Improvement in the photoprotective capability benefits the productivity of a yellow-green wheat mutant in N-deficient conditions


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Abstract

Wheat yellow-green mutant Jimai5265yg has a more efficient photosynthetic system and higher productivity than its wild type under N-deficient conditions. To understand the relationship between photosynthetic properties and the grain yield, we conducted a field experiment under different N application levels. Compared to wild type, the Jimai5265yg flag leaves had higher mesophyll conductance, photosynthetic N-use efficiency, and photorespiration in the field without N application. Chlorophyll a fluorescence analysis showed that PSII was more sensitive to photoinhibition due to lower nonphotochemical quenching (NPQ) and higher nonregulated heat dissipation. In N-deficient condition, the PSI acceptor side of Jimai5265yg was less reduced. We proposed that the photoinhibited PSII protected PSI from over-reduction through downregulation of electron transport. PCA analysis also indicated that PSI photoprotection and electron transport regulation were closely associated with grain yield. Our results suggested that the photoprotection mechanism of PSI independent of NPQ was critical for crop productivity.

Keywords: nitrogen application rate; photoprotection; photosynthetic N-use efficiency; wheat; yellow-green mutant.

Highlights

- Photochemical efficiency of PSI and PSII was higher in the mutant without N application
- The mutant had a more efficient photoprotective mechanism for PSI independent of NPQ
- Regulation of electron transport is related to PSI photoprotection and grain yield

Abbreviations: Cc – CO₂ concentration inside the chloroplast; Ci – intercellular CO₂ concentration; ETRI – electron transport rate of PSI; ETRII – electron transport rate of PSII; F₀ – minimum fluorescence; F₀′ – minimum fluorescence in the actinic light; Fm – maximum fluorescence; Fm′ – maximum fluorescence in the actinic light; Fc/Fm – maximum quantum efficiency of PSII photochemistry; g_m – mesophyll conductance; g_s – stomatal conductance; J_e – alternative electron flux; J_e(PCO) – electron flux to photorespiratory carbon oxidation; J_e(PCR) – electron flux to photosynthetic carbon reduction; J_max – light-saturated potential rate of electron transport; J_t – electron transport rate; L_d – limitation of biochemical capacity; L_m – limitation of mesophyll diffusion; LMA – leaf mass per area; L_s – limitation of stomatal diffusion; Narea – nitrogen content per unit area; Nmass – nitrogen content per unit mass; NO – nonregulated heat dissipation; NPQ – nonphotochemical quenching; P700 – primary electron donor of PSI; PIB – post-illumination burst; Pm or Pm′ – maximum P700 signal measured using saturation light pulse following short far-red pre-illumination in dark or light-adapted state; Pn – net photosynthetic rate; PNUE – photosynthetic N-use efficiency; q_P – PSII efficiency factor (the fraction of open centers); R_d – mitochondrial CO₂ release in the dark; R_l – light respiration rate; ROS – reactive oxygen species; V_c,max – maximum carboxylation rate limited by Rubisco; I^* – CO₂-compensation point; Φ_A/A – oxidation status of PSI acceptor site; Φ_A/D – oxidation status of PSI donor site; Φ_ND – quantum yield nonregulated heat dissipation; Φ_PQ – quantum yield of nonphotochemical quenching; Φ_PSI – quantum yield of PSI photochemistry; Φ_PSI – PSI operating efficiency (quantum yield of PSI photochemistry); Φ_PQ – quantum yield of open centers.

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Introduction

Nitrogen fertilizer is an essential factor for wheat production by influencing organ development and photosynthetic efficiency. A large amount of N is invested in the photosynthetic apparatus. Insufficient N application often leads to downregulation of photosynthesis (Byeon et al. 2021), while nitrogen fertilizer overapplication results in environmental costs and lowered nitrogen-use efficiencies (Tian et al. 2018). It has been reported that the rice cultivar with higher nitrogen-use efficiency had better photosynthetic performance (Kumari et al. 2018). It has been reported that the rice cultivar with higher nitrogen-use efficiency had better photosynthetic performance (Kumari et al. 2021). Photosynthetic capacity is highly influenced by leaf structure and components in the chloroplasts, which were related to leaf N content regulated by N application. Evaluation of photosynthetic properties of germplasms with diverse chloroplast components under different levels of N supply was a way to understand the relationship between leaf photosynthesis and nitrogen, which was a fundamental production–resource function for ecosystem functioning (Rotundo and Cipriotti 2017). Also, the selection of germplasms insensitive to low N application is a strategy for improving N utilization and sustainable agricultural development.

The photosynthetic structural components of the light reactions are genetically conserved and have a less natural genetic variation (Nunes-Nesi et al. 2016). However, many mutants in Chl content and antenna sizes have been identified indicating higher natural genetic variation. Ort suggested that optimizing light antenna size could serve as a potential engineering target (Ort et al. 2011, Nunes-Nesi et al. 2016). Recent research on global atmospheric changes showed that solar irradiance on the Earth's surface increased due to reduced atmospheric aerosol. Mutants with reduced leaf Chl content provided an effective solution to mitigate the high solar irradiance (Genesio et al. 2021). The Chl-less mutants of different crops, such as rice, wheat, maize, and soybean, have been studied. It is reasonable that the photosynthetic rate of Chl-deficient mutants is reduced due to the restricted light-harvesting complex (Terao and Katoh 1996). However, a typical study using soybean pale-green mutant Y11y11 showed that the photosynthetic efficiency and biomass accumulation were unaffected by the reduction of Chl content (Walker et al. 2018). Likewise, another soybean mutant (y9y9), rice mutant (yg1), Arabidopsis thaliana mutant (dlhcb2, hpe1), and cotton mutant (virescent) showed normal or higher photosynthetic capability compared to the corresponding wild type (Benedict et al. 1972, Li et al. 2013, Jin et al. 2016, Sakowska et al. 2018, Bielszynski et al. 2020).

Why is there such a counter-intuitive relationship between Chl content and photosynthetic rate? Studies using the above mutants provided four pieces of evidence and reasonings. Firstly, Chl-deficiency enables a more even light distribution among chloroplasts within leaves (Slattery and Ort 2021). Gradient light distribution within leaves restrains the function of chloroplasts buried in the lower part of the leaf. Photosynthetic efficiency in the lower chloroplasts increased by achieving more light in the Chl-less mutants. Thirdly, the absorbed light energy conversion efficiency improved in the Chl-less mutant. It has been documented that light energy absorbed by the Chl antennas increased the efficiency of photochemistry, resulting in the generation of reactive oxygen species (ROS) and dissipation as NPQ and heat (Ort et al. 2011). The process of photoprotection of PSII and PSI consumed the absorbed light energy that could otherwise be used for carbon assimilation (Slattery and Ort 2021). Fourthly, Chl-deficient mutants exhibited improved photosynthetic N-use efficiency (PNUE) (Gu et al. 2017). Excessive Chl in large light antenna complex was inactive and occupied abundant leaf N. Chl-deficient mutants optimized leaf N-use by investing more N into carboxylation and substrate regeneration processes (Genesio et al. 2021).

Our previous study characterized a yellow-green wheat mutant Jimai5265yg during tissue culture. Genetic analysis indicated that the trait of less Chl was controlled by recessive gene loci on chromosome 4. The mutant had a higher quantum yield of PSII photochemistry ($\Phi_{PSII}$) and mesophyll conductance ($g_m$), which contributed to the improved photosynthetic efficiency. Furthermore, the photosynthetic advantage was maintained in response to the N-deficient treatment in hydroponic culture. In addition to the enhanced CO$_2$ diffusion, the N-deficient mutant has a higher cyclic electron transport and photochemical activity of PSI, coping with photodamage (Li et al. 2021). N deficiency induced the increase of relative N content of the photosynthetic system in both wheat and rice (Hou et al. 2019). The higher PNUE of the N-deficient mutant could be attributed to the more optimal N-partitioning pattern within leaves. The biomass and yield of the Chl-deficient mutants slightly declined by the pleiotropic effect of genetic mutation (Genesio et al. 2021). However, the grain yield of Jimai5265yg increased by 18% compared to the wild type in the field without N application (Table 1S, supplement). Other agronomic traits, such as spike number and 1,000-grain mass did not exhibit a significant difference between the two genotypes. The grain number per spike rather than the other two yield components (thousand kernel mass and spike number) was the dominant contribution to the higher yield (Zheng et al. 2021). What are the differences in photosynthetic parameters between two genotypes in different N application levels at anthesis? Is there any relationship between photosynthetic properties and the increasing productivity for the mutant in the conditions without N application? Uncovering those problems might increase light-use efficiency and productivity through the genetic manipulation of the photosynthetic system.

In this study, we focused on the photosynthetic property of Jimai5265yg under different N-application conditions in the field at anthesis. Similar to the results of Zheng et al. (2021), N has little effect on the net
photosynthetic rate \( F_{\text{PSII}} \) in two genotypes. Jimai5265yg did not show apparent advantages over the wild type on \( P_{\text{h}} \) in the conditions without N application. However, PNUE and photorespiration of the mutant were higher. It has been reported that crop productivity was associated with the photoprotective mechanism (Kromdijk et al. 2016). So, we detected the PSI and PSII performance using chlorophyll \( a \) fluorescence. Despite the lower NPQ, the quantum yield of PSII and PSI chemistry in the mutant was higher in the conditions without N application. Though the photoinhibition of PSII was sensitive to N deficiency, the reduction level of PSI and PSII electron acceptors decreased. We suggested that the photoprotection of PSI and electron transport regulation might be associated with the higher gain yield of the mutant in the N-deficient conditions.

**Materials and methods**

**Plant material and growth conditions:** Wheat (Triticum aestivum L.) Jimai5265 and Jimai5265yg were used as experimental materials. According to the earlier study, the yellow-green mutant Jimai5265yg is a mutant line of wheat cultivar Jimai5265 with two chlorine mutations on chromosomes 4A and 4B (Wang et al. 2018). The mutant plants are yellow-green throughout the life span in the field.

The field N treatments were conducted at the reach farm (34°20’N, 108°24’E) of Northwest A&F University, Shaanxi Province, China. The altitude is 466.7 m, and soil composition is 36.5% clay, 61.1% silt, and 2.4% sand, pH of 8.4. The contents of soil organic matter, total nitrogen, total phosphorus, and total potassium were 13.09, 0.86, 0.71, and 14.76 g kg\(^{-1}\), respectively, and the annual average temperature is 12.9°C, and the frost-free period is 211 d. The annual total solar radiation is 48.057 J m\(^{-2}\)s\(^{-1}\). The lamps of the assimilation chamber were rapidly switched off and recorded automatically every second for 150 s. The absolute value of the minimum photosynthetic rate was \( R_{\text{c}} \) (Ayoub et al. 2011).

It was a complete randomized block field experiment with four N concentrations \([\text{Nc}: 0 \text{ kg ha}^{-1}, \text{N}_{240}: 120 \text{ kg ha}^{-1}, \text{N}_{360}: 240 \text{ kg ha}^{-1}, \text{N}_{400}: 360 \text{ kg ha}^{-1}] \) for Jimai5265 and Jimai5265yg, respectively, and three replicates were set for each level. Each plot with an area of 9 m\(^2\) (3 × 3 m) was separated by an isolated blank line of 30 cm. Calcium superphosphate (P\(_2\)O\(_5\): 16%, 120 kg ha\(^{-1}\)) was applied as basal fertilizer. Nitrogen fertilizer was applied as urea (N ≥ 46%) before sowing. Wheat was artificially sowed on 3 October 2018, at a planting density of 150 kg ha\(^{-1}\).

**Flag leaf area, mass, and nitrogen content:** Three representative wheat plants were randomly selected from each plot, and the whole flag leaves were cut. The cut leaves were fixed on the cardboard and scanned, and the flag leaf area was measured with ImageJ 1.53 software.

The flag leaves were treated at 105°C for 30 min and dried at 80°C for 12 h to determine the dry mass. The flag leaves dried to constant mass were cut into pieces and transferred into a desiccating tube. The nitrogen content of the leaves was digested with H\(_2\)SO\(_4\)-H\(_2\)O\(_2\) and determined using the flow analyzer (AutoAnalyzer 3, Germany).

**Chlorophyll \( a \) fluorescence (CF) measurement:** Chlorophyll \( a \) fluorescence of the flag leaves was measured using the Dual-PAM-100 (Heinz Walz GmbH, Germany) from 6 to 8 May 2019 from 9:00–12:00 and 15:00–18:00 h. The parameters \( F_{\text{m}}, F_{\text{m}'} \), and \( P_{\text{700}} \) were determined after 30-min darkness adaptation. \( F_{\text{m}'} \) and \( F_{\text{m}} \) were measured after continuous illumination with an actinic light of 1,300 \( \mu \text{mol} \text{(photon)} \text{ m}^{-2} \text{ s}^{-1} \). \( F_{\text{m}} \), \( F_{\text{PSII}}, q_{\text{m}}, q_{\text{NPQ}}, \Phi_{\text{PSII}}, \Phi_{\text{NPQ}} \), and \( \Phi_{\text{qP}} \) were calculated according to Oxborough and Baker (1997) and Kramer et al. (2004). Flag leaves of four individuals were measured for each genotype. A light curve was determined at a gradient of PAR [17, 58, 131, 213, 329, 500, 758; 1,177; 1,808; 2,804 \( \mu \text{mol} \text{(photon)} \text{ m}^{-2} \text{ s}^{-1} \)]. \( F_{\text{m}}' = F_{\text{v}}/F_{\text{m}} + F_{\text{m}}/F_{\text{F}}; F_{\text{v}}/F_{\text{m}} = (F_{\text{m}} - F_{\text{F}})/F_{\text{m}}, \Phi_{\text{PSII}} = (F_{\text{m}} - F_{\text{F}})/F_{\text{m}}; q_{\text{m}} = (F_{\text{m}} - F_{\text{F}})/F_{\text{m}}; q_{\text{NPQ}} = (F_{\text{m}} - F_{\text{F}})/F_{\text{m}}; q_{\text{NPQ}} = q_{\text{NPQ}}(F_{\text{F}})/F_{\text{m}}; \Phi_{\text{PSII}} = (F_{\text{m}}' - F_{\text{F}})/F_{\text{m}}; \Phi_{\text{ND}} = (F_{\text{m}} - P)/F_{\text{m}}; \Phi_{\text{NA}} = (P - F_{\text{m}})/F_{\text{m}}. \)

**Gas-exchange measurements:** At anthesis, the flag leaf gas exchange was measured using the Li-Cor 6400 portable photosynthesis system (LI-COR, Inc.). Three individuals of each genotype were measured. All measurements were made at a leaf temperature of 25°C and an air relative humidity of 40–60%. The CO\(_2\)-response curve (\(P_{\text{CO2}}/C_{\text{r}}\)) was determined under a series of reference CO\(_2\) concentrations (400, 300, 200, 100, 50, 400, 600, 800, 1,000, 1,200; 1,500; 1,800 \( \mu \text{mol} \text{ mol}^{-1} \)), while keeping PAR at 1,300 \( \mu \text{mol} \text{(photon)} \text{ m}^{-2} \text{ s}^{-1} \). Post-illumination burst (PIB) measurements were taken after light adaption when CO\(_2\) concentration was 400 \( \mu \text{mol} \text{ mol}^{-1} \). The lamps of the assimilation chamber were rapidly switched off and recorded automatically every second for 150 s. The absolute value of the minimum photosynthetic rate was \( P_{\text{m}} \), and the absolute steady-state value of the photosynthetic rate was \( P_{\text{e}} \). (Ayoub et al. 2011).

The maximum carboxylation rate limited by Rubisco (\(V_{\text{cmax}}\)), RuBP generation (\(J_{\text{ass}}\), CO\(_2\) concentration inside the chloroplast (\(C_{\text{i}}\)), and CO\(_2\)-compensation point (\(\Gamma^*\)) were calculated using the \( R \) package developed by Duursma and Remko based on the model of Farquhar et al. (1980) and Duursma (2015).

\( g_{\text{n}} \) and \( C_{\text{i}} \) were calculated according to Flexas et al. (2007), briefly described as follows. The linear electron transport rate \( (J) \) was estimated as:

\[
J_l = \Phi_{\text{PSII}} \times \alpha \times 0.5 \times \text{PPFD}
\]

where \( \alpha \) is the fraction of light absorbed by leaves between 0.5 and 0.95, which we estimated by the SPAD value determined by Zheng et al. (2021). The partition fraction of photons between PSI and PSII can be assumed as 0.5. \( \Phi_{\text{PSII}} \) was calculated by CF measurements.
The mesophyll conductance \((g_m)\) was calculated as follows:

\[
g_m = \frac{P_N}{C_i - \Gamma^* \left[ J_e + 8 \left( \frac{P_N + R_s}{P_N + R_a} \right) \right] - J_e - 4 \left( \frac{P_N + R_a}{P_N + R_s} \right)}
\]

(2)

where \(P_N, C_i, R_a\) and \(\Gamma^*\) were calculated from the CO₂-response curve \((P_N/C_i)\) using the \(R\) package. \(J_e\) was calculated by Eq. 1.

The \(C_i\) was determined as:

\[
C_i = C_s - \frac{P_N}{g_m}
\]

(3)

The limitation to \(P_N\) was following the approach of Jákli et al. (2017), which was based on the \(P_N/C_i\) curve, briefly described as follows:

\[
g_{sat} = \frac{\partial P_N}{\partial C_i} \quad L_s = g_m - \frac{g_{sat}}{\frac{\partial P_N}{\partial C_i}}
\]

(4)

\[
g_{sat} = \frac{\partial P_N}{\partial C_i} \quad L_m = g_m - \frac{g_{sat}}{\frac{\partial P_N}{\partial C_i}}
\]

(5)

\[
g_{sat} = \frac{\partial P_N}{\partial C_i} \quad L_a = g_m - \frac{g_{sat}}{\frac{\partial P_N}{\partial C_i}}
\]

(6)

where \(\partial P_N/\partial C_i\) is the slope of the \(P_N/C_i\) curves calculated over a range of 50–100 µmol mol⁻¹. \(g_{sat}\) is the total conductance to CO₂ diffusion from the ambient air into chloroplasts:

\[
g_{sat} = (1/g_s + 1/g_{sat})^{-1}
\]

(7)

The electron fluxes in the photosynthetic carbon reduction cycle \([J_{APCR}]\), photorespiratory carbon oxidation cycle \([J_{APCO}]\), and alternative electron flux \(J_a\) were calculated according to Gao et al. (2018):

\[
J_{APCR} = 4 \times \frac{P_N + R_a}{1 - C_i}
\]

(8)

\[
J_{APCO} = 2 \times \frac{\Gamma^*/C_i}{1 - \frac{J_e}{J_s}}
\]

(9)

\[
J_{APCO} = J_i - J_{APCR} - J_{APCO}
\]

(10)

where \(P_N, R_a, C_i\), and \(\Gamma^*\) were obtained from \(P_N/C_i\) curves.

**Statistical analysis:** The results are reported as the means with standard errors (SE). The significance of the results was checked by Duncan’s multiple range tests using IBM SPSS Statistics 21.

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**Results**

**Gas-exchange parameters and PNUE:** Low and moderate N applications \((N_{120} \text{ and } N_{240})\) could improve the \(P_N\) of both genotypes, but the \(P_N\) of \(Jimai5265yg\) was more sensitive to N applications (Table 1). There was no significant difference in \(P_N\) between the mutant and the wild type except for the \(N_{360}\) application. However, the PNUE of \(Jimai5265yg\) was significantly higher than that of the wild type in all N conditions, especially for \(N_0\) and \(N_{240}\) (17.1 and 19.9%, respectively). There was no significant difference between genotypes or N applications for \(V_{cmax}\) and \(J_{max}\). \(N_{360}\) excess increased the \(\Gamma^*\) of both genotypes, suggesting that excessive N enhanced photorespiration. PIB has been used to measure the photorespiration rate (Vines et al. 1983). The increase of PIB and \(R_t\) in both genotypes under \(N_{240}\) and \(N_{360}\) applications confirmed the increase of photorespiration by high N (Table 2). The \(\Gamma^*\) and \(R_t\) of \(Jimai5265yg\) also increased under \(N_0\) application without the increase of PIB (Tables 1, 2). However, the wild type had a significantly higher \(R_t\) than that of \(Jimai5265yg\) in the \(N_{360}\) condition (Table 2).

There was a significant nitrogen effect on \(L_m\) and \(L_s\) for two genotypes (Fig. 1). The difference between the two genotypes was more remarkable in response to low N \((N_0 \text{ and } N_{120})\) than to high N \((N_{240} \text{ and } N_{360})\) for \(L_m\). The \(L_m\) of \(Jimai5265yg\) was significantly lower than that of the wild type due to higher \(g_m\) and \(C_i\) under \(N_0\) application (Table 2). Correspondingly, \(L_s\) of \(Jimai5265yg\) was significantly higher than that of the wild type. In contrast, the \(L_m\) of the wild type was larger for low N applications due to reduced \(g_m\) and \(C_i\).

**Chlorophyll a fluorescence parameters:** The previous study showed that \(N_0\) and \(N_{360}\) induced the decline of SPAD value which indicated the Chl content (Zheng et al. 2021). Similarly, the minimal and maximal fluorescence \(F_0\) and \(F_{m}\) significantly decreased in response to \(N_0\) and \(N_{360}\) applications (Table 3). The level of \(F_0\) and \(F_{m}\) was significantly lower in \(Jimai5265yg\) than in its wild type for \(N_0\) and \(N_{240}\) application. The lower \(F_{m}\) might be associated with PSI photoinhibition damage. Two genotypes had a lower maximal efficiency of PSII photochemistry \((F_v/F_m)\) in the \(N_{360}\) condition. \(Jimai5265yg\) maintained a slightly higher \(F_v/F_m\) than that of the wild type, especially for the \(N_{240}\) application (Fig. 2). There was no significant difference in \(P_a\) between different N applications for both genotypes, but the mutant had a slightly lower \(P_a\) than the wild type, indicating that the active PSI reaction centers were insensitive to N treatment (Table 3). The quantum yield of PSII \((\Phi_{PSII})\) decreased with the increase of PAR (Fig. 2A). Compared to \(N_{120}\) and \(N_{240}\) applications, \(N_0\) and \(N_{360}\) applications induced the increase of \(\Phi_{PSII}\) and \(q_P\) in \(Jimai5265yg\), while only the \(N_{360}\) application increased \(\Phi_{PSII}\) and \(q_P\) of the wild type. So \(Jimai5265yg\) has a significantly higher \(\Phi_{PSII}\) and \(q_P\) than the wild type in the \(N_0\) condition (Fig. 2A, D). In contrast, \(Jimai5265yg\) has a significantly lower \(\Phi_{PSII}\) than that of the wild type in the \(N_0\) condition. Excessive
Table 1. The effects of different N applications on $P_C$, $C_{\text{max}}$, $\Gamma_\text{cmax}$, $\Gamma$, $V_{\text{max}}$, $g$, $C_{\text{Jimai5265yg}}$, and PNUE in Jimai5265 and Jimai5265yg at anthesis. $P_C$ – net photosynthetic rate; $C_{\text{Jimai5256}}$ – intercellular CO₂ concentration; $\Gamma_\text{cmax}$ – CO₂-compensation point; $V_{\text{max}}$ – maximum carboxylation rate limited by Rubisco; $g$ – the light-saturated potential rate of electron transport; $g$ – mesophyll conductance; $C_{\text{Jimai5256yg}}$ – photosynthetic N-use efficiency. Results are represented as mean ± standard error, $n = 3$. Means in a row followed by different lowercase letters are significantly different at $p \leq 0.05$ compared via Duncan’s multiple range tests. N – different N concentrations; G – genotype. NS – not significant; **$p \leq 0.01$; *$p \leq 0.05$.

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<tr>
<td>$P_C$ [µmol(CO₂) m⁻² s⁻¹]</td>
<td>24.457 ± 1.203 $^a$</td>
<td>24.278 ± 1.747 $^a$</td>
<td>24.641 ± 0.325 $^a$</td>
<td>26.515 ± 1.453 $^a$</td>
<td>25.759 ± 0.418 $^a$</td>
<td>29.898 ± 0.621 $^a$</td>
<td>24.029 ± 0.291 $^a$</td>
<td>26.639 ± 0.200 $^a$</td>
<td>*</td>
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<tr>
<td>$C_{\text{Jimai5256yg}}$ [µmol mol⁻¹]</td>
<td>45.033 ± 0.484 $^a$</td>
<td>64.084 ± 1.517 $^a$</td>
<td>46.272 ± 2.013 $^a$</td>
<td>47.967 ± 0.796 $^a$</td>
<td>47.967 ± 0.796 $^a$</td>
<td>60.148 ± 4.699 $^a$</td>
<td>53.958 ± 1.464 $^a$</td>
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<td>$V_{\text{max}}$ [µmol(CO₂) m⁻² s⁻¹]</td>
<td>66.099 ± 5.282 $^a$</td>
<td>50.014 ± 9.618 $^a$</td>
<td>77.265 ± 3.737 $^a$</td>
<td>85.949 ± 16.412 $^a$</td>
<td>88.924 ± 12.054 $^a$</td>
<td>70.052 ± 1.236 $^a$</td>
<td>63.505 ± 5.992 $^a$</td>
<td>75.081 ± 2.415 $^a$</td>
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<td>$g$ [µmol m⁻² s⁻¹]</td>
<td>0.227 ± 0.025 $^a$</td>
<td>0.461 ± 0.044 $^a$</td>
<td>0.255 ± 0.041 $^a$</td>
<td>0.291 ± 0.039 $^a$</td>
<td>0.276 ± 0.042 $^a$</td>
<td>0.313 ± 0.046 $^a$</td>
<td>0.274 ± 0.044 $^a$</td>
<td>0.285 ± 0.031 $^a$</td>
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<tr>
<td>$g$ [µmol m⁻² s⁻¹]</td>
<td>0.326 ± 0.003 $^a$</td>
<td>0.385 ± 0.029 $^a$</td>
<td>0.574 ± 0.214 $^a$</td>
<td>0.425 ± 0.048 $^a$</td>
<td>0.321 ± 0.071 $^a$</td>
<td>0.415 ± 0.143 $^a$</td>
<td>0.317 ± 0.037 $^a$</td>
<td>0.398 ± 0.003 $^a$</td>
<td>NS NS NS</td>
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<td>PNUE</td>
<td>3.709 ± 0.469 $^a$</td>
<td>4.476 ± 0.468 $^a$</td>
<td>3.694 ± 0.173 $^a$</td>
<td>3.991 ± 0.322 $^a$</td>
<td>3.281 ± 0.245 $^a$</td>
<td>4.071 ± 0.239 $^b$</td>
<td>3.418 ± 0.129 $^b$</td>
<td>3.51 ± 0.080 $^b$</td>
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Table 2. The effects of different N applications on PIB, $R_L$, and $R_d$ in Jimai5265 and Jimai5265yg at anthesis. PIB – post-illumination burst; $R_L$ – light respiration rate; $R_d$ – mitochondrial CO₂ release in the dark. Data are expressed as means ± standard error, $n = 3$. Lowercase letters following the data within the same row refer to a significant difference ($p \leq 0.05$), compared via Duncan’s multiple range tests. N – nitrogen treatment; G – genotype. NS – not significant; **$p \leq 0.01$; *$p \leq 0.05$.

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<tr>
<td>PIB [µmol(CO₂) m⁻² s⁻¹]</td>
<td>1.820 ± 0.137 $^a$</td>
<td>1.390 ± 0.225 $^a$</td>
<td>1.876 ± 0.074 $^a$</td>
<td>1.916 ± 0.330 $^a$</td>
<td>2.859 ± 0.114 $^a$</td>
<td>2.605 ± 0.004 $^a$</td>
<td>2.983 ± 0.151 $^a$</td>
<td>2.710 ± 0.294 $^a$</td>
<td>** NS NS</td>
<td></td>
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<tr>
<td>$R_L$ [µmol(CO₂) m⁻² s⁻¹]</td>
<td>1.506 ± 0.047 $^a$</td>
<td>1.324 ± 0.026 $^a$</td>
<td>1.473 ± 0.049 $^a$</td>
<td>1.596 ± 0.095 $^a$</td>
<td>2.642 ± 0.018 $^a$</td>
<td>2.126 ± 0.176 $^a$</td>
<td>2.568 ± 0.225 $^a$</td>
<td>2.200 ± 0.071 $^a$</td>
<td>** NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_d$ [µmol m⁻² s⁻¹]</td>
<td>1.700 ± 0.182 $^a$</td>
<td>2.471 ± 0.102 $^a$</td>
<td>1.479 ± 0.043 $a$</td>
<td>1.208 ± 0.010 $a$</td>
<td>0.761 ± 0.022 $a$</td>
<td>1.314 ± 0.133 $a$</td>
<td>2.340 ± 0.244 $a$</td>
<td>1.465 ± 0.440 $a$</td>
<td>** NS NS</td>
<td></td>
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N (N\textsubscript{360}) reduced the Φ\textsubscript{NPQ} of the wild type. N\textsubscript{120} and N\textsubscript{240} have a reverse effect on Φ\textsubscript{NO} in two genotypes (Fig. 2B, C). In the N\textsubscript{0} condition, Φ\textsubscript{NO} of Jimai5265yg was significantly higher than that of the wild type, while Φ\textsubscript{NO} of Jimai5265yg was significantly lower in the N\textsubscript{240} application (Fig. 2C).

Φ\textsubscript{PSI} was significantly higher in Jimai5265yg than that of the wild type under N\textsubscript{120} application when PAR was lower than 500 µmol(photon) m\textsuperscript{-2} s\textsuperscript{-1} (Fig. 2E). Φ\textsubscript{PSI} of Jimai5265yg was maintained higher when the PAR increased, except for 329 µmol(photon) m\textsuperscript{-2} s\textsuperscript{-1} in the N\textsubscript{0} and N\textsubscript{240} conditions. Compared with other three N applications, N\textsubscript{360} increased Φ\textsubscript{PSI} of the wild type as the PAR increased. There was no significant difference between Jimai5265yg and its wild type in Φ\textsubscript{ND} with few PAR exceptions (Fig. 2G). However, Φ\textsubscript{NA} of Jimai5265yg was significantly lower than that of the wild type under low N applications (N\textsubscript{0} and N\textsubscript{120}) when PAR was less than 329 µmol(photon) m\textsuperscript{-2} s\textsuperscript{-1} (Fig. 2F). Though extreme N applications (N\textsubscript{0} and N\textsubscript{360}) increased Φ\textsubscript{NA} in Jimai5265yg, the Φ\textsubscript{NA} of the mutant kept relatively lower than in the N\textsubscript{0} condition, indicating the decrease of PSI acceptor-side reduction status.

Electronic transport rate: The quantum efficiency and photoinhibition process of PSI and PSII was regulated by electronic transport. Compared to other N concentrations, N\textsubscript{360} increased the ETRI and ETRII of the wild type. Besides the N\textsubscript{360} condition, Jimai5265yg had higher ETRI and ETRII in the N\textsubscript{0} condition. As a result, the ETRI and ETRII were significantly higher in Jimai5265yg than those in the wild type in the N\textsubscript{0} condition (Fig. 3). Under all N concentrations, the mutant had a significantly higher Je(PCR) (Table 4), indicating the increase of electron transport rate.
PHOTOSYNTHESIS OF YELLOW-GREEN WHEAT MUTANT IN N-DEFICIENT CONDITIONS

Fig. 2. The light-response curves of parameters derived from chlorophyll fluorescence in flag leaves of Jimai5265 and Jimai5265yg at anthesis under four N concentrations. $\Phi_{\text{PSII}}$ – effective quantum yield of PSII (A); $\Phi_{\text{NPQ}}$ – quantum yield of nonphotochemical quenching (B); $\Phi_{\text{Ox}}$ – quantum yield of nonregulated energy loss in PSII (C); $q_P$ – photochemical quenching (D); $\Phi_{\text{PSI}}$ – effective quantum yield of PSI (E); $\Phi_{\text{A}}$ – redox poise of PSI acceptor site (F); $\Phi_{\text{D}}$ – redox poise of PSI donor site (G). The significant difference was calculated using Duncan’s multiple range test. The asterisk showed differences between the two genotypes within the same N concentration. NS – not significant; **$p \leq 0.01$; *$p \leq 0.05$. 

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positive values in PC2 and PC1, respectively, and followed a positive direction with the load score of PC1. N0 and N240 applications on the Jt, Jₑ(PCR), and Jₑ(PCO) of the two genotypes (Table 4). However, Jimai5265yg had significantly lower Jₑ, Jₑ(PCR), and Jₑ than the wild type in the N0 condition, indicating that the electron transport of Jimai5265yg was downregulated.

Principal component analyses: To determine the best set of dependent variables associated with each genotype and with the N applications, PCA was performed based on the original values of all variables. The first and the second components (PC1 and PC2) explained 40% and 25.5% variation, respectively (Fig. 4). Two genotypes were not separated by PCA, but Jimai5265yg and Jimai5265yg separately followed a positive direction with PN, gₑ, ETRI, Φₑ(PSII), gₛ, Φₑ(NO), Cₛ, and Jₑ(PCR). Other variables were positively related to both genotypes (Fig. 4A,C). N120 separated from N0, N240, and N360 and followed a positive direction with Cₛ, Φₑ(NO), Φₑ(PCO) Nₑ(PSII), Fₑ, Vₑ(max), which were negatively correlated with the load score of PC1. N0 and N360 applications exhibited positive values in PC2 and PC1, respectively, and followed a positive direction with Fₑ/Fₛ, Jₑ(PCR), qₛ, ETRII, Φₑ(PSII). The yield gain per spike was more closely related to Jₑ, Pₑ, Jₑ, Φₑ(NO), and PNUE.

Discussion

The parameters of gas exchange, chlorophyll fluorescence, and electron transport in two genotypes were studied under four N concentrations in the field. The findings presented here proved that photoprotection was crucial for crop productivity and could serve as a new breeding strategy. Although N deficiency decreased the photosynthesis per unit leaf area changed little during intensive breeding for most crops due to the inexpensive N fertilizer, which reduced the selection pressure for photosynthesis improvement (Jahn et al. 2011). The photosynthetic capacity of modern crops was sufficient to increase grain yield (Richards 2000). At anthesis, low or high N conditions might affect the net carbon gain per unit ground area rather than per unit leaf area due to the change of total leaf area. The total leaf area, leaf area duration, and N content were more critical for the grain yield. For early growth, photosynthesis was the determinant for leaf growth rather than the leaf area since the leaf had not been fully expanded (Liu et al. 2018). The photosynthetic rate was more likely to be influenced by N treatments during the seedling stage (Li et al. 2021). However, we observed a few differences in PN between N treatments for both genotypes at anthesis (Table 1). Similar to the previous study, PN of the late growth stage was less sensitive to N applications (Richards 2000). PN of Jimai5265yg was significantly higher than that of the wild type under N240 applications (Table 1), suggesting that the photosynthetic advantages of Jimai5265yg were dependent on the N application. However, PCA analysis revealed that the grain yield under N0 was not necessarily connected to the net photosynthetic rate (Fig. 4). According to the study by Richards (2000), the photosynthetic rate of the whole crop population was more closely related to the grain yield.

It has been found that some photosynthetic processes, such as stomatal conductance (gₛ) and maximum photosynthetic rate, were associated with grain yield formation (Fischer et al. 1998). We estimated the limitations (Lₑ, Lₑ, Lₛ) to photosynthesis under different N applications in quantification. Three limitations exhibited no significant difference under high N conditions (N240 and N360), but Lₑ and Lₛ increased for Jimai5265yg and the wild type respectively under low N conditions (N0 and N120) (Fig. 1).
The suboptimal conditions often induced the increase of mesophyll limitation (Brestic et al. 2018). The alteration of limitations to $P_N$ might be mediated by the change of mesophyll conductance ($g_m$) since the difference in $V_{\text{max}}$ and $J_{\text{max}}$ between the two genotypes was not significant (Table 1). Mesophyll conductance was affected by leaf thickness and anatomical structures related to LMA. Low N supply induced the accumulation of nonstructural chemical components and resulted in a higher LMA (Pan et al. 2011). However, Jimai5265yg has significantly higher $g_m$ than the wild type without the diversity in LMA in the N0 condition (Table 2, supplement), indicating that $g_m$ was influenced by other factors rather than LMA, such as the distribution or orientation of chloroplasts and other organelles (Xiong et al. 2016). Even if $g_m$ was high, the photosynthesis of Jimai5265yg was restricted by carboxylation efficiency in the N0 condition.

There is no remarkable superiority in net photosynthetic rate for Jimai5265yg in the N0 condition, but Jimai5265yg had a significantly higher PNUe. Leaf N concentration is often positively correlated with leaf respiration (Reich et al. 2008). The interaction between the photorespiratory pathway and N metabolism determined the leaf N status and the content of photosynthetic proteins (Wingler et al. 2000). Photorespiratory ammonia recycling was also dependent on mitochondria function and the products of chloroplasts (Champigny 1995). Jimai5265yg has a higher $T^*$ and $R_8$ in the N0 condition (Tables 1, 2). The rise of the CO2-compensation point indicated the increase in photorespiratory CO2 loss (Colman 1984). The increase of photorespiration in the rice ygl53 mutant helped balance the high C/N ratio induced by the high photosynthetic efficiency (Liang et al. 2021). We proposed that the higher PNUe of Jimai5265yg might be associated with higher photorespiration, which influenced the leaf N status and facilitated the internal nitrogen remobilization. Leaf N status and remobilization positively correlated with spike differentiation, pollen fertility, and the subsequent grain yield (Reynolds et al. 2012). PCA showed that the grain yield was closely related to PNUe rather than $P_N$. The greater grain number per spike for Jimai5265yg in the N0 condition observed by Zheng et al. (2021) might result from improved N reassimilation and remobilization efficiency.

Besides participating in the carbon and nitrogen cycles, the photorespiratory pathway was essential for protecting PSII from photoinhibition by consuming the excess photochemical energy and repairing the photodamaged PSII (Takahashi et al. 2007). $F_0$ had been used as the indicator for irreversible damage of PSII as the temperature increased. The rise of $F_0$ was related to the disconnection of LHCII from the core PSII center and the reduction of PSII (Pastenes and Horton 1999). The lower $F_0$ provided the basal protection of PSII in Jimai5265yg in the field where intense light and high temperature were inevitable (Table 3). The reduction of $F_0/F_m$ reflected the photoinhibition and damage of PSII complexes. N-deficiency caused a decrease in $F_0/F_m$ in many species, such as tea, Panax notoginseng, maize,
A. thaliana, and rice (Lin et al. 2016, Honoki et al. 2018, Zhang et al. 2020). Though a study of wheat indicated that Fv/Fm parameter was insensitive to N treatment, Gao et al. (2018) and our previous study showed a decrease in Fv/Fm for wheat seedlings when N was deficient (Li et al. 2021). In this study, Fv/Fm was lower in the N120 condition for two genotypes at anthesis (Table 3). Fv/Fm of Jimai5265yg was significantly higher than the wild type in the N240 condition, suggesting more activated PSII reaction centers and less susceptibility to photoinhibition. High irradiance increased susceptibility to photoinhibition of N-deficient plants, so we observed the light-response curve of qP (Fig. 2D), representing a proportion of the open PSII reaction centers (Lu and Zhang 2000). Many studies proved that low N decreased qP in maize, bean, and wheat (Antal et al. 2010, Jin et al. 2015, Kartseva et al. 2021). We observed similar results in the wild type for N0, N120, and N240 applications, but N deficiency (N0) increased qP of Jimai5265yg (Fig. 2D), suggesting that low N and extreme N deficiency had different effects on the mutant. It has been reported that the higher reduction level of the primary electron acceptor QA increased susceptibility to photoinhibition of PSII (Lu and Zhang 2000). The higher qP value of Jimai5265yg compared to the wild type under N0 application indicated a lower reduction state of QA and lower excitation pressure of the PSII acceptor side. Though there was less excitation energy transferred into the reaction centers, the PSI complex of Jimai5265yg was more damaged by photoinhibition in the N0 condition (lower ΦPSII). We observed stable Pm in response to different N treatments, suggesting that photochemically active PSI were unaffected. The photo-
inhibition of PSII was an effective protective mechanism for the PSI (Brestic et al. 2016).

Nonphotochemical quenching (NPQ) reflecting a thermal dissipation of excess light energy served as a protection of PSII against photoinhibition (Shimakawa and Miyake 2019). The lower Φ_{PSII} of Jimai5265yg in the N₀ condition might contribute to the higher photoinhibition of PSII. However, the quantum yield of the PSII photochemical process (Φ_{PSII}) in Jimai5265yg was significantly higher than the wild type in the N₀ condition (Fig. 2A). Insufficient or excess N supplies reduced the distribution of light energy to the active PSII reaction center (Φ_{PSII}) and increased energy flow to the nonphotochemical process (NPQ) (Jauffrais et al. 2016, Zhang et al. 2020). N-deficient wheat and maize showed a reduction in Φ_{PSII}, especially for the low N-sensitive cultivars (Lu and Zhang 2000, Gao et al. 2018). Excessive N decreased both Φ_{PSII} and Φ_{PSII} in Panax notoginseng (Cun et al. 2021). The PSII photochemical conversion efficiency depended on the excitation energy distribution in PSI complexes. It seems that more proportion of excitation energy was consumed by PSII photochemistry and lost through the nonregulated dissipative pathway (higher Φ_{PSII}) for Jimai5265yg in the N₀ condition. The higher PSII photochemical efficiency was a benefit for CO₂ assimilation, but the increase of NO was associated with a harmful longer lifetime of energy excitation (Laisk et al. 1997). Nonregulated energy dissipation comprised the thermal dissipation in nonfunctional PSIIIs and constitutive light-independent thermal dissipation processes (Stirbet and Govindjee 2011). Higher qₑ of Jimai5265yg induced by N₀ indicated less inactive PSIIIs, so the increase of Φ_{PSII} was associated with the constitutive light-independent thermal dissipation process (Fig. 2C,D). The previous study introduced the higher Φ_{PSII} as a more reduced PSII acceptor side or a larger proportion of closed PSII reaction centers; as a result, triplet Chl * and ROS formed and led to PSII photoinhibition (Samson et al. 2019). However, it seems the increase of NO was not dependent on the closure of PSI reaction centers in our experiments (qₑ) considering the complementarity of Φ_{PSII}, Φ_{PSII}, and Φ_{PSII} (Fig. 2A–D). Although the photochemical efficiency of PSII in Jimai5265yg improved under N₀ application, the capacity of PSI photoprotection decreased due to an increase of Φ_{PSII} at the expense of the harmless energy dissipation pathway NPQ.

The generation of NPQ was dependent on a trans-thylakoid proton gradient built up by linear and cyclic electron transport (CET) (Zivcak et al. 2014a). Our previous study showed that Jimai5265yg had higher CET than the wild type under the N-deficient condition at the seedling stage. The nonphotochemical energy dissipation was enhanced in Jimai5265yg due to the increase of oxidized P700 (Φ_{PSII}) (Li et al. 2021). The increase in the CET rate provided efficient protection against oxidative stress, such as heat, high light, and drought (Zivcak et al. 2014a,b). We did not observe a significant difference in CET (Φ_{PSII}/Φ_{PSII}) between the two genotypes, but the higher Φ_{PSII} of Jimai5265yg under N₀, N₁₂₀, and N₂₄₀ indicated that the photochemical efficiency of PSI increased (Fig. 2F). The photochemical reaction in PSI was limited by the oxidation status of the donor side (Φ_{PSII}) and the reduction status of the acceptor side (Φ_{PSII}). It has been documented that N deficiency induced the over-reduction of the PSI acceptor side and led to PSI photoinhibition. A wheat chlorina mutant ANK has a more reduced acceptor side of PSI and inactive PSI despite normal NPQ level (Brestic et al. 2016). In contrast, the PSI acceptor side of Jimai5265yg (Φ_{PSII}) was less reduced than that of the wild type at low light conditions with insufficient N applications (N₀ and N₁₂₀) (Fig. 2F), indicating that PSI was photoinhibited less. It has been assumed that downregulated electron transport protected the acceptor side of PSI against over-reduction. The photodamaged PSI prevented electron transfer to PSI and, therefore, further production of ROS (Tiikkanen and Aro 2014). According to our results, the PSI of Jimai5265yg was more susceptible to photodamage because of its lower capacity to trigger NPQ. Meanwhile, the PSII protected PSI against oxidative damage by adjusting electron flow.

Studies using wheat seedlings revealed that low or deficient N conditions reduced the electron transport rate due to the decreased carboxylation rate (Gao et al. 2018, Li et al. 2021). Consistently, we observed that Jₑ of Jimai5265yg was significantly lower in low and deficient N (N₀ and N₁₂₀) compared to N₃₆₀ and N₅₆₀ conditions (Table 4). However, the wild type had a higher Jₑ under the N₀ condition. As a result, the difference between the two genotypes on electron transport rate was more significant for the N₀ condition. Despite lower Jₑ, Jimai5265yg had a higher Jₑ, suggesting more electrons flow to the carboxylation process. The electron flow through PSII (ETRII), which was required by carboxylation and oxygenation, was also higher in the N₀ condition for Jimai5265yg (Fig. 3A). Photorespiratory or Mehler pathway could serve as another energy sink for dissipation of excess excitation energy under stress conditions (Wingler et al. 2000, Huang et al. 2019). The higher Jₑ and Φₑ indicated that photorespiration was essential for the excessive energy consumption of Jimai5265yg (Tables 1, 4). However, Jₑ is important for the wild type to prevent photoinhibition when Jₑ was higher in the N₀ condition. To eliminate excessive electrons, Jimai5265yg was dependent on reducing electron transport rate and photorespiration, while NPQ and other processes such as the Mehler pathway were more critical for its wild type to cope with N deficiency.

Conclusion and future perspective: Our results demonstrated that different N applications had little effect on the net photosynthetic rate at anthesis for two genotypes. The yellow-green mutant Jimai5265yg had a higher PNU, which might be associated with N metabolism regulated by the photorespiratory pathway. Despite the lower NPQ, Jimai5265yg had higher photochemical efficiency of PSI and PSII and lower reduction status of the PSI acceptor side. The photodamaged PSII protected PSI from over-reduction by regulating electronic transport. Besides, the increased photorespiration was the electron
sink for energy dissipation. Though the $P_h$ advantage of the mutant was not clear at anthesis, the protective capability against photodamage of PSI was enhanced under the N-deficient condition (N$_{S}$). It seems that photoprotection and its related electron transport of PSII and PSI might be associated with the productivity of the mutant in the N-deficient field. Our results may open new possibilities for improving grain yield or spike development of N-deficient wheat by manipulating the photoprotective mechanism. Photoprotection was dependent on the light-harvesting machinery composition and structure, thylakoid components, and the xanthophyll cycle, which has not been studied in $Jimai5265yg$. Further investigation on N absorption, metabolism, and remobilization in $Jimai5265yg$ may help explain why the mutant has a higher grain yield in the N-deficient field.

References


Laisk A., Oja V., Rasulov B. et al.: Quantum yields and rate constants of photochemical and nonphotochemical excitation
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