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Distribution of proteins and amino acids in milled and brown rice as affected by nitrogen fertilization and genotype

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ABSTRACT

A field experiment involving six japonica rice cultivars with contrasting agronomic traits and seven nitrogen (N) fertilizer treatments was performed to determine the effects of N and genotype on distribution of four kinds of grain proteins and amino acids in milled and brown rice. For brown and milled rice, albumin and globulin were controlled more by genotype than N treatments, whereas prolamin and glutelin were largely determined by N. Substantial genotypic differences in response of milled/brown (M/B) ratios of proteins to N treatments were detected. In comparison with large panicle cultivars, small panicle cultivars such as Wuyujing3 had the lower ratio and exhibited more stability under contrasting N treatments. N had significant influence on amino acid composition of brown and milled rice, with contents of the 17 amino acids measured increasing with elevated N rate. However, cysteine and methionine in brown rice and lysine and methionine in milled rice were not significantly affected by N. In addition, N had little effect on ratios of M/B for most of the amino acids.

1. Introduction

Rice provides a staple source of energy, protein, and other nutrients to half of the world population (Shih, 2004). Protein is the second most abundant component of rice grain, accounting for about 9% of its dry weight (Kennedy and Burlingame, 2003). Essentially, cereal proteins are classified into albumin, globulin, prolamin, and glutelin according to their solubility. Compared to other cereals, rice has relatively lower protein content but has higher protein quality due to its higher ratio of glutelin/prolamin (Kaul and Raghaviah, 1975). However, rice protein, like most cereal proteins, is deficient in the essential amino acid lysine, and hence is of poor nutritional quality.

Protein is a major factor in determining the texture, pasting capacity, and sensory characteristics of milled rice. Many studies found that protein plays a significant role in determining the functional properties of the starch, including inhibiting the swelling of starch granules, reducing the pasting and crystallizing capacities, and increasing the pasting temperature of the isolated rice starch (Shih, 2004). Clarifying the effect of protein on physicochemical properties of rice will help deepen insights into the biochemical basis of rice quality.

Protein in brown rice is more concentrated in the aleurone layer, the embryo, and the subaleurone layer of the endosperm compared to the deeper starchy endosperm (Ellis et al., 1987). Generally, prolamin is more abundant in the outer layers, whereas glutelin increases in proportion toward the center of the endosperm (Leesawatwong et al., 2005). Rice is mainly consumed as a polished grain, and milling has a strong effect on protein losses. Protein profile in rice grain is thus viewed as an important trait affecting relative proportions of protein fractions in milled rice.

Nitrogen (N) has a positive effect on grain protein accumulation, with N topdressing at the panicle development stage playing a major role (Borrell et al., 1999; Perez et al., 1996). However, little is known concerning the variations in distribution of protein fractions and amino acids in milled and brown rice among contrasting N treatments, the knowledge of which will be helpful for evaluating and clarifying the N effect on rice quality.

In the present study, six japonica rice cultivars with similar heading date but contrasting panicle size and cooking and eating quality were used and seven N treatments were undertaken. Grain albumin, globulin, prolamin, and glutelin were assayed and amino

Abbreviations: C, carbon; CK, control of fertilizer treatments; CV, coefficient of variation; HN, high nitrogen rate; LN, low nitrogen rate; MN, moderate nitrogen rate; N, nitrogen.

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acid compositions of two selected cultivars were analyzed. Our aims were to: (1) examine the N effect on protein distribution in milled rice and brown rice; and (2) identify genotypic differences in response to protein distribution as a result of N treatments.

2. Materials and methods

The field experiment was performed at an experimental station of Nanjing Agricultural University (Jiangning, Nanjing; 31°56′39″N, 118°59′13″E) in 2008. The design was a randomized split-plot design with seven N treatments split for six genotypes. There were four replications. According to our previous experiments in 2007 (Ning et al., 2009), six japonica rice cultivars differing in panicle size, heading date, and cooking and eating quality were selected (Table 1). Note that the eating quality of the six cultivars was evaluated by the consumers in Yangtze Delta, China (Du et al., 2008; Wan et al., 2005; Xu et al., 2006).

Seven N treatments were conducted as follows: (1) CK, no N fertilizer was applied during the whole growth stage, except that P and K fertilizer was applied before transplanting; (2) LN82 and LN55, low N rate (90 kg/ha); (3) MN82 and MN55, moderate N rate (180 kg/ha); (4) HN82 and HN55, high N rate (270 kg/ha). Note that fertilization modes of 55 and 82 mean the ratio of basal/topdressing fertilizer is 5:5 and 8:2, respectively.

The soil fertility and agronomic practices were described in Ning et al. (2009). Rice was sown in seedbeds on May 25, and transplanted on June 28, 2008. At maturity, about 100 panicles with similar maturity were harvested in each replication. The samples were naturally dried and dehulled. Brown rice was milled for 90 s with a JNMJ3 rice polisher (Taizhou Grain Industry Instrument Corp, Zhejiang, China), with ~10% outer layers being removed. Brown and milled rice were ground by a stainless steel grinder for 3 min and the resulting powders were used for chemical analysis.

Protein fractions were separated and analyzed according to the method reported by Liu et al. (2005). The basic scheme involved sequential extraction of rice powders as follows: water, yielding albumins; 10% NaCl, yielding globulins; 55% *n*-propanol, yielding prolamins; and Biuret reagent, yielding glutelins. Glutelin contents were measured by the Biuret method (Holme and Peck, 1998), while the contents of the other three fractions were determined using the Bradford reagent according to Bollag and Edelstein (1990). Amino acids were analyzed with an L-8900 High-Speed Amino Acid Analyzer (Hitachi Corp, Japan) after HCl (6 mol/L) hydrolysis. During acid hydrolysis, asparagine and glutamine are deamidated to aspartate and glutamate, respectively, rendering the acid and its corresponding amide indistinguishable. Tryptophan is destroyed under the standard hydrolysis conditions, and so is cysteine although to a lesser extent.

Samples were analyzed in triplicate and mean values were used for comparisons. Variance analysis was performed using the Data Processing System (DPS, Institution of Agricultural Entomology, Zhejiang University). Means were compared by the least significant difference (LSD) test ($P \leq 0.05$).

Table 1
Six cultivars used in this study and their agronomical traits in 2008.

Cultivars	Heading date	Spikelets/panicle[a]	Cooking and eating quality
Zaofeng9	August 20	126.0de	Excellent
Xudao4	August 20	149.9c	Fair
Wuyujing3	August 28	123.5e	Excellent
Ningjing2	August 28	167.3b	Fair
Ningjing1	September 4	131.8d	Good
9522	September 4	181.7a	Fair

[a] Data within a line followed by a different letter are significantly different ($P < 0.05$).

3. Results

3.1. N and genotype effect on protein fractions

Variance analysis showed significant effects of N, genotype, and their interaction on the four protein fractions in both brown and milled rice (Table 2). For brown and milled rice, albumin and globulin were controlled more by genotype than N treatments, whereas prolamin and glutelin were largely determined by N. For ratios of prolamin/glutelin, N showed smaller effects than genotype did (Table 2).

Significant genotypic variations were detected among the cultivars examined. For example, averaged glutelin content of brown rice across the seven N treatments was lowest for 9522 and highest for Xudao4, with a range of 5.84–7.14% (Table 3). N treatment had a positive effect on globulin, prolamin, and glutelin of both brown and milled rice (Tables 3 and 4). Generally, protein content increased progressively with N rate, and was higher for the treatment of 55 than that of 82 (Tables 3 and 4). For the HN55 treatment, averaged glutelin content of brown rice was 7.66%, being significantly higher than those of HN82 (7.00%) and CK (5.35%).

N showed similar effect on prolamin/glutelin ratio between brown rice and milled rice (Tables 3 and 4). Prolamin/glutelin was high for CK, intermediate for HN, and low for MN.

3.2. N and genotype effect on distribution of protein fractions

Ratios of milled/brown rice (M/B) were calculated to measure the protein distribution in rice grains. Variance analysis showed significant effect of N, genotype, and their interaction on M/B values of the four proteins, with genotype having the larger effect (Table 2).

Comparison of M/B values of the four proteins among the seven treatments confirmed their uneven distribution between milled and brown rice, with albumin showing the lowest value (29.90%), followed by globulin (77.81%), then prolamin (92.71%) and glutelin (93.32%), as shown in Table 5.

Averaged glutelin M/B values across the seven N treatments were 92.89%, 92.74%, and 97.22% for 9522, Ningjing2, and Xudao4, respectively, being relatively higher compared with those small panicle cultivars including Ningjing1 (91.96%), Wuyujing3 (92.95%), and Zaofeng9 (92.16%). Although no significant genotypic variations were detected for all the six cultivars, cultivars with large panicle tended to exhibit higher values for the major storage protein, glutelin (Table 5).

N treatment had significant influence on M/B values, but its effect depended on N rate and protein fractions. M/B of albumin and prolamin showed no clear trend among the seven treatments, while that of globulin increased with N rate (Table 5).

Further, differences in genotypic response of M/B values to N were detected for glutelin. Generally, cultivars with large panicle were more sensitive to N treatments than those with small panicle in terms of protein distribution. CV values were 4.42%, 6.4, and 6.79% for 9522, Ningjing2, and Xudao4, respectively, being higher than those of Ningjing1 (1.33%), Wuyujing3 (2.99%), and Zaofeng9 (2.78%). Of note, small panicle cultivars like Wuyujing3, Zaofeng9, and Ningjing1 are also of good cooking and eating quality.

3.3. N effect on distribution of amino acids between milled and brown rice

Our previous study showed that medium N rate (MN) at ~180 kg/ha can improve both grain yield and nutritional quality for rice production in the agro-ecological region of South Jiangsu (Ning et al., 2009). Considering that Ningjing1 and Ningjing2 differed in