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Acclimation of winter wheat (*Triticum aestivum*, cv. Yangmai 13) to low levels of solar irradiance

Y. ZHENG[*,**,+], B. MAI[*], R. WU[*], Y. FENG[*], A. SOFO[***], Y. NI[*], J. SUN[*], J. LI[*], and J. XU[*]

College of Environmental Science and Engineering, Nanjing University of Information Science & Technology (NUIST), Nanjing, 210044, PR China[*]
Jiangsu Key Laboratory of Meteorological Disaster - KLME, Nanjing, 210044, PR China[**]
Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100, Potenza, Italy[***]

Abstract

Winter wheat is a grass species widely planted in northern and central China, where the increase of aerosols, air pollutants and population density are causing significant reduction in solar irradiance. In order to investigate the adaptation of winter wheat (*Triticum aestivum* L., cv. Yangmai 13) to low irradiance conditions occurring in the downstream plain of the Yangtze River (China), plants were subjected to four solar irradiance treatments (100%, 60%, 40%, and 20% of environmental incident solar irradiance). Significant increases in chlorophyll (Chl) and xanthophyll (Xan) pigments, and decreases in Chl *a*/*b* and Xan/Chl ratios were observed in plants under low light. Light-response curves showed higher net photosynthetic rates (P_N) in fully irradiated plants, that also showed a higher light-compensation point. Shaded plants maintained high values of minimal fluorescence of dark-adapted state (F_o) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) that assess a lower degree of photoinhibition under low light. Reduced irradiance caused decreases in effective quantum yield of PSII photochemistry (Φ_{PSII}), electron transport rate (ETR), and nonphotochemical quenching coefficient (q_N), and the promotion of excitation pressure of PSII ($1 - q_P$). The activities of the antioxidant enzymes superoxide dismutase and peroxidase were high under reduced light whereas no light-dependent changes in catalase activity were observed. Thiobarbituric acid reactive species content and electrolyte leakage decreased under shaded plants that showed a lower photooxidative damage. The results suggest that winter wheat cv. Yangmai 13 is able to maintain a high photosynthetic efficiency under reduced solar irradiance and acclimates well to shading tolerance. The photosynthetic and antioxidant responses of winter wheat to low light levels could be important for winter wheat cultivation and productivity.

Additional key words: antioxidant enzymes; chlorophyll; electrolyte leakage; oxidative stress; photoinhibition.

Introduction

Shading conditions cause increases in chlorophyll (Chl), alter chloroplast structure, and hinder photosynthesis and stomatal conductance (Cavagnaro and Trione 2007, Sofo *et al.* 2010). Under low light, plants showed decreases in photosynthetic light-saturation point and light-compensation point (LCP), and this can allow them to capture and utilize less photons in a more efficient way (Dai *et al.* 2009). On the other hand, excessive radiation caused photoinhibition and photooxidative damage, with consequent reductions in the maximal quantum yield of PSII photochemistry (F_v/F_m) (García-Plazaola *et al.* 2004, Kreslavski *et al.* 2007). When suddenly exposed to higher

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[+]Corresponding author; phone: +86 025 58731433, email: zhengyf@nuist.edu.cn
Abbreviations: APX – ascorbate peroxidase; CAT – catalase; Chl – chlorophyll; CK – fully irradiated control plants; ETR – electron transport rate; F_0 – minimal fluorescence of dark-adapted state; F_m – maximum fluorescence of dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; L20 – 20% irradiated plants; L40 – 40% irradiated plants; L60 – 60% irradiated plants; LCP – light-compensation point; P_N – net photosynthetic rate; PAR – photosynthetically active radiation; POD – guaiacol-type peroxidase; PSII – photosystem II; q_N – nonphotochemical quenching coefficient; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive species; Xan – xanthophylls; Φ_{PSII} – effective quantum yield of PSII photochemistry; $1 - q_P$ – excitation pressure of PSII.
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light levels, the nonphotochemical quenching coefficient (q_N) of sun-exposed leaves increases to maximal levels in a short period, whereas it takes a much longer time in shaded leaves that have lower capacities of heat energy dissipation (Yang *et al.* 2005, Mu *et al.* 2010).

In general, plants adopt three main mechanisms to dissipate excess light energy and acclimate themselves to this stress condition: thermal dissipation by xanthophyll cycle, acceleration of photosynthetic apparatus with increases in effective quantum yield of PSII photochemistry (Φ_{PSII}), and scavenging of reactive oxygen species (ROS) deriving from over-excitation of the photosynthetic apparatus (Demmig-Adams *et al.* 1995, Kreslavski *et al.* 2007). ROS detoxification is carried out by several enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol-type peroxidase (POD) and catalase (CAT). In addition, concentrations of thiobarbituric acid reactive species (TBARS) are a reliable indicator of lipid peroxidation and plant oxidative stress (Sofo *et al.* 2010).

Winter wheat is a grass species widely planted in northern and central China, particularly in the downstream plain of the Yangtze River, where about 15% of the total wheat growing area is located. In the last five decades, air pollutants, aerosols and population density in this region increased dramatically and, as a result, solar irradiance decreased by more than 6% per decade (Li *et al.* 2005, Che *et al.* 2007). This value is significantly higher than the average global decrease of photosynthetic active radiation caused by air pollution, assessed as 1.3% a^{-1} (IPCC 2007). Light reduction significantly restrains growth and grain yield and quality of wheat crops (Li *et al.* 2005, Li *et al.* 2010). While some works reported reduction of photosynthesis and light-use efficiency in winter wheat (Guo *et al.* 2009, Mu *et al.* 2010), other authors found increases of these parameters (Li *et al.* 2010). Considering the urgency of the global dimming phenomenon and the economic importance of winter wheat, new studies are necessary.

Špundová *et al.* (2005) and Causin *et al.* (2009) reported that shading conditions increased the senescence rate, weakening of antioxidative protection and development of oxidative stress symptoms in leaves of wheat grown in greenhouse. Changes in photosynthetic performance and antioxidant response to shade treatments have been observed also in other species (Sofo *et al*. 2004, Zhou *et al*. 2010).

Thus, our working hypothesis is that the variation of photosynthesis might be associated with changes in the oxidative stress in leaves of this species. To test this hypothesis, we conducted a field experiment on plants of a poorly-studied winter wheat cultivar (*Triticum aestivum* L., cv. Yangmai 13) subjected to five different levels of environmental solar irradiance. The objectives of this study were: (*1*) to explore the photosynthetic response of winter wheat to low light, and (*2*) to reveal the possible relationship between photosynthesis and oxidative stress in plants grown under shading conditions.

Materials and methods

Plant material and experimental design: The experiment was conducted in field in an agro-meteorological experimental station located in NUIST campus, in the central Jiangsu Province, eastern China (32°14'N, 118°42'E). This site is characterized as having a subtropical monsoon climate, with mean annual temperature of 16.5°C, maximum temperature of 28.6°C in July, and minimum of 3.4°C in January. The annual precipitation varies generally within 737.3 and 1,658.3 mm, with an average value of 1,098.6 mm, the solar radiation is about 4,540 MJ m^{-2} yr^{-1}. Soil is of yellow-brown type having organic matter of 10.35 g kg^{-1}, pH 5.35, total nitrogen content of 0.55 g kg^{-1}, available phosphorus content of 4.46 mg kg^{-1}, and available potassium of 59.38 mg kg^{-1}. Winter wheat (*Triticum aestivum* L., cv. Yangmai 13) used in this experiment was obtained from Ldxiahe Agricultural Research Institute (Jiangsu, China). The seeds were selected for uniformity, and disinfected for 10 min in a $HgCl_2$ solution with a concentration of 1.0 g L^{-1}, and then repeatedly rinsed in deionized water. Seeds were planted in an open field on November 4, 2009. Prior to sowing, the soil mixture was fertilized with 692.3 kg ha^{-2} of nitrogen complex fertilizer (N_8-$P_2O_5$6-K_2O6*, Tianbu Co. Ltd.*, Shanghai, China).

The experimental design consisted of complete uniform plots. Each plot (4 × 4 m) had 1.5 m space as a buffer zone in order to prevent mutual interference. Starting from February 2009, plants were subjected to three shading treatments: 20, 40, and 60% of environmental incident irradiance (L20, L40 and L60, respectively), whereas no shading (100% of solar incident irradiance) was set as control (CK). Shading conditions were obtained by using one (L60), two (L40) or three (L20) layers of polyethylene neutral shading net, that uniformly reduced PAR intensity, as assessed by measures of solar irradiance with a portable spectroradiometer (*TBQ-2, JWF Ltd. Co.,* Shanghai, China) (Fig. 1). During the experimental period, the nets were kept about 50 cm above the wheat canopy, to ensure good ventilation, and they were large enough to fully cover the corresponding shaded plots. The temperature and relative humidity (Table 1) measured 10 cm above the wheat canopy were recorded hourly by a thermosensor attached to a *HOBO U23-001* data-logger (*Onset Computer Corp.,* Bourne, MA, USA).

Photosynthesis and Chl fluorescence: Measurements on five different plants per light treatment (n = 5) were performed *in vivo* on five fully expanded, horizontally positioned leaves from 09:00 to 11:00 h on a clear,

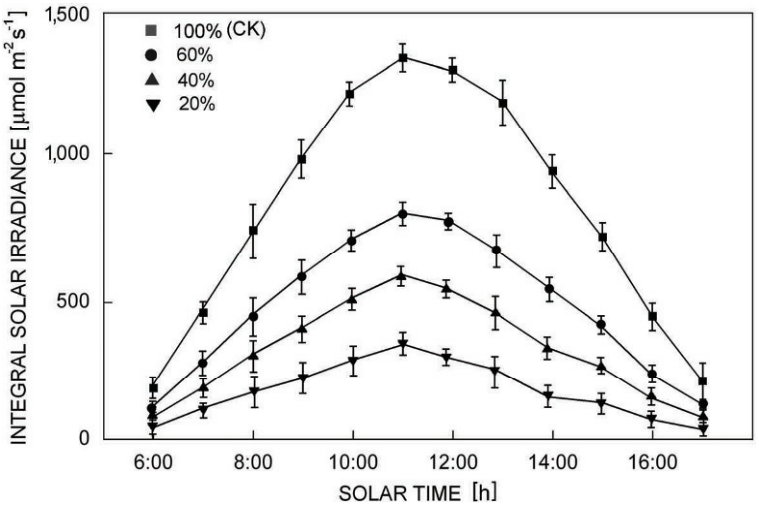

Fig. 1. Diurnal variation of integral solar irradiance (wavelength range = 400–700 nm) during the experimental period in *Triticum aestivum* L. (cv. Yangmai 13) not covered (100%, CK; ■), and covered with one (60%; ●), two (40%; ▲) or three (20%; ▼) layers of polyethylene neutral shading net. Data represent means ± SD (n = 15).

Table 1. Air temperature and relative humidity under different environmental irradiance treatments.

Environmental parameters	Irradiance [%]			
	100 (CK)	60	40	20
Air temperature [°C]	15.19	14.90	14.83	14.76
Relative humidity [%]	76.51	77.75	77.87	78.00

cloudless day (29 April 2010).

Photosynthetic light-response curves were carried out using a programmable, open-flow gas-exchange portable system (*LI-6400, Li-Cor, Inc.,* Lincoln, NE, USA) operated at 400 μmol s^{-1} air flow rate starting from the highest actinic light intensity (1,500 μmol PAR m^{-2} s^{-1}; 90% red fraction at a wavelength of 630 nm and a 10% blue fraction at 470 nm) to the complete darkness, at regular intervals of 15 min, in order to give the stomata time to equilibrate at each level. Net photosynthetic rate (P_N) was measured at 1,500; 1,320; 880, 616, 440, 176, 132, 88, 44, 18, and 0 μmol PAR m^{-2} s^{-1}. Leaf temperature, relative air humidity, and CO_2 concentration inside the leaf chamber were maintained at 25°C, 65%, and 400 μl L^{-1}, respectively. LCP for each light treatment was calculated as the x-intercept [μmol(PAR m^{-2} s^{-1}] of the PAR/P_N curve (Fig. 2), whose equation is P_N = [P_{max} (I – LCP) α_c]/[P_{max} + (I – LCP) α_c] (Drake and Read 1981), where P_{max} is the maximum photosynthetic rate, I is incident PAR, and α_c is the quantum efficiency calculated as the derivative of the initial part of the curve.

Chl fluorescence was measured using a leaf chamber fluorometer (*LI-6400-40, Li-Cor, Inc.,* Lincoln, NE, USA). Leaves were dark-adapted for 30 min and, for each light treatment, the following parameters were measured (Maxwell and Johnson 2000, Baker 2008): minimal fluorescence of dark-adapted state (F_o), obtained with open PSII centers following excitation by a weak light; and F_v/F_m, calculated as (F_m – F_o)/F_m, where F_m is the maximal fluorescence of dark-adapted state. On the basis of previous trials, suitable settings for the measuring light (intensity 480–1,500 μmol m^{-2} s^{-1}, modulation 0.25 kHz, filter 1 Hz, gain 10), and the flash saturating light pulse (duration 0.7 sec, intensity 4,200 μmol m^{-2} s^{-1}, modulation 20 kHz, filter 50 Hz) were used.

Light-adapted leaves from five plants for each light treatment (n = 5) were chosen for light-response curves. Light-response curves were recorded at 09:00–11:00 h using a 90% red–10% blue actinic light. Light curves were carried out starting from the highest intensity [2,425 μmol(PAR m^{-2} s^{-1}] to the complete darkness at regular intervals of 15 min, in order to give the stomata time to equilibrate at each level. At each level of light intensity, the following parameters were measured (Maxwell and Johnson 2000, Baker 2008): Φ_{PSII}, calculated as (F_m' – F_t)/F_m', where F_m' is the maximal fluorescence of light-adapted state and F_t is the steady-state fluorescence yield measured under actinic light; relative electron transport rate through PSII (ETR), given by $\Phi_{PSII} \times f \times I \times \alpha_{leaf}$, where f is the fraction of absorbed quanta used by PSII (0.5 for C_3 plants), I is incident PAR, and α_{leaf} is leaf absorptance calculated as α_{blue} B + α_{red} B (100 – B), where B is the percentage (10%) of incident blue light and α_{blue} and α_{red} are the measured absorptances in the blue (0.91) and red (0.84); q_N, calculated as (F_m – F_m')/(F_m – F_0'), where F_o' is the minimal fluorescence of light-adapted state measured by using far-red light pulse to drain electrons from PSII; and excitation pressure of PSII (1 – q_P), calculated as [1 – (F_m' – F_t)/(F_m' – F_0')]. On the basis of previous trials, suitable settings for the measuring light (intensity 2,400 μmol m^{-2} s^{-1}, modulation 15 kHz, filter 1 Hz, gain 10),

the flash saturating light pulse (duration 0.7 sec, intensity 4,200 μmol m^{-2} s^{-1}, modulation 20 kHz, filter 50 Hz), and the dark period with far-red light (duration 5 sec, far-red intensity 4,800 μmol m^{-2} s^{-1}, pre-time 1 s, post-time 1 s, modulation 0.25 kHz, filter 1 Hz) were used.

On the same measuring day, fresh leaves for each light treatment were detached and immediately used for the following analyses.

Photosynthetic pigments: Fresh leaves were ground in ice-cold 80% (v/v) acetone using a previously chilled mortar and pestle. Total contents of Chl *a*, Chl *b*, and total Chl were measured according to Moran (1982). The xanthophyll content was determined after Thayer and Björkman (1990).

Antioxidant enzyme activities: For SOD activity, fresh leaves (0.5 g) were homogenized at 0°C in 1 mL 50 mM potassium phosphate buffer (pH 7.8). Samples were centrifuged at 1,000 × *g* for 20 min at 0°C. The activity of SOD was measured spectrophotometrically at 560 nm by detecting the inhibition of nitro-blue tetrazolium reduction by SOD. The blue colour developed in the reaction was spectrophotometrically measured at 560 nm and the corresponding nonexposed samples were used as blank (Sofo *et al.* 2004). The volume of sample causing 50% inhibition in colour development min^{-1} as taken as one unit of SOD activity.

For CAT activity, fresh leaves (0.5 g) were ground at 0°C with 5 mL 20 mM KH_2PO_4 and the homogenate was centrifuged at 1,500 × *g* for 20 min at 0°C. This extraction was repeated three times. 2.5 mL of the enzyme extract was added with 2.5 mL 100 mM H_2O_2 and then placed into water bath at 30°C. After 10 min, the mixture was added with 2.5 mL of 10% H_2SO_4 and titrated with 100 mM $KMnO_4$ solution until a faint pink colour was obtained. The absorbance was determined at 240 nm. One unit of CAT activity corresponded to the amount of enzyme that decomposes 1 μmol(H_2O_2) min^{-1}.

POD activity was determined according to Dias and Costa (1983), with some modifications. Fresh leaves (0.5 g) were ground using a previously chilled mortar and pestle in 5 mL of 20 mM KH_2PO_4 at 0°C in an ice bath, and the homogenate was centrifuged at 1,000 × *g* for 10 min at 4°C. An aliquot of the supernatant (0.1 mL) was added to 4.9 mL of the reaction mixture (2.9 mL of 50 mM potassium phosphate buffer, pH 7.0; 1.0 mL of 2% H_2O_2; and 1.0 mL of 50 mM guaiacol solution). The reaction was stopped by applying 2.0 mL of 20% trichloroacetic acid to the reaction mixture. The absorbance was recorded every minute at 470 nm for 5 min, and the enzyme extract in boiling water for 5 min was taken as control. One unit of POD activity corresponded to the amount of enzyme that decomposes 1 μmol(guaiacol) min^{-1}.

Thiobarbituric acid reactive substances and electrolyte leakage: TBARS content was measured following the method of Heath (1968). Fresh leaves (0.5 g) were homogenized at 0°C in 5 mL of 5 % (w:v) trichloroacetic acid solution. The homogenate was centrifuged at 1,000 × *g* for 10 min. The supernatant (2 mL) was added with 2 mL of 0.67% (w:v) thiobarbituric acid. The mixture was incubated in boiling water for 30 min, and then cooled down by placing the reaction tubes in an ice bath. The sample was centrifuged again at 1,000 × *g* for 10 min, and then supernatant absorbance was measured at 450, 532, and 600 nm, respectively.

For the estimation of electrolyte leakage, leaf discs (1 cm^2) were rinsed and placed into glass beaker with 20 mL deionized water for 2 h at 20°C. After this step, leachate conductivity of water was measured (C). Successively, test tubes containing the discs were refilled with 20 ml and placed in boiling water bath for 15 min, cooled at 20°C, and after 24 h the electric conductivity of the external solution was measured (C'). The values of electrolyte leakage were calculated as C/(C + C'), according to Tarhanen *et al.* (1999).

Statistic analysis: All data were subjected to the one-way analysis of variance (*ANOVA*), and *Duncan*'s Multiple Range Test was used to determine the significance ($P \leq 0.05$) of differences between treatments using *Origin* statistical software (*Origin 8.0, Originlab Ltd. Co*., Northampton, USA). *Pearson*'s correlation analysis was used to determine the relationship between various parameters and irradiance treatments, and differences at $P \leq 0.05$ were calculated.

Results

Photosynthetic pigments and photosynthesis: The level of solar irradiance significantly caused changes in the photosynthetic pigment contents (Table 2). Chl *a* significantly ($P \leq 0.01$) increased with reducing irradiance. The levels of Chl *a* in L60 and L40 were significantly higher than those of CK, with maximum values (1.72 times higher than CK) in L20. Similar trends were found for both Chl *b* and Chl. The Chl *a*/*b* ratios significantly declined with decreasing solar irradiance, indicating that the increase of Chl *b* content was higher than that of Chl *a* (Table 2). Reduced irradiance resulted in increases ($P \leq 0.01$) in Xan content, and decreases ($P \leq 0.01$) in Xan/Chl. Compared to CK, Xan/Chl in L60 and L20 decreased by 8.0 and 13.0%, respectively.

The trends of P_N rapidly increased at PAR over 200 μmol m^{-2} s^{-1} and then increased slowly up to a PAR of 900 μmol m^{-2} s^{-1}, before reaching a plateau, regardless of the irradiance treatments (Fig. 2*A*). The maximum

Table 2. Effects of solar irradiance on the levels of chlorophylls (Chl) and xanthophylls (Xan) in leaves of winter wheat. Data represent means ± SD (n = 5). Significant differences between light treatments were tested *via ANOVA* and *Duncan*'s Multiple Range Test. The values in the same column with different letters differ significantly at $P \leq 0.05$.

Irradiance [%]	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Chl [mg g^{-1}(FM)]	Xan [mg g^{-1}(FM)]	Chl *a*/*b*	Xan/Chl
100 (CK)	1.37 ± 0.04^{c}	0.40 ± 0.01^{c}	1.77 ± 0.05^{c}	6.24 ± 0.02^{b}	3.41^{a}	3.52^{a}
60	1.92 ± 0.06^{b}	0.61 ± 0.03^{b}	2.53 ± 0.09^{b}	8.16 ± 0.02^{b}	3.16^{b}	3.23^{b}
40	1.99 ± 0.13^{b}	0.63 ± 0.04^{b}	2.63 ± 0.17^{b}	8.39 ± 0.06^{a}	3.14^{b}	3.19^{bc}
20	2.36 ± 0.15^{a}	0.81 ± 0.08^{a}	3.18 ± 0.23^{a}	9.71 ± 0.07^{a}	2.92^{c}	3.06^{c}

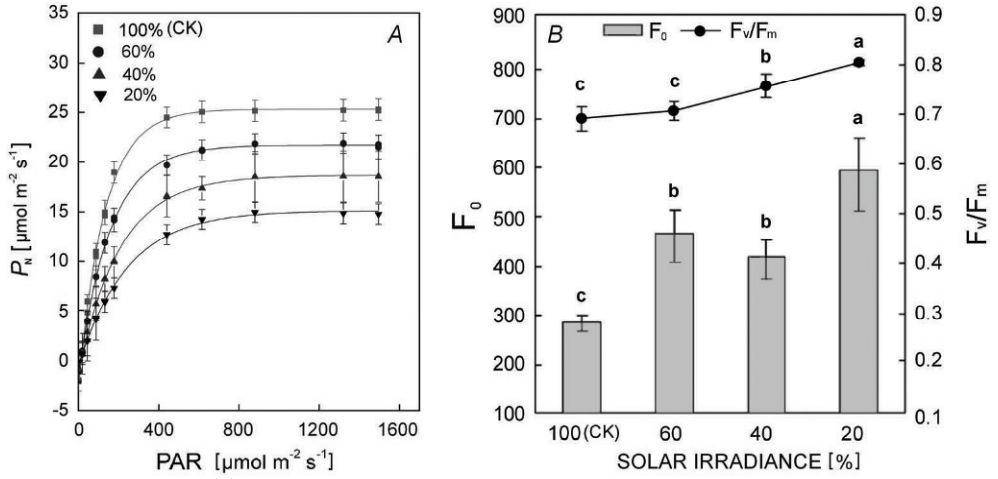

Fig. 2. *A*: Light-response curves of net photosynthetic rate (P_N) from leaves of *Triticum aestivum* L. (cv. Yangmai 13) exposed to 100% (CK) (■), 60% (●), 40% (▲), and 20% (▼) solar irradiance. Data represent means ± SD (n = 5). *B*: Minimal fluorescence of dark-adapted state (F_o), and maximal quantum yield of PSII photochemistry (F_v/F_m) in leaves of *Triticum aestivum* L. (cv. Yangmai 13) exposed to 100% (CK), 60%, 40%, and 20% solar irradiance. Data represent means ± SD (n = 5). Means followed by the *different letters* are significantly different at $P \leq 0.05$, according to *Duncan*'s Multiple Range Test.

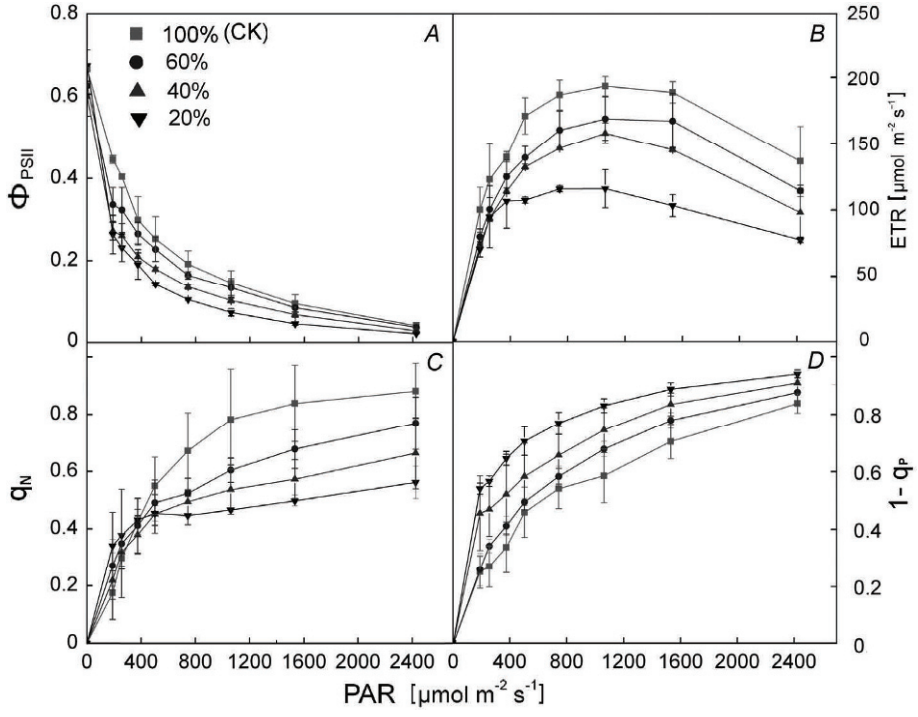

Fig. 3. Light-response curves of *A*: effective quantum yield of PSII photochemistry (Φ_{PSII}), *B*: electron transport rate (ETR), *C*: nonphotochemical quenching coefficient (q_N), and *D*: excitation pressure of PSII from leaves of *Triticum aestivum* L. (cv. Yangmai 13) exposed to 100% (CK) (■), 60% (●), 40% (▲), and 20% (▼) solar irradiance. Data represent means ± SD (n = 5).

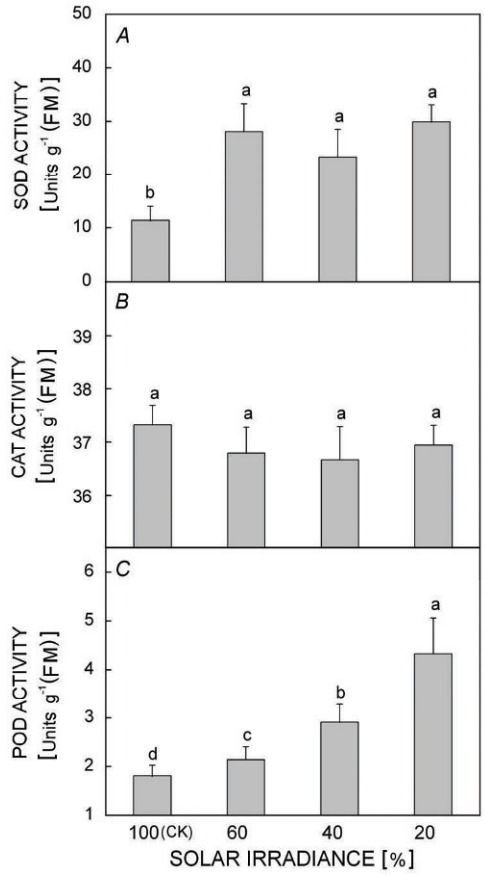

Fig. 4. Activities of *A*: superoxide dismutase (SOD), *B*: catalase (CAT), and *C*: guaiacol-type peroxidase (POD) in leaves of *Triticum aestivum* L. (cv. Yangmai 13) exposed to 100% (CK), 60%, 40%, and 20% solar irradiance. One unit of SOD activity corresponds to the amount of sample causing 50% inhibition in colour development min^{-1} at 560 nm. For POD and CAT, one unit corresponds to the amount of enzyme that decomposes 1 μmol of substrate min^{-1}. Data represent means ± SD (n = 5). Means followed by the *different letters* are significantly different at $P \leq 0.05$, according to *Duncan*'s Multiple Range Test.

value of P_N significantly decreased by 14.5 and 41.7% in L60 and L20, respectively, if compared to CK (Fig. 2*A*). The values of LCP decreased with reducing irradiance treatments. The highest value of LCP was observed in CK (7.3 μmol m^{-2} s^{-1}), whereas in L60, L40 and L20 it was 5.5, 3.5 and 1.9 μmol m^{-2} s^{-1}, respectively.

Chlorophyll fluorescence: The level of irradiance had a significant impact on the minimal fluorescence of dark-adapted state (F_o) and on F_v/F_m (Fig. 2). The values of F_0 increased with decreasing solar irradiance (Fig. 2*A*). In L60 and L40, F_0 values were not significantly different, but both of them were significantly higher than the value of CK. The treatment L20 showed the highest value of F_0, about two-fold that of CK. It is noteworthy that F_v/F_m significantly increased with reducing irradiance, ranging from 0.72 to 0.81 (Fig. 2*B*).

Decreases in Φ_{PSII}, and increases in ETR, q_N, and $1 - q_P$ were observed in all the treatments with increasing PAR (Fig. 3). The reduction of Φ_{PSII} was highest in L20 (Fig. 3*A*) and the differences between the treatments were not always significant ($P \leq 0.05$). However, above a PAR of 1,600 μmol m^{-2} s^{-1}, Φ_{PSII} was not statistically different ($P \leq 0.05$) in all the treatments (Fig. 3*A*). Independently from the light level, ETR values reached a maximum at PAR of about 1,000 μmol m^{-2} s^{-1}, and then decreased slowly at higher PAR values (Fig. 3*B*). The ETR light curves of all the treatments were quite coincident in a PAR range of 0–200 μmol m^{-2} s^{-1}, whereas at PAR values above 200 μmol m^{-2} s^{-1} significant reductions ($P \leq 0.05$) in ETR were observed, particularly in L20.

Generally, q_N values increased with increasing PAR in all the treatments (Fig. 3*C*). Within a PAR range of 0–400 μmol m^{-2} s^{-1}, q_N values were significantly higher ($P \leq 0.05$) under reduced light than in CK, while at PAR values above 400 μmol m^{-2} s^{-1} the trend was reversed, with CK showing lower values (Fig. 3*C*). The values of $1 - q_P$ increased gradually when PAR exceeded 200 μmol m^{-2} s^{-1}, with the highest values in L20 (Fig. 3*D*).

Antioxidant enzyme activities, lipid peroxidation, and membrane permeability: SOD activity increased significantly when plants were subjected to reduced irradiance treatments (Fig. 4). Compared to CK, SOD activities in L60 and L20 significantly increased by 104 and 162%, respectively. In contrast to SOD, CAT activity did not significantly change, whereas significant increases in POD activity were observed in all of the reduced irradiance treatments, particularly in L20.

Lipid peroxidation was significantly affected by the reduced irradiance treatments (Fig. 5*A*). TBARS in L60 and L20 decreased significantly, showing values of 33.8 and 42.7% lower than those of CK (Fig. 5*A*). Changes in lipid peroxidation and membrane damage occurred simultaneously, as electrolyte leakage was found to be reduced gradually with decreasing solar irradiance (Fig. 5*B*). Compared to CK, significant reductions of electrolyte leakage were observed in L40 and L20 (Fig. 5*B*).

Discussion

The increase in leaf Chl (Chl *a* and Chl *b*) is a response that can contribute to maximizing of the light harvesting capacity in low-light growth conditions, so allowing wheat plants to use light more effectively (Liu *et al.* 2007, Dai *et al.* 2009). We found significant increases in Chl *a*, Chl *b,* and total Chl in wheat leaves under reduced irradiance (Table 2). Furthermore, the Chl *a*/*b* ratio is a good indicator of plant acclimation to light intensity, as this value typically decreases when plants are subjected to low-light environment (Dai *et al.* 2007, Li *et al.* 2010).

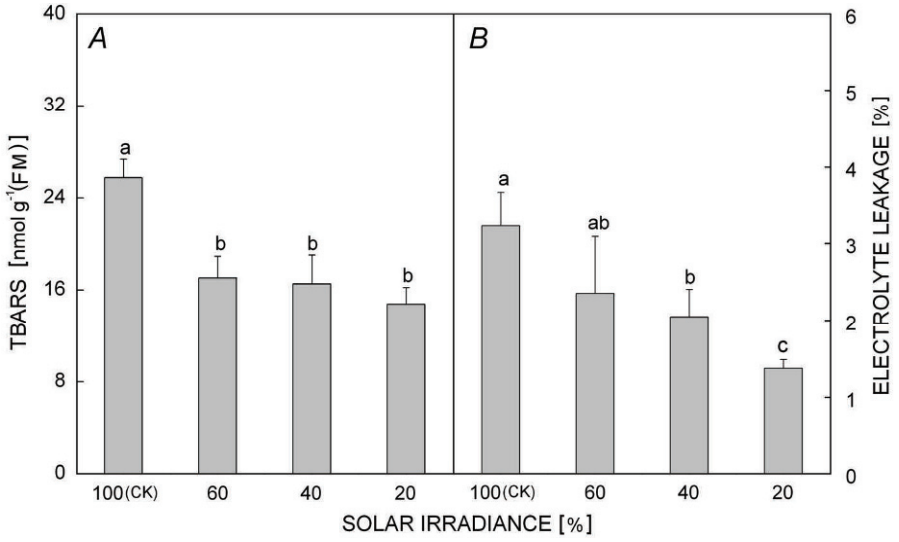

Fig. 5. *A*: Thiobarbituric acid reactive species (TBARS), and *B*: electrolyte leakage in leaves of *Triticum aestivum* L. (cv. Yangmai 13) exposed to 100% (CK), 60%, 40%, and 20% solar irradiance. Data represent means ± SD (n = 5). Means followed by the *different letters* are significantly different at $P \leq 0.05$, according to *Duncan*'s Multiple Range Test.

The reductions in Chl *a*/*b* found in the shaded plants were primarily caused by the higher increase of Chl *b* (Table 2). The capacity of acclimation to lower solar irradiance stresses was also confirmed by the significant decreases in Xan/Chl in plants under reduced light (Table 2). This suggests that plants under low light were able to finely regulate light absorption at expenses of thermal dissipation, and that a reduced need of photoprotection against triplet-state Chl occurred in shaded plants (Demmig-Adams *et al.* 1995, Heldt 2005).

Generally, it is known that net photosynthetic rate under light-saturation point declines with decreasing solar radiation (Chang *et al.* 2008). On the other hand, recent studies on winter wheat suggested that relatively low-intensity shading improves P_N and growth in this species (Li *et al.* 2010). In the present study, we found that P_N values increased with increasing PAR, with lower values present in the shading treatments (Fig. 2*A*). In particular, the decrease found in L60 were not marked if compared to CK, suggesting that plants are able to maintain a high photosynthetic rate also at 40% less environmental light. As LCP is the irradiance level at which there is sufficient light for net photosynthesis, low LCP values translate into the ability of a species to photosynthesize at low light intensities. In this study, we observed reductions in LCP in plants under low light (Fig. 2*A*) that were capable of better using light energy if compared to fully irradiated plants.

Decreases in photosynthetic efficiency due to photoinhibition or photooxidative damage in reaction center of PSII are usually measured by Chl fluorescence parameters (Baker 2008, Liu *et al.* 2007). In particular, the F_v/F_m ratio is often used to estimate the degree of photoinhibition determined by excessive light (Baker, 2008). Our results show that F_v/F_m increased in the shading treatments (Fig. 2*B*), implying that under high light the primary photochemical reactions of photosynthetic apparatus were damaged and photoinhibition occurred (Dai *et al.* 2007).

The variation of F_0 is closely correlated with the photosynthetic pigments and the characteristics of PSII reaction center, as an increase in F_0 might either be resulted from the increase in Chl content or from the impaired PSII system centre (Schnettger *et al.* 1994, Maxwell and Johnson 2000). We hypothesize that the high values of F_0 in the shading treatments could have been primarily due to the significant increases in Chl pigments (Table 2).

The lower values of effective quantum yield of PSII photochemistry (Φ_{PSII}) found in the shading treatments demonstrated that less light energy was utilized in photochemical reaction if compared to CK (Fig. 3*A*). It is noteworthy that the differences of Φ_{PSII} between treatments here observed are not so marked if compared to analogous experiments carried out on other winter wheat cultivars (Li *et al.* 2010, Mu *et al.* 2010). By contrary, fully irradiated plants experienced an excess of light energy, and this phenomenon was supported by their higher thermal dissipation (Fig. 3*C*), according to Demmig-Adams *et al.* (1995). The values of $1 - q_P$, higher in shaded plants (Fig. 3*D*), suggesting that a higher fraction of closed PSII reaction centers occurred under low light (Maxwell and Johnson 2000), with consequent lower excitation pressure on PSII and depressing effects on P_N (Fig. 2*A*). The significant reduction of relative electron transport rate (ETR) in the shading treatments supports this point (Fig. 4*B*).

High light levels may cause photoinhibition in plant leaves, increasing the production of ROS, toxic for cell metabolism (Rout and Shaw 2001). Superoxide radicals

($O_2^{\bullet-}$) are scavenged by SOD, and its enzymatic activity results in the formation of H_2O_2, then transformed into H_2O and O_2 by CAT, APX and other peroxidases (Li *et al.* 2000, Favaretto *et al.* 2011). In this experiment, higher values in SOD and POD activities were observed in the shading treatments (Fig. 4*A*), suggesting that wheat plants were well acclimated to the low light conditions and that the activities of these two light-sensitive enzymes can be depressed under environmental light, according to Casano *et al.* (1997). The paralleled significant decrement of TBARS concentration and electrolyte leakage in the reduced irradiance treatments indicated that a lower degree of oxidative damage occurred under low light (Fig. 5*A*) and that the membranes were not damaged as well (Fig. 5*B*).

In conclusion, our results demonstrated that *Triticum aestivum* L. (cv. Yangmai 13) has a good degree of shading tolerance and acclimation, and is able to cope with low solar irradiance better than other related winter wheat cultivars, such as Yangmai 158 and Yangmai 11 (Li *et al.* 2010, Mu *et al.* 2010). The higher contents of Chl found in the reduced irradiance treatments (Table 2) did not fully compensate the negative effect of reduced solar irradiance, thus P_N and Φ_{PSII} decreased in shaded plants (Fig. 2*A*). Yangmai 13 plants under reduced light did not present decreases in the efficiency of the photosynthetic and biochemical apparatus, as confirmed by their higher values of F_v/F_m (Fig. 2*B*), higher activities of antioxidant enzymes (Fig. 4), and lower levels of membrane damage (Fig. 5) if compared to fully irradiated plants. As any significant and widespread change in solar irradiance significantly affects agricultural production and field experiments on wheat subjected to reduced solar irradiance are scarce, our data on photosynthetic and antioxidant responses of winter wheat to low light levels could be important for winter wheat cultivation and productivity.

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