Nitrogen Fertilizer Increases Seed Protein and Milling Quality of Rice

M. Leesawatwong,[1–4] S. Jamjod,[2,3] J. Kuo,[1] B. Dell,[4] and B. Rerkasem[2]

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ABSTRACT

Rice grain breakage during milling is a problem in many parts of Asia. It has been suggested that nitrogen (N) fertilizer can improve the milling quality of rice. Therefore, this study investigates effects of N fertilization on grain N concentration, endosperm storage protein distribution, and milling quality of rice. Four Thai extra long grain commercial rice cultivars (KDML105, KLG1, PTT1, and CNT1) were grown at Chiang Mai University in the wet season of 2001 with 0 or 120 kg of N/ha at flowering. Anatomical sections showed that there was more storage protein accumulated in the lateral regions of polished grain of high N concentration than in grain of low N concentration. Percent (%) unbroken rice was positively correlated with relative abundance of storage protein in the lateral region of the endosperm in all cultivars. Applying N increased head rice N concentration in all cultivars, whereas % unbroken rice was increased in KLG1 and CNT1. KDML105 cultivar, on the other hand, already had high % unbroken rice and more abundant storage protein in the lateral region with the grain of low N concentration. It is hypothesized that high density of storage protein in the lateral region of the endosperm provides resilience and lessens grain breakage during milling. The additional protein may increase hardness in rice grains and thus could make the rice more resistant to breakage during milling. Furthermore, N fertilization may enhance the nutritional quality of rice grain by increasing the glutelin content, which is rich in lysine.

Application of nitrogen (N) fertilizer can affect rice yield and milling quality. Wopereis-Pura et al (2002) reported that applying 30 kg of N/ha at flowering increased grain yield by ≈0.4 and 1.0 ton/ha in the wet and dry seasons, respectively. Furthermore, the application of 75 kg of N/ha improved head rice of IR8 by 7% over the 0 kg N treatment (Fagade and Ojo 1977). Seetanum and De Datta (1973) showed that topdressing with N fertilizer at flowering increased % head rice and grain protein of IR8, IR20, RD1, and C4-63. However, responses in head rice yield (HRY) can be genotype dependent (Perez et al 1996). For example, Borrell et al (1999) found that HRY was positively related to grain N concentration in Lemont, whereas it was not related in Starbonnet. Furthermore, Nangju and De Datta (1970) and del Rosario et al (1968) suggested that N fertilization can increase the packing of protein matrix between endosperm starch, resulting in reduced grain breakage during milling, but definitive data of N fertilization effect on grain breakage are not yet available. This study investigates the effect of N fertilization on soluble protein concentration and storage protein distribution in the endosperm of four Thai commercial rice cultivars. The data are interpreted in relation to rice milling quality.

MATERIALS AND METHODS

Materials

Rough rice samples of the cultivars Khao Dawk Mali (KDML) 105, Pathum Thani (PTT) 1, Khlong Luang (KLG) 1, and Chainat (CNT) 1 were collected from a paddy field N experiment in the wet season of 2001. Two N (urea) treatments were applied, 0 kg (N0) and 120 kg of N/ha once at flowering (N120). There were three replicated plots (5 × 6 m) per cultivar per N treatment. The grain yield averaged 2.7 ton/ha with no significant difference between N treatments. Rough rice samples were harvested by hand at the same stage of physiological maturity (≈22–24% grain moisture content, wb) (Nangju and De Datta 1970; Berrio and Cuevas-Perez 1989) and air-dried at room temperature to a moisture concentration of 14% wb and were then stored in sealed plastic bags at ≈4°C.

[1] Centre for Microscopy and Microanalysis, University of Western Australia, Crawley 6009, Australia.

[2] Agronomy Department, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.

[3] Corresponding author: Phone: 665-394 4621. Fax: 665-321 0000. E-mail: agosjmjd@chiangmai.ac.th; g4368102@cm.edu

[4] School of Biological Sciences and Biotechnology, Murdoch University, Perth 6150, Australia.

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Milling Quality

Rough rice samples (100 g) were dehulled (Sheller series P-1, Ngek Seng Huat, Ltd., Thailand) and the resulting brown rice was weighed. Brown rice was polished for 30 sec (Miller series K-1, Ngek Seng Huat) to obtain milled rice. The milled rice was separated into head and broken rice and weighed. The % unbroken rice was calculated by HRY as milled rice yield.

N Concentration

The brown, head, and broken rice (5 g of each) were ground into flour using a stainless steel coffee grinder. Flour (2 g) was digested in conc. H_2SO_4 (Yoshida 1976) and analyzed for grain N concentration using a flow injection analyser (Quick-Chem 8000) after digestion by the Kjeldahl method.

Physical Properties

For each cultivar and N treatment, 100 individual brown rice grains were weighed and measured for length, width (length from dorsal to ventral sides), and thickness (length from both lateral sides). The L/W ratio was calculated and used to classify the grain categories of extra long grain (>7.50 mm), long grain (6.61–7.50 mm); medium grain (5.51–6.50 mm), and short grain (<5.50 mm). The L/W ratio was classified for grain shapes of slender (>3.0), medium (2.0–3.0), bold (1.1–2.0), and round (<1.1) (Jennings et al 1979).

Soluble Protein

Extraction methods of soluble protein were adapted from Juliano and Boulter (1976) and Villareal and Juliano (1978). Rice flour powder (0.25 g) was extracted with 5 mL of 0.5*M* NaCl for albumin-globulin by shaking for 1.5 hr, then the suspension was centrifuged at 734.5 × *g* for 10 min, the supernatant was kept, and the residue was extracted two times with 5 mL of 0.5*M* NaCl for 1 hr. The extracted residue was washed three times with distilled water and re-extracted two times for 30 min with 5 mL of 70% ethanol containing 0.6% β-mercaptoethanol for prolamin, and the suspension was then centrifuged at 734.5 × *g* for 10 min. The supernatant was kept and the residue was washed two times with distilled water. The washed residue was extracted for 2 hr with 0.5% SDS containing 0.6% β-mercaptoethanol and then centrifuged at 734.5 × *g* for 10 min. The supernatant was kept. The supernatants from all extractions were analyzed for protein concentration by the method of Bradford (1976).

Protein Distribution in Rice Endosperm

Central transverse slices (2 cm wide) of head rice were fixed in 2.5% gluteraldehyde in 0.05*M* phosphate buffer, pH 7.0, for 24 hr at 20°C and dehydrated in an alcohol series and infiltrated in glycol methacrylate (O'Brien and McCully 1981). Transverse-sections (2.5 μm) of three head rices from each cultivar and each N treatment were cut with glass knives using a Sorvall-microtome (JB-4). Sections were stained with 1% amido black 10B for storage protein. Stained slides were examined with a compound microscope (Axioskop II Plus, Zeiss, Germany) and representative areas were captured with a digital camera (Ziess Axiocam). The three images of each treatment (0.5 × 0.5 mm) were analyzed for storage protein distribution in the grain sections shown in Fig. 1.

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) in a randomized complete block design and simple linear regression. Significant different means were separated at $P < 0.05$ by the least significant difference (LSD) test. Statistical analyses were made with commercial software.

RESULTS

Milling Quality

The milled rice yield was 52% of rough rice, which was not significantly different between N treatments (data not shown). With no fertilization, the % unbroken rice was highest (95%) in KDML105, intermediate in PTT1 and KLG1, and lowest (79%) in CNT1 (Table I). Applying 120 kg of N/ha at flowering increased % unbroken rice of CNT1 to 89% and KLG1 from 85.5 to 90.5%. However, the N120 application had no effect on % unbroken rice in KDML105 and PTT1.

Grain N Concentration

Nitrogen fertilization significantly improved N concentrations in brown, head, and broken rice in all four cultivars (Table II). Applying 120 kg of N/ha at flowering increased grain N concentration from 1.3 to 2.0% N or higher. In general, CNT1 had low N concentrations in both head and broken rice at both levels of treatment. It appears that polishing tended to decrease grain N concentration in CNT1 much more than the other cultivars (data not shown).

Physical Properties of Rice Grain

Increasing N concentration did not significantly influence the physical properties of brown rice of the four cultivars (Table III). Length and diameter dimensions differed between cultivars. The grain size of all cultivars was classified as extra long with length of 7.5–8.0 mm, width of 2.0–2.4 mm, and thickness of 1.6–1.8 mm. The L/W ratio varied with cultivar and was the highest in KDML105 (3.60) and the lowest in KLG1 (3.35). The grain shape of all cultivars was classified as slender because the L/W ratio was >3 in all four cultivars.

TABLE I
Effect of N Treatment (N) on % Unbroken Rice of Four Thai Rice Cultivars (C)[a]

	% Unbroken Rice	
Cultivar	N0	N120
KDML105	95.0aA	93.5aA
PTT1	90.7aB	91.3aAB
KLG1	85.4aC	90.5bB
CNT1	79.1aD	89.2bB
	F-test	$LSD_{0.05}$
N × C	***	2.6

[a] Lower and upper case letters are for comparison between columns and rows, respectively. Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE II
Effect of N Treatment (N) on Grain N Concentration of Brown, Head, and Broken Rice Types (T)[a]

	N Concentration (%)	
Type	N0	N120
Brown rice	1.33b	2.03c
Head rice	1.27ab	2.00c
Broken rice	1.25a	2.05c
	F-test	$LSD_{0.05}$
N × T	*	0.06

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

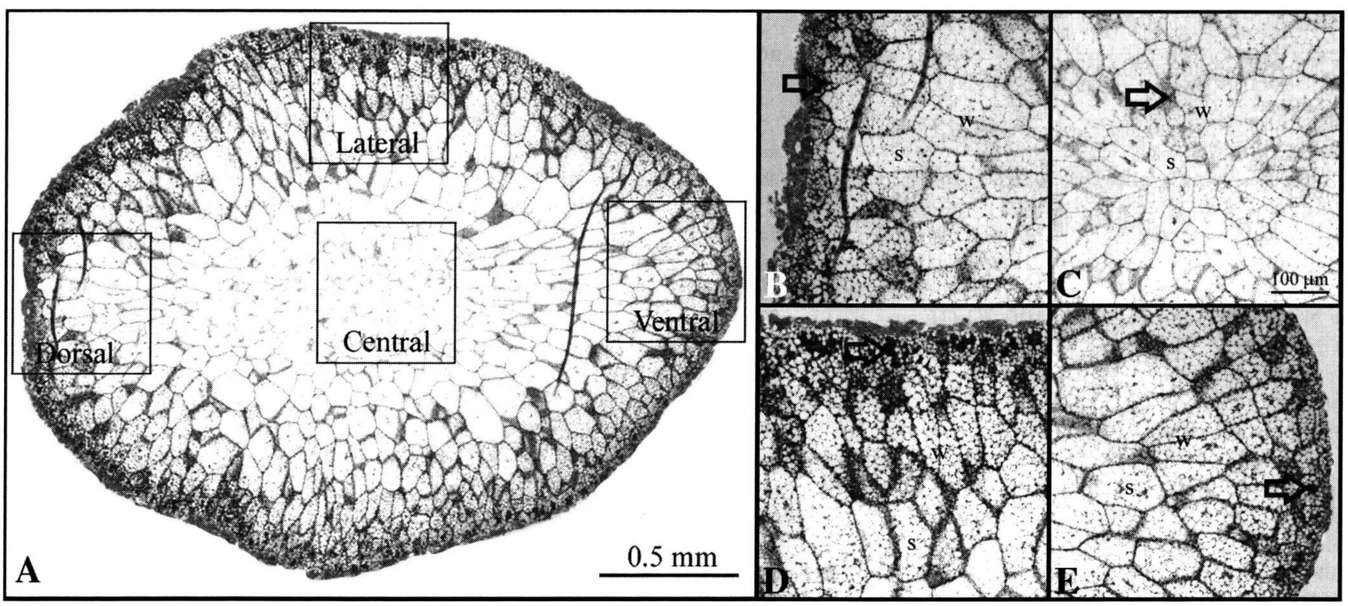

Fig. 1. Storage protein accumulation (positive staining with 1% amido black 10B [arrow]) in rice endosperm of low N KDML105. **A,** transverse section of rice endosperm; **B–E,** high magnification of dorsal, central, ventral, and lateral regions, respectively. W, cell wall; S, starch granules.

TABLE III
Effect of N Concentration (N) on Brown Rice Dimensions of Four Thai Rice Cultivars (C)[a]

	Grain Dimension (mm)											
	Length			Width			Thickness			L/W Ratio		
Cultivar	Low	High	Mean	Low	High	Mean	Low	High	Mean	Low	High	Mean
KDML105	7.53	7.47	7.50c	2.11	2.06	2.09d	1.66	1.64	1.65c	3.58	3.63	3.60a
PTT1	7.61	7.57	7.59bc	2.16	2.13	2.15c	1.75	1.74	1.74b	3.52	3.55	3.54b
KLG1	7.92	7.81	7.86a	2.38	2.32	2.35a	1.82	1.82	1.82a	3.33	3.37	3.35d
CNT1	7.74	7.73	7.74ab	2.22	2.22	2.22b	1.71	1.75	1.73b	3.50	3.49	3.49c
Mean	7.70	7.70		2.22	2.18		1.73	1.74		3.48	3.51	
	F-test	$LSD_{0.05}$		*F*-test	$LSD_{0.05}$		*F*-test	$LSD_{0.05}$		*F*-test	$LSD_{0.05}$	
N	ns	–		ns	–		ns	–		ns	–	
C	*	0.22		***	0.05		**	0.05		***	0.03	
N × C	ns	–		ns	–		ns	–		ns	–	

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE IV
Effect of N Concentration (N) on Individual Brown Rice Weight of Four Thai Rice Cultivars (C)[a]

	Individual Grain Weight (mg)		
Cultivar	Low N	High N	Mean
KDML105	19.6	19.3	19.5c
PTT1	22.1	21.6	21.8b
KLG1	25.2	25.1	25.2a
CNT1	22.9	22.8	22.8b
Mean	22.5	22.2	
	N	C	N × C
F-test	ns	***	ns
$LSD_{0.05}$	–	1.3	–

[a] Values followed by the same letter are not significantly different ($P < 0.05$).

Individual grain weight of brown rice was higher in KLG1 than the other three cultivars (Table IV) and did not differ between N concentrations. However, brown rice weight was associated with grain width and thickness but not with grain length as described in Equation 1 and also closely correlated with the L/W ratio as described in Equation 2.

$$BW = -0.32L^{ns} + 13.52W^{*} + 11.51T^{*} - 24.92\ (r^2 = 0.94^{***}) \quad (1)$$

$$BW = -20.08LW^{***} + 92.62\ (r^2 = 0.86^{***}) \quad (2)$$

where BW = brown rice weight, L = length, W = width, and T = thickness.

Soluble Proteins

Soluble proteins in brown, head, and broken grain were analyzed in three fractions: glutelin, prolamin and albumin-globulin. Nitrogen fertilizer increased soluble protein fractions differently, with some differences among the cultivars. The glutelin concentration of grain with high N concentration was double that of grain with low N concentration in KDML105, KLG1, and PTT1, and 2–3× in CNT1 (Table V). Without N fertilizer, the glutelin concentration was especially low in CNT1, being half or less of the glutelin concentration of the other three cultivars. Polishing increased the glutelin concentration in PTT1, CNT1, and KDML105 but not in KLG1. The prolamin concentration did not respond to grain N concentration in brown rice except for a small increase in KLG1 and CNT1 (Table VI). The effect of polishing on prolamin concentration of grain depended on grain N concentration and cultivar. With low N concentration, polishing decreased prolamin concentration in all cultivars. However, in grain of high N concentration, polishing increased prolamin concentration in KDML105 and CNT1 but depressed it in KLG1 and PTT1. The albumin-globulin concentration was higher in brown than in polished rice (Table VII). Brown, head, and broken rice with low N concentrations had depressed albumin-globulin concentrations in KDML105 but not in KLG1, PTT1, and CNT1.

Storage Protein in Endosperm

Anatomical sections (Fig. 1) showed that storage protein was more abundant in the lateral region of rice endosperm. Increasing N concentration increased the density of storage protein in all regions of the endosperm (Fig. 1, Table VIII). This was particularly evident in the lateral region in all cultivars (Fig. 2). Furthermore, at low N concentration, staining for storage protein was more intensive in KDML105 than in the other three cultivars (Table VIII, Fig. 2). The relative abundance of storage protein in the lateral region was correlated with % unbroken rice of all cultivars (Fig. 3).

DISCUSSION

The present study showed that applying 120 kg of N/ha at flowering increased % unbroken rice of CNT1 and KLG1. Similar findings have been documented for a number of other rice cultivars. Seetanun and De Datta (1973) showed that IR20 and RD1 had a higher % unbroken rice when N fertilizer was applied at heading than when N was applied at panicle initiation or at transplanting. Applying N fertilizer up to 120 kg/ha in the dry season improved milling quality of chalky cultivars IR8, IR5, and Sigadis, but not in C4-63, a nonchalky cultivar (Nangju and De Datta 1970). N fertilization did not affect % unbroken rice of KDML105 and PTT1. Both cultivars are similar to high HRY cultivars such as IR22 (Seetanun and De Datta 1973) and Starbonnet (Berrell et al 1999), where there were no further increases in HRY with N topdressing. These findings suggested that some cultivars grown with low amounts of N fertilizer may also have produced good HRY.

Nitrogen fertilization at flowering increased head rice N concentration in the four studied cultivars by up to 65%. De Datta et al (1972) and Perez et al (1996) reported that topdressing N at flowering increased head rice N concentration by 30–60%. Polishing, however, did not affect milled rice N concentration, except that it was slightly depressed in CNT1. Protein concentration was not significantly different in brown and head rice (Resurreccion et al 1979). Therefore N applications may be able to increase the nutritional quality of milled rice by increasing protein concentration.

Increased N concentration resulted in increased soluble protein fractions in the grain. Glutelin, the main storage protein of rice, was the soluble protein fraction most increased by N fertilization. Because glutelin is relatively rich in lysine (Juliano et al 1973), N fertilizer that can increase the nutritional value of rice by improving milled rice protein by 2% (from 7 to 9%) will double the protein intake in the Asian diet from 10 to 20%. The effect of polishing on soluble protein fractions differed among cultivars.

TABLE V
Effect of N Concentration (N) on Glutelin Concentration (G) of Brown, Head, and Broken Rice Types (T) of Four Thai Rice Cultivars[a]

		Glutelin Concentration (mg/g)		
Cultivar	**N Concentration**	**Brown Rice**	**Head Rice**	**Broken Rice**
KDML105	Low N	9.05aB	10.07bB	21.00cC
	High N	22.14aE	23.07aD	28.63bD
PTT1	Low N	8.32aB	12.67bC	18.43cC
	High N	20.51aDE	25.61bD	29.45bD
KLG1	Low N	11.27aC	10.77aBC	14.22bB
	High N	22.95aE	23.89aD	28.25aD
CNT1	Low N	3.79aA	6.62bA	10.63cA
	High N	17.51aD	21.23bD	26.88cD

	N	G	T	N × G	N × T	G × T	N × G × T
F-test[b]	*	***	***	**	***	***	*

[a] Lower and upper case letters are for comparison between columns and rows, respectively. Values followed by the same letter are not significantly different ($P < 0.05$).
[b] Data transformed by Log_{10}.

TABLE VI
Effect of N Concentration (N) on Prolamin Concentration (G) of Brown, Head, and Broken Rice Types (T) of Four Thai Rice Cultivars[a]

		Prolamin Concentration (mg/g)		
Cultivar	**N Concentration**	**Brown Rice**	**Head Rice**	**Broken Rice**
KDML105	Low N	2.60cBC	1.29aA	1.76bC
	High N	2.22aB	5.32bD	5.00bD
PTT1	Low N	1.90bAB	1.35aAB	1.35aB
	High N	2.30bBC	2.06abB	1.75aC
KLG1	Low N	1.72aA	1.37aAB	1.58aBC
	High N	2.37bBC	1.77aB	1.56aBC
CNT1	Low N	2.08bAC	1.09aA	0.93aA
	High N	2.83aC	3.98bC	5.25cD

	N	G	T	N × G	N × T	G × T	N × G × T
F-test[b]	***	***	ns	***	***	***	***

[a] Lower and upper case letters are for comparison between columns and rows, respectively. Values followed by the same letter are not significantly different ($P < 0.05$).
[b] Data transformed by Log_{10}.

TABLE VII
Effect of N Concentration (N) on Albumin-Globulin (G) Concentration of Brown, Head, and Broken Rice Types (T) of Four Thai Rice Cultivars[a]

		Albumin-Globulin Concentration (mg/g)			
Cultivar	**N Concentration**	**Brown Rice**	**Head Rice**	**Broken Rice**	**Mean**
KDML105	Low N	7.09	4.62	5.42	5.71D
	High N	4.07	2.75	2.95	3.26A
PTT1	Low N	6.49	4.08	4.43	5.00C
	High N	5.11	3.94	4.77	4.61C
KLG1	Low N	6.83	3.82	4.28	4.97C
	High N	6.19	4.25	4.45	4.97C
CNT1	Low N	5.67	3.34	3.42	4.14B
	High N	5.95	3.52	4.37	4.61C
	Low N	6.52cB	3.96aB	4.39bA	
	High N	5.33cA	3.62aA	4.14bA	

	N	G	T	N × G	N × T	G × T	N × G × T
F-test[b]	ns	**	***	***	*	ns	ns

[a] Lower and upper case letters are for comparison between columns and rows, respectively. Values followed by the same letter are not significantly different ($P < 0.05$).
[b] Data transformed by Log_{10}.

Polishing decreased the albumin-globulin concentration in all cultivars. However, polishing increased the glutelin concentration in PTT1 and CNT1, increased prolamin concentration in KDML105 and CNT1, but depressed the prolamin concentration in KLG1 and PTT1. Work undertaken by Cagampang et al (1966), Houston et al (1968), and others suggest that storage protein types are not evenly distributed across the grain, with albumin and globulin more abundant in the outer region of the grain (including the aleurone layers) and glutelin increasing in proportion toward the center of the endosperm. By contrast, prolamin is more evenly distributed across the endosperm. This work suggested that both glutelin and prolamin may have little influence on grain breakage. On the other hand, albumin-globulin could reduce grain breakage because they are more abundant in the peripheral region of rice grain.

The application of N fertilizer also increased the relative abundance of storage proteins in the endosperm. This was particularly evident in the lateral region, where breakage generally occurs during milling. Protein abundance, measured by image analysis, was correlated with grain breakage during milling ($r^2 = 0.46$*).

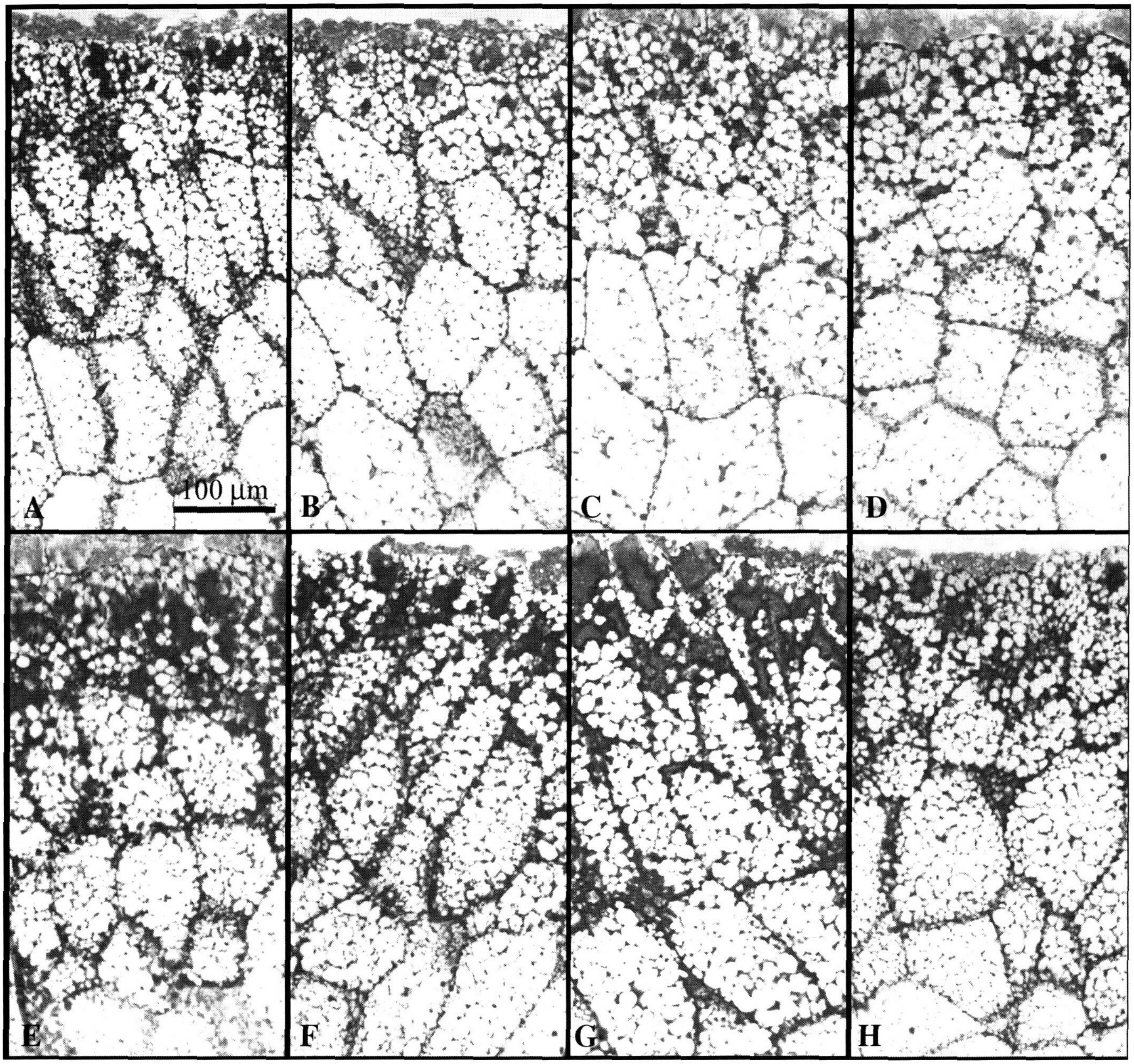

Fig. 2. Storage protein (positive staining with amido black 10B) distribution in the lateral region of rice endosperm of low N (**A–D**) and high N (**E–H**) KDML105 (**A, E**), PTT1 (**B, F**), KLG1 (**C, G**) and CNT1 (**D, H**).

TABLE VIII
Effect of N Concentration (N) on Storage Protein Distribution in Different Parts of Rice Endosperm of Four Thai Rice Cultivars (C)[a]

	Storage Protein Distribution in Rice Endosperm (%)[b]							
	Central		Dorsal		Ventral		Lateral	
Cultivar	Low N	High N	Low N	High N	Low N	High N	Low N	High N
KDML105	4	4	16bA	31aA	14	26	29bA	35aA
PTT1	5	5	17aA	21aB	21	28	22bB	36aA
KLG1	4	7	14bA	28aA	14	40	18bB	37aA
CNT1	4	8	18bA	27aA	13	26	19bB	33aA
Mean	4b	6a	16	27	15b	30a	22	35
	F-test	$LSD_{0.05}$	*F*-test	$LSD_{0.05}$	*F*-test	$LSD_{0.05}$	*F*-test	$LSD_{0.05}$
N	*	1.7	***	2.2	***	4.7	***	2.2
C	ns	–	*	3.1	ns	–	***	3.1
N × C	ns	–	*	4.4	*	9.4	***	4.4

[a] Lower and upper case letters are for comparison between columns and rows, respectively. Values followed by the same letter are not significantly different ($P < 0.05$).
[b] Mean values of protein distribution rating on the specific part of three rice grains.

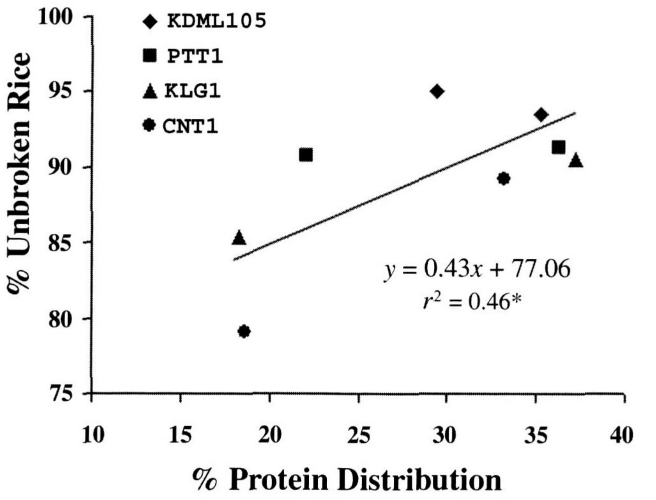

Fig. 3. Relationship between % protein distribution in the lateral portion with % unbroken rice of four Thai rice cultivars.

These conclusions are supported by the results of a small number of other workers who observed N fertilizer increases in head rice protein (Perez et al 1996). Furthermore, Nangju and De Datta (1970) and del Rosario et al (1968) found that head rice increased application of N fertilizer up to a certain level. They suggested that the effect of N on decreasing breakage might have been enhanced because protein bodies occupy the space between unpacked starch granules and thus function as a binder for starch. Studies in wheat suggest that the structure of the protein matrix surrounding starch granules (Barlow et al 1973; Stenvert and Kingswood 1977) and the interface between the protein matrix and starch granules (Greenwell and Schofield 1986; Greenblatt et al 1995) are the physicochemical bases of endosperm hardness. Although, we did not measure hardness in this study, we found the same association between concentration of protein bodies and resistance to milling breakage in rice. Increasing storage protein in the lateral region of rice grains may improve milling quality. This finding should be examined further, by identifying the factors controlling accumulation of storage protein in the lateral region of the grain.

CONCLUSIONS

In conclusion, the effect of N fertilizer on milling quality and HRY differed with cultivar. PTT1, KLG1, and CNT1 showed a positive response in grain N concentration and HRY, but KDML105 was not affected. KDML105, however, had more abundant storage protein in the lateral region of rice endosperm. The results suggest that high density of storage protein in the lateral region of the endosperm can affect breakage. Although the mechanism for reducing breakage is unknown, the additional protein may increase hardness in rice grains and thus could make the rice more resistant to breakage during milling. Furthermore, N fertilization may enhance the nutritional quality of rice grain by increasing the glutelin content, which is rich in lysine.

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