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The analysis of determining factors and evaluation of tolerance to photoinhibition in wheat (*Triticum aestivum* L.)

H. LI[*], Q. ZHENG[*], J. ZHANG[**], B. LI[*], and Z. LI[*,+]

State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China[*]
Zhaoxian Institute of Agricultural Sciences, Shijiazhuang 051530, China[**]

Abstract

Photoinhibition is a significant constraint for improvement of radiation-use efficiency and yield potential in cereal crops. In this work, attached fully expanded leaves of seedlings were used to assay the factors determining photoinhibition and for evaluation of tolerance to photoinhibition in wheat (*Triticum aestivum* L.). Our results showed that even 1 h under PPFD of 600 μmol(photon) m^{-2} s^{-1} could significantly reduce maximal quantum yield of PSII photochemistry (F_v/F_m) and performance index (PI) compared to low light [300 μmol(photon) m^{-2} s^{-1}]. The decrease of F_v/F_m and PI was more noticeable with the increase of light intensity; irradiance higher than 800 μmol(photon) m^{-2} s^{-1} resulted in photoinhibition. Compared to 25°C, lower (20°C) or higher temperature (≥ 35°C) aggravated photoinhibition, while slightly high temperature (28°) alleviated photoinhibition. At 25°C, irradiance of 1,000 μmol(photon) m^{-2} s^{-1} for 1 h was enough to cause photoinhibition and a significant decrease of F_v/F_m, PI, trapped energy flux, electron transport flux, and density of reaction center as well as increase of dissipated energy flux per cross section were observed. In addition, seedlings at 21–32 days after planting showed a relatively stable phenotype, while the younger or older seedlings indicated an increased susceptibility to photoinhibition, especially in senescing leaves. Finally, six wheat varieties with relative tolerance to photoinhibition were identified from 22 Chinese winter wheat varieties by exposing attached leaves of the 25-d old seedlings for 1 h to 1,000 μmol(photon) m^{-2} s^{-1} at 25°C. Therefore, our work established a possible method for development of new wheat varieties with enhanced tolerance to photoinhibition.

Additional keywords: chlorophyll fluorescence; high light; photoinhibition; *Triticum aestivum* L.

Introduction

Excessive light energy absorbed by plants under high light (HL) condition usually results in photoinhibition (Powles 1984, Long *et al.* 1994). It could lead to a substantial loss of carbon assimilation under natural conditions even without other stress (Ögren *et al.* 1992, Werner *et al*. 2001). Therefore, it is considered as a significant constraint for improvement of radiation-use efficiency (RUE) and yield potential in cereal crops such as winter wheat (Reynolds *et al.* 2000). Tolerance to photoinhibition in wheat was found being genotype-dependent (Wang 2000, Monneveux *et al.* 2003, Yang *et al.* 2006, Li *et al.* 2010, Chen *et al*. 2011). Thus, selection for varieties with enhanced tolerance to photoinhibition is an important approach toward breeding new high-yielding wheat varieties (Reynolds *et al.* 2000). However, it is time consuming, laborious, and low efficient to select photoinhibition-tolerant lines from many segregated progenies directly in the field due to the complex and dynamic environmental cues including light intensity, temperature, *etc*. Hence, establishment of a relatively precise and high throughput method may improve the selection efficiency for breeding new photoinhibition-tolerant wheat varieties.

In laboratories, photoinhibition was usually initiated by exposing detached leaves, leaf segments or whole plant to excessive irradiance for hours (Hetherington *et al*. 1989, Hurry and Huner 1992, Mishra and Singhal 1992, Öquist

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[+]Corresponding author; tel: 86 10 6480 6607; fax: 86 10 6480 6605; e-mail: zsli@genetics.ac.cn
Abbreviations: Chl – chlorophyll; DAP – days after planting; DI_o/CS – dissipated energy flux per cross section; ET_o/CS – electron transport flux per cross section; F_v/F_m – maximal quantum yield of PSII photochemistry; HL – high light; LL – low light; PI – performance index; PI_{ABS} – performance index on an absorption basis; RC/CS_m – density of reaction center per excited cross section; RUE – radiation-use efficiency; TR_o/CS – trapped energy flux per cross section; V_J – relative variable fluorescence intensity at the J-step.
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and Huner 1993, Lin *et al*. 1998, Ma *et al*. 2006, Yang *et al*. 2006, Chen *et al*. 2011). Jiao (1992) developed photoinhibitory treatment by exposing detached leaves immersed in water to sun light for several days to screen rice germplasms. Since the sun light is dynamic, the precision and repeatability of this method might be limited. Then, this method was improved by Li *et al*. (2010) using array lamps as a light source which can evenly provide PPFD about 1,000 μmol m^{-2} s^{-1} on leaf segments. Detached leaves or leaf segments may obtain relatively even light intensity, while attached leaves may get uneven light due to leaf angle and shade from other leaves. On the other hand, detached leaves may reflect less *in vivo* response relative to attached leaves since detached leaves prevent signal transduction from other parts. For example, compared to detached leaves, attached leaves may raise endogenous ABA concentration and then maintain relatively higher PSII efficiency under HL stress (Weng *et al*. 2011). Therefore, the objective in the present work was to analyze the influencing factors and establish a relative precise method with attached leaves for assessment of tolerance to photoinhibition in wheat genotypes.

Materials and methods

Plant growth: The winter wheat (*T. aestivum* cv. Jing 411) was used to establish photoinhibitory treatment methods in this experiment. Seeds were sterilized with 1.5% H_2O_2 overnight at room temperature. Then, the geminated seeds were transplanted in a plastic plate on nylon net in water. After being grown in a growth chamber (*HP1000GS*, *Wuhan Ruihua Instrument & Equipment Co. Ltd.*, China) for one week, the seedlings were transplanted in a plastic box with nutrition medium, consisting of 0.2 mM KH_2PO_3, 0.5 mM $MgSO_4$, 1.5 mM KCl, 1.5 mM $CaCl_2$, 0.1 mM FeEDTA, 2 mM $Ca(NO_3)_2$, 1×10^{-3} mM H_3BO_3, 1×10^{-4} mM $(NH_4)_6Mo_7O_{24}$, 5×10^{-4} mM $CuSO_4$, 1×10^{-3} mM $ZnSO_4$, and 1×10^{-3} mM $MnSO_4$, which was renewed every three days. The growth conditions were set as follows: day/night temperature of 20/15°C, PPFD of 300 μmol m^{-2} s^{-1} with 14-h photoperiod, and relative humidity of 60%. Unless specially pointing out, the attached fully expanded second leaves of seedlings, at the third leaf stage, were used for HL treatments. Additionally, the whole seedling plants were subjected to darkness for 0, 2, 4, 6, and 8 d before the HL treatment in order to study the influence of leaf senescence on photoinhibition.

For evaluation of the leaf position effect on photoinhibition, seeds of Jing 411 were planted in the super single cell cones *SC10* (*Stuewe and Sons Inc*., USA) which were filled with *Sunshine SB100* mix (*Sun Gro Horticulture Distribution Inc.*, USA) and fertilized with *Osmocote Exact 15–9–11* (*Scotts Sierra Horticultural Product Company*, USA). Then, they were transferred into the *RL98* trays (*Stuewe and Sons Inc.*, USA) and grown in a greenhouse where the growth conditions were set as follows: day/night temperature of 22–25/15–18°C, PPFD of 300–800 μmol m^{-2} s^{-1} with 14-h photoperiod, and relative humidity of 30–40%. At the sixth leaf stage, about 35 d after planting (DAP), all five fully expanded leaves, from first to fifth upmost leaves on the main stems, were subjected to PPFD of 1,400 μmol m^{-2} s^{-1} for 0.5 h. At least five leaves for each leaf position were measured and three independent experiments were carried out.

In addition, 22 Chinese winter wheat varieties or advanced lines were also used to assay the tolerance to photoinhibition (Table 1). Three independent experiments were carried out and in each experiment 3–4 plants were assayed.

Photoinhibitory treatment: By using two-side adhesive tape, the examined leaves were pasted on a flat paper in order to keep the upper surface vertically exposed to light source direction. Meanwhile, roots were grown in the nutrition medium as mentioned above. PPFD in a range of 600–1,900 μmol m^{-2} s^{-1} provided with LED-light source was used to determine optimal PPFD for the photoinhibitory treatment. For examination of temperature effects on photoinhibition, the temperature was kept between 20–42°C in a growth incubator (*E36HO*, *Percival Scientific*, USA) when leaves were treated for 1 h at PPFD of 800 μmol m^{-2} s^{-1}. The light source was provided with *GE* compact fluorescent light bulbs (*F55BX/840*, USA). The leaves were also exposed to PPFD of 1,000 μmol m^{-2} s^{-1} and then kept in darkness for hours to study the effects of HL duration on the recovery from photoinhibition. Additionally, the leaves of seedlings at 19–32 DAP were exposed to 1 h of 1,000 μmol(photon) m^{-2} s^{-1} in order to determine suitable leaf age for a photoinhibition evaluation.

Chlorophyll fluorescence: The chlorophyll (Chl) fluorescence parameters including F_v/F_m, PI (PI_{ABS}, performance index on an absorption basis), and JIP-test parameters, such as trapped energy flux per cross section (TR_o/CS), electron transport flux per cross section (ET_o/CS), dissipated energy flux per cross section (DI_o/CS), and density of reaction center per excited cross section (RC/CS_m) (Strasser *et al.* 1995, 2001, Rapacz 2007) were simultaneously obtained by using a *Handy-PEA* fluorometer (*Hansatech Instruments Ltd*., UK). After dark adaption for 20 min, which is sufficiently long to relax ompletely all nonphotochemical quenching of fluorescence, the middle parts of leaves were assayed immediately. The saturated flash light intensity was set at 3,000 μmol(photon) m^{-2} s^{-1} and the flash light duration was 1 s. Based on the shape of fluorescence rise of high time resolution (10 μs) which shows the steps O, J (F_{2ms}), I (F_{30ms}), and P (F_m), the JIP-test parameters were calculated as follows (Strasser *et al.* 1995, 2001, Rapacz 2007):

$TR_o/CS = (F_v/F_m)\ F_o$
$ET_o/CS = (F_v/F_m)\ [1 - (F_J - F_o)/(F_m - F_o)]\ F_o$
$DI_o/CS = F_o - TR_o/CS$
$RC/CS_m = [F_m/(F_v/F_m)]/\{M_o/[(F_J - F_o)/(F_m - F_o)]\}$
$PI_{ABS} = \{[1 - (F_o/F_m)]/(M_o/V_J)\}\ [(F_m - F_o)/F_o]\ [(1 - V_J)/V_J]$.

Table 1. The maximal quantum yield of PSII photochemistry (F_v/F_m), performance index (PI), trapped energy flux per cross section (TR_o/CS), electron transport flux per cross section (ET_o/CS), dissipated energy flux per cross section (DI_o/CS), and density of reaction center per excited cross section (RC/CS_m) in 22 Chinese winter wheat varieties exposed for 1 h to PPFD of 1,000 μmol m^{-2} s^{-1} at 25°C. Data were represented as means ± SE (n = 6–8). K-means cluster analysis were conducted with iterate and classify method using z-transformation of mean values of all the investigated fluorescence parameters. Mean values of clusters indicated by *different letters* are significantly different at $P<0.05$ according to LSD test. * Data were represented as mean ± SD.

Varieties	Release year	F_v/F_m	PI	TR_o/CS	ET_o/CS	DI_o/CS	RC/CS_m	Cluster
Xiaoyan 41	New line	0.730 ± 0.004^{a}	2.31 ± 0.17^{a}	304.2 ± 1.0^{ab}	199.4 ± 4.3^{a}	112.6 ± 2.6^{b}	681.6 ± 13.8^{a}	1
Xiaoyan 101	New line	0.722 ± 0.002^{ab}	2.15 ± 0.07^{ab}	303.4 ± 1.2^{ab}	197.4 ± 3.0^{a}	116.7 ± 0.9^{b}	668.7 ± 11.4^{a}	1
Fengdecunmai 1	2011	0.722 ± 0.006^{ab}	2.03 ± 0.09^{ab}	289.0 ± 4.6^{bc}	185.4 ± 5.4^{ab}	111.6 ± 3.4^{b}	626.5 ± 21.5^{ab}	1
Aikang 58	2005	0.720 ± 0.012^{ab}	2.04 ± 0.27^{ab}	280.0 ± 7.7^{bc}	177.0 ± 6.6^{b}	109.8 ± 8.2^{b}	604.9 ± 25.2^{b}	1
Wennong 14	2010	0.717 ± 0.005^{ab}	2.09 ± 0.11^{ab}	299.6 ± 1.9^{ab}	197.1 ± 5.3^{a}	118.3 ± 3.1^{b}	630.1 ± 16.5^{ab}	1
Linmai 2	2004	0.712 ± 0.011^{ab}	2.03 ± 0.33^{ab}	271.3 ± 2.8^{c}	172.9 ± 5.2^{bc}	110.2 ± 5.2^{b}	595.8 ± 42.2^{b}	1
Xiaoyan 60	New line	0.714 ± 0.004^{ab}	1.86 ± 0.15^{b}	302.1 ± 2.2^{ab}	194.1 ± 5.8^{ab}	121.4 ± 1.9^{ab}	603.7 ± 11.3^{b}	2
Yannong 19	2001	0.709 ± 0.006^{b}	1.96 ± 0.12^{ab}	302.5 ± 6.8^{ab}	200.5 ± 7.7^{a}	124.0 ± 3.9^{ab}	598.0 ± 22.8^{b}	2
Shannong 20	2010	0.707 ± 0.006^{b}	1.72 ± 0.15^{bc}	293.9 ± 5.0^{ab}	185.5 ± 9.6^{ab}	122.1 ± 5.1^{ab}	578.7 ± 8.5^{bc}	2
Misuimai	Unknown	0.706 ± 0.004^{bc}	1.74 ± 0.07^{bc}	287.6 ± 5.6^{bc}	185.5 ± 6.4^{ab}	119.9 ± 3.8^{b}	550.1 ± 8.6^{bc}	2
Tanmai 98	2009	0.706 ± 0.008^{bc}	1.87 ± 0.04^{b}	291.1 ± 2.6^{bc}	188.7 ± 4.3^{ab}	121.6 ± 4.1^{ab}	594.0 ± 23.4^{b}	2
Liangxing 99	2006	0.701 ± 0.003^{bc}	1.71 ± 0.04^{bc}	294.5 ± 3.3^{ab}	187.0 ± 4.4^{ab}	125.6 ± 2.7^{ab}	590.0 ± 14.4^{bc}	2
Jinfeng 4208	Unknown	0.701 ± 0.004^{bc}	1.74 ± 0.11^{bc}	298.0 ± 3.7^{ab}	193.0 ± 5.5^{ab}	127.3 ± 2.8^{ab}	565.0 ± 17.1^{bc}	2
Ke 181	Unknown	0.698 ± 0.008^{bc}	1.69 ± 0.18^{bc}	305.2 ± 4.1^{a}	196.3 ± 7.3^{a}	132.2 ± 4.0^{a}	572.6 ± 18.0^{bc}	2
Heng 0628	2008	0.697 ± 0.006^{bc}	1.70 ± 0.09^{bc}	296.2 ± 2.6^{ab}	191.8 ± 3.7^{ab}	128.6 ± 3.1^{ab}	559.3 ± 21.2^{bc}	2
Zhengmai 9405	2004	0.696 ± 0.016^{bc}	1.62 ± 0.27^{bc}	291.6 ± 5.6^{b}	180.6 ± 7.6^{b}	127.6 ± 8.3^{ab}	576.2 ± 51.0^{bc}	2
Lumai 13	1986	0.691 ± 0.008^{bc}	1.91 ± 0.18^{ab}	291.8 ± 3.4^{b}	197.3 ± 5.0^{a}	130.7 ± 3.9^{ab}	548.7 ± 20.9^{bc}	2
Nongda 340	Unknown	0.701 ± 0.006^{bc}	1.32 ± 0.06^{c}	280.6 ± 3.8^{bc}	160.8 ± 4.2^{c}	120.1 ± 3.6^{ab}	559.9 ± 17.2^{bc}	3
Han 00-7086	2007	0.690 ± 0.002^{bc}	1.41 ± 0.09^{c}	255.7 ± 4.7^{d}	152.3 ± 5.4^{c}	114.8 ± 2.1^{b}	509.6 ± 13.4^{c}	3
Jimai 20	2003	0.687 ± 0.004^{c}	1.39 ± 0.04^{c}	278.4 ± 6.3^{c}	170.2 ± 3.8^{bc}	126.6 ± 2.9^{ab}	519.8 ± 19.4^{c}	3
Yannong 23	2003	0.683 ± 0.004^{c}	1.69 ± 0.06^{bc}	270.3 ± 4.9^{c}	174.6 ± 2.8^{bc}	125.5 ± 2.4^{ab}	532.4 ± 14.8^{c}	3
Jinan 16	1998	0.679 ± 0.007^{c}	1.27 ± 0.05^{c}	272.0 ± 8.7^{c}	161.6 ± 3.5^{c}	128.1 ± 1.9^{ab}	508.8 ± 23.4^{c}	3
Cluster1*		0.721 ± 0.006^{a}	2.11 ± 0.11^{a}	291.3 ± 13.5^{a}	188.2 ± 11.5^{a}	113.2 ± 3.5^{a}	634.6 ± 34.2^{a}	
Cluster2*		0.702 ± 0.007^{b}	1.77 ± 0.11^{b}	295.9 ± 5.5^{a}	190.9 ± 6.0^{b}	125.5 ± 4.1^{b}	576.0 ± 19.0^{b}	
Cluster3*		0.688 ± 0.008^{c}	1.42 ± 0.16^{c}	271.4 ± 9.8^{b}	163.9 ± 8.7^{a}	123.0 ± 5.5^{b}	526.1 ± 21.2^{c}	

Chl content: The Chl content was measured directly by using a Chl meter *SPAD-502* (*Minolta*, Japan). In average of 10–15 readings from leaf base to leaf tip was recorded as a representative Chl content for each leaf. For each treatment, 4–6 leaves were measured.

Statistical analysis: Data were represented as mean ± standard error (SE) except for the data used for cluster analysis which were represented as mean ± standard deviation (SD). One way analysis of variance (*ANOVA*) analysis, multiple comparisons by LSD method, and K-means cluster analysis were conducted by using *SPSS* statistical software (*SPSS 13.0*). The mean values of the investigated fluorescence parameters were z-transformed before they were used for K-means cluster analysis which was carried out by using iterate and classify method. The number of clusters and maximum iterations were set as 3 and 10, respectively. Figures were conducted by using *Sigmaplot 10.0*.

Results

The effects of light intensity on photoinhibition: As shown in Table 1S (*supplement available online*), the values of F_v/F_m and PI significantly decreased after 1 h of 600 μmol(photon) m^{-2} s^{-1} compared to low light (LL, 300 μmol m^{-2} s^{-1}). The decrease of F_v/F_m and PI was further enlarged with the increase of light intensity, *e.g.*, PPFD ≥ 800 μmol m^{-2} s^{-1} resulted in photoinhibtion. The strongest PPFD (1,900 μmol m^{-2} s^{-1}) declined F_v/F_m and PI to 0.592 and 0.670, respectively, indicating that more damage due to photoinhibition occurred. Considering the extent of photoinhibition for different wheat genotypes, PPFD of 800–1,000 μmol m^{-2} s^{-1} was about three times higher than the growth light intensity and might be enough for evaluation of tolerance to photoinhibition in wheat varieties at the seedling stage.

The effects of temperature on photoinhibition: To explore the effects of temperature on photoinhibition, attached leaves in wheat seedling plants grown in LL were subjected for 1 h to PPFD of 800 μmol m^{-2} s^{-1} at different constant temperatures (Table 2S, *supplement available online*). Compared with the HL treatment at 25°C, the values of F_v/F_m and PI were significantly lower at lower temperature (20°C) and higher temperature (≥ 35°C), indicating that more severe photoinhibition took place under HL together with low or high temperature. However, at slightly high temperature (28°C), the F_v/F_m and PI were higher than those at 25°C, indicating that slightly higher temperature may decrease susceptibility to photoinhibition to some extent.

The effects of HL duration on photoinhibition: As illustrated in Fig. 1, the F_v/F_m and PI declined dramatically with prolonging HL duration. The decline of F_v/F_m seemed to occur in three phases: 10–30 min, 1 h, and 1.5–3 h according to differences in F_v/F_m values (Fig. 1*A*). Significant difference was observed between the three phases, reflecting the different photoinhibitory stress. Similar changes were observed in PI which amplitude was much more sensitive to HL than that of F_v/F_m (Fig. 1*B*). Consistent with the changes of F_v/F_m and PI, TR_o/CS (Fig. 2*A*), ET_o/CS (Fig. 2*B*), and RC/CS_m (Fig. 2*D*) declined, while DI_o/CS (Fig. 2*C*) increased significantly with prolonging photoinhibition. These dynamic changes in JIP-test parameters indicated that in accordance with the decrease of photosynthetic efficiency, the energy trapping flux, electron transport flux, and density of reaction center per activated cross section were also repressed, while thermal dissipation process was enhanced in response to photoinhition. When the HL-treated leaves were transferred back to darkness, the F_v/F_m increased gradually and ultimately restored approximately to the pretreatment level (Fig. 3). However, the time needed for dark recovery of F_v/F_m to the nontress level was related to HL duration, *e.g.*, 10–30 min of HL might need less than 10 h (Fig. 3*A*–*C*), 1–1.5 h of HL might need about 24 h (Fig. 3*D*,*E*), and 3 h of HL might need longer than 24 h for the recovery (Fig. 3*F*). Taken these results together, 1 h of the HL treatment seemed to be optimal duration for photoinhibitory treatment of wheat seedlings.

The effects of leaf age on photoinhibition: The leaves in wheat seedlings at 19–32 DAP were subjected to HL to determine the effects of leaf age on photoinhibition. As shown in Fig. 1S, before the HL treatment, the F_v/F_m ratio was not significantly different in the seedlings at 19–32 DAP (Fig. 1S*A*, *supplement available online*), while the PI at 19 DAP was significantly lower than those at 21–32 DAP (Fig. 1S*B*), suggesting that the photochemical efficiency was lower in seedlings at 19 DAP. After the HL treatment, both F_v/F_m and PI in seedlings at each DAP declined markedly in comparison with pretreatment level (Fig. 1S*C*,*D*). Additionally, they were both significantly lower at 19 DAP than those at 21–32 DAP, suggesting that younger leaves were more sensitive to HL than fully developed leaves. Therefore, the seedlings at 21–32 DAP might be suitable for photoinhibitory treatment due to a relative stable values of F_v/F_m and PI.

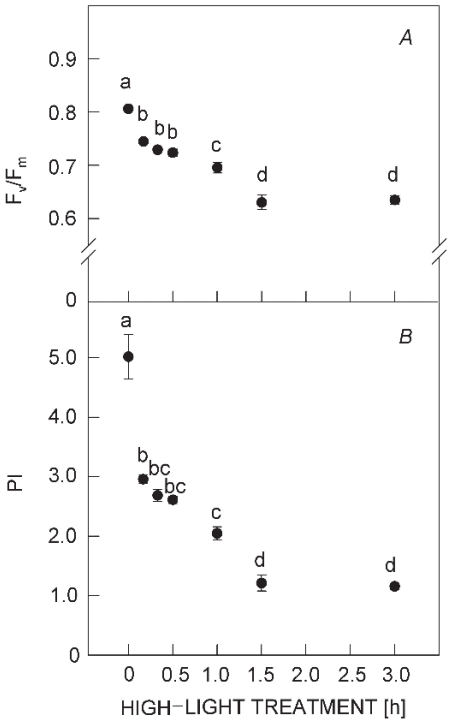

Fig. 1. Dynamic changes of maximal quantum yield of PSII photochemistry (F_v/F_m, *A*) and performance index (PI, *B*) in the attached fully expanded second leaves of winter wheat *Triticum aestivum* cv. Jing 411 subjected to 1,000 μmol m^{-2} s^{-1} PPFD. Data were represented as mean ± SE ($n = 6$). Measurements were carried out at the third-leaf stage. Mean values indicated by *different letters* are significantly different at $P<0.05$ according to LSD test.

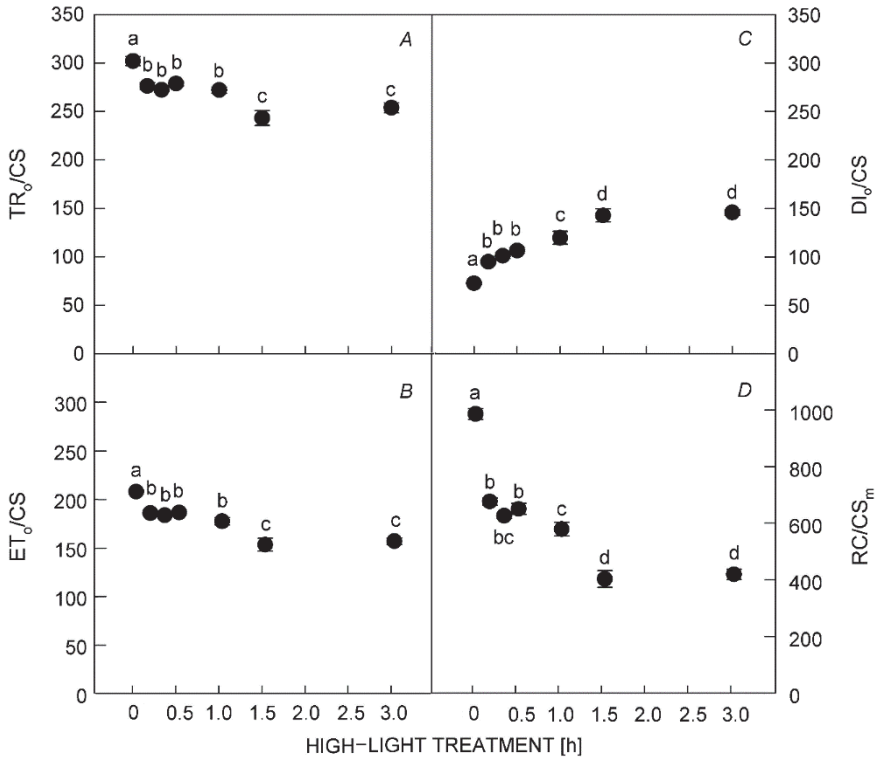

Fig. 2. Dynamic changes of the JIP-test parameters: trapped energy flux per cross section (TR_o/CS, *A*), electron transport flux per cross section (ET_o/CS, *B*), dissipated energy flux per cross section (DI_o/CS, *C*), and density of reaction center per excited cross section (RC/CS_m, *D*) in the attached fully expanded second leaves of winter wheat *Triticum aestivum* cv. Jing 411 subjected to 1,000 μmol m^{-2} s^{-1} PPFD. Data were presented as mean ± SE (n = 6). Measurements were carried out at the third-leaf stage. Mean values indicated by *different letters* are significantly different at P<0.05 according to LSD test.

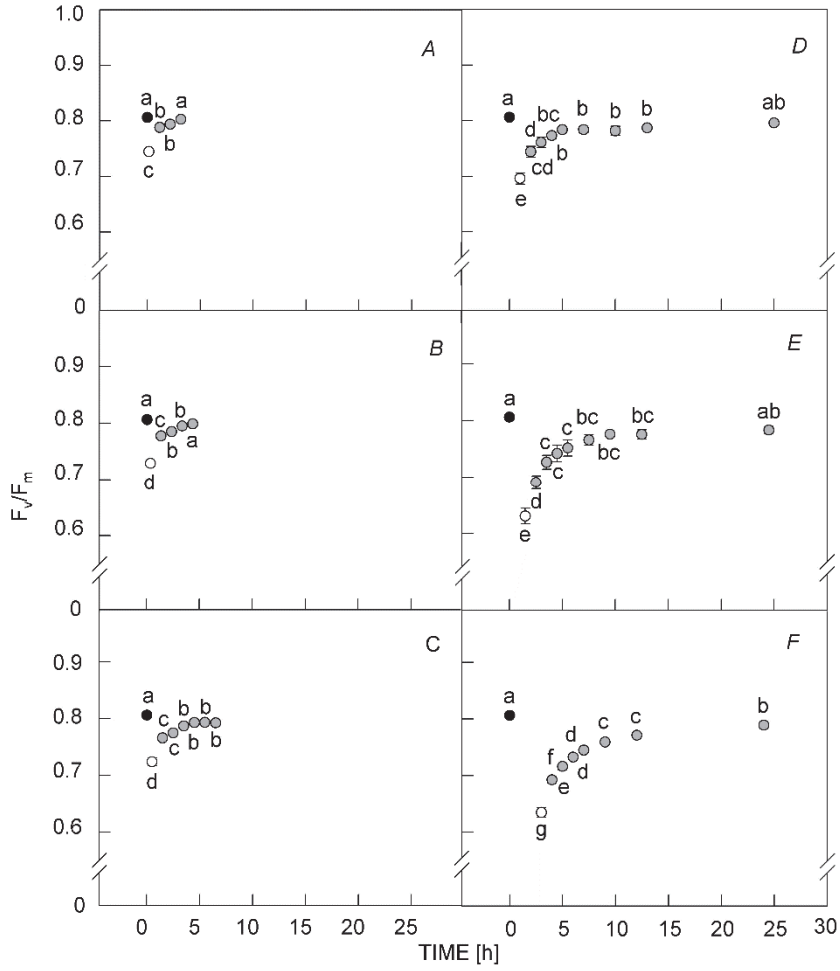

Fig. 3. The dark recovery of maximal quantum yield of PSII photochemistry (F_v/F_m) in the attached fully expanded second leaves of winter wheat *Triticum aestivum* cv. Jing411 subjected to PPFD of 1,000 μmol m^{-2} s^{-1} for 10 min (*A*), 20 min (*B*), 0.5 h (*C*), 1 h (*D*), 1.5 h (*E*), and 3 h (*F*) followed by darkness. Data were presented as mean ± SE (n = 6). Measurements were carried out at the third-leaf stage. The black circles indicate the pre-treatment control; the white circles indicate high-light treatment, while the grey circles indicate the restoration of F_v/F_m in darkness. Mean values indicated by *different letters* are significantly different at P<0.05 according to LSD test.

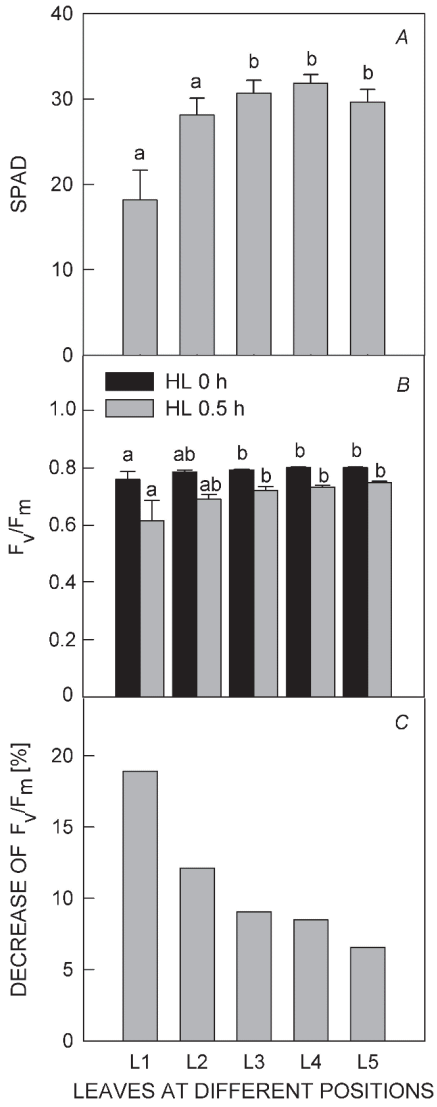

Fig. 4. The chlorophyll content (*A*), maximal quantum yield of PSII photochemistry (F_v/F_m, *B*), and the decrease of F_v/F_m (*C*) in leaves of different positions (L1–L5) in winter wheat *Triticum aestivum* cv. Jing411 subjected to PPFD of 1,400 μmol m^{-2} s^{-1} for 0.5 h. Data were presented as mean ± SE (n = 6). Mean values indicated by *different letters* are significantly different at P<0.05 according to LSD test.

The effects of leaf senescence on photoinhibition: In order to explore the effects of leaf senescence on photoinhibition, all five leaves in wheat plants at the sixth leaf stage were subjected to PPFD of 1,400 μmol m^{-2} s^{-1} for 0.5 h. The first leaves displayed senescent syndrome since both the Chl content (Fig. 4*A*) and F_v/F_m (Fig. 4*B*) were significantly lower than those of the other leaves before the HL treatment. After the HL treatment, F_v/F_m declined dramatically in all the five leaves at different leaf positions. Especially, the decrease of F_v/F_m was pronounced for the first leaves (Fig. 4*C*), followed by the second and the other leaves, indicating that the senescing leaves showed enhanced sensitivity to photoinhibition.

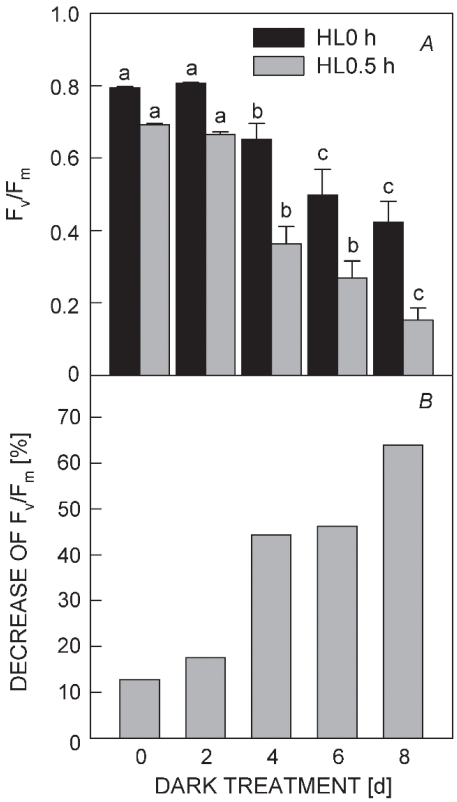

Fig. 5. The maximal quantum yield of PSII photochemistry (F_v/F_m, *A*) and the decrease of F_v/F_m (*B*) in the attached fully expanded second leaves of wheat seedlings *Triticum aestivum* cv. Jing411 exposed to PPFD of 1,000 μmol m^{-2} s^{-1} for 0.5 h after 0–8 d of darkness. Data were presented as mean ± SE (n = 6). Measurements were carried out at the third-leaf stage. Mean values indicated by *different letters* are significantly different at P<0.05 according to LSD test.

Additionally, the effects of dark-induced senescence on the photoinhibition was also evaluated. When the whole plants were subjected to darkness, their leaves turned yellow after 6 d, while F_v/F_m significantly decreased after 4 d of darkness (Fig. 5*A*). After the HL treatment, the F_v/F_m ratio decreased considerably and the decrease was pronounced from 4 d of darkness, when F_v/F_m was reduced by 44.3–63.9% (Fig. 5*B*). This experiment suggested that darkness longer than 4 d enhanced susceptibility to photoinhibition, while darkness shorter than 2 d seemed to have no effects on HL-induced photoinhibition.

Evaluation of wheat genotypes for tolerance to photoinhibtion: Twenty-two Chinese winter wheat varieties or advanced lines were assayed for tolerance to photoinhibition by exposing attached leaves in seedlings, grown under PPFD of 300 μmol m^{-2} s^{-1}, for 1 h to PPFD of 1,000 μmol m^{-2} s^{-1} at 25°C. Beforethe treatment, the values of F_v/F_m in these genotypes were all around 0.8, not significantly different, and also no significant difference was observed for the other fluorescence parameters (data not shown). However, after the HL treatment, the values of

F_v/F_m, PI, and other JIP-test parameters were significantly different in these genotypes (Table 1). According to the K-means cluster analysis, these 22 varieties can be classified as three clusters: cluster 1 included six varieties (Xiaoyan 41, Xiaoyan 101, Fengdecunmai 1, Aikang 58, Wennong 14, and Linmai 2), cluster 3 included five varieties (Nongda 340, Han 00-7086, Jimai 20, Yannong 23, and Jinan 16), and cluster 2 consisted of 11 varieties (Xiaoyan 60, Yannong 19, Shannong 20, Misuimai, Tanmai 98, Liangxing 99, Jinfeng 4208, Ke 181, Heng 0628, Zhengmai 9405, and Lumai 13). The wheat genotypes in cluster 1 conferred significantly higher F_v/F_m, PI, TR_o/CS, ET_o/CS, and RC/CS_m, but lower DI_o/CS than those in cluster 3, indicating that these varieties in cluster 1 maintained higher PSII photochemical efficiency, energy trapping flux, electron transport flux, and density of reaction center, but lower dissipated energy flux per activated cross section relative to cluster 3. Therefore, it seemed that wheat varieties in cluster 1 were tolerant to photoinhibition induced by short-term HL relative of those in cluster 3 which appeared to be susceptible to photoinhibition. For cluster 2 consisting of other 11 varieties, the values of PSII photochemical efficiency-related parameters, such as F_v/F_m, PI, and RC/CS_m, were between cluster 1 and cluster 3, indicating that they might show intermediate tolerance to photoinhibition.

Discussion

We analyzed the factors influencing photoinhibitory treatment and established a possible method to assess tolerance to photoinhibition in wheat. Under field conditions, photoinhibition is largely determined by light intensity intercepted by leaves which is dynamic due to clouds, time, season, *etc*. However, when carrying out HL-induced photoinhibition in a laboratory by using lamps as light source, a tradeoff should be considered between the simultaneous output of light intensity and heat and the input of electricity. For instance, in this work, PPFD of 800 μmol m^{-2} s^{-1} for 1 h was about 2.7 times higher than the growth light intensity (300 μmol m^{-2} s^{-1}) and could decline F_v/F_m from 0.793 to 0.709, resulting in photoinhibiton. Although 1 h of 1,900 μmol(photon) m^{-2} s^{-1} could decrease F_v/F_m and PI to 0.592 and 0.670, accounting for 74.7 and 84.5% of the LL control, respectively, the photoinhibition-induced damage on photosynthetic apparatus may exceed its repair capability. Hence highly exessive HL might be unsuitable for evaluation of tolerance to photoinhibition in various wheat genotypes. Our data suggested that PPFD of 800–1,000 μmol m^{-2} s^{-1}, about three times higher than the growth light intensity, might be suitable for screening photoinhibition-tolerant wheat genotypes at their seedling stage.

In the field, other stresses, such as low temperature, high temperature, and/or water deficit, usually accompany HL and exacerbate photoinhibition process (Al-Khatib and Paulsen 1989, Hetherington *et al*. 1989, Hurry and Huner 1992, Fuse *et al*. 1993, Yin *et al*. 2010). Therefore, it is important to keep suitable temperature when performing photoinhibitory treatments in a laboratory. In this work, compared with 25°C, the values of F_v/F_m and PI were significantly lower at lower (20°C) and higher temperature (> 35°C), but were higher at slightly high temperature (28°C), which was consistent with low and high temperature-induced photoinhibition (Al-Khatib and Paulsen 1989, Hetherington *et al*. 1989, Hurry and Huner 1992, Fuse *et al*. 1993,Yin *et al*. 2010). The high values of F_v/F_m and PI at slightly high temperature (28°C) may occur because of protection of photosynthetic apparatus due to high temperature tolerance.

Apart from light intensity and temperature, HL duration also largely determined photoinhibition. For instance, even 10–30 min under PPFD of 1,000 μmol m^{-2} s^{-1} significantly decreased F_v/F_m, PI, and other JIP-test fluorescence parameters (TR_o/CS, ET_o/CS, and RC/CS_m) and the decrease was enlarged with prolonging of HL treatment for 1–3 h. However, longer duration of the HL treatment needed more time to restore F_v/F_m to previous control level, suggesting that more severe photoinhibition took place during longer duration of HL treatment such as 1.5 h and 3 h. Considering evaluation efficiency and time needed to perform fluorescence measurement, 1 h of HL was suitable for photoinhibitory treatments to evaluate wheat genotypes.

In addition, wheat leaf longevity or developmental stage affects its response to photoinhibition. For instance, developmental leaf senescence (Lu *et al*. 2001, 2003) as well as newly developed or etiolated leaves (Mahmudov *et al*. 2005) were susceptible to photoinhibition. In this work, leaves in wheat seedlings at ≤ 19 DAP were sensitive to photoinhibition, while those at 21–32 DAP reached a relative stable results, indicating that leaves in seedlings at 21–32 DAP might be suitable for evaluation of tolerance to photoinhibition. As the senescing first leaves in wheat seedlings at sixth leaf stage and ≥ 4 d of dark-induced senescence enhanced susceptibility to photoinhibition, the senescing leaves are unsuitable for assessment of photoinhibitory responses of wheat genotypes.

In this work, 22 Chinese winter wheat varieties were evaluated for tolerance to photoinhibition. According to cluster analysis of the mean values for F_v/F_m, PI, and other JIP-test fluorescence parameters, these wheat varieties were classified as three clusters. Cluster 1 consisted of six varieties that conferred significantly higher photosynthetic efficiency-related parameters, such as F_v/F_m, PI, TR_o/CS, ET_o/CS, and RC/CS_m, but lower thermal energy dissipated flux (DI_o/CS) after photoinhibitory treatment relative of cluster 3 including five wheat varieties. Therefore, wheat varieties in cluster 1 appeared to be relatively tolerant compared to those in cluster 3. The wheat varieties in cluster 2, consisting of 11 wheat varieties, seemed to

confer intermediate tolerance to photoinhibition as the values of PSII photochemical efficiency-related F_v/F_m, PI, and RC/CS_m were between cluster 1 and cluster 3. To some extent, cluster analysis based on six JIP-test fluorescence parameters, involving PSII photochemical efficiency, energy trapping, electron transport, energy dissipation, and density of reaction center, might be more precise than only one parameter such as F_v/F_m in order to evaluate wheat genotypes. Our results suggested that most varieties from traditional breeding programs showed intermediate tolerance or susceptibility, but a small number of varieties showed tolerance. It indicates that during wheat breeding history, the tolerance to photoinhibition in wheat varieties was not efficiently selected. But interestingly, two tolerant varieties, Xioayan 101 and Xiaoyan 41 in cluster 1, [Xiaoyan 101 was derived from Xiaoyan 41 and Liangxing 99 (cluster 2)], suggest that it is possible to select new wheat varieties with enhanced tolerance from a lot of progenies by crossing photoinhibition-tolerant and intermediate tolerant varieties. Therefore, this work may provide useful method for screening for new wheat varieties with improved tolerance to photoinhibitory stress.

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