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Responses of photosynthetic characteristics and antioxidative metabolism in winter wheat to post-anthesis shading

C. XU[*], Y. YIN[*], R. CAI[*,**], P. WANG[*], Y. NI[*], J. GUO[*], E. CHEN[*], T. CAI[*], Z. CUI[*], T. LIU[*], D. YANG[*], and Z. WANG[*,+]

State National Key Laboratory of Crop Biology, Agronomy College, Shandong Agricultural University, Tai'an 271018, Shandong Province, China[*]
Life Science and Technology College, Hebei Normal University of Science & Technology, Changli 066600, Hebei Province, China[**]

Abstract

In a field experiment, two winter wheat (*Triticum aestivum* L.) cultivars, Tainong 18 (a large-spike cultivar) and Jinan 17 (a multiple-spike cultivar), were treated with 78% (S1), 50% (S2), and 10% (S3) of full sunshine (S0, control) from anthesis to maturity to determine the responses of photosynthetic characteristics and antioxidative enzyme activities in a flag leaf. Compared with S0 treatment, the chlorophyll (Chl) content and maximal efficiency of photosystem II (PSII) photochemistry (F_v/F_m) of flag leaves were enhanced in treatments S1 and S2. From 0 to 7 d post flowering, the Chl content and F_v/F_m in S3 were also higher than those in S0, but significantly lower than those in controls, respectively. With the increase of shading intensity, the effective quantum yield of PSII (Φ_{PSII}) was promoted; whereas, the ratio of Chl *a*/*b* declined. Compared with S0, treatments S2 and S3 significantly suppressed the activities of superoxide dismutase (SOD) and peroxidase (POD), net photosynthetic rate (P_N), and contents of total soluble sugar, nevertheless, S1 treatment showed positive effects on the above parameters. Under the same shading condition, Jinan 17 had larger Chl content and higher activities of PSII and antioxidative enzymes, but lower malondialdehyde (MDA) content than Tainong 18. The results indicated that multiple-spike cultivar was more advantageous for the Huang-Huai-Hai Plain, where shading problem occurs later during the growth period, than the large-spike cultivar, because of the lesser damage in a flag leaf and better photosynthetic function of the former one. Wheat plants under S1 shading condition had relatively high activities of antioxidative enzymes and a low degree of membrane lipid peroxidation, which was in favor of stress resistance, maintaining high P_N duration, and accumulation of photosynthates in wheat plants.

Additional key words: antioxidative metabolism; photosynthetic traits; shading; *Triticum aestivum* L.

Introduction

Grain yield in wheat is dependent on photosynthate production and allocation. Light intensity is one of the main factors affecting it. Huang-Huai-Hai plain is one of the main wheat production areas in China. Shading after flowering, which is caused by cloudy weather and by surrounding protective trees, often occurs on this plain. As the data show, solar radiation and sunshine duration have been declining continuously in the downstream plain of the Yellow River during last 50 years (Yan *et al*. 2003, Xu and Zhao 2005, Yang *et al*. 2005). The down-trend, which mainly happens in summer and winter, is still going on (Xu and Zhao 2005, Yang *et al*. 2005).

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[+]Corresponding author; tel.: +86-538-8241359, e-mail: zlwang@sdau.edu.cn
Abbreviations: APX – ascorbic acid peroxidase; AsA-GSH – ascorbate-glutathione; CAT – catalase; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DAA – days after anthesis; ETR – electron transport rate; F_m – maximum fluorescence; F_m' – maximum fluorescence of light-adapted state; F_s – steady state fluorescence; F_v/F_m – maximal efficiency of photosystem II photochemistry; F_0 – minimal fluorescence; FM – fresh mass; g_s – stomatal conductance; MDA – malondialdehyde; NBT – nitroblue tetrazolium; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; POD – peroxidase; PSII – photosystem II; ROS – reactive oxygen species; SOD – superoxide dismutase; Φ_{PSII} – effective quantum yield of photosystem II photochemistry.
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In addition, this plain is also the most important fruit and industrial tree production area. Thus, various intercropping systems of trees and wheat are developed to ensure simultaneously the production of wheat, fruit, and industrial trees. Poplar-wheat is one of the most important intercropping systems in this plain (Fang *et al*. 1999). Light intensity of canopy in this intercropping system is only 30.8–80.2% of a single culture field (Burgess *et al.* 2005, Yu *et al*. 2007).

Photosynthesis is the basis of a crop production and higher photosynthesis product accumulation is the premise of the crop yield (Arisnabarreta and Miralles 2008). Photosynthates of functional leaves, physiological activity of which directly influences the dry matter accumulation and allocation, contribute over 80% of dry matter to grain yield during grain filling in winter wheat (Murchie *et al*. 1999). Light intensity is an important factor affecting crop photosynthetic production (Guo *et al*. 2010). Changes in radiation influence both photosynthetic light- and carbon-use efficiency, and this ultimately affects the total grain yield (Bell *et al*. 2000, Jiang *et al*. 2002, Greenwald *et al*. 2006, Zhang *et al*. 2007). Most researchers studied the effect of light intensity on the growth and development of crops through shading (Bell *et al*. 2000, Jiang *et al*. 2002, Zhang *et al*. 2007, Guo *et al*. 2010). Both pre-anthesis and post-anthesis shading winter wheat caused a decrease in both dry matter accumulation and allocation to grain and then reduced the grain yield (Wang *et al*. 2003). Shading winter wheat from booting stages reduced the dry matter of the ears (Demotes-Mainard and Jeuffroy 2004). Shading also significantly impaired P_N *via* changes in the photosystem II (PSII) functioning (Burkey and Wells 1991, Mitchell *et al*. 1996, Mu *et al*. 2010), and it had been found that the Chl content of flag leaves increased and Chl *a*/*b* ratio decreased under such conditions (Dai *et al*. 2009). Meanwhile, the light intensity could significantly impair antioxidative metabolism in the plant (Schöner and Krause 1990). It was found that shading not only increased the throughput rate of O_2^- and the content of H_2O_2, but it also enhanced the activity of SOD, POD, and catalase (Huang *et al*. 2008). It should be noted that the activity and a cooperative relationship of antioxidative enzymes determined the stress resistance of crops (Ge *et al*. 2005). However, no evidence was given to indicate the relationship between photosynthetic characteristics and antioxidative metabolism in flag leaves of wheat under shading.

To date, most research studies have focused on the grain yield and a quality influenced by shading (Wang *et al*. 2003, Demotes-Mainard and Jeuffroy 2004, Estrada-Campuzano *et al*. 2008). However, the impact of shading on photosynthetic characteristics, antioxidative metabolism, and the relationship between both has not been documented in flag leaves of winter wheat. Therefore, our experiment was done in a field with two winter wheat (*Triticum aestivum* L.) cultivars of a different spike and kernel type. The objectives of the present study were: (*1*) to test the photosynthetic performance, total soluble sugar content and antioxidative metabolism under shading, and (*2*) to examine the response to shading in flag leaf of wheat cultivars with a different genotype, grown in the field, which might provide a theoretical basis for a selection of suitable cultivars for higher grain yield of winter wheat in the Huang-Huai-Hai plain of China.

Materials and methods

Field design: The field experiment was carried out from October 2010 to June 2011 (growing season) at Tai'an Experimental Station (36°09′N, 117°09′E) of Shandong Agricultural University, Tai'an, Shandong Province, China. The soil type was a loam containing 13.5 g kg^{-1} organic matter, 0.89 g kg^{-1} available N, 26.6 mg kg^{-1} available phosphate, and 75.2 mg kg^{-1} available K. Maize (*Zea mays* L.) was the previous crop and the straw was returned to the field. Fig. 1 shows the climate data from March to June in 2011.

Two high-yield winter wheat (*Triticum aestivum* L.) cultivars currently used in a local wheat production, Tainong 18 and Jinan 17, were used. Before sowing, 120 kg(N) ha^{-1}, 105 kg(P_2O_5) ha^{-1}, and 105 kg(K_2O) ha^{-1} were applied as a basal fertilizer and another 120 kg(N) ha^{-1} was top-dressed at the jointing stage. Seeds were sown on 12 October 2010 to produce a density of 180 plants m^{-2} with a row space of 0.25 m.

The top of wheat canopy was covered by different layers of black net screens starting from anthesis (12 May 2011) to maturity (14 June 2011) to provide three shading treatments, *i.e*. shading with 1, 2, and 3 layers of the screen, which blocked about 22% (S1), 50% (S2), and 90% (S3) of the full radiation above the canopy, respectively. No shading was set as the control (S0). The screens were more than 180 cm above the ground to ensure good ventilation and they were large enough to fully cover the corresponding shaded plots. The experiment was a split-plot design with shading as the main plot and wheat cultivar as subplot and 3 replicates for each. The size of each plot was 3 m × 5 m.

Sampling: During the anthesis, ears, flowered on the same day, were labeled for an uniformity in size and developmental stage. Flag leaves of the labelled plants in each treatment were sampled at 7-d intervals from the anthesis to 28 d after the anthesis (DAA). A part of the flag leaves was frozen immediately in liquid N, for at least 30 min and then stored at −40°C for Chl content, antioxidative enzyme activity, and MDA content assays. The second part was sterilized at 105°C, then oven-dried at 70°C and ground into powder for total soluble sugar

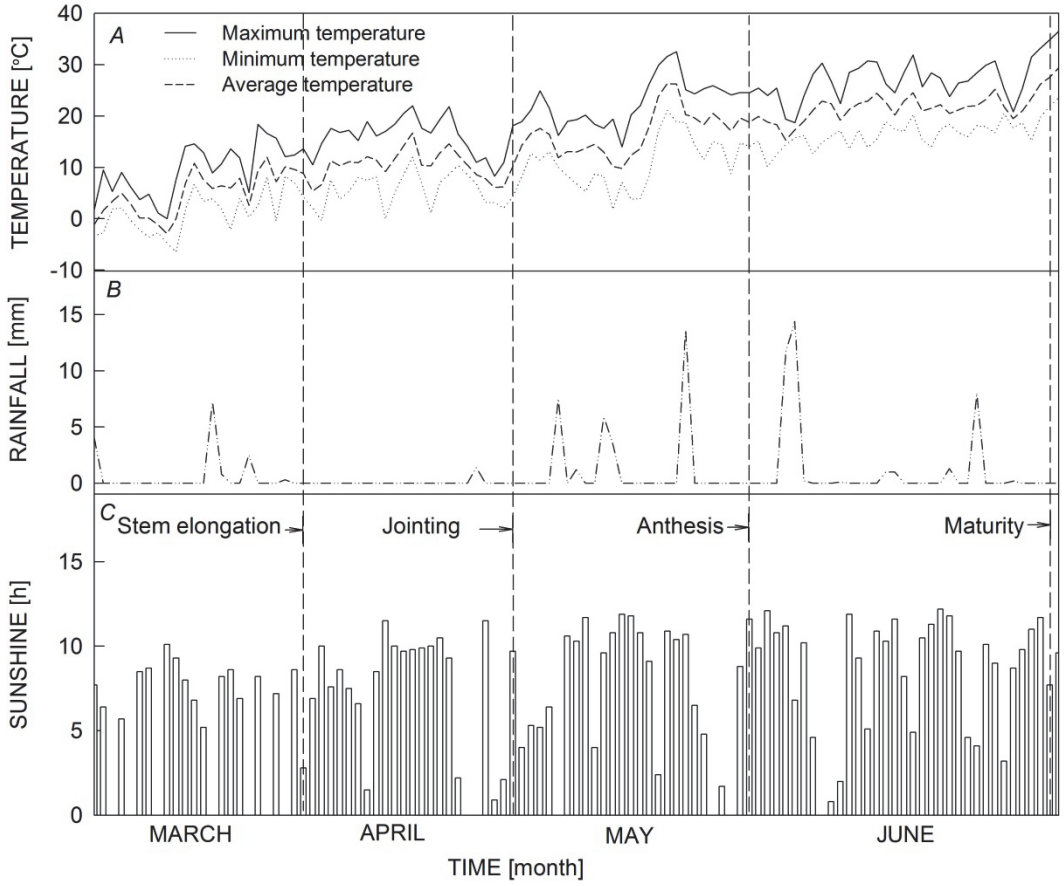

Fig. 1. Climate data (*A*, temperature; *B*, rainfall; and *C*, sunshine hours) from March to June 2011.

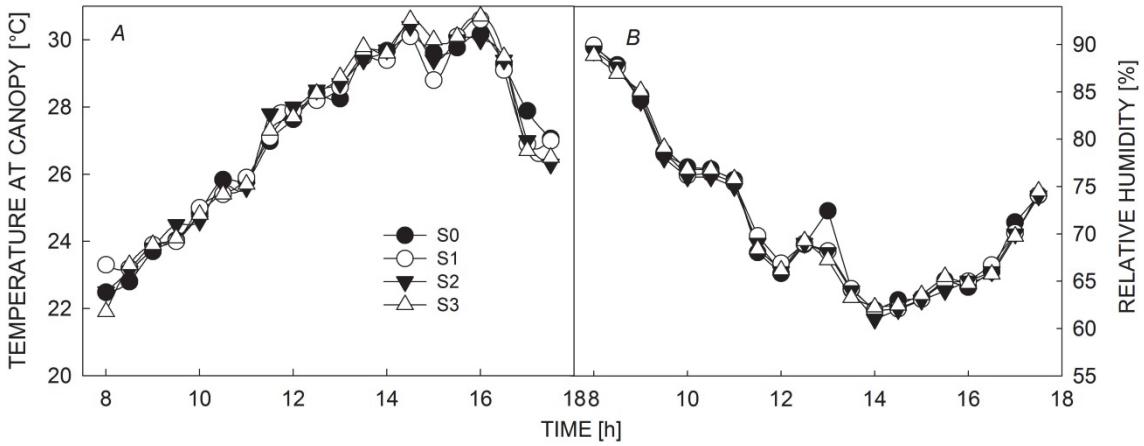

Fig. 2. Microclimate (*A*, temperature at canopy and *B*, relative humidity) in wheat canopy under shading at 20 DAA from March to June 2011. S0 refers to no shading (control); S1, S2, and S3 refer to shading treatments (78%, 50%, and 10% of full sunshine, respectively).

content measurement. The third part was left intact for detecting photosynthetic parameters and Chl fluorescence.

Microclimate in the field: The temperature at the canopy level and relative humidity under net screens were measured with a *HOBO H8 datalogger* (*MicroDAQ,* Contoocook, NH, USA). All the data were monitored every 30 min from 8:00 to 18:00 at 20 DAA. The data were shown in Fig. 2.

Chl content: Leaves (0.1 g) were sliced and incubated

with 25 ml of an extraction solution containing equal volumes of acetone and anhydrous ethanol. After complete extraction in the dark, Chl content and Chl *a*/*b* were colorimetrically analyzed according to Arnon (1949).

Photosynthetic parameters and Chl fluorescence parameters: P_N, stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of the flag leaf were measured with a portable photosynthesis system (*CIRAS-II, PP systems*, Hitchin, UK). The chamber was equipped with a red/blue LED light source. The PAR was set at 1,200 μmol m^{-2} s^{-1}. The measurements were carried out with an open system.

Chl fluorescence parameters of the flag leaves were measured with a pulse-modulated fluorometer (*FMS-2, Hansatech*, UK). The minimal and maximal fluorescence (F_0 and F_m) were determined after full-dark adaptation for 20 min. The steady-state fluorescence (F_s) and the maximum fluorescence in a light-adapted state (F_m') were determined under actinic light of 1,200 μmol m^{-2} s^{-1} for 10 min and an 1-s pulse of saturating radiation of 4,000 μmol m^{-2} s^{-1}, respectively. The F_v/F_m, Φ_{PSII}, and the electron transport rate (ETR) were calculated according to Li *et al*. (2007).

Total soluble sugar content: 0.05 g of powder, consisting of grounded flag leaves, was dispersed and mixed with 5 ml of deionized water, and then the extract was heated for 30 min at 100°C. After centrifugation for 5 min at 4,000 × *g* and a removal of the supernatant, the pellets were resuspended and reextracted twice. Three supernatants were combined in 25 ml volumetric flask and added to the volume by deionized water. Then, 1 ml of solution was taken into a glass tube, and 3 ml of anthrone reagent (4.5 mg anthrone in 88% sulfuric acid) were added. The sample was mixed and heated for 10 min at 100°C. The absorbance at 620 nm was measured after the sample was cooled down.

Antioxidative enzyme activity and MDA content: 0.5 g of leaves was homogenized in 5 cm^3 of a respective extraction buffer (50 mM phosphate buffered saline (PBS) + 0.4% polyvinylpyrrolidone (PVP), pH 7.0) in a prechilled mortar and pestle on ice. The homogenate was centrifuged at 10,000 × *g* for 30 min at 4°C and the supernatant was collected as a crude enzyme extract.

SOD (EC 1.15.1.1.) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) according to Giannopolitis and Ries (1977). One unit SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored spectrophotometrically (*UV-2401, Shimadzu Corp.*, Japan) at 560 nm. Activity was expressed as units (U) per gram of fresh leaf mass (FM).

POD (EC 1.11.1.7) activity was determined using the guaiacol oxidation method (Klapheck *et al*. 1990). Guaiacol oxidation was monitored spectrophotometrically for 60 s at 470 nm. The activity was expressed as units (guaiacol oxidation product per minute) per gram of FM of leaf tissue.

CAT (EC 1.11.1.6) activity was measured by monitoring the decrease in absorbance at 240 nm for 60 s as a consequence of H_2O_2 consumption (Aebi 1984). Activity was expressed as units (H_2O_2 decomposed per minute) per gram of FM of leaf tissue.

MDA was estimated by measuring the content of 2-thiobarbituric acid-reactive substances in a supernatant, prepared in 20% trichloracetic acid containing 0.5% 2-thiobarbituric acid, and heated at 95°C for 25 min. MDA content was then determined spectrophotometrically as the absorbance at 532 nm (A_{532}) and corrected for nonspecific turbidity at 600 nm (A_{600}).

Statistical analysis: The one-way analysis of variance (*ANOVA*) was used to test the significance among treatments or between cultivars.

Results

Chl content: The Chl contents in the flag leaf of S1 and S2 were higher than that in S0, and increased with increasing shading intensity. Before 7 DAA, the content of Chl was higher in the flag leaf of S3 than of S0, while it was lower after 14 DAA. At 28 DAA, the Chl content increased in the flag leaf of S1 and S2 by 10.3 and 24.7% in Tainong 18, and by 9.1 and 20.8% in Jinan 17, respectively. In addition, the Chl content decreased in the flag leaf in both Tainong 18 and Jinan 17 in S3 by 14.0 and 6.4%, respectively (Fig. 3*A*,*B*). However, the ratio of Chl *a*/*b* was lower under S2 and S3 compared with S0, and decreased with increasing shading intensity. The Chl *a*/*b* decreased in the flag leaf of Tainong 18 at S1 between 0 and 14 DAA, and in Jinan 17 between 0 and 7 DAA (Fig. 3*C*,*D*).

Chl fluorescence: The Φ_{PSII} and ETR in both wheat cultivars increased along with the increasing shading intensity (Fig. 4*C*–*F*). Meanwhile, the F_v/F_m of flag leaf increased under shading (except S3 after 14 DAA) in both cultivars (Fig. 4*A*,*B*). This indicated that S1 and S2 did not essentially damage the potential light conversion efficiency of PSII in wheat flag leaf, which could keep higher photochemical turnover efficiency and make full use of the absorbed irradiance compared with the control. However, the adaptability to the heavy shading (S3) lasted only shortly.

***P_N* and its correlative parameters**: Shading after anthesis showed different effect on g_s and C_i of the flag leaf in wheat. The g_s decreased significantly under

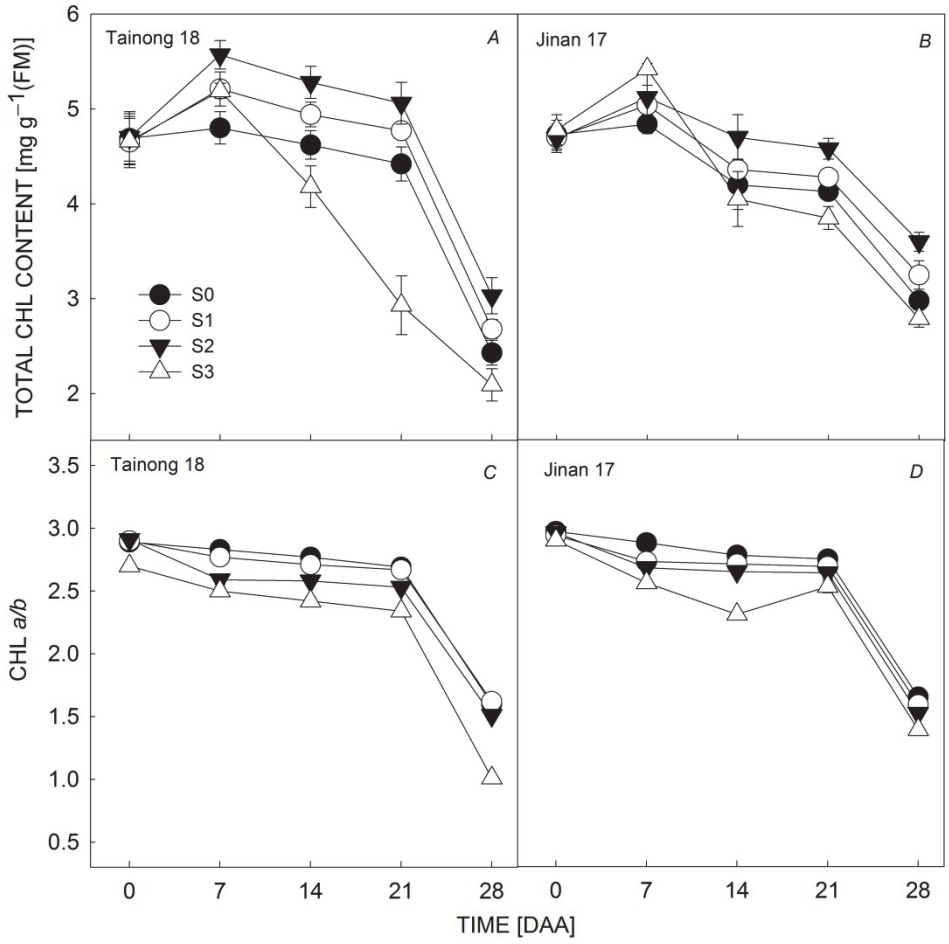

Fig. 3. Post-anthesis shading effects on chlorophyll (Chl) content (*A*,*B*) and Chl *a/b* (*C,D*) in flag leaf of winter wheat cultivars Tainong 18 and Jinan 17. S0 refers to no shading (control); S1, S2, and S3 refer to shading treatments (78%, 50%, and 10% of full sunshine, respectively). Data represent means ± SD of 3 replicates.

shading in both cultivars, while C_i increased. The effect on P_N of flag leaf was dependent on the level of shading applied and the effect was also cultivar-dependent. In both cultivars, P_N was significantly reduced in S2 and S3. During the grain filling phase, P_N decreased in Tainong 18 by 24.4 and 72.6% in S2 and S3, respectively. However, in Jinan 17, it was decreased by 17.4 and 65.8% in S2 and S3, respectively. There was no significant difference in the P_N of flag leaf between low-intensity light (S1) and the non-shading (S0) treatments. However, the P_N was higher in S0 than under S1 before 14 DAA, while it was higher in S1 compared with S0 after 21 DAA (Table 1). This indicated that low-intensity light (S1) could maintain photosynthesis at a higher level for a longer time in winter wheat. In addition, Jinan 17 was found to be more tolerant to shading than Tainong 18.

Total soluble sugar content: The total soluble sugar content was lower in the flag leaves in S1 than in S0 during the whole grain filling phase in both cultivars except for 14 to 21 DAA in Jinan 17. The total soluble sugar content was significantly reduced in both cultivars under S2 and S3 treatments. Under S2 and S3 treatments at 21 DAA, the total soluble sugar content significantly decreased in the flag leaves of Tainong 18 by 35.0 and 52.0%, respectively; in Jinan 17, the total soluble sugar contents significantly decreased in the flag leaves under S2 and S3 treatments at 21 DAA by 24.5 and 56.4%, respectively. Beyond that, the total soluble sugar content in Tainong 18 decreased by 67.2 and 88.2% at S2 and S3 treatments compared with S0 at 28 DAA, while it decreased by 68.2 and 79.6% in Jinan 17 (Fig. 5). The results indicated that low-intensity shading improved the synthesis of total soluble sugar and promoted a remobilization of photosynthates in Jinan 17 compared with Tainong 18.

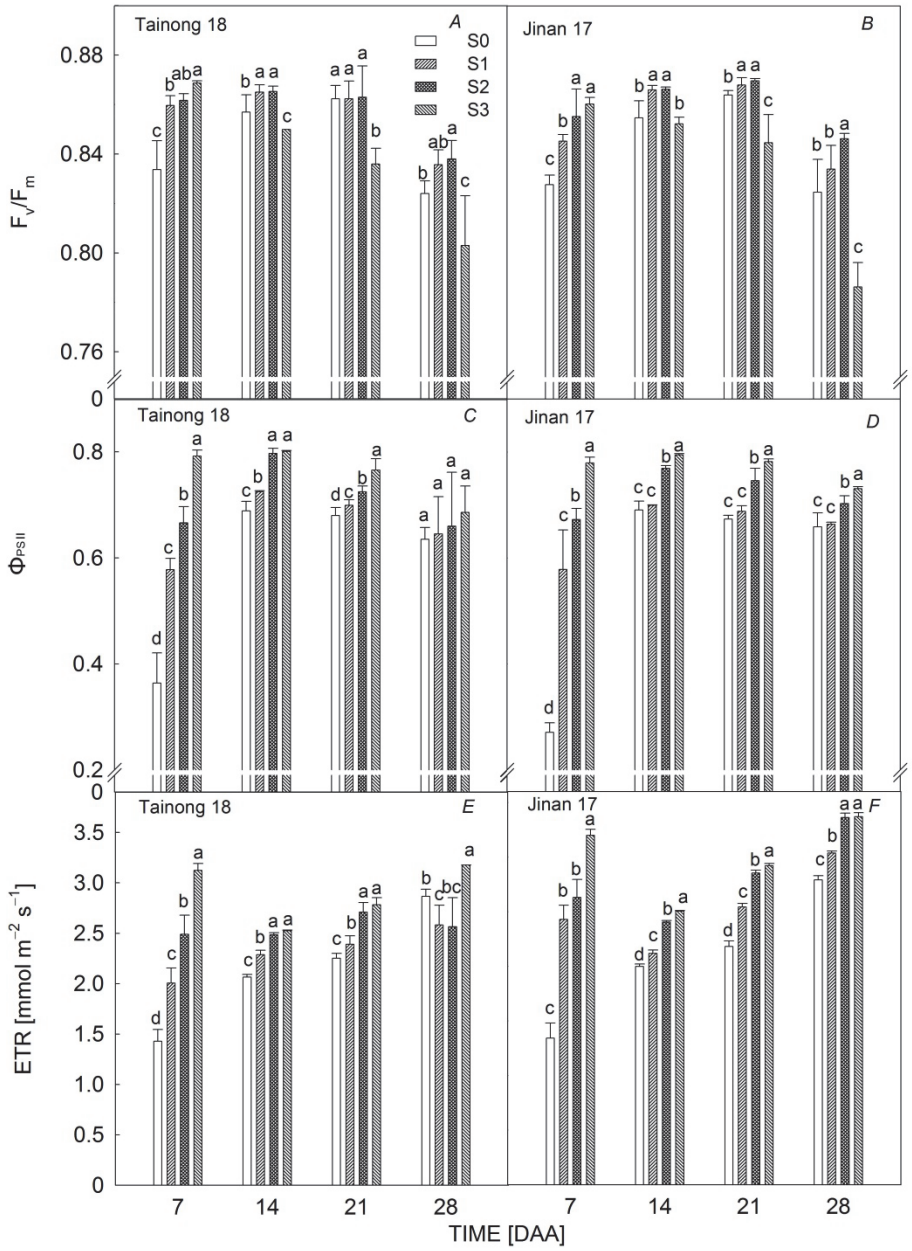

Fig. 4. Post-anthesis shading effects on maximal efficiency of photosystem II photochemistry (F_v/F_m) (*A,B*), effective quantum yield of photosystem II photochemistry (Φ_{PSII}) (*C,D*) and electron transport rate (ETR) (*E,F*) in the flag leaves of winter wheat cultivars Tainong 18 and Jinan 17. S0 refers to no shading (control); S1, S2, and S3 refer to shading treatments (78%, 50%, and 10% of full sunshine, respectively). Data represent means ± SD of 3 replicates. Different *small letters* in each group indicate significant differences at $P<0.05$.

Antioxidative enzyme activity: The SOD activity increased under S1 treatment in both cultivars, but decreased in S2 and S3 treatments. At 28 DAA, the activity of SOD increased by 3.0% in the flag leaves of S1 in Tainong 18 and 5.1% in Jinan 17. In addition, the SOD activity decreased by 31.7 and 71.9% in S2 and S3 treatments, while it decreased by 13.3 and 29.0% in Jinan 17 (Fig. 6*A*,*B*). In both cultivars, the POD activity and the SOD activity showed a similar trend under shading treatments (Fig. 6*C*,*D*). No significant difference was found in the activity of CAT under S0, S1, and S2 treatments, but the activity of CAT significantly decreased under S3 treatment. In addition, the activity of CAT in both cultivars showed a similar trend under shading conditions (Fig. 6*E*,*F*). These results suggested that relatively low-intensity shading (S1) could improve the activity of antioxidative enzymes.

Table 1. Effects of shading on net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in flag leaves of winter wheat cultivars Tainong 18 and Jinan 17 7, 14, 21, and 28 days after anthesis (DAA). S0 refers to no shading (control); S1, S2, and S3 refer to shading treatments (78%, 50% and 10% of full sunshine, respectively). Data represent means ± SD of 3 replicates. Different *small letters* in each column in same cultivar indicate significant differences at $P<0.05$.

Stage	Treatment	Tainong 18			Jinan 17		
		P_N [μmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]	P_N [μmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]
7 DAA	S0	18.87 ± 0.53^a	393.67 ± 26.23^a	40.67 ± 7.81^d	18.50 ± 0.63^a	504.33 ± 28.58^a	35.67 ± 11.53^d
	S1	17.73 ± 0.80^b	369.33 ± 6.08^b	78.33 ± 4.36^c	17.50 ± 0.60^a	454.00 ± 23.43^b	68.33 ± 9.54^c
	S2	14.67 ± 1.04^c	279.67 ± 23.64^c	95.33 ± 8.72^b	14.53 ± 0.44^b	290.67 ± 5.00^c	113.00 ± 3.00^b
	S3	5.43 ± 0.36^d	64.67 ± 7.00^d	103.00 ± 4.58^a	6.57 ± 1.65^c	77.67 ± 30.51^d	130.00 ± 4.58^a
14DAA	S0	19.37 ± 0.78^a	309.67 ± 14.73^a	100.67 ± 3.60^d	18.83 ± 0.10^a	264.33 ± 17.09^a	90.00 ± 10.39^d
	S1	18.20 ± 1.14^b	260.33 ± 2.65^b	123.67 ± 8.00^c	18.87 ± 1.06^a	269.00 ± 18.00^a	111.00 ± 15.40^c
	S2	13.56 ± 1.02^c	190.33 ± 5.57^c	150.33 ± 16.09^b	16.47 ± 2.52^b	206.33 ± 25.24^b	129.00 ± 7.55^b
	S3	2.17 ± 0.36^d	22.67 ± 3.61^d	183.66 ± 5.00^a	5.83 ± 1.06^c	99.33 ± 17.52^c	173.33 ± 7.00^a
21DAA	S0	17.67 ± 0.27^b	193.33 ± 19.08^b	78.00 ± 8.66^c	19.23 ± 2.36^a	199.00 ± 6.93^b	109.00 ± 6.00^c
	S1	19.40 ± 1.05^a	238.00 ± 21.00^a	135.33 ± 8.72^b	20.10 ± 0.46^a	235.33 ± 15.62^a	112.33 ± 13.00^c
	S2	12.67 ± 0.85^c	128.33 ± 16.37^c	156.00 ± 7.94^b	16.10 ± 0.30^b	160.33 ± 7.00^c	124.33 ± 16.37^b
	S3	1.00 ± 0.79^d	16.00 ± 3.46^d	246.67 ± 33.29^a	2.47 ± 0.61^c	23.00 ± 3.61^d	158.67 ± 6.08^a
28DAA	S0	10.60 ± 1.81^a	112.67 ± 17.52^b	132.67 ± 8.00^c	8.60 ± 2.21^b	105.00 ± 3.46^b	110.00 ± 11.36^b
	S1	11.93 ± 0.95^a	168.00 ± 30.05^a	216.67 ± 8.54^b	10.80 ± 0.97^a	150.00 ± 27.88^a	173.00 ± 15.88^a
	S2	6.07 ± 2.45^b	24.00 ± 7.94^c	232.33 ± 16.64^a	6.07 ± 1.23^c	89.33 ± 8.72^b	177.33 ± 11.79^a
	S3	-	-	-	-	-	-

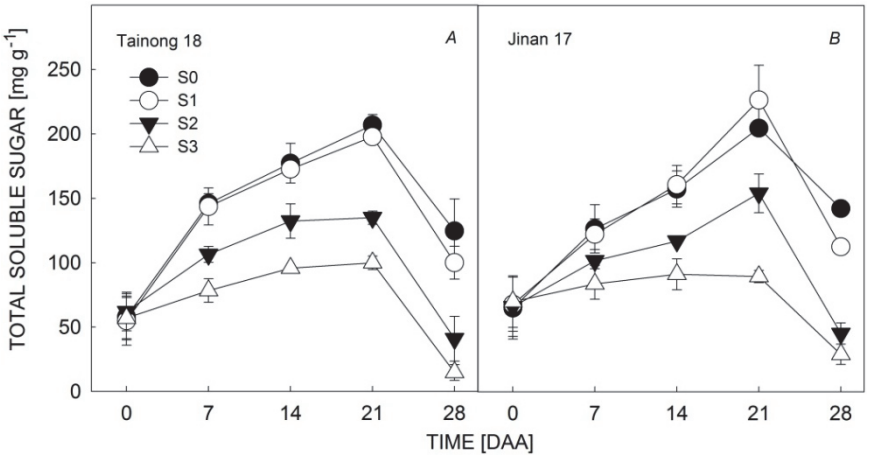

Fig. 5. Post-anthesis shading effects on total soluble sugar content in flag leaf of winter wheat cultivars Tainong 18 (*A*) and Jinan 17 (*B*). S0 refers to no shading (control); S1, S2, and S3 refer to shading treatments (78%, 50%, and 10% of full sunshine, respectively). Data represent means ± SD of 3 replicates.

MDA content: The MDA content in Tainong 18 increased by 9.4, 34.2, and 45.1% at S1, S2, and S3 treatments, respectively, compared with S0 at 28 DAA, while it increased by 6.2, 33.9, and 44.6% in Jinan 17 (Fig. 7). We also found the MDA content in the flag leaves increased sharply at 7 DAA in Tainong 18, while it increased rapidly at 21 DAA in Jinan 17. Heavy-intensity shading (S2 and S3) increased the degree of membrane lipid peroxidation of the flag leaves in both cultivars, however, no significant difference was observed between S1 and S0 during the grain filling phase. These showed that heavy-intensity shading (S2 and S3) accelerated aging of winter wheat.

Discussion

Effects of shading on photosynthetic characteristics and antioxidative metabolism of flag leaves: The structure and function of plant leaves are not only controlled by genetic factors, but also determined significantly by environmental factors, including light as a key element (Björkman *et al*. 1966, Monneveux *et al*. 2003). Most investigators had found that shading reduced P_N of plant leaves (Wang *et al*. 2003, Acreche *et al*. 2009, Guo *et al*. 2010, Mu *et al*. 2010). The present study indicated that the effect on P_N was dependent on the level of shading applied: heavy shading treatments (S2 and S3) were found to reduce P_N significantly, however, low-intensity shading (S1) decreased P_N between 0 and 14 DAA, but increased it between 21 and 28 DAA (Table 1). In agreement with the results found by Li *et al*. (2010a) on winter wheat, low-intensity shading improved P_N and the growth of winter wheat. This suggested that light intensity could change some physiological functions of plants.

At the same time, there are series of protective means in wheat plants to remove redundant excitation energy and to delay aging process of flag leaves after anthesis (Garnczarska *et al*. 2004). The antioxidants are compounds capable of quenching reactive oxygen species (ROS) without undergoing own conversion to destructive radicals (Iqbal *et al*. 2010). SOD is involved in the detoxification of O_2^-, however, SOD reactions lead to the formation of H_2O_2, which is removed by CAT together with the components of ascorbate-glutathione (AsA-GSH) cycle (Jiménez *et al*. 1997). It was reported that the activity of SOD and ascorbic acid peroxidase (APX, EC 1.11.1.11) increased in spinach under strong light and low-temperature treatments (Schöner and Krause 1990). In addition, the activity of SOD and CAT were also improved in flag leaves by a transfer of wheat from weak to high light (Mishra *et al*. 1995). All of these studies indicated that high light improves the capability of antioxidant systems in crops. On the other hand, recent studies on winter wheat found that the activity of SOD and POD were higher under shading treatments (Zheng *et al*. 2011). In the present study, the results indicated that the activity of SOD, POD, and CAT increased under 22% shading treatment, but decreased significantly under 50% and 90% shading treatments (Fig. 6). Instead, the content of MDA decreased under 22% shading treatment, but increased significantly under 50% and 90% shading treatments (Fig. 7). The activity of antioxidant enzymes affected directly CO_2 assimilation in flag leaf (Li *et al*. 2010b). Based on the present results, it is suggested that low-intensity shading (S1) improved stress resistance of winter wheat and helped to remove active oxygen, enhance physiological activity of leaves, which ensured a continuation of normal photosynthetic process (in fact, we found that S1 even increased P_N of flag leaf between 21 and 28 DAA).

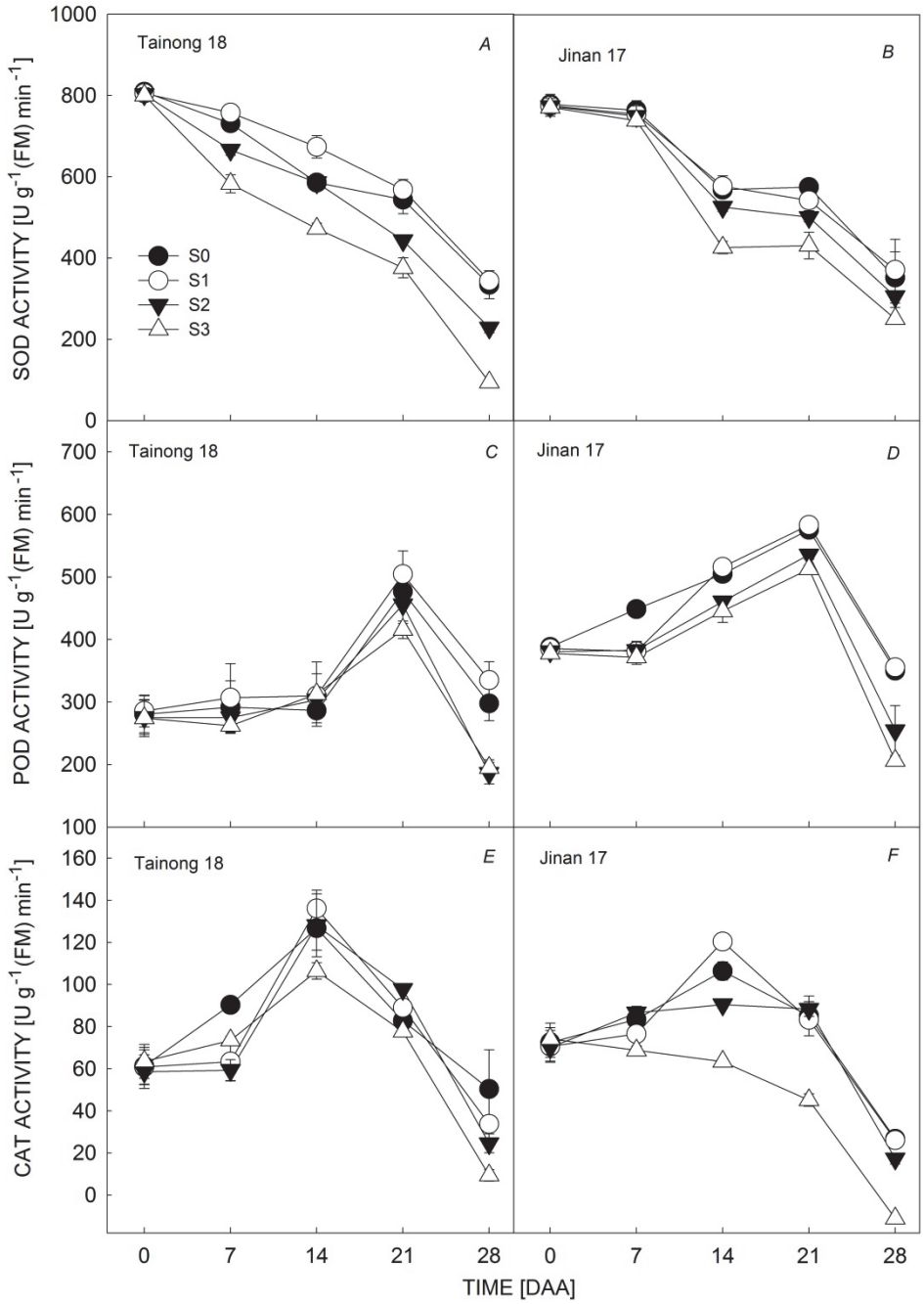

Fig. 6. Post-anthesis shading effects on superoxide dismutase (SOD) (*A,B*), peroxidase (POD) (*C,D*) and catalase (CAT) (*E,F*) activity in flag leaf of winter wheat cultivars Tainong 18 and Jinan 17. S0 refers to no shading (control); S1, S2, and S3 refer to shading treatments (78%, 50%, and 10% of full sunshine, respectively). Data represent means ± SD of 3 replicates.

The relationship between Chl and photosynthetic characteristics under shading: An increase in the photosynthetic pigment content may contribute to capture and use light more effectively (Shaver *et al*. 2008). A reduction in light intensity had been shown to alter chloroplast ultrastructure and Chl components (Evans and Poorter 2001). In addition, changes of light absorption, electron transport, and of the primary light energy conversion in PSII have been reported (Van Rensen and Curwiel 2000, Govindjee 2002, Minagawa and Takahashi 2004). In the present study, Chl contents increased under all shading treatments except S3 treatment (Fig. 3*A,B*). The increase in Chl *b* was faster than that of Chl *a*, resulting in reduced Chl *a*/*b* ratio (Fig. 3*C,D*). These findings were consistent with another report for shaded wheat (Zheng *et al*. 2011). Here, reductions in flag leaf P_N under shading were explained by the reduction in the ratio of Chl *a*/*b*. However, the change of Chl *a*/*b* was

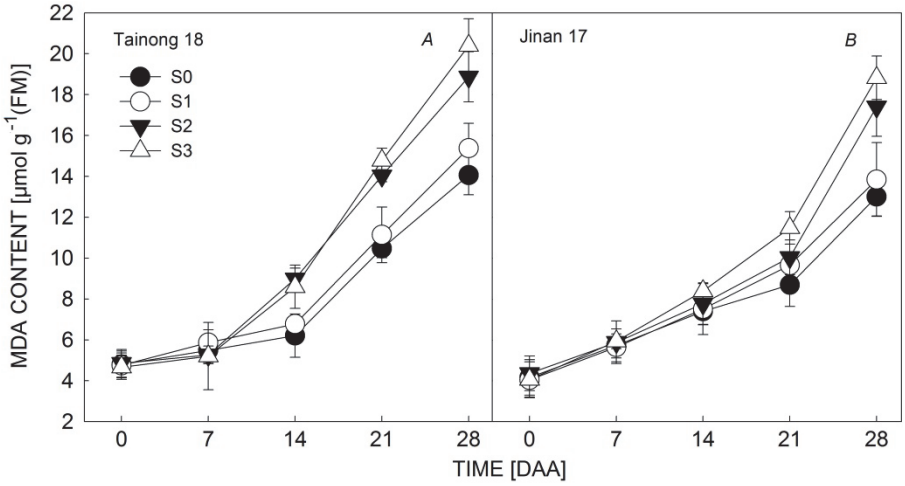

Fig. 7. Post-anthesis shading effects on malondialdehyde (MDA) content in flag leaf of winter wheat cultivars Tainong 18 (*A*) and Jinan 17 (*B*). S0 refers to no shading (control); S1, S2, and S3 refer to shading treatments (78%, 50%, and 10% of full sunshine, respectively). Data represent means ± SD of 3 replicates.

small in S1 treatment, while the total Chl content increased, which might lead to observation that no difference between S0 and S1 treatment was found in P_N of the flag leaves. In addition, the increase of total Chl content showed the self-regulation of pigment contents in plants under low-intensity shading treatment.

In the present study, the PSII was not essentially damaged, and it even became more active in the flag leaves under shading conditions as explained by increased F_v/F_m (except S3 treatment between 14 and 28 DAA), Φ_{PSII} and ETR (Fig. 4). The relative quantity of electrons passing through PSII in the dark-adapted leaves was higher (Li *et al*. 2010a). Low nonphotochemical quenching (NPQ) in shaded leaves indicated that less light energy absorbed by the antenna pigments in PSII was dispersed *via* heat (Guo *et al*. 2006). This is in agreement with the observation in this study that flag leaf P_N increased under S1, and that it decreased in S2 and S3 treatments of the heavy shading. This could suggest that low-intensity shading might contribute to more effective capture and use of light, which is a self-compensation in adverse situation in winter wheat.

Different spike and kernel type wheat cultivars in responses to shading: Protein synthesis in leaves was found to be controlled by the irradiation as well as soluble sugar content (Chi *et al*. 2001). In the present study, it was found that the total soluble sugar content decreased in the flag leaves under S2 and S3 treatments in both Tainong 18 and Jinan 17 (Fig. 5), which was consistent with reports for shaded wheat (Wang *et al*. 2003, Estrada-Campuzano *et al*. 2008) and other plants (Chi *et al*. 2001). However, it was not significantly changed in S1 treatment. In addition, there was a difference in total soluble sugar content in the flag leaves between Tainong 18 and Jinan 17 in responses to shading: Jinan 17 was found to be more tolerant to shading than Tainong 18. Total soluble sugar content losses in the flag leaves of Jinan 17 were much lower than that in Tainong 18 under heavy shading treatments (S2 and S3). These could be explained by: (*1*) the higher content of Chl and activity of PSII in the flag leaves of Jinan 17, which contributed to more effective capture and use of light; (*2*) the higher activity of antioxidative enzymes and lower content of MDA for Jinan 17 in the flag leaves, which prevented the flag leaves from damage, improved stress resistance and ensured further growth.

Conclusion: In our experimental conditions, wheat plants had relatively high activities of antioxidative enzymes and a low degree of membrane lipid peroxidation under relatively low-intensity shading condition, which was in favor of stress resistance, maintaining high P_N and the accumulation of photosynthates in wheat plants. Compared with the large-spike cultivar, the multiple-spike cultivar was found to be more suitable for the Huang-Huai-Hai Plain with shading problem at the late growth period due to the lesser damage of flag leaves and better photosynthetic function under such conditions.

References

Acreche, M.M., Briceno-Félix, G., Sánchez, J.A.M., Slafer, G.A.: Grain number determination in an old and a modern Mediterranean wheat as affected by preanthesis shading. – Crop Pasture Sci. **60**: 271-279, 2009.

Aebi, H.: Catalase in vitro. – Methods Enzymol. **105**: 121-126, 1984.

Arnon, D.I.: Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Bell, G.E., Danneberger, T.K., McMahon, M.J.: Spectral irradiance available for turfgrass growth in sun and shade. – Crop Sci. **40**: 189-195, 2000.

Björkman, O., Holmgen, P.: Photosynthetic adaptation to light intensity in plants native to shaded and exposed habitats. – Plant Physiol. **19**: 845-859, 1966.

Burgess, P.J., Incoll, L.D., Corry, D.T., Beaton, A., Hart, B.J.: Poplar (*Populus* spp.) growth and crop yields in a silvoarable experiment at three lowland sites in England. – Agroforest. Syst. **63**: 157-169, 2005.

Burkey, K.O., Wells, R.: Response of soybean photosynthesis and chloroplast membrane function to canopy development and mutual shading. – Plant Physiol. **97**: 245-252, 1991.

Chi, W., Wang, R.F., Zhang, C.L.: [Changes of photosynthetic characteristics of strawberry leaf under shading.] – Chin. J. Appl. Ecol. **12**: 566-568, 2001. [In Chin.]

Dai, Y., Shen, Z., Liu, Y., Wang, L., Hannaway, D., Lu, H.: Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. – Environ. Exp. Bot. **65**: 177-182, 2009.

Estrada-Campuzano, G., Miralles, D.J., Slafer, G.A.: Yield determination in triticale as affected by radiation in different development phases. – Eur. J. Agron. **28**: 597-605, 2008.

Evans, J.R., Poorter, H.: Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. – Plant Cell Environ. **24**: 755-767, 2001.

Fang, S., Xu, X., Lu, S., Tang, L.: Growth dynamics and biomass production in short-rotation poplar plantations: 6-year results for three clones at four spacings. – Biomass and Bioenergy **17**: 415-425, 1999.

Garnczarska, M., Bednarski, W., Morkunas, I.: Re-aeration-induced oxidative stress and antioxidative defenses in hypoxically pretreated lupine roots. – J. Plant Physiol. **161**: 415-422, 2004.

Ge, T.D., Sui, F.G., Bai, L.P., Lu, Y.Y., Zhou, G.S.: [Effects of water stress on the protective enzyme activities and lipid peroxidation in roots and leaves of summer maize.] – Sci. Agr. Sin. **38**: 922-928, 2005. [In Chin.]

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases. I. Occurrence in higher plants. – Plant Physiol. **59**: 309-314, 1977.

Govindjee: A role for a light-harvesting antenna complex of photosystem II in photoprotection. – Plant Cell **14**: 1663-1668, 2002.

Greenwald, R., Bergin, M.H., Xu, J., Cohan, D., Hoogenboom, G., Chameides, W.L.: The influence of aerosols on crop production: a study using the CERES crop model. – Agr. Syst. **89**: 390-413, 2006.

Guo, C.H., Gao, Z.Q., Miao, G.Y.: [Effect of shading at post flowering on photosynthetic characteristics of flag leaf and response of grain yield and quality to shading in wheat.] – Acta Agron. Sin. **36**: 673-679, 2010. [In Chin.]

Guo, H.X., Liu, W.Q., Shi, Y.C.: Effects of different nitrogen forms on photosynthetic rate and the chlorophyll fluorescence induction kinetics of flue-cured tobacco. – Photosynthetica **44**: 140-142, 2006.

Huang, J., Guo, S.R., Jiang, F.L., Chen, L., Wu, Z.: [Effects of shading treatment and regular light recovery on growth and reactive oxygen metabolism of Pak-choi.] – Acta Hort. Sin. **35**: 753-756, 2008. [In Chin.]

Iqbal, N., Masood, A., Nazar, R., Syeed, S., Khan, N.A..: Photosynthesis, growth and antioxidant metabolism in mustard (*Brassica juncea* L.) cultivars differing in cadmium tolerance. – Agr. Sci. Chin. **9**: 519-527, 2010.

Jiang, H., Wang, X.H., Deng, Q.Y., Yuan, L.P., Xu, D.Q.: Comparison of some photosynthetic characters between two hybrid rice combinations differing in yield potential. – Photosynthetica **40**: 133-137, 2002.

Jiménez, A., Hernandez, J.A., Del Río, L.A., Sevilla, F.: Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. – Plant Physiol. **114**: 275-284, 1997.

Klapheck, S., Zimmer, I., Cosse, H.: Scavenging of hydrogen peroxide in the endosperm of *Ricinus communis* by ascorbate peroxidase. – Plant Cell Physiol. **31**: 1005-1013, 1990.

Li, H., Jiang, D., Wollenweber, B., Dai, T., Cao, W.: Effects of shading on morphology, physiology and grain yield of winter wheat. – Eur. J. Agron. **33**: 267-275, 2010a.

Li, N.N., Li, H., Pei, Y.T., Shi, Y.H., Tian, Q.Z., Xie, L.J., Wang, S.L., Liu, X., Xu, F.J.: [Effects of allocations of row-spacing on photosynthetic characteristics and yield structure of winter wheat cultivars with different spike types.] – Sci. Agr. Sin. **43**: 2869-2878, 2010b. [In Chin.]

Li, P.M., Cai, R.G., Gao, H.Y., Peng, T., Wang, Z.L.: Partitioning of excitation energy in two wheat cultivars with different grain protein contents grown under three nitrogen applications in the field. – Physiol. Plant. **129**: 822-829, 2007.

Minagawa, J., Takahashi, Y.: Structure, function and assembly of photosystem II and its light-harvesting proteins. – Photosynth. Res. **82**: 241-263, 2004.

Mishra, N.P., Fatma, T., Singhal, G.S.: Development of anti-oxidative defense system of wheat seedlings in response to high light. – Physiol. Plant. **95**: 77-82, 1995

Mitchell, R.A.C., Gibbard, C.L., Mitchell, V.J., Lawlor, D.L.: Effects of shading in different developmental phases on biomass and grain yield of winter wheat at ambient and elevated CO_2. – Plant Cell Environ. **19**: 615-621, 1996.

Mu, H., Jiang, D., Wollenweber, B., Dai, T., Jing, Q., Cao, W.: Long-term low radiation decreases leaf photosynthesis, photochemical efficiency and grain yield in winter wheat. – J. Agron. Crop Sci. **196**: 38-47, 2010.

Murchie, E.H., Chen, Y.Z., Hubbart, S., Peng, S., Horton, P.: Interactions between senescence and leaf orientation determine in situ patterns of photosynthesis and photoinhibition in field-grown rice. – Plant Physiol. **119**: 553-564, 1999.

Monneveux, P., Pastenes, C., Reynolds, M.P.: Limitations to photosynthesis under light and heat stress in three high-yielding wheat genotypes. – J. Plant Physiol. **160**: 657-666, 2003.

Demotes-Mainard, S., Jeuffroy, M.-H.: Effects of nitrogen and radiation on dry matter and nitrogen accumulation in the spike of winter wheat. – Field Crops Res. **87**: 221-233, 2004.

Arisnabarreta, S., Miralles, D.J.: Radiation effects on potential number of grains per spike and biomass partitioning in two- and six-rowed near isogenic barley lines. – Field Crops Res. **107**: 203-210, 2008.

Shaver, J.M., Oldenburg, D.J., Bendich, A.J.: The structure of chloroplast DNA molecules and the effects of light on the amount of chloroplast DNA during development in *Medicago truncatula*. – Plant Physiol. **146**: 1064-1074, 2008.

Schöner, S., Krause, G.H.: Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. – Planta **180**: 383-389, 1990.

Van Rensen, J.J., Curwiel, V.B.: Multiple functions of photosystem II. – Indian J. Biochem. Biophys. **37**: 377-382, 2000.

Wang, Z., Yin, Y., He, M., Zhang, Y., Lu, S., Li, Q., Shi, S.: Allocation of photosynthates and grain growth of two wheat cultivars with different potential grain growth in response to pre- and post-anthesis shading. – J. Agron. Crop Sci. **189**: 280-285, 2003.

Xu, Z.X., Zhao, F.F.: [Variation of sunlight radiation duration in the Yellow River Basin.] – Resources Science **27**: 153-159, 2005. [In Chin.]

Yan, M.H., Deng, W., Chen, P.Q.: [Analysis of climate jumps in the Sanjiang Plain.] – Sci. Geograph. Sin. **23**: 661-667, 2003. [In Chin.]

Yang, X.M., Zeng, Y., Qiu, X.F., Jiang, A.J.: [The climatic change of global solar radiation over the Yellow River Basin during 1960-2000.] – Advances Atmospheric Sci. **16**: 243-248, 2005. [In Chin.]

Yu, C.J., Fang, S.Z., Luo, C.B.: [A preliminary study on poplar-wheat intercropping patterns.] – China Forest. Sci. Technol. **21**: 47-51, 2007. [In Chin.]

Zhang, C.J., Chu, H.J., Chen, G.X., Shi, D.W., Zuo, M., Wang, J., Lu, C.G., Wang, P., Chen, L.: Photosynthetic and biochemical activities in flag leaves of a newly developed superhigh-yield hybrid rice (*Oryza sativa*) and its parents during the reproductive stage. – J. Plant Res. **120**: 209-217, 2007.

Zheng, Y., Mai, B., Wu, R., Feng, Y., Sofo, A., Ni, Y., Sun, J., Li, J., Xu, J.: Acclimation of winter wheat (*Triticum aestivum*, cv. Yangmai 13) to low levels of solar irradiance. – Photosynthetica **49**: 426-434, 2011.