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Leaf-colour modification affects canopy photosynthesis, dry-matter accumulation and yield traits in rice

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ABSTRACT

Context: Recent research has proposed to modify leaf-colour traits to improve canopy photosynthesis (A_c) by allowing light penetration to lower layers of a dense canopy.
Research question: Whether and how enhanced light penetration can really increase A_c *and whether leaf-colour modification influences other growth-related traits remain unclear.*
Methods: Canopy light and nitrogen profile parameters (i.e., the extinction coefficient for light, K_L; and for nitrogen, K_N; and their ratio, K_N/K_L), A_c, and agronomic yield traits were examined in nine rice genotypes comprising different genetic backgrounds and their leaf-colour variants.
Results: Compared with stay-green (G) variants, yellow-leaf (Y) variants caused larger effects on crop growth and development: altered growth duration (increased in one genetic background while decreased in the other), lower tiller number, and reduced leaf area. As with G traits, a delayed senescence at the post-flowering stage was observed in Y variants, which was associated with nitrogen dynamics in plants. Although Y variants expectedly allowed more light penetration into lower layers of the canopy (i.e., lower K_L), the leaf-nitrogen profile, and thus, the leaf photosynthetic capacity (i.e., A_{max}) profile, did not necessarily follow more closely the light profile. Improved A_c and higher daily crop growth rate (CGR) were observed in the Y variant of one genetic background but not of the other, and the higher A_c or CGR were associated with improved leaf photosynthetic nitrogen use efficiency and higher canopy K_N values. The higher CGR during the grain-filling phase and resulting increased harvest index of this Y variant contributed to its greater grain productivity. Multiple regression analysis of the data of all nine genotypes indicated that the K_N:K_L ratio was the most important factor determining A_c and CGR.
Conclusions and implications: Leaf-colour modification can improve A_c, CGR and crop productivity only if other traits (especially N profile in the canopy) are adjusted synergistically. However, the observed diversity in phenotypic variations of multiple traits caused by leaf-colour modification implies the potential of exploiting breeding or crop management to improve rice biomass and yield.

1. Introduction

Rice is one of the most important crops in meeting diversified social demands by humans. First, grain production of rice plant accounts for ca. 21 % and ca. 50 % of human food calories for global and Asian consumption, respectively (Awika, 2011; Muthayya et al., 2014). Second, utilisation of rice husk and straw has been considered as one of the most promising sources of renewable energy to address the rapidly growing needs for alternative energy (Lim et al., 2012; Dungani et al., 2016). However, as the world's population and economy expand, the gap between the need for agricultural production and the actual level of productivity is likely to widen further in the near future (Zhu et al., 2008). The stagnation of rice productivity in recent decades is partly associated with the low efficiency of converting photosynthetically active radiation (PAR) into biomass accumulation, which is currently far below the theoretical maximum value of solar energy conversion efficiency (Long et al., 2015; Ort et al., 2015; Yin and Struik, 2015). One possible strategy to overcome this is to improve canopy photosynthesis (A_c) by modifying leaf-colour traits (i.e., stay-green and yellow-leaf traits). Stay-green traits are mainly meant to sustain canopy

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photosynthetic competence during later grain filling (Gregersen et al., 2013; Borrell et al., 2014), while yellow-leaf traits are applied to increase light penetration to lower layers and thus improve A_c (Ort et al., 2011).

Rice productivity during its entire life cycle depends on the duration of complete canopy cover and the capacity of canopy photosynthesis. Accelerating canopy closure during vegetative growth (i.e., improving early leaf area growth rate) or slowing down canopy senescence during the grain filling phase (i.e., introducing stay-green traits) may enhance light capture (Yin and Struik, 2015). For a closed canopy, however, undue increases in leaf area index (LAI) enhance self-shading (leaves in the lower parts of the canopy may be shaded by the upper leaves that receive most of incoming PAR), and therefore, could be detrimental to canopy photosynthesis (Hirose et al., 1997). Green leaves in the upper canopy of typical C_3 crop plants are usually light-saturated under full sunlight, whereas leaves deep down the canopy contribute less to A_c due to light starvation (Zhu et al., 2008; Walker et al., 2018). Adopting rice genotypes with reduced canopy chlorophyll content (relatively yellower leaves) has been proposed to improve A_c and biomass production by allowing more light penetration into the lower layers of the canopy (Ort et al., 2011). This concept has been previously examined in soybean (Pettigrew et al., 1989; Slattery et al., 2017; Walker et al., 2018), but little relevant information is available for rice, because yellow-leaf variants are rare in rice.

Besides the canopy light profile, the profile of leaf photosynthetic resources (leaf nitrogen in particular) also affects the carbon gain of the canopy by influencing photosynthesis of each individual layer (Hikosaka, 2014). The physiological mechanism behind this is that leaf photosynthetic capacity is closely related to nitrogen content (Evans, 1988, 1989). In an actual plant canopy, the nitrogen distribution is normally not uniform, but may acclimate to the light profile in such a way that the upper sunlit leaves have more nitrogen content than the lower, shaded ones (Field, 1983; Evans, 1993; Li et al., 2013). This spatial nitrogen distribution pattern in a plant canopy can be more efficient in accumulating photosynthate compared with that under uniform nitrogen distribution (Hirose and Werger, 1987), since it serves as an adaptive response to the light distribution within the canopy (Field, 1983). In fact, mathematical optimisation analysis has shown that the A_c is maximised if the light-saturated maximum leaf-photosynthesis (A_{max}) profile follows the light profile (Anten et al., 1995; Goudriaan, 1995). This requires that the photosynthetically active nitrogen extinction coefficient (K_N, defining the extent to which nitrogen gradually decreases with canopy depth) equals the light extinction coefficient (K_L, defining the extent to which photon flux density gradually attenuates with canopy depth) if A_{max} is linearly correlated to leaf nitrogen content. However, typically, K_N in an actual canopy is considerably lower than K_L, e.g., only $\sim$ 0.35 times K_L in kenaf (Archontoulis et al., 2011). Nevertheless, the expected decrease of K_L in yellow-leaf genotypes may make it possible to close the gap between light and nitrogen profile distribution, putting K_N closer to K_L, but whether this is the case and how leaf-colour traits would facilitate to maximise A_c remains to be investigated.

In addition to light and nitrogen profiles along the canopy depth, some agronomic and physiological characteristics of the canopy like LAI, tiller number, and canopy nitrogen content are variable among different genotypic backgrounds, growth stages, and even growing environments. These traits interact with each other and contribute to the variations in K_L and K_N (Yin et al., 2003; Ouyang et al., 2021). There is little information associated with investigating the impact of leaf-colour modification on these traits and exploring how these canopy traits affect biomass accumulation, grain production and yield components.

In this study, we used rice genotypes from different genetic backgrounds that had been modified to have either the stay-green trait or the yellow-leaf trait or both. We aimed (i) to examine whether and how A_c and dry matter accumulation can be improved by leaf-colour modification; (ii) to calculate the ratios of K_N to K_L among genotypes with contrasting chlorophyll content; and (iii) to identify the beneficial traits improving the crop yield and biomass production after leaf-colour modification.

2. Materials and methods

2.1. Plant material and growth conditions

Four genotypes of rice (*Oryza sativa* L.) were modified to have either the stay-green variant or the yellow-leaf variant, or both, creating a total of nine genotypes that were used in this study. The default genotypes belong to four different rice groups, representing four genetic backgrounds. Cv. Guanglingxiangnuo (GLXN) belonging to mid-season *japonica* rice material was modified via T-DNA insertion using the KOD-Plus-Mutagenesis Kit (Toyobo, Osaka, Japan) to substitute each position of the wild-type *Wx* gene sequence; then this gene fragment from the site-directed mutagenesis was inserted to construct the plasmids, which were introduced in EHA105 *Agrobacterium* strain cells by electroporation; finally, transgenic rice plants were generated by *Agrobacterium tumefaciens*-mediated transformation; the variant used in our study was selected from the homozygous single T-DNA insertion transgenic plant of Liu et al. (2014) as it holds a pleiotropic, stay-green trait. The other three rice materials were modified by radiation mutagenesis. To realise this, the seeds were incubated in a water bath at 33 °C for 48 h to induce germination to obtain high-frequency gene mutagenesis. Then they were irradiated with ^{60}Co γ-rays at 50 Gy; after 24 h, the seeds were sown under field conditions to obtain the first generation (M1); the leaf-colour traits were identified and screened from a population of M2 plants; the derived variants were genetically fixed through advancement to the M8 generation (Jiang et al., 2007). Mid-season *japonica* cv. Yandao 8 (YD) was modified to have a stay-green variant, an early *indica* cv. Zhefu 802 (ZF) was modified to have a yellow-leaf variant, and a *japonica* type cv. Wuyunjing 3 (WYJ) was modified to have both stay-green and yellow variants. Seeds of GLXN, YD, and WYJ cultivars were from Yangzhou University, and seeds of the ZF background were from Zhejiang University, China. After multiplying for many generations, these genotypes showed stability of the G and Y variant lines.

Two independent experiments were conducted, one in a greenhouse at Wageningen University & Research, Wageningen, the Netherlands (51°58′N, 05°40′E), and one at the field farm of Yangzhou University, Jiangsu Province, China (32°30′N, 119°25′E), respectively. The phenological information of days from sowing to different stages (i.e., beginning of stem elongation, heading, and maturity) of these genotypes in each experiment are listed in Table 1. The leaf chlorophyll contents of the nine genotypes in the field experiment are listed in Fig. S1.

2.2. Greenhouse experiment

The environmental conditions of the greenhouse were controlled. Light intensity inside was kept within 400–500 W m^{-2}; temperature was set at 26 ± 0.5 °C for the 12 h light period and at 23 ± 0.5 °C for the 12 h dark period; the CO_2 level was about 400 μmol mol^{-1} and the relative humidity was set at 65–75 %. Pre-germinated seeds of the nine genotypes were sown on 30 April 2019 in porous plastic trays, and then transplanted to pots (24 cm in diameter and 20 cm in height, and 7 litres in volume) at the 3rd leaf stage with two seedlings per pot. All pots were placed on movable lorries according to a randomised complete block design, with three replications per genotype. The soil used was a sandy loam with 84.5 mg alkali-hydrolysable N, 6.5 mg Olsen-P, and 229.7 mg exchangeable K. 1.5 g KH_2PO_4 was applied and incorporated with soil just before transplanting as basal fertiliser. Nitrogen in the form of urea (totally 1 g urea per pot) was applied at pre-transplanting, early tillering, and panicle initiation stage with the proportions being 50 %, 20 % and 30 %, respectively. Throughout the entire growth cycle, pots were frequently supplied with tap water, ensuring 2 cm of standing water on top of the soil surface. Drainage was only undertaken at a critical leaf-

Table 1

Duration of different phenological phases for rice genotypes of four genetic backgrounds, each including a default genotype (CK) and its greener-leaf variant (G) and/or yellower-leaf variant (Y), in two experiments.

Experiment	Background	Genotype	Days from sowing to beginning of stem elongation (d)	Days from sowing to heading (d)	Days from heading to maturity (d)	Days from sowing to maturity (d)
Greenhouse Experiment (2019)	GLXN	CK	68	74	58	132
		G	68	74	58	132
	YD	CK	43	50	55	105
		G	45	52	55	107
	WYJ	CK	47	55	48	103
		G	47	55	48	103
		Y	51	59	50	109
	ZF	CK	90	100	50	150
		Y	58	65	47	112
Field Experiment (2020)	GLXN	CK	86	96	77	173
		G	86	96	77	173
	YD	CK	77	85	77	162
		G	79	87	77	164
	WYJ	CK	85	95	64	159
		G	85	95	64	159
		Y	89	99	71	170
	ZF	CK	78	88	56	144
		Y	71	78	49	127

The sowing date in the greenhouse experiment was 30 April in 2019 with seedlings transplanted 20 d later; the sowing date in the field experiment was 27 May in 2020 with seedlings transplanted 30 d later.

age stage to control non-productive tillers, in line with the local standard practices of the rice growing area, where our field experiment was conducted.

2.3. Field experiment

Pre-germinated seeds were first sown on 27 May 2020 in paddy seedbed field plots, and then seedlings of the 3rd leaf stage in the seedbed were transplanted to the experimental field with a hill spacing of 0.25 m × 0.16 m and two seedlings per hill. A split-plot design was used with genetic backgrounds as the main plots and genotypes as the split plots, in a randomised block arrangement with three replicates. The main plots were separated by ridges at a 1-m width covered by plastic film inserted in the soil at a depth of 0.5 m. Each split-plot area was 20 m^2, and, thus, the size of each main plot for the WYJ background (the only one having three genotypes) was 60 m^2 while it was 40 m^2 for each of the other three backgrounds (i.e., GLXN, YD, and ZF). The soil used in the experiment was also a sandy loam (Typic Fluvaquent, Etisols [U.S. taxonomy]) with 25.5 g kg^{-1} organic matter, 103 mg kg^{-1} alkali-hydrolysable N, 33.4 mg kg^{-1} Olsen-P, and 70.5 mg kg^{-1} exchangeable K. One day before transplanting, phosphorus (30 kg ha^{-1} as single superphosphate) and potassium (40 kg ha^{-1} as KCl) as basal fertiliser were applied. 240 kg N ha^{-1}, in the form of urea, was applied at pre-transplanting, early tillering, and panicle initiation stage with the same proportion as in the greenhouse experiment. Weather data for the average air temperature and sunshine hours during the rice growing season were monitored at a weather station close to the experimental site and are shown in Fig. S2.

2.4. Canopy light and nitrogen distribution measurements

In the field experiment, the level of photosynthetically active radiation at various canopy depths was measured by using a 100-cm linear ceptometer (SunScan Systems SS1, Delta-T devices, Cambridge, UK) placed diagonally across the rows. The PAR measurements, at stem elongating, flowering (defined as 80 % panicles fully emerged from flag-leaf sheath and began flowering, ca. 7 d after heading stage), and 20 d after flowering stage, were taken every 10 cm from the top of the canopy to the ground level with three to four replicates in each plot for each genotype. All measurements were restricted to the time span from 11:00 to 13:00 under a clear sky. Next, leaves of five plants in the same canopy were dissected at the same 10-cm interval from the top canopy downwards according to the stratified-clipping method (Monsi and Saeki, 2005). Leaf area of each horizontal layer of the canopy was determined by using an area metre (Li-3100; Li-Cor, Lincoln, NE, USA). Afterwards, these leaf blades were placed in a forced air oven (UF 260, Memmert Corp., Germany) at 105 °C for 0.5 h and then dried at 75 °C for 72 h to a constant weight. After weighing, the leaf samples were ground into powder, which was then assessed for the leaf nitrogen concentration by the Kjeldahl apparatus (Kjeltec 8400, Foss Corp., Germany). The specific leaf nitrogen (SLN, leaf nitrogen content per unit leaf area) was then calculated.

2.5. Estimation of light and nitrogen extinction coefficients

Photon flux density was assumed to attenuate with the canopy depth according to the Lambert-Beer's law:

$$I_i = I_0 \exp(-K_L \mathrm{LAI}_i) \quad (1)$$

where LAI_i (m^2 green leaves m^{-2} ground) is the cumulative surface area of green leaves per unit ground area from the top of the canopy; I_i and I_0 (μmol m^{-2} s^{-1}) are the incoming photon flux values at depth i and above the canopy, respectively; K_L is the light extinction coefficient.

Following Anten et al. (1995), the vertical gradient of the SLN in the canopy was expressed as:

$$\mathrm{SLN}_i = (\mathrm{SLN}_0 - n_b)\exp(-K_N \mathrm{LAI}_i) + n_b \quad (2)$$

where SLN_i is the SLN (g N m^{-2}) of the leaves at the ith layer of the canopy; SLN_0 is the SLN of the leaves at the top of the canopy; K_N is the extinction coefficient for effective leaf nitrogen; n_b is the minimum leaf nitrogen for photosynthesis, at or below which photosynthesis is zero; an estimated value of 0.3 g N m^{-2} for n_b was adopted for all the rice genotypes from Yin and van Laar (2005). K_N and SLN_0 were estimated by fitting Eq. (2) to the measured data.

2.6. Plant growth measurements

In the greenhouse experiment, tiller number and leaf area were measured at heading (defined as the moment the first panicle of the whole plant emerged from the flag-leaf sheath), 20 d after heading, and

40 d after heading with sampling size of three to five pots per genotype. Above-ground dry weight for each genotype at the same moments and at maturity (defined as the moment the rachis of panicle turned yellow) was determined after oven drying at 75 °C for 72 h to constant weight.

In the field experiment, three extra time points for measurements were added: at 20 d after transplanting, 35 d after transplanting, and at stem-elongating, to better examine the dynamics of traits throughout the whole crop cycle. Plants of five hills randomly selected from each plot were sampled as one replicate at each measurement. Daily average crop growth rate (CGR) was calculated for each genotype as the difference in above-ground dry weight divided by the number of days between samplings. After that, the leaf part of the sample at each stage was used to measure the nitrogen concentration using the Kjeldahl apparatus (Kjeltec 8400, Foss Corp., Germany). Total canopy nitrogen content (N_C, g N m^{-2} ground), and canopy-averaged nitrogen per leaf area (SLN$_C$, g N m^{-2} leaf) were then calculated.

2.7. Leaf chlorophyll content and photosynthesis measurement

In the field experiment, leaf chlorophyll concentration was measured at tillering, stem-elongating, heading, 20 d after heading, and 40 d after heading, according to the method of Arnon (1949). The uppermost newly expanded leaves were ground to extract chlorophyll with 95 % ethanol at 60–65 °C, and then the chlorophyll content was determined using an ultraviolet spectrophotometer (Lambda 650, PerkinElmer, USA). Light-saturated leaf photosynthesis at ambient CO$_2$ level (A_{max}, μmol CO$_2$ m^{-2} s^{-1}) was measured on flag leaves at a light intensity of 2000 μmol photons m^{-2} s^{-1} using an open-path gas exchange system (Li-Cor 6400XT; Li-Cor Inc., Lincoln, NE, USA) at the flowering stage (7 d after the first panicle tip emerged from the flag-leaf sheath), with the flow rate set at 400 μmol s^{-1}. All measurements were carried out at a leaf temperature of 25 °C and a leaf-to-air vapour pressure deficit between 1.0 and 1.6 kPa. The leaf segments used for photosynthesis measurements were cut out and used to determine leaf nitrogen concentration by an element analyser (Vario Macro cube, Elementar, Germany) based on the micro-Dumas combustion method.

Canopy photosynthesis was measured using a static-chamber method at the same day (i.e., the flowering stage) as leaf photosynthesis measurements, which has been applied and validated in many previous studies (Welker et al., 2004; Bubier et al., 2007; Xia et al., 2009; Niu et al., 2010). Transparent PVG plates (Transmittance > 95 %) were used to make an assimilation chamber with dimensions 60 cm × 60 cm × 150 cm, and then connected with the gas exchange system (Li-Cor 6400XT; Li-Cor Inc., Lincoln, NE, USA) (Fig. S3; also see detailed information in Niu et al., 2008). Two electric fans (size: 120 mm × 120 mm × 25 mm) with rotation speeds of 6000–10, 000 rpm were built to promote air mixing continuously inside the chamber. Fifteen consecutive recordings of CO$_2$ and water vapour concentrations were taken on each frame at 10-s intervals during a 150-s period after steady-state conditions were achieved within the chamber. According to our field planting density, plants of totally eight hills (two rows × four hills) were enclosed in the chamber. We made this measurement for three to four times for each plot. All measurements were conducted from 10:00 to 12:00 a.m. at a clear sky during flowering, when the incoming solar photosynthetic flux density was between 1000 and 1200 μmol photon m^{-2} s^{-1}. The variation in air temperature inside the chamber was less than 0.2 °C during an average measurement period. The flux rates were determined from the time-course of the CO$_2$ concentration in the assimilation chamber. To correct for the effect of soil respiration, the measurement was conducted at the same spot with all above-ground parts of the plants in the chamber cut off. The net canopy photosynthesis rate ($A_{c,net}$, μmol CO$_2$ m^{-2} ground s^{-1}) was then calculated as the difference between the two measurements according to the soil-flux calculation procedure in the LI-6400 gas exchange system manual (LiCor Inc., Lincoln, NE, USA) or LI-8100A soil gas flux system manual (LiCor Inc., Lincoln, NE, USA).

2.8. Calculation of photosynthetic nitrogen use efficiency

Leaf photosynthetic nitrogen-use efficiency (PNUE) was defined as:

$$\mathrm{PNUE} = \frac{A_{\max}}{\mathrm{SLN} - n_\mathrm{b}} \tag{3}$$

where A_{max} (μmol CO$_2$ m^{-2} s^{-1}) is the maximum net photosynthesis rate of the flag leaf at a light intensity of 2000 μmol m^{-2} s^{-1} and a CO$_2$ concentration of 400 μmol mol^{-1}; SLN (g N m^{-2} leaf) is the specific leaf nitrogen, and n_b represents a base leaf nitrogen content as defined for Eq. (2) ($n_b = 0.3$ g N m^{-2} for rice; Yin and van Laar, 2005).

Canopy photosynthetic nitrogen-use efficiency (PNUE$_c$) was defined as:

$$\mathrm{PNUE_c} = \frac{A_\mathrm{c}}{(\mathrm{SLN_c} - n_\mathrm{b})\mathrm{LAI}} \tag{4}$$

where A_c (μmol CO$_2$ m^{-2} ground s^{-1}) is the canopy photosynthesis; SLN$_c$ (g N m^{-2} leaf) is the canopy-averaged specific leaf nitrogen (= N_c/LAI); and LAI (m^2 leaf m^{-2} ground) is the leaf area index.

2.9. Yield and yield-related traits at maturity

At maturity, yield, yield components, total above-ground biomass, and harvest index were determined in the greenhouse and field experiments. Grain yield and yield components were measured following the procedure as described by Yoshida et al. (1976). Aboveground plants sampled at maturity were separated into straw, filled and unfilled grains, and rachis. Dry weight of each part was determined by oven-drying to constant weight, then the harvest index was calculated as the ratio of filled grain weight to total above-ground weight. In the greenhouse experiment, plants of three pots were sampled as one replicate for each plot to determine the yield (g hill^{-1}) and yield components (i.e., number of panicles per hill, number of spikelets per panicle, filled-grain percentage, and 1000-grain weight). In the field experiment, grain yield (g m^{-2}) was determined from harvesting plants of 2 m^2 in each plot and adjusted to 14 % moisture. The yield components (i.e., number of panicles per m^2, number of spikelets per panicle, filled-grain percentage, and 1000-grain weight) were determined from randomly selected plants of ten hills (excluding the border ones) in each plot. The number of spikelets per panicle was calculated as total number of spikelets divided by the number of panicles. The filled-grains percentage was defined as the percentage of filled grains (specific gravity ≥ 1.06 g cm^{-3}) to the total number of spikelets. The 1000-grain weight was calculated as the filled grain weight divided by the number of the filled grains, then multiplied by 1000. The daily average grain-filling rate was calculated as the final grain yield (14 % moisture) divided by the days of duration from heading stage to maturity.

2.10. Data analysis

Nonlinear fitting was carried out using the GAUSS method in PROC NLIN of SAS (SAS Institute Inc., Cary, NC, USA) to estimate K_L and K_N in Eqs. (1) and (2). Significance of differences between a variant genotype and its default genotype was assessed using SPSS (version 25.0; SPSS Inc) based on the computed F values in each figure or table if applicable. Multiple linear regression analysis, where genetic backgrounds (GLXN, YD, WYJ, and ZF) were introduced as dummy variables, was conducted to identify which physiological variables (N_c versus K_N:K_L) was most important in determining A_c or CGR.

3. Results

3.1. Phenology, growth traits and canopy nitrogen content

The comparison of variant genotypes with their default (CK) genotypes showed that plant growth and development traits were more influenced by introducing yellow-leaf traits than introducing stay-green traits. The growth durations from sowing to maturity did not differ much between stay-green variants (G) and their CK genotypes in both experiments (Table 1). However, introducing yellow-leaf traits resulted in a completely different phenology, as the life cycle was prolonged in the yellow-leaf variant (Y) from the WYJ background whereas it was shortened in the Y genotype from the ZF background.

Fig. 1 presents time courses of above-ground dry weight and the daily average growth rate for the entire rice cycle in all genotypes. Overall, the patterns of the above-ground dry weight were similar in variants and their default genotypes, except for lower values in WYJ-Y

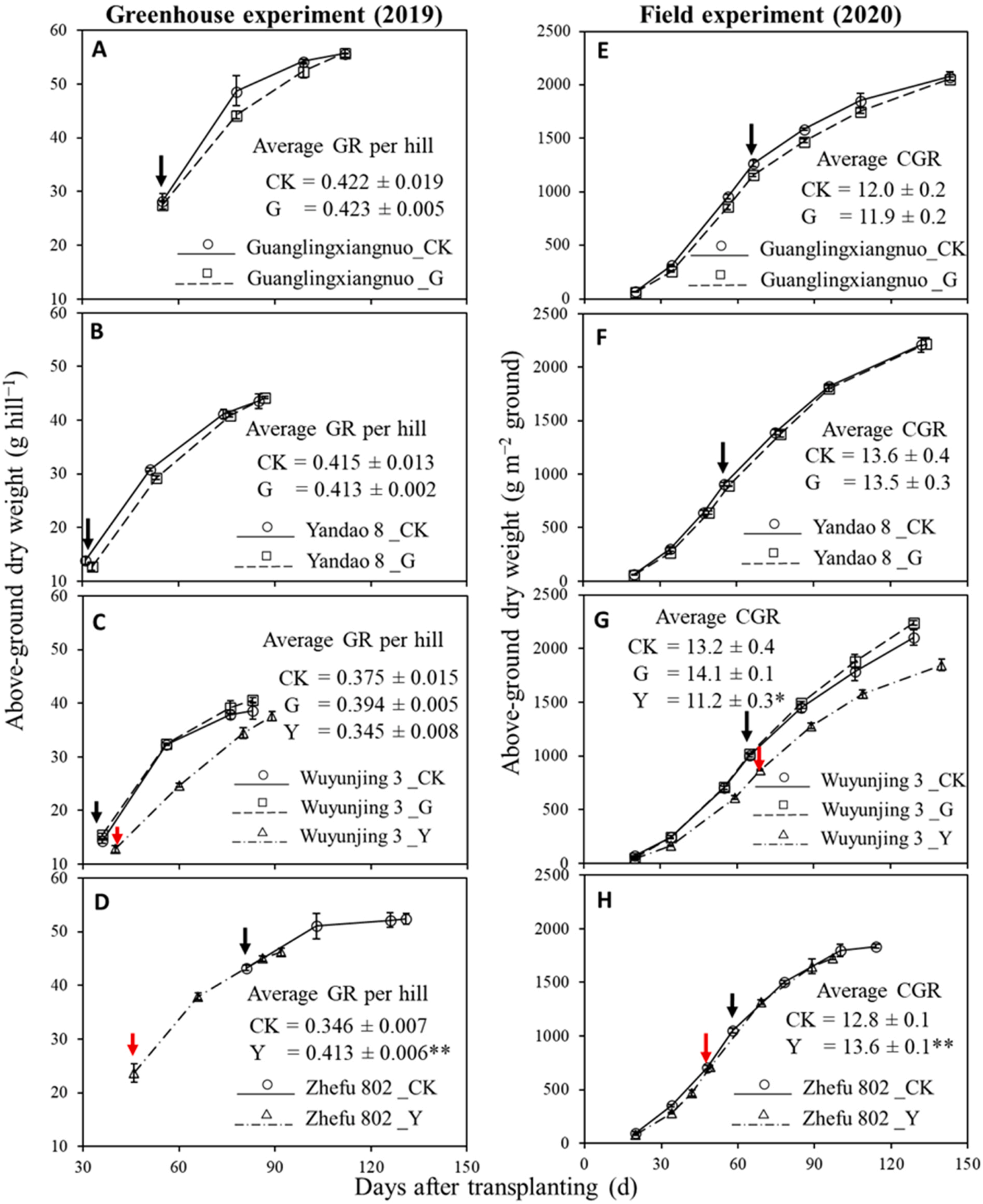

Fig. 1. The time course of rice above-ground dry weight in two experiments. Rice genotypes comprised four default rice genotypes (CK, circles) and their greener-leaf variants (G, squares) and/or yellower-leaf variants (Y, triangles). Each data point is shown as mean ± standard error of three to four replicates. Daily average growth rate per hill (GR per hill, g hill^{-1} d^{-1} in greenhouse experiment) and daily average crop growth rate (CGR, g m^{-2} ground d^{-1} in field experiment) for the entire life cycle were listed in each panel with a significant difference between variant genotype and its default genotype shown by asterisks: *$P < 0.05$, **$P < 0.01$. Black arrows indicate the heading dates of default genotypes and stay-green variants, and red arrows represent those of yellow-leaf variants.

compared with WYJ-CK. In general, the daily growth rate (i.e., the slope between two sampling dates) declined after heading in all genotypes. To avoid the possible confounding effect of the changed growth durations, we analysed the daily average growth rate of the entire growth duration. The daily growth rate was significantly affected only in Y-variants, with an increase for ZF-Y in both experiments and a decrease in WYJ-Y in the field experiment.

Tiller number and leaf area were influenced more by the Y modification than by the G modification (Figs. 2, 3). Across genetic backgrounds, tiller number was lower in Y variants than in their CK genotypes (Fig. 2). Data from the field experiment indicated a similar growth of LAI for genotypes from the same background at early

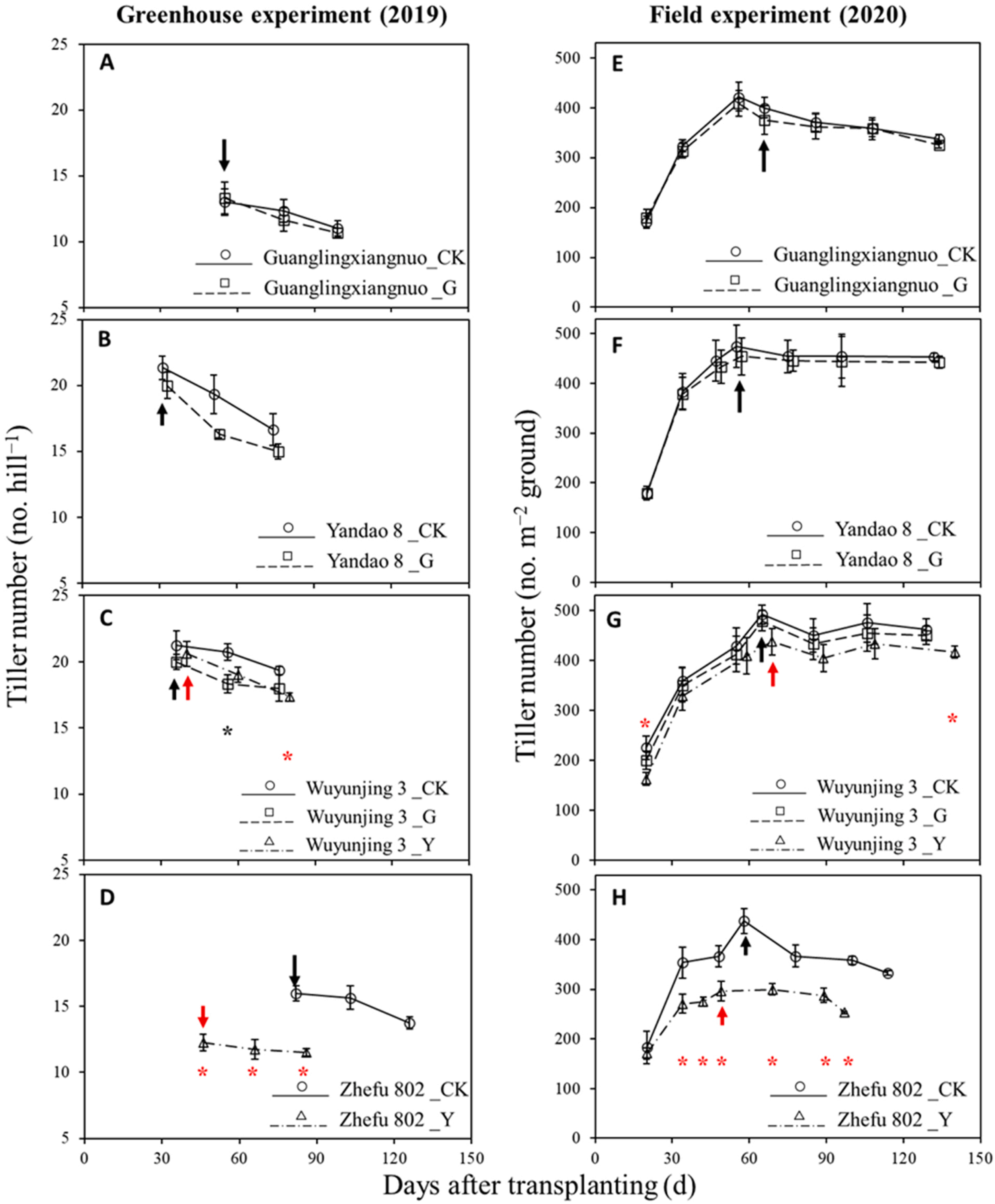

Fig. 2. The time course of tiller number of rice genotypes in two experiments. Genotypes comprised four default genotypes (CK, circles) and their greener-leaf variants (G, squares) and/or yellower-leaf variants (Y, triangles). Each data point is shown as mean ± standard error of three to four replicates. A significant difference ($P < 0.05$) between variant genotype and its default genotype at each sampling stage is shown by asterisks in different colours, with black and red for stay-green variants and yellow-leaf variants, respectively. Black arrows indicate the heading dates of default genotypes and stay-green variants, and red arrows represent those of yellow-leaf variants.

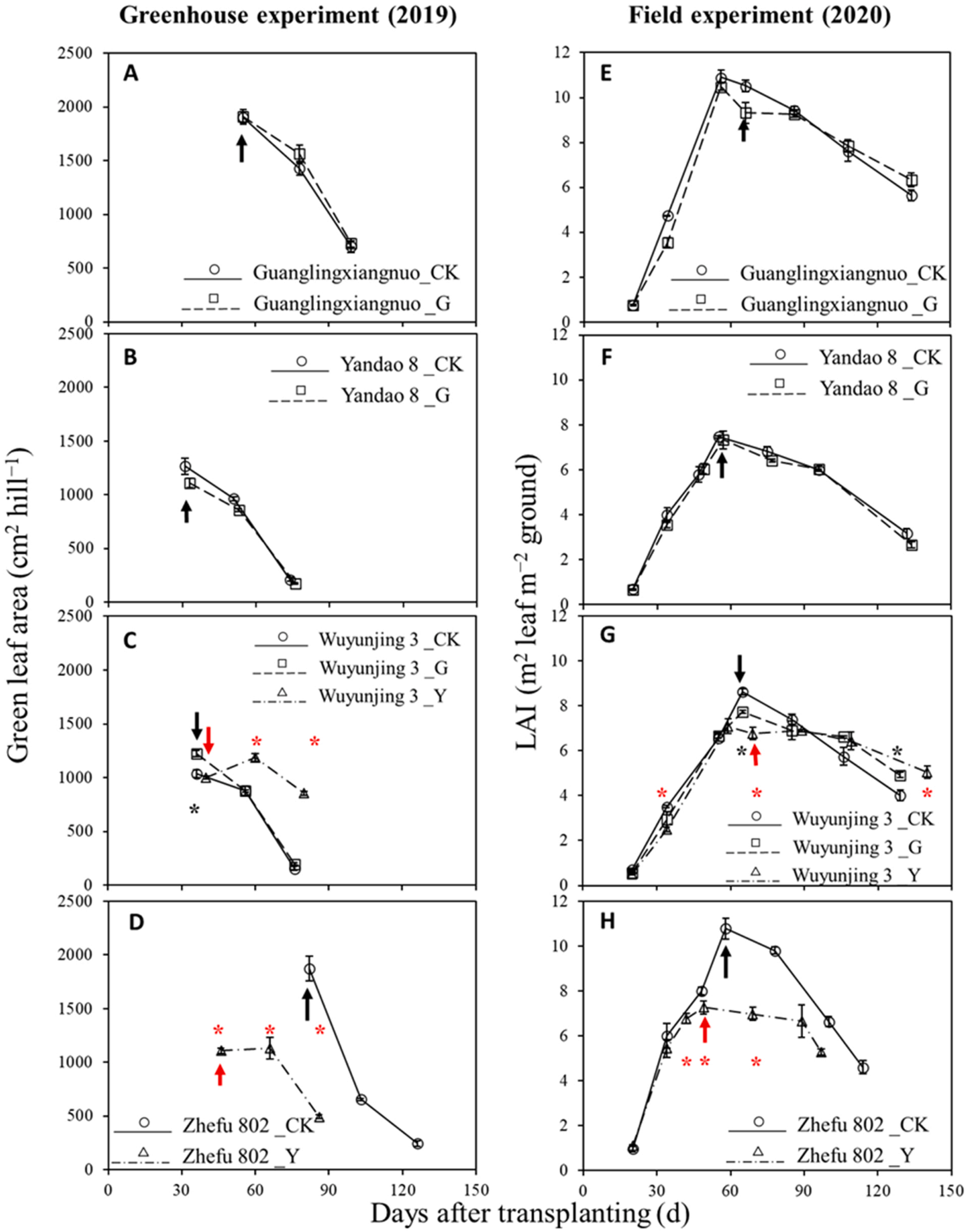

Fig. 3. The time course of leaf area in rice genotypes. Leaf area is shown in leaf area per hill for the greenhouse experiment (A–D) and in leaf area index (LAI) for the field experiment (E–H). Genotypes comprised four default rice genotypes (CK, circles) and their greener-leaf variants (G, squares) and/or yellower-leaf variants (Y, triangles). Each data point represents the mean ± standard error of three to four replicates. A significant difference ($P < 0.05$) between variant genotype and its default genotype at each sampling stage is shown by asterisks in different colours, with black and red for stay-green variants and yellow-leaf variants, respectively. Black arrows indicate the heading dates of default genotypes and stay-green variants, and red arrows represent those of yellow-leaf variants.

vegetative stage (Fig. 3). However, the peak of growth curves of LAI for Y variants was less sharp around flowering, followed by a slower decline when approaching maturity, compared with G variants and CK genotypes.

The pattern of total canopy nitrogen content in green leaves (N_c) in the field experiment for each genotype across stages (Fig. 4) was similar to that of LAI, fast increasing at early stages and then decreasing after peaking around the heading stage. Canopy-averaged nitrogen per leaf area (SLN_c) was higher in the variants, especially in Y variants during the reproductive phase (Fig. 4G–H). It is worth mentioning that ZF-Y began to manifest a higher SLN_c at vegetative growth stage (ca. 30 d after transplanting).

3.2. Contrasting impact of yellow-leaf modification on yield and yield-related traits

In both experiments, yield and yield-related traits did not significantly differ between G and their CK genotypes, except that the filled-grain percentage was significantly reduced in GLXN-G (Table 2). For Y variants, panicle number and filled-grain percentage were lower while number of spikelets per panicle was higher than for CK counterparts across experiments. Compared with its CK genotype, ZF-Y also showed significantly higher 1000-grain weight, daily grain-filling rate and harvest index, which contributed to a higher final yield, despite a lower total biomass. On the contrary, WYJ-Y exhibited lower daily grain-filling rate in both experiments, and a remarkably lower yield and total biomass gain in the field experiment.

3.3. Yellow-leaf variants had significantly higher K_N:K_L than default genotypes

Canopy extinction coefficients for light (K_L) and for nitrogen (K_N), and the specific leaf nitrogen at the top of the canopy (SLN_0) (Table 3) were derived from the vertical profile of relative values of photosynthetically active radiation and canopy leaf nitrogen, respectively (Fig. 5). Overall, G modification did not result in a significant change in light and nitrogen profiles in comparison with the CK genotypes, although the K_N:K_L ratio was slightly higher in G than in CK genotypes when approaching maturity. For Y variants, more light penetrated into the lower layers of the canopy, as reflected by an average of 33 % and 42 % lower K_L for Y variants than for the CK genotypes from WYJ and ZF background, respectively, across stages. With the aging of canopy leaves, K_L increased and the rates of increase in Y variants were faster than those in their CK counterparts towards the early grain-filling stage. SLN_0 and K_N did not significantly differ between Y and CK genotypes for the WYJ background ($P > 0.05$), except for a slightly lower value of SLN_0 in the Y variant at the stem-elongating stage (Table 3). However, higher values of SLN_0 (20 % increase) and K_N (25 % increase) were observed for the ZF-Y genotype than its CK genotype, across three stages (Fig. 5). Across stages, both Y variants had a significantly higher K_N:K_L ratio than their CK counterparts (Table 3; Fig. S4).

To check the reliability of our estimates for canopy light and N profiles, we used the unpublished data collected from a pre-experiment using only ZF genotypes conducted under field conditions in 2016, in which we measured their light profile and N profile at the flowering stage. Parameters characterising the profiles, K_L and K_N, had very similar values (Fig. S5) to those we obtained from the 2020 full experiment for the two genotypes (Table 3).

3.4. Differences in leaf and canopy photosynthesis among yellow-leaf variants

Light saturated net flag-leaf photosynthesis (A_{max}) and net canopy photosynthesis (A_c) were examined at flowering stage in the field experiment (Fig. 6). In general, variation in A_c across genotypes was significantly associated with variation in A_{max} ($R^2 = 0.68$; Fig. 7A), while A_{max} was highly correlated with its leaf nitrogen content ($R^2 = 0.60$; Fig. 7B). The photosynthetic performance at both leaf and canopy level were not changed much by G modification. However, for Y modification, opposite trends were found relative to their CK from different backgrounds: the modification caused ca. 12 % decreases in both A_{max} and A_c in the WYJ background, while it caused ca. 32 % and 38 % increases in the ZF background, although leaf N content and canopy-averaged N content were increased in both Y variants. This difference in the effect of Y-modification between the two backgrounds is also directly reflected in Fig. 7A, where A_c is plotted against A_{max}. Because of this difference, the photosynthetic nitrogen use efficiencies (PNUE) at the leaf and canopy level were both significantly increased in ZF-Y, while the leaf PNUE was significantly decreased in WYJ-Y (Fig. 6C, F).

3.5. K_N:K_L ratio as a main determinant of canopy photosynthesis and daily growth rate

We calculated daily CGR around flowering from measured biomass at two stages, i.e., heading and 20 d after heading. Across genotypes, CGR around flowering was positively correlated with A_c (measured at flowering) ($R^2 = 0.77$; Fig. 8). Theoretically, canopy productivity depends on: (i) overall canopy photosynthetic potential, represented here by N_c (which is the product of SLN_c and LAI), and (ii) the distribution of photosynthetic resources (nitrogen) in the canopy, represented by the K_N:K_L ratio (Yin and Struik, 2015). Our data showed that A_c or CGR was highly associated with the K_N:K_L ratio, although data points representing WYJ-Y clearly deviated from the linear relationships (Fig. 9). To examine which factor (i.e., N_c or K_N:K_L) contributed most to the variations in A_c and CGR (thus biomass gain) and whether the effect was caused by the genetic background, we conducted a multiple linear regression, where A_c or CGR was regressed against N_c and K_N:K_L with genetic backgrounds (GLXN, YD, WYJ-CK&G, WYJ-Y, ZF) introduced as dummy variables (Table 4). The genotypes in the WYJ background were divided into two groups (WYJ-CK&G and WYJ-Y) to account for the earlier indicated negative effects of the Y modification on A_c or CGR in this WYJ background. Regression analysis showed that the variation in the K_N:K_L ratio contributed most to the variations in both A_c and CGR among all genotypes ($P < 0.001$), while N_c exerted some positive effect but was not the determining factor accounting for the variance in A_c and CGR in our study ($P > 0.05$). Nevertheless, the analysis also showed that CGR differed significantly among genetic backgrounds, with CGR of YD, WYJ-CK&G and ZF being higher, and that of WYJ-Y being lower, than that of GLXN. Similar trends in genetic differences were found for A_c.

4. Discussion

4.1. Variation in leaf photosynthesis as a result of leaf-colour modification affects canopy photosynthesis

Of the two kinds of leaf-colour modification, stay-green (G) modification had less influence on leaf photosynthesis than yellow-leaf (Y) modification (Fig. 6). In general, light-saturated leaf photosynthesis (A_{max}) is closely related to leaf nitrogen content (Hikosaka, 2010), in line with our result in Fig. 7B. To be more specific, A_{max} is related to the nitrogen invested in various photosynthetic enzymes that determine the photosynthetic capacity (Harley et al., 1992; Makino et al., 1994; Gu et al., 2012). Leaf-colour modification, as an artificial means to affect photosynthetic nitrogen distribution within the leaf, particularly alters the nitrogen investment in chlorophyll content (Fig. S1). This change in leaf chlorophyll content influences the photosynthetic competence by adjusting the light absorption (Evans, 1996). In this study, we examined the variation in leaf photosynthetic nitrogen use efficiency (PNUE), presented as A_{max} per unit nitrogen content (see Eq. (3)), which resulted from the changes in the photosynthetic nitrogen content and photosynthetic nitrogen distribution within the leaf. For the two Y variants, an

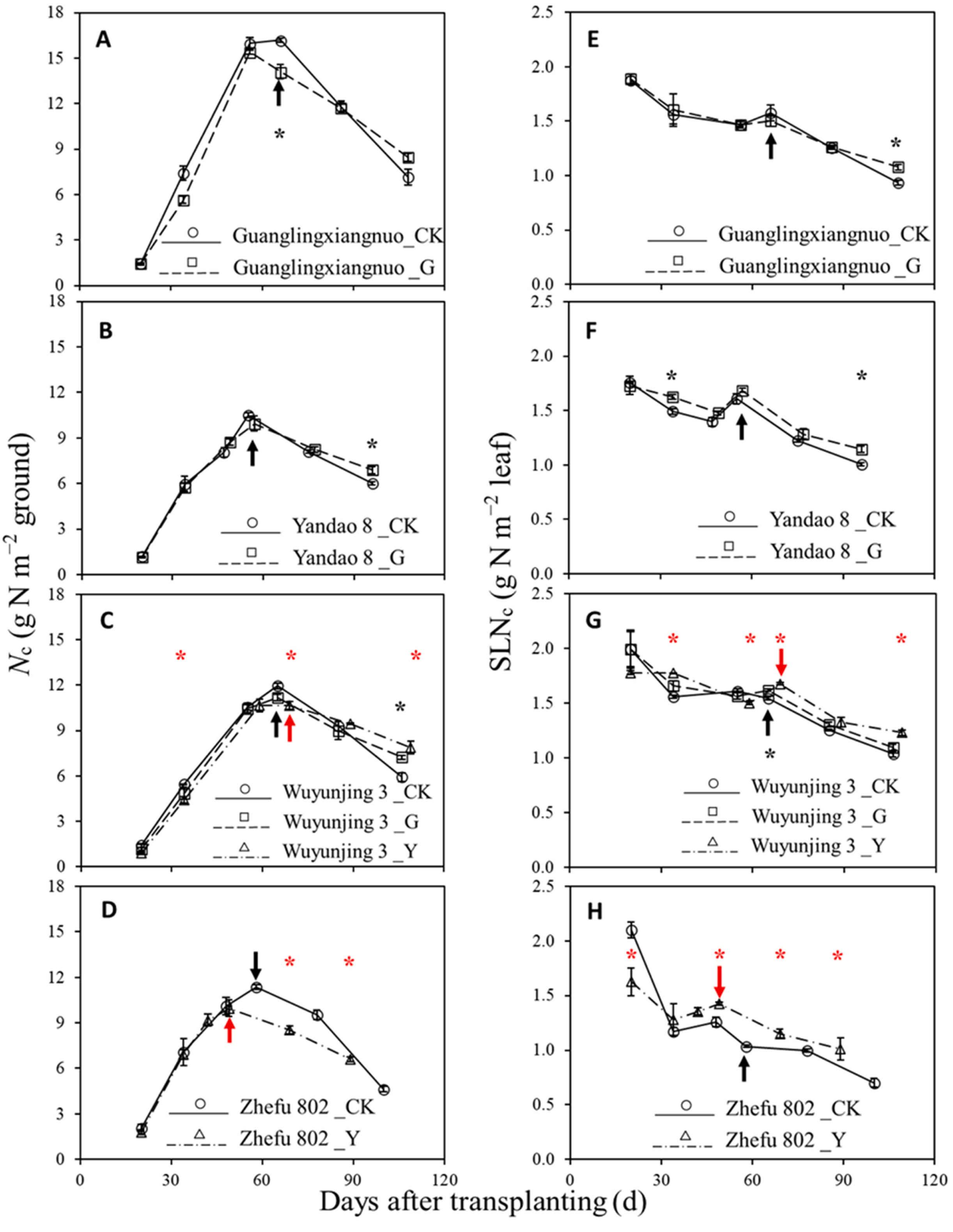

Fig. 4. The time course of crop nitrogen status in the field experiment. Canopy total nitrogen content (N_c, A-D) and canopy-averaged specific leaf nitrogen (SLN$_c$, E-H) of green leaves are shown for four default rice genotypes (CK, circles) and their greener-leaf variants (G, squares) and/or yellower-leaf variants (Y, triangles). Each data point represents the mean ± standard error of three to four replicates. A significant difference ($P < 0.05$) between variant genotype and its default genotype at each sampling stage is shown by asterisks in different colours, with black and red for stay-green variants and yellow-leaf variants, respectively. Black arrows indicate the heading dates of default genotypes and stay-green variants, and red arrows represent those of yellow-leaf variants.

Table 2
Yield and yield related traits (mean with its standard error of three or four replicates in brackets) for rice genotypes of four genetic backgrounds, each including a default genotype (CK) and its greener-leaf variant (G) and/or yellower-leaf variant (Y), in two experiments.

Experiment	Background	Genotype	Yield components				Grain yield	Grain-filling rate	Total biomass	Harvest index
			Panicle number	Spikelets per panicle	Filled-grain	1000-grain weight				
Greenhouse			(no. hill^{-1})	(no.)	(%)	(g)	(g hill^{-1})	(g hill^{-1} d^{-1})	(g hill^{-1})	(g g^{-1})
Experiment	GLXN	CK	10.3 (0.3)	93 (2)	98.2 (0.2)	27.8 (0.8)	26.1 (0.4)	0.450 (0.007)	60.0 (0.8)	0.374 (0.008)
(2019)		G	10.0 (0.4)	98 (4)	**97.3 (0.2)***	28.1 (0.2)	26.8 (0.7)	0.462 (0.012)	60.4 (1.0)	0.382 (0.010)
	YD	CK	15.7 (0.7)	67 (3)	94.1 (2.1)	28.7 (0.1)	28.0 (0.4)	0.510 (0.008)	44.4 (1.3)	0.542 (0.006)
		G	14.7 (0.3)	73 (2)	91.9 (1.8)	28.0 (0.3)	27.5 (0.5)	0.500 (0.010)	43.0 (0.2)	0.559 (0.003)
	WYJ	CK	17.3 (0.3)	53 (1)	90.0 (0.5)	29.9 (0.5)	24.5 (0.4)	0.510 (0.009)	40.4 (1.5)	0.521 (0.001)
		G	17.0 (0.6)	54 (1)	91.8 (0.8)	30.2 (0.1)	25.4 (0.5)	0.529 (0.010)	41.6 (0.5)	0.524 (0.003)
		Y	**15.7 (0.3)***	**58 (1)****	**87.9 (0.1)***	29.1 (0.5)	23.4 (0.6)	**0.468 (0.011)***	39.5 (0.9)	0.509 (0.009)
	ZF	CK	14.3 (0.7)	92 (4)	97.6 (0.1)	24.7 (0.2)	31.2 (0.3)	0.612 (0.005)	59.3 (1.1)	0.453 (0.007)
		Y	**11.3 (0.7)***	**139 (2)*****	**80.6 (3.4)** **	**25.6 (0.2)** *	32.5 (1.2)	**0.690 (0.025)***	**52.2 (0.5)** **	**0.534 (0.016)** **
Field			(no. m^{-2} ground)	(no.)	(%)	(g)	(g m^{-2})	(g m^{-2} d^{-1})	(g m^{-2})	(g g^{-1})
Experiment	GLXN	CK	338 (6)	107 (3)	87.4 (0.7)	27.1 (0.2)	823 (11)	10.7 (0.14)	2082 (37)	0.339 (0.004)
(2020)		G	325 (6)	113 (2)	**81.2 (0.7)** **	26.9 (0.3)	800 (22)	10.4 (0.29)	2056 (40)	0.336 (0.006)
	YD	CK	453 (2)	126 (3)	69.2 (1.2)	27.5 (0.1)	1076 (11)	14.0 (0.14)	2208 (67)	0.422 (0.011)
		G	443 (4)	127 (2)	74.3 (1.7)	27.4 (0.6)	1128 (32)	14.6 (0.42)	2222 (51)	0.441 (0.002)
	WYJ	CK	462 (11)	94 (2)	87.6 (2.2)	29.2 (0.2)	1055 (6)	16.5 (0.10)	2104 (71)	0.446 (0.007)
		G	450 (8)	100 (1)	86.9 (0.6)	30.1 (0.2)	1117 (26)	17.5 (0.40)	2237 (22)	0.450 (0.005)
		Y	**417 (4)***	**102 (0)***	**72.8 (2.0)** **	28.7 (0.4)	**857 (27)** **	**12.1 (0.38)*****	**1846 (55)***	0.429 (0.012)
	ZF	CK	373 (4)	110 (1)	74.1 (2.1)	24.0 (0.3)	704 (27)	12.6 (0.48)	1836 (20)	0.342 (0.013)
		Y	**293 (3)****	**176 (1)*****	**64.1 (1.4)***	**25.3 (0.2)** *	**795 (7)***	**16.2 (0.14)****	**1686 (16)** **	**0.417 (0.007)** **

Data of a variant genotype significantly different from those of its default genotype (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) are shown in **bold**.

Table 3
Values (mean with its standard error in brackets) of leaf area index (LAI), and canopy light and nitrogen distribution parameters for rice genotypes of four genetic backgrounds, each including a default genotype (CK) and its greener-leaf variants (G) and/or yellower-leaf variants (Y), measured at three stages in the field experiment.

Stage	Background	Genotype	LAI (m^2 leaf m^{-2} ground)	K_L (m^2 ground m^{-2} leaf)	SLN$_0$ (g N m^{-2} leaf)	K_N (m^2 ground m^{-2} leaf)	K_N/K_L (–)
Stem elongating stage	GLXN	CK	10.92 (0.29)	0.384 (0.046)	1.69 (0.03)	0.026 (0.004)	0.067 (0.004)
		G	10.50 (0.17)	0.417 (0.053)	1.64 (0.01)	0.020 (0.002)	**0.048 (0.004)***
	YD	CK	5.77 (0.35)	0.431 (0.004)	1.70 (0.03)	0.065 (0.001)	0.152 (0.004)
		G	6.04 (0.21)	0.459 (0.022)	1.79 (0.01)	0.060 (0.006)	0.132 (0.016)
	WYJ	CK	6.57 (0.16)	0.382 (0.023)	1.85 (0.01)	0.041 (0.003)	0.108 (0.008)
		G	6.68 (0.12)	0.403 (0.027)	1.82 (0.04)	0.044 (0.002)	0.109 (0.004)
		Y	7.09 (0.31)	**0.291 (0.001)***	**1.76 (0.02)***	0.042 (0.003)	**0.150 (0.013)***
	ZF	CK	7.21 (0.05)	0.517 (0.086)	1.77 (0.03)	0.099 (0.003)	0.202 (0.033)
		Y	6.78 (0.23)	**0.246 (0.002)***	**1.99 (0.03)****	0.113 (0.009)	**0.460 (0.035)****
Flowering stage	GLXN	CK	10.53 (0.26)	0.460 (0.028)	1.85 (0.03)	0.033 (0.003)	0.073 (0.011)
		G	**9.32 (0.22)***	0.446 (0.050)	1.77 (0.01)	0.036 (0.007)	0.080 (0.006)
	YD	CK	7.46 (0.05)	0.462 (0.041)	2.13 (0.04)	0.104 (0.012)	0.225 (0.007)
		G	7.33 (0.40)	0.448 (0.040)	2.17 (0.04)	0.098 (0.006)	0.223 (0.027)
	WYJ	CK	8.62 (0.13)	0.451 (0.001)	1.97 (0.04)	0.081 (0.001)	0.180 (0.002)
		G	**7.72 (0.07)****	0.447 (0.034)	2.03 (0.02)	0.084 (0.004)	0.189 (0.012)
		Y	**6.77 (0.27)****	**0.237 (0.016)*****	2.07 (0.05)	0.079 (0.004)	**0.334 (0.028)****
	ZF	CK	10.79 (0.47)	0.696 (0.062)	1.67 (0.07)	0.109 (0.002)	0.160 (0.018)
		Y	**7.28 (0.30)*****	**0.378 (0.017)****	**2.14 (0.06)****	**0.133 (0.005)***	**0.351 (0.005)*****
20 d after flowering stage	GLXN	CK	9.44 (0.18)	0.529 (0.025)	1.54 (0.02)	0.044 (0.003)	0.083 (0.009)
		G	9.25 (0.14)	0.544 (0.018)	1.57 (0.03)	0.047 (0.001)	0.086 (0.002)
	YD	CK	6.82 (0.20)	0.738 (0.023)	1.72 (0.03)	0.113 (0.005)	0.153 (0.007)
		G	6.41 (0.08)	0.717 (0.003)	1.68 (0.02)	0.118 (0.002)	0.164 (0.004)
	WYJ	CK	7.40 (0.23)	0.627 (0.035)	1.69 (0.02)	0.084 (0.003)	0.134 (0.002)
		G	6.90 (0.42)	0.616 (0.024)	1.73 (0.01)	0.087 (0.009)	0.141 (0.009)
		Y	6.90 (0.05)	**0.458 (0.031)***	1.70 (0.04)	0.089 (0.008)	**0.198 (0.026)***
	ZF	CK	9.82 (0.15)	0.823 (0.046)	1.41 (0.02)	0.084 (0.001)	0.103 (0.005)
		Y	**6.98 (0.29)*****	**0.591 (0.021)***	**1.69 (0.08)***	**0.116 (0.008)***	**0.197 (0.010)*****

LAI, Leaf area index; KL, canopy light extinction coefficient; SLN0, specific leaf nitrogen at the top of the canopy; KN, canopy nitrogen extinction coefficient.
Data of a variant genotype significantly different from those of its default genotype (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) are shown in **bold**.

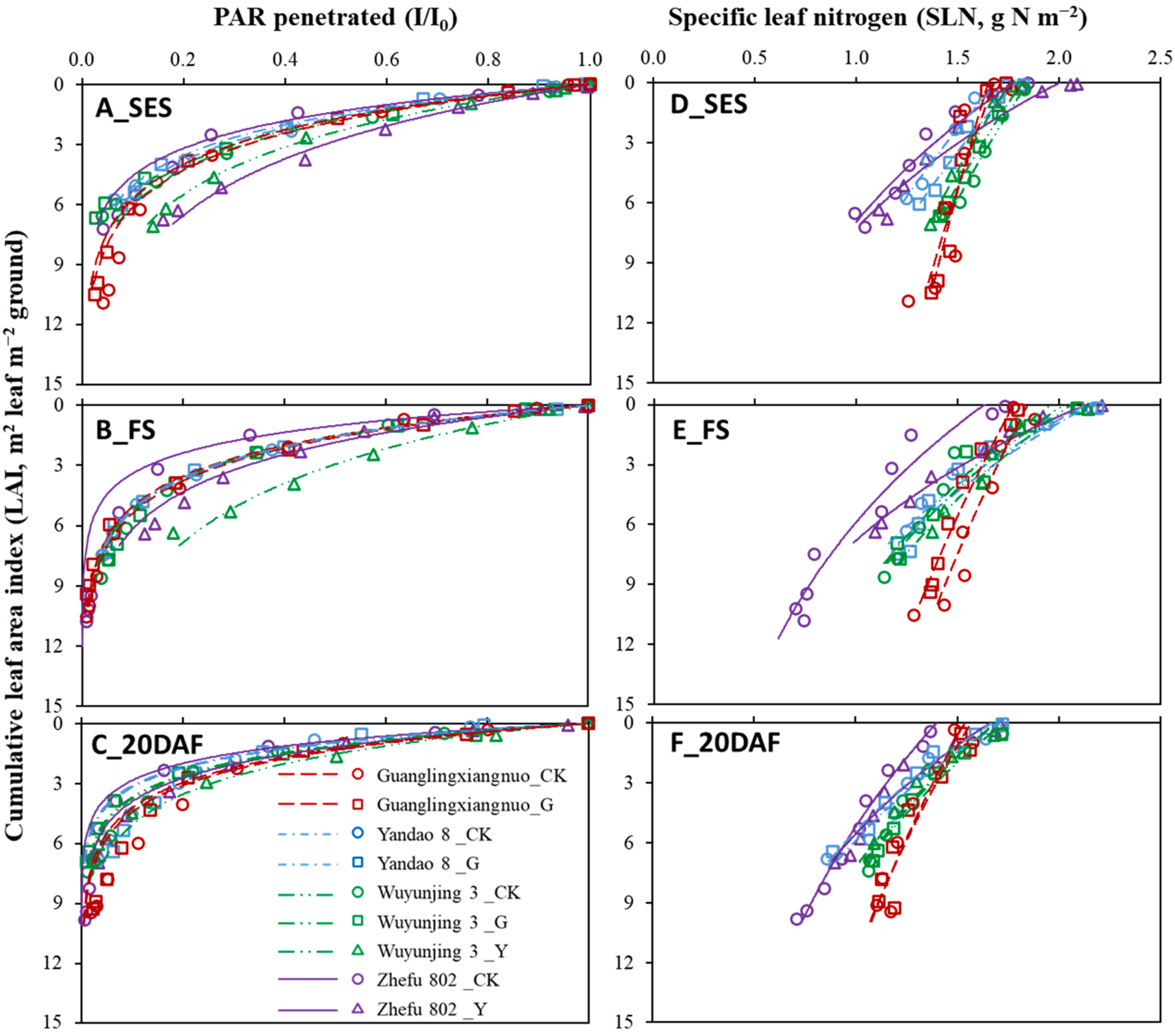

Fig. 5. Vertical light and nitrogen profile in canopies of rice genotypes in the field experiment. The canopy relative photosynthetically active radiation profile (PAR, A–C) and canopy leaf nitrogen profile (SLN, D–F) are plotted against cumulative leaf area index (LAI) counted from the top of the canopy for four default rice genotypes (CK, circles) and their greener-leaf variants (G, squares) and/or yellower-leaf variants (Y, triangles) at three stages: SES, stem-elongating stage (A and D); FS, flowering stage (B and E); 20DAF, 20 d after flowering stage (C and F). Each data point represents the mean of three to four replicates. Curves for light profiles are drawn from Eq. (1) with parameter K_L fitted, while curves for nitrogen profiles are drawn from Eq. (2) with parameters SLN_0 and K_N fitted for each genotype. Cv. Guanglingxiangnuo is in red; cv. Yandao 8 is in blue; cv. Wuyunjing 3 is in green and cv. Zhefu 802 is in violet.

opposite effect on A_{max} and PNUE for the flag leaf was observed (decrease in WYJ-Y and increase in ZF-Y), suggesting discrepant effects of Y modification on the leaf nitrogen partitioning.

It was reported that exploiting the genetic variation in A_{max} can contribute to improving canopy photosynthesis (A_c) when other factors are constant (Teng et al., 2004; Gu et al., 2014). This is understandable since A_c is the spatial integration of leaf photosynthesis over canopy layers. Our results showed that leaf-colour modification similarly had an implication on canopy photosynthesis. In this case, the significantly different effects of Y modification on A_{max} were carried over to the canopy scale, as reflected by a good relationship among genotypes between A_{max} and A_c ($R^2 = 0.68$; Fig. 7A). However, the dependence of A_c on A_{max} appeared to be genetic background specific, on the basis of the understanding that A_c also depends on the relative nitrogen to light profiles in the canopy as discussed below.

4.2. Importance of the closeness between canopy nitrogen and light profiles in determining canopy photosynthesis

When upscaling photosynthesis from leaf to canopy level, factors like the extent to which photosynthetic N resources acclimate to light also matter (Yin and Struik, 2015). According to an optimisation theory, A_c can be maximised when the leaf nitrogen distribution within the canopy is acclimated to the light distribution (i.e., K_N:$K_L = 1$) if A_{max} is linearly related to SLN (Field, 1983; Hirose and Werger, 1987; Goudriaan, 1995; Sands, 1995). For an actual canopy, nitrogen distribution is more uniform than light distribution, and the gradients of both light and nitrogen are highly affected by genotypic variation (Gu et al., 2017a; Ouyang et al., 2021). This was consistent with our results in that a clear difference was observed among different genetic backgrounds (Fig. 5). For example, compared with genotypes from other backgrounds, the ones from the GLXN background distributed more nitrogen resource to the lower layers of canopy (smaller K_N). Our results also showed an extremely steeper light gradient than the nitrogen gradient in a canopy. This gave rise to a severe mismatch in the distribution of light and nitrogen resources in the canopy, as indicated by a K_N:K_L ratio even below 0.1 for the GLXN background (Table 3). Overall, the observed K_N:K_L ratio, either across genotypes or across stages for our default genotypes, was not even close to the theoretical optimum (Table 3). Such low K_N:K_L ratios mainly resulted from the very low K_N. The low K_N:K_L ratios may

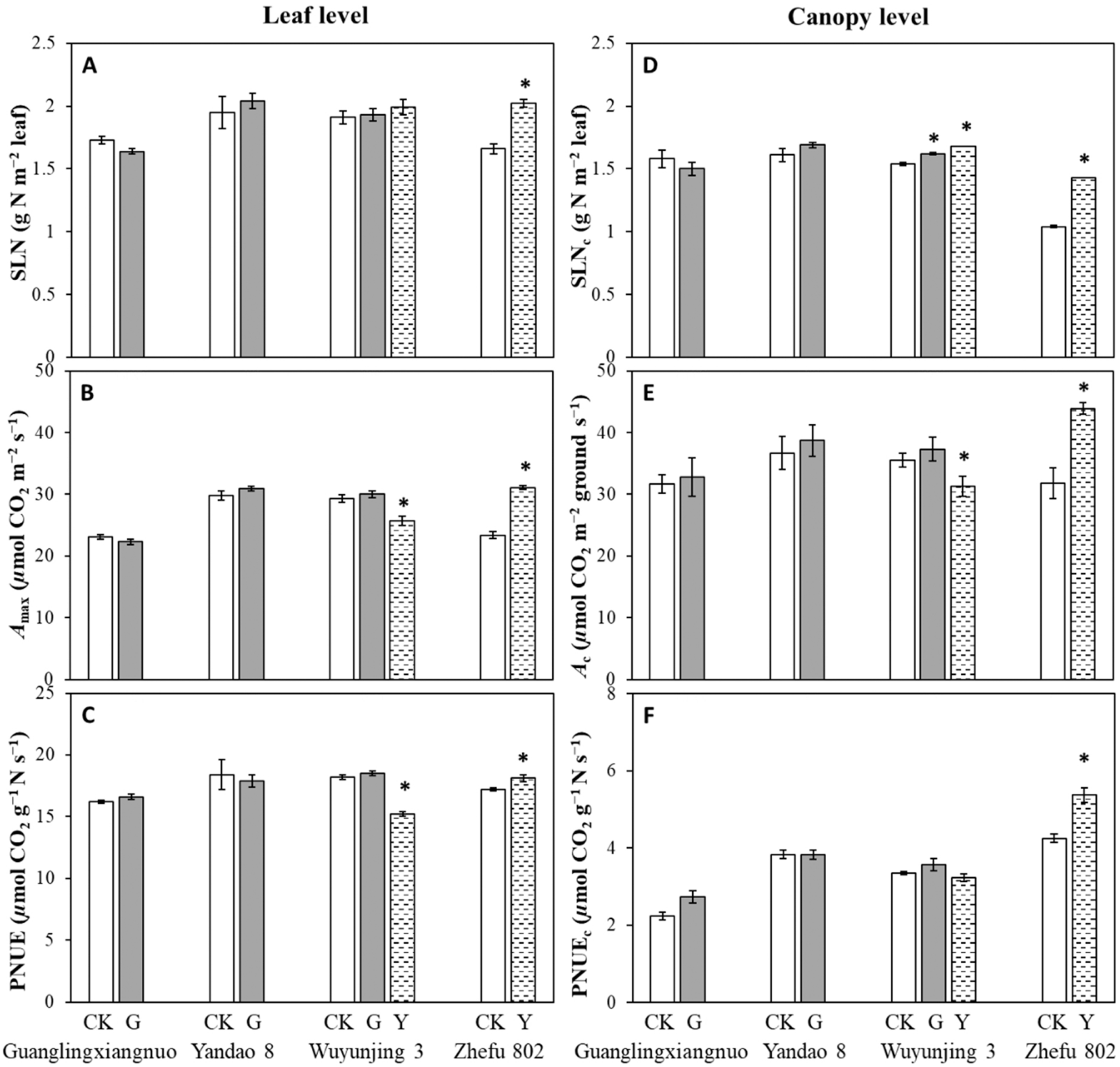

Fig. 6. Photosynthetic characteristics of rice genotypes at the flowering stage in the field experiment. Both leaf (A–C) and canopy (D–F) photosynthetic parameters for four default rice genotypes (CK, white bars) and their greener-leaf variants (G, grey bars) and/or yellower-leaf variants (Y, dashed bars) are shown. Specific leaf nitrogen (SLN, A), light saturated leaf photosynthesis (A_{max}, B), leaf photosynthetic nitrogen-use efficiency (PNUE, C), canopy-averaged specific leaf nitrogen (SLN$_c$, D), canopy photosynthesis (A_c, E), and canopy photosynthetic nitrogen-use efficiency (PNUE$_c$, F) represent means ± standard errors of three or four replicates. PNUE and PNUE$_c$ were calculated based on Eq. (3) and Eq. (4), respectively. The asterisks (*) indicate statistical significance at the $P < 0.05$ level between variant genotype and its default genotype.

reflect the possibilities that: (i) K_L varies diurnally and seasonally whereas the acclimation of nitrogen in the canopy always lags behind; (ii) A_{max} responds to leaf nitrogen content nonlinearly, in a diminishing-return manner as shown by Evans (1989); and (iii) the distribution of nitrogen in canopies is only partially driven by light distribution.

Lowering the canopy chlorophyll content proposed to improve light penetration to lower leaves has an implication on canopy photosynthesis as such manipulation decreases K_L, thereby increasing the K_N:K_L ratio. Our results with Y variants (especially with the ZF-Y genotype) confirmed this, as evidenced by higher K_N:K_L ratios in Y variants (especially in the ZF-Y genotype) (Table 3; Fig. S4). Compared with its CK genotype, ZF-Y exhibited a phenotypic plasticity in nitrogen adaptation to the light environment with a steeper nitrogen gradient along the canopy (i.e., higher SLN$_0$ and K_N) (Fig. 5 and S5). This adaptive change in ZF-Y may partly result from its low tillering ability and LAI (Figs. 2 and 3), which overcame the weakness as occurred in its CK genotype where high LAI diluted leaf nitrogen content after the stem-elongating stage (Fig. S6). This result supports the findings of Moreau et al. (2012) that acclimation of the leaf nitrogen profile to the light profile is probably a whole-plant process as it was related to leaf canopy size. The resulting increase of K_N in ZF-Y, representing a higher efficiency in nitrogen use at the whole plant canopy, led to a further enhancement in A_c. This is supported by the multiple regression analysis showing that an increased K_N:K_L ratio was most significant parameter in improving A_c (Table 4). However, this effect was observed to a much less extent in the Y genotype from the WYJ background (Fig. 9). Therefore, reducing leaf and canopy chlorophyll alone (i.e., decline in K_L) may not necessarily contribute to A_c, unless a corresponding improvement in nitrogen partitioning is achieved at canopy level.

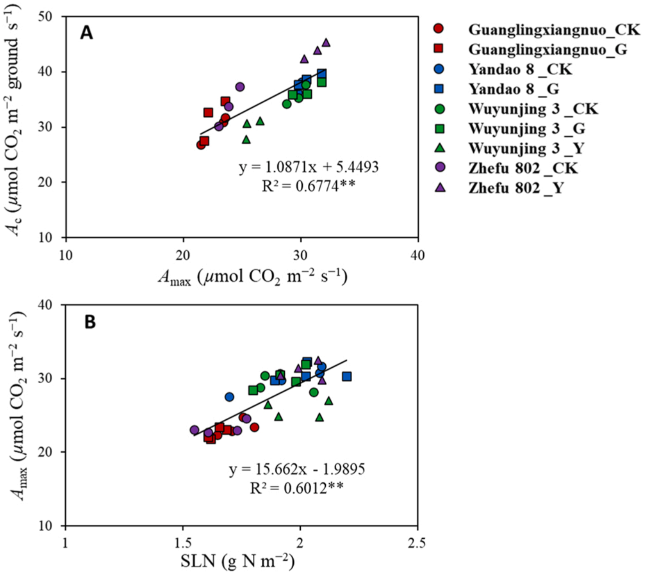

Fig. 7. Relations of photosynthetic parameters of rice genotypes in the field experiment. A: Relationship between canopy photosynthesis (A_c) and light saturated leaf photosynthesis (A_{max}) at the flowering stage. B: Relationship between A_{max} and specific leaf nitrogen (SLN). Linear regressions were fitted for all genotypes with three or four replicates. The significance of each correlation is shown by asterisks: $^{**}P < 0.01$. Cv. Guanglingxiangnuo is in red; cv. Yandao 8 is in blue; cv. Wuyunjing 3 is in green and cv. Zhefu 802 is in violet. CK in circles represent default rice genotypes; G in squares represent greener-leaf variants and Y in triangles represent yellower-leaf variants.

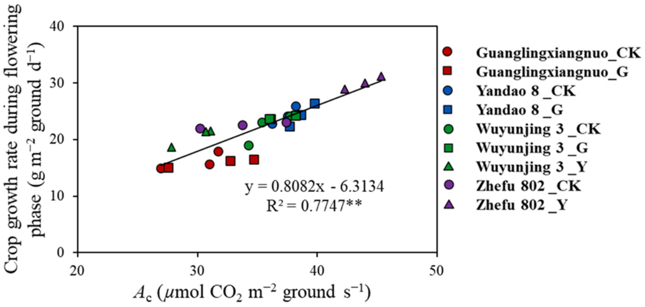

Fig. 8. Relationship between daily crop growth rate during flowering phase and canopy photosynthetic rate at the flowering stage (A_c). The daily average crop growth rate during flowering phase was calculated from dry weights obtained from sampling intervals between heading and 20 d after heading stages. Linear regressions were fitted for all genotypes, based on data of three individual replicates. The significance of correlation is shown by asterisks: $^{**}P < 0.01$. Cv. Guanglingxiangnuo is in red; cv. Yandao 8 is in blue; cv. Wuyunjing 3 is in green and cv. Zhefu 802 is in violet. CK in circles represent default rice genotypes; G in squares represent greener-leaf variants and Y in triangles represent yellower-leaf variants.

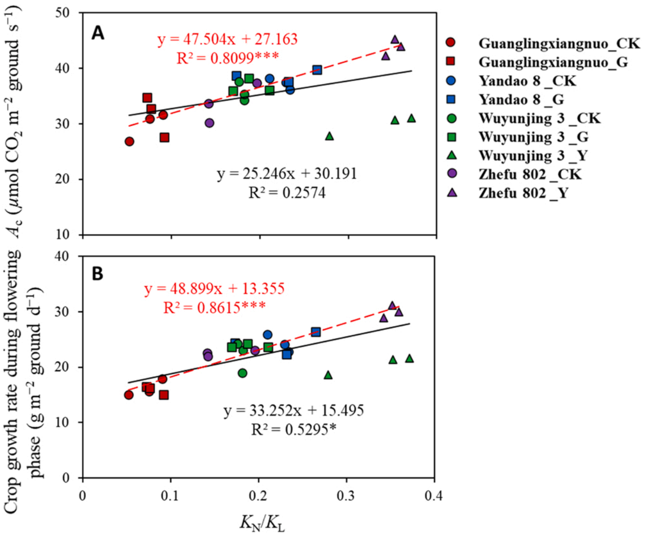

Fig. 9. Canopy photosynthesis or crop growth rate in relation to the canopy nitrogen to light extinction coefficient ratio (K_N/K_L). Canopy photosynthesis at flowering stage (A_c) is shown in Panel A, while daily average crop growth rate during flowering phase (shown in Panel B) was calculated from sampling intervals between heading and 20 d after heading stages in the field experiment. Linear regressions were fitted to pooled data of three replicates for all genotypes (black and full line). The red and dotted line represents the regression excluding the yellow-leaf variant from Wuyunjing background (see the text). The equation colours match the line colours. The significance of each correlation is shown by asterisks: $^{*}P < 0.05$, $^{***}P < 0.001$. Cv. Guanglingxiangnuo is in red; cv. Yandao 8 is in blue; cv. Wuyunjing 3 is in green and cv. Zhefu 802 is in violet. CK in circles represent default rice genotypes; G in squares represent greener-leaf variants and Y in triangles represent yellower-leaf variants.

4.3. Opportunities for improving dry matter and yield by leaf-colour modification

The influence of leaf-colour modification on A_c eventually contributed to the dry matter accumulation, which was indicated by the high association between A_c and daily average CGR ($R^2 = 0.77$; Fig. 8). Leaf-colour modification is expected to improve dry matter accumulation either by extending the canopy duration (G traits) (Gregersen et al., 2013; Borrell et al., 2014) or by allowing more light penetrated into the lower canopy (Y traits) (Ort et al., 2011; Gu et al., 2017b). Given that reducing canopy chlorophyll alone may not necessarily resulted in an improved A_c, opportunities may exist by an optimisation of the relevant photosynthetic traits. For example, our results from the multiple regression analysis (Table 4) suggested that A_c in the WYJ-Y genotype would have increased by some 13.47 (= 12.7 + 0.77) μmol CO$_2$ m^{-2} ground s^{-1} if it had improved properties at leaf (higher A_{max} and PNUE) as well as canopy (e.g. K_N:K_L ratio) scales as the ZF-Y genotype. At this stage, we are not sure whether the ZF-Y genotype represents an absolutely optimised state, and it is highly likely that there is a room for the ZF-Y genotype to be further optimised for its photosynthetic characteristics.

Our study showed that leaf modification not only affected leaf and canopy photosynthesis as discussed above, but also pleiotropically impacted other growth and developmental traits. In general, the impact of Y modification across the whole growth duration was more comprehensive than that of G modification as it affected other development or growth traits to a larger extent (Table 1). Opposite effect of Y variants on growth duration observed in WYJ vs ZF backgrounds (Table 1) indicated that the plant's developmental sensitivity to photoperiod or to temperature may also have been changed differently, as photoperiod and temperature are major environmental factors affecting rice phenology (Vergara et al., 1966; Yin et al., 1997; Saito et al., 2009). The ZF-Y genotype showed fewer days to heading, indicating higher proportion of younger leaves, which could be beneficial for A_c and PNUE$_c$. On the other hand, less advantage of Y genotypes than their default rice genotype or G variants as a result of lower ability of light absorptance by leaf (Evans, 1996; Melis, 2009), may be responsible for the lower CGR of both Y variants before the establishment of a complete canopy (Fig. 1). In addition, the obvious decrease in number of tillers (Fig. 2) could also have contributed to the low CGR in Y variants, as the CGR can be significantly affected by tillers number (Hossain et al., 2011). Fewer tillers lead to lower leaf area index (LAI) (Zhong et al., 2002, 2003), and our data also showed that Y variants had a lower LAI although they reached their maximum LAI earlier (Fig. 3). Hence, one option to improve dry matter accumulation after Y modifications could be via

Table 4

Multiple regression analysis of canopy photosynthesis at flowering stage (A_c, μmol CO_2 m^{-2} ground s^{-1}) or daily crop growth rate (CGR, g m^{-2} ground d^{-1}) during flowering phase calculated from sampling intervals between heading and 20 d after heading stages as a function of genetic backgrounds (GLXN, YD, WYJ-CK&G, WYJ-Y, ZF), total canopy nitrogen content in green leaves (N_c, g N m^{-2} leaf), and K_N:K_L ratio (A_c or CGR $= b_0 + b_1\text{GLXN} + b_2\text{YD} + b_3\text{WYJ-CK\&G} + b_4\text{WYJ-Y} + b_5\text{ZF} + b_6N_c + b_7K_N{:}K_L$), based on overall data of nine rice genotypes.

Dependent variable	Intercept b_0	Regression coefficient							R^2	No. of data points
		$b_1^{§}$	$b_2^{§}$	$b_3^{§}$	$b_4^{§}$	$b_5^{§}$	b_6	b_7		
A_c	17.82	0	0.98	0.91	− 12.70***	0.77	0.57	55.01***	0.87	27
CGR	4.51	0	4.14**	3.91**	− 3.58	5.53**	0.56	40.07***	0.93	27

Significance level: ** $P < 0.01$, *** $P < 0.001$.

§ Genetic backgrounds (GLXN, YD, WYJ-CK&G, WYJ-Y, ZF) as a category variable were quantified by introducing five dummy variables, with the GLXN as the reference level; so, the regression coefficient b_1 was nil while values of b_2–b_5 indicate the effect on A_c or CGR in different genetic backgrounds, respectively, relative to the GLXN background; to differentiate the effects of Y trait from the CK and G in the WYJ background on A_c and CGR (see the text), an additional dummy variable (WYJ-Y) was introduced in regression analysis.

crop management such as increasing planting density (e.g., a previous study of Gu et al., 2017b).

Y variants also showed a longer duration of its maximum LAI compared with their CK genotypes (Fig. 3), which contributed to an efficient canopy light interception along the growing season for rice. A similar trend was observed for total canopy nitrogen content in green leaves (N_c) in the field experiment for each genotype across stages (Fig. 4), as expected given the close relationship between LAI and N_c (Fig. S6) as quantitatively described by Yin et al. (2003). Interestingly, both Y variants exhibited a slower decline of crop growth rate between two sampling dates during late grain-filling phase under abundant light environment (Fig. 1), suggesting a delayed senescence effect, which is expected in stay-green cases (Christopher et al., 2008; Wu et al., 2018; Zhao et al., 2019) and was also observed in our tested G variants (Fig. 1). Such a pattern in CGR could be related to nitrogen dynamics in Fig. 4. Among Y variants, only ZF-Y maintained a higher capacity of dry matter assimilation after the establishment of the canopy (due to the optimised nitrogen distribution at leaf and canopy level as discussed above). Such adjustment compensated for its relatively weaker dry matter accumulation in the early stages and led to a significantly enhanced CGR for the entire life cycle in relative to its CK genotype (Fig. 1). Although total biomass gain of ZF-Y was constrained by the shortened growth duration (Table 1), its superior assimilate accumulation during the grain-filling phase and associated higher harvest index was responsible for its greater yield productivity (Table 2). The larger sink size (notably increased spikelet numbers) may also play a role as greater sink demanding for greater dry matter production can have a feedback effect on assimilate production (Dingkuhn et al., 2020). Further study on the impact of leaf-colour modification on carbon and nitrogen source-sink balance is needed to explain our observed difference in yield and biomass accumulation during grain-filling phase.

5. Conclusion

In general, the impact of yellow-leaf (Y) modifications was greater than that of stay-green (G) ones. In the Y variants, light could penetrate more into the lower parts of the canopy (i.e., significantly reduced K_L), but the photosynthetic capacity (i.e., A_{max}) profile did not necessarily follow more closely to the light profile. Compared with its default genotype, the increase of A_c and CGR in ZF-Y was attributed to the higher nitrogen partitioning efficiency at both leaf (i.e., improved leaf PNUE) and canopy (i.e., steeper nitrogen gradient with more nitrogen content in the upper leaf layer of the canopy) level. The restricted leaf area and lower tiller number in Y variants maintained the higher nitrogen concentration per unit leaf area, which delayed senescence as occurred in the G variants. All these contributed to the higher CGR during the grain-filling phase and grain production per day in ZF-Y, although its total biomass accumulation was limited by shorter growth duration. Our results suggest that leaf-colour modification not only altered canopy light penetration, but also caused variations in phenology and other morphophysiological characteristics. Whether this arose from genetic linkages or due to the randomness of radiation mutagenesis is unclear at this stage. Nevertheless, the altered traits appeared to be stable, which can be co-exploited by plant breeding for improving rice biomass and yield. Further studies are needed to understand the impact of leaf-colour modification on carbon and nitrogen source-sink relationships during grain filling.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2022.108746.

References

Anten, N.P.R., Schieving, F., Werger, M.J.A., 1995. Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C_3 and C_4 mono- and dicotyledonous species. Oecologia 101 (4), 504–513. https://doi.org/10.1007/BF00329431.

Archontoulis, S.V., Vos, J., Yin, X., Bastiaans, L., Danalatos, N.G., Struik, P.C., 2011. Temporal dynamics of light and nitrogen vertical distributions in canopies of sunflower, kenaf and cynara. Field Crops Res. 122 (3), 186–198. https://doi.org/10.1016/j.fcr.2011.03.008.

Arnon, D., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24 (1) https://doi.org/10.1104/pp.24.1.1.

Awika, J.M., 2011. Chapter 1. Major cereal grains production and use around the world. In: Awika, J.M., Piironen, V., Bean, S. (Eds.), Advances in Cereal Science: Implications to Food Processing and Health Promotion, 1089. American Chemical Society, ACS Symposium Series, pp. 1–13. https://doi.org/10.1021/bk-2011-1089.ch001.

Borrell, A.K., van Oosterom, E.J., Mullet, J.E., George-Jaeggli, B., Jordan, D.R., Klein, P.E., Hammer, G.L., 2014. Stay-green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns. New Phytol. 203 (3), 817–830. https://doi.org/10.1111/nph.12869.

Bubier, J.L., Moore, T.R., Bledzki, L.A., 2007. Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. Glob. Change Biol. 13 (6), 1168–1186. https://doi.org/10.1111/j.1365-2486.2007.01346.x.

Christopher, J.T., Manschadi, A.M., Hammer, G.L., Borrell, A.K., 2008. Developmental and physiological traits associated with high yield and stay-green phenotype in wheat. Aust. J. Agric. Res. 59 (4), 354–364. https://doi.org/10.1071/AR07193.

Dingkuhn, M., Luquet, D., Fabre, D., Muller, B., Yin, X., Paul, M.J., 2020. The case for improving crop carbon sink strength or plasticity for a CO_2-rich future. Curr. Opin. Plant Biol. 56, 259–272. https://doi.org/10.1016/j.pbi.2020.05.012.

Dungani, R., Karina, M., Subyakto, Sulaeman, A., Hermawan, D., Hadiyane, A., 2016. Agricultural waste fibers towards sustainability and advanced utilization: a review. Asian J. Plant Sci. 15 (1/2), 42–55. https://doi.org/10.3923/ajps.2016.42.55.

Evans, J.R., 1988. Acclimation by the thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. Funct. Plant Biol. 15 (2), 93–106. https://doi.org/10.1071/PP9880093.

Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78 (1), 9–19. https://doi.org/10.1007/BF00377192.

Evans, J.R., 1993. Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. II. Stability through time and comparison with a theoretical optimum. Funct. Plant Biol. 20 (1), 69–82. https://doi.org/10.1071/PP9930069.

Evans, J.R., 1996. Chapter: developmental constraints on photosynthesis: effects of light and nutrition. In: Baker, N.R. (Ed.), Photosynthesis and the Environment, Advances in Photosynthesis and Respiration, 5. Springer, Dordrecht, pp. 281–304. https://doi.org/10.1007/0-306-48135-9_11.

Field, C., 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. Oecologia 56 (2–3), 341–347. https://doi.org/10.1007/BF00379710.

Goudriaan, J., 1995. Optimization of nitrogen distribution and of leaf area index for maximum canopy photosynthesis rate. In: Thiyagarajan, T.M., ten Berge, H.F.M., Wopereis, M.C.S. (Eds.), Nitrogen Management Studies in Irrigated Rice. AB-DLO and TPE-WAU. SARP Research Proceedings, pp. 85–97.

Gregersen, P.L., Culetic, A., Boschian, L., Krupinska, K., 2013. Plant senescence and crop productivity. Plant Mol. Biol. 82 (6), 603–622. https://doi.org/10.1007/s11103-013-0013-8.

Gu, J., Yin, X., Stomph, T.J., Struik, P.C., 2014. Can exploiting natural genetic variation in leaf photosynthesis contribute to increasing rice productivity? A simulation analysis. Plant Cell Environ. 37 (1), 22–34. https://doi.org/10.1111/pce.12173.

Gu, J., Yin, X., Stomph, T.J., Wang, H., Struik, P.C., 2012. Physiological basis of genetic variation in leaf photosynthesis among rice (Oryza sativa L.) introgression lines under drought and well-watered conditions. J. Exp. Bot. 63 (14), 5137–5153. https://doi.org/10.1093/jxb/ers170.

Gu, J., Zhou, Z., Li, Z., Chen, Y., Wang, Z., Zhang, H., 2017b. Rice (Oryza sativa L.) with reduced chlorophyll content exhibit higher photosynthetic rate and efficiency, improved canopy light distribution, and greater yields than normally pigmented plants. Field Crops Res. 200, 58–70. https://doi.org/10.1016/j.fcr.2016.10.008.

Gu, J., Chen, Y., Zhang, H., Li, Z., Zhou, Q., Yu, C., Kong, X., Liu, L., Wang, Z., Yang, J., 2017a. Canopy light and nitrogen distributions are related to grain yield and nitrogen use efficiency in rice. Field Crops Res. 206, 74–85. https://doi.org/10.1016/j.fcr.2017.02.021.

Harley, P.C., Thomas, R.B., Reynolds, J.F., Strain, B.R., 1992. Modelling photosynthesis of cotton grown in elevated CO_2. Plant Cell Environ. 15 (3), 271–282. https://doi.org/10.1111/j.1365-3040.1992.tb00974.x.

Hikosaka, K., 2010. Mechanisms underlying interspecific variation in photosynthetic capacity across wild plant species. Plant Biotechnol. 27 (3), 223–229. https://doi.org/10.5511/plantbiotechnology.27.223.

Hikosaka, K., 2014. Optimal nitrogen distribution within a leaf canopy under direct and diffuse light. Plant Cell Environ. 37 (9), 2077–2085. https://doi.org/10.1111/pce.12291.

Hirose, T., Werger, M.J.A., 1987. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. Oecologia 72 (4), 520–526. https://doi.org/10.1007/BF00378977.

Hirose, T., Ackerly, D.D., Traw, M.B., Ramseier, D., Bazzaz, F.A., 1997. CO_2 elevation, canopy photosynthesis, and optimal leaf area index. Ecology 78 (8), 2339–2350. https://doi.org/10.1890/0012-9658(1997)078[2339:CECPAL]2.0.CO;2.

Hossain, M.A., Sarkar, M.A.R., Paul, S.K., 2011. Growth analysis of late transplant aman rice (cv. BR 23) raised from tiller seedlings. Libyan Agric. Res. Cen. J. Int. 2 (6), 265–273.

Jiang, H., Li, M., Liang, N., Yan, H., Wei, Y., Xu, X., Wu, G., 2007. Molecular cloning and function analysis of the stay green gene in rice. Plant J. 52 (2), 197–209. https://doi.org/10.1111/j.1365-313X.2007.03221.x.

Li, H., Zhao, C., Huang, W., Yang, G., 2013. Non-uniform vertical nitrogen distribution within plant canopy and its estimation by remote sensing: a review. Field Crops Res. 142, 75–84. https://doi.org/10.1016/j.fcr.2012.11.017.

Lim, J.S., Abdul Manan, Z., Wan Alwi, S.R., Hashim, H., 2012. A review on utilisation of biomass from rice industry as a source of renewable energy. Renew. Sustain. Energy Rev. 16 (5), 3084–3094. https://doi.org/10.1016/j.rser.2012.02.051.

Liu, D., Wang, W., Cai, X., 2014. Modulation of amylose content by structure-based modification of Os GBSS 1 activity in rice (*Oryza sativa* L.). Plant Biotechnol. J. 12 (9), 1297–1307. https://doi.org/10.1111/pbi.12228.

Long, S.P., Marshall-Colon, A., Zhu, X.G., 2015. Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell 161 (1), 56–66. https://doi.org/10.1016/j.cell.2015.03.019.

Makino, A., Nakano, H., Mae, T., 1994. Responses of ribulose-1,5-bisphosphate carboxylase, cytochrome f, and sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis. Plant Physiol. 105 (1), 173–179. https://doi.org/10.1104/pp.105.1.173.

Melis, A., 2009. Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant Sci. 177 (4), 272–280. https://doi.org/10.1016/j.plantsci.2009.06.005.

Monsi, M., Saeki, T., 2005. On the factor light in plant communities and its importance for matter production. Ann. Bot. 95 (3), 549–567. https://doi.org/10.1093/aob/mci052.

Moreau, D., Allard, V., Gaju, O., le Gouis, J., John Foulkes, M., Martre, P., 2012. Acclimation of leaf nitrogen to vertical light gradient at anthesis in wheat is a whole-plant process that scales with the size of the canopy. Plant Physiol. 160 (3), 1479–1490. https://doi.org/10.1104/pp.112.199935.

Muthayya, S., Sugimoto, J.D., Montgomery, S., Maberly, G.F., 2014. An overview of global rice production, supply, trade, and consumption. Ann. N. Y. Acad. Sci. 1324 (1), 7–14. https://doi.org/10.1111/nyas.12540.

Niu, S., Wu, M., Han, Y., Xia, J., Li, L., Wan, S., 2008. Water-mediated responses of ecosystem carbon fluxes to climatic change in a temperate steppe. New Phytol. 177 (1), 209–219. https://doi.org/10.1111/j.1469-8137.2007.02237.x.

Niu, S., Wu, M., Han, Y., Xia, J., Zhang, Z., Yang, H., Wan, S., 2010. Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. Glob. Change Biol. 16 (1), 144–155. https://doi.org/10.1111/j.1365-2486.2009.01894.x.

Ort, D.R., Zhu, X., Melis, A., 2011. Optimizing antenna size to maximize photosynthetic efficiency. Plant Physiol. 155 (1), 79–85. https://doi.org/10.1104/pp.110.165886.

Ort, D.R., Merchant, S.S., Alric, J., Barkan, A., Blankenship, R.E., Bock, R., Croce, R., Hanson, M.R., Hibberd, J.M., Long, S.P., Moore, T.A., Moroney, J., Niyogi, K.K., Parry, M.A.J., Peralta-Yahya, P.P., Prince, R.C., Redding, K.E., Spalding, M.H., van Wijk, K.J., Vermaas, W.F.J., von Caemmerer, S., Weber, A.P.M., Yeates, T.O., Yuan, J.S., Zhu, X.G., 2015. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc. Natl. Acad. Sci. USA 112 (28), 8529–8536. https://doi.org/10.1073/pnas.1424031112.

Ouyang, W., Yin, X., Yang, J., Struik, P.C., 2021. Roles of canopy architecture and nitrogen distribution in the better performance of an aerobic than a lowland rice cultivar under water deficit. Field Crops Res. 271, 108257 https://doi.org/10.1016/j.fcr.2021.108257.

Pettigrew, W.T., Hesketh, J.D., Peters, D.B., Woolley, J.T., 1989. Characterization of canopy photosynthesis of chlorophyll-deficient soybean isolines. Crop Sci. 29 (4), 1025–1029. https://doi.org/10.2135/cropsci1989.0011183×002900040040x.

Saito, H., Yuan, Q., Okumoto, Y., Doi, K., Yoshimura, A., Inoue, H., Teraishi, M., Tsukiyama, T., Tanisaka, T., 2009. Multiple alleles at Early flowering 1 locus making variation in the basic vegetative growth period in rice (Oryza sativa L.). Theor. Appl. Genet. 119 (2), 315–323. https://doi.org/10.1007/s00122-009-1040-3.

Sands, P.J., 1995. Modelling canopy production. I. Optimal distribution of photosynthetic resources. Funct. Plant Biol. 22 (4), 593–601. https://doi.org/10.1071/PP9950593.

Slattery, R.A., Vanloocke, A., Bernacchi, C.J., Zhu, X.G., Ort, D.R., 2017. Photosynthesis, light use efficiency, and yield of reduced-chlorophyll soybean mutants in field conditions. Front. Plant Sci. 8, 549. https://doi.org/10.3389/fpls.2017.00549.

Teng, S., Qian, Q., Zeng, D., Kunihiro, Y., Fujimoto, K., Huang, D., Zhu, L., 2004. QTL analysis of leaf photosynthetic rate and related physiological traits in rice (Oryza sativa L.). Euphytica 135 (1), 1–7. https://doi.org/10.1023/B:EUPH.0000009487.89270.e9.

Vergara, B.S., Tanaka, A., Lilis, R., Puranabhavung, S., 1966. Relationship between growth duration and grain yield of rice plants. Soil Sci. Plant Nutr. 12 (1), 31–39. https://doi.org/10.1080/00380768.1966.10431180.

Walker, B.J., Drewry, D.T., Slattery, R.A., VanLoocke, A., Cho, Y.B., Ort, D.R., 2018. Chlorophyll can be reduced in crop canopies with little penalty to photosynthesis. Plant Physiol. 176 (2), 1215–1232. https://doi.org/10.1104/pp.17.01401.

Welker, J.M., Fahnestock, J.T., Henry, G.H.R., O'Dea, K.W., Chimner, R.A., 2004. CO_2 exchange in three Canadian High Arctic ecosystems: response to long-term experimental warming. Glob. Change Biol. 10 (12), 1981–1995. https://doi.org/10.1111/j.1365-2486.2004.00857.x.

Wu, H., Xiang, J., Zhang, Y., Zhang, Y., Peng, S., Chen, H., Zhu, D., 2018. Effects of post-anthesis nitrogen uptake and translocation on photosynthetic production and rice yield. Sci. Rep. 8, 12891. https://doi.org/10.1038/s41598-018-31267-y.

Xia, J., Niu, S., Wan, S., 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. Glob. Change Biol. 15 (6), 1544–1556. https://doi.org/10.1111/j.1365-2486.2008.01807.x.

Yin, X., van Laar, H.H., 2005. Crop Systems Dynamics: An Ecophysiological Simulation Model of Genotype-by-environment Interactions. Wageningen Academic Publishers, THE NETHERLANDS. https://doi.org/10.3920/978-90-8686-539-0.

Yin, X., Struik, P.C., 2015. Constraints to the potential efficiency of converting solar radiation into phytoenergy in annual crops: from leaf biochemistry to canopy physiology and crop ecology. J. Exp. Bot. 66 (21), 6535–6549. https://doi.org/10.1093/jxb/erv371.

Yin, X., Lantinga, E.A., Schapendonk, A.H.C.M., Zhong, X., 2003. Some quantitative relationships between leaf area index and canopy nitrogen content and distribution. Ann. Bot. 91 (7), 893–903. https://doi.org/10.1093/aob/mcg096.

Yin, X., Kropff, M.J., Horie, T., Nakagawa, H., Centeno, H.G., Zhu, D., Goudriaan, J., 1997. A model for photothermal responses of flowering in rice I. Model description and parameterization. Field Crops Res. 51 (3), 189–200. https://doi.org/10.1016/S0378-4290(96)03456-9.

Yoshida, S., Forno, D.A., Cock, J.H., Gomez, K.A., 1976. Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute, The Philippines, pp. 24–79.

Zhao, Y., Qiang, C., Wang, X., Chen, Y., Deng, J., Jiang, C., Sun, X., Chen, H., Li, J., Piao, W., Zhu, X., Zhang, Z., Zhang, H., Li, Z., Li, J., 2019. New alleles for chlorophyll content and stay-green traits revealed by a genome wide association study in rice (*Oryza sativa*). Sci. Rep. 9, 2541. https://doi.org/10.1038/s41598-019-39280-5.

Zhong, X., Peng, S., Sanico, A.L., Liu, H., 2003. Quantifying the interactive effect of leaf nitrogen and leaf area on tillering of rice. J. Plant Nutr. 26 (6), 1203–1222. https://doi.org/10.1081/PLN-120020365.

Zhong, X., Peng, S., Sheehy, J.E., Visperas, R.M., Liu, H., 2002. Relationship between tillering and leaf area index: quantifying critical leaf area index for tillering in rice. J. Agric. Sci. 138 (3), 269–279. https://doi.org/10.1017/S0021859601001903.

Zhu, X.G., Long, S.P., Ort, D.R., 2008. What is the maximum efficiency with which photosynthesis can convert solar energy into biomass? Curr. Opin. Biotechnol. 19 (2), 153–159. https://doi.org/10.1016/j.copbio.2008.02.004.