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Response of Grain Yield and Milled-Rice Protein Content to Nitrogen Topdress Timing at Panicle Initiation Stage of Rice

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ABSTRACT : Response of grain yield and milled-rice protein content to nitrogen topdress (N) timing at panicle initiation stage (PIS) is critical for quantifying real-time N requirement for target grain yield and milled-rice protein content. Two split-split-plot experiments with three replications, one in 2004 and the other in 2005, were conducted in Experimental Farm, Seoul National University, Suwon, Korea. The experiments included three N rates at tillering stage (TS), three N timing treatments at panicle initiation stage (PIS) and two rice cultivars. The N rates at TS, N timing at PIS, and rice cultivars were randomly assigned to main plot, sub plot, and sub-sub plot, respectively. Results showed that the delayed N application at PIS reduced grain yield in 2004 and increased milled-rice protein content in both years significantly at 0.05 probability level. The calculated optimum N timing at PIS from pooled data by N rates and rice cultivars in two years was at 28 days before heading (DBH). However, real-time of N timing at PIS was dependent on plant growth and N status around PIS that in turn was dependent on applied N rates at TS. The optimum N timing at PIS was at 30 DBH for no N treatments at TS while at 27 DBH for 3.6 and 7.2 kg N/10a treatments and at 27 and 29 DBH for Hwaseongbyeo and Daeanbyeo, respectively. In general, earlier applied N at PIS resulted in lower milled-rice protein content but the highest grain yield was expected to be obtained when N topdress at PIS was applied at the time when shoot N concentration started to drop below about 23 mg/g due to dilution effect after transplanting. In conclusion, the results of our experiments imply that the currently recommended N topdress time (24DBH) at PIS in Korea should be reconsidered for the higher grain yield and the better quality of rice.

***Keywords*: rice, nitrogen, yield, protein, timing**

Rice grain yield has increased in last several decades to about 5.3 ton per hectare per crop but it is equivalent to only 60% of climate-adjusted yield potential in Asia (Matthews *et al.*, 1995). Therefore, development of new rice cultivars with high yield potential and resistance in parallel with 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).

Topdress N is split into several times for applications to meet the demands of the rice plant and to secure the high grain yield and N use efficiency. Based on the critical stages of rice yield and yield components formation, N fertilizer has been recommended to be applied in several splits: at transplanting for crop recovery after transplanting, at TS for increasing tiller and panicle numbers, 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).

The N split application for ordinary rice cropping had been recommended to be 50% at transplanting, 20% at TS, 20% at PIS, and 10% at full heading in Korea until it was changed recently into 50, 20, 30, and 0%, respectively. N split-topdress at full heading stage is recommended not to apply because it increases the protein content of milled-rice, leading to poor eating quality of cooked rice.

Although N topdressing at PIS in Korea has been recommended to apply at 24 days before heading (DBH) that is correspondent to the time for spikelet differentiation, there have been some arguments about the timing of N topdress at PIS. Matsushima (1995) discussed from his experiment conducted in Japan in 1973 that the earlier N application at 48 to 28 DBH usually resulted in higher number of spikelet per hills than N application at 24 DBH but N application should be timed after 13 DBH for the high percentage of grain ripening. Overall, the grain yield per hill was lower in N application at the period from 43 to 18 DBH compared to the earlier and later N application. The lowest grain yield and ripened grain ratio was observed at the treatment with N application at 33 DBH that corresponds to the growth stage of neck-node differentiation and the lower internode of the culm begins elongation. N application at neck-node differentiation stage brought the higher number of spikelets per unit area, higher number of unfertilized caryopses, greater susceptibility to lodging, and lower starch accumulation before heading but none of these factors played a definitive role for the yield decline. In the field, the most distinguished

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in the treatment with N application at 33 DBH was the growth pattern of the upper leaves that was usually long, curved in shape, and droopy. Following the discussion, he recommended to develop the ideal rice cultivar with the uppermost two or three short and erect leaves and apply N at early vegetative growth and heading stages. However, Murayama (1995) 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 the earlier N application (at PIS).

Relationship between milled-rice protein content and grain quality has been reported (Chae and Jun, 2002; 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 in general milled-rice protein content showed negative correlation with palatability score of cooked rice. Similarly, Ishima *et al.* (1974) found highly negative correlation between protein content in polished rice and taste evaluation score. This means an increase in milled-rice protein content reduces eating quality of rice.

The recent guideline that milled-rice protein content is desirable to be below 6.5 % on the basis of 14% moisture content for good eating quality rice and the successful development of ideal japonica rice cultivars that have short stature, erect leaves, and high resistance to lodging and pest in Korea lead to the reevaluation of N topdress timing at PIS. Therefore the, objectives of our study were (1) to test the effect of different N topdress time around PIS in combination with variable N rates at TS on rice grain yield and protein content and (2) to determine the optimum N topdress timing at PIS for the high rice grain yield and quality.

MATERIALS AND METHODS

Site description and experimental design

Two experiments, one in 2004 and the other in 2005, were conducted at Experimental Farm, 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 experiments included three N rates at TS (0, 3.6, and 7.2 kg/10a), three N timing treatments: 10 days before PIS (PIS-10), at PIS (PIS), and 10 days after PIS (PIS+10), and two rice cultivars of medium maturity: Hwaseongbyeo (V1) and Daeanbyeo (V2). It is noted from Table 1 that the word PIS was 24 days before heading as common date for N application at panicle initiation stage of rice in Korea. The details of experimental design were presented in Table 1.

The experiments were split-split-plot design in which N treatments at TS, PIS, and rice varieties were randomly assigned to main plot, sub plot and sub-sub plot of 30 m^2. Rice was transplanted manually at the spacing of 0.15 m × 0.30 m with 3 seedlings per hill on 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 and analysis

In 2004, five hills per plot were collected on the 9th, 22nd, and 29th July before N application for measuring crop growth and N status parameters including shoot dry weight (SDW, kg/10a), shoot N concentration (SNC, mg/g), and shoot N content (SND, kg N in shoot/10a). At harvest (16th October), two samples, five hills for measuring yield components (total number of spikelets, number of filled and unfilled spikelets, and 1000-grain weight) and shoot N concentration and content and 72 hills for grain yield (kg/10a) and milled-rice protein (%) measurement, were collected from each plot. In 2005, the same procedures were used but sampling dates were the 12th, 21st, and 31st July for observing crop growth and N status parameters at PIS before N application and on the 9th October for yield, yield component, plant and grain N analysis. Shoot and milled-rice N concentration was analyzed by Kejeltec Auto 1035 System (Tecator, Sweden) and milled-rice N concentration (%) was converted into milled-rice protein content (%) by multiplying with factor of 5.95.

Data analysis

Data collected from two years (2004-2005) were sub-

Table 1. Treatment design of the experiment in 2004-2005.

Applied N at transplanting[†]	Applied N at tillering stage	N timing at PIS	Rice variety
4.8	0, 3.6, 7.2	PIS-10	V1, V2
4.8	0, 3.6, 7.2	PIS	V1, V2
4.8	0, 3.6, 7.2	PIS+10	V1, V2

[†] Applied N (kg/10a); V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively; PIS, PIS-10, PIS+10 were treatments with N application at PIS, 10 days before and after PIS, respectively.

jected 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 TS and N timing at PIS, rice cultivars and their interaction) using PROC GLM procedure in SAS 8.1 (SAS Inc., USA). Because the expected and actual dates before heading were different from year to year and rice cultivars, the applied N timing treatments (PIS, PIS-10 and PIS+10) were recalculated to N timing at actual days before heading (DBH) of each cultivar. After that the optimum timing for each rice cultivar at different applied N levels at TS was calculated from stepwise multiple regression equations to predict grain yield or milled-rice protein content using linear and quadratic term of DBH. The same procedure was applied for determining the optimum crop variables for N topdress timing at PIS.

RESULTS AND DISCUSSION

Actual date for N topdressing before heading

As the ordinary time for N topdress at PIS of rice falls on 24 DBH in Korea, the expected dates for N application were 14, 24 and 34 DBH for PIS–10, PIS and PIS+10 treatments, respectively. However, the expected dates for N application were different from the actual dates because of variation in heading date between two rice cultivars from a year to the other. The difference between expected and actual dates for N application also resulted from a requirement that canopy reflectance must be measured before N application for other purposes in the two experimental years. Because canopy reflectance measurement required sunny and cloudless sky day, it was delayed sometimes due to bad weather and therefore, N application was delayed too. The actual dates for N application of N timing treatments was calculated and presented in Table 2.

In general, compared to the expected dates, N was applied earlier at PIS in 2005 than in 2004 for all N timing treatments. Between two rice cultivars, N was applied earlier for Daeanbyeo than Hwaseongbyeo. The difference in DBH among N topdress timing treatments in two years and two rice cultivars was considered in the data analysis.

Rice grain and milled-rice protein content

In 2004, N topdress time had significant effect on grain yield and milled-rice protein content ($P<0.01$) (Table 3a). Among N timing treatments, delayed N application at PIS (treatment PIS+10) had significantly lower grain yield and higher milled-rice protein content for both rice cultivars than the other two N timing treatments. Even though statistical analysis indicated that no significant difference in grain yield of treatment PIS–10 and PIS for both rice cultivars, maximum yield was obtained at the treatment of PIS-10 (31 DBH) for Hwaseongbyeo and at the treatment of PIS (23 DBH) for Daeanbyeo. This was in agreement with significant interaction between N timing and rice cultivars ($P<0.05$) in variance analysis. Applied N at TS increased grain yield significantly but not protein contents.

In 2005, even though grain yield of the delayed N applica-

Table 2. Actual day of N topdress in days before heading for the three N timing treatments at PIS in 2004-2005.

N Timing	Rice cultivar	N application date at PIS	Heading date	N topdress in DBH[†]
The 2004 experiment				
PIS–10 (Expected 34 DBH)[†]	Hwaseongbyeo	13 July	13 August	31
	Daeanbyeo	13 July	17 August	35
PIS (Expected 24 DBH)	Hwaseongbyeo	25 July	13 August	19
	Daeanbyeo	25 July	17 August	23
PIS+10 (Expected 14 DBH)	Hwaseongbyeo	1 August	13 August	12
	Daeanbyeo	1 August	17 August	16
The 2005 experiment				
PIS–10 (Expected 34 DBH)	Hwaseongbyeo	15 July	18 August	34
	Daeanbyeo	15 July	21 August	37
PIS (Expected 24 DBH)	Hwaseongbyeo	23 July	18 August	26
	Daeanbyeo	23 July	21 August	29
PIS+10 (Expected 14 DBH)	Hwaseongbyeo	31 July	18 August	18
	Daeanbyeo	31 July	21 August	21

[†] DBH: days before heading.

Table 3a. Effect of nitrogen (3.6 kg/10a) topdress timing at panicle initiation stage on grain yield and milled-rice protein content in 2004.

N treatment		Grain yield (kg/10a)			Milled-rice protein (%)		
Ntill[†]	Timing[§]	V1[‡]	V1	Mean	V1	V2	Mean
No N	PIS-10	694.0	786.8	740.4	6.30	6.24	6.27
	PIS	669.4	798.5	734.0	6.60	6.68	6.64
	PIS+10	526.5	725.9	626.2	6.97	7.11	7.04
	Average	***630.0***	***770.4***	***700.2***	***6.62***	***6.68***	***6.65***
3.6 kg/10a	PIS-10	736.5	813.8	775.2	6.46	6.44	6.45
	PIS	697.1	804.0	750.6	6.79	6.70	6.75
	PIS+10	592.8	748.0	670.4	7.06	6.68	6.87
	Average	***675.5***	***788.6***	***732.1***	***6.77***	***6.61***	***6.69***
7.2 kg/10a	PIS-10	764.0	780.3	772.2	6.28	6.27	6.28
	PIS	775.0	845.8	810.4	6.74	6.86	6.80
	PIS+10	637.1	751.0	694.1	7.29	7.26	7.28
	Average	***725.4***	***792.4***	***758.9***	***6.77***	***6.80***	***6.79***
Pooled	PIS-10	731.5a	793.6a	762.6a	6.35c	6.32c	6.33c
	PIS	713.8a	816.1a	765.0a	6.71b	6.75b	6.73b
	PIS+10	585.5b	741.6b	663.6b	7.11a	7.02a	7.06a
	LSD.05	***34.6***	***29.8***	***24.4***	***0.26***	***0.25***	***0.17***
ANOVA (Probability level)							
Cultivar				0.00			0.74
Ntill				0.01			0.32
Timing				0.00			0.00
Ntill × Cultivar				0.06			0.47
Ntill × Timing				0.05			0.07
Cultivar × Timing				0.02			0.78

[†] Ntill was N rate applied at tillering stage (kg/10a).
[‡] V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively.
[§] PIS–10, PIS, PIS+10 were N topdress with 3.6 kg N/10a at 10 days before PIS, at PIS and at 10 days after PIS, respectively.

tion at PIS (PIS+10) was lower than that of treatment PIS and PIS–10 but not significant (Table 3b). No significant difference among N timing treatments in 2005 might have resulted from the fact that applied N date for all N timing treatments was earlier than the expected dates, ranging from 18-34 DBH for Hwaseongbyeo and 21-37 DBH for Daeanbyeo (Table 2). Milled-rice protein content of the delayed N application treatment (PIS+10) was significantly higher than two other N timing treatments (PIS and PIS-10).

The significant effect of rice cultivar and interaction between applied N rates at TS and N timing at PIS on grain yield in both years (Tables 3a and 3b) revealed that grain yield of each rice cultivar responded differently to N timing at different N rate applied at TS. Therefore, the optimum N topdress time should be determined in consideration of rice cultivar and crop growth and nutrition status at the time of N application. Additionally, the delayed N application at PIS, in general, was not favorable for securing both the high grain yield and the low protein content of Hwaseongbyeo and Daeanbyeo cultivars.

Yield components

Total number of spikelets per square meter, filled or unfilled spikelet ratio, and grain weight are three rice yield components that are directly used for grain yield calculation by Eq. 1.

$$\text{Grain yield (kg/10a)} \tag{1}$$
$$= \text{number of spikelets/m}^2 \times \text{ripening ratio} \times \text{grain weight}$$

Table 3b. Effect of nitrogen (3.6 kg/10a) topdress timing at panicle initiation stage on grain yield and milled-rice protein content in 2005.

N treatment		Grain yield (kg/10a)			Milled-rice protein (%)		
Ntill†	Timing§	V1‡	V2	Mean	V1	V2	Mean
No N	PIS-10	663.4	681.4	672.4	6.68	6.80	6.74
	PIS	618.4	713.3	665.9	6.65	6.99	6.82
	PIS+10	601.3	645.1	623.2	7.16	7.05	7.11
	Average	***627.7***	***679.9***	***653.8***	***6.83***	***6.95***	***6.89***
3.6 kg/10a	PIS-10	638.6	677.3	658.0	6.44	6.65	6.55
	PIS	667.8	733.7	700.8	6.64	6.82	6.73
	PIS+10	646.2	704.3	675.3	6.93	6.98	6.96
	Average	***650.9***	***705.1***	***678.0***	***6.67***	***6.82***	***6.75***
7.2 kg/10a	PIS-10	685.0	731.3	708.2	6.66	6.70	6.68
	PIS	682.6	719.6	701.1	6.84	6.80b	6.82
	PIS+10	710.2	731.6	720.9	7.22	7.16	7.19
	Average	***692.6***	***727.5***	***710.1***	***6.91***	***6.93***	***6.90***
Pooled	PIS-10	662.3	696.7	679.5	6.59	6.72b	6.66b
	PIS	656.3	722.2	689.2	6.71	6.87b	6.79b
	PIS+10	652.6	693.7	673.1	7.10	7.06a	7.08a
	LSD.05	***NS***	***NS***	***NS***	***NS***	***0.18***	***0.16***
ANOVA (probability level)							
Cultivar				0.00			0.26
Ntill				0.04			0.42
Timing				0.53			0.00
Ntill × Cultivar				0.66			0.59
Ntill × Timing				0.04			0.66
Cultivar × Timing				0.38			0.50

†Ntill was N rate applied at tillering stage (kg/10a).
‡V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively.
§PIS – 10, PIS, PIS+10 were N topdress with 3.6 kg N/10a at 10 days before PIS, at PIS and at 10 days after PIS, respectively.

Among yield components, number of spikelets mainly depends on rice growth and nutrition status before booting stage of rice (Cui & Lee, 2002; Ishii, 1995) while grain weight are more dependent on crop growth and nutrition status at reproductive (PIS to heading) and ripening period. Ishii (1995) showed that 50% reduction in radiation by shading at vegetative stage significantly reduced total number of spikelets from 41.6 (1000/m^2) to 38.3 (1000/m^2) but did not significantly reduce 1000-grain weight. On the other hand, 50% reduction in radiation at grain filling stage did not significantly reduce number of spikelets but reduced 1000-grain weight from 20.0 g (100% radiation) to 19.5 g (50% radiation). Moreover, 50% reduction in radiation at reproductive stage (from PIS to heading) significantly reduced both number of spikelets and 1000-grain weight. Therefore, comparing yield components among N timing treatments could reveal growth and nutrition limitation in a given important growth stage affecting rice grain yield.

In 2004, N topdress timing had significant effect on total number of spikelets (Table 4a). In general, the earlier applied N at PIS had the higher number of spikelets regardless of applied N rates at TS, indicating that without N application at PIS, crop N nutrition was not sufficient for spikelet formation even at high applied N rate at TS (7.2 kg N/10a). Unfilled spikelet percentage was highest with earlier applied N at PIS (PIS-10) but not statistically significant. Moreover, grain weight was statistically lower in the PIS–10 treatment than the PIS and PIS+10 treatments. A common relationship among total number of spikelets, unfilled spikelet percentage and grain weight in relation to N timing found in the

Table 4a. Effect of nitrogen (3.6 kg/10a) topdress timing at panicle initiation stage on yield component parameters in 2004.

Treatment design		Total number of spikelets (1000/m²)			Unfilled spikelets (%)			1000-grain weight (g)		
Ntill[†]	Timing[‡]	V1[§]	V2	Mean	V1	V2	Mean	V1	V2	Mean
No N	PIS-10	28.66	31.64	30.15	9.81	7.51	8.66	26.85	26.92	26.89
	PIS	25.96	30.45	28.21	8.50	7.43	7.97	28.16	28.36	28.26
	PIS+10	20.87	27.59	24.23	8.01	7.04	7.53	27.50	28.30	27.90
	Average	***25.16***	***29.89***	***27.53***	***8.77***	***7.33***	***8.05***	***27.50***	***27.86***	***27.68***
3.6 kg/10a	PIS-10	30.89	32.95	31.92	9.94	7.44	8.69	26.49	26.69	26.59
	PIS	27.24	31.41	29.33	8.76	8.43	8.60	28.13	28.03	28.08
	PIS+10	23.34	29.06	26.20	8.24	8.07	8.16	27.69	28.02	27.86
	Average	***27.16***	***31.14***	***29.15***	***8.98***	***7.98***	***8.48***	***27.44***	***27.58***	***27.51***
7.2 kg/10a	PIS-10	32.01	32.10	32.06	10.15	7.49	8.82	26.57	26.27	26.42
	PIS	31.85	32.81	32.33	9.37	7.75	8.56	26.89	27.95	27.42
	PIS+10	25.68	29.13	27.41	7.16	6.71	6.94	26.73	27.67	27.20
	Average	***29.85***	***31.35***	***30.60***	***8.89***	***7.32***	***8.11***	***26.73***	***27.30***	***27.01***
Pooled	PIS-10	30.52a	32.23a	31.38a	9.97	7.48	8.73	26.64b	26.62b	26.63b
	PIS	28.35b	31.56a	29.96b	8.87	7.87	8.37	27.73a	28.11a	27.92a
	PIS+10	23.30c	28.60b	25.95c	7.80	7.27	7.54	27.31a	28.00a	27.66a
	LSD.05	***1.62***	***1.49***	***1.25***	***NS***	***NS***	***NS***	***0.54***	***0.35***	***0.33***
ANOVA (Probability level)										
Cultivar				0.00			0.01			0.02
Ntill				0.01			0.80			0.25
Timing				0.00			0.03			0.00
Ntill × Cultivar				0.07			0.85			0.50
Ntill × Timing				0.38			0.60			0.71
Cultivar × Timing				0.05			0.19			0.17

[†] Ntill was N rate applied at tillering stage (kg/10a).
[‡] PIS–10, PIS, PIS+10 were N topdress with 3.6 kg N/10a at 10 days before PIS, at PIS and at 10 days after PIS, respectively.
[§] V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively.

experiment was that early applied N at PIS resulted in higher spikelet number, higher unfilled spikelet percent and lower grain weight. However, this relationship was not applicable for 1000-grain weight of Hwaseongbyeo rice cultivar at treatments without N or 3.6 kg N/10a applied at TS in 2004. In this case, the treatment PIS+10 with delayed N application until 12 DBH (Table 4a) had lower number of spikelets and unfilled spikelet percent than two other N timing treatments (PIS and PIS–10) but grain weight was also lower. This was not the same as expected that late N application would result in lower spikelet number but higher grain weight due to high crop photosynthesis after heading. In this case very late N application at PIS would have reduced photosynthesis very much until N application and the lack of photosynthesis product and nitrogen in this duration might have reduced the spikelet size, leading to low grain weight. Ishii (1995) indicated that crop photosynthesis and dry mater accumulation in reproductive period (PIS to heading) were important in increasing number of spikelets and grain weight.

Response of total number of spikelets of Hwaseongbyeo rice cultivar to N timing showed the same trend in both 2004 and 2005 experiments regardless of applied N rates at TS (Table 4a and 4b). The average total number of spikelets for this rice cultivar was 30.53, 28.35, and 23.30 (1000/m²) for N topdress timing treatments with N application at 31, 19, 12 DBH, respectively in 2004 and 29.74, 28.01 and 25.72 (1000/m²) for N timing treatments with applied N at 34, 26, and 18 DBH, respectively in 2005. On the other hand, the response of total number of spikelets to N topdress timing of

Table 4b. Effect of nitrogen (3.6 kg/10a) topdress timing at panicle initiation stage on yield component parameters in 2005.

Treatment design		Total number of spikelets ($1000/m^2$)			Unfilled spikelets (%)			1000-grain weight (g)		
Ntill[†]	Timing[‡]	V1[§]	V2	Mean	V1	V2	Mean	V1	V2	Mean
No N	PIS-10	29.1	27.4	28.3	12.8	7.0	9.9	26.4	26.7	26.6
	PIS	26.2	29.6	27.9	14.9	11.7	13.3	27.7	27.3	27.5
	PIS+10	23.3	25.3	24.3	9.9	10.1	10.0	28.7	28.4	28.6
	Average	***26.2***	***27.4***	***26.8***	***12.5***	***9.6***	***11.1***	***27.6***	***27.5***	***27.5***
3.6 kg/10a	PIS-10	28.2	28.7	28.4	14.0	10.9	12.5	26.3	26.6	26.5
	PIS	28.2	31.4	29.8	14.1	13.4	13.8	27.6	27.0	27.3
	PIS+10	25.0	27.1	26.0	10.5	10.0	10.3	29.0	28.9	29.0
	Average	***27.1***	***29.0***	***28.1***	***12.9***	***11.5***	***12.2***	***27.7***	***27.5***	***27.6***
7.2 kg/10a	PIS-10	31.9	31.2	31.5	16.3	12.2	14.2	25.7	26.9	26.3
	PIS	29.6	32.9	31.3	15.4	18.3	16.9	27.6	26.8	27.2
	PIS+10	28.9	31.3	30.1	14.8	15.0	14.9	29.0	27.6	28.3
	Average	***30.1***	***31.8***	***31.0***	***15.5***	***15.2***	***15.3***	***27.4***	***27.1***	***27.3***
Pooled	PIS-10	29.74a	29.08b	29.41a	14.36	10.03b	12.20b	26.14c	26.71b	26.43c
	PIS	28.01ab	31.30a	29.66a	14.82	14.49a	14.66a	27.67b	27.03b	27.35b
	PIS+10	25.72b	27.88b	26.80b	11.73	11.72b	11.73b	28.90a	28.30a	28.60a
	LSD.05	***2.95***	***1.71***	***1.76***	***NS***	***2.44***	***1.93***	***0.99***	***0.99***	***0.64***
ANOVA (Probability level)										
Cultivar				0.04			0.07			0.43
Ntill				0.02			0.17			0.75
Timing				0.01			0.14			0.00
Ntill × Cultivar				0.93			0.42			0.93
Ntill × Timing				0.52			0.92			0.93
Cultivar × Timing				0.10			0.07			0.16

[†] Ntill was N rate applied at tillering stage (kg/10a).

[‡] PIS–10, PIS, PIS+10 were N topdress with 3.6 kg N/10a at 10 days before PIS, at PIS and at 10 days after PIS, respectively.

[§] V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively.

Daeanbyeo rice cultivar in 2005 was different from that in 2004 and from those of Hwaseongbyeo rice cultivar. In 2005, Daeanbyeo had the highest total number of spikelets at treatment with N applied at PIS (29 DBH) than treatment PIS–10 (37DBH) and PIS+10 (21 DBH) regardless of applied N rates at TS. The difference in the response of total number of spikelets to N topdress timing of Daeanbyeo rice cultivar in 2005 may have resulted from the difference in actual date of applied N. Possibly 37 DBH would be too early for N application at PIS to sufficiently increase the total number of spikelets in rice production.

The relationship between total number of spikelets and unfilled spikelets was similar in 2005 to that in 2004: the higher total number of spikelets and the higher unfilled-spikelet percent. Matsushima (1995) have discussed the negative effect of total number of spikelets on the percentage of ripened grain in three-year experiment in Japan. On an average over rice cultivars and applied N rates at TS, 1000-grain weight was 26.43, 27.35, and 28.60 (g) for treatments with applied N timing at PIS–10, PIS and PIS+10, respectively in 2005.

Shoot nitrogen concentration and content

Shoot N concentration and content at harvest were measured in the experiments because the variation in rice SNC

Table 5a. Effect of nitrogen (3.6 kg/10a) topdress timing at panicle initiation stage on shoot nitrogen concentration and content in 2004.

N treatment		Shoot N concentration (mg/g)			Shoot N content (kg/10a)		
Ntill[a]	Timing[‡]	V1[§]	V2	Mean	V1	V2	Mean
No N	PIS-10	7.61	7.52	7.56	9.99	11.16	10.58
	PIS	8.49	8.20	8.35	11.52	12.40	11.96
	PIS+10	7.70	8.45	8.08	8.83	11.61	10.22
	Average	***7.93***	***8.06***	***8.00***	***10.11***	***11.72***	***10.92***
3.6 kg/10a	PIS-10	7.66	7.66	7.66	11.31	12.00	11.66
	PIS	8.78	8.36	8.57	12.54	13.17	12.86
	PIS+10	8.14	7.77	7.96	10.46	11.16	10.81
	Average	***8.19***	***7.93***	***8.06***	***11.44***	***12.11***	***11.78***
7.2 kg/10a	PIS-10	7.99	8.44	8.21	13.10	12.71	12.90
	PIS	8.04	8.64	8.34	12.69	13.82	13.26
	PIS+10	8.40	9.29	8.84	11.45	13.67	12.56
	Average	***8.14***	***8.79***	***8.46***	***12.41***	***13.40***	***12.91***
Pooled	PIS-10	7.75bc	7.87b	7.81b	11.47a	11.96b	11.71b
	PIS	8.44a	8.40a	8.42a	12.25a	13.13a	12.69a
	PIS+10	8.08ba	8.50a	8.29a	10.25b	12.14b	11.20b
	LSD.05	***0.54***	***0.51***	***0.34***	***1.20***	***0.87***	***0.71***
ANOVA (Probability level)							
Ntill				0.30			0.03
Timing				0.04			0.01
Cultivar				0.25			0.00
Ntill × Cultivar				0.05			0.47
Ntill × Timing				0.33			0.67
Cultivar × Timing				0.41			0.19

[†] Ntill was N rate applied at tillering stage (kg/10a).

[‡] PIS–10, PIS, PIS+10 were N topdress with 3.6 kg N/10a at 10 days before PIS, at PIS and at 10 days after PIS, respectively.

[§] V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively.

and SND among treatments was closely related to grain yield, yield components (Matsushinma, 1995) and protein content (Murayama, 1995). In 2004, N timing treatments at PIS showed significant effect on both SNC and SND at harvest (Table 5a). Shoot N concentration of early applied N at PIS treatment (7.81 mg/g) was significantly lower than 8.42 and 8.29 mg/g of treatments with applied N at PIS and PIS+10, respectively but SND of early applied N at PIS treatment (11.71 kg/10a) was not significantly different from 11.20 kg/10a of treatment with late N application at PIS.

Comparing the values of SNC and SND averaged over N rates at TS, we found that SNC of treatment with N application at PIS was not significantly different from that of treatment with N application at PIS+10 but SND of the former was consistently higher than the latter in 2004 (Table 5a). It resulted from the fact that late N application at PIS resulted in lower biomass accumulation at harvest (data was not presented). The applied N at TS in 2004 did not significantly increase SNC but increased SND. On an average, applied N at TS increased SND from 10.92 kg/10a (no N application) to 11.78 and 12.91 kg/10a for treatments with 3.6 and 7.2 kg N/10a applied at TS, respectively.

In 2005, the variation in SNC and SND was very similar to that of 2004 but analysis of variance showed no statistically significant difference among N timing treatments (Tables 5a and 5b). The reason for no consistent difference in SNC and SND between late N application at PIS and two other N timing treatments in 2005 might have resulted from

Table 5b. Effect of nitrogen (3.6 kg/10a) topdress timing at panicle initiation stage on shoot nitrogen concentration and content in 2005.

N treatment		Shoot N concentration (mg/g)			Shoot N content (kg/10a)		
Ntill[†]	Timing[‡]	V1[§]	V2	Mean	V1	V2	Mean
No N	PIS-10	8.68	7.93	8.31	11.24	10.25	10.74
	PIS	9.03	8.37	8.7	11.04	10.73	10.89
	PIS+10	8.38	8.16	8.27	9.29	9.96	9.63
	Average	***8.70***	***8.15***	***8.43***	***10.52***	***10.31***	***10.42***
3.6 kg/10a	PIS-10	8.05	8.34	8.19	10.89	11.55	11.22
	PIS	8.78	9.05	8.92	12.03	12.27	12.15
	PIS+10	8.63	8.61	8.62	10.59	11.21	10.9
	Average	***8.49***	***8.67***	***8.58***	***11.17***	***11.68***	***11.42***
7.2 kg/10a	PIS-10	8.77	8.14	8.46	12.71	11.67	12.19
	PIS	9.37	8.55	8.96	12.72	12.38	12.55
	PIS+10	9.95	9.04	9.5	13.66	13.54	13.6
	Average	***9.36***	***8.58***	***8.97***	***13.03***	***12.53***	***12.78***
Pooled	PIS-10	8.50	8.14b	8.32b	11.61	11.15	11.38
	PIS	9.06	8.66a	8.86a	11.93	11.80	11.86
	PIS+10	8.99	8.60a	8.80a	11.18	11.57	11.38
	LSD.05	***NS***	***0.42***	***0.29***	***NS***	***NS***	***NS***
ANOVA (Probability level)							
Ntill				0.45			0.05
Timing				0.11			0.25
Cultivar				0.00			0.62
Ntill × Cultivar				0.01			0.04
Ntill × Timing				0.29			0.20
Cultivar × Timing				0.54			0.75

[†] Ntill was N rate applied at tillering stage (kg/10a).
[‡] PIS–10, PIS, PIS+10 were N topdress with 3.6 kg N/10a at 10 days before PIS, at PIS and at 10 days after PIS, respectively.
[§] V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively.

the earlier N topdress than the expected date of N timing treatments. In 2005, the treatment PIS+10 had earlier N application at 18-21 DBH than at 12-16 DBH of 2004 and also at the expected date of 14 DBH. This means that we did not really have treatment with late N application at PIS in 2005. Similar to 2004, applied N rate at TS showed significantly increased SND but not SNC.

Optimum nitrogen timing at PIS

Due to the difference in actual date of applied N at PIS among N timing treatments and rice cultivars, analysis of variance could not be employed for determining the optimum N topdress timing at PIS. Therefore, stepwise multiple linear regression analysis (forward selection at $P<0.05$) was applied to derive the relationship of grain yield and milled-rice protein content with the actual DBH of N topdress (Table 6a and Table 6b).

Instead of actual grain yield and milled-rice protein content, relative yield (ratio of the fertilized-plot yield to the unfertilized-plot yield) or relative protein content (ratio of the fertilized-plot protein to the unfertilized-plot protein) were used to eliminate the effect of year to year variation. These relative values improved the coefficient of determination (R^2) of the regression models to predict grain yield and protein content using DBH from 0.21 and 0.49 to 0.49 and 0.57, respectively (Table 6a). Stepwise multiple regression analysis using linear and quadratic terms of DBH as independent variables selected both linear and quadratic terms for the model to predict grain yield (Eq. 2 and 3) but only

Table 6a. Rice grain yield and milled-rice protein content in response to nitrogen topdress timing at PIS in 2004-2005 and the optimum time for N topdress at PIS (input data were means over three replications from two years, three N timing treatments, three N rates at tillering stage and two rice cultivars, n = 36).

Stepwise regression equations		R^2	Optimum N timing (DBH)[‡]
$\text{Yield} = 390.4 + 24.6\text{DBH}^b - 0.44\text{DBH}^2$	(Eq. 2)	0.21	28
$\text{Relative yield}^{\dagger} = 0.56 + 0.048\text{DBH} - 0.00086\text{DBH}^2$	(Eq. 3)	0.49	28
$\text{Milled-rice Protein} = 7.41 - 0.025\text{DBH}$	(Eq. 4)	0.49	N/A
$\text{Relative milled-rice protein} = 1.19 - 0.0049\text{DBH}$	(Eq. 5)	0.57	N/A

[†] Relative yield and protein were ratio of fertilized-plot yield and protein content to unfertilized-plot yield and protein content, respectively.
[‡] DBH was actual days of applied N before heading; N/A: non applicable.

Table 6b. Dependence of the optimum N topdress timing at PIS on N rates at tillering stage and rice cultivar.

Factor treatment	Stepwise regression equations		R^2	Optimum N timing (DBH[§])
	N rate at tillering stage (kg/10a)			
0	$\text{RY}^{\dagger} = 0.35 + 0.058\text{DBH} - 0.00098\text{DBH}^2$	(Eq. 6)	0.81	30
3.6	$\text{RY} = 0.54 + 0.052\text{DBH} - 0.00095\text{DBH}^2$	(Eq. 7)	0.81	27
7.2	$\text{RY} = 0.58 + 0.053\text{DBH} - 0.00097\ \text{DBH}^2$	(Eq. 8)	0.49	27
	Rice cultivar			
V1[‡]	$\text{RY} = 0.36 + 0.0666\text{DBH} - 0.00122\ \text{DBH}^2$	(Eq. 9)	0.58	27
V2	$\text{RY} = 0.52 + 0.0506\text{DBH} - 0.00088\text{DBH2}$	(Eq. 10)	0.54	29

[†] RY was ratio of fertilized-plot yield to unfertilized-plot yield.
[‡] V1 and V2 were Hwaseongbyeo and Daeanbyeo, respectively.
[§] DBH was days of applied N before heading.

linear term for the model to predict milled-rice protein content (Eq. 4 and 5). Equations 4 and 5 indicate that the earlier we apply topdress-N at PIS the lower protein content we may obtain. The lower grain N accumulation in the earlier N application was discussed by Murayama (1995). On the contrary to the milled-rice protein content, grain yield showed the quadratic response to DBH of N topdress at PIS. Thus, the optimum N topdress timing at PIS for the highest grain yield was calculated from the equations 2 and 3 as 28 DBH (Table 6a and Fig. 1). The optimum topdress N timing (28 DBH) at PIS calculated from our experiment was four days earlier than the currently recommended N timing at PIS in Korean (24 DBH). This might have resulted from the fact that the recommended N timing at PIS in Korea might have been drawn from the experiments with older rice cultivars. The optimum N timing for the older cultivars with tall and easily lodging characteristics might be later than the optimum N timing for Hwaseongbyeo and Daeanbyeo in our experiment, the current cultivars with short stature and lodging resistance.

Considering the previous analysis of variance that the grain yield of each rice cultivar responded to panicle N topdress timing differently at the different topdress N rates at TS, we did stepwise multiple regression analysis (forward selection with $P < 0.05$) to relate grain yield to actual DBH (N topdress time at PIS) for each topdress N level at TS and for each rice cultivar (Table 6b). The analysis of regression again verified the different response of grain yield of different rice cultivars at different topdress N rates at TS to the actual DBH of N topdress time at PIS. From the regression equations 6 to 7 for predicting grain yield by DBH, the optimum topdress N timing at PIS was calculated as 30 DBH for the treatment without N topdress at TS and 27 DBH for the treatments with 3.6 and 7.2 kg N/10a topdress at TS (Table 6b and Fig. 2). Similarly, the optimum topdress N timing at PIS was estimated differently as 27 and 29 DBH for Hwaseongbyeo and Daeanbyeo, respectively. The different optimum N timing for the different topdress N rates at TS might be related to the crop growth and N nutrition status at PIS that was in turn affected by topdress N rate at TS. The optimum N timing of 30 DBH for the treatment without N application at TS compared to 27 DBH for the treatments with N topdress at TS indicated that the real-time for N topdress at PIS possibly depended on the crop growth and N nutrition status at PIS: the poorer the crop growth and N nutrition status is the earlier N topdress at PIS is required for

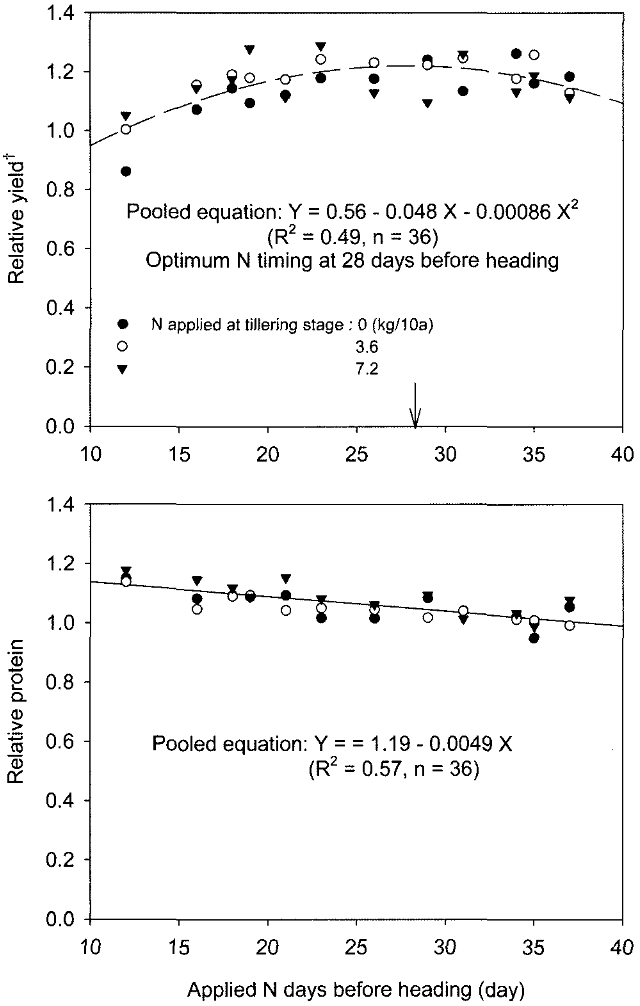

Fig. 1. Response of relative grain yield and milled rice protein content to N topdress date in days before heading.
†*Relative yield and milled-rice protein content were defined as ratio of fertilized-plot to unfertilized-plot values.*

higher yield.

The total number of spikelets and 1000-grain weight also showed the quadratic response to the N topdress time (in DBH) at PIS like grain yield. The optimum topdress N timing for the highest total number of spikelets and the highest grain weight was calculated as 31 DBH and 18 DBH, respectively (Fig. 3), being different from each other and from that (28 DBH for the data pooled across all the treatments) for the highest grain yield. The calculated 31 DBH and 18 DBH for the highest total number of spikelets and grain weight coincide with panicle initiation stage and late spikelet initiation stage of rice, respectively. Kumura (1995) also found that optimum topdress N for maximum total number of spikelets was earlier than that for the maximum grain yield. He indicated that topdress N time for the highest total number of spikelets resulted in low percentage of ripened grain (%).

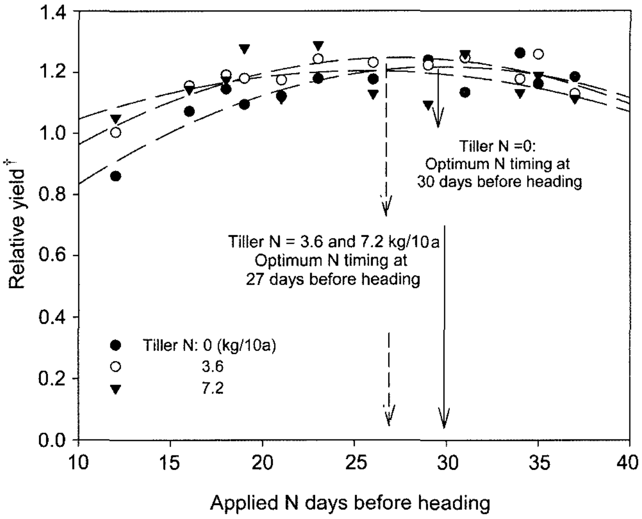

Fig. 2. Response of relative grain yield to N topdress date in days before heading for each topdress N rate at tillering stage. The details of response at each N level at tillering stage was presented by Eq. 6 to Eq. 8 in Table 6b.
†*Relative yield was defined as ratio of fertilized-plot to unfertilized-plot yield values.*

The high grain weight due to late applied N at 18 DBH might be related to the lower number of spikelets (limited sink) and the increased photosynthesis product after heading (increased source), therefore, promoting grain filling process. However, N topdress at later than 18 DBH reduced grain weight (Fig. 3). This reduction might have resulted from the reduced hull size due to the limited supply of N and photosynthate at the critical stage for hull growth (Ishii, 1995).

Optimum nitrogen concentration for real-time N application at PIS

As discussed in the above section that the optimum N timing depended on the crop growth and N nutrition status at the time of N topdress at PIS, we plotted grain yield and protein content versus SNC measured just prior to N topdress at PIS–10, PIS, and PIS+10 to find the optimum N concentration for the optimum grain yield and milled-rice protein content (Fig. 4).

Milled-rice protein content had negative linear relationship with SNC measured just prior to N topdress at PIS. This means that the higher SNC before N topdress at PIS is, the lower protein content is obtained at harvest. This relationship was possibly not common relationship between SNC at PIS and milled-rice protein content at harvest found in other publications (Kim, 2004; Nguyen, 2005). This relationship might be indirect relationship between the SNC at PIS and

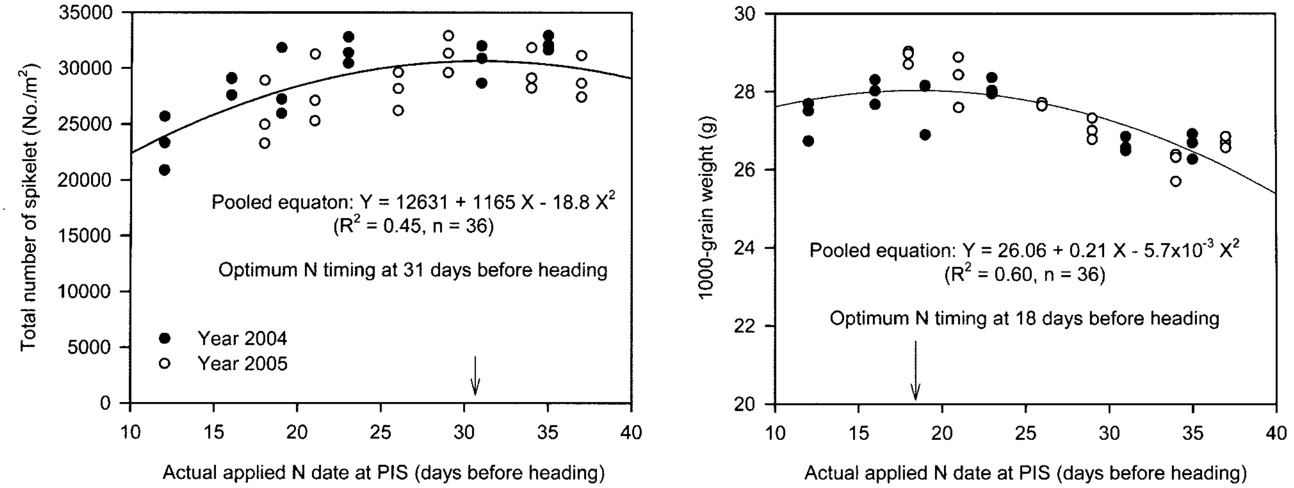

Fig. 3. Response of total number of spikelets and 1000-grain weight to actual N topdress date in days before heading.

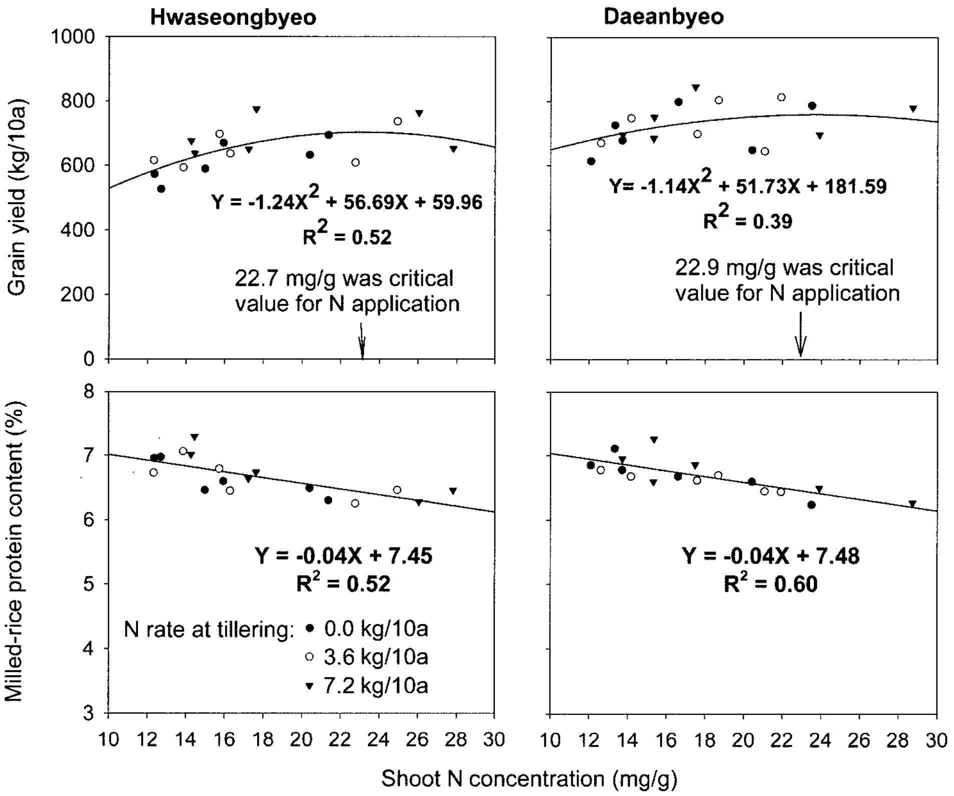

Fig. 4. Response of grain yield and milled-rice protein content to shoot N concentration measured just before N application of three N timing treatments at PIS (Data were means over three replications).

the grain yield and milled-rice protein content because all of them were directly affected by the N topdress date. The effect of N topdress date on grain yield and protein content was discussed in the above section. And the temporal decline of SNC after topdressing N at TS has been intensively reported by Kim (2004), Cui & Lee (2002), and Sheehy *et al.* (1998). From the indirect relationship between SNC before applied N of three N timing treatment and grain yield, the calculated optimum shoot N concentration for the timing of N topdress at PIS to obtain the maximum grain yield was 22.7 and 22.9 mg/g for Hwaseongbyeo and Daeanbyeo, respectively (Fig. 4). This means that topdress N at PIS is required to be applied at the time that shoot N concentration drops below those values due to the dilution effect after N topdress at TS.

CONCLUSION

Earlier applied N at PIS resulted in lower protein content but the highest grain yield was obtained when topdress N at PIS was applied at 28 days before heading in general. However, optimum N timing was also dependent on plant growth and N nutrition status at PIS and rice cultivars. The optimum N timing for the highest grain yield at PIS was at 30 DBH for no N treatments and at 27 DBH for the treatments with 3.6 and 7.2 kg N/10a applied at TS while at 27 and 29 DBH for Hwaseongbyeo and Daeanbyeo, respectively. These results imply that the N topdress time of 24 DBH that has been recommended in Korea based on the past experiments with old cultivars should be reconsidered for the improvement of grain yield and rice quality as well.

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