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Protein Content of Rice Grain as Affected by Nitrogen Fertilizer and Some Triazines and Substituted Ureas[1]

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ABSTRACT

Field experiments were conducted to determine whether varietal differences in protein content exist and to study the use of cultural practices — plant density, time of N application, and application of chemicals — for increasing protein content of rices (*Oryza sativa* L.).

Results demonstrated that a high-yielding improved line 'IR480-5-9' has high protein content in the grain. The protein content of brown rice of IR480-5-9 varied from 9.5 to 10.2% without application of N. The highest protein of 'IR480,' was about 12.5% while that of 'IR8' was 9.8%. In another experiment, the protein content of IR480 was 10.2% while that of 'IR22' was 8.6%. Both rices had similar grain yields (7.8 metric tons/ha).

In another experiment, the protein content of several rice cultivars was significantly higher when 150 kg/ha N was applied half at planting and half at heading than when all N was applied at planting.

Simetryne [2,4-bis (ethyl amino)-6-(methyl thio)-S-triazine], at 0.5 kg/ha active ingredient, applied either as granular or liquid formulation at panicle initiation or at heading stage of the rice crop, significantly increased the protein content and protein yield of IR22 compared with the fertilized control. Three chemicals, tenoran [N'-4 (4-chlorophenoxy) phenyl-N,N-dimethyl urea], CP 17029 [2,4 bis (3 methoxy-propyl amino)-6-(methyl thio)-S-triazine], and benzomarc (N-benzoyl-N-dichloro-3,4-phenyl N'-N'-dimethyl urea) also increased protein content of rice grain without significantly affecting grain yields and yield components.

Additional index words: **Brown rice, Unfilled grains, Panicle initiation, Heading, Flowering, Protease activity, Tiller, Topdressing, Protein yield.**

IN many Asian countries rice (*Oryza sativa* L.) is an important source of protein. But common rices generally have low protein content particularly if they do not receive adequate N fertilizer. There is sufficient evidence that N application increases the protein content of rice grain (6, 8, 11, 12, 15). Rice breeders are attempting to combine high yield potential with good milling quality and high protein content in the rice grain in their breeding programs (2, 3, 10).

Several attempts to increase the protein content of the rice grain have been made by using herbicides used at sub-toxic levels. For example, Ries, Schwiezer, and Chmiel (13) reported increases in the protein content of several crops by applying simazine [2-chloro-4,6-bis (ethyl amino-S-triazine)]. Vergara, Miller, and Avelino (14) subsequently reported, however, that an increase in the protein content of the rice grain caused by simazine application was associated with a decrease in grain yield that resulted from an increase in unfilled grains. Recently, Allinson (1) reported that the application of simazine above 0.28 kg/ha active ingredient reduced the dry matter yields of reed canarygrass (*Phalaris arundinacea* L.). In addition, he reported that compared with a single application, split applications of simazine lowered the protein contents of the first and the second harvests but raised the protein content of the third harvest.

We conducted a series of experiments to study the varietal differences in protein content in rice grain and to determine proper cultural practices such as plant density, time of N application, and use of chemicals for high-protein rices.

MATERIALS AND METHODS

In field experiments at the International Rice Research Institute (IRRI) and three experimental stations of the Philippine Bureau of Plant Industry (BPI) in the 1969 wet season, we applied N as ammonium sulfate at 30 kg/ha increments from 0 to 150 kg/ha. Except for 20 kg/ha N, which we topdressed at panicle initiation, we incorporated the N during the last harrowing. We planted 'IR8' and an experimental selection 'IR480-5-9' in a split plot design with three replications. Nitrogen levels served as the main plots, and varieties, as the subplots.

In another series of field experiments at the IRRI farm (Maahas clay: pH 6.0; organic matter 2%; cation exchange capacity 45 meq/100 g soil; principal clay mineral, montmorillo-

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nite) we studied the effects of plant density, N fertilization, and chemical application on the protein content of rice grain.

During 1969, to study the effects of plant density on protein content, we broadcast IR8 at 100 kg/ha and transplanted it at one seedling per hill at spacings of 20 × 20, 40 × 40, 50 × 50, and 100 × 100 cm. We grew the crop without fertilizer using a randomized complete block design with three replications.

In a second experiment, in the 1970 dry season, we used the improved varieties 'IR20,' 'IR22,' and 'RD1' to study the effects of time of application on protein content of the rice grain. We applied N as ammonium sulfate at 150 kg/ha N at planting, at planting and panicle initiation, and at planting and heading. We used a split-split plot design with three replications. Times of N application served as main plots, varieties as the subplots, and the time of harvest as the sub-subplots.

In a third experiment, in 1970 and 1971, we screened several triazines and substituted ureas for their possible effects on the protein content of the rice grain.

We tested IR22 in the 1970 crop season. In the dry season we applied 150 kg/ha N as ammonium sulfate — 100 kg/ha during land preparation and 50 kg/ha at panicle initiation. In the wet season we applied 60 kg/ha N during land preparation and topdressed 20 kg/ha N in all plots. An additional treatment was imposed without any fertilizer or chemical. We used the randomized complete block design, with each treatment having four replications.

In the 1971 dry season we grew IR22 and the line 'IR480-5-9' in plots treated with 150 kg/ha N applied uniformly during land preparation. Additional treatments were imposed: no fertilizer and 150 kg/ha N applied in three split doses (100 kg/ha during land preparation, 25 kg/ha at panicle initiation, and 25 kg/ha at heading). We used a split-plot design, with varieties as the main plots and chemical treatments as the subplots.

We measured detailed yield components for the 1970 crop seasons and determined grain yields for all seasons. The grain yield data are presented as rough rice at 14% moisture. We obtained the protein content of the brown rice by determining the N content (Kjeldahl method) and multiplying it by the factor 5.95 (7).

RESULTS AND DISCUSSION

In the experiments on varietal differences in protein content, the brown rice of IR480-5-9 had between 9.5 and 10.2% protein without applied N. Its highest protein content was about 12.5%, while that of IR8 was 9.8% (Fig. 1). Subsequently, a study of the source of high N content in high-protein rice by IRRI chemists showed that IR480-5-9 had higher protease activity per tiller, both at flowering and 21 days later, than four other varieties (10).

The grain yields of IR480-5-9 varied from 3.4 to 3.8 tons/ha without fertilizer. The maximum grain yield in these experiments was about 5.8 tons/ha, a normal grain yield for a wet-season crop. IR480-5-9 had lower grain yield than did IR8. IR480-5-9 tillers less than IR8, which may account for its somewhat lower grain yield. High tillering is an important character for high grain yield in rice in the tropics. Furthermore, bacterial leaf blight affected IR480-5-9 more than IR8, which may also explain its lower grain yield. Under disease-free condition IR480-5-9 produced 21 tons/ha of rough rice in three crops within a calendar year, which is close to the record yield obtained with three crops of IR8 (9).

These data show that the high-yielding line, IR480-5-9, has high protein content. Subsequent studies revealed that IR480-5-9 is the highest-yielding among the high-protein rices in the world collection of 7,760 rices and more than 2,000 rices of improved plant type (3). Since IR480-5-9 has good grain quality, it may serve as an excellent parent in breeding for high-protein rice.

The studies on plant density showed that at wider spacings the protein content of IR8 brown rice was higher, but grain yields were lower than at narrower spacings (Fig. 2). At wider spacings more N was available to the plants and, therefore, the protein content in rice grain was increased. But the grain yields decreased at wider spacings because fewer panicles were produced per unit area. The results also indicate that the protein content in the rice grain is lower in the dry season than in the wet season (Fig. 2). This is true in many experiments conducted on our farm.

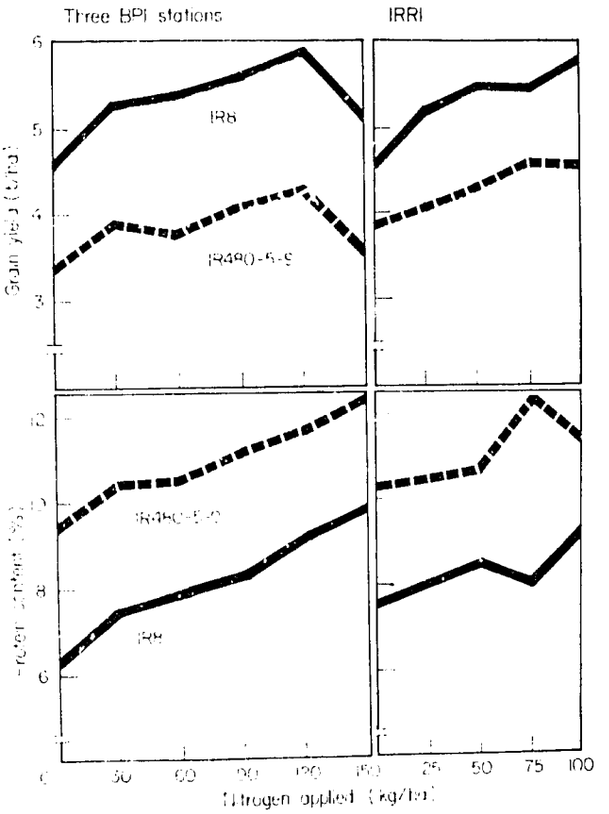

Fig. 1. Effect of N level on grain yield (rough rice) and protein content of brown rice at IRRI and at other locations (average of three Bureau of Plant Industry stations). 1969 wet season.

In the experiments on timing of N application, IR20, IR22, and RD1 had significantly higher protein content than IR8 (Table 1). Applying 150 kg/ha N in split doses at planting and panicle initiation, or at planting and heading, resulted in significantly higher protein contents than applying all N during planting (Table 1). Topdressing at heading gave significantly higher protein content but significantly lower grain yield than topdressing at panicle initiation. When the N was applied in split doses at planting and heading, the grain yields were similar to those obtained when the entire amount was applied during transplanting.

Split application is already recommended under farm conditions when the water levels in paddies cannot be controlled well (6). The increased protein content obtained from topdressing N is an additional reason for recommending split application.

In the experiments with herbicides in the 1970 dry season, simetryne [2,4-bis (ethyl amino)-6-(methyl thio)-S-triazine] at 0.5 kg/ha active ingredient applied

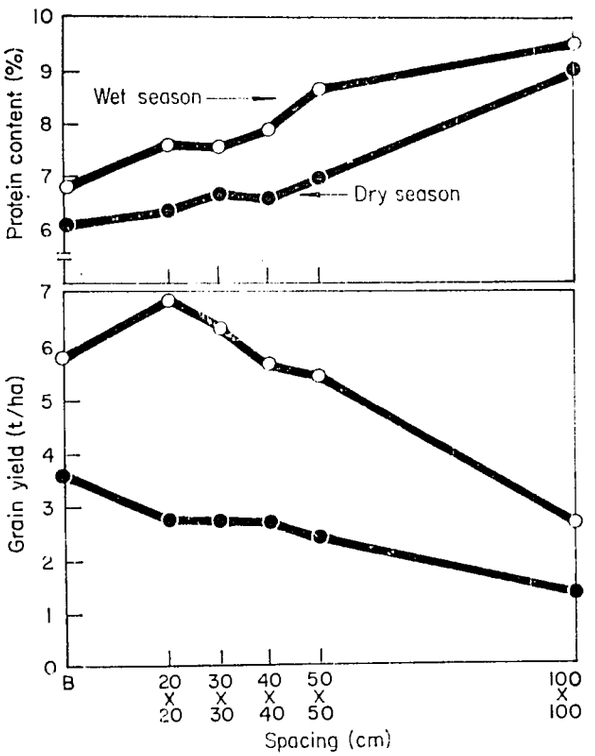

Fig. 2. Effect of plant density (B = broadcast; other spacings are transplanted) on grain yield and protein content of brown rice without fertilizer nitrogen (variety IR8). IRRI, 1969.

at complete heading significantly increased the protein content of IR22 compared with the fertilized control (Table 2). The amino acid composition, particularly the lysine content of protein of the simetryne-treated brown rice (3.6%), was similar to that of the fertilized control (3.7%).

Simazine, applied at 1.0 kg/ha, also significantly increased the protein content in the rice grain as it did in an earlier study (13), but it significantly reduced the grain yield, as also occurred in studies by Vergara et al. (14). At 0.5 kg/ha, however, the differences in protein content and grain yields due to simazine treatments were not significant.

In the 1970 wet season, simetryne, at 0.5 kg/ha active ingredient applied at panicle initiation, increased

Table 1. Effect of applying 150 kg/ha N at different times on the grain yield and the percentage of brown rice protein in four varieties (average of 15 times of harvest). IRRI, 1970 dry season.

[illegible]

Table 2. Effect of liquid formulations of Simazine and Simetryne on protein content of rice grain and grain yield of IR22. IRRI, 1970 dry season.

[illegible]

the protein content of rice grain without significantly decreasing the grain yield compared with the fertilized control (Table 3). But simetryne in this season was effective when applied at panicle initiation rather than at complete heading. Simazine, at 0.5 kg/ha, when applied at panicle initiation, was not toxic to IR22, and significantly increased IR22's protein content and protein yield. In our experiments the sterility (percentage of unfilled grains) caused by simazine applied at panicle initiation was not greater than that of the fertilized control (Table 3); it was considerably lower than that reported earlier (14). Probably the granular formulation of simazine used in this experiment had a lower toxicity than the liquid formulation used in earlier experiments (14). Furthermore, we used 0.5 kg/ha active ingredient, whereas a minimum rate, 1.25 kg/ha active ingredient, was used in the experiment in California with the 'Earlirose' cultivar.

Table 3. Effects of granular chemicals on the protein content of rice grain, grain yield, and yield components of IR22. IRRI, 1970 wet season.

Chemical	Application: Rate, kg/ha	Application: Time*	Toxicity rating†	No. of spikelets, 10^3/sq m	Wt. of 100 grains, g	Unfilled grains, %	Grain yield t/ha	Protein content, %	Protein yield: Yield, kg/ha	Protein yield: Relative yield‡
Simazine	0.5	PI	0	[illegible]	2.2	[illegible]	[illegible]	9.8	[illegible]	[illegible]
Simazine	1.0	CH	4	21	2.1	35	2.6	10.9	[illegible]	[illegible]
Simetryne	0.5	PI	3	[illegible]	2.3	10	4.4	10.3	455	117
Simetryne	1.0	CH	2	[illegible]	2.2	15	3.8	10.1	[illegible]	[illegible]
Tenoran	1.0	PI	0	20	2.3	6	4.3	9.2	394	98
CP 17029	0.5	B	0	23	2.3	9	4.8	9.2	424	108
Diuron	1.0	B	7	16	2.0	62	1.1	13.8	151	38
Monuron	1.0	B	9	16	1.9	84	0.4	14.8	[illegible]	17
Benzomarc	0.5	CH	0	24	2.3	9	4.5	9.5	430	111
Fertilizer treatments:										
Unfertilized control		-	-	18	2.3	7	3.5	7.9	272	63
Fertilized control§			-	24	2.3	6	4.4	8.8	387	100
LSD, 5%				4	0.1	14	0.5	1.0	46	
CV, %				13	4	16	10	6	11	

* PI = panicle initiation; B = booting; CH = complete heading. † Toxicity scale: 0 = no toxicity; 10 = complete kill. ‡ Protein yield with fertilized control = 100 (80 kg/ha N = 100). § All plots received 60 kg/ha N during land preparation and 20 kg/ha N at panicle initiation except the unfertilized control.

Table 4. Effect of chemicals and nitrogen fertilizer on protein content, protein yield, and grain yield of IR22. IRRI, 1971 dry season.

Chemical	Application: Rate, kg/ha	Application: Time*	Grain yield, t/ha	Brown rice protein: Content,	Brown rice protein: Yield, kg/ha
[illegible]	[illegible]	CH	[illegible]	9.5	503
[illegible]	0.5	B	[illegible]	9.4	599
[illegible]	[illegible]	CH	[illegible]	9.2	602
[illegible]	0.5	CH	[illegible]	9.7	647
[illegible]	[illegible]	B	[illegible]	11.9	260
Fertilizer treatments:					
Unfertilized control	-	-	[illegible]	[illegible]	337
Fertilized control†	-	-	[illegible]	8.6	524
Fertilized control‡	-	-	[illegible]	8.5	521
LSD, 5%	-	-	[illegible]	0.7	63
CV,**	-	-	[illegible]	5	9

* B = booting; CH = complete heading; PI = panicle initiation.
† Plots received [illegible] kg/ha nitrogen during land preparation including those treated with chemicals.
‡ Plots received [illegible] kg/ha nitrogen during land preparation, 25 kg/ha each at panicle initiation and at heading.

Table 5. Effects of chemicals and nitrogen fertilizer on the protein content, protein yield, and grain yield of the IR480-5-9 line. IRRI, 1971 dry season.

Chemical	Application: Rate, kg/ha	Application: Time*	Grain yield, t/ha	Brown rice protein: Content,	Brown rice protein: Yield, kg/ha
Simetryne	0.5	PI	[illegible]	11.2	652
Tenoran	0.5	B	[illegible]	11.2	669
Benzomarc	0.5	CH	[illegible]	11.0	665
Monuron	1.0	B	2.2	14.4	244
Fertilizer treatments:					
Unfertilized control	-	-	4.7	8.2	276
Fertilized control†	-	-	[illegible]	10.2	610
Fertilized control‡	-	-	[illegible]	10.8	601
LSD, 5%	-	-	0.8	0.8	82
CV,**	-	-	9	5	11

* B = booting; CH = complete heading; PI = panicle initiation.
† Plots received 150 kg/ha nitrogen during land preparation including those treated with chemicals.
‡ Plots received 150 kg/ha nitrogen during land preparation, 25 kg/ha N each at panicle initiation and at heading.

Other chemicals that increased protein content without reducing the grain yields were benzomarc (N-benzoyl-N-dichloro-3,4-phenyl N'-N'-dimethyl urea), tenoran [N'-4(4-chlorophenoxy) phenyl-N,N-dimethyl urea], and CP 17029 [2,4 bis (3 methoxy-propyl amino)-6-(methyl thio)-S-triazine] (Table 3).

The results indicate that even chemicals in the triazine group will increase the protein yield in rice. Toxicity to the rice crop must, however, be minimized by using proper formulation, rate, and time of application for these chemicals.

Monuron [3(p-chlorophenyl)-1,1-dimethyl urea] and diuron [3-(3,4-dichlorophenyl)1,1-dimethyl urea] substantially increased the protein content of brown rice but reduced the grain yields, primarily because they caused a higher percentage of unfilled grains and a lower 100-grain weight as compared with the fertilized control (Table 3).

In the 1971 dry season the chemicals simetryne, tenoran, CP 17029, and benzomarc caused significant increase of one percentage point in the protein content of IR22 compared with the fertilized control (Table 1). These four chemical treatments also increased the protein yields significantly. Monuron, a chemical belonging to the substituted urea group, decreased protein yield by substantially reducing the grain yield. The crop suffered severe toxicity.

Fertilizer application alone increased the protein content of IR480-5-9 by 2 to 2.4 percentage points and the grain yield by 3 tons/ha compared with the unfertilized treatment (Table 5). The application of simetryne, tenoran, and benzomarc significantly increased the protein content of IR480-5-9 without significantly decreasing the grain yields. The increase in protein yields obtained with these chemical treatments were not significant, however (Table 5).

Studies have shown that high-protein rices are nutritionally superior to rices with normal protein content in feeding trials with both human beings (5) and rats (4). This is because of the higher levels of essential amino acids in the high protein rice at the same rice intake. The varietal differences in increase in protein content and increase in protein yield through fertilizer and chemical treatments are worth noting. IR480-5-9 produced higher protein content and protein yield in most of the fertilizer and chemical treatments than IR22. Grain yields were similar, however, confirming the superiority of IR480-5-9 as a potential parent in breeding for high-yielding and high-protein rices.

Future high-yielding varieties should have higher protein contents without decreased grain yield. Our results indicate that the protein content of the rice grain may be further increased through the application of certain chemicals.

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