Effect of N Top-Dressing on Protein Content in Japonica and Indica Rice Grains

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ABSTRACT

The accumulation and distribution of ^{15}N in the protein and protein fractions of rice grains were investigated by N topdressing at three stages: panicle initiation, heading (HD), and HD+20 days. A traditional tall indica and a semidwarf japonica rice variety treated with two levels of basal N were used for the investigation. Topdressing of N increased crude protein and each protein fraction in the grain. The amount of increase was considerably greater in the indica than in the japonica variety. The top-dressed ^{15}N partitioned most effectively to the glutelin fraction when it was applied at the HD stage. On the other hand, ^{15}N applied at HD+20 days contributed more to increased prolamin. Top-dressing of N was effective in obtaining rice grains with high quality protein (high glutelin content) in the indica variety, especially when N was top-dressed at HD and HD+20 days.

The ecological diversification of rice (*Oryza sativa* L.) has resulted in two major ecogeographic races: indica, adapted to the tropics, and japonica, adapted to temperate regions. Unlike japonica, indica has been developed in less favorable environmental and cultural conditions: low soil fertility, lodging, high pest incidence, etc. Although there is much information about the effect of nitrogen application on growth and yield in these varieties, little is known about the resulting grain quality, particularly about the protein content. Although rice is generally less important than some other grains as a source of protein, it is significant for the people whose daily food consumption is dominated by rice. In many countries, particularly, in South and Southeast Asia, people depend on rice as their major source of protein. Hence, it is important to obtain rice with high levels of good-quality protein.

Research is currently under way to promote more efficient ways of N fertilization by choosing the best application stage to achieve higher protein production. In rice cultivation, N fertilizer is commonly applied in two parts: two-thirds during land preparation or at the four-to-five leaf stage as a basal dressing and one-third at the reproductive stage as a topdressing. The time of N topdressing, the period from reproductive to early ripening stage, plays a major role in determining the protein content of the rice grain. Although several studies have reported the effect of N topdressing on protein content of rice (Patrick et al 1974, Islam et al 1990, Souza et al 1993), the particular effect of N topdressing has not been assessed because of priming (Hauck et al 1976) or added N interaction (Jenkinson et al 1985) with soil N.

Among cereals, rice contains better-quality protein because the major reserve protein is glutelin, whereas prolamin predominates in other cereals (Cagampang et al 1966, Del Rosario et al 1968, Hauston and Mohammad 1970). An improvement in protein content does not necessarily imply the improvement of biological value in rice grains unless it involves an increase in the glutelin content. Therefore, a strategy for improvement of grain protein by N fertilization should consider not only the amount of protein but also its fractions in the grain.

In the present study, the effects of ^{15}N fertilizer top-dressed at the stages of panicle initiation (PI), heading (HD), and HD+20 days were assessed. ^{15}N accumulation and distribution in protein and protein fractions were studied using a traditional tall indica and a semidwarf japonica rice variety.

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MATERIALS AND METHODS

A pot experiment by soil culture was conducted using Hinohikari, a semidwarf, erect japonica rice variety, most widely cultivated in southern Japan, and Hadsaduri, a tall indica variety with a weak stem and a long and slender-awned grain.

The dormancy of Hadsaduri was broken by soaking seeds in water (40°C) continuously supplied with air for 24 hr (Hayashi 1977). A pot was filled with 3 kg of shirasu soil, a pyroclastic flow soil widely distributed in Kagoshima prefecture, Japan. The soil contained nitrogen at 0.7 g/kg and carbon at 6.8 g/kg; the pH was 4.76 (KCl). The soil pH was adjusted to 5.5 by the addition of dolomite (423 mg/kg of fresh soil).

Two levels of basal N fertilizer (0.5 g of N per pot for the lower level and 1.0 g for the higher), as $(NH_4)_2SO_4$, were used in the experiment. In addition to basal dressing, 0.2 g of ^{15}N fertilizer per pot (2.69 atom % excess N) as $(NH_4)_2SO_4$ was applied to each at the panicle initiation (PI) stage, the heading (HD) stage, and at HD+20 days. Phosphorus and potassium fertilizer, 0.50 g of P_2O_5 as super phosphate and 0.5g of K_2O as potassium chloride, respectively, were applied to each pot as basal dressing. Four seedlings (at 23 days after emergence) were transplanted into each pot. Each treatment was replicated three times.

Plants were harvested at the maturity stage. The unfilled grains were separated using water, and the filled grains were exposed to sunlight for drying. Grains were hulled by using an automatic rice husker and ground in a vibrating sample miller. The meal obtained from the grains was used to determine crude protein (N × 5.95). Protein fractionation was by the Osborne method modified by Frances et al (1952) and Ozaki (1949).

Three grams of the ground rice was put into a glass-stoppered centrifuge tube and extracted with 50 ml of diethyl ether. After decantation, the sample was allowed to stand for 5–6 hr to allow the remaining ether to dry out. To extract the albumin, about 30–35 ml of water was added, and the sample was mixed well by an automatic laboratory mixer and allowed to stand overnight. The mixture was centrifuged at 10,000 rpm for 10 min. The centrifugate was decanted directly into a 500-ml Kjeldahl flask. The residue was then washed twice with 30–35 ml of water and centrifuged; the centrifugate was added to the previous extract.

The same procedure was followed to extract the globulin with 2.5% NaCl and the prolamin with 70% ethanol. The fourth and last extraction was made with 0.2% NaOH to extract the glutelin. The extraction procedure employed for the previous three solvents was altered only as follows. After two subsequent extractions with 0.2% NaOH (overnight), the residue was washed twice with water. After the fourth extraction, all of the residue was trans-

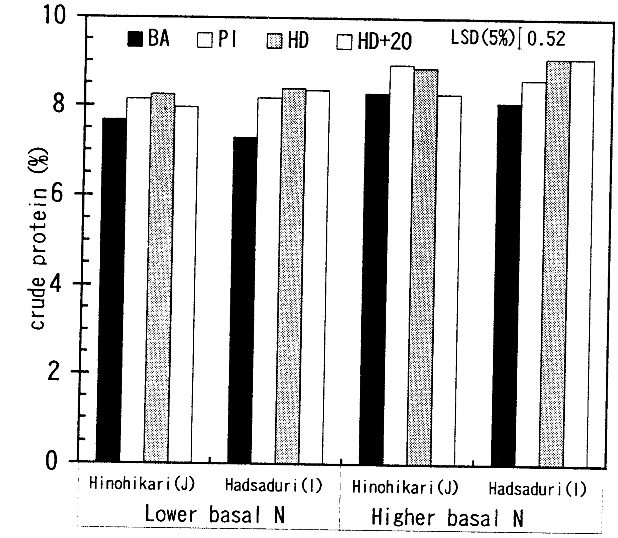

Fig. 1. Crude protein content in rice as affected by stages of N top-dressing at lower basal N (0.5 g/pot) and at higher basal N (1.0 g/pot). J = japonica; I = indica; BA = basal only; PI = panicle initiation stage; HD = heading stage; HD+20 days = heading + 20 days. Amount of top-dressed N (g/pot) = 0.2.

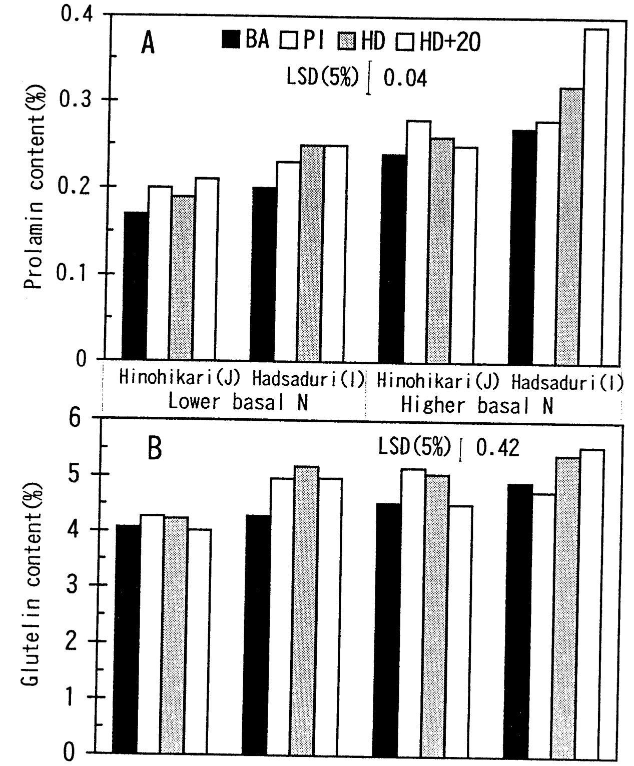

Fig. 2. Prolamin (**A**) and glutelin (**B**) content in rice as affected by stages of N top-dressing at lower basal N (0.5 g/pot) and at higher basal N (1.0 g/pot). J = japonica; I = indica; BA = basal only; PI = panicle initiation stage; HD = heading stage; HD+20 days = heading + 20 days. Amount of top-dressed N (g/pot) = 0.2.

ferred into a 500-ml Kjeldahl flask and used to determine the insoluble fraction of the N. Each treatment was replicated three times. The Kjeldahl method was used to determine N content in all fractions.

The ^{15}N in each fraction was determined by emission spectrometry. The ^{15}N excess (%) was calculated by subtracting the natural abundance from the estimated value. The amount of top-dressed N (mg/g of dry matter) was calculated as follows:

$$\frac{^{15}\text{N atom \% excess of sample}}{^{15}\text{N atom \% excess of fertilizer}} \times \text{total N per gram of sample}$$

The data were analyzed using analysis of variance, and the mean differences were assessed by the least significant difference method at the 5% level of significance (IRRISTAT 1991).

RESULTS AND DISCUSSION

The effects of applying N topdressing at different growth stages on the crude protein and the protein fractions were evaluated by comparing the value of the selected parameter with topdressing and its value without topdressing.

Crude Protein Content

The crude protein content (%) of Hinohikari (japonica) and Hadsaduri (indica) as affected by stage at application of topdressing is shown in Figure 1. Topdressing affected the crude protein content of the indica variety more than that of the japonica variety in both levels of basal N. The amount of increase was considerably higher in the indica than in the japonica variety. Early application of N topdressing (at PI and HD) in japonica and late application in indica were found to be more effective for grain protein production.

Protein Fractionation

The Osborne protein fractionation procedure, when performed as outlined by Ozaki (1949), extracted about 44% of the crude protein. This low efficiency of the procedure was also reported by Padhye and Salunkhe (1979) and Russell and Kymal (1956). The major reason for the low extraction is the insoluble nature of rice protein, especially glutelin, which constitutes a major portion of the storage protein in rice grains. However, using a modified procedure, we extracted 70–80% of the crude protein, of which about 75% was the glutelin fraction. The present study emphasized the glutelin fraction, which constitutes the largest portion of protein in polished rice.

The amount of prolamin and glutelin fractions as affected by time of topdressing application is shown in Figure 2. At both levels of basal N, the time of topdressing did not greatly affect the albumin content (except for HD+20 days at the lower basal level) (data not presented). However, the japonica variety contained a higher amount of albumin than the indica variety.

The N topdressing affected the prolamin content of the grain in the indica more than in the japonica variety, especially at later stages. Topdressing of N at PI and HD stages increased the glutelin content of the grains in japonica rice, although the effect at the lower basal N level was slight. In the indica rice, the glutelin of the grains was increased over all stages of N application at the lower basal N level. On the other hand, the effect was observed only at HD and HD+20 days with higher basal N.

Top-Dressed N: Accumulation and Distribution

The ^{15}N excess (%) in the grain and in the various protein fractions at the different stages of topdressing is shown in Table I. The ^{15}N excess in the grain was the highest when N was top-dressed at the HD stage. ^{15}N excess in all the protein fractions of grains in japonica were the highest at the HD stage and lowest at HD+20 days. On the other hand, the protein fractions in indica showed high values at HD and HD+20 days, especially for prolamin at HD+20 days. The result could be related to the biosynthesis of proteins during development of the rice seed. Yamagata et al (1982) demonstrated that glutelin synthesis occurs earlier than prolamin synthesis during rice seed development. Therefore, it is understandable that the prolamin fraction showed higher ^{15}N excess when fertilizer was applied at HD+20 days.

TABLE I
^{15}N Excess (%) in Grain and in Protein Fractions of Rice

Basal N Level and Variety	Stage of Application of Nitrogen Topdressing[a]	Grain	Protein Fraction Albumin	Globulin	Prolamin	Glutelin
Lower basal N level[b]						
Hinohikari						
(japonica)	PI	0.65	0.62	0.66	0.56	0.68
	HD	0.83	0.76	0.87	0.71	0.91
	HD+20 days	0.35	0.38	0.36	0.54	0.35
Hadsaduri						
(indica)	PI	0.54	0.54	0.57	0.48	0.47
	HD	0.63	0.68	0.71	0.59	0.73
	HD+20 days	0.58	0.54	0.63	0.75	0.63
Higher basal N level						
Hinohikari	PI	0.43	0.43	0.40	0.38	0.44
	HD	0.65	0.56	0.63	0.52	0.65
	HD+20 days	0.27	0.35	0.23	0.35	0.15
Hadsaduri	PI	0.36	0.41	0.39	0.38	0.42
	HD	0.57	0.55	0.55	0.49	0.49
	HD+20 days	0.54	0.51	0.57	0.75	0.56
LSD (5%)		0.11	0.11	0.18	0.15	0.10

[a] In addition to basal N (0.5 g/pot lower and 1.0 g/pot higher), 0.2 (g/pot) ^{15}N fertilizer N was top-dressed. PI = panicle initiation. HD = heading.
[b] Lower level, 0.5 g of N/pot; higher level, 1.0 g/pot.

TABLE II
Amount of Top-Dressed Nitrogen (mg/g of dry matter) in Protein Fractions of Rice

Basal N Level and Variety	Stage of Application of Nitrogen Topdressing[a]	Grain	Protein Fraction Albumin	Globulin	Prolamin	Glutelin
Lower basal N level[b]						
Hinohikari						
(japonica)	PI	3.31	0.42	0.29	0.07	1.81
	HD	4.24	0.53	0.47	0.08	2.39
	HD+20 days	1.76	0.35	0.17	0.07	0.88
Hadsaduri						
(indica)	PI	2.73	0.29	0.22	0.07	1.44
	HD	3.27	0.33	0.32	0.09	2.36
	HD+20 days	3.03	0.28	0.29	0.12	1.95
Higher basal N level						
Hinohikari	PI	2.38	0.36	0.21	0.07	1.41
	HD	3.59	0.38	0.37	0.08	2.04
	HD+20 days	1.37	0.24	0.11	0.06	0.42
Hadsaduri	PI	1.93	0.30	0.18	0.07	1.47
	HD	3.22	0.25	0.27	0.10	1.66
	HD+20 days	3.09	0.27	0.31	0.18	1.94
LSD (5%)		0.60	0.08	0.08	0.03	0.35

[a] In addition to basal N (0.5 g/pot lower and 1.0 g/pot higher), 0.2 (g/pot) ^{15}N fertilizer N was top-dressed. PI = panicle initiation. HD = heading.
[b] Lower level, 0.5 g of N/pot; higher level, 1.0 g/pot.

The content of top-dressed N in the grain and in the different protein fractions (dry matter basis) is presented in Table II. Both varieties exhibited the highest accumulation of top-dressed N in the grain when the fertilizer was applied at the HD stage at both levels of basal N. However, the japonica variety accumulated more N than the indica variety when N was applied at either the PI or the HD stages, whereas top-dressed N was accumulated in larger amount in the indica variety when N was applied at HD+20 days.

The amount of top-dressed N in the prolamin fraction of the japonica variety differed slightly among the dressing application stages and the levels of basal N. Increase basal N in the japonica variety decreased the contribution of top-dressed N in the glutelin (Table II), which could be because basal application supplied enough N.

The prolamin fraction in the indica variety showed the highest partitioning of top-dressed N at HD+20 days, and it was the highest at higher basal N. The glutelin of the indica variety at the lower basal N level was contributed most by top-dressed N applied at the HD stage. The partition of top-dressed N decreased when higher basal N was supplied.

Most albumins and globulins in rice seed are removed by polishing. Therefore the protein of polished rice consists of glutelin and prolamin. Glutelin, which is synthesized in a spherical-shaped protein body (PB) I, has a higher biological value. On the other hand, prolamin, which is synthesized in PB II (crystalline), has lower biological value (Horikoshi and Morita 1982). The higher biological value of glutelin has also been reported by many other researchers (e.g., Padhye and Salunkhe 1979). The present study revealed that topdressing of N was effective for obtaining rice with high-quality proteins (i.e., those with high glutelin content) in the indica variety, especially when N was top-dressed at HD and HD+20 days.

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