Effect of Different Nitrogen Doses on the Storage Proteins and Palatability of Rice Grains of Primary and Secondary Rachis Branches

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Abstract: The effects of applied nitrogen (N) doses on crude protein and amylose accumulation in rice grains on primary and secondary rachis branches and their palatability were examined. The crude protein content of rice grains on both primary (PRB) and secondary rachis branches (SRB) increased with the increase in N supply showing a strong negative correlation with palatability. The correlation coefficient between protein content of the grains on PRB and SRB was –0.92 and –0.98, respectively. Although the amylose content also decreased with increasing N supply, the change was not significant on either PRB or SRB. However, it showed a good correlation with palatability; i.e., the correlation coefficient on PRB and SRB was 0.93 and 0.84, respectively. Analysis of protein by SDS-PAGE showed that the contents of protein bodies PB-I and PB-II, were correlated with palatability, but the latter had a higher correlation. The grains on PRB showed a higher palatability than those on SRB due to the difference in the accumulation pattern of proteins and amylose. These results suggest that when breeding for varieties with good palatability, the plants with more grains on PRB should be selected and by maintaining the nitrogen level in the field, we can control protein accumulation and thus can improve the palatability of rice crop.

Key words: Amylose, Grain protein, Nitrogen supply, Palatability, Protein bodies, Rice quality, Rice rachis branch.
parameters are known to be directly related to certain physicochemical properties of the endosperm, such as the amylose content, protein content and pasting viscosity (Matsue, 1993). Therefore, an improvement of the rice grain quality should be a major concern of scientists to meet the standards of the international market. The objective of this study was to determine the effect of amount of nitrogen supply on protein and amylose accumulation and palatability of the rice grains of primary and secondary rachis branches.

Materials and Methods

1. Rice culture
   This experiment was conducted in the experimental field of Jeollabuk-do Agricultural Research and Extension Service (JBARES), Iksan, Korea. Seeds of rice genotype Nampyong were sown on 28 May 2010 in nursery boxes with 448 holes (16 mm in diameter; 25 mm depth). Three seeds were sown per hole, and all of the holes had been filled with nursery soil for rice seedlings. The seedlings were grown for 34 days and were then transplanted in the field. Each plot had twenty rows each ten meter long. The field layout was a randomized block design with three replicates. Each hill supported 3–5 plants, and the plant-to-plant and row-to-row spacings were 30 × 15 cm and 30 cm, respectively. Phosphorus and potassium were applied at the rates of 7 and 8 kg 10 a⁻¹, respectively. To assess the effect of nitrogen on the grain proteins, nitrogen was applied at the rate of 0, 5, 9 and 13 kg 10 a⁻¹.

2. Quantification of storage proteins by SDS-PAGE
   Protein bodies were observed in the endosperm cells of Nampyong white rice that was polished to 90% of the brown rice weight using a milling machine. The polished grains of the primary and secondary rachis branches of panicles treated with different doses of nitrogen fertilizer (0, 5, 9 and 13 kg 10 a⁻¹) were collected separately after harvesting. These polished grains were ground 3 times with Percellys 24 (Bertin, France), at 6,000 rpm for 30 s and 100 mg of the ground sample was used for the extraction. The rice protein bodies were extracted with five volumes of an ice-cold solution containing 200 mM Tris-HCl (pH 7.5), 5% (w/v) SDS and 5% 2-mercaptoethanol. After standing overnight at 4°C, the homogenate was boiled for 3 min and centrifuged for 30 min at 30,000 rpm (Kato-Noguchi, 2000). The supernatant was mixed with an equal volume of Tris-HCl buffer (pH 6.8) containing 20% (v/v) glycerol, 5% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.1% (w/v) bromophenol blue, and SDS-PAGE was performed according to the Laemmli method (1970) using 12% polyacrylamide gels. The gels were stained with coomassie brilliant blue R-250, and protein content of each band was measured using a densitometer (GS-800; Bio-Rad, USA).

3. Observation of protein bodies using a scanning electron microscope
   The brown rice samples collected from all of the treatments were observed using scanning electron microscopy (SEM) after cutting the grains at the middle. The samples were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and washed with the same buffer three times. The post-fixation was conducted in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) for two hrs, and washed with distilled water thrice. The samples were dehydrated in a graded series of ethyl alcohol (50, 70, 80, 95 and 100% for 30 min; in 100% 3 times) at room temperature. The specimens were treated with isoamyl acetate three times for twenty min and dried in a critical-point drier with liquid carbon dioxide as the transitional fluid. The samples were coated with a thin layer of gold using a sputter coater under an argon atmosphere. The specimens were observed with a scanning electron microscope (JSM-5410LV; JEOL, Japan).

4. Observations and statistical analysis
   At the time of harvest, twenty hills were randomly selected and collected for observations. The crude protein and amylose content of the rice grains were analyzed using a component analyzer (Infratec 1241, FOSS, Sweden). The palatability was evaluated with a Mido Meter (MB-90A, Toyo Rice Cleaning Machine Co, Ltd., Japan) using 33 g of milled rice per sample (Hwang et al., 2004). All of the experimental data were subjected to a mean variance analysis with SAS (ver. 9.2). Duncan’s new multiple range test at a 5% level (DMRT) (Gomez and Gomez, 1984) was used to separate the means to determine the significant effects.
Results

With the increase of the N supply, a significant increase in the crude protein content of PRB and SRB was observed (Fig. 1, Table 1). When the N supply increased from 9 to 13 kg 10 a<sup>-1</sup>, no significant increase was observed in the protein content of grains on the PRB, whereas increased significantly those on the SRB. The crude protein content of the SRB was higher than that of PRB in all the N treatments and control. Conversely, the amylose content was significantly higher in the PRB than SRB, but was not significantly influenced by the increase of the N supply. The palatability was significantly higher on PRB than on SRB, and the palatability of the grains decreased significantly on both PRB and SRB with increased N. The maximum decrease was observed when the N supply increased from 0 to 5 kg 10 a<sup>-1</sup> (6.9 and 4.9 units), followed by 9 to 13 kg 10 a<sup>-1</sup> (3 and 2.8 units) in the PRB and SRB, respectively, whereas the change was slight when N supply increased from 5 to 9 kg 10 a<sup>-1</sup> on both PRB and SRB (1.0 and −0.2 units, respectively; Table 1). The palatability showed good negative correlation with the crude protein content (−0.92 and −0.98) and a positive correlation with amylose content (0.93 and 0.84) in both PRB and SRB, respectively. In the PRB, the correlation between the palatability and amylose was higher than the SRB, however a higher correlation was observed between the palatability and total proteins in the SRB. Amylose and total protein levels also showed good negative correlations in both PRB and SRB (−0.75 and −0.85, respectively; Table 3).

The main storage proteins of PB-I and PB-II were quantified in the white rice grains of the PRB and SRB (10% polished) using SDS-PAGE (Fig. 2). In grains,
protein content of PB-II was much higher than PB-I on both PRB and SRB. With increasing N supply, protein accumulation increased in PB-I and PB-II complexes of grains on both rachis branches. A maximum increase in the PB-I and PB-II protein accumulation in grains on both PRB and SRB was observed when N was supplied at the rate of 13 kg 10 a⁻¹. For PB-I, the percent increase was higher in the PRB than SRB; however, in the case of PB-II, the percent increase was much higher in the SRB than PRB in all of the treatments (Table 2). PB-II showed a higher correlation (−0.80 and −0.86) with the palatability than PB-I (−0.75 and −0.77) in grains on both the PRB and SRB. The amylose content showed a good negative correlation with PB-I and PB-II (−0.68 and −0.92) in the SRB, whereas a very weak correlation (−0.46 and −0.53) was observed in the PRB (Table 3).

Discussion

Crude protein content was higher in grains on SRB than the PRB, and increased with the increasing N supply. The rate of increase was higher on SRB than PRB (Table 1). It seems that, under a low external nitrogen supply, the rice plants preferentially allocate more nitrogen to the SRB; when the N supply is increased (to 5 and 9 kg 10 a⁻¹), the plants distribute more nitrogen to the PRB (18 and 28%) in comparison to the SRB (16 and 23%). However, if nitrogen is available in surplus, the plants then reallocate more nitrogen toward the SRB (31%) than the PRB (29%). Similar findings were reported by Dong et al. (2007) who found that the crude protein content was increased in the PRB and SRB and that higher protein levels accumulated in the SRB with increases in the amount of nitrogen applied. A similar result was also reported by Zhao et al. (2004) who found that the crude protein content was increased with increased nitrogen application. The amylose content of the grains on the PRB was found to be significantly higher than on the SRB, but no significant decrease was observed with the increased of N. Conversely, Dong et al. (2007) reported that the amylose content increased when the nitrogen was applied at the rates of 0, 120 and 240 kg ha⁻¹. The dissimilar results may be due to the sharp increase of the nitrogen application in contrast to our treatments.

Both in the grains of PRB and SRB, the palatability decreased with the increase N and showed a highly negative correlation with the crude protein and positive correlation with the amylose content, i.e., −0.92 and −0.98 (protein) and 0.93 and 0.84 (amylose), respectively (Table 3). The crude protein content showed a highly negative correlation with amylose both for the PRB and SRB, suggesting that the protein accumulation decreases the amylose accumulation under a high nitrogen supply. The grains on the PRB were superior in palatability to those on the SRB, which suggests that not only the low crude protein content but also the high amylose content are responsible for the high palatability of rice grains. SEM observations further strengthened the above findings (Fig. 1). It was observed that the cooked texture of the rice with a high protein crude content tends to be harder and chewier than that of grains with low protein (Primo et al., 1962; Tamaki et al., 1989). Kim et al. (2009) also reported that all of the sensory evaluation components were largely affected by a change in the crude protein content of brown rice. In a previous report, a decrease in the amylose content had been observed concurrent with an increase in the crude protein content with nitrogen application or uptake (Prakash et al., 2002). Our observations were similar in that the grains on the PRB exhibited a lower crude protein content than those on the SRB at any position within the panicle. The amylose content of the grains on the PRB with superior palatability was higher than that on the SRB. These results suggested that, within a single cultivar under the same cultural conditions, the palatability of cooked grains with a high amylose content and amylogram values was superior to that of the grains with a low amylose content and amylogram values (Matsue et al., 1994, 1995).

The storage protein content data according to the SDS-PAGE analysis showed that the protein content of PB-II was much higher than PB-I, in grains both on PRB and SRB (Table 2). The storage proteins of rice seed are accumulated in two types of particles called protein bodies. PB-I stores indigestible prolamins, whereas PB-II mainly contains digestible proteins glutelins and globulins (Tanaka et al., 1980; Kumagai et al., 2006). Glutelin is the major storage protein of rice and accounts for 80% of the total protein found in the rice grain, which is why the protein content of PB-II was higher than PB-I. However, the total protein content and percent increase of PB-II was more than PB-I, and, although indigestible, the proteins of PB-I were equally important for the palatability of the rice.

The palatability had a negative correlation with PB-I and PB-II in grains both on PRB and SRB. Correlation values suggested that PB-II was more correlated with the palatability; therefore, the increase protein accumulation in PB-II was more responsible for the decrease in the palatability of the grains on both PRB and SRB. In the grains of SRB, PB-I and PB-II showed good negative correlations with amylose. With the increase of N supply, the concentration of protein bodies increased and the amylose content decreased on both PRB and SRB. This result indicated that an increase in the nitrogen level acts as a signal for the plant, stimulating protein accumulation in the protein bodies and decreasing the amylose accumulation during grain filling, which altered metabolism, causing a decrease in the palatability of the SRB grains. Dong et al. (2007) reported that the starch branching enzyme (Q-enzyme) activity was increased when the nitrogen level was increased from 0 kg ha⁻¹ to 120 kg
Moreover, the activity of Q-enzyme was significantly and negatively correlated with the amylose content, suggesting that nitrogen application could regulate the activity of Q-enzyme, thereby affecting the physical and chemical properties of rice. These results manifested that the increase in nitrogen level had a different effect on PRB and SRB; therefore, two different mechanisms might be involved in PRB and SRB. However, how the nitrogen level affects the accumulation of protein and amylose in the grains on PRB and SRB needs to be examined further.

**Conclusion**

From the above discussion it is clear that the crude protein, amylose content, PB-I, PB-II and number of grains on PRB and SRB are important parameters and are mainly responsible for the palatability. The palatability of the entire ear could be influenced by the ratio of the number of grains of the primary and secondary rachis branches, which could lower the overall palatability of the ear. This suggests that, firstly, varieties with more grains on the primary rachis branches of their panicles should be selected for cultivation. Secondly, we can further improve the palatability of rice by the control of protein accumulation by maintaining optimum nitrogen level in the field.

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