CO$_2$ assimilation rates (P_N) (*A*,*B*), stomatal conductance (g_s) (*C*,*D*), and mesophyll conductance (g_m) (*E*,*F*) under well-watered (WW) and drought and recovery (DR) treatments in upland (*A*,*C*,*E*) and pima cotton (*B*,*D*,*F*). Means and errors of at least three plants per treatment and species are shown. The period of drought acclimation (from day eleven to nineteen) is indicated by *the dark grey area*, and the days under rewatering by *the pale grey area*.

The intrinsic water-use efficiency (WUE$_i$, *i.e.*, the ratio of P_N to g_s) does not appear to have any changes during the drought and recovery compared to well-watered treatment, except on the 7th day after rewatering (the 25th of the whole experiment) in upland cotton (Fig. 4*A*). In contrast, drought significantly enhanced WUE$_i$ in pima cotton, despite WUE$_i$ was reduced to the level of well-watered treatment after rewatering (Fig. 4*B*). To further explain why WUE$_i$ in upland and pima cotton had different responses to drought, we assessed the dynamic changes of V_{cmax}/g_s and g_m/g_s under drought and recovery, and their relationships with WUE$_i$. We found that compared with well-watered treatment, drought imposition increased V_{cmax}/g_s on the 10th day of the whole experiment in upland cotton, and on the 7th day in pima cotton (Fig. 4*C*,*D*). No effects of drought imposition on g_m/g_s were observed in upland and pima cotton, except on the 10th of the whole experiment in pima cotton. Rewatering decreased V_{cmax}/g_s and g_m/g_s to the level of well-watered treatments. Pooling all data under different treatments, we observed significant correlations between WUE$_i$ and V_{cmax}/g_s and g_m/g_s, respectively, in upland and pima cottons (Fig. 5).

To further analyze the overall effects of a drought cycle on the two cotton species, the average values of photosynthetic parameters during the whole experiment

Table 1. Mean values during the whole experiment and at the 14th day after imposing drought of net photosynthetic rate (P_N), stomatal conductance (g_s), mesophyll conductance (g_m), and maximum carboxylation rate (V_{cmax}) in upland and pima cotton under well-watered (WW) and drought and recovery (DR) treatments. Values are means ± SE. *Different letters* indicate significant differences at the 0.05 probability level.

	Treatments	P_N (mean) [µmol m^{-2} s^{-1}]	P_N (14th d) [µmol m^{-2} s^{-1}]	g_s (mean) [mol(H$_2$O) m^{-2} s^{-1}]	g_s (14th d) [mol(H$_2$O) m^{-2} s^{-1}]	g_m (mean) [mol(CO$_2$) m^{-2} s^{-1}]	g_m (14th d) [mol(CO$_2$) m^{-2} s^{-1}]	V_{cmax} (mean) [µmol m^{-2} s^{-1}]	V_{cmax} (14th d) [µmol m^{-2} s^{-1}]
Upland cotton	WW	16.91 ± 0.62^a	19.44 ± 0.62^a	0.21 ± 0.01^a	0.22 ± 0.01^a	0.37 ± 0.03^a	0.34 ± 0.02^a	93.32 ± 4.18^a	104.02 ± 12.76^a
	DR	13.95 ± 0.53^b	10.50 ± 1.30^b	0.17 ± 0.01^b	0.11 ± 0.02^b	0.33 ± 0.02^a	0.29 ± 0.10^a	76.69 ± 3.89^b	59.87 ± 2.69^b
Pima cotton	WW	13.62 ± 0.53^a	12.91 ± 0.37^a	0.16 ± 0.01^a	0.13 ± 0.01^a	0.43 ± 0.03^a	0.73 ± 0.07^a	82.14 ± 3.09^a	75.28 ± 3.69^a
	DR	13.00 ± 0.65^a	8.97 ± 0.24^b	0.13 ± 0.01^b	0.06 ± 0.00^b	0.42 ± 0.03^a	0.52 ± 0.11^a	84.83 ± 3.28^a	80.85 ± 3.74^a

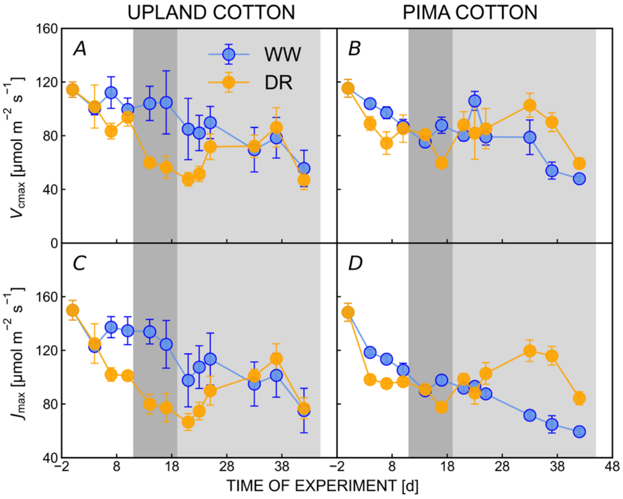

Fig. 3. Variation during the experiment of the maximum carboxylation rate (V_{cmax}) (*A*,*B*) and the maximum electron transfer rate (J_{max}) (*C*,*D*) under well-watered (WW) and drought and recovery (DR) treatments in upland (*A*,*C*) and pima cotton (*B*,*D*). Means and errors of at least three plants per treatment and species are shown. The period of drought acclimation (from day eleven to nineteen) is indicated by *the dark grey area*, and the days under rewatering by *the pale grey area.*

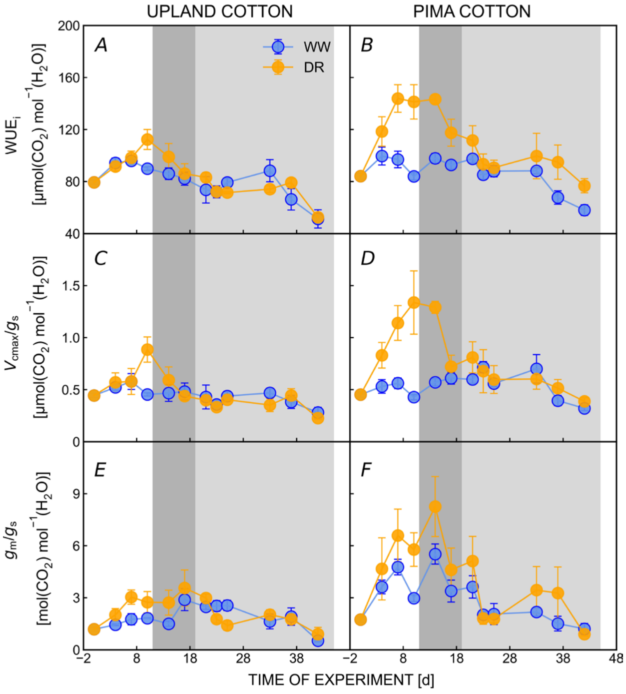

Fig. 4. Variation during the experiment of intrinsic water-use efficiency (WUE$_i$) (*A*,*B*), the ratio of the maximum carboxylation rate (V_{cmax}) to stomatal conductance (g_s) (V_{cmax}/g_s) (*C*,*D*), and the ratio of mesophyll conductance (g_m) to g_s (g_m/g_s) (*E*,*F*) under well-watered (WW) and drought and recovery (DR) treatments in upland (*A*,*C*,*E*) and pima cotton (*B*,*D*,*F*). Means and errors of at least three plants per treatment and species are shown. The period of drought acclimation (from day eleven to nineteen) is indicated by *the dark grey area*, and the days under rewatering by *the pale grey area*.

and the lowest values under drought were shown in Table 1. Upland cotton had a larger decrease in averaged P_N, g_s, and V_{cmax} under drought relative to pima cotton, indicative that the process of drought and rewatering had less effect on photosynthesis in pima cotton than in upland cotton.

Photosynthesis limitation: Over the experiment period (42 d), the photosynthesis of well-watered plants declined by 51 and 59% in upland and pima cotton, respectively, which was mostly caused by the decrease of biochemical and stomatal conductance (Fig. 6*A*,*C*). Compared to the young leaves at the beginning of the experiment, leaf aging increased MCL on the 25th day in upland cotton and on the 21th day in pima cotton and also increased BL on the 14th day for upland and pima cotton. With the aging of leaves, BL gradually dominated in both species, but MCL and SL could not be ignored.

For the drought-dependent photosynthesis limitation, SL increased sharply on the 7th day in upland cotton and on the 4th day in pima cotton and then continued to increase during the drought imposition (Fig. 6*B*,*D*); BL slowly increased at the beginning of drought imposition in upland and pima cotton; drought imposition increased MCL on the 10th day in upland cotton and the 4th day in pima cotton, but MCL was still lower dramatically than SL and BL. During the acclimation, SL decreased but MCL almost unchanged in upland and pima cotton; SL in pima cotton decreased faster than that in upland cotton; BL almost reached their maximum on the 14th and 17th day in upland and pima cotton, respectively; BL and SL dominated the limitation of photosynthesis in upland cotton while only SL dominated in pima cotton. During the recovery, the total limitation of photosynthesis rapidly decreased and then restored to the control level. Upland cotton had a higher photosynthesis limitation than pima cotton during the drought cycle.

Discussion

Leaf aging induced a progressive decline in photosynthesis in well-watered pima cotton

Net CO_2 assimilation rate (P_N) in well-watered upland cotton remained constant between the onset of the experiment and day 25th, thereafter progressively declined (Fig. 2*A*). Such decline was likely the symptom of leaf aging with increased BL, as reflected by V_{cmax} and J_{max} showing declining patterns similar to those of P_N, as observed in other species (*e.g.*, Flexas *et al.* 2009, Cano *et al.* 2014). In contrast, P_N declined progressively during the whole experiment for well-watered pima cotton (Fig. 2*B*), which is consistent with the apparently faster leaf senescence observed for this species in this experiment. Pima cotton has larger internodes on the stem than upland cotton, which causes the mature leaf (used for measurements in the present study) to go down from the functional to the nonfunctional leaf position more quickly as the new leaves continue to mature. The relatively rapid downward movement of the leaf might be due to the fact that pima cotton needs to reallocate nutrients to

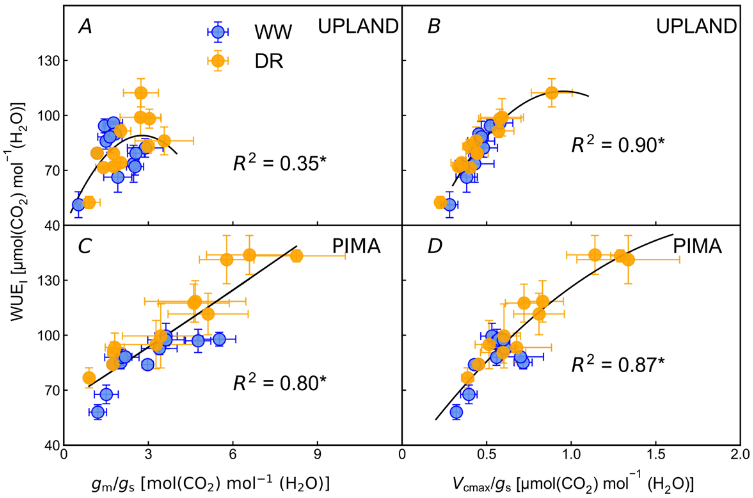

Fig. 5. The relationship between intrinsic water-use efficiency (WUE$_i$) and the ratio of mesophyll conductance (g_m) to stomatal conductance (g_s) (g_m/g_s) (*A*,*C*) and maximum carboxylation rate (V_{cmax}) to g_s (V_{cmax}/g_s) (*B*,*D*), respectively, by pooling the well-watered (WW) and drought and recovery (DR) data together in upland (*A*,*B*) and pima cotton (*C*,*D*). Means and errors of at least three plants per treatment and species are shown. *The solid lines* are the nonlinear regressions; * indicates significance at the 0.05 probability level.

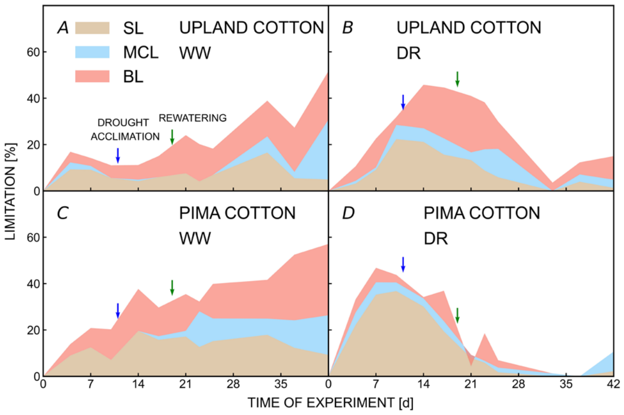

Fig. 6. Quantitative limitation of photosynthesis under well-watered (WW) (*A*,*C*), and drought and recovery (DR) (*B*,*D*) treatments in upland (*A*,*B*) and pima cotton (*C*,*D*). SL, MCL, and BL denote for stomatal, mesophyll, and biochemical limitations, respectively. *The blue arrows* indicate the beginning of the drought acclimation period; *the green arrow* the beginning of the recovery period.

the youngest leaves, flowers, or cotton bolls to complete its short life span under sufficient water resources. Previous studies in several woody species showed that with leaf aging, photosynthesis becomes limited by biochemistry rather than CO_2 diffusions (Grassi and Magnani 2005, Flexas *et al.* 2009). However, in the present study, in addition to the dominant BL, MCL and SL were also playing important roles in the aging-driven photosynthesis decline in both cotton species, possibly because leaf aging decreased leaf thickness and ratio of palisade tissue thickness to leaf thickness (Gao *et al.* 2012), changed the size and structures of chloroplast (Mulisch and Krupinska 2013) and stoma morphology, thereby resulting in decreased g_m and g_s during aging.

Surprisingly, the effects of leaf aging during the whole experiment on photosynthetic characteristics are similar (P_N) to or even larger (g_m, V_{cmax}, and J_{max}) than those caused by drought imposition in both cotton species. It is likely because drought can inhibit plants growth (Pace *et al.* 1999) and thus avoid rapid senescence of leaves. That is why the photosynthetic capacity parameters (V_{cmax} and J_{max}) could recover rapidly after rewatering, and were significantly higher than those under well-watered treatment, especially in pima cotton. This keeps us in mind that the position selection of measured leaf is an issue that must be considered but has been ignored by most researchers when evaluating the effects of drought on plants.

The maintenance of photosynthetic capacity under drought contributes to the rapid recovery of photosynthesis upon rewatering in pima cotton

Leaf photosynthetic responses to drought and rewatering have been analyzed in the upland and pima cotton. Numerous studies (Flexas *et al.* 2002, 2004, 2006, 2009; Galmés *et al.* 2007, Cano *et al.* 2013, 2014) have shown that the decline in P_N by drought was mainly due to the synergistic decrease of g_s and g_m. However, in this study, only the decreased g_s dominated the decline of P_N under drought in both cotton species (Fig. 2), while the role of V_{cmax} and J_{max} was also critical, especially in upland cotton (Fig. 3). Upon rewatering, photosynthetic characteristic parameters (*i.e.*, P_N, g_s, V_{cmax}, and J_{max}) could be recovered to or above well-watered values, which suggests that drought did not cause the irreparable damage to cotton photosynthesis. The recovery of CO_2 diffusion and biochemical characteristics can also be regarded as evidence of the high resilience of the photosynthetic apparatus under short-term drought conditions in both cotton species.

Compared to upland cotton, pima cotton showed more sensitivity to drought treatment, which was indicated by faster decreases in g_s. Many studies demonstrated that the decrease of g_s is the primary response of photosynthesis to drought (Faria *et al.* 1998, Chaves *et al.* 2002, 2003; Flexas *et al.* 2007), which could quickly minimize water loss under drought conditions. In contrast, V_{cmax} and J_{max} in pima cotton were less affected by drought relative to those in upland cotton. The maintenance of photosynthetic capacity in drought pima cotton could facilitate the recovery of photosynthesis once rehydrated when the stomata are allowed to open, which can be proved by the fact that pima cotton had a faster upregulation of P_N and g_s compared to upland cotton. This result may explain at least to some extent the often-observed phenomenon that stressed plants upon recovery grow faster than unstressed control plants. Overall, the regulation behavior of pima cotton on photosynthetic characteristics under drought and recovery seems to be in line with the water-regulation strategies of isohydric plants to a certain extent, *i.e.*, they effectively control the water loss of tissues through stomatal closure to avoid drought-induced hydraulic failure (David *et al.* 2007). Also, they may sustain photosynthetic capacity to achieve the goal of rapidly improving carbon acquisition and assimilation upon rewatering. Instead, upland cotton might be more like anisohydric plant since it has a more stable and stronger photosystems under drought to cope with mild or moderate drought (Yi *et al.* 2016b).

From the average values of cumulative photosynthetic parameters during the whole experiment (Table 1), ultimately the overall drought cycle had less effect on pima cotton than upland cotton. This is because the fast upregulation of photosynthetic characteristic parameters during recovery period mostly compensated for the loss caused by drought period in pima cotton. Therefore, studying the whole process of drought and recovery could provide more valuable guidance for improving photosynthetic productivity than just studying drought in an environment with frequent drought cycles.

The ratio of V_{cmax} to g_s dominates the changes of WUE_i in the whole process of drought and recovery

We observed prominent positive correlation of WUE_i with both V_{cmax}/g_s and g_m/g_s when all leaves were pooled together in upland and pima cotton (Fig. 5), demonstrating that WUE_i is regulated by both CO_2 diffusion and photosynthetic capacity. g_m and V_{cmax} are two critical photosynthetic characteristics affecting carbon acquisition and assimilation, independent of water loss (Han *et al.* 2016). Despite their maintenance would be capable of alleviating the decline of P_N and potentially WUE_i under drought, the sustainability of WUE_i seems to be only achieved when there are no parallel changes in g_s (Flexas *et al.* 2013). In our study, there was no obvious difference in g_m/g_s between drought and well-watered treatments in both cotton species, which indicates that g_m/g_s is not the dominant factor for the difference in response of WUE_i to drought among cotton species. Instead, under drought, the slower reduction of V_{cmax} than g_s contributes to the increase of WUE_i in pima cotton, while a nearly proportional decline in g_s and V_{cmax} leads to the consistency of WUE_i between drought and well-watered treatments in upland cotton. Meanwhile, compared to the well-watered treatment, the higher photosynthetic capacity parameters are conducive to the maintenance of WUE_i after rewatering in pima cotton. Thus, V_{cmax}, as well as g_s, dominate the changes of WUE_i during drought and recovery periods in both cotton species.

Conclusion: In summary, the leaves of pima cotton age faster than those of upland cotton under well-watered treatment, leading to a greater decrease of photosynthesis. Interestingly, drought can inhibit leaf aging of pima cotton, possibly because pima cotton needs to reallocate nutrients to the flowers and cotton bolls, rather than to develop new leaves under limited water resources. Under drought, upland cotton has a more stable photosystem than pima cotton, proved by a slower decrease of photosynthesis. However, an overall drought cycle has smaller effect on photosynthesis of pima cotton than that of upland cotton, which is because of the maintenance of V_{cmax} and its coordination with g_s during drought and recovery. Meanwhile, the difference in response of WUE_i to drought among cotton species can be attributed to different V_{cmax}/g_s. Furthermore, studying the whole process of drought and recovery has more valuable guiding significance for improving photosynthetic productivity than just studying drought in changing environmental conditions. Note that all data analyses were based on the assumption that well-watered and drought plants age at the same rate. This assumption deserves separate investigation in the future.

References

Bernacchi C.J., Portis A.R., Nakano H. *et al.*: Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis *in vivo*. – Plant Physiol. **130**: 1992-1998, 2002.

Björkman O., Demmig B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Blum A.: Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. – Field Crop. Res. **112**: 119-123, 2009.

Cano F.J., López R., Warren C.R.: Implications of the mesophyll conductance to CO_2 for photosynthesis and water-use efficiency during long-term water stress and recovery in two contrasting *Eucalyptus* species. – Plant Cell Environ. **37**: 2470-2490, 2014.

Cano F.J., Sánchez-Gómez D., Rodríguez-Calcerrada J. *et al.*: Effects of drought on mesophyll conductance and photosynthetic limitations at different tree canopy layers. – Plant Cell Environ. **36**: 1961-1980, 2013.

Chaves M.M., Flexas J., Pinheiro C.: Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. – Ann. Bot.-London **103**: 551-560, 2009.

Chaves M.M., Maroco J.P., Pereira J.S.: Understanding plant responses to drought – from genes to the whole plant. – Funct. Plant Biol. **30**: 239-264, 2003.

Chaves M.M., Pereira J.S., Maroco J. *et al.*: How plants cope with water stress in the field? Photosynthesis and growth. – Ann. Bot.-London **89**: 907-916, 2002.

Costa J.M., Ortuño M.F., Chaves M.M.: Deficit irrigation as a strategy to save water: physiology and potential application to horticulture. – J. Integr. Plant Biol. **49**: 1421-1434, 2007.

David T.S., Henriques M.O., Kurz-Besson C. *et al.*: Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. – Tree Physiol. **27**: 793-803, 2007.

Faria T., Silvério D., Breia E. *et al.*: Differences in the response of carbon assimilation to summer stress (water deficits, high light and temperature) in four Mediterranean tree species. – Physiol. Plantarum **102**: 419-428, 1998.

Farquhar G.D., von Caemmerer S., Berry J.A.: A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. – Planta **149**: 78-90, 1980.

Flexas J., Barbour M.M., Brendel O. *et al*.: Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. – Plant Sci. **193-194**: 70-84, 2012a.

Flexas J., Barón M., Bota J. *et al*.: Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandieri*·× *V. rupestris*). – J. Exp. Bot. **60**: 2361-2377, 2009.

Flexas J., Bota J., Escalona J.M. *et al*.: Effects of drought on photosynthesis in grapevine under field conditions: an evaluation of stomatal and mesophyll limitations. – Funct. Plant Biol. **29**: 461-471, 2002.

Flexas J., Bota J., Loreto F. *et al*.: Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. – Plant Biol. **6**: 269-279, 2004.

Flexas J., Cano F.J., Carriquí M. *et al*.: CO_2 diffusion inside photosynthetic organs. – In: Adams III W.W., Terashima I. (ed.): The Leaf: A Platform for Performing Photosynthesis. Vol. 44. Pp. 163-208. Springer, Cham 2018.

Flexas J., Díaz-Espejo A., Conesa M.A. *et al*.: Mesophyll conductance to CO_2 and Rubisco as targets for improving intrinsic water use efficiency in C_3 plants. – Plant Cell Environ. **39**: 965-982, 2016.

Flexas J., Díaz-Espejo A., Galmés J. *et al*.: Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. – Plant Cell Environ. **30**: 1284-1298, 2007.

Flexas J., Gallé A., Galmés J. *et al*.: The response of photosynthesis to soil water stress. – In: Aroca R. (ed.): Plant Responses to Drought Stress. Pp. 129-144. Springer, Berlin-Heidelberg 2012b.

Flexas J., Galmés J., Gallé A. *et al*.: Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. – Aust. J. Grape Wine Res. **16**: 106-121, 2010.

Flexas J., Medrano H.: Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. – Ann. Bot.-London **89**: 183-189, 2002.

Flexas J., Niinemets Ü., Gallé A. *et al*.: Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. – Photosynth. Res. **117**: 45-59, 2013.

Flexas J., Ribas-Carbó M., Bota J. *et al*.: Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. – New Phytol. **172**: 73-82, 2006.

Gago J., Douthe C., Florez-Sarasa I. *et al*.: Opportunities for improving leaf water use efficiency under climate change conditions. – Plant Sci. **226**: 108-119, 2014.

Gallé A., Florez-Sarasa I., El Aououad H., Flexas J.: The Mediterranean evergreen *Quercus ilex* and the semi-deciduous *Cistus albidus* differ in their leaf gas exchange regulation and acclimation to repeated drought and re-watering cycles. – J. Exp. Bot. **62**: 5207-5216, 2011.

Gallé A., Florez-Sarasa L., Tomas M. *et al*.: The role of mesophyll conductance during water stress and recovery in tobacco (*Nicotiana sylvestris*): acclimation or limitation? – J. Exp. Bot. **60**: 2379-2390, 2009.

Galmés J., Medrano H., Flexas J.: Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. – New Phytol. **175**: 81-93, 2007.

Galmés J., Ochogavía J.M., Gago J. *et al*.: Leaf responses to drought stress in Mediterranean accessions of *Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameter. – Plant Cell Environ. **36**: 920-935, 2013.

Gao X.-L., Gao J.-F., Feng B.-L. *et al*.: [Anatomical structure of leaf in different mung bean varieties.] – Acta Agron. Sin. **38**: 181-185, 2012. [In Chinese]

Gilbert M.E., Pou A., Zwieniecki M.A., Holbrook N.M.: On measuring the response of mesophyll conductance to carbon dioxide with the variable *J* method. – J. Exp. Bot. **63**: 413-425, 2012.

Grassi G., Magnani F.: Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. – Plant Cell Environ. **28**: 834-849, 2005.

Han J., Lei Z., Zhang Y. *et al*.: Drought-introduced variability of mesophyll conductance in *Gossypium* and its relationship with leaf anatomy. – Physiol. Plantarum **166**: 873-887, 2018.

Han J.-M., Meng H.-F., Wang S.-Y. *et al*.: Variability of mesophyll conductance and its relationship with water use efficiency in cotton leaves under drought pretreatment. – J. Plant Physiol. **194**: 61-71, 2016.

Harley P.C., Loreto F., Di Marco G., Sharkey T.D.: Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by the analysis of the response of photosynthesis to CO_2. – Plant Physiol. **98**: 1429-1436, 1992.

Hoagland D.R., Arnon D.I.: The Water-Culture Method for Growing Plants without Soil. Circular 347. Pp. 32. College of Agriculture, University of California, Berkeley 1950.

Lawson T., Blatt M.R.: Stomatal size, speed and responsiveness impact on photosynthesis and water use efficiency. – Plant Physiol. **164**: 1556-1570, 2014.

Long S.P., Marshall-Colon A., Zhu X.-G.: Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. – Cell **161**: 56-66, 2015.

Loriaux S.D., Avenson T.J., Welles J.M. *et al*.: Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity. – Plant Cell Physiol. **36**: 1755-1770, 2013.

McAusland L., Vialet-Chabrand S., Davey P. *et al*.: Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. – New Phytol. **211**: 1209-1220, 2016.

Morison J.I.L., Baker N.R., Mullineaux P.M., Davies W.J.: Improving water use in crop production. – Philos. T. Roy. Soc. B **363**: 639-658, 2008.

Mulisch M., Krupinska K.: Ultrastructural analyses of senescence associated dismantling of chloroplasts revisited. – In: Biswal B., Krupinska K., Biswal U. (ed.): Plastid Development in Leaves during Growth and Senescence. Advances in Photosynthesis and Respiration (Including Bioenergy and Related Processes). Vol. 36. Pp. 307-335. Springer, Dordrecht 2013.

Nadal M., Flexas J.: Variation in photosynthetic characteristics with growth form in a water-limited scenario: Implications for assimilation rates and water use efficiency in crops. – Agr. Water Manage. **216**: 457-472, 2019.

Niinemets Ü., Cescatti A., Rodeghiero M., Tosens T.: Leaf internal diffusion conductance limits photosynthesis more strongly in older leaves of Mediterranean evergreen broad-leaved species. – Plant Cell Environ. **28**: 1552-1566, 2005.

Pace P.F., Cralle H.T., El-Halawany S.H.M. *et al*.: Drought-induced changes in shoot and root growth of young cotton plants. – J. Cotton Sci. **3**: 183-187, 1999.

Piel C., Frak E., Le Roux X., Genty B.: Effect of local irradiance on CO_2 transfer conductance of mesophyll in walnut. – J. Exp. Bot. **53**: 2423-2430, 2002.

Schreiber U.: Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. – In: Papageorgiou G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration. Pp. 279-319. Springer, Dordrecht 2004.

Schwalm C.R., Anderegg W.R.L., Michalak A.M. *et al.*: Global patterns of drought recovery. – Nature **548**: 202-205, 2017.

Sharkey T.D.: O_2-insensitive photosynthesis in C_3 plants 1: its occurrence and a possible explanation. – Plant Physiol. **78**: 71-75, 1985.

Sharkey T.D.: What gas exchange data can tell us about photosynthesis. – Plant Cell Environ. **39**: 1161-1163, 2016.

Sharkey T.D., Bernacchi C.J., Farquhar G.D., Singsaas E.L.: Fitting photosynthetic carbon dioxide response curves for C_3 leaves. – Plant Cell Environ. **30**: 1035-1040, 2007.

Sinclair T.R., Rufty T.W.: Nitrogen and water resources commonly limit crop yield increases, not necessarily plant genetics. – Glob. Food Secur. **1**: 94-98, 2012.

Singh S.K., Reddy K.R.: Regulation of photosynthesis, fluorescence, stomatal conductance and water-use efficiency of cowpea (*Vigna unguiculata* [L.] Walp.) under drought. – J. Photoch. Photobio. B **105**: 40-50, 2011.

Thimmanaik S., Kumar S.G., Kumari G.J. *et al.*: Photosynthesis and the enzymes of photosynthetic carbon reduction cycle in mulberry during water stress and recovery. – Photosynthetica **40**: 233-236, 2002.

Tomás M., Flexas J., Copolovici L. *et al.*: Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: quantitative limitations and scaling up by models. – J. Exp. Bot. **64**: 2269-2281, 2013.

Vadez V., Kholova J., Medina S. *et al.*: Transpiration efficiency: new insights into an old story. – J. Exp. Bot. **65**: 6141-6153, 2014.

von Caemmerer S.: Biochemical Models of Leaf Photosynthesis. Pp. 165. CSIRO Publishing, Collingwood 2000.

Wu A., Brider J., Busch F.A. *et al.*: A cross-scale analysis to understand and quantify the effects of photosynthetic enhancement on crop growth and yield across environments. – Plant Cell Environ. **46**: 23-44, 2023.

Xiong D.: Leaf anatomy does not explain the large variability of mesophyll conductance across C_3 crop species. – Plant J. **113**: 1035-1048, 2023.

Yi X.-P., Zhang Y.-L., Yao H.-S. *et al.*: Different strategies of acclimation of photosynthesis, electron transport and antioxidative activity in leaves of two cotton species to water deficit. – Funct. Plant Biol. **43**: 448-460, 2016a.

Yi X.-P., Zhang Y.-L., Yao H.-S. *et al.*: Rapid recovery of photosynthetic rate following soil water deficit and re-watering in cotton plants (*Gossypium herbaceum* L.) is related to the stability of the photosystems. – J. Plant Physiol. **194**: 23-34, 2016b.

Zhou S., Duursma R.A., Medlyn B.E. *et al.*: How should we model plant responses to drought? An analysis of stomatal and non-stomatal responses to water stress. – Agr. Forest Meteorol. **182-183**: 204-214, 2013.

Zhou S., Medlyn B., Santiago S. *et al.*: Short-term water stress impacts on stomatal, mesophyll, and biochemical limitations to photosynthesis differ consistently among tree species from contrasting climates. – Tree Physiol. **34**: 1035-1046, 2014.

Zhu K., Yuan F.H., Wang A.Z. *et al.*: Stomatal, mesophyll and biochemical limitations to soil drought and rewatering in relation to intrinsic water-use efficiency in Manchurian ash and Mongolian oak. – Photosynthetica **59**: 49-60, 2021.