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基于快速叶绿素荧光参数的不同基因型棉花叶片衰老研究

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The rapid chlorophyll a fluorescence characteristics of different cotton genotypes reflect differences in leaf senescence*

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Abstract: The light reactions of photosystem Ⅱ (PSⅡ) is greatly associated with the photosynthetic capacity. In order to capture more detailed information describing the status of PSⅡ during leaf senescence and rapidly screen cotton (*Gossypium* L.) genotypes with different duration of photosynthetic capacity, the PSⅡ photochemistry of the first leaves counted from the stem top of three cotton genotypes (‘Baimian1’ ‘Baimian5’ and ‘DP99B’) presented different leaf senescence progresses in production were examined by chlorophyll a fluorescence (Chl F) analysis during leaf senescence. The results showed that ‘Baimian1’ ‘Baimian5’ and ‘DP99B’ were late, intermediate and early aging types, respectively, based on the performance index of light absorption (PI_{ABS}). The three genotypes complied with the similar patterns in electrical transferring inhibition accompanying leaf senescence. The depletion of oxygen-evolving complex (OEC) was obvious at the late growth stage. The inhibition of the acceptor side of PSⅡ was greater than that of the donor side. The electron flow that through the light reactions of photosystem Ⅱ and photosystem Ⅰ was significantly limited accompanying leaf senescence. With the duration of leaf senescence, the energy distributed to thermal dissipation and the primary quinone electron acceptors of PSⅡ (Q_A) restoration increased, and correspondingly the energy used to transport an electron into the electron transport chain beyond Q_A^- (the reduction state of Q_A) declined. However, three cotton genotypes showed greater and greater electron transferring inhibition, except the number of Q_A reduction events between time=0 and time to reach maximal fluorescence, in the order of ‘DP99B’>‘Baimian5’>‘Baimian1’ with the duration of leaf senescence. It can be seen that the chlorophyll fluorescence characteristics can quickly and noninvasively reflect the senescence and the internal physiological mechanism of leaf senescence among different cotton genotypes.

Keywords: Cotton; Leaf senescence; Chlorophyll a fluorescence; Photosystem Ⅱ; Rapid chlorophyll a fluorescence parameter

Chinese Library Classification: S562 **Open Science Identity:**

基于快速叶绿素荧光参数的不同基因型棉花叶片衰老研究*

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摘 要: 光系统Ⅱ(PSⅡ)的光反应与作物光合能力密切相关。为了获得更多关于叶片衰老过程中 PSⅡ状态的详细信息并快速筛选出具有不同叶片光合功能持续时间的棉花基因型, 本试验利用快速叶绿素荧光技术研究了生产上报道叶片衰老快慢不同的 3 种棉花基因型倒一叶衰老过程中的 PSⅡ的光化学反应。结果显示, 基于光

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吸收的性能指数(PI_{ABS})将‘百棉 1 号’‘百棉 5 号’和‘DP99B’分为晚衰型、中间型和早衰型。3 种基因型棉花品种叶片衰老过程中电子传递受抑制情况遵循同样的规则。放氧复合体(OEC)在生育后期大量降解; PSⅡ受体侧的抑制情况要大于供体侧; 叶片衰老显著限制光系统Ⅱ-光系统 I 间的电子传递; 随着叶片衰老, 用于热耗散和还原初级醌受体(Q_A)的能量增加, 而用于 PSⅡ电子传递链中 Q_A 还原(Q_A^-)之后电子传递的能量下降。但是叶片衰老过程中, 电子传递受抑制程度(除荧光开始到荧光最大时间段之间的 Q_A 还原次数之外)为‘DP99B’>‘百棉 5 号’>‘百棉 1 号’。由此可知, 不同棉花基因型的叶绿素荧光特征可快速、无损地反映叶片衰老的快慢及内在生理机制。

关键词: 棉花; 叶片衰老; 叶绿素荧光; 光系统Ⅱ(PSⅡ); 快速叶绿素荧光参数

Cotton (*Gossypium* spp.) has infinite reproductive properties, and is an important cash crop. Appropriate senescence can improve fiber yield and quality of cotton by withstanding adverse weather conditions, efficiently utilizing material resources and energy during the growing season (Chen et al., 2018). Premature senescence generally leads to yield loss of about 10%, even more than 20% (Dong et al., 2005; Wright, 1999), and has been commonly in many cotton-growing countries (Dong et al., 2006; Dong et al., 2005) due to imbalance of source and sink (Wright, 1999) or poor ability to take up nutrients from soil in late season (Brouder and Cassman, 1990) and so on.

Leaf senescence can dramatically affect crop production by reducing photosynthetic capacity and influencing dry matter transfer from senescing leaves to harvestable organs (Gregersen et al., 2008; Rajcan et al., 1999; Rajcan and Tollenaar, 1999a; Rajcan and Tollenaar, 1999b). Research shows that the disassembly of the photosynthetic apparatus (PSA) within chloroplasts of plants and the electron flow that through the light reactions of photosystem Ⅱ (PSⅡ) and photosystem Ⅰ (PSⅠ) are greatly associated with the photosynthetic capacity decline (Grover, 1993; Smart, 1994; Weng et al., 2005; Wingler et al., 2004). Furthermore, it has been proved that PSⅡ is more susceptible to senescence than PSⅠ in senescent leaves (Grover et al., 1986). Kautsky and Hirsch (1931) firstly proposed the possibility using fluorescence methods for PSA analysis, because they founded there was a close relationship between chlorophyll fluorescence and primary reaction of photosynthesis.

Chlorophyll a fluorescence (Chl F) analysis is noninvasive, fast and precise, and has been widely used for assessment of the physiological state of PSA of different species under different environment conditions (Baker and Rosenqvist, 2004; DeEll et al., 1999; Wang et al., 2016). The most commonly and widely used Chl F analyses are performed by the saturation pulse method, which uses the darkness adapted leaf samples to obtain different parameters characterizing the steady-state status of the PSA (Baker, 2008; Lazár, 2015). In the last two decades, because fast Chl F rise kinetics OJIP which showed a typical O-J-I-P phase and OJIP test analysis can capture more detailed information on the structure and function of the PSA, primarily PSⅡ *in vivo* plant, it was developed to rapidly evaluate the physiological state of PSA in a high number of field grown plants (Goltsev et al., 2016) based on the energy flux theory in photosynthetic membranes (Strasser and Strasser, 1995). The OJIP test reflects the behavior of PSⅡ function, which includes the energy flow in PSⅡ and electron transport from H_2O to the final electron acceptors of PSⅠ (Ivanov et al., 2008; Papageorgiou, 2013). Wang et al (2016) examined the PSⅡ photochemistry by Chl F analysis in high-yield rice (*Oryza sativa*) ‘LYPJ’ flag leaves during senescence and found that natural senescence inhibited oxygen-evolving complex (OEC)-PSⅡ electron transport, also significantly limited the PSⅡ-PSⅠ electron flow. Paunov et al. (2018) analyzed the effect of cadmium and zinc on Chl F in durum wheat (*Triticum turgidum* var. *durum*) and found that both metals disturbed photosynthetic electron transport processes, which led to a 4- to 5-fold suppression of the efficiency of energy transformation in PSⅡ. Urbano Bron et al. (2004) used Chl F as a tool to evaluate the ripening of ‘Golden’ papaya fruit (*Carica papaya*), finding that maximal chlorophyll fluorescence (F_M) and minimal chlorophyll fluorescence (F_O) showed strong correlation with changes in skin color and fruit firmness, which suggested a relationship between the fluorescence and the level of papaya fruit senescence. Although many researches about the Chl F parameters in senescence leaves have been reported, more detailed information describing the status of PSⅡ by the fast induced fluorescence during leaf senescence is still unclear (Lu et al., 2002; Tang et al., 2015). And there is no report about Chl F in different cotton genotypes. In order to capture more detailed information describing the status of PSⅡ during leaf senescence and rapidly screen cotton genotypes with different duration of photosynthetic capacity, we observed the change of PSⅡ status using fast induced Chl F analysis during leaf senescence of different cotton genotypes under the same weather condition.

1 Materials and Methods

1.1 Cotton materials

Three insect-resistant transgenic cotton genotypes were used for this study. They are 'Baimian1', bred by Henan Institute of Science and Technology with a performance of early-maturing but slow leaf senescence (Hu et al., 2014; Wang et al., 2020); 'DP99B' bred by the Monsanto Company and officially registered in Hebei Province in 2000 with a performance of early-maturing and fast leaf senescence (Zhang et al., 2007; Wang et al., 2020); 'Baimian5' bred by Henan Institute of Science and Technology with a performance of early-maturing but middle leaf senescence.

1.2 Experimental design

A field experiment was conducted at the experimental station of Henan Institute of Science and Technology in Xinxiang, Henan Province, China (35°18′N, 113°55′E). A randomized experiment was designed with three cotton genotypes and three replicates in sandy soil with pH 8.5, 6.0 g·kg^{-1} organic matter, 18.6 mg·kg^{-1} available nitrogen, 16.2 mg·kg^{-1} available phosphorus, 158.5 mg·kg^{-1} available potassium in 2012. The cotton population was 4.5×10^4 plants·hm^{-2} for three genotypes. Before planting the land was plowed and irrigated. The three different cotton genotypes had a conventional field management, planted in 20 April and topped in 15 July. The weather conditions of the study area were provided in Table 1.

Table 1 The weather conditions of Xinxiang, Henan, China in 2012 and from 1961 to 2011

Time	Temperature (℃)		Rainfall (mm)		Sunshine (h)	
	2012	Average from 1961 to 2011	2012	Average from 1961 to 2011	2012	Average from 1961 to 2011
Early July	26.69	27.32	54.74	55.30	62.75	52.20
Middle July	27.07	28.47	51.15	11.10	61.70	66.40
Late July	27.53	29.55	50.62	14.30	72.32	78.80
Early August	27.26	27.00	52.08	44.70	68.21	62.00
Middle August	25.89	25.70	41.82	36.40	64.16	43.90
Late August	24.78	24.99	31.65	0.40	71.37	55.80
Early September	22.80	23.56	27.95	22.30	58.06	50.90
Middle September	21.12	20.64	22.35	22.80	58.69	68.60
Late September	25.56	20.59	16.94	8.70	59.08	58.20
Early October	17.25	19.92	12.14	0.40	57.46	73.10

1.3 Chlorophyll fluorescence measurements

From 20 July (after topping) to 11 October (harvest time), polyphasic Chl F transient (OJIP) of the first leaves counted from the stem top of each cotton genotypes were measured with a portable fluorometer (Handy PEA, Hansatech, UK) by according to methods of Strasser et al. (1995) in the afternoon (16:00–17:00) at approximately 20 days intervals. Each cotton genotypes contained six leaves with the similar growth condition. Before measurements, leaves were dark-adapted for 30 min. Then OJIP transient was induced by 1 second pulses of red light (650 nm, 3500 μmol·m^{-2}·s^{-1}). The OJIP test (Strasser et al., 2004) was used to analyze each OJIP transient. The formulae and explanation of the technical data of the OJIP curves, as well as the selected OJIP test parameters used in this study was list blow (Table 2).

1.4 Statistical analysis

Duncan test was used to determine the differences among genotypes at $P<0.05$ with SPSS (version 22.0; IBM). Charts were generated using Excel 2003 and Origin 9.0 software (OriginLab Inc., USA). Data presented in the figures and tables are means of six biological replicates.

2 Results

2.1 Identification of photosynthetic function duration of three cotton genotypes

When the main stem nodes above the uppermost white flower at the first node of fruit branch was equal to 5, it indicates the growth and development of cotton has entered the physiological decline stage. In present study, the physiological decline periods of three cotton genotypes were basically the same between 19 July and 26 July (Table 3). 'DP99B' has a significantly less main stem nodes above the uppermost white flower at the first node of fruit branch than that of 'Baimian1' between 28 June and 19 July, indicated that the reproductive growth process of 'DP99B' was faster than Baimian1 in this stage.

Table 2 Definitions of measured and calculated chlorophyll a fluorescence parameters used in the experiment

Terms and formulae	Description
F_t	Fluorescence at time after onset of actinic illumination
F_O~$F_{50\mu s}$	Minimum fluorescence, when all PSⅡ reaction centers (RCs) are open.
F_J	Fluorescence at the J-step (2 ms) of the OJIP transient
F_I	Fluorescence at the I-step (30 ms) of the OJIP transient
F_M	Maximum recorded fluorescence at the P-step when all RCs are closed.
F_K	Fluorescence at the K-step (300 μs) of the OJIP transient
$V_J=(F_J-F_O)/(F_M-F_O)$	Relative variable fluorescence at the phase J of the fluorescence induction curve
$V_I=(F_I-F_O)/(F_M-F_O)$	Relative variable fluorescence at the phase I of the fluorescence induction curve
$W_K=(F_K-F_O)/(F_J-F_O)$	Represent the damage to oxygen-evolving complex (OEC)
$F_V=F_M-F_O$	Maximal variable fluorescence
$\Phi P_O=TR_O/ABS=F_V/F_M=[1-(F_O/F_M)]$	Maximum quantum yield of primary photochemistry, TR_O is the trapped energy flu, ABS is the absorption energy flu
$\Phi E_O=ET_O/ABS=\Phi P_O\times\Psi o$	Quantum yield (at time = 0) for electron transport from Q_A^- to plastoquinone, ET_O is the energy flu used to electron transport
$\Psi o=ET_O/TR_O=1-V_J$	Probability (at time = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-.
$\Phi D_O=DI_O/ABS=1-\Phi P_O=(F_O/F_M)$	Thermal dissipation quantum yield, DT_O is the energy flu used to thermal dissipation
$\delta R_O=(1-V_I)/(1-V_J)$	Efficiency/probability with which an electron from the intersystem electron
$\Phi R_O=\Phi P_O\times\Psi o\times\delta R_O$	Quantum yield of reduction of end electron acceptors of PSI
$S_m=Area/F_V$	Normalized total complementary area above the OJIP transient
$M_O=4\times[(F_{300\mu s}-F_{50\mu s})/(F_M-F_{50\mu s})]$	Approximated initial slope (in·ms^{-1}) of the fluorescent transient, relating to the closure rate of reaction centers
$N=S_m/S_s=S_m\times M_O\times(1/V_J)$	Turnover number: number of the primary quinone electron acceptors of PSⅡ (Q_A) reduction events between time = 0 and time to reach F_M, where S_m is normalized total complementary area above the OJIP transient (reflecting multiple-turnover Q_A reduction events), S_s is normalized total complementary area corresponding only to the O-J phase (reflecting single-turnover Q_A reduction events).
$RC/CS_O=-\Phi P_O\times(V_J/M_O)\times F_O$	Density of active PSⅡ reaction center (RC), CS denotes cross section (at time = 0)
$ABS/RC=(M_O/V_J)/[1-(F_O/F_M)]$	Absorption flux per RC corresponding directly to its apparent antenna size-ratio between chlorophyll in antenna and chlorophyll in reaction center (RC)
$TR_O/RC=M_O\times(1/V_J)$	Trapped (maximum) energy flux (leading to Q_A reduction) per reaction center (RC) (at time = 0)
$ET_O/RC=(M_O/V_J)\times(1-V_J)$	Electron transport flux from Q_A^- to plastoquinone per RC at time = 0
$DI_O/RC=ABS/RC-TR_O/RC$	Dissipation energy flux per PSⅡ RC at time = 0
$PI_{ABS}=(RC/ABS)\times[\Phi P_O/(1-\Phi P_O)]\times[\Psi o/(1-\Psi o)]$	Performance index on absorption basis

Table 3 Main stem nodes number above the uppermost white flower at the first node of fruit branch of different cotton genotypes in 2012

Genotype	Date (month-day)				
	06-28	07-05	07-12	07-19	07-26
Baimian1	9.4a	7.5a	7.2a	6.6a	3.8a
Baimian5	8.4b	7.1a	6.7b	5.9b	3.8a
DP99B	8.3b	6.7b	6.8b	6.0b	3.8a

Different lowercase letters in the same column indicated significant differences among different cotton genotypes at $P<0.05$.

The performance index based on light energy absorption (PI_{ABS}) is a photosynthesis performance index that reflects the overall photosynthetic activity of PSⅡ. Therefore, the variations of PI_{ABS} in three cotton genotypes were observed every 20 days from 21 July based on the results of the main stem nodes above the uppermost white flower at the first node of fruit branch (Table 4). There was no obvious difference in the value of PI_{ABS} in three genotypes of cotton on 21 July. With the duration of leaf senescence, PI_{ABS} declined gradually in three cotton genotypes. On 10 August, obvious difference of PI_{ABS} appeared in 'DP99B' compared with the other cotton genotypes. Then, obvious difference of PI_{ABS} continued in 'DP99B' compared with the other cotton genotypes. At last, 'Baimian1' decreased 57.13%, 'DP99B' decreased 89.66%, 'Baimian5' decreased 76.17% compared with the PI_{ABS} on 21 July. And obvious difference of PI_{ABS} appeared between those cotton genotypes on 11 October. These results indicated that the process of leaf senescence in three cotton genotypes were different. The senescence of leaves in 'DP99B' was faster than that in the other cotton genotypes. And the rate of leaf senescence in 'DP99B' was the fastest, then was 'Baimian5', 'Baimian1' was the slowest. Taken together, the functional period of leaf in 'Baimian1' was longest, 'DP99B' was shortest, 'Baimian5' was middle.

Table 4 Trends of the performance index based on light energy absorption (PI_{ABS}) with time for different cotton genotypes in 2012

Date (month-day)	Baimian1	Baimian5	DP99B
07-21	70.82a	73.07a	74.69a
08-10	63.71a	59.98a	47.62b
08-30	59.28a	58.65a	43.94b
09-20	38.39a	38.22a	28.23b
10-11	30.36a	17.41b	7.72c

Different lowercase letters in the same line at the same date indicated significant differences among genotypes at $P<0.05$.

2.2 Chlorophyll a fluorescence OJIP transient in cotton leaves of three genotypes

The state of the light-dependent photosynthetic processes in three cotton genotypes were analyzed with OJIP transient during the leaf senescence (Fig. 1). Characteristic difference existed at each phase (O−J, J−I, and I−P phase) of the OJIP fluorescence. The O−J phase denotes gradual reduction of Q_A which is the primary electron acceptor in PSⅡ. The K-phase will appear before J point when the donor site of PSⅡ is injured. The J−I phase mainly reflect the reduction of the intersystem electron carriers. The I−P phase reflects the reduction of PSⅠ electron acceptor (Strasser et al., 2004; Yusuf et al., 2010).

In this study, the relative fluorescence intensity of those cotton genotypes almost exhibited the uptrend with the duration of leaf senescence at the O−J phase, but the uptrend was not obvious in ‘Baimian1’ and ‘Baimian5’ until 20 September. After 20 September, ‘Baimian1’ had no obvious change, ‘Baimian5’ and ‘DP99B’ had an increase at the O−J phase. This indicated a large number of Q_A^- accumulate in PSⅡ reaction centers (RCs) due to the blocking of the electron transport from Q_A to the secondary quinone electron acceptors of PSⅡ (Q_B) with the senescence of cotton leaves. But difference among the three cotton genotypes on electron transport was great especially after 20 September. At the J−I phase, the relative fluorescence intensity of three cotton genotypes also increased differently with the duration of leaf senescence especially in ‘DP99B’. This indicated the reduction of the intersystem electron carriers had been influenced greatly, but the difference existed in the three cotton genotypes. At the I−P phase, the relative fluorescence intensity of those cotton genotypes gradually declined with the duration of leaf senescence because there was reduction in the net Chl-a content of leaves with onset of senescence (Wang et al., 2014).

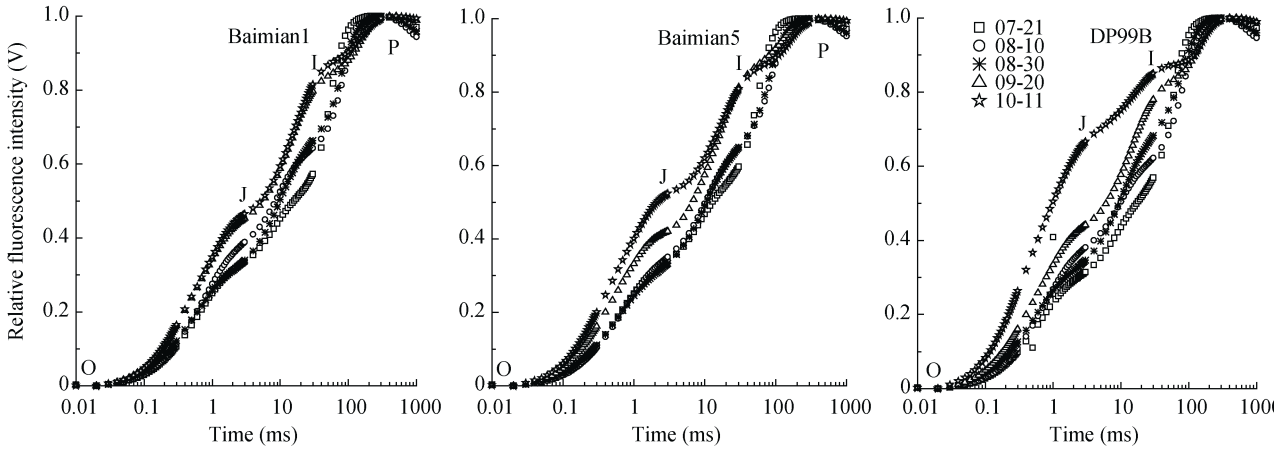

Fig. 1 Double normalized chlorophyll fluorescence curves of the first leaves from top of three cotton genotypes

Compared with the fluorescence intensity on 21 July, F_O increased by 2.84%, 22.91%, 26.29% and F_P reduced by 4.98%, 7.15%, 23.86% respectively in ‘Baimian1’, ‘Baimian5’ and ‘DP99B’ on 11 October. These results probably indicated that the components of photosynthetic apparatus of three cotton genotypes were differently injured during the leaf senescence. And this result was accord with the above result of PI_{ABS} (Table 4).

2.3 Changes of reaction center, donor and acceptor side of PSⅡ in leaves of three cotton genotypes

More parameters involved the reaction center, electron transport at donor and acceptor sides of PSⅡ were analyzed. The variation of RC/CS_O and W_K demonstrate the changes in the reaction center and donor side of PSⅡ, respectively, were shown in Fig. 2. Parameters such as M_O, S_m, V_I, V_J, N and Ψo primarily reflect changes in the acceptor site of PSⅡ were shown as a spider plot (Fig. 3).

With the duration of leaf senescence, the RC/CS_O of the three cotton genotypes declined greatly before 30 August, then it increased on 20 September, which may be caused by the weather condition during late August. On the whole, the RC/CS_O of the three cotton genotypes showed descending trend. It can be seen that those active reaction centers were susceptible to

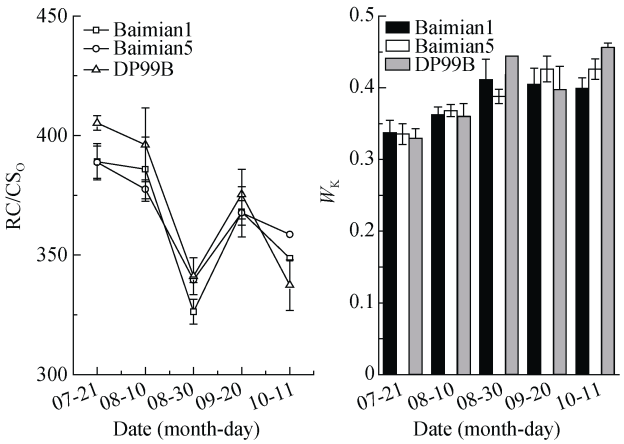

Fig. 2　Variation of the values of RC/CS$_O$ and W_K in three cotton genotypes during leaf senescence

disruption reversibly, but the overall trends of RC/CS$_O$ were decline with the duration of leave senescence. Compared with the highest values of RC/CS$_O$, the RC/CS$_O$ values of 'Baimian1' 'Baimian5' and 'DP99B' on 11 October decreased by 10.37%, 7.77% and 16.75%, respectively. And different cotton genotypes had similar change on the active reaction centers during leaf senescence. The reduction of active reaction centers number of those cotton genotypes indicated that less energy was used to drive electron transport. With the duration of the leaf senescence, the trends of W_K of three cotton genotypes were increasing, the values of W_K changed greatly after 10 August. At last, the W_K of 'DP99B' was significantly greater than that of 'Baimian1'. And there were evident differences between 'DP99B' and 'Baimian1', but 'Baimian5' had no significantly difference with 'DP99B' and 'Baimian1' on 11 October. Compared with the value of W_K on 21 July, the W_K on 11 October in 'Baimian1' 'Baimian5' and 'DP99B' increased by 18.40%, 26.95% and 38.53%, respectively. This indicated that the oxygen-evolving complex (OEC) was damaged greatly at the late growth stage, while the damaged extents of the OEC were different in three cotton genotypes even at the same growing environment.

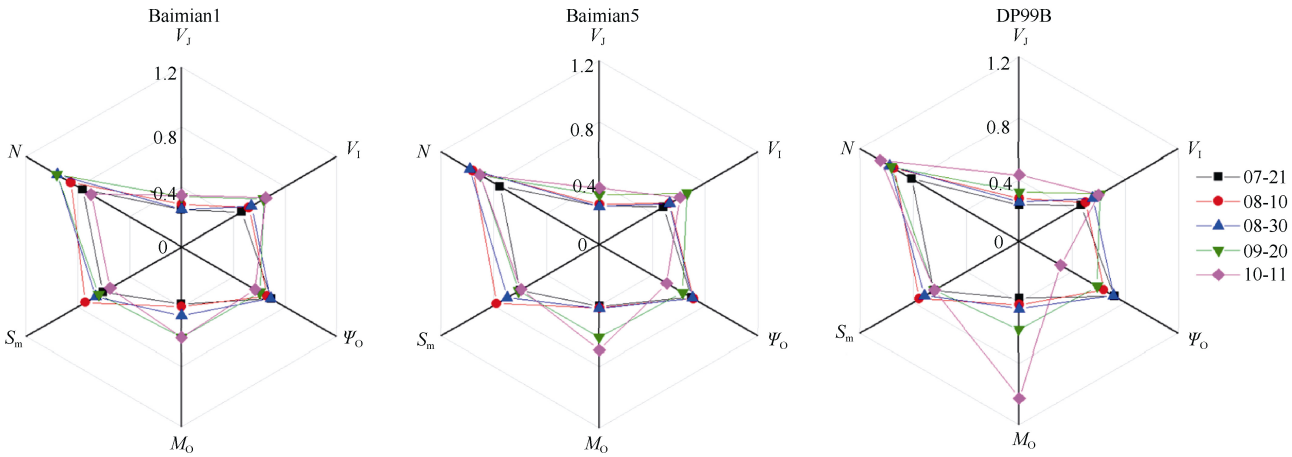

Fig. 3　Variation of parameters at acceptor side of PSⅡ of three cotton genotypes during leaf senescence

The V_J of three cotton genotypes increased during the leaf senescence. The V_I of 'Baimian1' always increased, while the V_I of 'Baimian5' and 'DP99B' first increased then declined during the leaf senescence. The V_J of 'Baimian1' 'Baimian5' and 'DP99B' increased by 27.28%, 46.57% and 45.80%, respectively; and the V_I of 'Baimian1', 'Baimian5' and 'DP99B' increased by 28.74%, 25.78% and 22.18%, respectively, on 11 October, compared with those on 21 July. It indicated that the electron transport had stronger inhibition in 'Baimian5' and 'DP99B' than that in 'Baimian1' at J phase. While the strength of inhibition was 'Baimian1' > 'Baimian5' > 'DP99B' at Ⅰ phase.

The values of S_m and N were normalized by dividing 50. With the duration of leaf senescence, the S_m of three cotton genotypes had an upward trend followed by a downward trend. While the obvious difference of S_m only existed in 'Baimian1' and 'DP99B' on 11 October. And the S_m of 'DP99B' was larger than 'Baimian1' on 11 October. It indicated that the plastoquinone (PQ) sink degraded rapidly at late senescence stage, but 'DP99B' need more energy than 'Baimian1' to restore the acceptor side of PSⅡ. With the duration of the leaf senescence, probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (at time=0) (Ψ_O) decreased, which indicated that the relative amount of an electron into the electron transport chain beyond Q_A^- had a decline trend with the leaf senescence. Among three cotton genotypes, the descent degree of Ψ_O in 'Baimian1' was least. Because of the decline of Ψ_O, more energy was used to restore Q_A, and accelerated the reduction of Q_A (M_O). Compared with the value of M_O on 21 July, the M_O on 11 October in 'Baimian1' 'Baimian5' and 'DP99B' increased by 58.79%, 71.23% and 175.01%, respectively. With the

increase of M_O, the behavior of the number of Q_A reduction events between time = 0 and time to reach F_M (N) in three cotton genotypes were different, 'Baimian1' and 'Baimian5' first increased then decreased, 'DP99B' increased always. It indicated that the openness of active reaction centers at time = 2 ms in three cotton genotypes was different with the decline of the functional activity of PSII during the leaf senescence, resulting the difference in light energy utilization.

2.4 Variations of PSⅡ and PSⅠ efficiency and excitation energy dissipation in cotton leaves of three genotypes

With the duration of the leaf senescence, the ΦP_O, ΦE_O and ΦR_O decreased, while ΦD_O increased. On 11 October, there were evident differences among those cotton genotypes in PSⅡ and PSⅠ efficiency. Among those genotypes, the range of change in energy efficiency was 'DP99B' > 'Baimian5' > 'Baimian1' from 20 July to 11 October. This indicated that with the cotton leaf senescence, the functional activity of PSⅡ and PSⅠ could be maintained until 11 October, accompanied the increase of the thermal dissipation quantum yield (Table 5).

With the duration of leaf senescence, the ABS/RC, TR_O/RC and DI_O/RC of three cotton genotypes increased rapidly. The ET_O/RC of three cotton genotypes first had the same trend with ABS/RC, TR_O/RC and DI_O/RC before 30 August, then decreased gradually. This showed that with the increase of ABS/RC more energy of per reaction center were trapped and dissipated at later period of leaf senescence accompanied with maximum electron transport flux (further than Q_A^-) per PSⅡ reaction center decrease. And there were significant differences of energy allocation between 'Baimian1' and 'DP99B' only on 11 October. Among three cotton genotypes, 'DP99B' has the largest light absorption flux, trapped energy flux, dissipation energy flux per PSⅡ reaction center and the least maximum electron transport flux (further than Q_A^-) per PSⅡ reaction center (Table 5).

Table 5 Mean values of PSⅡ reaction center numbers and energy allocation of three cotton genotypes in different days

Parameter	Date (month-day)	Genotype			Parameter	Date (month-day)	Genotype		
		Baimian1	Baimian5	DP99B			Baimian1	Baimian5	DP99B
ΦP_O	07-21	0.82a	0.82a	0.82a	ABS/RC	07-21	1.57a	1.59a	1.54a
	08-10	0.81a	0.82a	0.81a		08-10	1.46a	1.50a	1.43a
	08-30	0.82a	0.82a	0.82a		08-30	1.78a	1.78a	1.81a
	09-20	0.81a	0.83a	0.79b		09-20	1.84a	1.85a	1.79a
	10-11	0.80a	0.76b	0.71c		10-11	1.85b	2.00b	2.25a
ΦE_O	07-21	0.57a	0.57a	0.58a	TR_O/RC	07-21	1.28a	1.30a	1.27a
	08-10	0.54a	0.56a	0.52a		08-10	1.19a	1.18a	1.16a
	08-30	0.57a	0.58a	0.56a		08-30	1.42a	1.37a	1.46a
	09-20	0.49a	0.51a	0.45a		09-20	1.45a	1.48a	1.46a
	10-11	0.46a	0.39b	0.21c		10-11	1.46b	1.51ab	1.58a
ΦD_O	07-21	0.18a	0.18a	0.18a	ET_O/RC	07-21	0.89a	0.90a	0.91a
	08-10	0.19a	0.19a	0.19a		08-10	0.77a	0.81a	0.76a
	08-30	0.19a	0.18a	0.19a		08-30	1.01a	0.99a	1.02a
	09-20	0.19b	0.18b	0.22a		09-20	0.84a	0.91a	0.83a
	10-11	0.20 c	0.24 b	0.32 a		10-11	0.80a	0.73ab	0.52b
ΦR_O	07-21	0.40 a	0.39 a	0.40 a	DI_O/RC	07-21	0.29a	0.29a	0.28a
	08-10	0.36 a	0.38 a	0.36 a		08-10	0.27a	0.27a	0.27a
	08-30	0.35 a	0.38 a	0.34 a		08-30	0.33a	0.30a	0.33a
	09-20	0.27a	0.26a	0.26a		09-20	0.36a	0.35a	0.38a
	10-11	0.24a	0.24a	0.15b		10-11	0.39c	0.49b	0.73a

Different lowercase letters in the same line at the same date indicated significant differences among genotypes at $P<0.05$.

3 Discussion

3.1 Senescence being reflected by PI_{ABS} and differences exist among three genotypes

Leaf senescence is an integrated response of leaf cells to age information and other internal and environmental signals, early leaf senescence may decrease yield in crop plants by limiting the growth phase (Lim et al., 2007). Senescence may differ between genotypes because the internal factors, especially the senescence related genes, and the external factors including light, temperature, water availability, carbon dioxide, nutrients and so on (Chen et al., 2018). In this paper, the three cotton genotypes enter into the senescence periods at the time between 19 July and 26 July which were proved by the main stem nodes above the uppermost white flower at the first node of fruit branch under the same environment (Table 3).

The disassembly of the photosynthetic apparatus within chloroplast and the concomitant decrease in photosynthetic activity of PSⅠ and PSⅡ, especially PSⅡ, are the most remarkable events in leaf senescence (Grover and Mohanty, 1992; Grover et al., 1986; Woolhouse, 1987). PI_{ABS} is shown to be well correlated with photosynthetic capacity which can be measured as CO_2 assimilation (Heerden et al., 2003; Ripley et al., 2004). PI_{ABS} is more sensitive than commonly used parameter maximum quantum yield of photosystem Ⅱ (F_V/F_M) (Živčák et al., 2008), very susceptible to environmental stress, so it has been used to differentiate genotypes by their responses to different abiotic stresses including natural senescence (Boureima et al., 2012; Holland et al., 2014; Kalaji et al., 2014). Chen and Dong (2016) proposed that there were three senescence performance categories including normal, premature and late senility in cotton. In this research, three genotypes showed distinctly leaf senescence differences being expressed by PI_{ABS} (Table 4), which could classify 'Baimian1' 'Baimian5' and 'DP99B' into late, middle and early senescence types. Furthermore, the senescence of leaves in 'DP99B' not only began earlier, but also expanded faster than that in 'Baimian1' and 'Baimian5' (Table 4), which was identical to the phenomenon observed at the yield level, and also explained why 'DP99B' usually had lower fiber yield than 'Baimian1' (Wang et al., 2020).

3.2 Different electron transport exist in different senescence performance

The senescence pattern which affects photosynthesis is variable and differences among cultivars with different genotypes (Falqueto et al., 2009). Anything affecting photosynthesis can affect the intensity of Chl F, so Chl F can be used as a versatile tool to sense environmental and physiological changes of plants (Guo and Tan, 2015). The shape of the fluorescence rise kinetics (OJIP) is highly dependent on the physiological conditions. In the present study, the major difference during natural senescence for different cotton genotypes occurred in the J−P phase, indicating major difference existed at the intersystem electron carriers and PSⅠ electron acceptors among different genotypes. Furthermore, during the leaf senescence the PSⅡ−PSⅠ electron flow was also significantly limited. Among those cotton genotypes, the variation of fluorescence intensity about 'DP99B' was the biggest, 'Baimian1' was the least, 'Baimian5' was the middle at the J−P phase on 11 October compared with 21 July (Fig. 1).

In order to further identify the damage site and sensitive functions during the photosynthetic electron transport within senescent leaf, we calculated several OJIP parameters. The OJIP parameters are quantitative analysis of the OJIP transient (Chen et al., 2016), helping account the different steps and phases of the OJIP transient with the redox states of PSⅡ and realize the efficiencies of electron transfer through the intersystem chain to the end electron acceptors at the PSⅠ acceptor side, concomitantly (Pollastrini et al., 2014; Tsimilli-Michael and Strasser, 2008). W_K is a relative measure of inactivation of OEC. With the duration of leaf senescence, the overall trends of W_K in three cotton genotypes were increasing (Fig. 2). At the same time, the inhibition of the acceptor side of PSⅡ was greater than that of the donor side, which can be proved by the bigger increase of V_J and V_I than W_K in three cotton genotypes. Among those genotypes, 'DP99B' had the biggest increase of W_K, 'Baimian5' had the biggest increase of V_J, 'Baimian1' had the biggest increase of V_I (Fig. 2 and Fig. 3).

By analyzing the energy efficiency and acceptor side of PSⅡ, we found that ΦP_O, ΦE_O and ΦR_O declined greatly with the duration of leaf senescence, while ΦD_O increased greatly on 11 October with 'DP99B' the largest, 'Baimian1' the least, 'Baimian5' in middle (Table 5). This was related with the decline of the number of reactive centers per excited cross section. The reactive centers were disrupted during the senescence of leaves accompanied with the increase of ABS/RC, TR_O/RC, DI_O/RC and decline of ET_O/RC at the later season (Fig. 2 and Table 5). This indicated that when cotton leaf senesced, an electron into the electron transport chain beyond Q_A^- declined and thermal dissipation quantum yield increased, among which 'DP99B' had the largest variation, 'Baimian1' the least, and 'Baimian5' was in the middle. Although more energy was used to restore Q_A, but the behavior of the number of Q_A reduction events (N) in three cotton genotypes were different (Fig. 3). This may be due to the openness differences of active reaction

centers at time = 2 ms in three different cotton genotypes during the senescence of leaves, resulting in the difference in light energy utilization.

4 Conclusion

The Chl F analysis proved that three cotton genotypes showed distinctly different leaf-senescing progresses with ‘Baimian1’ > ‘Baimian5’ > ‘DP99B’ in functional period of leaf, which in accordance with the empirical observation in the field. However, they complied with the similar patterns in electrical transferring inhibition along with leaf senescence. For example, the depletion of OEC was greatly obvious at the late growth stage, while the inhibition of the acceptor side of PSⅡ was greater than that of the donor side. The energy distributed to thermal dissipation and Q_A restoration increased, and correspondingly the energy used to transport an electron into the electron transport chain beyond Q_A^- declined. However, three cotton genotypes showed greater and greater electron transferring inhibition except for the number of Q_A reduction events between time = 0 and time to reach F_M in sequences of ‘DP99B’ > ‘Baimian5’ > ‘Baimian1 with the duration of leaf senescence.

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