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Breeding for high protein content in rice

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The IRRI world collection of rice varieties was screened for protein content and varieties with high protein were identified for use in crosses to IR8. These crosses were carried through the F_8 plant generation. Lines were identified which gave 30 percent higher protein content than IR8 check plots and about 70 percent of the rough rice yield of IR8. IRRI breeding lines screened from yield trials were identified which produced 18 to 23 percent higher protein than IR8 check plots but which did not differ significantly in grain yield. Information now available indicates that high yielding varieties with improved plant type can be developed that will produce 20 to 25 percent higher protein than IR8. High protein varieties must yield as well as other varieties if they are to be accepted by farmers. Therefore, they must have high levels of disease and insect resistance along with other essential traits. A close plant spacing and basal nitrogen fertilization was found to reduce environmental variability of protein content.

INTRODUCTION

Rice is a major source of food protein in Asia and other countries where the daily intake of rice is high. Its value as a protein source is enhanced by its high lysine content relative to other cereal grains. The main limitation of rice as a protein source is its low protein content (6 to 8 percent). Any increase in protein content would result in a substantial increase in protein intake by large numbers of consumers provided the quality of the protein is not impaired. For these reasons, breeding for increased protein content is an extremely important objective. Studies on protein content began at IRRI in 1962 (IRRI [1963]). A specific hybridization program was not undertaken until 1967 because of a lack of understanding of the many factors affecting protein content, including the availability of parent varieties with genetically high protein content.

If varieties with high protein content are to be accepted by farmers they must have a grain yield potential and grain quality at least equal to the varieties they would replace. Consequently high protein varieties must possess all the characteristics that breeders are attempting to incorporate into modern semidwarf varieties.

Numerous studies have been conducted at IRRI on factors affecting the quantity and quality of protein in brown rice (IRRI, [1963], [1964], [1965], 1966,

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1967*a*, 1967*b*, 1968, 1970*a*, 1971). Most of the early work was centered in the chemistry department where much information on the knowledge of rice protein was obtained. At present, the breeding program and other phases of the protein improvement work are carried out in a coordinated program among several IRRI departments. A contract with the National Institutes of Health (Contract PH-43-67-726), which started in 1967, resulted in the expansion of the program to its present level.

PROTEIN SCREENING PROCEDURES

We measure protein in brown rice in all screening trials rather than in milled rice since the protein contents of brown rice and of milled rice are highly correlated (Juliano et al., 1964). Furthermore, it is difficult to remove bran uniformly in milling. For example, in a 1968 variety-fertilizer experiment (IRRI, 1968), the brown rice protein content of four improved plant type varieties averaged 8.2 percent and that of the milled rice, 7.3 percent. High protein rice tends to have a lower percentage loss in protein in milling than low protein rice (IRRI, 1970*a*).

Grain samples used for evaluating the protein content of individual plants from pedigree rows are based on 10 fully developed brown-rice grains selected at random from the grain produced by a single plant. This sampling technique appears to be reasonably reliable. Usually three to six plants are taken from each pedigree row. Likewise 20 brown-rice grains are used to determine the protein content of samples from yield trials. Any yield trial sample that shows a high or a low value is re-examined. A more reliable technique for sampling yield trial plots would be useful. Samples are ground in a Wig-L-Bug amalgamator so small samples must be used.

In preparing experimental plots for protein experiments, every effort should be made to maintain soil uniformity. Land to be used should be planted to a single variety the season before. A relatively low nitrogen fertilizer rate should be used on the experiments as well as on the preceding rice crop.

SCREENING THE WORLD COLLECTION

The IRRI world collection of rice varieties was screened for protein content starting in 1966 to identify genetically high protein varieties to be used in the breeding program (Juliano et al., 1968). The mean protein values of the 7,760 varieties ranged from 5 to 17 percent, with an overall mean of 10.6 percent. There were 126 accessions which showed at least 13.5 percent protein based on brown rice samples (wet basis), or a mean of 14 percent for two different crops. Six varieties, selected from the 126 accessions, showed high protein content through four crop seasons (Table 1). They were subsequently used as parents in crosses with IR8 made in 1967. Aside from BPI-76 and BPI-76-1 none of the 126 high protein varieties screened from the world collection have value as commercial varieties in the Philippines. BPI-76-1 was crossed to IR8 and

Table 1. Protein content (of brown rice at 12% moisture) of six varieties screened from the IRRI world collection that were used as parents in crosses with IR8.

Variety	Origin	Protein (%)			
		Planting seed	1966 wet season	1967 Dry season	1967 Wet season
Chok-jye-bi-chal	Korea	15.1	15.0	15.4	15.5
Chow-sung	Korea	15.3		14.6	15.4
Santo	Korea	16.7	14.2	13.8	13.7
Rikuto Norin 20	Japan	14.5	13.5	14.3	14.0
Omirt 39	Hungary	14.7	13.6	12.8	14.3
Crythroceros Korn	Korea	16.2	11.5	13.5	14.3

IR12-178 (Mon Chim Vang A x I-geo-tze) and selections from both crosses were screened along with lines from the other high protein crosses.

HYBRIDIZATION PROGRAM

The six high protein parent varieties listed in Table 1 are all japonica varieties, except Omirt 39 from Hungary which is classified as an indica variety (IRRI, 1970*b*). These six varieties came from temperate regions and are not adapted to a tropical climate. They mature very early, total dry matter production is low and they are susceptible to several important diseases and insects. The extremely high protein content of the six varieties may be due in part to an environment-genotype interaction brought about by their low grain yield and low total dry matter production. On the other hand, these varieties appear to possess genes for high protein since some of their hybrid progeny show higher than normal protein content combined with improved plant type and higher total dry matter production. The six high protein crosses have been advanced through the F_8 generation. Most of the lines appear to be fixed for most plant and grain characters and many have been tested in replicated yield trials.

There was a wide segregation of plant types coming from the crosses. Some sterility was encountered but this was not a serious problem. Rigid selection pressure for improved plant type and high protein content was exercised each generation starting with the F_2 plant generation. While protein contents as high as the high protein parents were seldom observed, there were many lines that produced consistently higher protein than the IR8 check rows. The range and average protein content of plants selected from each generation are shown for the IR1103 cross in Table 2. The data for IR1100, IR1101, IR1102, IR1104, and IR1105 were similar. Many of the hybrid lines gave higher protein values than IR8 check plants each generation. Likewise, the average protein content of the plant selections made from these crosses was higher than that of the IR8 check row plants in each generation (F_3 to F_9) for each cross.

Table 2. Summary of single-plant brown rice protein analyses (at 12% moisture) of lines from high protein cross IR1103.

Year	Season	Generation	Protein content (%) IR1103[a] Range		Mean	IR8[b] Range		Mean
1967	Wet	F_2	16.0		16.0	10.0		10.0
1968	Dry	F_3	6.8	12.8	9.7	6.3	9.9	8.0
1968	Wet	F_4	7.2	14.0	10.1	6.4	11.3	8.4
1969	Dry	F_5	6.5	15.9	10.8	7.1	11.1	8.7
1969	Wet	F_6	7.9	15.3	11.2	7.6	10.1	8.7
1970	Dry	F_7	7.0	15.0	10.4	7.5	10.8	8.7
1970	Wet	F_8	6.9	15.2	10.6	6.8	11.7	8.4
1971	Dry	F_9	5.7	13.2	9.7	6.4	9.2	7.5

[a]IR8 x Chow-sung. [b]Check rows.

Table 3. Summary of protein content (brown rice at 12% moisture) and other data on high protein lines and IR8 grown in pedigree rows.

Line	Seeding to heading (days)	Protein content (%) F_3 - F_8	F_9 Avg. of two plants	F_9 Bulk sample	F_9 rough rice yield (g/plant)	F_9 fertility (%)
IR8[a]	104	8.5	7.5	7.5	46.2	89
			IR8 x Chow-sung			
IR1103-1-3	102	10.7	10.6	11.1	44.4	94
IR1103-2-3-1-3	100	11.0	10.7	10.7	49.1	88
IR1103-2-3-1-5	102	10.6	10.6	11.1	39.6	90
IR1103-15-8-4	109	11.6	11.4	11.3	32.1	84
IR1103-15-8-5	105	11.6	10.1	10.7	45.5	87
IR1103-52-2	103	10.9	9.5	9.2	32.5	89
IR1103-66-4	85	10.7	10.0	10.5	33.6	89
IR1103-68-6	104	11.0	9.9	10.3	36.6	85
			IR8 x Crythroceros Korn			
IR1104-107-2	107	9.9	8.6	7.6	52.1	89
			IR8 x Rikuto Norin 20			
IR1100-67-5	103	13.0	9.9	10.1	35.4	92
			IR8 x Omirt 39			
IR1101-14-3	85	9.8	7.7	8.3	44.7	83
IR1101-18-3	95	9.9	9.1	8.7	34.3	91
IR1101-29-3	79	12.7	9.2	10.5	35.1	80
IR1101-38-4	76	12.0	10.8	10.5	41.4	87
IR1101-44-3	70	12.8	11.9	11.4	37.8	87
IR1101-45-2	80	12.3	11.1	10.9	30.5	79

[a]Protein values are averages of plants from IR8 check rows grown in nursery each season.

The average protein contents of selected lines based on the F_2 to F_8 seed generation are shown in Table 3. The protein content of six lines exceeded that of the IR8 check plants by 3.5 to 4.5 percentage points or from 41.2 to 52.9 percent. In the F_9 generation these lines exceeded IR8 by from 1.7 to 4.4 percentage points or 22.7 to 58.7 percent. The average rough rice yield per plant of the six lines averaged 77 percent of the yield of IR8. There were four lines which averaged about the same rough rice yield as IR8 and averaged 3.1 percentage points higher protein (41.3 percent more).

ENVIRONMENT AND PROTEIN CONTENT

Considerable variability in protein content is caused by environmental factors such as season of the year (wet or dry season), plant population density, and time and rate of nitrogen fertilizer applied. Usually protein content is higher during the wet season than during the dry season, possibly because grain yields are higher during the dry season.

IR8 plants showed differences in protein content of as much as 75 percent within a season when a 30 x 30 cm plant spacing and split application of nitrogen fertilizer (basal and top dressing) were used. We reduced this variability in the 1971 dry season by using a closer plant spacing (20 x 20 cm) and by applying all nitrogen fertilizer basally before transplanting. The combined effect of the closer plant spacing and the basal N treatment was a reduction in protein content and reduced variability as shown in Tables 2 and 3. In the future, we will plant pedigree nurseries at a spacing of 20 x 20 cm and possibly grow as many as three rows of each selection rather than just a single row. Plants to be evaluated for protein content would be saved from the center row of each line.

Table 4 shows the effect of plant population density on protein content in an experiment conducted by the IRRI agronomy department. The protein content of IR8 varied from 6.1 to 9.0 percent in the dry season and from 6.9 to 9.5 percent in the wet season. IR127-80-1, a low tillering variety, showed an even greater variation between a close spacing and wide spacing.

Table 4. Percentage of brown rice protein at 12% moisture of IR8 and IR127-80-1 (unfertilized) at different plant densities. (Data are averages of three replications.)

	Protein (%)			
	Dry season		Wet season	
Plant spacing (cm)	IR8	IR127-80-1	IR8	IR127-80-1
Broadcast	6.1	6.0	6.9	7.5
20 x 20	6.4	6.4	7.6	7.8
30 x 30	6.7	7.5	7.6	8.4
40 x 40	6.6	8.6	7.9	8.5
50 x 50	7.0	9.3	8.7	9.0
100 x 100	9.0	10.9	9.5	11.3

Table 5. Percentage of brown rice protein of two varieties as affected by time of nitrogen application[a] IRRI, 1969-1970.

Application time	1969 wet season[b]		1970 dry season[c]	
	IR8	IR20	IR8	IR20
Basal	8.8	9.2	8.3	8.9
50% basal and 50% at panicle initiation	8.6	9.6	8.6	9.2
50% basal and 50% at heading	9.0	9.5	8.9	9.9

[a]Data are averages over 15 times of harvest each with three replications. [b]60 kg/ha N applied. LSD (5%) = 0.7. [c]150 kg/ha N applied. LSD (5%) = 0.5.

Late application of nitrogen fertilizer tends to increase protein content and variability as well. Trials by the IRRI agronomy department (IRRI, 1971, p. 131-132) have shown the wide differences that occur in protein content between basal application and split application (Table 5). In our future pedigree nurseries and in preliminary yield trials nitrogen will be applied basally. Lines found to have higher protein at moderate nitrogen levels should eventually be tested at high levels and with split application to determine their overall response. Rate of nitrogen applied and protein content appear to be positively correlated.

SCREENING YIELD TRIALS FOR PROTEIN CONTENT

The varieties and breeding lines grown in most yield trials at IRRI are screened for protein content. From this screening program, IR480-5-9 (Nahng Mon S4/2 x Taichung Native 1) was identified by the agronomy department as a high protein line (IRRI, 1971). IR480-5-9 yielded 6.7 t/ha in a replicated variety yield trial at IRRI in 1967 dry season in which IR8 yielded 7.2 t/ha. This line is not suitable for farm production in the Philippines because of susceptibility to bacterial leaf blight, leafhoppers, and planthoppers. The higher protein content of IR480-5-9 has been confirmed in later experiments. Other lines showing high protein content are IR160-27-3 (Nahng Mon S4 x Taichung Native 1) and IR667-98 (IR8 x [Yukara x Taichung Native 1]). A sister line of IR160-27-3 has been named Sinaloa A68 in Mexico and is being grown commercially, but no information is available on the comparative protein content of Sinaloa A68 and other commercial varieties. The lines from Nahng Mon S4 crosses produce satisfactory milled rice yields and they have attractive grain appearance.

IR667-98, a line with IR8 plant type being widely tested in Korea, gave higher protein content than leading japonica varieties in several 1970 variety-fertilizer trials conducted in Korea as well as in two IRRI yield trials in the 1971 dry season. Further testing of IR667-98 is required to substantiate these findings.

YIELD OF HIGH PROTEIN LINES

Selected F_8 plant lines from high protein crosses, grown in a single-plot observational yield trial are shown in Table 6. A 20- x 20-cm plant spacing was used in this test and all nitrogen was applied basally. The IR8 check plots yielded 4.75 to 5.64 t/ha and with protein contents from 6.0 to 6.6 percent. Several lines in this test approached the yield of IR8 and had considerably higher protein contents than IR8.

Many of the same lines were grown in a replicated yield trial (Table 7). A 20- x 20-cm plant spacing was used and 120 kg/ha N was applied basally. Six hybrid lines in this test did not differ significantly in yield of rough rice from IR8, but had a significantly higher protein content: IR160-27-3, IR773A1-36-2, BPI-76-1, two IR1006 (IR8 x BPI-76-1) lines, and an irradiated IR8 line obtained from the Philippine Atomic Research Center.

In the same replicated yield trial there were six lines from the high protein crosses (IR1100 to IR1105) which averaged 30.1 percent higher protein than IR8 but their average yield was 31 percent lower than IR8. They are shown in Table 7 along with four other lines from the high protein crosses that averaged only 8.2 percent higher protein than IR8 with grain yields 24.1 percent lower than IR8. The two groups of lines did not show statistically significant differences in grain yield but showed highly significant differences in protein content. Apparently this significant difference in protein content is genetically controlled.

The results of five 1971 dry season yield trials in which IR8, IR480-5-9, and IR160-27-3 were grown are shown in Table 8. The average yields of IR480-5-9

Table 6. Grain yield, protein (at 12% moisture), and other data from breeding lines and varieties grown in single observational yield trial plots. IRRI, 1971 dry season.

Line no.	Seeding to heading (days)	Panicles (no.)	Plant ht (cm)	Yield: Rough rice (t/ha)	Yield: Brown rice (t/ha)	Brown rice protein (kg/ha)	Protein content (%): Brown rice	Protein content (%): Milled rice	Amylose content (%)	Gel. temp.[b]
IR1102-25-3[c]	93	13	112	4.23	3.19	322	10.1	8.8	25.6	L
IR1103-64-4	103	12	82	3.54	2.72	169	6.2	5.0	26.9	L
IR1104-16-2	76	8	76	3.53	2.69	250	9.3	8.8	27.4	
IR480-5-9[a]	96	10	87	4.71	3.52	282	8.0	7.3	22.9	L
IR8[a]	104	13	73	5.15	3.95	253	6.2	5.37	28.5	L
IR160-25-3[a]	104	11	88	4.85	3.66	294	8.03	6.98	24.0	L
IR1105-15-7[d]	77	12	73	4.50	3.56	285	8.0	7.1	25.4	L
IR1100-111-2	102	13	90	4.23	3.21	266	8.8	8.3	Waxy	L
IR1103-1-3-7	96	11	103	4.71	3.71	338	9.1	8.1	28.4	L
IR1103-15-8	108	9	87	3.57	2.75	209	7.6	6.6	27.9	L
IR1103-1-3-5	97	11	99	4.28	3.40	265	7.8	7.6	28.5	L
IR667-98-1	88	11	75	5.53	4.30	353	8.2	7.0	19.1	L

[a]Average of four plots. [b]Gelatinization temperature, L = low. [c]IR8 x Santo. [d]IR8 x Chok-jye-bi-chal.

Table 7. Average rough and brown rice yields and protein content (at 12% moisture) of brown and milled rice and other data of lines grown in replicated yield trial, IRRI, 1971 dry season.

Line no.	Seeding to heading (days)	Panicles (no.)	Plant ht (cm)	Yield: Rough rice (t/ha)	Yield: Brown rice (t/ha)	Brown rice protein (kg/ha)	Protein content (%): Brown rice	Protein content (%): Milled rice	Amylose content (%)	Gel. temp.[b]
IR1100-18-3	74	13	96	4.16	3.24	330	10.2	9.3	25.6	L
IR1101-64-1	73	15	87	4.97	38.1	362	9.5	8.9	22.8	L
IR8[a]	104	12	93	7.01	5.35	392	7.3	6.7	27.8	L
IR773A1-36-2	89	12	85	6.61	5.10	428	8.4	7.8	23.4	L
IR1103-49-4	99	12	89	4.91	3.81	366	9.6	8.4	27.6	L
IR1006-26-1	99	11	96	6.92	5.46	453	8.3	7.6	27.6	L
IR1006-33-1	100	11	95	6.35	4.91	393	8.0	6.9	27.4	L
IR480-5-9	90	10	92	6.04	4.57	416	9.1	8.4	21.8	L
IR160-27-3	97	10	99	7.02	5.50	468	8.5	7.8	23.1	L
IR1103-1-3	95	12	111	4.90	3.89	350	9.0	8.3	25.9	L
IR1103-2-3	89	15	93	5.13	3.29	303	9.2	8.8	26.5	L
IR1103-15-8	101	10	91	4.61	3.57	350	9.8	8.7	24.8	L
BPI-76-1	92	10	129	6.02	4.58	394	8.6	7.8	25.1	H/I
IR1103-52-2	95	12	83	6.73	5.26	400	7.6	6.6	18.0	L
IR1103-64-4	101	13	99	6.54	5.22	360	6.9	6.0	28.2	L

[a]Average of three plots. [b]Gelatinization temperatare, L = low, H/I = high/intermediate.

and IR160-27-3 do not differ significantly from IR8 but they exceed IR8 in protein content by a margin of 23 and 18 percent, respectively.

HIGH PROTEIN CONTENT AND OTHER GRAIN PROPERTIES

A taste-panel evaluation of cooked milled rice of some high protein breeding lines conducted by Dr. Luz U. Oñate of the University of the Philippines, College of Agriculture, showed no significant differences in color scores among lines from the same cross that differed by as much as 4 percentage points of protein in the raw grain. The lines compared had similar amylose content (IRRI, 1971). Presumably, an increase in protein content of 2 percent has a negligible effect on the color of milled rice.

Amino acid analysis of F_4 brown rice grains of the lines with the lowest and the highest protein contents among the six crosses, IR1100 to IR1105, showed a decrease in lysine and tryptophan values as protein content increased (IRRI, 1971). It appears that increases in protein content caused by either genetic or environmental factors have similar effects on the amino acid composition of rice protein. Milled rice of F_7 seeds of five lines with 11.4 to 11.8% protein contained 3.3 to 4.0% lysine, 3.4 to 4.1% threonine, 0.9 to 1.2% tryptophan, and 3.0 to 4.6% sulfur amino acids in the protein (IRRI, 1971). These results show that if both parents have normal lysine content in their protein, an increase of 2 percentage points in protein does not necessarily result in a lowering in lysine content, since the lines with similar protein content

Table 8. Summary of mean rough and brown rice yields, protein content (at 12% moisture), milling and other data on IR8 and two high protein lines. IRRI, 1971 dry season.

Line	Yield			Protein (%)				
	Rough rice (t/ha)	Brown rice (t/ha)	Brown rice protein (kg/ha)	Brown rice	Milled rice	Brown rice (%)	Total milled rice (%)	Head rice (%)
			Replicated yield trial[a] (120 kg/ha N)					
IR8	7.02	5.51	408	7.4	7.0	78.5	67.9	49.6
IR160-27-3	6.56	5.06	430	8.5	7.8	77.1	67.8	44.9
IR480-5-9	6.04	4.57	402	9.1	8.4	75.6	65.5	50.6
			Variety x fertilizer trial[b] (60 kg/ha N)					
IR8	7.30	5.69	410	7.2	6.7	77.9	68.1	47.9
IR160-27-3	6.86	5.24	409	7.8	7.6	76.4	69.3	35.8
IR480-5-9	6.31	4.75	413	8.7	8.4	75.2	67.2	44.9
			Variety x fertilizer trial[b] (120 kg/ha N)					
IR8	7.13	5.52	475	8.6	8.0	77.8	68.3	41.0
IR160-27-3	7.07	5.43	554	10.2	9.9	76.8	68.5	39.3
IR480-5-9	7.50	5.68	602	10.6	10.3	75.7	67.1	51.3
			Variety x spacing x fertilizer experiment[c] (120 kg/ha N)					
IR8	5.64	4.37	315	7.2		77.5	68.4	52.4
IR160-27-3	6.19	4.77	415	8.7	8.2	77.1	70.1	48.2
IR480-5-9	6.31	4.81	433	9.0	8.3	76.3	68.4	53.6
			Observational yield trial[a] (80 kg/ha N)					
IR8	5.14	3.94	213	6.2	5.4	76.7	66.9	46.7
IR160-27-3	4.85	3.66	289	7.9	6.9	75.4	66.8	38.7
IR480-5-9	4.71	3.52	282	8.0	7.5	74.7	64.4	41.8
			Mean of five trials					
IR8	6.40	4.97	358	7.3				
IR160-27-3	6.24	4.84	412	8.6				
IR480-5-9	6.08	4.59	416	9.0				

[a]Four replications. [b]Three replications. [c]Agronomy department trial.

show a range in lysine content and one may select progenies with high lysine values.

When high protein lines of divergent origin are combined in further hybridizations there may be a further increase in protein content. Crosses are being made which combine the different high protein sources. These crosses also include combinations with high yielding, disease- and insect-resistant lines. An increase in protein content of 25 percent without any reduction in grain yield appears to be a reachable goal.

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Discussion: Breeding for high protein content in rice

P. A. LIEUW-KIE-SONG: Have you analyzed F_1 material for protein content?

H. M. Beachell: No analysis was done on F_1 seeds.

A. O. ABIFARIN: How much of the brown rice protein is left after milling?

B. O. Juliano: Brown rice does not exceed milled rice in protein content by more than one percentage point. With brown rice of 8 percent protein, removal of 10 percent by weight as bran-polish during milling leaves about 84 percent of brown rice protein in the milled rice. At about 11 percent protein, the loss of brown rice protein during milling is only about 12 percent based on 10 percent weight removal, leaving 88 percent of its protein in milled rice.

E. A. SIDDIQ: Do you find differences in the levels of protein increase among varieties with increasing rates of applied nitrogen? If so, is it anyway related to the endosperm texture (packing of starch granules)?

B. O. Juliano: Yes, increased protein content from applied fertilizer nitrogen improves grain translucency and the hardness of any variety.

T. H. JOHNSTON: Breeding for higher protein content is also an important phase of the rice improvement programs in the U.S. Determining the protein and lysine content of promising lines is a routine part of the quality testing procedure. Several U.S. researchers have reported that the protein content of brown rice in different crosses did not appear to be simply inherited. Studies on F_2 plants and F_3 lines indicated that the heritability of protein content was low.