Photosynthetic and chlorophyll fluorescence regulation of upland cotton (*Gossypium hirsutum* L.) under drought conditions

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Abstract

To investigate physiological characteristics of photosynthesis at different stages of growth, the transgenic cultivar Lumianyan28 was treated under two conditions with relative water content of < 60% (drought treatment) and 70-80% (well watered control), respectively. Results obtained from leaves of main stem showed that drought decreased the transpiration efficiency and inner transpiration efficiency of functional leaves which refers to the forth main stem leave from top before tip pruning, and thereafter, refers to the third one or the second one in the later period. It can be inferred that plants were most sensitive to soil drought at the initial bloom stage. Due to the reduction of thylakoid stacking, the content of chlorophyll a/b and net photosynthetic rate of the water-stressed plants were significantly lower than those of the well-watered control plants at the initial bloom stage. The transpiration rate of the functional leaves under drought treatment was remarkably higher than those of control plants at the bud stage, showing the relative significance of transpiration. The photosynthetic electron transport rate (ETR) of the main stem leaves correlated with the net photosynthetic rate under drought treatment (correlation coefficient=0.907*). However, such a correlation was not detected in the well-watered leaves. These results suggested that regulating photosynthetic system at crucial stages was the defense response of cotton plant to drought. However, this ability was very limited and progressively reduced along with prolonged drought.

Keywords: electronic transmission rate, fluorescence, functional leaves, photosynthesis, transpiration rate.

Abbreviations: Pn-Net photosynthetic rate; Ci-intercellular CO₂ concentration; GPD -leaf stomatal conductance; Tr-transpiration rate; Fo-Initial fluorescence; Fv/Fm-maximal photochemical efficiency; ΦPSII-Quantum yield; ETR-electronic transport rate; qP-photochemical quenching; NPQ-Non-photochemical quenching.

Introduction

Drought, the main limiting factor for plant growth and yield, has increasingly influenced crop production with the rise in global climate changes (Elisabeth et al., 2009). Cotton, one of the main crops in the Huang-Huai-Hai Plain where the annual precipitation is less than ideal, is prone to drought stress. Soil water deficits often reduce plant growth partly by reducing photosynthesis and photosynthetic mechanism has been one hot spot in crop leaves subjected to drought conditions. Non-stomatal factors and stomatal factors played different roles at the regulation of photosynthetic rate in different drought conditions (Singh and Reddy, 2011 ). Some have shown that stomatal closure and increased mesophyll resistance frequently play a dominant role in decreased CO₂ assimilation during drought stress (Cornic, 2000; Flexas et al., 2002; De Souza et al., 2005). Stomatal closure and reduction of leaf internalCO₂ concentration result mainly in decreased photosynthetic rates under mild or moderate water stress (Cornic, 2000; Flexas et al., 2004). Ennahl and Earl (2005) discussed limitation factors of cotton leaves were increased under severe water stress in a greenhouse, and re-wetting of severely stressed plants completely reversed the diffusive limitation (CO₂ concentration in the chloroplast returned to control levels). However, re-watering failed to stop the falling of the leaf net photosynthetic carbon assimilation due to prolonged chloroplast-level inhibition. Under field and laboratory conditions, more insight has been gained into the stomatal and/or mesophyll mechanisms involved in the modulation of photosynthesis, flowering time and leave architectural plasticity under conditions of limited water availability (Van Heerden et al., 2007; Karkanis et al., 2011). Another study has shown that under drought during the flowering and boll-setting periods, photosynthetic indexes apparently decrease but the photosynthetic pigment content increase (Liu et al., 2008). Also, there are researches showed drought induced a decrease in light absorbed by the PSII antennae, but enhanced electron transport flux for light energy utilization (Zhang et al., 2011). The dynamic changes in chlorophyll fluorescence are a direct reflection of photosynthesis in crops (Maxwell, 2000). It is postulated that a transient increase in chlorophyll a fluorescence is an indicator of the primary reactions of photosynthesis (Las’ar, 2006; Zhu et al., 2005). Fluorescence parameters are a good reflection of photosynthesis and can be used to analyze the impact of stress on photosynthesis quickly, precisely, and non-destructively (Fracheboud and Leipner, 2003; Longenberger et al., 2009). Previous studies related to physiological characterization of photosynthesis under drought have focused on short-time drought or drought at some specific growth periods. However, changes in and relationship between photosynthesis and fluorescence parameters in cotton in a continuing field drought environment during all stages of the
growth process have not been studied in detail. In order to provide a theoretical basis for the implementing strategies of water-saving irrigation and to improve photosynthetic function in breeding drought-resistant cultivars, we thus systematically investigated the dynamic changes of photosynthesis and fluorescence parameters of cotton plants.

Results and discussion

Chlorophyll content and Chlorophyll a/b

The variation of chlorophyll contents was characterized by a single peak for plants in either the drought or well-watered control during the entire growth period (Fig. 1). For the drought treatment, the value peaked on July 7 (the initial bloom stage), and it was significantly higher than that for the control treatment \( (F = 554.685^{**}) \). The difference is likely due to a reduction in leaf water content. For the control treatment leaves, the peak appeared on August 3 (boll stage) and it remained significantly higher \( (F = 648.010^{**}) \), thereafter \( (F = 71.930^{*}, \text{August 16}; F = 56.733^{*}, \text{September 1}) \). The chlorophyll content of drought-stressed leaves changed over time and decreased earlier than that of control plants, providing the condition of premature. In the early stages of growth, no obvious differences in the chlorophyll a/b values were seen between the two treatments (Fig. 2). However, the chlorophyll a/b value was significantly lower \( (F = 156.626^{**}) \) than control leaves’ on July 7 (the initial flowering stage). These results showed that the stacking of the thylakoids was weakened, and the light harvesting competence and the photosynthetic capability of the chloroplasts deteriorated. The chlorophyll a/b values of the drought-stressed leaves were all significantly lower than those of control, except on August 3 when it was significantly higher \( (F = 23.526^{*}) \), showing the extent of thylakoid stacking was reduced in drought-stressed which resulted in a decreased photosynthesis.

Photosynthesis

\( Pn \) at the seedling stage (June 4) was significantly higher \( (F=7.461^{*}, \text{Fig.3}) \) for the drought-stressed leaves than that for the control leaves. This is likely because a higher chlorophyll content and higher intercellular \( \text{CO}_2 \) concentration existed in the drought-stressed leaves, and they resisted early drought stress by activating plant defenses over a brief time span. At other stages, however, the \( Pn \) in the water-stressed leaves was significantly lower than those in the control leaves. It was suggested that part of the photosynthesis was controlled by non-stomatal factors (Escalona et al., 1999), namely, the photosynthetic activity of mesophyll cells. It is well known that extreme changes in some environmental factors will cause a decrease in leaf stomatal conductance \( (\text{Cond}) \), ribulose diphosphate carboxylase, and phosphoenolpyruvate carboxylase activity and in the \( \text{CO}_2 \) assimilation ability. These changes are accompanied by decrease in \( Pn \) (Farquhar and Sharkey, 1982). Two peaks appeared in the \( \text{Cond} \) curve under

**Table 1.** Pearson correlation between the \( Pn \) and \( \text{ETR} \) of functional leaves under drought and control treatments

<table>
<thead>
<tr>
<th>items</th>
<th>( Pn )-Drought</th>
<th>( \text{ETR} )-Drought</th>
<th>( Pn )-Control</th>
<th>( \text{ETR} )-Control</th>
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<tr>
<td>( Pn )-Drought</td>
<td>1.000</td>
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<tr>
<td>( \text{ETR} )-Drought</td>
<td>0.907*</td>
<td>1.000</td>
<td>0.792</td>
<td>0.335</td>
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<tr>
<td>( Pn )-Control</td>
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<td>0.487</td>
<td>1.000</td>
<td>--</td>
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<tr>
<td>( \text{ETR} )-Control</td>
<td>0.627</td>
<td>0.335</td>
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<td>1.000</td>
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*Significant at the 0.05 probability level. \( Pn \)-Drought, \( Pn \)-Control: Net photosynthetic rate of cotton under drought and control treatment, respectively; \( \text{ETR} \)-Drought, \( \text{ETR} \)-Control: Electronic transport rate of cotton drought and control treatment, respectively.

Fig. 1. the chlorophyll content of cotton functional leaves under drought treatment. ** indicates highly significantly different \( (P \leq 0.01) \) and * presents significantly different \( (P \leq 0.05) \).

Fig. 2. the chlorophyll a/b of cotton functional leaves under drought treatment. ** indicates highly significantly different \( (P \leq 0.01) \) and * presents significantly different \( (P \leq 0.05) \).

Fig. 3. \( Pn \) of cotton functional leaves under drought treatment. ** indicates highly significantly different \( (P \leq 0.01) \) and * presents significantly different \( (P \leq 0.05) \).
drought treatment, but only one peak was detected under control condition (Fig. 4). At the initial bloom stage, July 7, the maximum difference appeared between the two treatments, with the normal leaf stomatal conductance significantly higher for control leaves than for those of the drought-stressed leaves (F = 20.065*). This indicated that cotton, which is sensitive to water-stress especially in the initial bloom stage, would close its stomata once experiencing drought in order to decrease water evaporation. After the boll stage (August 3), the value under drought treatment was significantly lower, possibly because of premature senescence resulting from the shortened growth phase of cotton plants due to drought stress. Fig. 5 shows that, except the three stages (June 4, July 7, and September 1, respectively) Ci of main stem leaves under the drought treatment was significantly higher than those of control. These results indicated that, under drought the assimilation of CO₂ in the main stem leaves was reduced, the activity of photosynthetic enzyme was affected and Ci increased. Stomatal conductance is the main mechanism of regulating transpiration (Kramer, 1983; Isoda and Wang, 2002; Wang et al., 2004). Tr of cotton leaves under the two treatments presented an odd peak curve (Fig. 6): ascending first and then descending. At the bud stage, Tr of drought-stressed leaves was significantly higher (F = 9.695*, June 23) than that of control leaves, reflecting the resistance of cotton to early drought stress. The peak values of the Tr under both of the two treatments appeared at the initial flowering stage, but were significantly different (F = 24.927**, July 7). Hereafter, Tr under drought condition was all lower than that of control, and the differences were noted at the final stages of plant growth (F = 95.615**, August 16; F = 18.043*, September 1). These results could be related to the possibilities that the stomata became increasingly resistant to diffusion and/or because of senescence.

Transpiration efficiency and inner leaf transpiration efficiency

The transpiration efficiency of the leaves (Tel) was measured using the Pn/Tr ratio. Fig. 7 showed that the Tel of the drought-stressed leaves was lower than that of the control leaves, and significant differences were seen at the bud stage and the blooming-bolling stages (F = 42.588**, June 27; F = 11.787*, July 20; F = 107.831**, August 3; F = 176.137**, August 16). The lowest Tel values for the two treatments were seen during the initial flowering stage (July 7), which was related to the greater water loss that caused a higher Tr in the leaves. The maximum inner Tel for both of the treatments appeared on June 4 and it decreased thereafter (Fig.8). The inner Tel values under drought treatment was significantly lower than that of the control (F = 18.298**, June 23; F = 6.753*, July 20; F = 203.532**, August 16). However, it was significantly higher than that of the control leaves (F = 8.396*) on July 7 (the initial flowering stage). These results could be explained by the lower stomatal conductance that probably resulted from the partial closure of stomata under drought stress. It should be noted here that Tel was a physiological yardstick representing water-use efficiency at the leaf level (Blum, 2009), and we can evaluate stress degree of cotton according to changing trend of Tel during all the growth stage. But effective use of water should be taken into account under limited water condition as a major target for yield improvement.

Initial fluorescence and maximal photochemical efficiency

Fig. 9 shows that the Fo of drought-stressed leaves from main-stem was higher than that of the control leaves for almost the entire growth period. Significantly difference appeared at the initial bloom stage (F = 27.321**) when the leaves suffered from severe drought stress, and the PSII reaction center structure was severely damaged. Thereafter, the Fo decreased under both of the treatments although significant difference was not detected. On August 16 (the terminal stage), the control leaves showed a higher value, indicating that the chlorophyll system was seriously damaged. Fv/Fm decreased during later stages of growth under water stress and no significant differences existed in prometaphase (Fig. 10). However, the Fv/Fm value under the drought treatment was lower than that of the control on July 7 (the initial bloom stage) (F = 9.754*) when the cotton plants were subjected to extreme drought.
Quantum yield and electronic transport sufficiency

Photosynthetic electron transportation is promoted during the onset of drought stress in cotton due to the increased efficiency of the open PSII reaction centers (Massacci et al., 2008). \( \Phi_{\text{PSII}} \) was significantly lower in the drought-stressed leaves than in the control leaves since July 7 (Fig. 11) when the PSII non-cycle electron transport efficiency or light energy capture efficiency decreased significantly. Prior to that time, there were no obvious differences. ETR, representing the apparent quantum yield, was not significantly different in plants between the two different treatments before July 20 (blooming period), possibly because of the photosynthetic regulation in the plant itself (Fig. 12). At the initial bloom stage (F = 45.626**) and thereafter, however, the ETR under the drought treatment was significantly lower, indicating that drought stress influenced the photosynthetic electron transport mainly in the mid and late growth stages.

Photochemical quenching and non-photochemical quenching

The value of \( q_P \) in the drought-stressed leaves was significantly higher (F = 24.456**) and then decreased slightly (Fig. 13) at the bud stage (June 23), indicating that soil drought increased the transportation energy of photosynthetic electronic so as to hasten the plant growth to enter the reproductive stage. However, \( q_P \) of the control leaves did not show an obvious increase at the same time period, and no significant difference existed between the two treatments at other periods. NPQ of the drought-stressed leaves was significantly lower than that of the control leaves at the initial bloom stage (July 7, Fig. 14). This was a stage when the cotton was sensitive to drought stress and the photo-protection capability was very weak. The NPQ of the drought-stressed cotton on August 16, at the boll opening stage was significantly higher than that of the control (F = 339.856**), which can be explained by the asynchronous cotton growth and the great disparity in the structural function of leaves between the two treatments. No obvious differences existed between the two treatments at other growth stages.

Correlation analysis of the \( P_n \) and ETR

Table 1 shows that \( P_n \) of leaves (\( P_n \)-Dought) was significantly positively correlated with the ETR under drought treatment (ETR-Dought) (n=6, r = 0.907*), and the result coincide to the previous research (Xu et al., 2007). However, no significant correlation, as indicated by Pearson’s correlation coefficients, was found between \( P_n \) and ETR under control treatment (\( P_n \)-control and ETR-control). These results indicated that drought affected the electronic transportation and photosynthesis of cotton main stem leaves, which in turn affected the development and yield of plants. Moreover, chlorophyll fluorescence was also correlated to genotypes and drought management treatments (Patil et al., 2011). Next, combination of factors such as physiology variation and photosynthetic response gene expression should be considered in order to deeply understand the intricate adaptive mechanism of cotton plants under drought condition.

Materials and methods

Experimental design

The experiment was carried out in rainproof shelters (arc-shaped steel prop covered with a plastic film) installed to control RWC of soil (Kang et al., 2000). And this installation located at the Teaching Experiment Base (38°38’N, 115°E) at Hebei Agricultural University in 2008 and 2009. Two different water treatments were set up: (1) drought and (2) control. For the drought treatment, plants were grown in rainproof installations controlled by using an electrically operated valve to coat or open the plastic film so as to avoid natural rainfall and achieve artificial water control. Meanwhile, waterproof membrane was set with 80cm soil depth to avoid horizontal flow of groundwater between treatments. Plants within rainproof were ventilated to keep similar temperature between two different treatments. The relative water content (RWC), maintained at 50% ± 5%, was monitored using the oven drying method in the 0–80 cm soil layer at regular growth stages. For control plants, the RWC in the 0–80 cm soil layer was
maintained at 75% ± 5% by regular irrigation throughout the experiment. Three replicates were used for each of the two treatments, with plot size of 73.5 m², a density of 45,000 plants per ha, and a paired row space of 80 cm in a wide row and 70 cm in a narrow row. The soil type was loamy. Luminayan28, a new variety of conventional genetic modified cotton carried Bt CryIA grown extensively in the Huanghe valley, was used as the experimental crop. Seeds were grown directly into holes of ditch. Fertilizers (organic fertilizer (2250 kg/hm²), potassium chloride (225 kg/hm²), diammonium phosphate (375 kg/hm²), and urea (375 kg/hm²)) were applied during the preparation of the experimental plots. Except for the water treatments, the experiments were managed with practices used for high-yielding crops in the field.

Physiological measurements and sampling

The representative main stem leaves were packed in an ice box between 7 and 8 am in the morning and taken to the laboratory for chlorophyll content analysis. Three replicates, each consisting of 0.1 g leaves, were allocated into 3 test tubes. The samples were dissolved in 10 mL of 95% ethanol for 24 h. The supernatant obtained was used to assay the chlorophyll content using Colorimetric Determination with a U-2001 spectrophotometer (HITACHI) at 665 nm, 649 nm, and 470 nm colorimetric wavelengths (Zhao, 2000). Fully unfolded leaves (functional leaves) were selected and used to measure net photosynthesis rate, stomatal conductance, intercellular CO₂ concentration, transpiration rate by using the Li-6400 portable photosynthesis system as the mode of open flow gas exchange system. The process was repeated 5 times for each sample. Chlorophyll fluorescence parameters of the same functional leaves were measured using the FMS2 fluorometer (Hansatech) concurrently with measuring the photosynthesis rate. The measurements were repeated 5 times and the mean was taken for each sample. The procedure used was as follows: (1) select the appropriate position of the blade, (2) clamp using a clip, and (3) measure stable fluorescence (Fs) under actual growth irradiance after vertical sunlight irradiation for 5 min. Maximal fluorescence was subsequently assayed in light adaptation, and strong saturated pulse light (4,000 µmol m⁻² s⁻¹, pulse time, 0.7 s) was supplied. After 20 min of dark adaptation, original fluorescence was measured using a weak light intensity; subsequently, strong saturated light was provided and maximum fluorescence was measured.

Data analysis

All data were statistically analysed by using a single factor random block design with three replicates according to Excel 2003, DPS v2000, and SPSS17.0. The Duncan’s new Multiple Range (DMR) test at 5% probability level was used to test the differences among mean values. Significant differences had been labeled on the basis of DMR.
Conclusions

Cotton plants showed some protection ability at the early drought stage of water stress, although the Tel and inner Tel of cotton functional leaves were both decreased. The initial flowering stage is a crucial time for cotton growth when water requirements increase for chlorophyll content increasing, chlorophyll a/b values decreased significantly. Tr, as a chief driver of passive absorption of water, showed the effects of drought stress occurred relatively early. Moreover, a notable correlation existed between ETR and Ph of cotton subjected to soil drought stress throughout the growing period. Thus, there would appear to be the potential to influence cotton yield under drought stress.

Acknowledgments

We are grateful to Professor Kai Xiao, Mengyu Liu, Baodi Dong, Yunzhou Qiao and Chanhai Shi for their technical assistance. This research was financially supported by NSFC (Grant No. 30771267), Natural Science Foundation of Hebei Province (Nos.C2008000250, C2007000444, C2012503003, C2011503003). National High-tech R&D Program (863 Program) (No. 2008AAA102113), and National Technology Support Project (2008BAD95B13-04).

References

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


