Nitrogen fertilizer alters milling quality and protein distribution in head rice

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Abstract

Nitrogen (N) application is often essential for maintaining rice grain yield. Although many studies have indicated that application of N fertilizer increased the milling quality of rice grain, the underlying process remains unclear. This study examined protein distribution in the endosperm of four common Thai rice cultivars with low (1.40?0.02 %) and high (2.18?0.04 %) head rice N concentration and relationship to percentage of head rice yield (the percentage of whole milled rice). The grain of four cultivars, KDML105, HKL1, HPTT1 and CN1, grown in the wet season of 2001 with nil and 120 kg N ha^{-1} at flowering were evaluated for milling quality and storage protein distribution in the endosperm. Head rice recovery varied with cultivar and N fertilizer treatment. Without N fertilizer, there was less endosperm breakage in KDML105 and HPTT1 than CN1 and HKL1 and this was attributed to higher protein packaging in the former cultivars in the lateral part of the endosperm. The addition of N fertilizer increased lateral protein accumulation in CN1 and HKL1 and there was a corresponding decrease in endosperm breakage.

Media Summary

The study shows that increasing endosperm storage protein reduces wastage during milling, resulting in increased returns to farmers.

Key Words

Rice (*Oryza sativa* L.), Nitrogen, Endosperm storage protein, Milling quality

Introduction

In rice production, milling quality is an increasingly important factor determining the income of farmers. The market value of rough rice is based, to a large extent, on its milling quality and milling yield. Milling quality is defined as the head rice recovery after milling (Brorsen *et al.*, 1984). Generally, the head rice fraction contains all of the whole rice and rice greater than 3/4 in length. Head rice is the primary factor that determines prices in world rice markets. Many studies have been conducted to investigate factors affecting milling quality, including genetics (Jongkaewwattana *et al.,* 1993; Nangju and De Datta, 1970), field management (Jongkaewwattana, 1990; Yoshida, 1981) and environmental conditions during crop growth (Henderson, 1954; Yoshida and Hara, 1976).

Nitrogen fertilization is one management tool that affects rice yield and milling quality. Seetanun and De Datta (1973) reported that topdressing with N at flowering increased head rice yield of IR8, IR20, RD1 and C4-63, and this was associated with higher protein content in milled rice. Applying N fertilizer close to booting can enhance photosynthetic capacity during the grain filling period, leading to an increase in head rice yield (Japanese Food Agency, 1998). Wopereis-Pura *et al.* (2002) showed that grain yield increased with the addition of 30 kg N ha^{-1} at booting by about 5 and 25% during the wet and dry seasons, respectively. In spite of these findings, knowledge of how N fertilizer application can reduce endosperm breakage during milling remains unclear. The objective of this study was to determine effects of N fertilizer application on head rice N concentration, protein distribution in the endosperm and milling quality of four Thai commercial rice cultivars.

Materials and Methods

Four cultivars (Khao Dawk Mali (KDML) 105, Hawm Khlong Luang (HKL) 1, Hawm Pathum Thani (HPTT) 1 and Chainat (CN) 1) were grown in the Multiple Cropping Centre Experiment Station, Chiang Mai, in the wet season 2001 with nil and 120 kg N ha^{-1} at flowering and rough rice was hand-harvested at maturity. Rough rice (100 g samples) dried at room temperature to a moisture content of 14% (wet basis), was dehulled (sheller series P-1, Ngek Seng Huat LTD., Thailand) and the resulting brown rice was polished for 30 sec (miller series K-1, Ngek Seng Huat LTD., Thailand) to obtain milled rice. The milled rice was separated manually into head and broken rice (<3/4 in length). Head rice was analyzed for N concentration by titration after Kjeldahl digestion (Yoshida *et al.*, 1976). Head rice N concentration and percentage of head rice were analyzed by analysis of variance (ANOVA) and regression. Significantly different means were separated at $P = 0.05$ by the least significant difference (LSD) test.

Five head rice kernels from each of the four genotypes with low and high head rice N concentrations were trimmed and fixed in 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0 at room temperature. The samples were dehydrated in an alcohol series and embedded in glycol methacrylate (O'Brien and McCully, 1981). Transverse sections (2.5 ?m) were cut through centrally on a Sorvall-microtome (JB-4) and stained with 1% amido black 10B for storage protein. Slides were examined in an Axioskop II plus (Zeiss, Germany) compound microscope and representative areas were captured with a Ziess Axiocam digital camera.

Results

Head rice N concentrations of the four cultivars were similar (average 1.4%) with no N fertilizer but increased by 50-60% with the application of 120 kg N ha^{-1} at flowering in all cultivars (Table 1). There was a positive correlation (r = 0.89*) between head rice N concentration and head rice yield in CN1 and HKL1 but not in KDML105 and HPTT1 (r = -0.05).

Table 1. Effect of N treatment on head rice N concentration (%), percentage of head rice and relative storage protein distribution in the lateral, dorsal and ventral regions of head rice endosperm of four Thai rice cultivars.

Cultivar	N treatment (kg N ha^{-1})	Head rice N concentration (%)	Percentage of head rice	Peripheral protein distribution score*		
				Lateral	Dorsal	Ventral
KDML105	0	1.43a	95.0a	4	1	1
	120	2.21b	93.5ab	6	3	3
HPTT1	0	1.35a	90.7c	4	1	1
	120	2.18b	91.3bc	6	3	3
HKL1	0	1.43a	85.4d	3	1	1
	120	2.26b	90.5c	6	3	3

CN1	0	1.37a	79.1e	2	1	1
	120	2.08b	89.2c	4	3	3
F-test (LSD $_{0.05}$)	N	*** (0.07)	*** (1.3)			
	Cultivar	* (0.09)	*** (1.9)			
	N x Cultivar	ns	*** (2.6)			

The different letters indicate significant difference at *P* = 0.05.
* Relative score for protein abundance based on intensity of staining by amido black: 1 = few protein matrixes to 6 = numerous protein matrixes

Most of the storage protein in the milled rice was located in the peripheral part of the endosperm (Figure 1). Storage protein showed up clearly with 1% amido black 10B positive staining as matrixes between the starch granules. Nitrogen fertilizer increased the score for protein in the three peripheral regions examined (Table1). There was no obvious difference between cultivars in the relative distribution of protein matrix in the dorsal and ventral regions at the same N supply. However, the amount of storage protein in the lateral region was affected by both cultivar and N fertilizer (Table 1). Without N fertilizer, the score for lateral protein accumulation was highest in KDML105 and HPTT1, followed by HKL1, and CN1. KDML105 and HPTT1 had higher lateral protein scores even though grain N concentration was up to 22% lower than the other two cultivars (data not presented). The lateral storage protein score correlated with head rice N concentration (r = 0.91**) and percentage of head rice (r = 0.69*) for all four cultivars.

The results of our study suggest that the greater abundance of insoluble protein store into the peripheral region of the endosperm was closely associated with less endosperm breakage during milling. However, two contrasting cases were apparent. Firstly, histological examination suggests that KDML105 and HPTT1 preferentially allocated more protein into the lateral region at low N supply, and application of N fertilizer did not reduce breakage even though rice N concentration increased. Secondly, breakage in CN1 and HKL1 was reduced with an apparent increase in protein storage due to N fertilization. These findings suggest that there is likely to be a threshold level of protein accumulation in the peripheral part of the grain that is required to prevent breakage during milling. We suggest that in some cultivars, this level is sensitive to external N supply. These conclusions are also supported by the results of other workers who have observed increases in head rice and milled rice protein (Perez *et al.*, 1996). Furthermore, Cagampang *et al.* (1966) found that brown rice with higher protein content was more resistant to abrasive milling than brown rice with lower protein content in the same cultivar. The lack of association of head rice yield and N concentration observed in KDML105 and HPTT1 has previously been reported for C4-63 (Nangju and De Datta, 1970) and IR22 (Seetanun and De Datta, 1973). Further work is required to determine whether these cultivars also have abundant protein matrixes in the lateral parts of the grain as shown for KDML105 and HPTT1 at low N supply.

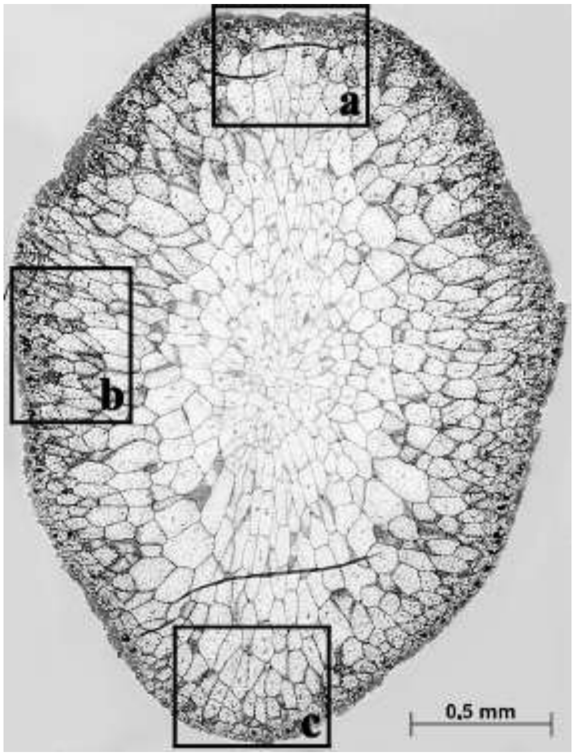

Figure 1. Storage protein (positively staining by 1% amido black 10B) distribution in rice endosperm (transverse section) of KDML105 with low grain N concentration [dorsal (a), lateral (b) and ventral region (c)].

Conclusion

Application of N fertilizer increased head rice N and the distribution of storage protein into the peripheral part of the endosperm in the studied cultivars. The lateral protein scores were closely correlated with head rice N concentration and percentage of head rice indicating a possible indirect effect of storage protein in preventing endosperm breakage during milling. Genotypic variation in the relationship between storage protein distribution in rice endosperm and endosperm breakage is being investigated.

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