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Comparison of Photosynthetic Characteristics and Antioxidant Systems in Different Wheat Strains

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Abstract To gain better wheat (*Triticum aestivum* L.) varieties for planting in Sichuan province, we compared the differences in the photosystem II (PSII) and antioxidant defense system at the blooming stage in 20 different new wheat strains collected from the wheat regional trial of Sichuan province in the year 2015. According to all measured data, we found that one of the strains, CD012J1, presented the highest photosynthetic and antioxidant enzyme activities, and a lower level of reactive oxygen species (ROS) than other wheat strains. In contrast, Chuan12147 had a lower photosynthetic rate and accumulated a higher level of ROS compared with other wheat strains. At the same time, we also found that wheat strains SH1103, 12C, XK322-1, and 13B4 had better photosynthetic capacity compared with Chuan12147. Correlation analysis indicated that wheat yield was significantly correlated with chlorophyll fluorescence parameters: the maximum efficiency of PSII photochemistry (F_v/F_m), the quantum yield of PSII electron transport, and the non-photochemical quenching coefficient. In addition, immunoblotting analysis indicated that Chuan12147 presented the highest levels of PsbS protein in six different wheat strains. Taken together, our results suggest that CD012J1 and Chuan12147 have the best and worst photosynthetic capacity and antioxidant systems, respectively. Moreover, F_v/F_m and PsbS protein could be used as a marker in breeding of wheat varieties.

Keywords Wheat (*Triticum aestivum* L.) · Chlorophyll fluorescence · Antioxidant enzymes · Reactive oxygen species · Thylakoid proteins

Introduction

Wheat (*Triticum aestivum* L.) is the major grain crop and staple food in the world. It occupies a central position in agriculture and economy and tends to grow in cooler temperate climates. In China, the total wheat production during 2014–2015 reached 13.0 million tons according to the data obtained from National Bureau of Statistics. Wheat is a rich source of carbohydrates and contains many proteins, vitamins such as vitamin A, C, and E, and minerals like calcium (Ca), iron (Fe), phosphorus (P), kalium (K), and magnesium (Mg) (Khan and Zeb 2007). However, its yield and grain quality are highly impacted by many factors: cultivar and different environmental stresses such as high light, drought stress, fungal diseases and so on (Wahid and others 2007; Kocheva and others 2009; Chen and others 2015). A previous study indicated that disease usually leads to a 13–34% loss of wheat yield, but losses may be as high as 50% when the flag leaf suffers from severe disease during the heading and filling stages (Griffey and others 1993). Wheat grain quality is highly influenced by climatic conditions, cropping year, process of harvest, and

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storage conditions (Amjad and others 2010). Therefore, it is essential to breed good wheat varieties for yield and grain quality.

It is well known that more than 90% of crop biomass is derived from photosynthesis, which is one of the most fundamental processes sustaining all forms of life on earth. Although a lack of correlation between photosynthesis and plant yield has been reported frequently, previous studies indicated that enhanced photosynthesis at the level of the single leaf may increase plant yields, irrespective of other genetic factors (Long and others 2006; Makino 2011). Elevated CO_2 experiments also showed a close relationship between enhanced photosynthesis, biomass, and yield (Makino 2011; Ainsworth and Long 2005). A recent study showed that ear photosynthesis could contribute towards increasing grain yield (Sanchez-Bragado and others 2016). However, photosynthesis is often limited by many abiotic and biotic stresses that occur under natural conditions (Yang and others 2008; Brestic and others 2012; Zhang and others 2015). As the most important part of the photosystem, photosystem II (PSII) is a large multisubunit pigment–protein complex found in the thylakoid membranes of algae, cyanobacteria, and plants (Pagliano and others 2013). PSII is sensitive and vulnerable to environmental stresses in the field (Brestic and others 2012). A previous study showed that the content of D1 protein, as the core member of PSII, significantly decreased in grain filling wheat plants exposed to high light and high temperature co-stress (Zhao and others 2011). Usually, high or low photosynthetic activity is determined to be a very important indicator of quality in wheat varieties. Therefore, different wheat varieties can be selected on the basis of photosynthetic characteristics in wheat plants. However, much less attention has been paid to the correlation between wheat yield and PSII photochemistry.

In recent years, it has become apparent that reactive oxygen species (ROS) play an important role in plant growth, development and especially response to biotic and abiotic environmental stresses. ROS include free radicals such as superoxide anions ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$), as well as nonradical molecules like hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). PSI and PSII which form the core of the light-harvesting system in the thylakoids of chloroplast are the major sources of ROS production (Asada 2006). Although ROS are mainly produced by PSI, the light-induced production of various types of ROS was demonstrated in PSII (Pospisil 2009). Moreover, it has been known that the indirect effects of environmental stresses caused damage to PSII and were closely associated with the production of ROS (Landjeva and others 2008). Some studies have indicated that ROS is involved in destroying D1 protein and inhibits the repair of photodamaged PSII by suppressing the de novo synthesis of the D1 protein (Murata and others 2007; Yamamoto and others 2008). It is well known that various environmental stresses lead to excessive accumulation of ROS causing progressive oxidative damage, and subsequently decrease the photosynthetic capacity of plants, finally resulting in decreased yield (Sharma and others 2012; Chen and others 2015). Therefore, excellent wheat varieties can be found by comparing the accumulation of ROS and the antioxidant capacity.

In this study, we compared differences in ROS accumulation, the activities of antioxidant enzymes and nonenzymatic antioxidants, photochemical efficiency, and the levels of thylakoid membrane proteins in 20 different wheat strains, which are currently under regional cultivation trials in Sichuan province. They are representatives of advantageous agronomic traits and will become the candidate lines of new wheat varieties in Sichuan province in the future. The present study is designed to evaluate the photosynthetic capacity of wheat strains by chlorophyll fluorescence and the antioxidant system. In addition, we also hope to find several markers for rapid selection of excellent wheat varieties and finally obtain some excellent wheat strains for planting in Sichuan or even in the entire country.

Materials and Methods

Plant Materials

Seeds of 20 new wheat strains were obtained from seven organizations from the Sichuan province (Table 1). Field experiments were conducted in the same field at the experimental farm of Sichuan Agricultural University in Ya'an (29°59′N, 103°00′E), Sichuan Province, China in 2015 and 2016. The field climate was subtropical humid, with an average annual temperature of 14.5 °C and a mean annual rainfall of 1693.8 mm. The soil is clayey textured soil with 15.36 g/kg organic matter, 1.27 g/kg total nitrogen (N), 37.52 mg/kg available phosphorus (P), and 95.33 mg/kg available potassium (K) in the 0–20 cm soil layer. It is about 60 cm for the depth to water table in our experimental field. At the blooming stage, wheat leaves were packed in polyethylene bags and brought back to the laboratory in the morning. The secondary leaves were used for the measurements of various parameters. Representative samples of each strains were prepared for physiological and biochemical analyses.

Chlorophyll Contents, Leaf Water Status, Soluble Sugar, Proline Content, and 1000-Grain Weight

The contents of chlorophyll (Chl) *a* and *b* were assayed by taking fresh leaf samples (0.5 g) randomly from selected wheat plants. The samples were homogenized

Table 1 The data of wheat strains chosen in the present experiment

Number	Wheat strains	1000-grain weight (g)	Sources
1	SH1103	38.5	Sichuan Academy of Agricultural Sciences
2	Chuan12147	30.7	Sichuan Academy of Agricultural Sciences
3	12 C	38.8	Chengdu Institute of Biology, Chinese Academy of Sciences
4	MR11-12	37.6	Mianyang Institute of Agricultural Sciences
5	XK322-1	39.8	Southwest University of Science and Technology
6	SQ-18	37.9	Sichuan Academy of Agricultural Sciences
7	B12-6	37.9	Sichuan Agricultural University
8	12N921	30.4	Sichuan Agricultural University
9	Y12-2721	33.2	Sichuan Agricultural University
10	CD012J1	45.5	Chengdu Institute of Biology, Chinese Academy of Sciences
11	Nan12pingB985	29.4	Nanchong Academy of Agricultural Sciences
12	MR11-19	34.3	Mianyang Institute of Agricultural Sciences
13	Chuan13015	31.6	Sichuan Academy of Agricultural Sciences
14	13P2-14	32.5	Sichuan Academy of Agricultural Sciences
15	13HW7-14	34.1	Sichuan Academy of Agricultural Sciences
16	Gy07316	35.8	Guangyuan Institute of Agricultural Sciences
17	13B4	36.5	Chengdu Institute of Biology, Chinese Academy of Sciences
18	XK322-4	34.6	Southwest University of Science and Technology
19	Chuan13ping6	34.0	Sichuan Academy of Agricultural Sciences
20	W7	34.5	Chengdu Institute of Biology, Chinese Academy of Sciences

and extracted with 80% (v/v) acetone for 30 min at room temperature. The extracts were filtered through two layers of filter paper. Then the absorbance was measured at 645 and 663 nm using a UV–visible spectrophotometer (Hitachi-U2000, Tokyo, Japan). Chlorophyll contents were calculated using equations proposed by Porra and others (1989). Relative water content (RWC) of the wheat leaf was measured according to Li and others (2014). The formula is as follows: RWC = (fresh weight − dry weight)/(turgid weight − dry weight) × 100%. The turgid weight was determined after keeping the leaves in distilled water at 4 °C overnight in darkness, until they reached a constant weight. Dry weight was determined after the turgid leaves were kept at 85 °C in an oven for 24 h.

Water-soluble carbohydrates were extracted from fresh leaves (0.5 g) using an 80% ethanol solution followed by a hot water extraction. Soluble sugar was colorimetrically quantified after reacting with anthrone reagent according to the method of Thomas (1977). Absorbance was recorded at 485 nm using a UV–visible spectrophotometer. The corresponding content was determined against a standard curve prepared by using a glucose solution. Determination of free proline content was done according to the previous method (Bates and others 1973). Leaf samples (0.5 g) from 20 wheat strains were homogenized in 3% (w/v) sulfosalicylic acid, and then the homogenate was filtered through filter paper. Then, glacial acetic acid and ninhydrin reagent were added to an aliquot of the filtrate. The reaction mixture was boiled at 100 °C for 1 h in water bath. Finally, the reaction was stopped by using an ice bath. The mixture was extracted with toluene and the absorbance of the fraction with toluene aspired from the liquid phase was taken at 520 nm using a UV spectrophotometer.

Twenty wheat strains was harvested manually at full maturity and threshed with a combine. Then, the mass of 1000 grains was calculated.

Lipid Peroxidation and Electrolyte Leakage

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) level using the thiobarbituric acid (TBA) reaction as described by Chen and others (2015). For MDA extraction, fresh leaves (0.5 g) were homogenized with 5 mL of 5% (w/v) tri-chloro acetic acid (TCA). The homogenate was centrifuged for 10 min at 2500×*g*. For every 2 mL of the supernatant, 2 mL of 5% TCA containing 0.67% TBA was added. The mixture was heated at 95 °C for 30 min and then rapidly cooled on an ice bath. Afterwards, the mixture was centrifuged at 4 °C for 10 min at 5000×*g*. The content of MDA was calculated from the difference of the absorbance of the supernatant at 532 and 600 nm spectrophotometrically. Electrolyte leakage was measured with a conductivity meter (DDSJ-308A, Shanghai Precision Instruments Co., Ltd., China) according to the method of Chen and others (2015). After measuring the conductivity, the samples were heated for 15 min at 95 °C

in water bath to achieve a 100% electrolyte leakage. The relative electrolyte leakage was calculated as the ratio of the initial conductivity to the absolute conductivity (boiled at 95 °C for 30 min).

Assay of Reactive Oxygen Species

The rate of $O_2^{\cdot-}$ production was determined according to Elstner and Heupel (1976) by monitoring the nitrate formation from hydroxylamine with some modifications. Fresh leaf samples (0.5 g) were homogenized with 3 mL of 65 mM phosphate buffer (pH 7.8), 0.1 mL of 10 mM hydroxylamine hydrochloride, and 1 mL supernatant. After incubation at 25 °C for 15 min, ethyl ether in the same volume was added and centrifuged at 1500×*g* for 10 min. The production rate of $O_2^{\cdot-}$ was calculated by a standard curve with $NO_2^{\cdot-}$ form the chemical reaction of $O_2^{\cdot-}$ and hydroxylamine. H_2O_2 content was assayed by the method described by Okuda and Sagisaka (1991) with slight modifications. Fresh leaf samples (0.5 g) were cut into small pieces and homogenized in an ice bath with 5 mL of 0.1% (w/v) TCA. After centrifugation at 12,000×*g* for 20 min at 4 °C, 0.5 mL of supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide (KI) were mixed. The absorbance of the mixture was recorded at 390 nm. Finally, the concentration of H_2O_2 was calculated using a standard curve plotted with known concentration of H_2O_2.

Histochemical assay for superoxide anion radicals ($O_2^{\cdot-}$) and H_2O_2 was visually detected with nitro blue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB) in detached leaf segments, respectively, as previously described method by Chen and others (2015). Briefly, leaf samples were excised at the base with a razor blade and immersed in 6 mM NBT solution containing 50 mM Hepes buffer (pH 7.5) for 2 h or 5 mM DAB solution containing 10 mM morpholineethanesulfonic acid (MES) buffer (pH 3.8) for 8 h in the dark. Pigments in detached leaves were then extracted using boiling ethanol (90%) for 0.5–2 h by shaking in a water bath. At least three leaves were used for each treatment.

Antioxidative Enzyme Extraction and Assays

For the antioxidative enzyme assays, fresh leaf tissues (0.5 g) were ground with 5 mL ice-cold 25 mM Hepes buffer (pH 7.8) containing 0.2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM ascorbate, and 2% polyvinylpyrrolidone (PVP) using a chilled mortar and pestle. The homogenates were centrifuged at 12,000×*g* for 30 min at 4 °C and the supernatant was used for the determination of enzyme activities. All steps in extracting the enzymes were carried out at 4 °C. Peroxidase (POD) activity was measured according to the procedure of Egley and others (1983). Superoxide dismutase (SOD) activity was determined as described by Giannopolitis and Ries (1977). Catalase (CAT) activity was based on the method described by Cakmak and Marschner (1992). Ascorbate peroxidase (APX) activity was assayed by monitoring the rate of ascorbate oxidation at 290 nm as described previously (Nakano and Asada 1980). Glutathione peroxidase (GPX) activity was determined by using the consecutive glutathione reductase reaction according to Flohe and Gunzler (1984). Glutathione reductase (GR) activity was measured as a decrease in the absorbance at 340 nm due to NADPH oxidation following Foyer and Halliwell (1976).

Assay of Antioxidants

Contents of reduced ascorbic acid/dehydroascorbate (AsA/DHA) were measured in extracts according to the method of Kampfenkel and others (1995). The measurement is based on the reduction of Fe^{3+} to Fe^{2+} by AsA. Then, Fe^{2+} forms complexes with bipyridyl giving a pink color that absorbs at 525 nm. The contents of AsA and AsA+DHA were calculated using L-ascorbate as the standard. DHA content was determined using total ascorbate minus AsA. Reduced glutathione/oxidized glutathione (GSH/GSSG) contents were determined as described by Griffith (1980).

Analysis of Chlorophyll Fluorescence

Chlorophyll fluorescence was measured with an imaging fluorometer (the Imaging PAM M-Series Chlorophyll Fluorescence System, Heinz-Walz Instruments, Effeltrich, Germany) according to the instructions provided by the manufacturer. All wheat plants were kept in the dark for 30 min before fluorescence measurements. Then the secondary leaf was used for the chlorophyll fluorescence measurements. The actinic light intensity was 400 μmol/m^2/s^1, and the saturated flash intensity was 8000 μmol/m^2/s^1. The minimal fluorescence level (F_0) with all PSII reaction centers open and the maximal fluorescence level (F_m) with all PSII reaction centers closed were measured with dark-adapted leaves, from which the maximum efficiency of PSII photochemistry (F_v/F_m) value was obtained. The representative image data acquired were normalized to a false color scale. Then, the photochemical quenching (qP), the quantum yield of PSII electron transport (ΦPSII), and the non-photochemical quenching (NPQ) were obtained based on fluorescence parameters measured in both light- and dark-adapted leaves according to Maxwell and Johnson (2000). The quantum yield of non-regulated energy dissipation Y(NO) is calculated according to Kramer and others (2004). The non-photochemical quenching coefficient (qN) compares the F_0 and F_m levels before and after illumination (Havaux and others 1991).

Isolation of Thylakoid Proteins and Immunoblotting Analyses

Thylakoid membranes from six representative wheat leaves were isolated as described (Chen and others 2016a). The Chl concentrations of the thylakoid membranes were determined as described previously by Porra and others (1989). Isolated thylakoid membrane proteins were separated by SDS-PAGE (6% acrylamide stacking gel + 15% separation gel + 6 M urea) (Laemmli 1970) and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilone, Millipore, Darmstadt, Germany). For specific antibodies against the D1, D2, CP43, Lhcb1, Lhcb2, Lhcb3, Lhcb4, Lhcb5, Lhcb6, PsbS, Lhca1, Lhca2, Lhca3, and Lhca4 proteins, blocking was performed with 5% skimmed milk. These protein antibodies were obtained from Agrisera (Umea, Sweden). Then, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Bio-Rad Comp. Hercules, CA, USA) and developed using a chemiluminescent detection system (ECL, GE Healthcare, Buckinghamshire, United Kingdom). Quantitative analysis of PsbS protein was performed using Quantity One software (Bio-Rad Comp. Hercules, CA, USA).

Statistical Analysis

All data presented are the mean values ± standard deviation (SD). For each measurement, at least four independent replicates were performed. All data were statistically analyzed using the SPSS Statistics 19.0 software (IBM, Chicago, IL, USA), and the means were compared using Duncan's multiplication range test at the 0.05 level of significance.

Results

Chlorophyll (Chl) Content, RWC, Soluble Sugar, and Proline Content

As shown in Fig. 1a, the total Chl content was different in 20 wheat strains. Chl content in SH1103, Chuan12147, 12 C, MR11-12, B12-6, 12N921, Y12-2721, and Chuan13015 was significantly lower than that in XK322-1, CD012J1, Nan12pingB985, MR11-19, 13HW7-14, Gy07316, 13B4, XK322-4, Chuan13ping6, and W7 ($p < 0.05$). Although there was no significant difference in RWC among many different wheat strains, a significant decrease by 8% in RWC was observed in Chuan12147 compared with CD012J1 in the field ($p < 0.05$) (Fig. 1b). In addition, we also found that the contents of soluble sugar and proline in Chuan12147 and CD012J1 were nearly the

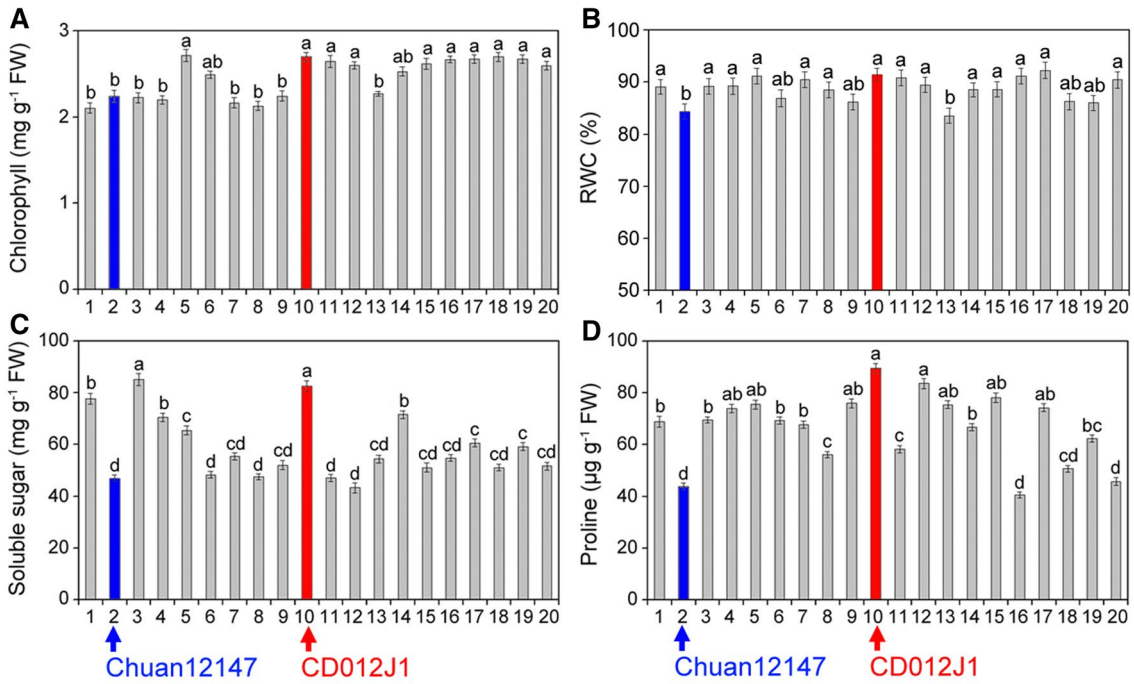

Fig. 1 Chlorophyll content (**a**), relative water content (RWC) (**b**), soluble sugar (**c**), and proline (**d**) of 20 wheat strains in the field. Values are mean ± SD from four independent biological replicates ($n = 4$). Statistical analysis was performed using Duncan's multiplication range test. *Different letters* depict significant differences among 20 wheat strains ($P < 0.05$)

lowest and highest, respectively (Fig. 1c and d). Moreover, compared to CD012J1, 1000-grain weight decreased by 32.5% in Chuan12147 (Table 1), indicating CD012J1 had higher grain yield compared to other wheat strains because a strong positive correlation is established between 1000-grain weight and wheat yield (Hadjichristodoulou 1990).

ROS Accumulation and Lipid Peroxidation in Wheat Leaves

To examine the levels of ROS production among 20 different wheat strains grown in the field, quantitative levels of the two major ROS species, $O_2^{\cdot-}$ and H_2O_2, were determined. Although the levels of $O_2^{\cdot-}$ and H_2O_2 were different, significant $O_2^{\cdot-}$ production rate and H_2O_2 content were shown in Chuan12147 (Fig. 2a and b). Compared to Chuan12147, CD012J1 presented very low levels of $O_2^{\cdot-}$ and H_2O_2 (72.9 and 55.5%, respectively). In addition, $O_2^{\cdot-}$ production rate and H_2O_2 content in SH1103, 12 C, XK322-1, and 13B4 were also lower than that of Chuan12147. Furthermore, we also examined the degree of oxidative damage in leaves of different wheat strains by investigating the level of lipid peroxidation (Barclay and Mckersie 1994). As shown in Fig. 2c, d, the concentrations of MDA and electrolyte leakage in Chuan12147 were also significantly higher than that of SH1103, 12C, XK322-1, CD012J1, and 13B4. Compared to CD012J1, the MDA and electrolyte leakage in leaves markedly increased by 89.1 and 63.9% in Chuan12147, respectively.

Based on the above results, we analyzed the accumulation of $O_2^{\cdot-}$ and H_2O_2 by histochemical staining of the leaves using NBT and DAB at the blooming stage in 20 wheat strains, respectively. In comparison, the accumulation of $O_2^{\cdot-}$ and H_2O_2 was more pronounced in Chuan12147 than that of SH1103, 12C, XK322-1, CD012J1, and 13B4 (Figs. 3, 4).

Antioxidant Defense System

The plant antioxidant defense system consists of antioxidant enzymes and non-enzymatic antioxidants. The activities of antioxidant enzymes in 20 different wheat strains are presented in Fig. 5. All antioxidant enzyme activities in Chuan12147 were nearly the lowest among 20 wheat strains. Compared to Chuan12147, the activities of six antioxidant enzymes (POD, SOD, CAT, APX, GPX, and GR) in CD012J1 significantly increased by 155.7, 40.5, 39.6, 226.5, 193.5, and 111.8%, respectively. Relative to other wheat strains, the highest activities of POD, APX, and GR were observed in CD012J1 (Fig. 5a, d and f). It

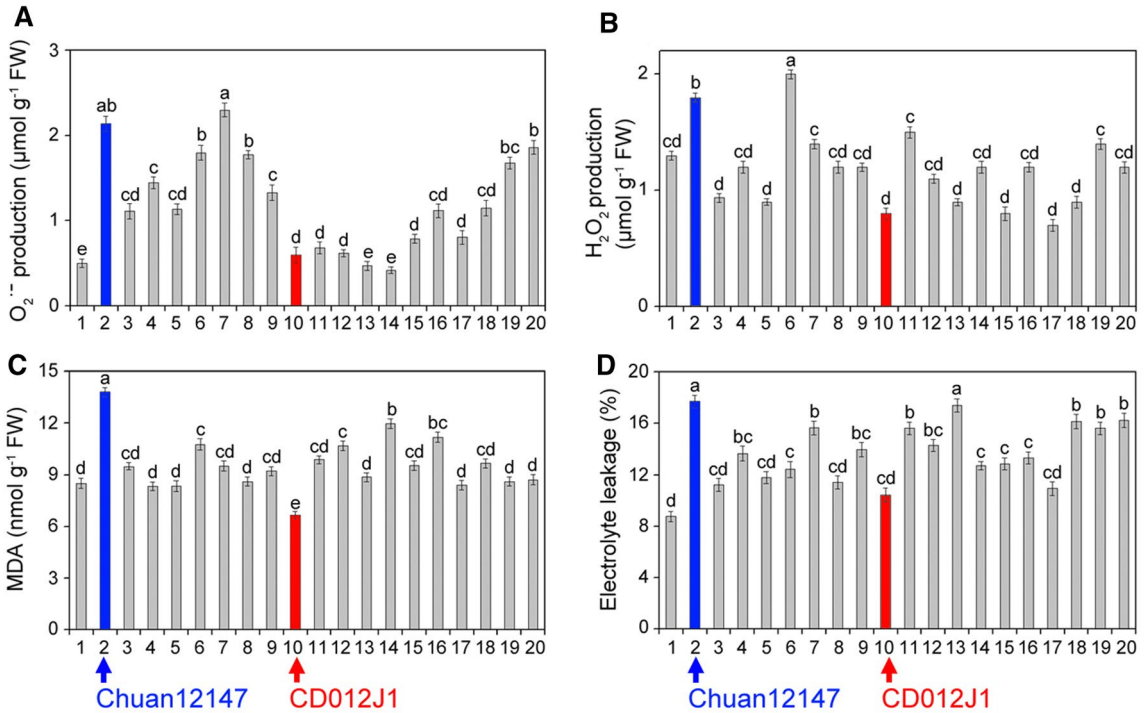

Fig. 2 Superoxide anion radicals ($O_2^{\cdot-}$) production rate (**a**), hydrogen peroxide (H_2O_2) contents (**b**), malondialdehyde (MDA) content (**c**), and electrolyte leakage (**d**) in wheat leaves at the blooming stage in the field. Values are expressed as the means ± SD from four independent biological replicates ($n = 4$). Means denoted by the *different letter* significantly differed at $p < 0.05$ according to Duncan's multiplication range test

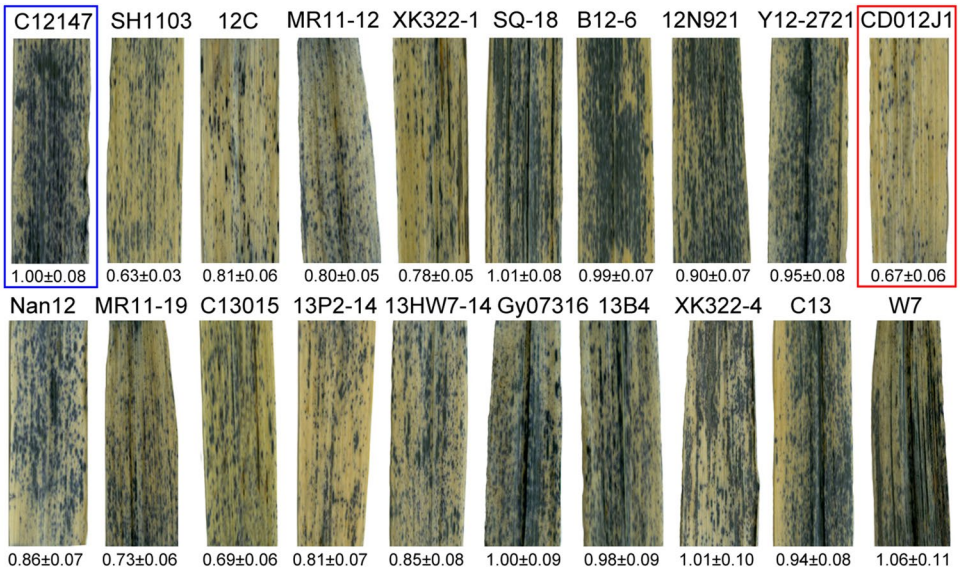

Fig. 3 Histochemical assays for superoxide anion radicals ($O_2^{\cdot-}$) by nitro blue tetrazolium (NBT) staining in 20 wheat strains. Quantitative values (±SD) are shown below the individual images

Fig. 4 Histochemical assays for hydrogen peroxide (H_2O_2) by 3,3-diaminobenzidine (DAB) staining in 20 wheat strains. Quantitative values (±SD) are shown below the individual images

was noteworthy that, in comparison with Chuan12147, the activities of six antioxidant enzymes in SH1103, 12C, XK322-1, and 13B4 were also higher. Contents of non-enzymatic antioxidants (AsA, DHA, GSH, and GSSG) are shown in Fig. 6. CD012J1 showed a higher level of AsA and DHA compared to other wheat strains. Similarly, the content of GSH in this strain was significantly higher than that in Chuan12147.

Difference in Chlorophyll Fluorescence

PSII photochemistry of 20 different wheat strains was examined by a modulated imaging fluorometer. As shown in Supplementary Fig. S1, the maximum photochemical efficiency of PSII in the dark-adapted leaves (F_v/F_m) in Chuan12147 and Chuan13015 was significantly lower than that in other wheat strains. Compared to CD012J1, the F_v/F_m value in Chuan12147 decreased by 16.9%. Furthermore, we also found that the quantum yield of ΦPSII in Chuan12147 was the lowest among 20 wheat strains and had a significant decrease by 48.9% compared with CD012J1 (Supplementary Fig. S2). Opposite to the values of ΦPSII, the level of NPQ in Chuan12147 was obviously higher than that in other wheat strains and had a remarkable increase by 180.0% relative to CD012J1 (Supplementary Fig. S3). Pearson correlation analysis suggested that F_v/F_m was the best correlation with grain yield in these parameters of 20 wheat strains (Table 2). At the same time,

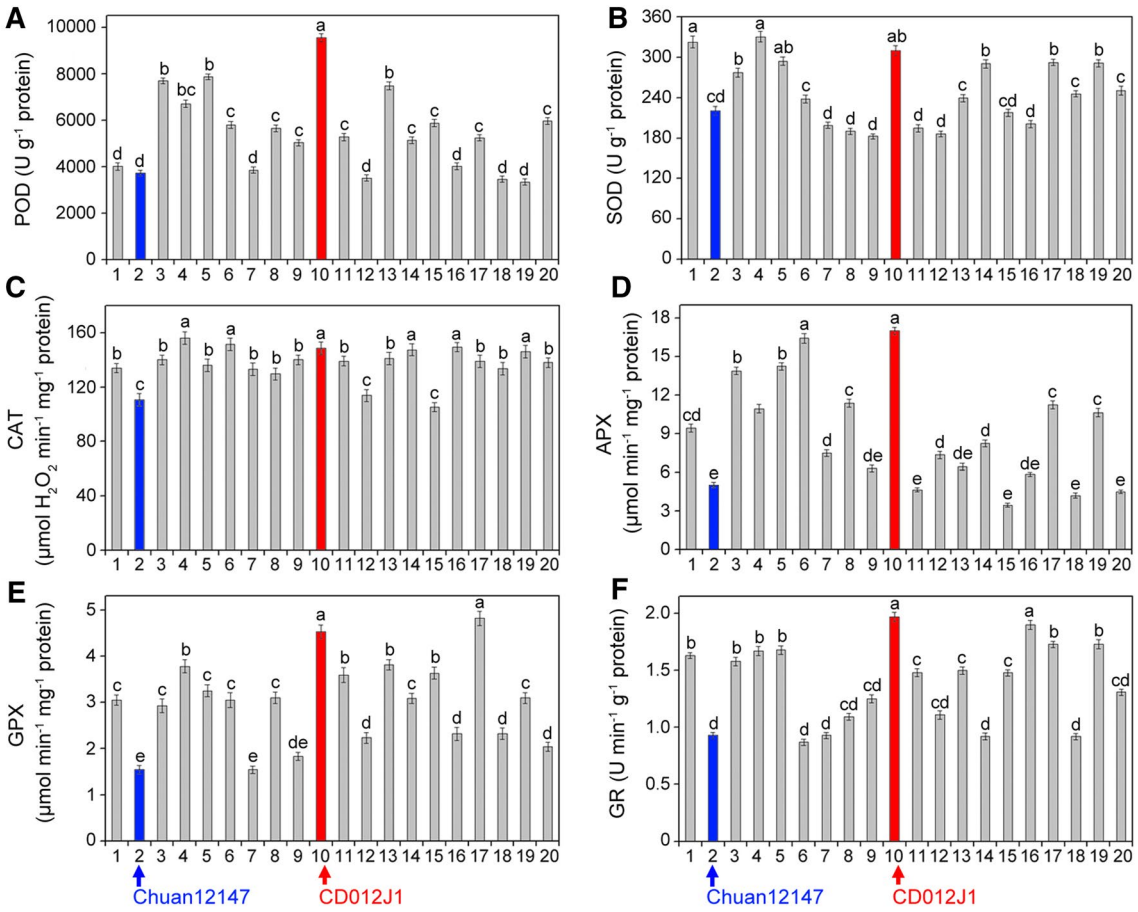

Fig. 5 The *POD*, peroxidase (**a**); *SOD*, superoxide dismutase (**b**); *CAT*, catalase; (**c**); *APX*, ascorbate peroxidase (**d**); *GPX*, glutathione peroxidase (**e**); and *GR*, glutathione reductase (**f**) in 20 wheat plants. *Bars* represent standard deviations from four independent biological replicates ($n=4$). *Different letters* depict significant differences among different wheat strains ($p<0.05$) according to Duncan's multiplication range test

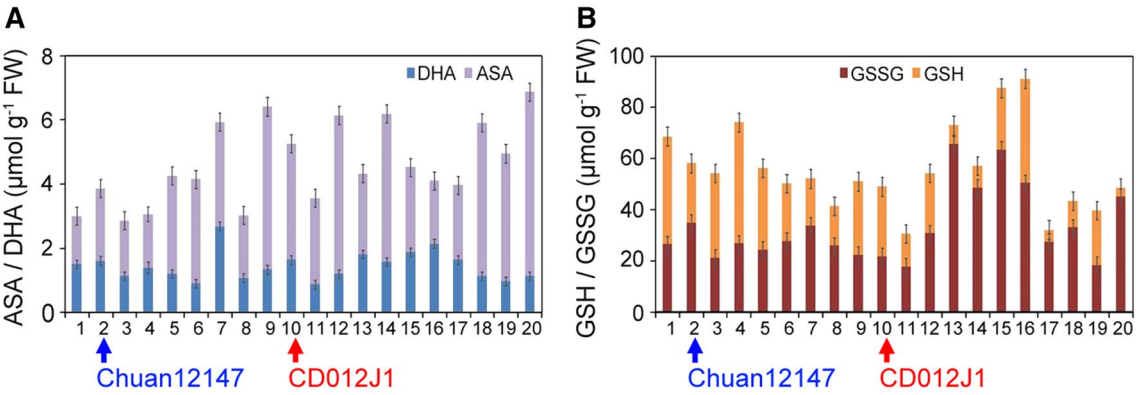

Fig. 6 The content of AsA (**a**), DHA (**b**), GSH (**c**), and GSSG (**d**) in different wheat strains at the blooming stage. *Bars* represent standard deviations from four independent biological replicates ($n=4$)

we also found that NPQ and ΦPSII had a better correlation with wheat grain yield. In addition, we analyzed image data of F_v/F_m, ΦPSII, NPQ, qP, Y(NO), and qN between Chuan12147 and CD012J1. Compared to Chuan12147, CD012J1 presented higher levels of ΦPSII and qP. However, the values of Y(NO) and qN in CD012J1 decreased by 222.4 and 29.8% compared to Chuan12147, respectively (Fig. 7).

Table 2 Results of two-way analysis of variance (ANOVA) of wheat 1000-grain weight and the following parameters

Dependent variable	Pearson correlation coefficient	Dependent variable	Pearson correlation coefficient
F_v/F_m	0.722**	SOD	0.352
ΦPSII	0.597**	POD	0.394
NPQ	−0.61**	CAT	0.062
Relative water content	0.484*	GPX	-0.095
qP	0.433	APX	0.338
H_2O_2	0.412	GR	-0.125
$O_2^{\cdot-}$	0.244	DHAR	-0.113
Chlorophyll	−0.226	GSH	0.348
MDA	0.006	AsA	0.019
Electrolyte leakage	0.157	Total protein content	-0.252

Numbers represent *F* values at 5% level

*$p < 0.05$

**$p < 0.01$

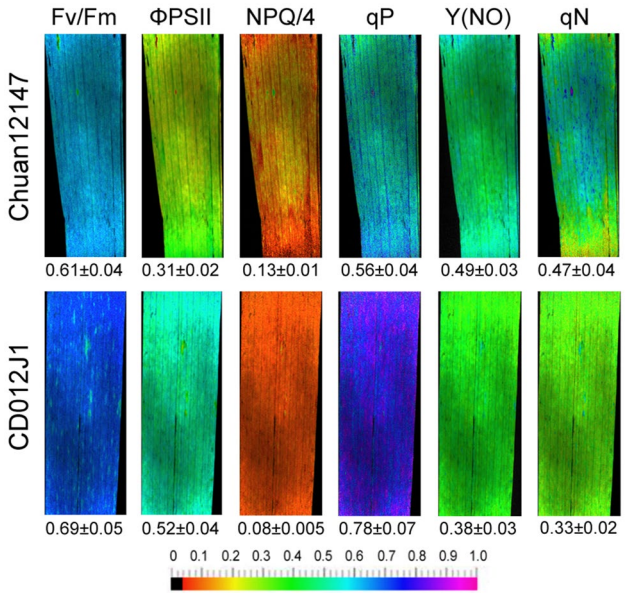

Fig. 7 Chlorophyll fluorescence parameters (F_v/F_m, maximum efficiency of PSII photochemistry. *qP*, photochemical quenching; *NPQ/4*, non-photochemical quenching; *ΦPSII*, quantum yield of PSII electron transport; *Y(NO)*, quantum yield of non-regulated energy dissipation; *qN*, non-photochemical quenching coefficient) in Chuan12147 and CD012J1. Quantitative values (±SD) are shown below the individual fluorescence images

Content of Thylakoid Membrane Proteins

To detect the differences in the levels of thylakoid proteins, the thylakoid polypetide composition was investigated by immunoblot analysis in six representative wheat strains (SH1103, Chuan12147, 12 C, XK322-1, CD012J1, and 13B4) (Fig. 8). The reason for selecting six representative wheat strains was because they had the better or worse photosynthetic capacity among 20 wheat strains. We found that the levels of almost all the analyzed thylakoid proteins showed no detectable changes in these wheat strains. Surprisingly, the level of PsbS protein was obviously increased in Chuan12147 compared with the other five wheat strains (Fig. 8c). Interestingly, although the level of the CP29 protein did not change in any of the plants, phosphorylation of the CP29 protein was observed in these wheat plants (Fig. 8a).

Discussion

It is well known that wheat plants suffer from various environmental stresses in the field, resulting in severe yield losses. Therefore, the use of excellent wheat cultivars is the most cost-effective approach in planting. Many studies have indicated that resistant wheat varieties have high photosynthetic efficiency and better antioxidant systems than non-resistant wheat in response to environmental stresses (Grzesiak and others 2003; Marcińska and others 2012; Chen and others 2015). Here, we compared the photosynthetic characteristics and antioxidant systems in 20 new wheat strains from the wheat regional trial of Sichuan province.

Photosynthetic pigments are a commonly observed index in comparing photosynthetic activity because a reduction in chlorophyll content occurs in higher plants normally under stressful conditions (Wang and others 2014, Su and others 2014; Chen and others 2015). The stress-induced alterations in chlorophyll content may be due to impaired biosynthesis or accelerated pigment degradation (Perveen and others 2010; Brestic and others 2016). Our results indicated that chlorophyll content in Chuan12147 was significantly

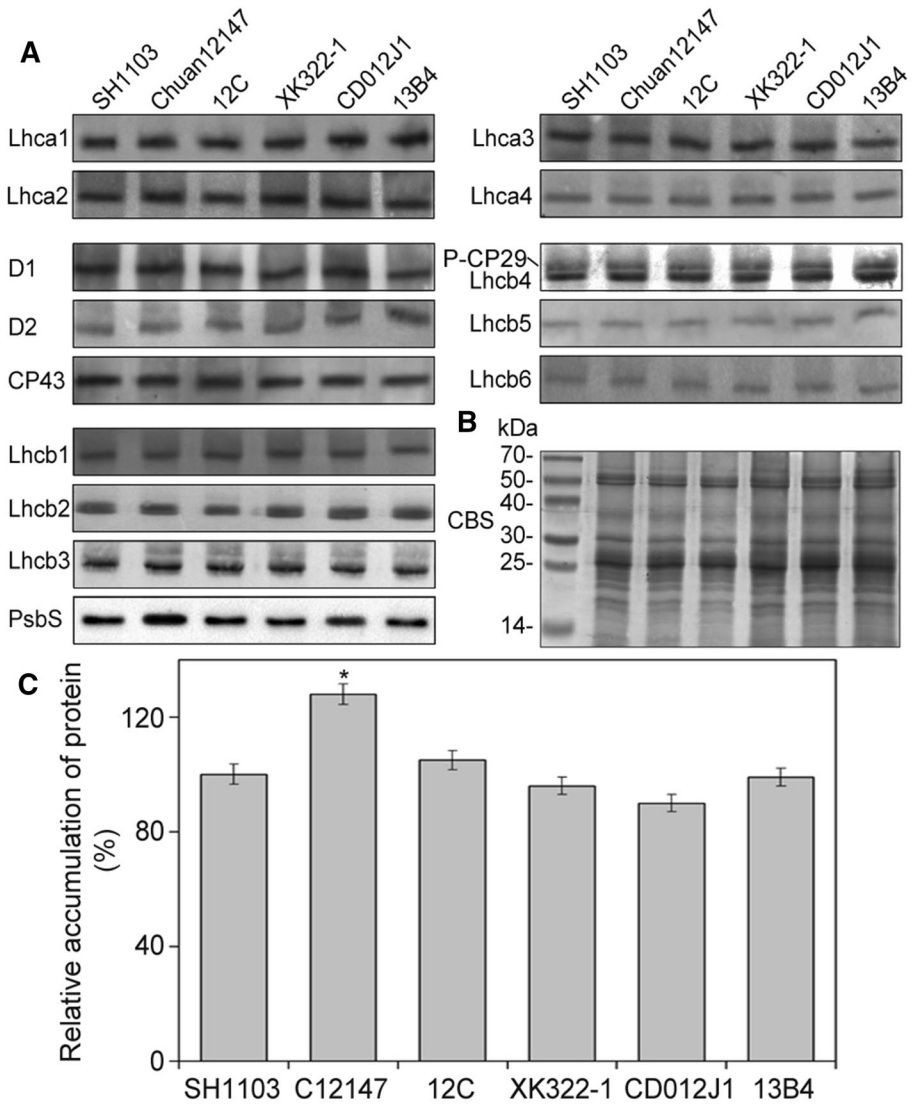

Fig. 8 Immunoblot analyses of thylakoid membrane proteins obtained from six representative wheat strains. Immunoblot analyses of thylakoid proteins were performed using antibodies specific for representative photosystem I (PSI) and photosystem II (PSII) (**a**). The SDS–PAGE results after Coomassie blue staining (CBS) are shown in the right panel (**b**). One microgram of total chlorophyll was loaded into each electrophoretic lane. **c** Quantitative data for PsbS protein. Results are presented relative to the content of SH1103 (100%). Significantly different values are indicated with an asterisk when $p < 0.05$ according to Duncan's multiplication range test. Values are means ± SD from three independent biological replicates

low. During the blooming stage, the low chlorophyll content in the field could be attributed to the enhancement of chlorophyll degradation (Park and Paek 2007). Thus, it could be suggested that CD012J1 probably has a more effective protective system against degradation of chlorophyll in the field.

Plants usually protect themselves using different physiological and biochemical strategies in response to various environmental stresses (Zivcak and others 2009). Some osmolytes such as proline and soluble sugars are widespread throughout the plant kingdom. Proline and soluble sugars are the two most important organic solutes under stressful conditions (Pérez-Alfocea and Larher 1995). Many studies have indicated that free proline levels increase in response to drought (Rampino and others 2006; Chorfi and Taibi 2011; Olsovska and others 2016). In our experiment, a high level of proline and soluble sugars was found in CD012J1 in the field, indicating that CD012J1 has a high resistance because the resistant plants can improve their resistance by increasing the content of permeability material (Marcińska and others 2012).

Under natural conditions, cellular homeostasis is disrupted and electrons are transferred to molecular O_2 to form ROS, thereby inducing oxidative stress and photoinhibition (Gill and Tuteja 2010). The results from the present experiment showed that CD012J1 accumulated lower concentrations of ROS compared to other wheat strains in the field. Low concentrations of ROS are known to act as signal molecules initiating several protective resistance mechanisms against pathogens, drought, and heat stress (Horváth and others 2007; Chen and others 2015). Here, we confirmed that the protective antioxidant system was activated in CD012J1, which subsequently changed the levels of ROS and avoided oxidative damage. It is well known that ROS species are also reportedly involved in lipid peroxidation, which in turn results in the injury

of cell membranes (Smirnoff 1993). The low content of $O_2^{\cdot-}$ and H_2O_2 in CD012J1 could be identified by the accumulation of MDA and electrolyte leakage (Halliwell and Gutteridge 1984). In this study, our results indicated that CD012J1 may have a better ROS-scavenging system in the field.

To detoxify excessive ROS species accumulation, plants have developed an antioxidant defense system (Bowler and others 1992; Larkindale and Huang 2004). The coordinated action of antioxidant enzymes and antioxidant compounds eliminate or reduce oxidative damage. In the present study, pronounced differences in these antioxidant enzyme activities were observed in 20 wheat strains. The activities of six anxioxidant enzymes (POD, SOD, CAT, APX, GPX, and GR) were markedly high in CD012J1. In contrast, these antioxidant enzyme activities were low in Chuan12147. In addition, the AsA-GSH cycle is one of the important protection systems against ROS in different cell compartments (Noctor and Foyer 1998). The high AsA and GSH content in CD012J1 also indicated that CD012J1 has a more effective system in oxidative protection. Therefore, the results suggested that these antioxidant enzymes and non-enzymatic antioxidants can alleviate oxidative damage by scavenging excessive accumulation of ROS in CD012J1.

Chlorophyll fluorescence has been proven to be a useful, non-invasive tool for the study of different aspects of photosynthesis (Ashraf and Harris 2013; Kalaji and others 2017). F_v/F_m, NPQ, ETR, and ΦPSII are usually used as sensitive indicators of plant photosynthetic performance (Baker 2008; Sun and others 2016). In the present study, when different wheat plants were exposed to field conditions at the blooming stage, CD012J1 presented significantly high values of Fv/Fm and ΦPSII, and a low level of NPQ, which dissipates light energy and decreases the efficiency of photochemical reactions of photosynthesis (Graßes and others 2002). However, there was a reverse result in Chuan12147. The results were further identified by the value of Y(NO) and qN. Y (NO) describes the quantum yield of non-regulated energy dissipation in PSII. Large values of Y(NO) reflect a suboptimal capacity of photoprotective reactions in Chuan12147 (Bürling and others 2010). qN is related to pH-dependent limitations in the chloroplast, carotenoid quenching of exactions, phosphate-availability limitations, and heat dissipation. It is the most sensitive index in evaluating the earlier stress. These findings indicated that CD012J1 has a higher photosynthetic capacity relative to other wheat strains in the field. In addition, our results indicated that chlorophyll fluorescence was significantly correlated with crop yield, especially F_v/F_m, suggesting that photosynthetic parameters were probably a good marker in breeding of wheat varieties. However, there were no studies about using chlorophyll fluorescence as a marker of crop yield presently.

Previous studies have indicated that PsbS protein plays an important role as a kinetic regulator in the energy dissipation process (Johnson and Ruban 2010; Chen and others 2016b). In this study, Chuan12147 had obviously high amounts of PsbS, which is involved in inducing the formation of NPQ. It was consistent with the results from the level of NPQ, suggesting that PsbS protein might be an effective marker in breeding of wheat varieties. In addition, our study indicated that CP29 was strongly phosphorylated in six wheat strains in the field, suggesting that CP29 phosphorylation plays an important role in monocots. Previous studies showed that phosphorylation of the CP29 protein in thylakoid membranes may be involved in a number of responses to a changing environment (Chen and others 2009, 2015).

Conclusions

In this experiment, we have determined that CD012J1 and Chuan12147 have the best and the worst photosynthetic capacity and antioxidant defense systems among 20 wheat strains by comparing the changes in ROS levels, PSII photochemistry, and the activities of antioxidant enzymes and non-enzymatic antioxidants, respectively. In addition, our results show that the level of PsbS protein is significantly correlated with NPQ, which has not been observed in previous studies. Furthermore, our results indicate that Fv/Fm and PsbS protein may be suitable markers in breeding of wheat varieties. Taken together, CD012J1 is the best strain to be planted in Sichuan. In addition to the CD012J1 strain, we propose that SH1103, 12C, XK322-1, and 13B4 are also suitable for planting in Sichuan.

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Compliance with Ethical Standards

Conflict of interest Authors declare no conflict of interest.

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