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Response of Grain Yield and Milled-Rice Protein Content to Nitrogen Rates Applied at Different Growth Stages of Rice

Hung The Nguyen*, Min-Ho Kim, Lan Thi Nguyen***, and Byun-Woo Lee**†**

**Faculty of Resource and Environment Management, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen City, Vietnam*
***Department of Plant Science, College of Agriculture and Life Science, Seoul National University, Republic of Korea*
****Faculty of Agronomy, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen City, Vietnam*

ABSTRACT : Response of grain yield and milled-rice protein content to nitrogen (N) rates at various growth stages is critical for quantifying real-time and real-amount of applied N requirement for target grain yield and protein content. An experiment including 10 N rate treatments at transplanting, tillering and panicle initiation stages with four rice cultivars in 2003, 6 N treatments with two rice cultivars in 2004 and 2005 was conducted. Increase of N rates at PIS significantly increased both grain yield and milled-rice protein content but increase of N rates at tillering stage significantly increased grain yield but not milled-rice protein content. Therefore, high grain yield and low milled-rice protein content would be difficult to obtain only by adjusting N rates at PIS. Internal N use efficiency (INUE) was 60.5 kg grain/kg N accumulation on an average over N treatments, cultivars, and experimental years, showing considerable reduction especially at high shoot N accumulation in the experimental year of low sunshine duration. Milled-rice protein content tended to increase almost linearly with increasing shoot N accumulation, but it revealed big variation even at the same shoot N accumulation at harvest. Milled-rice protein content decreased with increasing INUE. N accumulation in the milled rice increased at an almost constant proportion of 45.5 percent of the shoot N accumulated at harvest, showing slight decresing proportion with the increasing shoot N accumulation.

Keywords: rice, nitrogen, yield, protein

Rice is the most important in food crop in most of the Asian countries. However, it is suggested that the current average rice yield of 5.3 ton per hectare per crop is equivalent to only 60% of climate-adjusted yield potential in Asia (Matthews *et al.*, 1995). Therefore, improvement of new rice cultivars with high yield potential and resistance in parallel with the improved nutrient management, especially nitrogen (N) fertilizer management would largely contribute to rice yield improvement in near future (Cassman *et al.*, 1998; Peng *et al.*, 2005; Dobermann *et al.*, 2002).

Effect of N fertilizer in diverse environmental conditions on plant growth and N status, N use efficiency, yield and yield components, and milled-rice protein content have been intensively studied and reported worldwide (Ramasamy *et al.*, 1997; Cassman *et al.*, 1998; Dobermann *et al.*, 2002; Peng *et al.*, 2005; Kim, 2004; Nguyen, 2005). They agreed that the effect of applied N fertilizer on plant growth and yield was high but variable among locations (spatial variation) and time (temporal variation) and that is why current research conducted in many countries has focused on solving limited current widespread blanket fertilizer recommendation for large rice production area in several years.

The optimum N rate and timing are dependent on crop N demand, which possibly in turn is dependent on crop N nutrition status at a given crop growth and development stages that are important for grain yield formation. Based on critical stages for rice growth and development, N fertilizer has been recommended to be applied in several splits: at transplanting for crop recovery after transplanting, at tillering stage for increasing tiller and panicle number, at panicle initiation stage (PIS) for increasing number of spikelets per panicle, and at full flowering for increasing 1000-grain weight and ripening percentage (De Datta, 1981). Yanagisawa *et al.* (1967) reported the difference in recovery efficiency of N fertilizer at different time of application. He found that the highest N recovery efficiency was 55% of N applied at 15 days before heading, and 49% at flowering, 34% at tillering and 7% at transplanting. Patnaik & Broadbent (1967) showed that 18% of tracer ^{15}N was absorbed when N was applied at maximum tillering stage and 45% when N was applied at booting stage. Kim (2004) measured N recovery using modern high-yielding rice cultivar (Hwaseongbyeo) in Korea during two years: 2001-2002. In two years 2001-2002, nitrogen recovery efficiency from the study was 34% of applied N at transplanting and tillering stages which was significantly lower than 66.3 and 69.6% of N applied at PIS in year 2002 and 2003, respectively. Although there was variation in N recovery efficiency at different time of application, common N split application has

†Corresponding author: (Phone) +82-2-880-4544 (E-mail) leebw@snu.ac.kr <Received February 15, 2006>

been recommended to be 50% at transplanting, 20% tillering stage, and 30% at PIS in Korea.

Nitrogen application at different growth stages showed different effect on rice yield and yield components, and grain quality (Matsushima, 1995; Kim, 2004). Matsushima (1995) reported that N application at 48 to 28 days before heading (DBH) usually resulted in higher number of spikelets per hills than N application at 24 DBH but for high percentage of ripening grains N application should be after 13 DBH. However, he also indicated that N absorbed from late N application (at booting or heading) could be translocated directly into grain, resulting in much higher protein content in comparison to earlier N application.

Relationship between milled-rice protein content and grain quality has been reported (Ishima *et al.*, 1974; Taira, 1995). Chikubu *et al.* (1985) measured milled-rice protein content and palatability of cooked rice by five parameters (appearance, aroma, taste, stickiness, hardness) and found that milled-rice protein content had negative correlation coefficient with appearance (-0.42), aroma (-0.35), taste (-0.43), stickiness (-0.44) and positive correlation coefficient with hardness (0.41). Similarly, Ishima *et al.* (1974) found high negative correlation between protein content in polished rice and taste evaluation score. In overall milled-rice protein content showed negative correlation with palatability score of cooked rice. This means an increase in milled-rice protein content reduces eating quality of rice.

As a result, understanding the effect of N rates applied at various growth stages on rice grain yield and protein content is useful for determining optimum N rate and timing for high grain yield and quality of high-yielding rice cultivars for rice production in Korea. Therefore, the objective of this study was to test the effect of different N fertilizer rates at transplanting, TS, and PIS and their interaction on rice grain yield and milled-rice protein content.

MATERIALS AND METHODS

Site description and experimental design

Three experiments, one in year 2003, one in year 2004 and the other in year 2005, were conducted on area of 3,000 m^2 at Experimental Station, Seoul National University, Suwon, Korea. The soil of the field had texture of sandy clay loam, CEC of 11.9 $cmol^{(+)}\ kg^{-1}$; organic matter of 14.4 mg/g; total N of 0.75 mg/g, and pH of 5.4. The year 2003 experiment included 10 nitrogen (N) treatments and four rice varieties while the experiments in year 2004 and 2005 included 6 N treatments and two rice varieties. The details of experimental design were presented in Table 1.

It is noted from Table 1 that N fertilizer was applied at 14 days after transplanting (tillering stage) and 24 days before heading (PIS) as commonly recommended in Korea. The experiments were done with split-split-plot design in which N treatment at tillering stage and at PIS, and rice varieties were randomly assigned to main plots, sub plots and sub-sub plots, respectively. Plot size was 24 m^2 in year 2003 and 30 m^2 in year 2004 and 2005. Rice was transplanted manually at the spacing of 0.15 m×0.30 m with 3 seedlings per hill on 20th May 2003, 23rd May 2004 and 30th May 2005. The whole experimental field was applied with the same amount of 8.0 kg P_2O_5 + 4.8 kg K_2O/10a at transplanting and 2.4 kg K_2O/10a at PIS. The other management techniques for the whole field such as land preparation, weeding, water supplies, etc. were applied homogeneously.

Plant sampling

At harvest (12th October 2003, 16th October 2004 and 9th October 2005) two samples of five hills for measuring yield components (total number of spikelets, number of filled and

Table 1. Treatment design in 2003-2005 experiments.

Applied N rate (kg/10a)			Rice variety†
Transplanting	Tillering stage	Panicle initiation stage	
		In 2003	
0	0	0	V1, V2, V3, V4
4.8	0	0, 3.6, 7.2	V1, V2, V3, V4
4.8	3.6	0, 3.6, 7.2	V1, V2, V3, V4
4.8	7.2	0, 3.6, 7.2	V1, V2, V3, V4
		In 2004-2005	
4.8	0	0, 3.6	V1, V5
4.8	3.6	0, 3.6	V1, V5
4.8	7.2	0, 3.6	V1, V5

†V1-V5 were Hwaseongbyeo, SNU-SG1, Juanbyeo, Surabyeo, and Daeanbyeo, respectively.

unfilled spikelets, and 1000-grain weight) and 72 hills for grain yield (kg/10a) and milled-rice protein (%) measurement, were collected from each plot. The whole rice shoot and milled-rice samples were dried at 72°C for 72 hours and ground for N analysis by Kejeltec Auto 1035 System (Tecator, Sweden) for shoot N concentration and N uptake determination. The milled-rice N concentration (%) was converted into milled-protein content (%) by multiplying conversion factor of 5.95.

Data analysis

Data collected from three years (2003-2005) were subjected to ANOVA and contrast analyses for presenting treatment means over three blocks in combination with significant levels of treatment effect (effect of N rates applied at transplanting, TS and PIS, rice varieties and their interaction) using PROC GLM procedure in SAS 8.1 (SAS Inc., USA).

RESULTS AND DISCUSSION

Weather variation in three experimental years (2003-2005)

Weather variation, especially variation in sunshine duration and mean temperature during some critical growth and development stages of rice, is important information for explaining the response of rice grain yield and milled-rice protein content to applied N rate and timing. The variation in some selected weather parameters in year 2003-2005 was presented in Table 2.

The total sunshine duration was higher in year 2004 than those in year 2003 and 2005 (Table 2) even though there was not much difference in duration of sunshine hours from transplanting to PIS. Therefore, it is expected that rice growth and crop biomass, and nutrition accumulation from transplanting to PIS were not much different among three years. However, the much lower sunshine hours from PIS to harvest in year 2003 or from PIS to heading in year 2005 than those in year 2004 could result in different response of grain yield and protein content to applied N rate among three years. Mean temperature was lower in all growth stages in 2003 than in 2004 and 2005 (Table 2).

Rice grain yield and milled-rice protein content

Effect of N rates applied at transplanting and tillering stage

Table 3a showed the effect of applied N at different rice growth stages (at transplanting, TS, and PIS) on rice grain yield and protein content in 2003. In general, no applied N treatment at any stages gained very low grain yield ranging from 359 kg/10a (cv. Surabyeo) to 390 kg/10a (cv. Juanbyeo) and milled-rice protein content ranging from 6.70% (cv. Juanbyeo) to 6.97% (cv. Hwaseongbyeo). The very low grain yield regardless of rice cultivars under no N application in the experiment indicated that soil N of the experimental field with total soil N of 0.75mg/g soil was not fertile enough for proper rice crop growth and development. The low grain yield from no N application treatment was also obtained at the same field in year 2001 and 2002 (Kim, 2004). Although no N treatment (treatment 1) had lower grain yield, it had higher milled-rice protein content than those of treatments with applied N at transplanting and tillering but without applied N at PIS (treatments 2, 5, and 8). The higher grain yield and lower milled-rice protein content of those treatments compared to no N treatment suggested that early N application could promote vegetative growth before PIS and this accumulation of plant biomass and nutrition before PIS had an important effect on grain yield formation and a dilution effect on milled-rice protein content.

Comparing grain yield and milled-rice protein content of treatment 1 (no N at any stages) and treatment 2 (4.8 kg N/10a at transplanting and no N at tillering and PIS) indicated the highly positive effect of applied N at transplanting on grain yield and negative effect on milled-rice protein content in all four rice cultivars. On an average, applied 4.8 kg N/

Table 2. Weather data during main growth stages of rice in year 2003-2005.

Year	Transplanting to PIS	PIS to heading	Heading to harvest	Total
		Sunshine duration (hour)		
2003	282.5	110.2	101.6	494.3
2004	254.7	181.6	186.9	623.2
2005	260.0	87.2	189.1	536.3
		Average temperature (°C)		
2003	22.1	24.5	21.9	22.5
2004	22.5	27.8	22.6	23.5
2005	23.6	26.7	22.6	23.8

Table 3a. Effect of applied N rates at transplanting, tillering and panicle initiation stages on rice grain yield and milled-rice protein content in 2003.

Ttreat. No.	N rate(a, b, c)[†]	Grain yield (kg/10a)				Mean	Milled-rice protein content (%)				
		V1[‡]	V2	V3	V4		V1	V2	V3	V4	Mean
1:	N(0, 0, 0)	379	389	390	359	379	6.97	6.81	6.70	6.71	6.80
2:	N (4.8, 0, 0)	518	502	490	454	491	6.45	6.59	6.47	6.44	6.49
3:	N (4.8, 0, 3.6)	588	616	638	558	600	6.96	7.13	7.04	6.97	7.03
4:	N (4.8, 0, 7.2)	644	674	724	599	660	6.84	8.46	7.47	7.90	7.67
5:	N (4.8, 3.6, 0)	539	540	562	509	538	6.35	6.31	6.44	6.35	6.36
6:	N (4.8, 3.6, 3.6)	646	674	679	631	657	6.97	7.24	7.24	7.30	7.19
7:	N (4.8, 3.6, 7.2)	676	704	715	680	694	7.49	8.28	7.82	7.66	7.81
8:	N (4.8, 7.2, 0)	561	585	581	558	571	6.11	6.58	6.41	6.48	6.40
9:	N (4.8, 7.2, 3.6)	633	708	722	705	692	7.30	7.60	7.10	6.86	7.22
10:	N (4.8, 7.2, 7.2)	727	725	710	720	720	7.34	8.14	7.79	7.72	7.75
N rate at tillering stage (pooled by applied N rates at PIS)											
	0	583	597	617	537	584	6.75	7.39	7.00	7.10	7.06
	3.6	620	639	652	607	630	6.94	7.28	7.17	7.10	7.12
	7.2	640	673	671	661	661	6.92	7.44	7.10	7.02	7.12
N rate at PIS (Pooled by applied N rates at tillering stage)											
	0	539	542	544	507	533	6.30	6.49	6.44	6.42	6.42
	3.6	622	666	680	631	650	7.08	7.32	7.13	7.04	7.14
	7.2	682	701	716	666	691	7.22	8.29	7.69	7.76	7.74
	Means	*614*	*636*	*647*	*601*	*625*	*6.87*	*7.37*	*7.09*	*7.07*	*7.10*
ANOVA (probability level)											
Basal N		0.00	0.01	0.00	0.02	0.00	0.07	0.50	0.40	0.28	0.35
Tiller N[§]		0.01	0.00	0.01	0.00	0.00	0.79	0.45	0.66	0.65	0.99
Panicle N		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tiller N × Panicle N		0.67	0.61	0.04	0.95	0.17	0.03	0.18	0.54	0.35	0.25
Cultivar						0.00					0.00
Cultivar × Tiller N						0.03					0.59
Cultivar × Panicle N						0.37					0.00

[†]N(a, b, c) means a, b, c kg N/10a applied at transplanting, tillering stage and PIS, respectively.
[‡]V1, V2, V3, and V4 were Hwaseongbyeo, SNU-SG1, Juanbyeo and Surabyeo, respectively.
[§]Tiller and panicle N were N applied at TS and PIS, respectively.

10a increased grain yield by 112 kg/10a and obtained agronomic N use efficiency of 23.3 kg grain/kg applied N. Effect of applied N at tillering stage without applied N at PIS on grain yield and milled-rice protein content could be seen by comparing treatment 2, 5 and 8. Similar to the effect of applied N at transplanting, N applied at transplanting stage reduced protein content from 6.45% (no N) to 6.35% (3.6 kg N/10a) and 6.11% (7.2 kg N/10a) and increased grain yield from 491 kg/10a to 538 and 571 kg/10a for treatments with 3.6 and 7.2 kg applied N/10a, respectively (average over rice cultivars). Although N applied at transplanting and tillering stage showed similar effect (positive effect on grain yield and negative effect on milled-rice protein content), the effect of applied N at tillering was much smaller than that of applied N at transplanting. N use efficiencies of 3.6 and 7.2 kg N/10a treatments at tillering stage were 13.1 and 11.1 kg grain/kg applied N, respectively and both of them were much lower than 23.3 kg grain/kg N applied at transplant-

Table 3b. Effect of applied N fertilizer at tillering and panicle initiation stages on rice grain yield and milled-rice protein content in 2004.

N application (kg/10a)		Grain yield (kg/10a)			Milled-rice protein (%)		
Tiller N[†]	Panicle N	V1	V5	Mean	V1	V5	Mean
0	0	551.8	577.0	564.4	6.06	6.57	6.32
	3.6	669.4	798.5	734.0	6.60	6.68	6.64
	Average	***610.6***	***687.8***	***649.2***	***6.33***	***6.63***	***6.48***
3.6	0	590.8	646.8	618.8	6.21	6.39	6.30
	3.6	697.1	804.0	750.6	6.79	6.70	6.75
	Average	***644.0***	***725.4***	***684.7***	***6.50***	***6.55***	***6.52***
7.2	0	605.9	655.9	630.9	6.20	6.34	6.27
	3.6	775.0	845.8	810.4	6.74	6.86	6.80
	Average	***690.5***	***750.8***	***720.7***	***6.47***	***6.60***	***6.53***
Pooled	0	582.8	626.6	604.7	6.16	6.43	6.30
	3.6	713.8	816.1	765.0	6.71	6.75	6.73
ANOVA (probability level)							
Tiller N				0.03			0.26
Panicle N				0.00			0.02
Cultivar				0.00			0.41
Tiller N × Cultivar				0.62			0.25
Panicle N × Cultivar				0.59			0.43
Tiller N × Panicle N				0.13			0.45

[†]Tiller N: N (kg/10a) fertilizer applied at tillering stage; Panicle N: N (kg/10a) applied at panicle initiation stage; V1 and V5 were Hwaseongbyeo and Daeanbyeo rice cultivars, respectively.

ing. Analysis of variance indicated that only grain yield increased significantly with an increase of N rates applied at tillering even though there were trends to decrease milled-rice protein content with an increase of applied N rates at TS in 2003.

Similar to the 2003 experiment, applied N rates at tillering stage significantly increased rice grain yield ($P<0.05$) but not milled-rice protein content in 2004 (Table 3b) and 2005 (Table 3c). Rice grain yield increased from 649.2 kg/10a of treatment without N applied at tillering stage to 684.7 kg/10a and 720.7 kg/10a of treatments with 3.6 and 7.2 kg N applied at TS, respectively, resulting in N agronomic use efficiency of about 10 kg grain/kg applied N at TS in 2004 (Table 3b). The agronomic N use efficiency of about 8 kg grain/kg applied N at TS was obtained in the 2005 experiment (Table 3c).

Effect of N rates applied at PIS

An increase of N rates at PIS from 0 to 3.6 and 7.2 kg/10a resulted in an increase of grain yield from 533 to 650 and 691 kg/10a, achieving agronomic N use efficiencies of about 32.5 and 21.9 kg grain/kg applied N at 3.6 and 7.2 kg N/10a levels, respectively in 2003 (Table 3a). These values of agronomic N use efficiencies were higher than those of N applied at tillering. However, applied N at PIS also increased milled-rice protein content significantly. Milled-rice protein content increased from 6.42% of treatment without N to 7.14% and 7.74% of treatments with 3.6 and 7.2 kg N/10a at PIS, respectively. No significant interaction between applied N rate at tillering stage and PIS was observed for grain yield and milled rice protein content.

Data from 2004 and 2005 experiments (Table 3b and 3c) confirmed that an increase of N fertilizer rates applied at PIS from 0 to 3.6 kg/10a significantly increased rice grain yield ($P<0.01$) but at the same time significantly increased milled-rice protein content ($P<0.05$). Therefore, high grain yield and low protein content may not be obtained in rice production by adjusting only N fertilizer rate at PIS.

Rice cultivar effect

Grain yield and milled-rice protein content were significantly different among four rice cultivars used in 2003 experiment. On an average, grain yields of testing cultivars were in descending order of Juanbyeo > SNU-SG1 >

Table 3c. Effect of applied N fertilizer at tillering and panicle initiation stages on rice grain yield and milled-rice protein content in 2005.

N application (kg/10a)		Grain yield (kg/10a)			Milled-rice protein (%)		
Tiller N†	Panicle N	V1	V5	Mean	V1	V5	Mean
0	0	525.4	575.0	550.2	6.55	6.45	6.50
	3.6	618.4	713.3	665.8	6.65	6.99	6.82
	Average	***571.9***	***644.1***	***608.0***	***6.60***	***6.72***	***6.66***
3.6	0	542.2	599.5	570.8	6.36	6.71	6.54
	3.6	667.8	733.7	700.7	6.64	6.82	6.73
	Average	***605.0***	***666.6***	***635.8***	***6.50***	***6.76***	***6.63***
7.2	0	604.2	656.5	630.4	6.45	6.21	6.33
	3.6	682.6	719.6	701.1	6.84	6.80	6.82
	Average	***643.4***	***688.1***	***665.7***	***6.65***	***6.51***	***6.58***
Pooled	0	557.2	610.3	583.8	6.45	6.46	6.46
	3.6	656.3	722.2	689.2	6.71	6.87	6.79
ANOVA (probability level)							
Tiller N				0.04			0.95
Panicle N				0.00			0.00
Cultivar				0.00			0.04
Tiller N × Cultivar				0.50			0.40
Panicle N × Cultivar				0.09			0.12
Tiller N × Panicle N				0.04			0.51

†Tiller N: N (kg/10a) fertilizer applied at tillering stage; Panicle N: N (kg/10a) applied at panicle initiation stage; V1 and V5 were Hwaseongbyeo and Daeanbyeo rice cultivars, respectively.

Hwaseongbyeo > Surabyeo but milled-rice protein content of testing cultivars were ranked in different order: SNU-SG1 > Juanbyeo > Surabyeo > Hwaseongbyeo. Significant interaction between rice cultivar and applied N rate at tillering stage for grain yield ($P<0.05$) indicated that grain yield of different cultivars responded differently to the applied N rates at tillering stage. Among the tested rice cultivars, SNU-SG1 and Juanbyeo had higher yield and milled-rice protein content than those of Surabyeo and Hwaseongbyeo. Daeanbyeo showed significantly higher grain yield than Hwaseongbyeo in both 2004 and 2005 experiment. Daeanbyeo also showed higher milled-rice protein content than Hwaseongbyeo but not significantly in 2004.

In general, the lower N use efficiency of applied N at tillering stage than that of applied N at transplanting might have resulted from the method of N application. Before transplanting, N was applied and mixed with soil during soil preparation. Therefore, it was expected that NH_4^+ hydrolyzed from urea would be adsorbed mostly by soil colloids and lower N loss through leaching and volatilization. On the other hand, N fertilizer at tillering was applied on the soil surface without mixing with soil in our experiment. As a result, a large percentage of applied N might be lost from NH_3 volatilization and denitrification as discussed by Raun *et al.* (2002). The lower agronomic N use efficiency of N application at transplanting and tillering stage than N application at PIS may partly result from the difference in N recovery efficiency when N applied at different rice growth stages. Nishizawa (1995) indicated that N uptake velocity depended on growth stages of rice. The N uptake velocity was slow after transplanting and gradually increased and reached maximum value before heading stage and then dropped rapidly during the maturing period. The low N uptake velocity at early growth period may result from insufficient rice root system but the slow N uptake after heading from root and leaf senescence. The highly positive effect of N application at PIS on milled-rice protein content in our study was in agreement with discussion of Murayama (1995). Murayama (1995) indicated that the later N application would result in higher N accumulation in grain because some of the absorbed N from fertilizer might be translocated directly into grain but absorbed N at early growth stage was distributed at first into actively functioning leaves and roots and then redistributed into panicles. As a consequence, a lim-

Table 4. Year variation analysis for yield and protein content of cv. Hwaseongbyeo over three experimental years (2003-2005).

Applied N at (kg/10a)		Grain yield (kg/10a)				Milled-rice protein content (%)			
Tillering	PIS	2003	2004	2005	Mean	2003	2004	2005	mean
0	0	518.1	551.8	525.4	551.8	6.45	6.06	6.55	6.35
	3.6	587.8	669.4	618.4	625.2	6.96	6.60	6.65	6.74
	Average	***553.0***	***610.6***	***571.9***	***588.5***	***6.71***	***6.33***	***6.60***	***6.55***
3.6	0	539.2	590.8	542.2	557.4	6.35	6.21	6.36	6.31
	3.6	646.4	697.1	667.8	670.4	6.97	6.79	6.64	6.80
	Average	***592.8***	***644.0***	***605.0***	***613.9***	***6.66***	***6.50***	***6.50***	***6.55***
7.2	0	561	605.9	604.2	590.3	6.11	6.20	6.45	6.25
	3.6	633.3	775	682.6	697	7.30	6.74	6.84	6.96
	Average	***597.2***	***690.5***	***643.4***	***643.7***	***6.70***	***6.47***	***6.65***	***6.60***
Pooled	0	539.4	582.8	557.3	566.5	6.30	6.16	6.45	6.30
	3.6	622.5	713.8	656.3	664.2	7.08	6.71	6.71	6.83
	Average	***581.0***	***648.3***	***606.8***	***615.4***	***6.69***	***6.43***	***6.58***	***6.57***
ANOVA (probability level)									
Year					0.01				0.19
Ntill†					0.02				0.81
Npi					0.00				0.00
Ntill × Npi					0.37				0.24
Ntill × Year					0.70				0.81
Npi × Year					0.64				0.04

†Ntill and Npi were N (kg/10a) applied at tillering stage and PIS, respectively.

ited amount of N applied at early stage reached the panicles.

Year variation analysis over three experimental years (2003-2005)

Year variation analysis was done with the data from three levels of applied N at tillering stage (0, 3.6 and 7.2 kg/10a), two applied N levels at PIS (0 and 3.6 kg/10a) and cv. Hwaseongbyeo because only these treatments were repeated for three years in our study (Table 4). Year factor significantly affected grain yield ($P < 0.01$) but not protein content. On an average, grain yield was the lowest in 2003 (581.0 kg/10a) in comparison to 648.3 kg/10a in 2004 and 606.8 kg/10a in 2005. Year to year variation of grain yield was in good agreement with the variation in sunshine duration of whole growth period and of that from PIS to harvest in three experimental years. The sunshine duration in 2003, 2004, and 2005 were 494.3, 623.2, and 536.3 hours for the whole plant growth period and 211.8, 368.5, and 276.3 hours for the period from PIS to harvest, respectively (Table 2). There was not much difference in milled-rice protein content among three years but large difference between two applied N rates at PIS, revealing potential use of milled-rice protein content for N topdressing prescription at PIS. Similar to analysis of variance using one year data, applied N at tillering showed significant effect on grain yield but not on milled-rice protein content and applied N at PIS significantly increased both grain yield and milled-rice protein content. Analysis of variance using data from three years again confirmed that high grain yield and low milled-rice protein content could not be obtained by only adjusting the applied N rates at PIS. Other cultivation measures such as adjusting applied N rates at tillering and transplanting, and the timing of N topdressing at PIS may play an important role for obtaining high rice yield and quality.

Plant N concentration and accumalation at harvest

Effect of applied N rates at transplanting and tillering stage

Applied N at transplanting did not significantly increase shoot N concentration but shoot N content at harvest in 2003 ($P<0.01$) (Table 5a). Treatment 1 without N applied at transplanting had shoot N content ranging from 5.39 to 5.67 kg/10a for four rice cultivars. The applied N of 4.8 kg/10a (treatment 2) significantly increased the shoot N content averaged over four rice cultivars to 7.52 kg/10a compared to 5.53 kg/10a of no N treatment and obtained N recovery efficiency of

Table 5a. Effect of applied N rates at transplanting, tillering, and panicle initiation stage (PIS) on shoot N concentration and accumulation at harvest in 2003.

Treat N rate(a, b, c)†. No	Shoot N concentration (mg/g)					Shoot N accumulation (kg/10a)				
	V1‡	V2	V3	V4	Mean	V1	V2	V3	V4	Mean
1: N(0, 0, 0)	7.90	8.45	7.96	8.58	8.22	5.67	5.51	5.54	5.39	5.53
2: N (4.8, 0, 0)	8.22	8.64	7.98	8.60	8.36	8.13	7.82	7.09	7.05	7.52
3: N (4.8, 0, 3.6)	9.18	9.35	9.15	8.76	9.11	9.83	9.92	10.21	8.33	9.57
4: N (4.8, 0, 7.2)	9.22	9.88	9.92	10.27	9.82	10.74	11.67	12.98	11.06	11.61
5: N (4.8, 3.6, 0)	7.81	8.18	8.09	8.23	8.08	8.00	7.71	8.43	7.64	7.94
6: N (4.8, 3.6, 3.6)	8.81	10.22	9.20	8.97	9.30	10.37	12.05	11.20	9.85	10.87
7: N (4.8, 3.6, 7.2)	10.68	11.32	10.09	10.24	10.58	13.27	15.02	13.29	12.36	13.48
8: N (4.8, 7.2, 0)	7.72	8.73	8.45	8.48	8.35	8.21	9.03	9.13	8.45	8.71
9: N (4.8, 7.2, 3.6)	8.81	9.77	9.72	9.43	9.43	10.27	12.91	12.90	11.79	11.97
10: N (4.8, 7.2, 7.2)	9.76	10.41	9.45	10.26	9.97	13.29	14.08	12.14	13.75	13.32
Applied N rate at tillering stage (Pooled over N rates at PIS)										
0	8.87	9.29	9.02	9.21	9.10	9.57	9.80	10.09	8.81	9.57
3.6	9.10	9.91	9.13	9.15	9.32	10.54	11.59	10.97	9.95	10.76
7.2	8.77	9.63	9.21	9.39	9.25	10.59	12.01	11.39	11.33	11.33
Applied N rate at PIS (Pooled over N rates at tillering stage)										
0	7.92	8.52	8.17	8.44	8.26	8.11	8.19	8.22	7.71	8.06
3.6	8.93	9.78	9.36	9.05	9.28	10.16	11.63	11.44	9.99	10.80
7.2	9.89	10.54	9.82	10.26	10.12	12.43	13.59	12.80	12.39	12.80
Means	*8.91*	*9.61*	*9.12*	*9.25*	*9.22*	*10.23*	*11.13*	*10.82*	*10.03*	*10.55*
ANOVA (probability level)										
Basal N	0.58	0.65	0.96	0.97	0.68	0.03	0.02	0.01	0.16	0.01
Tiller N§	0.54	0.03	0.68	0.74	0.41	0.05	0.00	0.00	0.00	0.00
Panicle N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tiller N × Panicle N	0.18	0.04	0.22	0.84	0.25	0.69	0.46	0.00	0.94	0.73
Cultivar					0.00					0.00
Cultivar × Tiller N					0.44					0.14
Cultivar × Panicle N					0.39					0.17

†N (a, b, c) means a, b, c kg N/10a applied at transplanting, tillering stage and PIS, respectively.
‡V1, V2, V3, and V4 were Hwaseongbyeo, SNU-SG1, Juanbyeo and Surabyeo, respectively.
§Tiller and panicle N were N applied at tillering stage and PIS, respectively.

41.5%. Similar to applied N at transplanting, N applied at tillering stage also did not significantly increased shoot N concentration but increased shoot N content ($P<0.01$). However, the effect of applied N at tillering on plant N uptake was not as high as applied N at transplanting in terms of N recovery efficiency. On average, the treatments with applied N of 3.6 and 7.2 kg/10a obtained plant N accumulation of 10.76 and 11.33 kg/10a, respectively, compared to 9.57 kg/10a of no applied N at TS treatment. Therefore, N recovery efficiencies were 33.1 and 24.4% for the treatments with 3.6 and 7.2 kg N applied at tillering stage, respectively. No significant difference in shoot N concentration and content among different N rates applied at tillering stage was found in the 2004 and 2005 experiments. N recovery efficiencies were 21.1 and 17.6% for treatments with 3.6 and 7.2 kg N applied at TS, respectively in 2004 (Table 5b) and the higher N recovery efficiency of 27.5 and 23.8% for treatments with 3.6 and 7.2 kg N applied at TS in 2005 (Table 5c).

Effect of applied N at PIS

Dissimilar to early applied N before PIS, N applied at PIS significantly increased both shoot N concentration and accumulation ($P<0.01$) in three experimental years (Tables 5a-c). The high N accumulation in rice plant due to high N applied

Table 5b. Effect of applied N fertilizer at tillering and panicle initiation stages on shoot N concentration and accumulation at harvest in 2004.

N application (kg/10a)		Shoot N concentration (mg/g)			Shoot N accumulation (kg/10a)		
Tiller N†	Panicle N	V1	V5	Mean	V1	V5	Mean
0	0	7.24	7.61	7.43	08.65	8.84	8.75
	3.6	8.49	8.20	8.35	11.52	12.40	11.96
	Average	***7.87***	***7.91***	***7.89***	***10.09***	***10.62***	***10.35***
3.6	0	7.45	7.41	7.43	9.21	9.49	9.35
	3.6	8.78	8.36	8.57	12.54	13.17	12.86
	Average	***8.12***	***7.88***	***8.00***	***10.88***	***11.33***	***11.11***
7.2	0	7.11	7.72	7.41	9.78	10.17	9.98
	3.6	8.04	8.64	8.34	12.69	13.82	13.26
	Average	***7.57***	***8.18***	***7.88***	***11.24***	***12.00***	***11.62***
Pooled	0	7.27	7.58	7.42	9.21	9.50	9.36
	3.6	8.44	8.40	8.42	12.25	13.13	12.69
ANOVA (probability level)							
Tiller N				0.66			0.19
Panicle N				0.00			0.00
Cultivar				0.37			0.06
Tiller N × Cultivar				0.10			0.81
Panicle N × Cultivar				0.25			0.73
Tiller N × Panicle N				0.78			0.14

†Tiller N: N (kg/10a) fertilizer applied at tillering stage; Panicle N: N (kg/10a) applied at panicle initiation stage; V1 and V5 were Hwaseongbyeo and Daeanbyeo rice cultivars, respectively.

Table 5c. Effect of applied N fertilizer at tillering and panicle initiation stages on shoot N concentration and accumulation at harvest in 2005.

N application (kg/10a)		Shoot N concentration (mg/g)			Shoot N accumulation (kg/10a)		
Tiller N†	Panicle N	V1	V5	Mean	V1	V5	Mean
0	0	7.40	7.16	7.28	8.29	7.80	8.05
	3.6	9.03	8.37	8.70	11.04	10.73	10.89
	Average	***8.22***	***7.76***	***7.99***	***9.67***	***9.27***	***9.47***
3.6	0	7.07	7.56	7.32	8.46	9.10	8.78
	3.6	8.78	9.05	8.92	12.03	12.27	12.15
	Average	***7.93***	***8.31***	***8.12***	***10.24***	***10.69***	***10.46***
7.2	0	7.70	7.07	7.39	9.88	9.72	9.80
	3.6	9.37	8.55	8.96	12.72	12.38	12.55
	Average	***8.54***	***7.81***	***8.17***	***11.30***	***11.05***	***11.18***
Pooled	0	7.39	7.26	7.33	8.88	8.87	8.88
	3.6	9.06	8.66	8.86	11.93	11.80	11.86
ANOVA (probability level)							
Tiller N				0.72			0.09
Panicle N				0.00			0.00
Cultivar				0.29			0.66
Tiller N × Cultivar				0.04			0.33
Panicle N × Cultivar				0.23			0.38
Tiller N × Panicle N				0.64			0.30

†Tiller N: N (kg/10a) fertilizer applied at tillering stage; Panicle N: N (kg/10a) applied at panicle initiation stage; V1 and V5 were Hwaseongbyeo and Daeanbyeo rice cultivars, respectively.

at PIS was possibly a reason for high milled-rice protein content. Recovery efficiencies of 3.6 kg N/10a applied at PIS were very high (>76 %) in all three years. The higher effect of applied N at PIS on shoot N concentration, content, and N recovery efficiencies than that of early applied N before PIS could explain the higher effect of the former on grain yield and milled-rice protein content than the later.

Effect of rice cultivars

Even though there were significant differences in shoot N concentration and content among cultivars, no significant interaction between rice cultivar and N rates applied at TS and PIS in 2003 could be found (Table 5a). SNU-SG1 had the highest shoot N concentration (Table 5a) that was in agreement with its highest milled-rice protein content (Table 3a). Two rice cultivars, SNU-SG1 and Juanbyeo had higher shoot N accumulation (Table 5a) and also had higher grain yield (Table 3a) than two other rice cultivars, Hwaseongbyeo and Surabyeo. This might reveal the highly positive relationship between shoot N concentration at harvest and milled-rice protein content and between shoot N content at harvest and grain yield. In 2004-2005, no statistically significant difference in shoot N concentration and content at harvest between cv. Hwaseongbyeo and cv. Daeanbyeo was obtained.

Relationship among crop variables at harvest

Shoot N concentration and milled-rice protein content

Pooled data from three experimental years (2003-2005)

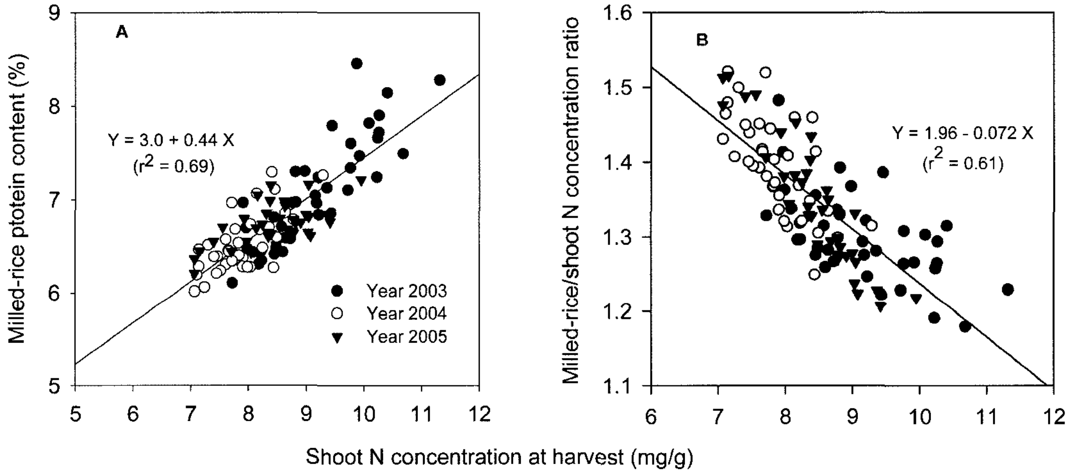

Fig. 1. Relationship between plant N concentration and milled-rice protein content and milled-rice/shoot N concentration ratio in three experimental years (2003-2005).

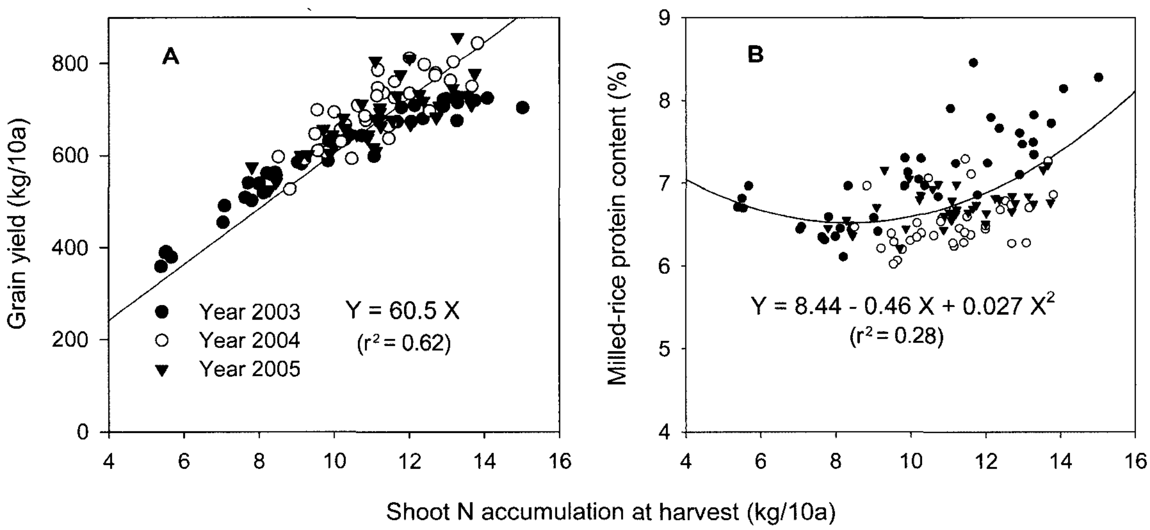

Fig. 2. Relationship between shoot N accumulation at harvest and grain yield (A) and milled-rice protein content (B) in three experimental years (2003-2005).

including five rice cultivars and various N treatments at TS and PIS showed that shoot N concentration at harvest had high correlation with milled-rice protein content (Fig. 1A). Fig. 1A indicated that there was a general balance in N concentration in grain and whole plant: the higher shoot N concentration, the higher grain N concentration. However, partition of N between grain and straw evidently depended on plant N concentration (Fig. 1B). The higher ratio of milled-rice N concentration to shoot N concentration was at lower plant N concentration (Fig. 1B). This means more N in vegetative part was partitioned to grain if rice was insufficient in N nutrition at grain filling duration.

Shoot N accumulation, grain yield and milled-rice protein content

Shoot N accumulation at harvest and grain yield had high correlation with each other. Variation in plant N accumulation due to difference in rice cultivars, N fertilizers and years explained 77% of variation in grain yield (Fig. 2A). The regression equation to predict grain yield by shoot N content at harvest was Y = 60.5 X (R^2 = 0.62), indicating that on average internal N use efficiency of rice crop was 60.5 kg grain/ kg N accumulation. The response of grain yield to N accumulation in year 2003 (filled circle) was also different from the last two experiment years 2004-2005 (unfilled circle and triangle shapes). In year 2003, due to low sunshine duration at grain filling period (Table 2) grain yield showed quadratic response to N accumulation while linear response was visualized in year 2004 and 2005 when sunshine duration was high at grain filling period. Milled-rice protein content showed different response to plant N accumulation in comparison to grain yield (Fig. 2B). The milled-rice protein content was at a narrower range in year 2004 and 2005 than in year 2003. The different range in milled-rice protein content might have resulted from higher applied N rates at PIS and lower sunshine duration in year 2003 than in year 2004-2005. Milled-rice protein content had negative correlation with internal N use efficiency (Fig. 3), indicating that unfavorable growth condition that limited internal N use efficiency might cause high grain N concentration. Unfavorable growth conditions such as low sunshine duration during grain filling and rain-fed rice cultivation resulted in low internal N use efficiency (Hossain *et al.*, 2005), while low sunshine duration during grain filling caused high milled-rice protein content (Kim, 2004).

Accumulation of N in shoot and milled-rice

Shoot N accumulation had close linear relationship with N

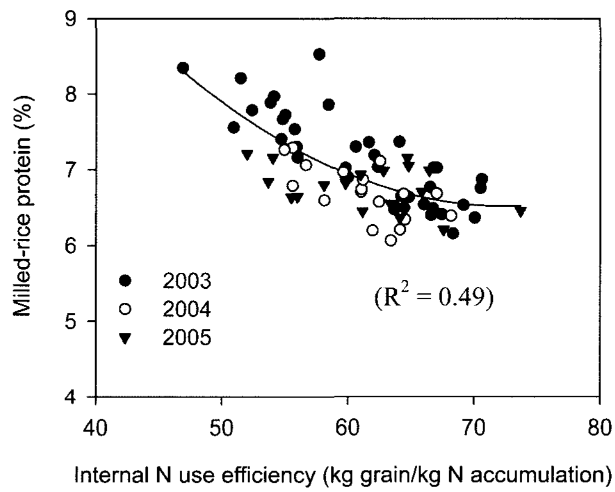

Fig. 3. Relationship between milled-rice protein content and internal N use efficiency.

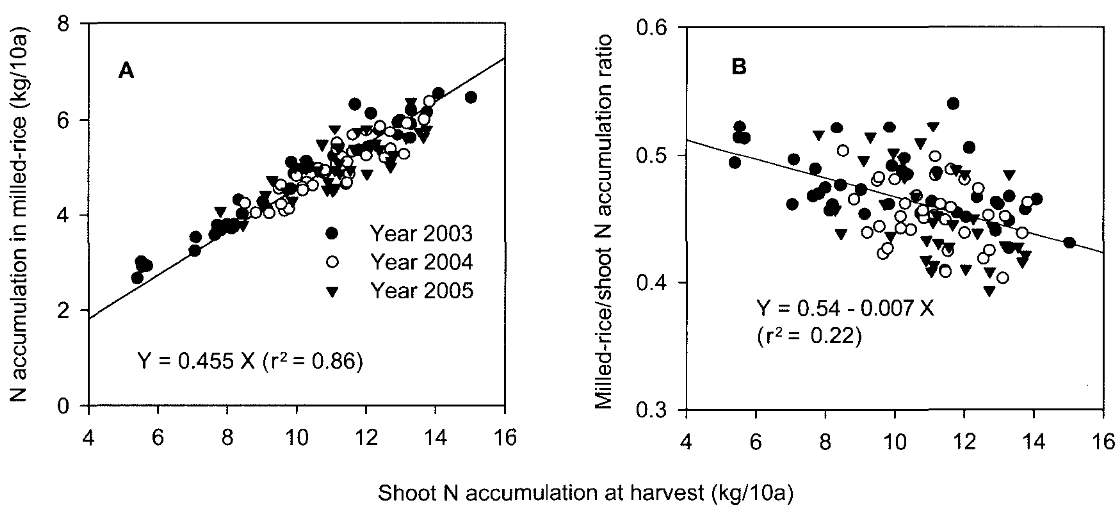

Fig. 4. Relationship between N accumulation in shoot and grain in three experimental years (2003-2005).

accumulation in milled-rice (Fig. 4A). This suggested that increased shoot N accumulation at harvest resulted in higher yield of protein in rice production. The regression equation to predict N accumulation in milled-rice by shoot N accumulation at harvest was Y = 0.455 X ($r^2 = 0.86$). In addition, the milled rice/shoot N accumulation ratio was dependent on total shoot N accumulation at harvest. The higher shoot N accumulation at harvest resulted in the lower percentage of N translocation to grain from vegetative part (Fig. 4B). This was in agreement with discussion in the above section that more N was partitioned to grain if rice plant was insufficient in plant N nutrition.

CONCLUSION

The N application at transplanting, tillering stage, and PIS resulted in different N uptake and agronomic N use efficiencies of which the N application at PIS had the higher agronomic N use efficiency and N recovery efficiency than those applied at transplanting and tillering stage. The N application at PIS significantly increased both grain yield and protein content but applied N at tillering stage significantly increased grain yield but not protein content. Therefore, it was considered difficult to obtain high rice grain yield and low protein content at the same time by only adjusting N rates at PIS.

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