Effects of Carbon and Nitrogen Fertilisers on Rice Quality of the OsNRT2.3b-Overexpressing Line

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Abstract: Excessive nitrogen fertiliser use reduces nitrogen use efficiency and causes significant damage to the environment. Carbon fertilisers have the advantage of improving soil fertility; however, the effects of carbon and nitrogen fertilisers on rice yield and quality are not clear. In this study, the nitrogen-efficient line (OsNRT2.3b-overexpressing [O8]) and wild type (WT) were treated with different levels of nitrogen and carbon fertilisers under field conditions to study the effects of different fertilisation treatments on rice quality. The results showed that the appearance, nutrition, and taste qualities of O8 were generally high compared with WT under various fertilisation treatment conditions in 2019 and 2020. Compared with 90 kg/ha and 270 kg/ha nitrogen fertiliser, a single application of 90 kg/ha and 270 kg/ha carbon fertiliser significantly reduced the protein content of O8 by approximately 37.08% and 35.50% in 2019 and 2020, respectively, compared with WT, and improved the eating quality of O8 and WT. However, the replacement of nitrogen fertiliser with 20% carbon fertiliser did not improve the eating quality of O8 and WT compared with a single application of nitrogen fertiliser. This study identifies a high-quality gene, OsNRT2.3b, for breeding high-quality rice and provides a theoretical basis for obtaining high-quality rice and molecular breeding.

Keywords: OsNRT2.3b; rice quality; nitrogen fertiliser; carbon fertiliser; rice

1. Introduction

Rice (Oryza sativa L.) is the main food crop globally and the staple food for approximately half of the world’s population [1,2]. Improving rice yield and nutritional quality focuses on strategies to fulfil future food demands and improve human health [3]. High-yield rice is no longer the only goal of breeders. Producers and consumers can significantly increase the value of rice by improving food quality. While seeking high yield, rice quality has also become a vital selection indicator for breeders.

Breeding high-yield and high-quality rice is a significant goal for crop geneticists and rice breeders. Rice yields have greatly improved in recent decades owing to the discovery and application of “green revolution genes” and “heterosis” [4–8]. With the improvement in living standards and the increase in the global rice trade, the demand for high-quality rice is increasing [9]. Therefore, cultivating high-quality rice is key to meeting the market demand [10]. There are four main parts to the comprehensive evaluation of rice quality: milling, appearance, nutritional, and cooking and eating qualities [10,11].
The milling quality of rice includes brown rice rate (BRR), milled rice rate (MRR), and head milled rice rate (HRR). These indicators can indicate the ability of rice grains to withstand husking and polishing without breakage and with high recovery, determining the final yield and breakage of milled rice. The appearance quality of rice usually includes indicators such as grain shape, chalkiness degree (ChD), and chalky rice rate (ChR). These indicators of appearance directly determine the market value of rice and are closely related to grain yield and head grain yield. The quantity and quality of starch, protein, vitamins, minerals, and other beneficial substances (such as iron) can affect the nutritional quality of rice [12]. Indicators of cooking and eating quality can indirectly reflect the characteristics and palatability of rice, such as amylose content (AC), gel consistency (GC), and alkali spreading value (ASV) [13].

In addition to being related to ecological conditions, rice quality is mainly affected by field fertiliser control technology [14]. Excessive use of inorganic fertilisers reduces soil fertility and causes enormous environmental pressure [15]. Nitrogen is a primary macronutrient for rice growth and development, affecting rice yield and quality [16]. Nitrogen has the flexibility to regulate the mineral composition of rice grains [17–19]. The availability of nitrogen in the soil can affect the selenium content in rice [17]. Nitrogen fertilisation increases the levels of copper, iron, manganese, and zinc in rice grains and increases phosphorus reactivation [19,20]. With the increase in nitrogen fertiliser application amount, the nitrogen utilisation efficiency decreases, resulting in wasted resources and aggravating environmental pollution [21,22]. Therefore, the use of nitrogen-efficient materials to improve rice quality is critical.

However, the high rice yield cannot be completely separated from the contribution of nitrogen fertilisers in the near future. Carbon fertilisers have the advantages of being time-effective and improving soil fertility. The microorganisms in carbon fertilisers can decompose and convert nutrients that are difficult to absorb by rice into adequate nutrients that are easily absorbed, increase the content of organic matter in the soil, and improve the yield and quality of rice [23–25]. However, the nutrient content of carbon fertilisers is relatively low and cannot meet the growing needs of rice; hence, the combined application of inorganic and carbon fertilisers is required [26]. Carbon fertilisers can shorten the optimal cooking time, increase the amylose content, and reduce the crude protein content. Carbon fertilisers can improve the quality of rice and improve the internal quality of rice, such as by reducing the chalkiness of rice, thereby improving the palatability and quality of rice [27,28].

In addition to the influence of environmental factors on rice quality, a typical quantitative trait, genes also affect rice quality by affecting milling, appearance, cooking, and eating qualities. In rice, nitrogen uptake and transport-related protein genes indirectly or directly affect nitrogen uptake and utilisation to regulate rice quality. OsLHT1 can improve nitrogen use efficiency (NUE) by increasing amino acid transport from roots to shoots, thereby improving rice quality [29]. The truncated dep1 allele can improve rice yield and NUE under low-nitrogen conditions and can affect rice quality [30]. Overexpression of the pH-sensing nitrate transporter OsNRT2.3b can increase the uptake of nitrogen, phosphorous, and iron and improve NUE in rice grown under different nitrogen supply environments [31]. Nitrogen, phosphorous, and iron are essential nutrients for rice and have important effects on its growth, yield, and quality [9,12,32,33].

Many factors affect rice quality, such as changes in nutrient uptake. The combination of carbon and inorganic fertilisers has been used to improve crop yield and quality [34,35] and is conducive to sustainable agricultural development. However, the effect of OsNRT2.3b on rice quality under different fertilisation conditions has not yet been reported. Therefore, in this study, the OsNRT2.3b-overexpressing line (O8) and wild type (WT) were used to study the impact of OsNRT2.3b on rice quality under different fertilisation treatment conditions to reveal the effects of OsNRT2.3b on rice quality.
2. Materials and Methods

2.1. Transgenic Lines

The OsNRT2.3b-overexpressing line (O8) and wild type (WT) used in this study are described in detail by Fan et al. [31]. Briefly, the Ubi promoter facilitates the open reading frames of OsNRT2.3b and is transformed into rice (Oryza sativa L.) using Agrobacterium-mediated methods [31,36].

2.2. Field Experiment

In 2019 and 2020, O8 and WT seedlings were grown in plots at the experimental site of the Anhui Science and Technology University, Fengyang, Anhui, China. The experimental area is in a subtropical monsoon climate zone with an average annual temperature of 14.9 °C, an average annual drop in water volume between 840–920 mm, and a frost-free period of 212 days a year. The soil in the experimental plots is cinnamon soil, and the chemical properties of the soils include available nitrogen content, 0.49 g/kg; total phosphorous content, 0.58 g/kg; available phosphorous content, 5.11 g/kg; available potassium, 3.94 \times 10^{-3} g/kg; organic matter, 12.81 g/kg; and pH 7.21.

The O8 and WT lines were planted under different fertiliser treatments, including 90 kg/ha carbon fertiliser (C1), 90 kg/ha nitrogen fertiliser (N1), 20% carbon fertiliser instead of nitrogen fertiliser (90 kg/ha) (N1C1-20%), 270 kg/ha nitrogen fertiliser (N2), 270 kg/ha carbon fertiliser (C2), and 20% carbon fertiliser instead of nitrogen fertiliser (270 kg/ha) (N2C2-20%). Nitrogen fertiliser was applied to the field at three stages: before transplanting, at the tillering stage, and just before the heading stage, at 50%, 20%, and 30%, respectively, and carbon, phosphate, and potassium fertilisers were applied before transplanting. The area of each plot was 3.75 m² (3 m × 1.25 m), and the depth of the plot was 1.2 m. Brick concrete was used to isolate the plots to prevent the exchange of water and fertiliser between the plots.

2.3. Nitrogen Use Efficiency

Plant samples were taken at the maturity and blooming stages under different fertiliser treatments and fixed at 105 °C for 20 min. Each plant was collected in triplicate. The samples were dried at 50 °C for 7 days, and the dry weight was measured. The tissues were ground to a powder. The total nitrogen concentration was determined using a Dumas nitrogen analyser (VELP, Italy). Agronomic nitrogen use efficiency (ANUE), nitrogen recovery efficiency (NRE), and post-anthesis N uptake (PNUE) were calculated as below.

\[
ANUE = \frac{\text{yield of N treatment} - \text{yield of low N treatment}}{\text{N supply}}.
\]

\[
NRE = \frac{\text{total N accumulation at maturity of N treatment} - \text{total N accumulation at maturity of low N treatment}}{\text{N supply}}.
\]

\[
PNUE = \text{total N accumulation at maturity} - \text{total N accumulation at blooming stage}.
\]

2.4. Grain Quality Measurements and Data Analysis

The O8 and WT seeds were harvested from the different fertiliser treatments at the mature stage for grain quality measurements. Each plant was collected four times. The rice appearance quality traits of aspect ratio, chalky rice ratio, and chalkiness degree were measured using the Hangzhou Wanshen SC-E rice appearance quality tester. Amylose content, gel consistency, and alkali spreading value were measured using a Diode Array 7200. The protein content of rice was determined using a Kjeldahl analyser (FOSS TECATOR Kjeltec8400).

The harvested rice was threshed with a thresher, and the seeds were subsequently air-dried, hulled with a rice husker (SY88-TH), and then degerminated with a whitening machine (BLH-3120) to obtain polished rice for calculating roughness and precision.

2.5. Statistical Analysis

Excel 2016 and IBM SPSS Statistics 19 (Chicago, IL, USA) were used for data processing and analyses. For the milling, appearance, and cooking and eating qualities of O8 and WT with different fertilisation treatments, the data were pooled for calculation of means and
standard errors (SE) and analysed by one-way analysis of variance (ANOVA) followed by the LSD test at $p \leq 0.05$ to determine the statistical significance of the differences between O8 and WT. IBM SPSS Statistics 19 was used for ANOVA between treatments. Two-way ANOVA was used for rice materials and different fertilisation treatments, and their interaction was determined by Duncan’s multiple range test ($p \leq 0.05$).

3. Results

3.1. NUE of the OsNRT2.3b-Overexpressing Line under Different Fertiliser Treatments

Nitrogen is one of the essential nutrients necessary for rice growth, providing the support needed for photosynthesis. It has a significant impact on rice growth, yield, and quality. The practical application of nitrogen fertilisers can increase rice biomass and yield and improve the nutritional value of rice. However, the effects of nitrogen fertiliser application and fertiliser management methods on yield and quality have two aspects. Appropriate fertiliser application and proper fertiliser management can increase rice yield and improve rice quality. In contrast, excessive nitrogen fertiliser application and unreasonable management methods can lead to reduced rice yield and quality.

To explore the effects of nitrogen-efficient rice and different fertilisation strategies on rice quality, the OsNRT2.3b-overexpressing line (O8) and wild type (WT) were planted under different fertilisation conditions: 90 kg/ha (N1) and 270 kg/ha (N2) nitrogen fertiliser, 90 kg/ha (C1) and 270 kg/ha (C2) carbon fertiliser, and 20% carbon fertiliser instead of nitrogen fertiliser (C1N1-20% and C2N2-20%). Fertilisers were applied in batches during four different periods: before transplanting, transplanting, tillering, and heading stages (Table 1). The growth period of O8 was longer than that of WT in 2019 and 2020: approximately 47 and 17 days in 2019 and 2020, respectively (Table 1). Compared with the WT, with carbon fertiliser treatment, the agronomic nitrogen use efficiency (ANUE) and nitrogen recovery efficiency (NRE) of O8 were increased under different fertiliser treatments, with increases between 70.56% and 155.49% and between 122.89% and 180.86%, respectively (Figure 1A,B). Nitrogen fertiliser, a critical factor in regulating rice quality, mainly affects rice quality at the grain-filling stage of quality formation. Post-anthesis nitrogen uptake (PNUE) plays an essential role in photosynthetic assimilation and grain-filling [37]. Therefore, we analysed the PNUE of O8 and WT plants under different fertiliser conditions. Compared with the WT, the PNUE of O8 was reduced under the low concentration fertiliser condition and increased by approximately 74.63% under the other fertiliser treatments (Figure 1C). Therefore, the O8 line has a higher nitrogen use efficiency than that of the WT.

Table 1. Timetables of cultivation during the rice growing stage.

<table>
<thead>
<tr>
<th>Fertilization Period</th>
<th>Time</th>
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<tbody>
<tr>
<td>Before transplanting</td>
<td>20 June 2019</td>
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<td>Transplanting stage</td>
<td>21 June 2019</td>
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<tr>
<td>Tiller stage</td>
<td>13 July 2019</td>
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<td>Heading stage</td>
<td>13 August 2019</td>
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<td>Harvest grain</td>
<td>7 September 2019 (WT)</td>
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<td>24 October 2019 (O8)</td>
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<td>20 June 2020</td>
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<td>12 July 2020</td>
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<td>13 August 2020</td>
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<td>7 September 2019</td>
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<td></td>
<td>1 October 2020 (WT)</td>
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<td></td>
<td>18 October 2020 (O8)</td>
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3.2. Effects of Different Fertiliser Treatments and OsNRT2.3b Overexpression on Milling Quality

The milling quality determines the economic value of rice from the mill to the market. To determine the effects of OsNRT2.3b and different fertilisers on milling quality, we obtained the milling quality traits of O8 and WT plants under different fertiliser treatments in 2019 and 2020. Compared with WT, the brown rice rate (BRR) of O8 considerably increased by approximately 2.10% in 2019 and 3.67% in 2020 under all fertiliser treatments, but not with C1 in 2019 (Figure 2A). Compared with WT, the milled rice rate (MRR) of O8 substantially increased by approximately 3.52% in 2019 and 5.02% in 2020 under all fertiliser treatments (Figure 2B). Compared with WT, the head milled rice rate (HRR) of O8 considerably increased by approximately 6.03% in 2019 and 13.24% in 2020 under...
all fertiliser treatments, but no difference was observed under the C2 treatment in 2019; however, it increased dramatically under all fertiliser treatments in 2020 (Figure 2C). Fertiliser treatment, OsNRT2.3b overexpression, and their interactions resulted in significant or extremely significant effects on milling quality (Table 2). Therefore, the results indicate that the rice milling quality was affected by both genetic and environmental factors, with genetic factors having a more significant effect.

**Figure 1.** Increased nitrogen use efficiency in overexpression OsNRT2.3b line under different fertilisation treatments. Comparison of (A) agronomic nitrogen use efficiency (ANUE), (B) nitrogen recovery efficiency (NRE), and (C) post-anthesis N uptake (PANU) between overexpression OsNRT2.3b line (O8) and wild-type (WT). n = 3. N, nitrogen fertilizer treatment. C, carbon fertilizer treatment. CN-20%, 20% carbon fertilizer instead of nitrogen fertilizer. N1: treatment with 90 kg/ha nitrogen fertilizer. C1: treatment with 90 kg/ha carbon fertilizer. N1C1-20%: 20% carbon fertilizer instead of nitrogen fertilizer (90 kg/ha). N2: treatment with 270 kg/ha nitrogen fertilizer; C2: treatment with 270 kg/ha carbon fertilizer. N2C2-20%: 20% carbon fertilizer instead of nitrogen fertilizer (270 kg/ha). The different letters indicate a significant difference between O8 and WT (p < 0.05).

**Figure 2.** Milling quality trait of overexpression OsNRT2.3b line and wild type. Brown rice rate in two years, 2019 and 2020 (A), milled rice rate in two years, 2019 and 2020 (B), and head milled rice rate in two years, 2019 and 2020 (C) of overexpression OsNRT2.3b line (O8) and wild type (WT) under different fertiliser treatment conditions. N1: treatment with 90 kg/ha nitrogen fertilizer. C1: treatment with 90 kg/ha carbon fertilizer. N1C1-20%: 20% carbon fertilizer instead of nitrogen fertilizer (90 kg/ha). N2: treatment with 270 kg/ha nitrogen fertilizer; C2: treatment with 270 kg/ha carbon fertilizer. N2C2-20%: 20% carbon fertilizer instead of nitrogen fertilizer (270 kg/ha). Significant differences between O8 and WT are indicated by different letters (p < 0.05).
Table 2. Correlation analysis of different fertilizer treatments on quality of O8 and WT.

<table>
<thead>
<tr>
<th>Year</th>
<th>Factors</th>
<th>BRR</th>
<th>MRR</th>
<th>HRR</th>
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<td>2019</td>
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<td>2020</td>
<td>Fertilizer treatment (F)</td>
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Note: BRR: Brown Rice Rate, MRR: Milling Rice Rate, HRR: Head milling Rice Rate, ChR: Chalky Rice Rate, ChD: Chalkiness Degree, AC: Amylose Content, PC: Protein Content, ASV: Alkali Spreading Value, GC: Gel Consistency. ** Significant at \( p < 0.01 \). * Significant at \( p < 0.05 \). ns, no significant difference.

3.3. Effects of Different Fertiliser Treatments and OsNRT2.3b Overexpression on the Appearance Quality

The appearance quality of rice changes in a complex manner with changes in gene expression and fertiliser application. Grain morphology was statistically analysed at maturity under the different fertiliser treatments (Figure 3A). A previous study reported that grain size influences rice quality, such as appearance, milling, and cooking and eating qualities [8]. Compared with WT, OsNRT2.3b overexpression significantly improved the aspect ratio by approximately 24.54% in 2019 and 25.85% in 2020, and different fertiliser treatments showed no significant differences in the two years (Figure 3B).

Figure 3. Grain phenotype of overexpression OsNRT2.3b line and wild type. (A) Grain phenotype of overexpression OsNRT2.3b lines (O8) and wild type (WT) under different fertilizer treatment conditions.
Aspect ratio (B), chalky grain rate (C), and chalkiness degree (D) of O8 and WT under different fertilizer treatment conditions in 2019 and 2020. N1: treatment with 90 kg/ha nitrogen fertilizer. C1: treatment with 90 kg/ha carbon fertilizer. N1C1-20%: 20% carbon fertilizer instead of nitrogen fertilizer (90 kg/ha). N2: treatment with 270 kg/ha nitrogen fertilizer. C2: treatment with 270 kg/ha carbon fertilizer. N2C2-20%: 20% carbon fertilizer instead of nitrogen fertilizer (270 kg/ha). Significant differences between O8 and WT are indicated by different letters (p < 0.05).

To further explore the effects of different fertilisation treatments and OsNRT2.3b overexpression on the appearance quality, we examined the chalky rice rate (ChR) and chalkiness degree (ChD). Compared with the WT, the ChR of O8 was not different under the N1 and N2C2-20% treatments in 2019 but was significantly reduced under other fertiliser treatments, with a decrease of 67.18%. (Figure 3C). In 2020, the ChR of O8 was significantly reduced by 19.91% under all fertiliser treatments (Figure 3C). Under all the fertilisation treatments, except N1C1-20% in 2019, compared with WT, the ChD of O8 was reduced by approximately 52.27% in 2019 and 25.29% in 2020 (Figure 3D). The slow-acting effect of carbon fertiliser may have led to a decrease in ChR and ChD in O8 compared with WT when nitrogen and carbon fertilisers were applied together in 2020 (Figure 3C,D). Fertiliser treatment, OsNRT2.3b overexpression, and their interactions resulted in significant or extremely significant differences in appearance quality (Table 2). Fertiliser treatment and OsNRT2.3b overexpression together affected ChR and ChD.

3.4. Effects of Different Fertiliser Treatments and OsNRT2.3b Overexpression on Cooking and Eating Quality

To evaluate the effect of OsNRT2.3b overexpression on the nutritional value of rice under different fertiliser treatments, cooking and eating quality traits were tested. Compared with the WT, the amylose content (AC) of O8 increased by approximately 27.02% in 2019 and 25.56% in 2020 under the different fertiliser treatments (Figure 4A). OsNRT2.3b overexpression reduced protein content (PC) by approximately 35.76% in 2019 and 38.94% in 2020 under fertiliser treatments (Figure 4B). Under all the fertiliser treatments, except C2 in 2019 and C2 and N2C2-20% in 2020, the gel consistency (GC) of O8, compared with WT, increased by approximately 35.29% in 2019 and 24.30% in 2020 (Figure 4C). OsNRT2.3b overexpression reduced the alkali spreading value (ASV) under all fertiliser treatments, but there were no differences under the N1 and N2C2-20% treatment in 2019 (Figure 4D). Fertiliser treatment, OsNRT2.3b overexpression, and their interactions resulted in significant or extremely significant differences in cooking and eating quality (Table 2). Thus, our results indicated that the cooking and eating quality were affected by both genetic and environmental factors, and the genetic factor, OsNRT2.3b overexpression, had a considerable influence.

3.5. Correlation Analysis of All Fertiliser Treatments and OsNRT2.3b Overexpression on Quality

As shown in Figure 5, there was a significant or extremely significant positive correlation between MRR and BRR; HRR and BRR or MRR; AC and BRR, MRR, HRR, or AR; PC and ChR or ChD; and GC and MRR under the different fertiliser treatments in 2019 and 2020. There were significant or extremely significant negative correlations between ChD and MRR or AR; AC and ChD; PC and BRR, MRR, and AR or AC; and ASV and MRR, AR, or AC under different fertiliser treatments in 2019 and 2020. The difference in significance between the various indicators in 2019 and 2020 may be caused by the difference in fertilisation and growth periods (Table 1). After changing the fertilisation strategy, the hierarchical clustering between the quality indicators and different fertiliser treatments also changed, such as AC and MRR; PC and ChD or AC; and GC and BRR, MRR, or HRR in 2019 and 2020 (Figure 6). The effect of carbon fertiliser on rice quality was more significant and lasting than that of nitrogen fertiliser (Figure 6). Therefore, the rice quality was affected by nitrogen and carbon fertilisers.
Agriculture 2022, 12, x FOR PEER REVIEW

Figure 4. Cooking and eating quality of overexpression OsNRT2.3b line and wild type. Amylose content (A), protein content (B), gel consistency (C), and alkali spreading value (D) of overexpression OsNRT2.3b line (O8) and wild type (WT) under different fertilizer treatment conditions in two years, 2019 and 2020. N1: treatment with 90 kg/ha nitrogen fertilizer. C1: treatment with 90 kg/ha carbon fertilizer. N1C1-20%: 20% carbon fertilizer instead of nitrogen fertilizer (90 kg/ha). N2: treatment with 270 kg/ha nitrogen fertilizer. C2: treatment with 270 kg/ha carbon fertilizer. N2C2-20%: 20% carbon fertilizer instead of nitrogen fertilizer (270 kg/ha). Significant differences between O8 and WT are indicated by different letters (p < 0.05).

Figure 5. Pearson’s correlation analysis of the correlation of quality indicators of O8 and WT under different fertilizer treatments. Correlation analysis of different fertiliser treatments on the quality of O8 and WT in 2019 (A) and 2020 (B). BRR: Brown Rice Rate, MRR: Milling Rice Rate, HRR: Head milling Rice Rate, AR: Aspect ratio, ChR: Chalky Rice Rate, ChD: Chalkiness Degree, AC: Amylose Content, PC: Protein Content, ASV: Alkali Spreading Value, GC: Gel Consistency. ** Significant at p < 0.01. * Significant at p < 0.05.
or HRR in 2019 and 2020 (Figure 6). The effect of carbon fertiliser on rice quality was more significant and lasting than that of nitrogen fertiliser (Figure 6). Therefore, the rice quality was affected by nitrogen and carbon fertilisers.

Figure 5. Pearson’s correlation analysis of the correlation of quality indicators of O8 and WT under different fertilizer treatments. Correlation analysis of different fertiliser treatments on the quality of O8 and WT in 2019 (A) and 2020 (B). BRR: Brown Rice Rate, MRR: Milling Rice Rate, HRR: Head milling Rice Rate, AR: Aspect ratio, ChR: Chalky Rice Rate, ChD: Chalkiness Degree, AC: Amylose Content, PC: Protein Content, ASV: Alkali Spreling Value, GC: Gel Consistency. ** Significant at p < 0.01. * Significant at p < 0.05.

Figure 6. Hierarchical clustering of quality indicators of O8 and WT under different fertilizer treatments. Hierarchical clustering of quality indicators of O8 and WT under different fertilizer treatment in 2019 (A) and 2020 (B). BRR, brown rice rate; MRR, milling rice rate; HRR, head milling rice rate; AR, aspect ratio; ChR, chalky rice rate; ChD, chalkiness degree; AC, amylose content; PC, protein content; ASV, alkali seeding value; GC, gel consistency.

4. Discussion

Human health requires foods with high nutritional value. Rice is a significant source of carbohydrates, providing at least 20% dietary protein, 3% dietary fat, and other essential nutrients [38]. With the continuous improvement in living standards, improving rice quality is an urgent problem that needs to be solved in rice breeding. Rice quality is controlled by genetics and is closely related to fertilisation type, which is a complex issue that concerns consumers and breeders [39].

4.1. Nitrogen Affects the Formation of Rice Quality

Rice quality is based on the physiological and biochemical metabolism of internal substances in rice. Genetic factors mainly dominate rice quality, but it is also regulated by the environment and cultivation measures. Nitrogen level and fertiliser application have been demonstrated to regulate the protein, iron, and zinc levels in rice grains [40,41]. Nitrogen fertiliser is an essential factor in determining rice quality.

Nitrogen is a major element required for rice growth and development. Appropriate nitrogen can improve rice quality [42,43]; however, excessive nitrogen can reduce rice quality by affecting grain morphogenesis [44]. Nitrogen is a major component of the protein; it accounts for 8% of rice grains and contains essential amino acids. Therefore, improving NUE and rice quality while increasing the rice yield is necessary. Nitrogen-efficient materials (O8) are of great significance for improving the quality of rice (Figures 1–4). The protein content plays an essential role in determining rice quality [39,44]. Protein content can be affected by fertiliser type, nitrogen fertiliser, and carbon fertiliser (Figure 4B). However, protein content is a typical quantitative trait with a complex genetic structure and is easily affected by nitrogen fertiliser application in the later growth stage [39]. A previous study demonstrated that increasing the nitrogen supply during wheat and rice growth promotes N accumulation and protein content, but excessive application reduces protein content in grains [45]. Overexpression of OsNRT2.3b decreased the protein content in rice grains (Figure 4B), and the absorbed nitrogen may have been used for the reproductive growth of rice, thereby delaying the growth period (Table 1). Protein content, which is propor-
tional to the degree of chalkiness and inversely proportional to gel consistency, affects rice quality. Nitrogen fertiliser availability affects appearance and cooking and eating qualities (Figures 3 and 4). Amylose content is a crucial factor in determining the appearance and taste quality of rice. The influence of OsNRT2.3b is similar to that reported for OsGS1.1b, a functional transcript of OsGS1.1, a NUE-associated gene [46], in terms of rice quality, increased NUE, and regulation of rice quality by affecting the amylose content of rice (Figures 1 and 4A).

In addition, the increase in gel consistency increased the taste and quality of rice. Increasing nitrogen fertiliser application increases nitrogen accumulation in rice plants and affects carbon and nitrogen metabolism [47]. The energy of photosynthetic products can be continuously transported to rice grains, increasing the grain filling degree and nitrogen content and affecting rice quality.

4.2. Carbon Fertilisers Improve Rice Quality

Carbon fertilisers can directly provide various nutrients for rice, create a good soil ecological environment for microorganisms, promote the reproduction of soil microorganisms, accelerate the decomposition of soil organic matter, increase soil nutrient content, improve soil physical and chemical properties and structure, and improve rice quality.

Rice quality mainly includes grain appearance, milling quality, nutritional quality, and cooking and eating quality [48,49]. The appearance quality of rice primarily affects its commercial value, milling quality, and nutritional value [50]. Studies have shown that rice milling quality is mainly determined by genetic characteristics, whereas appearance quality is related to fertiliser type, nutrient release, and nutrient utilisation. The application of carbon fertiliser can reduce the appearance quality, such as chalkiness degree and chalky rice rate, improving the rice milling quality under low carbon fertiliser conditions (Figures 2 and 3). Although carbon fertiliser is a slow-release fertiliser, the nutrient release rate is slow, and there is no significant difference when nitrogen fertilisers are replaced.

Nutritional and cooking quality are the two most important indicators that consumers generally pay attention to. Amylose content, gel consistency, and protein content are the main properties that determine the eating and cooking quality of rice [51,52]. Under nitrogen fertiliser conditions, the application of carbon fertiliser will be more beneficial for improving the gel consistency of efficient nitrogen lines than amylose content (Figure 4A,C). In addition, carbon fertilisers reduced the protein content of the grains, thereby improving the quality of rice (Figure 4B). Therefore, although nitrogen fertiliser is an essential element for enhancing rice yield, carbon fertilisers can contribute to improving rice quality.

4.3. OsNRT2.3b Improves Rice Quality under Different Fertiliser Treatment Conditions

OsNRT2.3b is a transcript of OsNRT2.3, a high-affinity nitrate transport protein gene [31]. Nitrogen, phosphorous, and iron are essential nutrients for rice and affect rice growth, yield, and quality [12,32–34]. Overexpression of OsNRT2.3b increases NUE (Figure 1) and improves nutrient uptake balance, such as that of nitrogen, phosphorous, and iron, in rice grown in different nitrogen supply environments [31,53]. Studies have shown that elevated CO₂ concentrations caused by the greenhouse effect and economic development exacerbate the adverse effects of low nitrogen on grain quality. However, the efficient use of nitrogen fertilisers in crops reduces these adverse effects [54]. Overexpression of OsNRT2.3b increases intercellular CO₂ levels, inhibits the photorespiratory rate, and increases photosynthesis [17,55,56], which might affect grain filling. Owing to the delay in the reproductive period, overexpression of OsNRT2.3b reduced grain filling under different fertiliser treatments in 2019 and 2020 (Figure 3, Table 1). Therefore, the grain quality of O8 was better than that of WT.

In addition, enhancing the expression of OsNRT2.3b is reported to inhibit the absorption, mobilisation, and distribution of phenanthrene which is toxic to humans, thereby reducing the phenanthrene content in rice grains [57]. Therefore, the appearance, nutrition, and taste quality of O8 were higher than those of the WT under different fertilisation treatment conditions (Figures 3 and 4). Increasing the expression of OsNRT2.3b resulted
in a high nutritional value under different fertiliser treatments. In addition, the starch digestibility of brown rice is very slow, and some starch is not converted into sugar and reaches the large intestine intact; therefore, eating brown rice can reduce the risk of diabetes [58,59]. Overexpression of OsNRT2.3b increased the brown rice rate in both years (Figure 2A), which may be highly beneficial for people at risk of developing diabetes.

5. Conclusions

Genetic and fertilisation conditions jointly regulate the quality of rice. Nitrogen fertiliser is the primary means of improving rice yield. The application of nitrogen and carbon fertilisers in a reasonable ratio can improve the physical and chemical properties of soil and the quality of rice. In addition, the application of nitrogen-efficient and high-quality materials can reduce the use of nitrogen fertilisers and improve the quality of rice. Therefore, OsNRT2.3b can be used as a genetic marker for high-quality and high-yield breeding in molecular breeding. This study provides a theoretical basis for breeders and field managers to cultivate high-yield and high-quality rice varieties.

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