agronomy

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Grain Quality Affected by Introducing Photorespiratory Bypasses into Rice

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Citation: Zhang, C.; Zhong, X.; Lin, D.; Wu, K.; Wu, Z.; Zhang, Z.; Peng, X. Grain Quality Affected by Introducing Photorespiratory Bypasses into Rice. *Agronomy* **2022**, *12*, 566. https://doi.org/10.3390/agronomy12030566

Academic Editors: Gianni Barcaccia, Alessandro Vannozzi and Fabio Palumbo

Received: 7 February 2022
Accepted: 21 February 2022
Published: 24 February 2022

Abstract: Grain quality is a critical component of high-yielding varieties to ensure acceptance by an ever-increasing population and living standards. During the past years, several photorespiration bypasses have been introduced into C3 plants, among which our GOC and GCGT bypasses exhibit increased photosynthesis and yield in rice. However, to the best of our knowledge, there are still no reports referring to effects of the bypasses on grain quality. Thus, the objective of this study is to determine the effect of GOC and GCGT bypasses on grain quality, and the mechanism of how photorespiratory bypasses affect grain quality was also investigated. Compared with the WT of Zhonghua 11, GOC4 and GCGT20 plants had higher nutritional quality and cooking quality as grain protein content was significantly increased by 11.27% and 14.97%, and alkali spreading value was significantly increased by 7.6% and 4.63%, respectively, whereas appearance quality appears to be negatively affected since the chalky rice rate was increased by 32.6% and 68%, respectively. Analyses also demonstrated that the changes in grain quality may result from the increased total nitrogen and constrained carbohydrate transport in the transgenic plants. Altogether, the results not only suggest that the increased photosynthesis and yield by introducing the photorespiratory bypasses can significantly affect grain quality parameters for rice, either positively or negatively, but also imply that the coordination of source–sink transport may play important roles in grain quality formation for high-yielding crops via increased photosynthetic efficiency.

Keywords: photorespiration bypass; grain quality; nitrogen; rice

1. Introduction

Rice (*Oryza sativa* L.) is a staple food feeding more than half of the population in the world. With the global population continuing to rise, there is a great need to increase crop yield. It is projected that the global food production needs to be doubled by 2050 [1,2]. Improvement of photosynthetic efficiency is regarded as a major feasible option to increase crop yield potential [3]. In C3 plants, photorespiration may consume 25–30% of photosynthates, thereby markedly decreasing photosynthetic efficiency [4,5], such that photorespiration is commonly seen as a very wasteful metabolic process. Therefore, mitigating the negative effects of photorespiration remains an important bioengineering target to improve the photosynthesis and yields of crops to meet demands of growing population [4,6]. In recent years, synthetic biology has been well-applied to biological systems, and several photorespiration bypasses have been introduced into C3 plants; Kebeish et al. (2007) [7] designed the first chloroplastic photorespiratory bypass via diverting glycolate into glycerate to form a synthetic CO_2 concentrating mechanism, which significantly increased photosynthesis and biomass in *Arabidopsis*. Subsequently, similar bypasses were reported in *camelina sativa*, tobacco, and other crops [8–10]. We have successfully expressed two different synthetic photorespiration bypasses, i.e., GOC and GCGT, in rice. The GOC bypass completely oxidizes glycolate into CO_2, which was catalyzed by three rice-self-originating enzymes, i.e., glycolate oxidase (OsGLO3), oxalate oxidase (OsOXO3), and catalase (OsCAT), while the GCGT bypass consists of four enzymes including *Oryza sativa* glycolate oxidase (OsGLO1) and *Escherichia coli* catalase (EcCAT), glyoxylate carboligase (EcGCL), and tartronic semialdehyde reductase (EcTSR), which can redirect 75% of carbon from glycolate metabolism to the Calvin cycle. GOC and GCGT rice plants showed increased biomass and yield both in the greenhouse and fields [11,12]. With the continuous improvement in economic and living standards, rice quality has attracted more attention and breeders and cultivators aiming to breed high quality rice varieties to satisfy people's demands. However, some studies have shown that deterioration of grain quality occurs in modern high-yielding rice varieties [13]. Therefore, in order to improve both quality and crop yields, it is necessary to understand the key quality traits of crops, so that they can be selected in the breeding plan and controlled by proper crop managements.

Generally, grain quality is not only controlled by varietal genotypes but also affected by environmental factors such as crop management, soil types, and climatic conditions during rice growth [14,15]. Carbon and nitrogen metabolisms are crucial to both the yield and quality of rice grains, and their products contained in starch and proteins account for nearly 87% of the dry matter in brown rice [16]. Nitrogen is a crucial nutrient, and applying nitrogen fertilizers is an important management factor that affects both grain yield and quality [17–19]. However, more than 50% of nitrogen fertilizers applied to the field have not been absorbed and utilized by crops and consequently cause soil acidification and environmental pollution, which seriously threaten humans' health [20]. Excessive use of nitrogen fertilizers also contributes to poor eating and cooking quality of the rice grain, such as hard texture and dull color [13,21]. Crop yields depend on carbohydrate synthesized through photosynthesis and their utilization at the sink organs [22]. It has been shown that source–sink balance impacted rice quality due to significant reduction in N and nonstructural carbohydrate (NSC) translocation [23,24]. As a product of photosynthesis, starch is the major source of nutrition for humans, and its components and structure have been reported to affect rice cooking quality [25].

Grain quality traits encompass milling quality, appearance quality, cooking quality, and nutritional quality, etc. [26,27]. Milling quality is mainly determined by brown rice rate, milled rice rate, and head milled rice rate. Nutritional quality is crucial because rice is the main source of protein and micronutrients, and protein content is an important factor to evaluate the rice nutritional quality [28]. Appearance quality is mainly evaluated by chalkiness degree and chalky rice rate [29]. Chalk refers to an opaque area in the grain, which is formed from loosely arranged starch granules. Starch is mainly composed of amylopectin and amylose, and amylose content is considered the most important factor that determines the cooking quality of rice [30]. Meanwhile, the alkali spreading value and the starch pasting properties also play important roles in rice cooking quality [31,32].

For all the reported photorespiratory bypass plants, focus is mainly placed on their biomass and yield, and few concerned their grain quality. While our earlier work has demonstrated that introducing GOC and GCGT photorespiration bypasses significantly increased the rice biomass and yield [11,12], their influence on grain quality remains an interesting topic. The objective of this study is to understand whether GOC and GCGT photorespiration bypasses can influence rice grain quality and to further explore the possible mechanism of how high photosynthetic efficiency affects rice grain quality.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The field experiments were carried out in the experimental farm on the campus of the South China Agricultural University, Guangzhou, China, during the rice growing season (March to June) in 2021. Two transgenic plants, GOC4 and GCGT20, which had been reported in the previous articles [11,12], were used as the plant materials in this experiment. Meanwhile, rice plants (cv. Zhonghua11) were used as the WT experimental material. A randomized block design was arranged for field trials under natural conditions with a planting density of 15 × 15 cm per plant in a 25 m^2 (5 m × 5 m) plot. There were four biological replicates per line, and each replicate contained 50 seedlings.

2.2. Rice Quality Analysis

Rice plants were hand-harvested on 7 June, and the rice seeds were air dried and stored at room temperature for three months for quality analysis according to the method of Mo et al. (2015) [33]. There are four biological replicates per line, and each replicate includes about 500 g rice grains. The rice grains were dehusked with a rice huller (Taizhou Grain Instrument Factory: Taizhou, China) to calculate the brown rice rate and subsequently polished using a Jingmi testing rice grader (Taizhou Grain Instrument Factory: Taizhou, China) to calculate the milled rice rate and head milled rice rate. The rice appearance detection analyzer (SC-E, Wseen: Hangzhou, China) was used to detect the chalky rice rate and chalkiness degree. The amylose content, alkali spreading value, and protein content were determined using an Infratec 1241 grain analyzer (Foss: Hilleroed, Denmark). The starch pasting properties were detected by a rapid viscosity analyzer (RVA, Model RVA-3D, Perten: Beijing, China) to evaluate the texture of the corresponding cooked rice [34,35]. The starch viscosity parameters include peak viscosity, hot paste viscosity, cool paste viscosity, breakdown value, and pasting temperature.

2.3. Total Nitrogen Content Analysis

Rice plants were sampled and separated into flag leaf, stem sheath, and panicle at the blooming and maturity stages, respectively. After that, the samples were treated under 105 °C to rapidly denature and inactivate the enzymes and then dried at 80 °C until they reached a constant weight. Subsequently, the dried samples were ground into powder to measure the total nitrogen content with multi-N/C 2100 S (Analytik Jena: Jena, Germany).

2.4. Total Soluble Protein and Chlorophyll Content Analysis

The total soluble protein content was determined according to the method of Liang et al. (2020) [36] with appropriate modifications. Briefly, 5 uL of supernatant from the leaf extracts was added to 2 mL of Coomassie Brilliant Blue G-250, and the OD595 value was recorded after 8 min. The standard curve was made with bovine serum albumin, and the corresponding protein content was calculated according to the standard curve. The leaf chlorophyll content was estimated by a SPAD meter (SPAD-502 Plus) [37,38].

2.5. Statistical Analysis

The statistically significant differences of all data in this study were analyzed on one-way ANOVA using the SPSS 26.0 (IBM: Armonk, NY, USA) statistical software program followed by Duncan's test. The data reached significant statistical difference when $p < 0.05$.

3. Results

3.1. Milling and Nutritional Quality

Brown rice rate, milled rice rate, and head milled rice rate are three key milling traits for evaluating milling quality [28]. The results showed that there were no significant differences in the brown rice rate and milled rice rate between GOC4, GCGT20, and WT rice grains (Figure 1A,B). In contrast, the head milled rice rate was shown to be decreased in GOC4 and GCGT20 relative to WT (Figure 1C), meaning that milling quality of the transgenic grains was decreased to some extent. Grain storage protein is an important nutrient for human health [31]. As shown in Figure 1D, the grain protein content values in GOC4 and GCGT20 were 7.6% and 7.9%, being significantly increased by 11.27% and 14.97%, respectively, as compared to WT (6.8%). The higher protein content implicates that significant improvement occurs for nutritional quality of the transgenic rice grain.

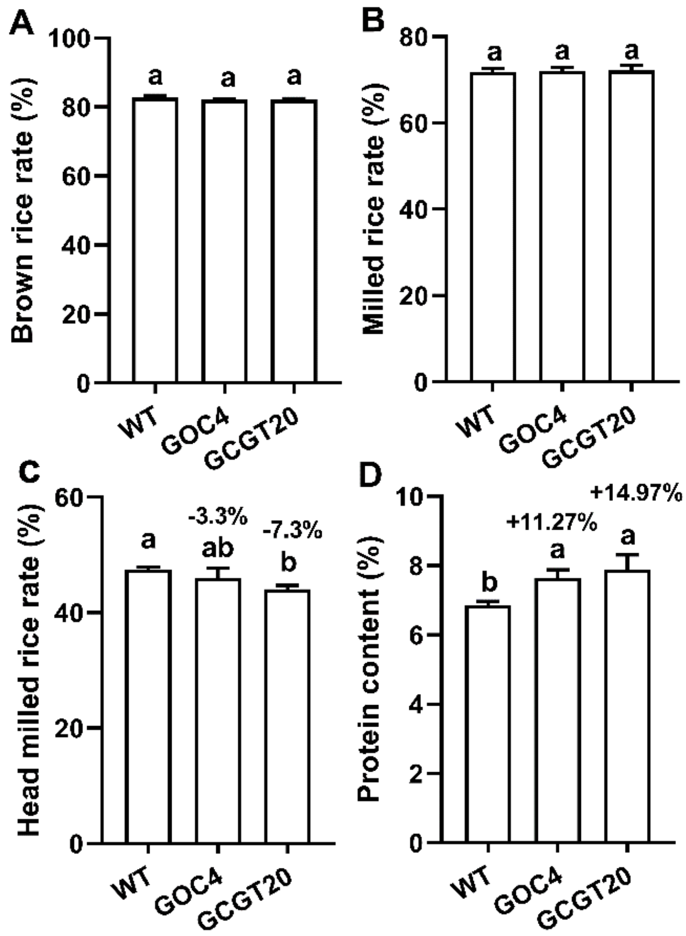

Figure 1. Milling and nutritional quality of GOC4, GCGT20, and WT rice grains. (**A**) Brown rice rate, (**B**) milled rice rate, (**C**) head rice rate, and (**D**) protein content. Data are presented as the mean ± SD according to one-way ANOVA followed by a Duncan's test for means comparison. Different letters indicate significant differences ($n = 4$, $p < 0.05$).

3.2. Appearance Quality

Grain chalkiness is the undesirable appearance quality that affect rice market value [39]. As shown in Figure 2A,B, the grains from GOC4 and GCGT20 transgenic lines exhibit apparent chalkiness. Further quantitative analysis found that their grain chalky rice rates were 9.9% and 12.5%, being increased by 36.8% and 68%, respectively, as compared with WT (7.5%), while there were no significant differences in chalkiness degree (Figure 2C,D). Overall, the rice grain appearance quality is decreased for photorespiration bypasses plants relative to WT.

3.3. Cooking Quality

Amylose content, alkali spreading value, and starch viscosity curve are three useful parameters for evaluating the cooking quality of rice [30,40]. As shown in Figure 3B, no significant differences were observed in amylose content. Meanwhile, the RVA curves were analyzed, and starch viscosity parameters including peak viscosity, hot paste viscosity, cool paste viscosity, breakdown value, and pasting temperature were all not significantly altered for GOC4 and GCGT20 grains, indicating that the GOC and GCGT bypasses have no influence on rice starch pasting properties (Figure 3A, Table 1). In contrast, the grain alkali spreading value for GOC4 and GCGT20 was significantly increased by 7.6% and 4.6%, respectively (Figure 3C), implying that the cooking quality was improved for photorespiration bypasses' rice grains.

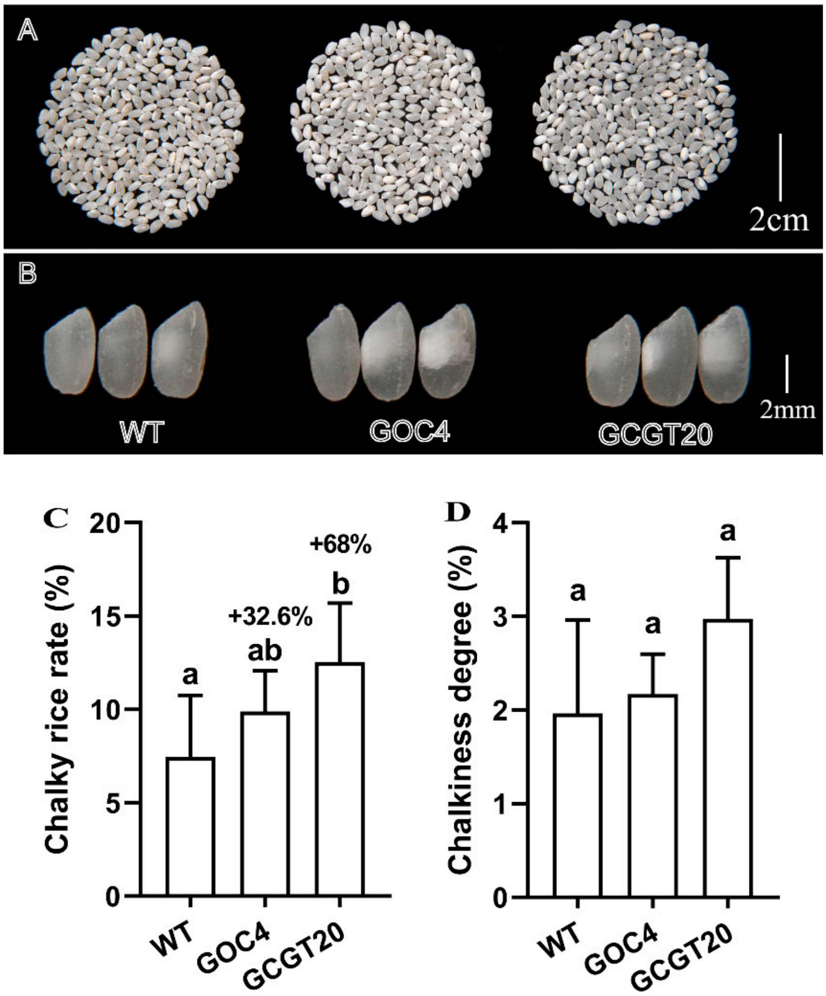

Figure 2. Appearance quality of GOC4, GCGT20, and WT rice grains. The appearance quality includes (**A**,**B**) the performance of the milled grain, (**C**) chalky rice rate, and (**D**) chalkiness degree. Data are presented as the mean ± SD according to one-way ANOVA followed by a Duncan's test for means comparison. Different letters indicate significant differences ($n = 4$, $p < 0.05$).

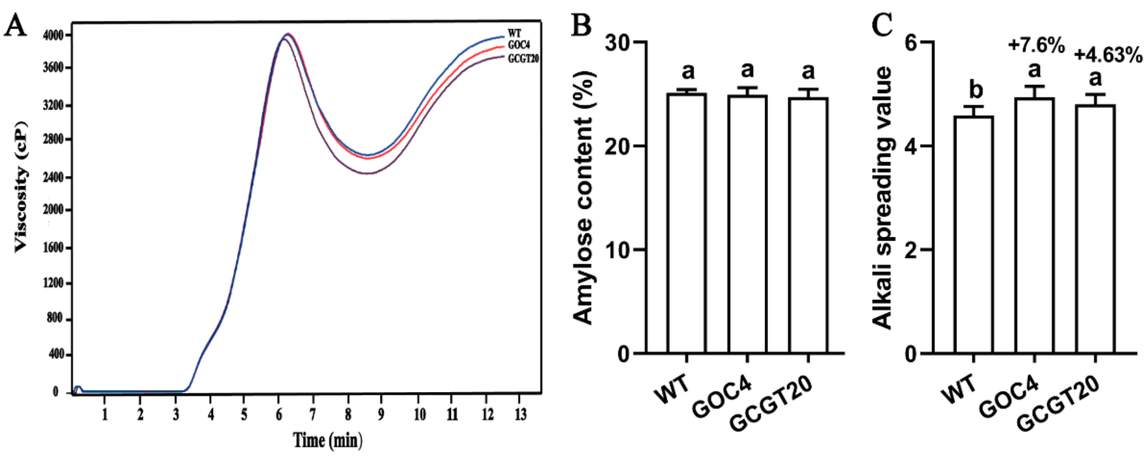

Figure 3. Cooking quality of GOC4, GCGT20, and WT rice grains. The cooking quality includes (**A**) the starch RVA curve, (**B**) amylose content, and (**C**) alkali spreading value. Data are presented as the mean ± SD according to one-way ANOVA followed by a Duncan's test for means comparison. Different letters indicate significant differences ($n = 4$, $p < 0.05$).

Table 1. Starch viscosity parameters of GOC4, GCGT20, and WT rice grains.

	Peak Viscosity (cP)	Hot Paste Viscosity (cP)	Breakdown Value (cP)	Cool Paste Viscosity (cP)	Pasting Temperature (°C)
WT	3988 ± 154 [a]	2705 ± 139 [a]	1283 ± 140 [a]	3997 ± 135 [a]	79 ± 0.5 [a]
GOC4	3961 ± 57 [a]	2721 ± 80 [a]	1241 ± 106 [a]	3995 ± 58 [a]	79 ± 0.4 [a]
GCGT20	3906 ± 69 [a]	2550 ± 112 [a]	1355 ± 147 [a]	3823 ± 102 [a]	78 ± 0.0 [a]

Note: data are presented as the mean ± SD and according to one-way ANOVA followed with a Duncan's test for means comparison. The same letter indicates no significant difference (n = 4, p > 0.05).

3.4. Total Nitrogen Contents

Nitrogen is an important nutrient that affects grain quality; thus, we further determined the total nitrogen contents in GOC4, GCGT20, and WT plants. As shown in Figure 4, the total nitrogen content of GOC4 and GCGT20 transgenic leaves, stem sheaths, and panicles were all higher than WT at the blooming and maturity stages. At the blooming stage, the total nitrogen content in flag leaves was significantly increased by 17.5% and 18.3%, respectively: 19.4% and 13.9% in stem sheathes and 13.4% and 11.1% in panicles (Figure 4A–C). At the maturity stage, increases in flag leaves were 5.8% and 10.7%, respectively: 16.5% and 18.7% in stem sheathes and 10.1% and 15% in panicles (Figure 4D–F). Overall, it may be concluded that introducing photorespiration bypasses largely up-regulated the total nitrogen content in rice.

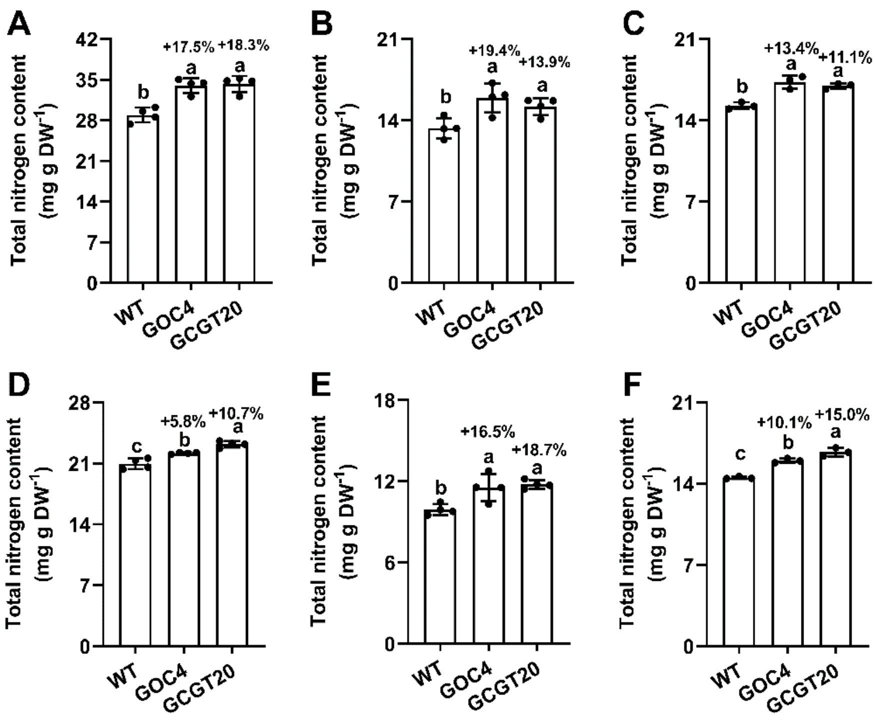

Figure 4. Total nitrogen contents of flag leaf, stem sheath, and panicle in GOC4, GCGT20, and WT plants at blooming and maturity stages. (**A**) Flag leaves at blooming stage; (**B**) stem sheath at blooming stage; (**C**) panicle at blooming stage; (**D**) flag leaves at maturity stage; (**E**) stem sheath at maturity stage; (**F**) panicle at maturity stage. Data are presented as the mean ± SD according to one-way ANOVA followed by a Duncan's test for means comparison. Different letters indicate significant differences (n = 4, p < 0.05).

3.5. Total Soluble Protein and Chlorophyll Contents

Since nitrogen is closely related to soluble protein and chlorophyll contents in plants [16,41], these two parameters were further detected in flag leaves at the blooming stage. A chlorophyll meter (SPAD) was used to estimate chlorophyll content [37]. As shown in Figure 5A, the total soluble protein in flag leaves of GOC4 and GCGT20 transgenic plants were significantly improved by 15.9% and 21%, respectively. Additionally, the SPAD values were 11.5% and 8.7% higher than WT (Figure 5B).

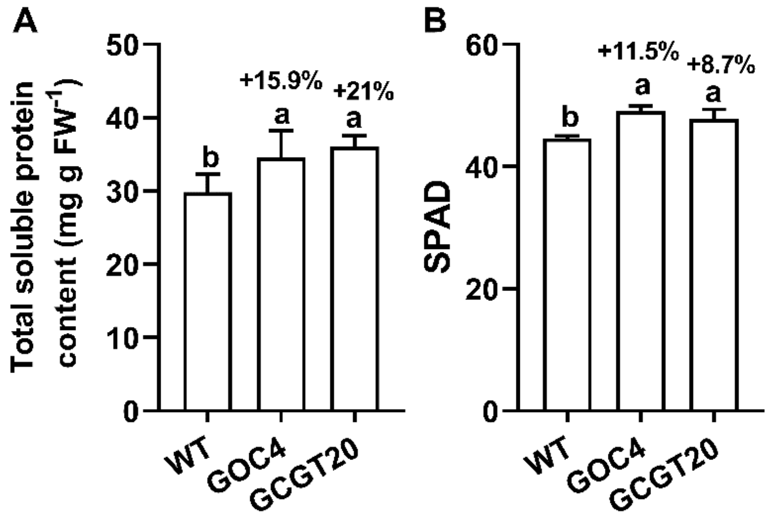

Figure 5. Total soluble protein content and chlorophyll contents for GOC4, GCGT20, and WT plants. (**A**) Total soluble protein content; (**B**) chlorophyll content is estimated by SPAD. Data are presented as the mean ± SD and according to one-way ANOVA followed by a Duncan's test for means comparison. Different letters indicate significant differences (n = 4, p < 0.05).

4. Discussion

Two photorespiratory bypasses, i.e., GOC and GCGT, have been successfully introduced into rice in our previous work [11,12]. Although the two bypasses are distinct in terms of their carbon and energy metabolisms, very similar results were achieved for both transgenic plants, with the net photosynthetic rate (Pn) being increased by 15–22% and 6–16%, biomass by 14–35% and 16–28%, and yield by 7–27% and 13–27%, respectively [11,12]. According to the energy demand calculated by Peterhansel et al. (2013) [42], the GOC bypass is much more wasteful than the GCGT bypass; 20 ATPs are needed for the GOC bypass, while only 11.75 ATPs are needed for the GCGT, which is even 0.5 ATP less than for native photorespiration. Additionally, in terms of carbon economy, GOC completely oxidizes glycolate to CO_2, whereas GCGT can recover 75% of carbon from glycolate into the Calvin cycle, just like native photorespiration. It has been considered that yields and quality are negatively related, although no genetic linkage has been established [43]. High quality without high yield is widely observed in crop breeding science [44,45], and many popular high-yielding cultivars and hybrids have relatively poor quality [13]. As the main staple, rice production must meet increasing demands for both quantity and quality to ensure an appropriate level of sustainability. Therefore, determining the influence of photorespiration bypasses on grain quality would be helpful for further understanding of the mechanistic relationship between high yield and quality for rice.

Starch is the main component for rice endosperm, which accounts for 80–90% of grain weight, thus its composition and structure are the key factors that determine rice cooking quality [46]. Starch is composed of different proportions of amylose and amylopectin, and cooking quality is generally assessed by amylose content, which can be divided into low (3–19%), intermediate (20–25%), and high (>25%) [30,47]. Like the WT of Zhonghua11, both GOC4 and GCGT20 were shown to belong to intermediate amylose rice variety (Figure 3B). Starch pasting properties are significantly correlated with amylose content, as amylose can inhibit starch granules from swelling and help maintain the structure of swollen granules [48,49]. In this research, the grain amylose content of GOC4 and GCGT20 was not altered, and their starch pasting properties were also not different from WT (Figure 3B, Table 1). Gelatinization temperature is a critical parameter for evaluating rice cooking quality. This temperature is the threshold point for driving transition from starch granules to gel and usually estimated by measuring the alkali spreading value [50,51]. The increased alkali spreading value (Figure 3C) implicates a lower gelatinization temperature and thereby an improved cooking quality for GOC4 and GCGT20 rice grains as compared to WT.

Rice storage protein accounts for 7–10% of grain weight; thus, its content affects rice nutritional quality, among which glutelin is superior to other storage proteins because it is easier for humans to digest [52]. The milled *japonica* rice protein content is around 6.8% [53], and it is generally accepted that higher protein content (>9%) has inferior palatability. In this research, the protein content in GOC4 and GCGT20 was significantly increased (Figure 1D), indicating an improved nutritional quality for the bypass grains. Appropriate nitrogen management is necessary to obtain high yield and high quality [19,54]. It has been well-known that increasing the total nitrogen application and the proportion of nitrogen fertilizers would increase protein content while reducing the amylose content and starch pasting properties of rice grains [55,56]. There have been reports to show that eating and cooking quality were diminished mainly because of the higher protein content in rice [28]. Nitrogen is a key component for chloroplasts and also closely related to chlorophyll content [57,58]. The current data found that in the two bypass rice plants, both total nitrogen and chlorophyll contents were significantly increased in leaves, stem sheathes, and panicles (Figures 4 and 5B). The increased total N content may be attributed to an increased nitrogen use efficiency due to decreased ammonia emission as native photorespiration is partially diverted inside chloroplasts. It has been previously predicted that plants with photorespiration bypasses may increase nitrogen use efficiency by up to 15% [8,59,60]. The higher grain protein content may be explained by the increased grain nitrogen concentration [54]. Therefore, it can be reasonably speculated that bypassing glycolate metabolism may have improved the nitrogen use efficiency to boost C3 crop yield accompanied with increased N content in plants, thereby leading to the increased grain protein content. Excessive N fertilizer application partially accounts for the poor eating and cooking quality of the rice grains, and thus developing crops with high nitrogen use efficiency is essential for the sustainability of agriculture [13].

Grain chalkiness is one of the most important factors affecting rice appearance, milling, and eating quality [61]. It is generally accepted that there is a negative correlation between grain protein content and appearance quality; rice grain with higher protein content leads to an increased chalkiness degree [28]. The chalky rice rate of COC4 and GCGT20 is largely increased relative to WT (Figure 2C). Grains with increased chalkiness are more prone to breakage during milling [62,63], which is consistent with our results that the head milled rice rate is decreased for GOC4 and GCGT20 grains (Figure 1C). Altogether, these results demonstrated that the increase of grain protein content improved the nutritional quality but affected the milling quality and appearance quality to some extent. Starch, as a product of photosynthesis, is the main storage substance of panicles [64,65]. The increased photosynthesis in the bypass plants significantly increased starch content in leaves [11,12]. The accumulation of grain starch is actually a result of the transportation of photosynthates from the leaves (source) to the particles (sink), and the developmental and filling process of amyloplasts in endosperm cells can affect the rice yield and quality [66]. The increased grain chalkiness appears to be triggered by poor filling of starch granules or an imbalance in the adjustment of starch degradation pathway during grain filling [11,12,67]. Both physiological and transcriptomic analyses suggested that the photosynthetic carbohydrates were not transported to grains in an efficient way in GCGT transgenic plants [12]. Therefore, the increased chalky rice rate in transgenic plants may be closely related to constrained carbohydrate transport. There has been reports demonstrating that the occurrence of chalky kernels is affected by sink–source status [24]. In contrast to our results, however, down-regulation or mutation of GWD1 (Glucan, Water-Dikinase 1) caused over-accumulation of starch in plant leaves but significantly reduced the degree of chalkiness and the percentage of chalky rice grains [68]. Due to the complex interaction between genes and growth environments, the physiological and molecular mechanism of chalk formation is still far from clear [67,69]. There have been a number of reports indicating that nitrogen fertilizers increase translucency but decrease the chalky rice rate. Chalkiness degree was decreased with increasing nitrogen content [69], consistent with the results of Qiao et al. (2011) [70]. Therefore, the increased chalky rice rate in our two bypass plants may be mainly due to the constrained carbohydrate transport. Taken together, the coordinated interaction between carbon and nitrogen metabolism may directly affect the growth and development of crops and the rice yield and quality, and it is more practically significant to be able to achieve both high yield and high quality of rice through a reasonable regulation of carbon and nitrogen metabolism in crops.

5. Conclusions

Compared with WT of Zhonghua 11, GOC4 and GCGT20 plants had higher grain protein and alkali spreading value, indicating that nutritional quality and cooking quality are improved in rice, whereas appearance quality is decreased due to the increased chalky rice rate. The study demonstrated that the photorespiration bypasses could affect rice grain quality, and the increased total nitrogen content and constrained carbohydrate transport may explain how the bypasses affect rice grain quality. Altogether, this study extends the research of photorespiration bypasses on grain quality and implied that the coordination of source–sink transport may play an important role in grain quality formation for high-yielding rice.

Author Contributions: X.P., C.Z. and Z.Z. conceived and designed the project. C.Z., X.Z., K.W., Z.W. and D.L. performed the experiments. C.Z. analyzed the data. X.P. and C.Z. wrote the original manuscript. X.P., revised and approved the final version of the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program of China (2020YFA0907600), the Major Program of Guangdong Basic and Applied Research (grant no. 2019B030302006) and the National Natural Science Foundation of China (grant nos. 31770256).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are provided as figures and table which are included in this paper.

Conflicts of Interest: The authors declare no conflict of interest.

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