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Comparative study of photosynthetic capacity in lower leaves in the canopy of dwarf and semidwarf wheat

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Abstract

Semidwarf wheat contributes to significant yield increase worldwide, however, few dwarf cultivars of wheat are cultivated due to grain yield penalty. In this study, a new dwarf wheat *Triticum aestivum* L., cv. Xiaoyan 101, was investigated to explore its photosynthetic performance and yield potential. In the comparison of the semidwarf wheat cultivars, Jing 411 and Xiaoyan 101, although the first three leaves (including flag leaves) did not differ significantly in both genotypes, Xiaoyan 101 conferred a higher content of photosynthetic pigments and higher photochemical efficiency but had lower contents of hydrogen peroxide and malondialdehyde in lower leaves in the canopy. In addition, the antioxidant enzymes-encoding genes were upregulated while the senescence-associated genes (*TaSAG3*, *TaSAG5*, *TaSAG7*, and *TaSAG12*) were downregulated in lower leaves in the canopy of Xiaoyan 101. Ultimately, Xiaoyan 101 produced approximate or even higher grain yield than the local semidwarf wheat varieties. Therefore, it is possible to breed dwarf wheat with enhanced photosynthetic activity but without yield sacrifice.

Keywords: canopy photosynthesis; chlorophyll fluorescence; gene expression; *Triticum aestivum* L.

Introduction

Since the 1960s, semidwarf wheat varieties have contributed to a significant yield increase, which is well-known as the 'Green Revolution'. The yield increase of semidwarf varieties is ascribed to improved lodging resistance and photosynthate partition to grains (Youssefian *et al.* 1992, Flintham *et al.* 1997, Bishop and Bugbee 1998). Wheat plant height is controlled by reduced height (*Rht*) genes. More than 25 *Rht*s have been designated and can be grouped as gibberellin acid (GA)-insensitive genes (*GAI*), such as *Rht-B1b*, *Rht-B1c*, *Rht-D1b*, *Rht-D1c*, and GA-responsive genes (*GAR*), such as *Rht4*, *Rht5*, *Rht6*, *Rht8*, *Rht24*, *etc.* (Tian *et al.* 2017). Most of the *Rht*s were identified from

Highlights

- A new dwarf wheat line Xiaoyan 101 without yield penalty was developed
- No reduction of photosynthetic activity occured in lower leaves of Xiaoyan 101
- The lower leaves in Xiaoyan 101 did not promote leaf senescence

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Abbreviations: APX – ascorbate peroxidase; Car – carotenoids; CAT – catalase; Cu/ZnSOD – copper/zinc superoxide dismutase; DAA – day after anthesis; DHAR – dehydroascorbate reductase; DI_0/CS – dissipated energy flux per cross-section; ET_0/CS – electron transport flux per cross-section; F_v/F_m – maximal quantum yield of PSII photochemistry; GR – glutathione reductase; LAI – leaf area index; MDA – malondialdehyde; MDAR – monodehydroascorbate reductase; MnSOD – manganese superoxide dismutase; PI – performance index; POD – peroxidase; qPCR – quantitative polymerase chain reaction; RC/CS_m – density of reaction center per excited cross-section; *Rht* – reduced height gene; RUE – radiation-use efficiency; *SAG* – senescence-associated gene; TCA – trichloroacetic acid; TR_0/CS – trapped energy flux per cross-section.

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mutants, but only a few *Rht*s, such as *Rht-B1b*, *Rht-D1b*, *Rht8*, and *Rht24*, are widely utilized in modern wheat varieties all over the world (Gale and Youssefian 1985, Worland *et al*. 1998, Gasperini *et al*. 2012, Tian *et al*. 2017, Würschum *et al*. 2017, Hayat *et al*. 2019). For instance, *Rht-B1b*, *Rht-D1b*, and *Rht8* accounted for 28.7, 56.6, and 69.0% of the investigated 129 wheat varieties from the Huang-Huai Valley, China, wheat growth region (Zhang *et al*. 2016). The reduction of plant height is dependent upon the types and number of *Rht*s (Lanning *et al*. 2012, Zhang *et al*. 2016).

*Rht*s have profound impacts on agronomic traits of wheat, such as reduction in plant height and leaf area, an increase of harvest index and grain number per spike, and delay of canopy closure (Gent and Kiyomoto 1998). Although semidwarf wheat varieties usually yielded more than tall wheat varieties, the effects of *Rht*s on photosynthesis at the leaf or canopy level are controversial. Several pieces of evidence showed that the semidwarf or dwarf wheat varieties had a higher photosynthetic rate than the tall wheat varieties (Pearman *et al*. 1979, Kulshrestha and Tsunoda 1981, Bishop and Bugbee 1998). For instance, semidwarf wheat with *Rht-B1b* or *Rht-D1b* performed higher photosynthetic capacity than that of the near-isogenic tall wheat, which was ascribed to a greater stomatal density and possibly reduced leaf cell size in the semidwarf wheat (LeCain *et al*. 1989, Morgan *et al*. 1990). Recent studies indicated that the dwarf wheat with *Rht-B1c* also exhibits better photosynthetic capacity than that of the near-isogenic tall wheat under water deficit (Nenova *et al*. 2014), cadmium stress (Dobrikova *et al*. 2017), and salt stress (Jusovic *et al*. 2018).

However, opposite evidence also exists for a positive contribution of *Rht*s to photosynthesis. For example, Rawson and Evans (1971) found that dwarf wheat had lower single-leaf photosynthetic rates and higher respiration rates in comparison with tall wheat varieties. Gale *et al*. (1974) showed that photosynthetic rates were not correlated with winter or spring habit, plant height, or gibberellin sensitivity but were correlated with leaf area. Moreover, Fischer *et al*. (1981) observed a positive correlation between photosynthetic activity four weeks before anthesis and plant height at maturity. In addition, the dwarf wheat with *Rht-B1b* + *Rht-D1b* contributed fewer photosynthates to grains compared with the tall and semidwarf near-isogenic lines with *Rht-B1b* or *Rht-D1b* (Kiyomoto and Gent 1989). Additionally, no difference was observed in photosynthetic rates between the tall and the corresponding near-isogenic semidwarf wheat with *Rht-B1b* (Nenova *et al*. 2014). Jobson *et al*. (2019) found that *Rht-B1b* even had negative effects and reduced photosynthetic rate and chlorophyll (Chl) content at anthesis in flag leaves as well as reduced grain protein and seed mass at 14 d after anthesis (DAA) under field conditions. However, a very recent QTL analysis demonstrated a genetic relation between *Rht8* and Chl content and between *Rht-B1b* and Chl *a* fluorescence parameters (An *et al*. 2022), indicative of the possibility to breed dwarf wheat with enhanced photosynthetic efficiency.

In comparison with tall wheat varieties, semidwarf and dwarf wheat varieties retard canopy closure and impose on canopy photosynthesis (Gent and Kiyomoto 1998). During stem elongation, the biomass, light interception, and canopy photosynthesis of tall wheat were 20% greater than those of the near-isogenic dwarf lines with *Rht-B1b* + *Rht-D1b*. However, after spike emergence, no difference was found in canopy light interception and photosynthesis between the tall and dwarf near-isogenic wheat lines (Gent 1995). Consistently, Miralles and Slafer (1997) found that the biomass in dwarf wheat with *Rht-B1b* + *Rht-D1b* at anthesis was reduced by 22% compared with the near-isogenic tall and semidwarf lines with *Rht-B1b* or *Rht-D1b*. In addition, the radiation-use efficiency (RUE) in dwarf wheat during pre-anthesis was the lowest compared with the near-isogenic tall and semidwarf wheat. However, at maturity, no significant difference was observed in the accumulated biomass and the proportion of incoming radiation intercepted by the canopy during pre- and post-anthesis between dwarf wheat with *Rht-B1b* + *Rht-D1b* and tall wheat (Miralles and Slafer 1997).

Hence, it seems difficult to draw a decisive conclusion on the effects of *Rht*s on photosynthetic activity. Dwarf wheat may reduce light interception, gas exchange, and biomass accumulation at the canopy level (Gent 1995). In addition, the lower leaves in the canopy shaded by upper leaves senesce earlier due to the activation of senescence-associated genes (*SAG*s) (Sakuraba *et al*. 2014), which may be pronounced in dwarf wheat. A 10-year study of wheat varieties differing in plant height showed that yield was positively related to leaf area duration (Borojevic and Williams 1982). However, there were very few studies carried out on the effects of *Rht*s on leaf senescence (Zhang *et al*. 2019), especially for lower leaves of dwarf wheat in the canopy. In addition, whether the short architecture of dwarf wheat has a negative influence on photosynthetic capacity in lower leaves in the canopy remains unclear. Several years ago, we initiated a project to breed new wheat varieties with improved photosynthetic efficiency. As the output of the project, Xiaoyan 101 has a stable short stature with plant height ranging between 55 and 65 cm, which was usually 10–30 cm shorter than most cultivated wheat varieties in major Chinese winter wheat-growing regions. However, its grain yield was approximate to or even higher than that of the semidwarf local varieties. Understanding the photosynthetic characters of Xiaoyan 101 at leaf and canopy level may favor wheat RUE improvement. The upper three leaves are of great importance for grain filling, which determines cereal yield potential. In this study, the objective was to compare photosynthesis-related traits in the top three (four) leaves in the canopy of Xiaoyan 101 and a semidwarf wheat Jing 411 with a plant height of 85–100 cm to ascertain if the lower leaves in the canopy of dwarf wheat reduce photosynthetic capacity.

Materials and methods

Plant material and growth conditions: Two Chinese winter wheat cultivars, *Triticum aestivum* cv. Xiaoyan 101 and Jing 411, with contrasting plant heights were used in this study. Xiaoyan 101 was developed from a cross between two semidwarf wheat varieties, Xiaoyan 81 and Liangxing 99. Xiaoyan 81, released in 2005, exhibits a high harvest index (Li *et al.* 2008). Liangxing 99, released in 2006, is a high-yielding and widely cultivated variety in the Huang-Huai Valley China wheat-growing region. Jing 411 is a semidwarf wheat variety that confers *Rht-D1b* (Zhou *et al.* 2007). It was released in 1992 and is a high-yielding winter wheat variety in the North China wheat-growing region. In addition, two local varieties, Gaoyou 2018 and Shixin 828, widely planted in Hebei province, were also included in this study. Gaoyou 2018 with high-yielding potential and good quality and Shixin 828 with high-yielding potential were released in 2005 and 2013, respectively.

The field experiments were performed at experimental stations in Changping district (Beijing) (116°24'45"E, 40°16'3"N) and Zhaoxian county (Hebei province, China) (114°49'12"E, 37°50'4"N), respectively. Usually, seeds were sown at a seedling rate of 300 seeds m^{-2} at the end of September in Beijing and around 10 October in Zhaoxian by using a plot seeder (*Plotseed TC*, *Wintersteiger*, Austria). Each variety was grown in three randomized blocks and each plot was six 12-m-long rows spaced by 0.2 m. During the growing seasons, no water and nutritional deficiencies occurred, and regular management was carried out. In the next early June, grain seeds were harvested by using a harvester (*Classic Plus*, *Wintersteiger*, Austria).

Leaf area index (LAI) and other agronomical traits: At 0–28 DAA, LAI was measured on overcast days with a single-sensor mode by using an *LAI-2000* canopy analyzer (*Li-Cor*, *Inc*., Lincoln, NE, USA) according to Malone *et al.* (2002). This instrument estimates LAI as a function of intercepted diffuse solar radiation at the top and bottom of the canopy. Three repeats of one above the canopy and four ground readings between the two middle rows were carried out to compute LAI for each plot. A 90° view cap was applied to the optics to avoid the influence of the operator and the adjacent plots. The standard outputs (five rings) were recomputed to estimate the four rings LAI using *FV2000* data processing software according to the manufacturer's instruction.

The plant height, biomass, harvest index, spike number, kernel number per spike, and thousand-kernel mass were surveyed on five randomized one square-meter plots harvested manually for each variety.

Content of photosynthetic pigments: At 2 DAA, the upper four leaves were randomly collected from the main stems in the canopies of Xiaoyan 101 and Jing 411 and stored in a –80°C refrigerator. Generally, 8–10 representatively healthy leaves at each position collected for each variety were used for photosynthesis pigments, MDA, H_2O_2, and gene expression assays. All measurements were carried out for three biological and four technical repeats.

The content of Chl (*a*+*b*), Chl *a*, Chl *b*, and carotenoids (Car) as well as the ratios of Chl (*a*+*b*)/Car and Chl *a*/*b* were determined as described previously (Arnon 1949, Lichtenthaler and Wellburn 1983). For extraction of photosynthetic pigments, 0.05-g leaf samples were added into 1.5-mL Eppendorf tubes containing 1 mL of 80% acetone, followed by maintaining in darkness at room temperature for 3 d. Until the leaf samples turned white, the extracts were spectrophotometrically assayed at 470, 645, 646, and 663 nm by using a microplate reader (*SpectraMax190*, *Molecular Devices*, USA).

Chl *a* fluorescence measurement: At 2 DAA, the top four leaves from the main stems in the canopy were measured for Chl *a* fluorescence. Ten leaves were measured for each positioned leaf for each variety. The Chl *a* fluorescence was measured by using a *Handy-PEA* fluorometer (*Hansatech Instruments Ltd*., UK). The middle parts of leaves were measured immediately after dark adaption for 30 min. When measuring, the saturated flash-light intensity was set at PPFD of 3,000 μmol m^{-2} s^{-1}, and the flash-light duration was 1 s. The JIP-test parameters include the maximal quantum yield of PSII photochemistry (F_v/F_m), performance index (PI), trapped energy flux per cross-section (TR_0/CS), electron transport flux per cross-section (ET_0/CS), dissipated energy flux per cross-section (DI_0/CS), and density of reaction center per excited cross-section (RC/CS_m) and were directly obtained from the software *PEA Plus* (*version 1.10*). These parameters were described in detail by Strasser *et al.* (1995).

Malondialdehyde (MDA) and H_2O_2 content: The content of MDA was assayed according to Ledwożyw *et al.* (1986). After homogenization of 0.05-g leaf samples with a *TissueLyser II* (*Qiagen*, USA), MDA and soluble sugar were extracted with 1 mL of 5% trichloroacetic acid (TCA) and were determined by recording the absorbance at 450, 532, and 600 nm, respectively (*SpectraMax 190*, *Molecular Devices*, USA). MDA concentration was computed as $6.45 \times (A_{532nm} - A_{600nm}) - 0.56 \times A_{450nm}$ according to Zhao *et al.* (1994). The content of H_2O_2 was determined according to Velikova *et al.* (2000). H_2O_2 was extracted from 0.5-g leaf samples with 2 mL of 0.1% TCA. After the reaction solution including 0.5 mL of the supernatant, 1 mL of 1 M potassium iodide, and 1.5 mL of 10 mM potassium phosphate (pH 7.0) was incubated in darkness for one hour, the concentration of H_2O_2 was calculated from the absorbance at 390 nm (*SpectraMax 190*, *Molecular Devices*, USA) according to a standard curve with different concentrations of H_2O_2.

Expressional analysis of genes involving antioxidant system and *SAG*s: Total RNA was extracted with *TRIzol Reagent* (*Thermo Fisher Scientific*, USA) according to the standard protocol. The first-strand cDNA was synthesized by using *SuperScript IV Reverse Transcriptase* (*Thermo Fisher Scientific*, USA) with 2 μg of total RNA. Quantitative polymerase chain reaction (qPCR) of gene expression was performed using *StepOnePlus^{TM}* Real-Time PCR Systems (*Thermo Fisher Scientific*, USA). The qPCR reaction was conducted in a 10-μL reaction solution consisting of 2 μL of cDNA, 5 μL of 2× *PowerUp SYBR Green Master Mix* (*Thermo Fisher Scientific*, USA), and 0.2 μL of gene-specific primers following a three-step PCR program.

TaActin was used as an internal reference gene according to Uauy *et al.* (2006). The qPCR primers for genes encoding antioxidant enzymes and *SAG*s used in this study had been described previously by Li *et al.* (2014) and Liu *et al.* (2019). For each sample, four technique replicates and three biological repeats were conducted. The relative expression levels were calculated with the comparative threshold cycle of qPCR (C_T) method according to Schmittgen and Livak (2008).

Gas-exchange parameters measurement: The CO_2 assimilation rate (P_N) was measured at 5, 12, and 23 DAA in the morning (9:00–11:00 h) and at noon (12:00–14:00 h) on attached leaves with a portable gas-exchange instrument (*GFS-3000*, *Walz*, Germany). When measuring, the light intensity, CO_2 concentration, and flow rate were set at PPFD of 800 μmol m^{-2} s^{-1}, 400 μmol mol^{-1}, and 750 μmol s^{-1}, respectively. The ambient temperature and relative humidity were 31.1 ± 2.0°C and 44.7 ± 23.1%, respectively. Eight leaves were measured for each of the top three leaves from the main stems randomly selected in the canopy for each variety.

Data analysis: Data were represented as mean ± SD. One-way analysis of variance (*ANOVA*), multiple comparative analysis, and independent *T*-test were performed using the *SPSS* software (*version 19.0*, *IBM*). Figure plotting was carried out with the *SigmaPlot* software (*version 10.0*).

Results

Plant height and yield performance of dwarf wheat Xiaoyan 101: Xiaoyan 101 performed as a dwarf phenotype while Jing 411 behaved semidwarf stature in the field (Fig. 1*A*). The plant height in Xiaoyan 101 was usually around 60 cm, 25 cm lower than that in Jing 411 in Beijing. As shown in Fig. 1*B*, the lengths of the upper four stem internodes in Xiaoyan 101 were reduced by 31.9, 28.1, 39.2, and 54% accordingly in comparison with those in Jing 411. However, the ear length in Xiaoyan 101 was 10.3% higher than that in Jing 411. No significant difference was observed for the lengths of other internodes and the upper four leaves between Xiaoyan 101 and Jing 411. In Xiaoyan 101, the flag leaves were wider, but the antepenultimate and the fourth leaves were narrower compared with Jing 411. Comparatively, the ratio of length to width in the flag leaves was significantly lower, while in the fourth leaf, it was higher in Xiaoyan 101. Additionally, the leaf area in the fourth leaf in Xiaoyan 101 was significantly lower than that in Jing 411 (Fig. 1S, *supplement*). Collectively, the short stature of Xiaoyan 101 comes from shortened lengths of the upper four stem internodes.

After anthesis, no significant difference was observed for LAI between Xiaoyan 101 and Jing 411 although the LAI in Xiaoyan 101 at 0–5 DAA was slightly lower than that in Jing 411 (Table 1). The fresh mass per plant in Xiaoyan 101 was significantly lower than that in Jing 411 at 0 DAA. However, the difference was not significant at 17 and 32 DAA (Fig. 2S, *supplement*). Comparatively, Xiaoyan 101 had slightly more spikes and a higher yield than Jing 411 across years. Further comparisons of yield component traits between Xiaoyan 101 and three widely cultivated local semidwarf wheat varieties, Jing 411, Gaoyou 2018, and Shixin 828, were conducted in Beijing and Zhaoxian across three years. As shown in Table 2, the plant height in Xiaoyan 101 was 64.9% of Jing 411 in 2016 in Beijing, 82.0 and 83.3% of Gaoyou 2018 in Zhaoxian in 2017 and 2018, and 82.9% of Shixin 828 in Zhaoxian in 2018 (Table 2).

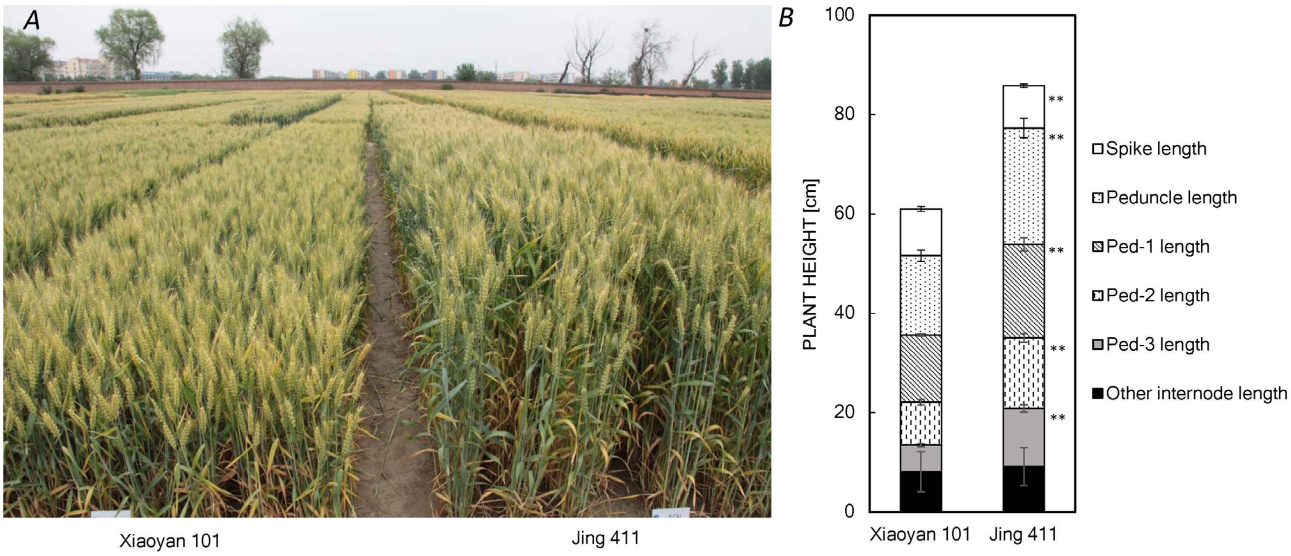

Fig. 1. Field performance (*A*) and stem internode length (*B*) of Xiaoyan 101 and Jing 411 at the grain-filling stage. Ped-1 – the internode immediately below the peduncle; Ped-2 – the second internode below the peduncle; Ped-3 – the third internode below the peduncle. Data represent mean ± SD. ** denotes a significant difference at *P*<0.01.

Table 1. Leaf area index (LAI), spike number, and yield in Xiaoyan 101 and Jing 411. Data represent mean ± SD. * denotes significant difference at $P<0.05$. DAA – days after anthesis; na – data not available.

Year	DAA	LAI		Spike number [m^{-2}]		Yield [t ha^{-1}]	
		Xiaoyan 101	Jing 411	Xiaoyan 101	Jing 411	Xiaoyan 101	Jing 411
2012	0	3.51 ± 0.31	4.10 ± 0.63	400.7 ± 20.0	342 ± 26.9*	6.00 ± 0.11	5.70 ± 0.20
	15	4.80 ± 0.12	4.79 ± 0.31				
2013	0	2.97 ± 0.24	3.49 ± 0.40	545.3 ± 43.6	456.7 ± 39.0	6.70 ± 0.22	6.32 ± 0.08
2014	5	4.58 ± 0.23	4.69 ± 0.27	na	na	7.34 ± 0.03	5.58 ± 0.67*
	14	4.85 ± 0.19	4.87 ± 0.12				
	20	4.78 ± 0.85	4.78 ± 0.07				
	28	4.83 ± 0.18	4.35 ± 0.04				

Table 2. Comparison of yield-related traits between Xiaoyan 101 and the widely cultivated local wheat varieties. Data represent mean ± SD. *The different letters* denote the significant difference at $P<0.05$. The widely planted varieties Jing 411, Gaoyou 2018, and Shixin 828 were used as controls.

Trait	2016 Beijing		2017 Zhaoxian		2018 Zhaoxian		
	Xiaoyan 101	Jing 411	Xiaoyan 101	Gaoyou 2018	Xiaoyan 101	Gaoyou 2018	Shixin 828
Plant height [cm]	57.7 ± 3.3^{a}	88.9 ± 3.2^{b}	64.1 ± 0.7^{a}	78.2 ± 5.8^{b}	60.2 ± 3.1^{b}	72.2 ± 5.6$^{a,\#}$	72.6 ± 2.4^{a}
Biomass [g m^{-2}]	1,519.8 ± 43.0^{a}	1,511.1 ± 176.0^{a}	2,159.6 ± 149.3^{a}	2,144.7 ± 153.2^{a}	1,390.5 ± 57.7^{b}	1,540.0 ± 32.7^{a}	1,341.3 ± 69^{b}
Grain mass [g m^{-2}]	763.4 ± 19.7^{a}	654.5 ± 71.6^{a}	1028.5 ± 54.2^{a}	918.2 ± 55.8^{b}	645.4 ± 25.3^{a}	627.2 ± 28.3^{a}	587.7 ± 23.0^{b}
Yield [t ha^{-1}]	7.63 ± 0.20^{a}	6.55 ± 0.72^{a}	10.29 ± 0.54^{a}	9.18 ± 0.56^{b}	6.45 ± 0.25^{a}	6.27 ± 0.28^{a}	5.88 ± 0.23^{b}
Harvest index	0.503 ± 0.025^{a}	0.434 ± 0.018^{b}	0.477 ± 0.010^{a}	0.429 ± 0.013^{b}	0.464 ± 0.016^{a}	0.408 ± 0.023^{c}	0.439 ± 0.015^{b}
Spike number [m^{-2}]	549.2 ± 22.3^{a}	553.3 ± 99.2^{a}	863.0 ± 46.1^{a}	819.6 ± 29.3^{a}	728.2 ± 33.0^{a}	764.6 ± 92.8^{a}	604.6 ± 50.9^{b}
Kernel number per spike	38.6 ± 2.6^{a}	33.4 ± 5.7^{a}	25.3 ± 0.7^{a}	25.4 ± 2.4^{b}	21.8 ± 2.1^{b}	23.6 ± 3.9^{b}	30.4 ± 3.9^{a}
Thousand-kernel mass [g]	49.5 ± 3.3^{a}	44.0 ± 3.8^{b}	46.6 ± 1.0^{a}	44.3 ± 1.8^{a}	43.8 ± 1.0^{a}	37.1 ± 1.8^{b}	37.4 ± 0.9^{b}

In 2016 and 2017, no significant difference was observed for biomass between Xiaoyan 101 and Jing 411/Gaoyou 2018. However, in 2018, the biomass in Xiaoyan 101 was significantly lower than that in Gaoyou 2018, but it was not significantly different from Shixin 828. The yield of Xiaoyan 101 was significantly higher than that of Gaoyou 2018 and Shixin 828 in 2017 and 2018, respectively. It was slightly higher than Jing 411 and Gaoyou 2018 in 2016 and 2018, respectively, although the difference was not significant. The harvest index in Xiaoyan 101 was consistently and significantly higher than all the three wheat varieties across years and locations. The spike number in Xiaoyan 101 was not significantly different from the three varieties, except it was significantly higher than Shixin 828 in 2018. Similarly, no significant difference was found for the kernel number per spike between Xiaoyan 101 and the three varieties except that it was significantly lower than Shixin 828 in 2018. The thousand-kernel mass in Xiaoyan 101 was significantly higher than the three semidwarf wheat varieties.

The content of photosynthetic pigments: The LAI in Xiaoyan 101 was slightly lower than that in Jing 411 at 0–5 DAA, indicating that Xiaoyan 101 appeared to retard canopy closure. The top four leaves from Xiaoyan 101 and Jing 411 at 2 DAA were investigated to determine the effects of short stature on canopy photosynthetic capacity and leaf senescence pattern of lower leaves. The top four leaves in the canopy were investigated as a probe reflecting canopy photosynthesis. As illustrated in Fig. 2, the content of Chl (a+b), Chl a, and Car in the antepenultimate and fourth leaves in the canopy of Xiaoyan 101 was significantly higher than that of Jing 411. In addition, the Chl b content and the ratio of Chl (a+b)/Car in the fourth leaf of Xiaoyan 101 were also significantly higher than that of Jing 411. However, the ratio of Chl a/b in the fourth leaf of Xiaoyan 101 was significantly lower than that of Jing 411. Hence, it appeared that lower leaves in the canopy of Xiaoyan 101 had more photosynthetic pigments relative to the semidwarf variety Jing 411.

Chl *a* fluorescence parameters: At 2 DAA, the Chl a fluorescence parameters were measured in the top four leaves in the canopies of Xiaoyan 101 and Jing 411 to ascertain if the dwarfism of Xiaoyan 101 reduces photochemical efficiency in lower leaves. As shown in Fig. 3*F*, the values of RC/CS$_{m}$ in all the top four leaves of Xiaoyan 101 were significantly higher than those of Jing 411, and the difference was enlarged for the fourth leaf. In addition, the values of PI in the top four leaves except for flag leaves in Xiaoyan 101 were also significantly higher than those of Jing 411 (Fig. 3*B*). Moreover, in

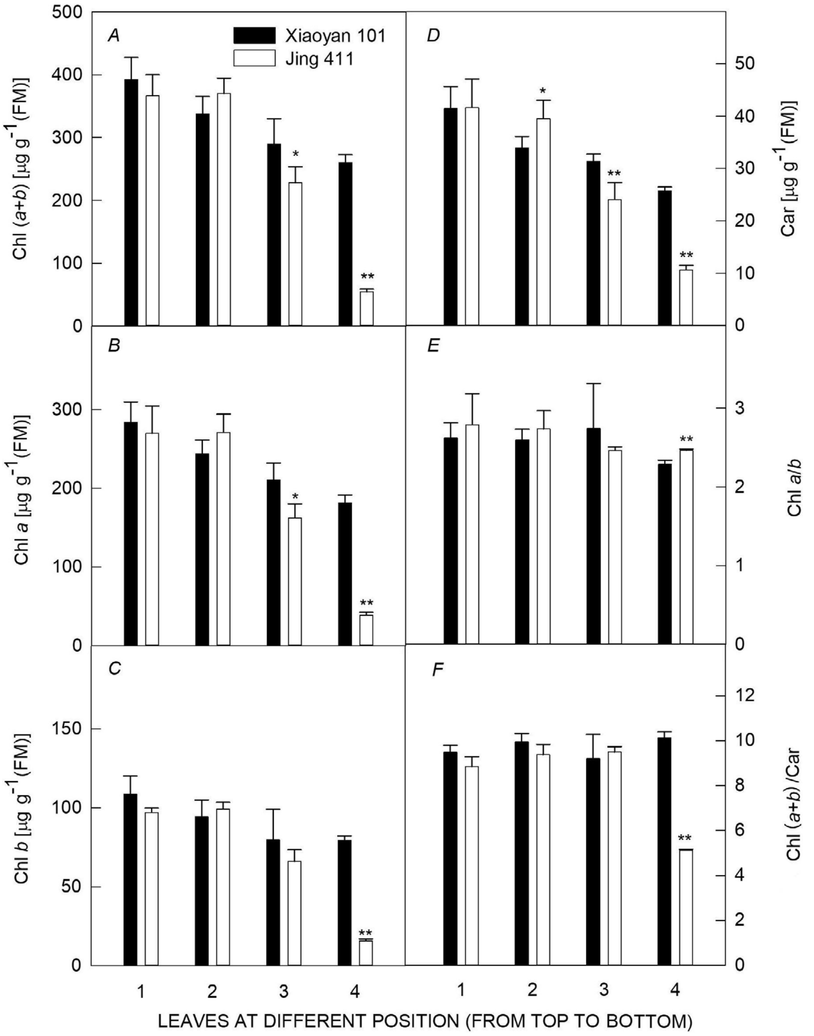

Fig. 2. The content of total chlorophyll [Chl (*a*+*b*)] (*A*), chlorophyll *a* (Chl *a*) (*B*), chlorophyll *b* (Chl *b*) (*C*), and carotenoids (Car) (*D*) and ratios of Chl *a*/*b* (*E*) and Chl (*a*+*b*)/Car (*F*) in the top four leaves in the canopy of Xiaoyan 101 and Jing 411. Data represent mean ± SD. * and ** denote a significant difference at $P<0.05$ and $P<0.01$, respectively.

comparison with Jing 411, the ET_0/CS in the lower leaves (antepenultimate and the fourth leaves) in the canopy of Xiaoyan 101 was significantly higher (Fig. 3*D*). However, for F_v/F_m (Fig. 3*A*), TR_0/CS (Fig. 3*C*), and DI_0/CS (Fig. 3*E*) in all top four leaves, no significant difference was found between Xiaoyan 101 and Jing 411. Collectively, Xiaoyan 101 had higher values of PI, ET_0/CS, and RC/CS_m in lower leaves than Jing 411, suggesting that the lower leaves in Xiaoyan 101 maintained higher electron transport flux and density of reaction centers per excited cross-section, and thus higher photochemical efficiency of PSII. Similar results were found for the antepenultimate leaves at 12 DAA (Fig. 3S, *supplement*). Not only PI, ET_0/CS, and RC/CS_m, but also TR_0/CS in the antepenultimate leaves of Xiaoyan 101 were significantly higher than those in Jing 411 at 12 DAA. However, no significant difference was observed in the flag and penultimate leaves between both genotypes. Therefore, it seemed that the short stature of Xiaoyan 101 maintained the high photochemical efficiency of PSII in the lower leaves, especially in the antepenultimate and the fourth leaves, in comparison with the semidwarf wheat Jing 411.

The content of MDA and H_2O_2: At 2 DAA, the content of MDA and H_2O_2 in the top four leaves in the canopies of Xiaoyan 101 and Jing 411 were determined and illustrated in Fig. 4. The MDA content in the top four leaves (except the antepenultimate leaves) of Xiaoyan 101 was significantly lower than that of Jing 411 (Fig. 4*B*). Additionally, the H_2O_2 content in the penultimate leaves was significantly higher, but in the fourth leaves, it was lower in Xiaoyan 101 compared to that in Jing 411 (Fig. 4*A*). Relatively, the lower leaves, especially the fourth leaves in Xiaoyan 101, accumulated less MDA and H_2O_2, suggesting that the lower leaves of Xiaoyan 101 were not prone to suffering from oxidative stress compared with Jing 411. On the contrary, the lower leaves in semidwarf wheat Jing 411 suffered severe oxidative stress and produced more membrane lipid peroxide.

Expression of genes encoding antioxidant enzymes: Expression levels of genes encoding wheat antioxidant enzymes including copper/zinc superoxide dismutase (Cu/ZnSOD), manganese superoxide dismutase (MnSOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase

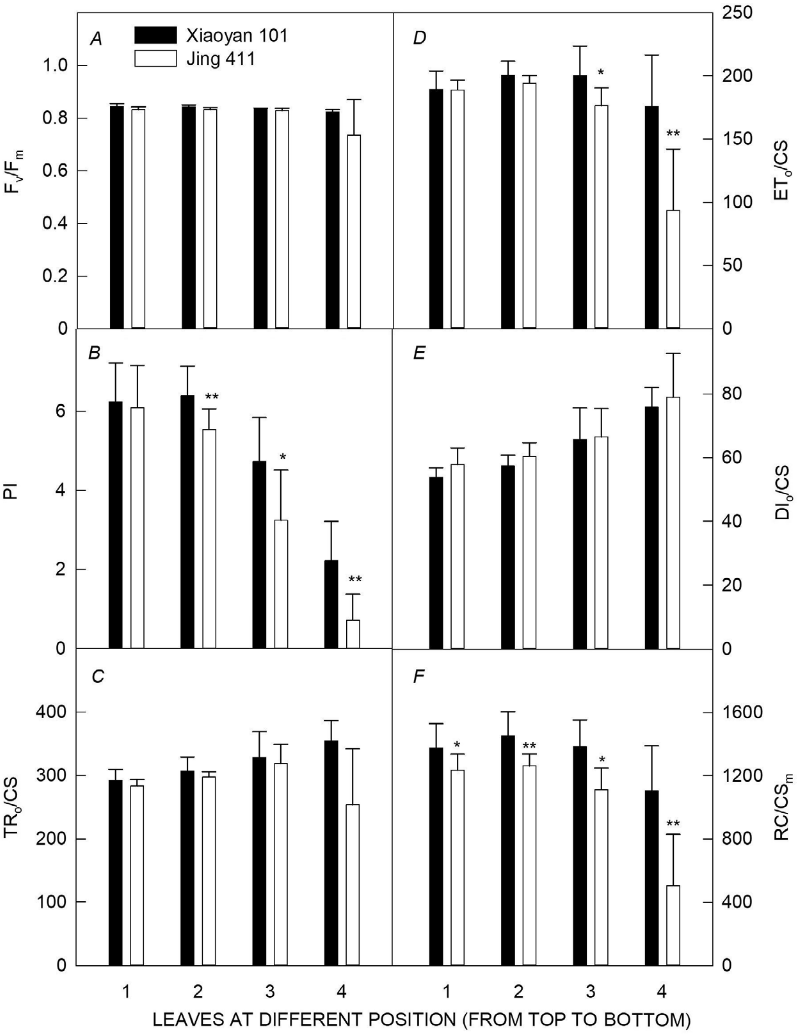

Fig. 3. The chlorophyll *a* fluorescence parameters in the top four leaves in the canopy of Xiaoyan 101 and Jing 411. Maximum photochemical efficiency of PSII (F_v/F_m) (*A*); performance index (PI) (*B*); trapped energy flux per cross-section (TR_0/CS) (*C*); electron transport flux per cross-section (ET_0/CS) (*D*); dissipated energy flux per cross-section (DI_0/CS) (*E*); density of reaction center per excited cross-section (RC/CS_m) (*F*). * and ** denote significant difference at $P<0.05$ and $P<0.01$, respectively.

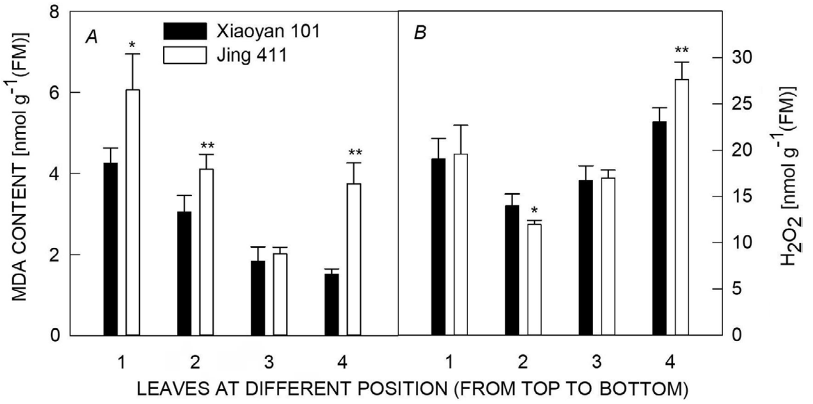

Fig. 4. The content of malondialdehyde (MDA (*A*) and hydrogen peroxide (*B*) in the top four leaves in the canopy of Xiaoyan 101 and Jing 411. Data represent mean ± SD. * and ** denote a significant difference at $P<0.05$ and $P<0.01$, respectively.

(POD), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR), and glutathione reductase (GR) in the top four leaves in canopies of Xiaoyan 101 and Jing 411 were determined at 2 DAA. As illustrated in Fig. 5, the transcripts of *TaCu/ZnSOD* (Fig. 5*A*), *TaCAT* (Fig. 5*C*), *TaAPX* (Fig. 5*D*), and *TaDHAR* (Fig. 5*F*) were significantly lower in the flag and penultimate leaves but were higher in the antepenultimate and fourth leaves in Xiaoyan 101 than those in Jing 411. In addition, the expression levels of *TaMnSOD* (Fig. 5*B*), *TaPOD* (Fig. 5*E*), and *TaMDAR* (Fig. 5*G*) in the top three leaves were significantly lower but were higher in the fourth leaves in Xiaoyan 101 compared with Jing 411. In addition, the mRNA levels of *TaGR* were significantly lower in flag leaves but were higher in the antepenultimate and fourth leaves in Xiaoyan 101 in comparison with

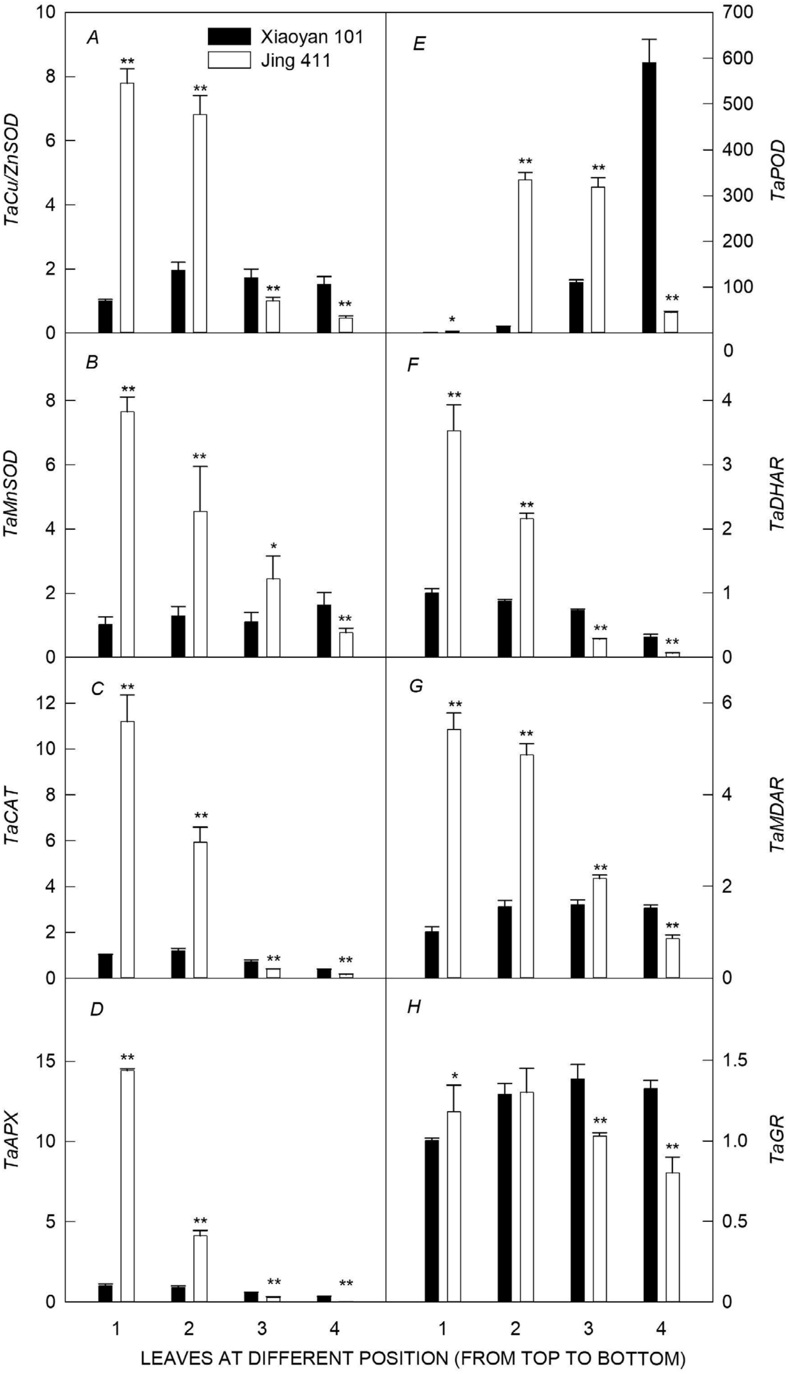

Fig. 5. Expression of genes encoding antioxidant enzymes in the top four leaves in the canopy of Xiaoyan 101 and Jing 411. Copper/zinc superoxide dismutase (*TaCu/ZnSOD*) (*A*); manganese superoxide dismutase (*TaMnSOD*) (*B*); catalase (*TaCAT*) (*C*), ascorbate peroxidase (*TaAPX*) (*D*); peroxidase (*TaPOD*) (*E*); dehydroascorbate reductase (*TaDHAR*) (*F*); monodehydroascorbate reductase (*TaMDAR*) (*G*); glutathione reductase (*TaGR*) (*H*). Data represent mean ± SD. * and ** denote significant difference at $P<0.05$ and $P<0.01$, respectively.

those in Jing 411 (Fig. 5*H*). From top to bottom leaves, the mRNA levels of *TaCu/ZnSOD*, *TaMnSOD*, *TaCAT*, *TaAPX*, *TaDHAR*, and *TaMDAR* declined drastically in Jing 411 while they changed little in Xiaoyan 101. Interestingly, the expression levels of *TaPOD* in Xiaoyan 101 elevated continuously and drastically, while they peaked in the penultimate and antepenultimate leaves in Jing 411. Compared to flag leaves, the expression levels of *TaGR* in lower leaves increased in Xiaoyan 101 but decreased in Jing 411. Taken together, the higher expression of genes encoding antioxidant enzymes in the lower leaves of Xiaoyan 101 may be associated with the high photochemical efficiency of PSII.

Expression of wheat *SAG*s: To study the effects of the dwarfism of Xiaoyan 101 on leaf senescence in lower leaves, the expression levels of four *TaSAGs* in the top four leaves were determined at 2 DAA. As illustrated in Fig. 6, the expression levels of *TaSAG3* (Fig. 6*A*), *TaSAG5* (Fig. 6*B*), and *TaSAG7* (Fig. 6*C*) were significantly lower in the top four leaves except flag leaves in Xiaoyan 101 than those in Jing 411. The mRNA transcripts of *TaSAG12*

(Fig. 6*D*) in the flag and penultimate leaves were higher, but they were significantly lower in the antepenultimate and fourth leaves in Xiaoyan 101 than those in Jing 411. Collectively, the consistently lower expression of *TaSAG3*, *TaSAG5*, *TaSAG7*, and *TaSAG12* in lower leaves in the canopy of Xiaoyan 101 demonstrated that senescence of lower leaves in Xiaoyan 101 appeared to be delayed relative to Jing 411.

The CO_2 assimilation rates: To explore the effects of the short architecture of Xiaoyan 101 on the photosynthetic rate in the lower leaves in the canopy during the grain-filling stage, the CO_2 assimilation rate in the top three leaves from the canopies of Xiaoyan 101 and Jing 411 was measured both in the morning and at noon at 5, 12, and 23 DAA (Fig. 7). As the fourth leaves were difficult to measure, they were excluded for CO_2 assimilation rate measurement. The P_N in flag leaves of Xiaoyan 101 was significantly higher than that of Jing 411 at 5 DAA when measured in the morning (Fig. 7*A*). In addition, it was significantly higher in Xiaoyan 101 than in Jing 411 at all three measuring dates at noon (Fig. 7*D–F*). However, no significant difference was observed for the penultimate leaves between Xiaoyan 101 and Jing 411. For the antepenultimate leaves, the CO_2 assimilation rate in Xiaoyan 101 was significantly lower at 5 DAA in the morning (Fig. 7*A*) but was higher at 23 DAA at noon compared with Jing 411 (Fig. 7*F*). It seemed that no significant reduction in P_N occurred in lower leaves of Xiaoyan 101 except at 5 DAA in the morning compared with Jing 411. However, the higher P_N in the flag leaves of Xiaoyan 101 in high light (at noon) may contribute to its good yield performance to some extent.

Discussion

In major wheat-growing regions in China such as the Huang-Huai Valley, the plant height of most cultivated wheat varieties usually ranged from 70 to 100 cm, most of which are at risk of lodging under favorable conditions. Although many dwarf wheat lines with a plant height of lower than 65 cm have been reported (Bugbee and Koerner 1997, Bishop and Bugbee 1998), very few dwarf wheats had been utilized in wheat production for lower yield potential. Is it possible to breed new dwarf wheat lines with a plant height of lower than 70 cm, for instance, 55–65 cm, without yield sacrifice? Several years ago, we successfully obtained a new dwarf line, Xiaoyan 101, through conventional breeding. It displayed stable short stature, for example, its plant height ranged between 57.7 and 64.1 cm across three years and two locations (Fig. 1, Table 2). In comparison with a semidwarf wheat, Jing 411, which is a high-yielding variety in North China, the top four internode stem lengths of Xiaoyan 101 were reduced drastically, especially the fourth internode length showing the largest reduction. However, the spike length of Xiaoyan 101 increased by 10.3% compared to Jing 411. After anthesis, no significant difference was observed for LAI and biomass between Xiaoyan 101 and Jing 411, which was consistent with previous studies (Gent 1995, Miralles and Slafer 1997). However, the spike number per square meter in Xiaoyan 101 was even higher than that in Jing 411, resulting in the high yield potential of Xiaoyan 101. Further comparisons with the widely cultivated local varieties in Beijing and Zhaoxian, Xiaoyan 101 yielded more than Jing 411 in Beijing, Gaoyou 2018 and Shixin 828 in Zhaoxian, suggesting that the dwarfism of

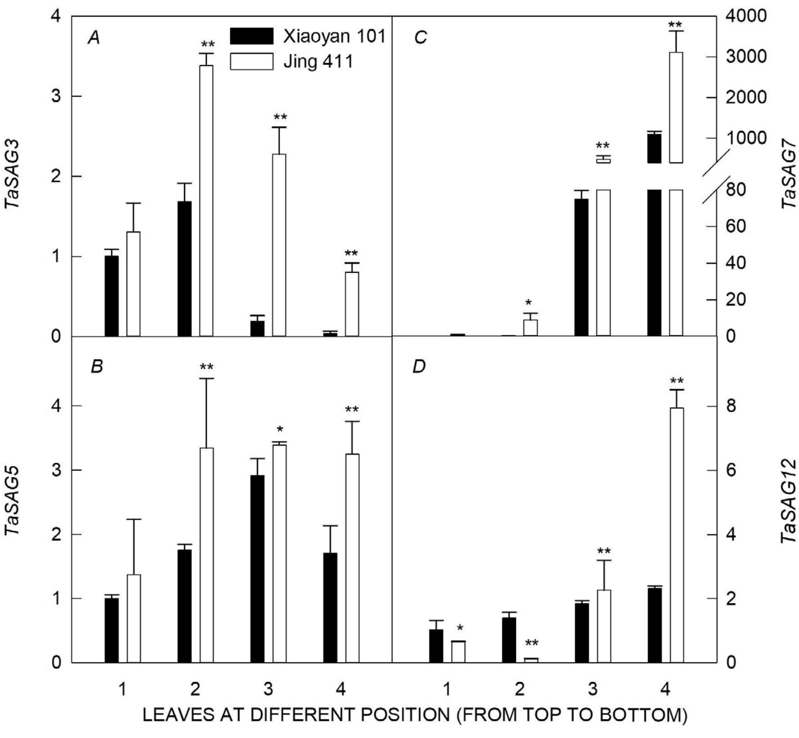

Fig. 6. Expression of wheat senescence-associated genes (*TaSAGs*) in the top four leaves in the canopy of Xiaoyan 101 and Jing 411. Data represent mean ± SD. * and ** denote significant difference at $P<0.05$ and $P<0.01$, respectively.

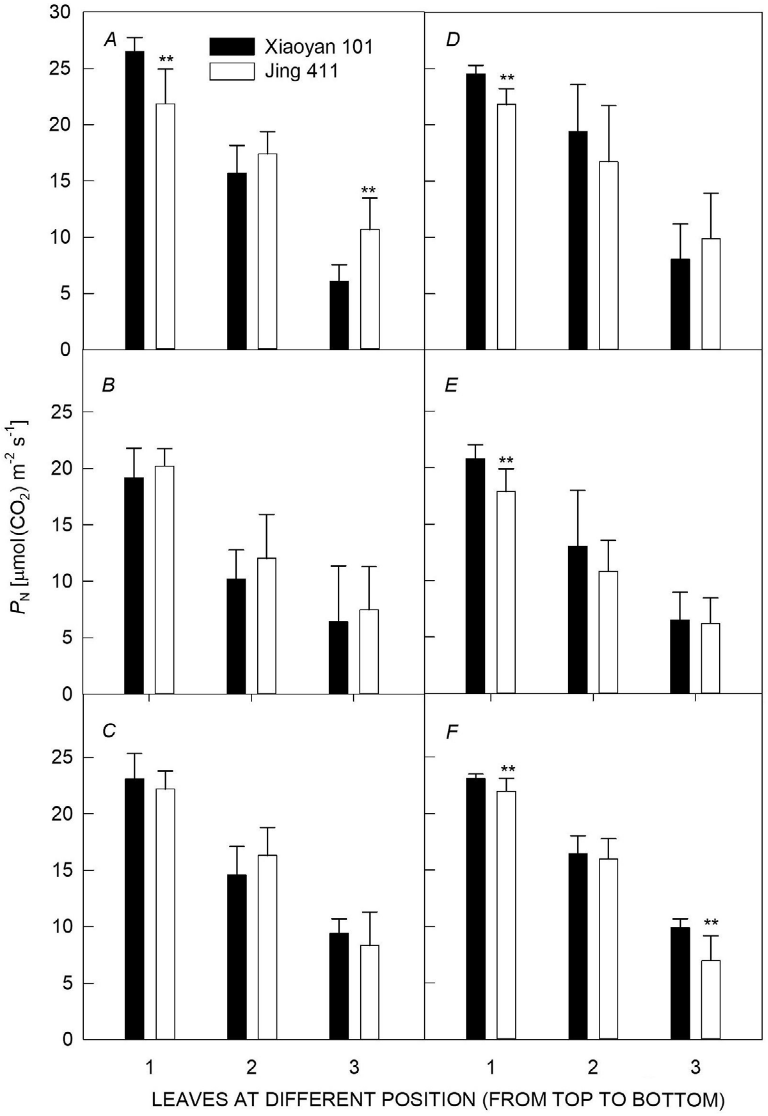

Fig. 7. The CO_2 assimilation rate in the top three leaves in the canopy of Xiaoyan 101 and Jing 411 in the morning (*A–C*) and at noon (*D–F*) at 5 d (*A*,*D*), 12 d (*B*,*E*), and 23 d after anthesis (*C*,*F*), respectively. ** denotes significant difference at $P<0.01$.

Xiaoyan 101 did not result in significant yield loss. Therefore, Xiaoyan 101 has the potential to be planted in well-watered regions where full of lodging risk for semidwarf wheat varieties exists. The high-yielding characteristics in Xiaoyan 101 resulted from the enhancement of harvest index and thousand-grain mass, which was consistent with the historical breeding of wheat since the 'Green Revolution' (Gent and Kiyomoto 1985, 1998).

As biomass is determined by leaf area index, leaf area duration, and photosynthesis at leaf and canopy levels, the photosynthetic capacity of lower leaves besides flag leaves in the canopy plays important roles in canopy establishment and accumulated biomass at maturity. For a long time, wheat photosynthesis has been extensively studied with flag leaves, which was considered a reliable predictor of source capacity and the main source of carbon for grain filling. However, the photosynthesis of the lower leaves in the canopy is not well understood. It was presumed that the short stature of dwarf wheat may reduce light interception, canopy photosynthesis, and thus leading to lower biomass (Gent 1995). In addition, *Rht*s were found to increase the light attenuation coefficient in the canopy (Miralles and Slafer 1997), which may reduce the photosynthetic capacity and longevity of lower leaves in the canopy. To determine if dwarf wheat has reduced photosynthetic capacity in lower leaves during the grain-filling stage, Xiaoyan 101 and Jing 411 were compared in this study. As shown in Fig. 7, the P_N in the penultimate and antepenultimate leaves in the canopy of Xiaoyan 101 was only significantly lower than those of Jing 411 at 5 DAA but no significant difference was observed at 12 and 23 DAA in the morning. At noon, it was even significantly higher in the lower leaves of Xiaoyan 101 than that of Jing 411 at 23 DAA, but no significant difference was observed at 5 and 12 DAA. Therefore, it appeared that no obvious or consistent reduction in CO_2 assimilation rate was observed for Xiaoyan 101 for the whole day during the grain-filling stage. Further assay of photosynthetic pigments showed that the content of Chls and carotenoids in the antepenultimate and fourth leaves of

Xiaoyan 101 was significantly higher than that of Jing 411 (Fig. 2), which was consistent with higher PI, ET_0/CS, and RC/CS_m in Xiaoyan 101 (Fig. 3). In addition, the expression levels of the genes encoding antioxidant enzymes in lower leaves, especially in the antepenultimate and fourth leaves in the canopy of Xiaoyan 101, were significantly higher than those of Jing 411. In addition, the expression of *TaSAGs* in lower leaves in the canopy of Xiaoyan 101 was significantly lower than those of Jing 411, suggesting that the lower leaves in Xiaoyan 101 senesced slowly relative to Jing 411. Collectively, for the dwarf wheat Xiaoyan 101, the lower leaves in the canopy had higher photochemical efficiency, antioxidant capacity, and longer leaf longevity in comparison to the semidwarf wheat Jing 411, which may account for its high-yielding potential to some extent.

As the plant height of Xiaoyan 101 was gibberellin (GA)-responsive (data not shown), the *Rht* gene in Xiaoyan 101 should not be the *GAI Rht*s, such as *Rht-B1b*, *Rht-B1c*, *Rht-D1b*, or *Rht-D1c*, *etc*. Genotyping with a simple sequence repeat (SSR) marker *WMS261*, a diagnostic marker for *Rht8* (Chebotar *et al*. 2001), indicated that no expected size of amplicon was produced from Xiaoyan 101, which suggested that the dwarfism of Xiaoyan 101 should not be regulated by *ht8* (data not shown). Tian *et al*. (2017) reported that Xiaoyan 81 and Liangxing 99 carried while Jing 411 did not carry *Rht24*. Therefore, as an offspring line of Xiaoyan 81 and Liangxing 99, Xiaoyan 101 was presumed to confer *Rht24*. Hence, its dwarf phenotype should be regulated by *Rht24* at least partly. Further genotyping by using wheat 660K single-nucleotide polymorphism (SNP) arrays through bulked segregant analysis (BSA) demonstrated that the largest proportion of the differential SNPs was located on chromosome 2B. Furthermore, the largest proportion of differential SNPs was mapped in the 10-Mb physical genome region of 657,080,548–666,936,360 on 2B where 110 differential SNPs resided (data not shown). Previously, *Rht4* had been mapped to 2B with an SSR marker *WMC317* by Ellis *et al*. (2005). Furthermore, it was confirmed by Zanke *et al*. (2014) with SSR markers *GWM1399* and *GWM619*, which were separated by *WMC317*. Through primer sequence blast against the Chinese Spring reference genome sequence, *WMC317* was located in a physical genome region of 784,343,192–784,343,720 on 2B, which was 120 Mb downstream of the *Rht* mapping region in Xiaoyan 101. Therefore, it seemed that the *Rht* gene on 2B regulating the dwarfism of Xiaoyan 101 is not probably *Rht4*. Quantitative trait locus (QTL) and genome-wide association study (GWAS) analysis of plant height suggested that besides the designated 25 *Rht*s, many novel *Rht* loci are still not characterized in wheat (Zanke *et al*. 2014, Tian *et al*. 2017). Further research is needed to understand the *Rht* gene on 2B in Xiaoyan 101.

This study demonstrated that the photosynthetic capacity in lower leaves of Xiaoyan 101 was not reduced due to dwarfism compared to Jing 411. On the contrary, the lower leaves in Xiaoyan 101 had a higher content of photosynthetic pigments and photochemical efficiency but lower content of H_2O_2 and MDA than those in Jing 411. In addition, the expression levels of the genes encoding the antioxidant enzymes were significantly higher, while the expression levels of *TaSAG3*, *TaSAG5*, *TaSAG7*, and *TaSAG12* were lower in lower leaves in Xiaoyan 101 than those in Jing 411. Therefore, it is possible to breed dwarf wheat varieties with improved photosynthetic capacity in lower leaves but without yield sacrifice and thus they can be used as dwarf wheat parents in wheat breeding.

Conclusion: In conclusion, here we reported an advanced dwarf wheat line Xiaoyan 101 with enhanced photosynthetic activity in lower leaves of the canopy but without yield penalty. The high photosynthetic activity in lower leaves was associated with less accumulation of ROS and improved leaf longevity. Therefore, it is possible to breed dwarf wheat without yield penalty but reduced lodging risk through the improvement of the photosynthetic capacity of lower leaves in the canopy.

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