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Study on Mutational Manipulation of Protein Characteristics in Rice

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Summary. A large number of induced mutants isolated from *indica* and *japonica* varieties of rice were screened for two different aspects of nutritive quality, namely crude protein content and protein distribution pattern in the endosperm. The study revealed a wide variation for both characteristics. There was no consistent relationship between protein content and test-grain weight or spikelet sterility. It appears from the present study that both these characteristics of protein are easily amenable to mutagen induced changes. The potential use of these characteristics in rice breeding is discussed.

Although the protein quality of rice is decidedly superior to that of all other cereals, the major nutritional limitation of rice is its low protein content. Increasing the quantity and quality of protein is potentially the most feasible way to combat malnutrition in rice-eating countries. Extensive screening of rice collections in recent years has revealed the existence of genotypes with much higher contents of protein than the known range of 6 to 9 percent. As well as the wide variability available in nature, it has been demonstrated that variability for protein characteristics could be generated within the confines of a superior and well adapted genotype through induced mutagenesis (cf. Tanaka, 1969, Tanaka and Tamura, 1968, Haq *et al.*, 1970, and Siddiq *et al.*, 1970). With the object of identifying genotypes with higher content and better distribution of protein in the endosperm, a number of induced mutants of rice were screened and the results are summarized in this paper.

Material and Methods

Induced true-breeding mutants of a few rice varieties belonging to the subspecies *indica* (IR-8 and Taichung (Native)-1) and *japonica* (Taichung-65 and Tainan-3) of *Oryza sativa* were used.

Study of nutritional quality was restricted to the estimation of protein content and its pattern of distribution in the endosperm. The protein content for each mutant line was estimated as the average of three plant values obtained by the macro-Kjeldahl method.

Three grains of uniform maturity were taken from each mutant for studying the protein distribution pattern in the endosperm. The grains were fixed in Carnoy's fluid for 48 hours and stored in 70 percent alcohol. After repeated washings in water, transverse sections were cut along the mid-endosperm using a "Weck" blade and were stained for two minutes in one percent aqueous solution of Bromophenol blue. The sections were then dehydrated by passing through ascending grades of alcohol/water, clove oil and xylene, and finally were mounted in Euperol. The distribution of protein bodies was studied under a light microscope. The scoring was done as follows.

The cross-section of the mid-endosperm was partitioned arbitrarily into four major zones, namely, a) peripheral b) sub-peripheral c) middle and d) inner. Depending on the size of the protein bodies and their density, each zone was scored in the following manner:

**** Zone characterized by having bigger bodies of protein either coalesced or closely arranged.

*** Zone characterized by having protein bodies of medium size scattered sparsely or less closely arranged.

** Zone characterized by small bodies of protein scattered sparsely or less closely arranged.

* Zone characterized by granule-like tiny bodies of protein scattered sparsely.

Results

65 true-breeding mutants, isolated following treatments with both physical and chemical mutagens of four different rice varieties (T (N)-I, IR-8, Taichung-65 and Tainan-3), were studied for protein content, test grain weight and percentage of seed sterility. The study, in general, revealed a wide variation in protein content (Table 1). Among the mutants of T-65, compactoid-1 had the lowest protein content of 8.25 per cent, while dwarf-6 had 13.05 per cent compared with 10.15 per cent in the control. Similarly, the mutants of Tainan-3 also showed considerable variation in protein content, the range being between 8.10 and 12.71 per cent. Protein content ranged from 7.71 in beaked-1 to 14.31 per cent in tetragonal grain mutants of T(N)1 compared with 10.50 per cent in the control. Study of a limited number of mutants of IR-8 showed a very narrow variation, the range being between 9.77 and 10.67 per cent.

Correlation coefficients between protein content and test grain weight or percentage of seed sterility are given in Table 2. The data suggested no significant correlations between protein content and yield components such as 1000 grain weight and degree of seed sterility in the mutants of T-65, IR-8 and T(N)-1. However, in mutants of Tainan-3 there was a significant negative correlation (−0.707) between protein content and seed sterility, whereas the protein

Table 1. *Percentage of protein content, seed sterility, and test grain weight in mutants*

No.	Mutants	Per cent of protein content (wet basis)	1,000 grain weight (grams)	% seed sterility
	Taichung-65			
1.	Control	10.15	26.80	12.69
2.	Dwarf-2	10.90	18.80	12.79
3.	Dwarf-3	9.65	18.80	12.09
4.	Dwarf-4	10.25	18.80	13.14
5.	Dwarf-5	11.27	19.20	8.26
6.	Dwarf-6	13.05	18.80	12.19
7.	Dwarf-7	11.78	19.20	8.92
8.	Dwarf-8	9.08	19.20	13.42
9.	Dwarf-9	10.47	20.00	17.72
10.	Dwarf-10	10.50	25.20	5.55
11.	Dwarf-11	10.78	26.00	14.93
12.	Dwarf-12	10.48	17.20	10.47
13.	Dwarf-13	10.75	19.60	8.28
14.	Dwarf-14	10.47	19.20	5.44
15.	Dwarf-15	9.35	22.40	5.25
16.	Dwarf-16	10.23	19.60	9.37
17.	Dwarf-17	9.51	18.80	14.62
18.	Dwarf-18	9.46	18.00	13.20
19.	Dwarf-19	10.66	16.30	11.77
20.	Dwarf-20	10.30	25.60	10.36
21.	Indica grain-2	9.31	25.60	4.11
22.	Indica grain-3	9.59	20.00	33.22
23.	Indica grain-5	9.81	24.00	33.22
24.	Indica grain-6	10.12	24.40	30.59
25.	Indica plant	10.33	23.20	20.88
26.	Compactoid-1	8.25	24.40	11.81
27.	Compactoid-2	10.23	24.40	13.67
28.	Loose panicle	10.15	26.40	5.84
29.	Matching	10.15	15.60	23.14
30.	Open panicle	8.67	25.60	9.00
31.	Reduced grain size	9.73	19.20	5.34
32.	Bold grain	8.37	29.20	24.19
33.	Brittle	9.86	24.40	26.03
34.	Shattering	8.63	22.80	5.14
	Tainan-3			
1.	Control	9.56	22.80	5.48
2.	Indica grain-3	9.47	24.00	21.23
3.	Indica grain-5	8.10	20.00	33.33
4.	Erectoid-1	9.53	17.60	13.72
5.	Erectoid-4	9.65	17.20	5.45
6.	Erectoid-5	10.60	19.20	1.66
7.	Bold grain	10.71	23.20	16.96
8.	Compactoid	8.60	22.00	10.79
9.	Promising panicle-1	8.88	22.40	19.63
10.	Promising panicle-2	8.99	21.20	25.60
11.	Grassy-1	11.08	14.00	5.60
12.	Grassy-2	11.93	13.60	4.90
13.	Grassy-3	12.34	12.80	5.40
14.	Grassy-4	12.71	16.40	1.19

Table 1 (continued)

No.	Mutants	Per cent of protein content (wet basis)	1,000 grain weight (grams)	% seed sterility
	Taichung (Native)-1			
1.	Control	10.50	19.60	13.90
2.	Tall	9.00	18.40	12.74
3.	Japonica grain-1	8.98	18.40	8.15
4.	Japonica grain-2	8.53	20.00	8.36
5.	Japonica grain-4	10.48	15.20	7.29
6.	Japonica grain-5	11.26	16.00	16.84
7.	Beaked-1	7.71	20.80	21.98
8.	Beaked-3	10.86	22.00	27.53
9.	Beaked-4	9.58	21.20	21.29
10.	Hooded-2	10.85	26.40	29.68
11.	Hooded-3	11.29	21.60	21.89
12.	Hooded-8	9.92	23.20	15.43
13.	Boat leaf	13.28	18.40	11.42
14.	Broad leaf-1	9.76	21.20	13.18
15.	Broad leaf-3	11.09	20.40	22.75
16.	Tetragonal	14.31	23.20	26.22
17.	Horizontal flag leaf	12.26	21.20	9.17
	IR-8			
1.	Control	9.71	26.80	18.05
2.	Fine grain-M5	10.67	23.60	23.42
3.	Peculiar dwarf	10.63	28.40	35.36
4.	Awned	9.77	28.40	11.51

content and 1000 grain weight showed a negative correlation (−0.365) in the mutants of T-65. Thus, no consistent association was observed between protein content and test weight or seed sterility.

Using the cross-sections of the mid-endosperm, the protein distribution pattern was scored in zones on the basis of the size and density of the protein bodies (Table 3). The study indicated that there was practically no variation in the size and intensity of the protein bodies in the peripheral and inner zones of all the mutants. Significant variation was, however, noticed in the sub-peripheral and middle zones in most of the mutants studied (Figs. 1—5).

In T-65, the majority of the mutants had loosely arranged, small protein bodies in the sub-peripheral layer and medium-sized bodies in a few dwarf mutants. In the middle zone, a majority of the mutants possessed granule-like bodies scattered all over, while a few mutants, such as dwarf-4, 11, 15 and *indica* grain-3, had small protein bodies. Unlike T-65, the mutants of Tainan-3 were characterized by small granular bodies in the subperipheral zone. In the middle zone, most of them had only granule-like protein bodies scattered sparsely, but mutants such

Table 2. *Correlation between protein content and seed weight and protein content and seed sterility*

Parentage of mutants	d.f.	Correlation coefficient: Protein content and seed sterility	Correlation coefficient: Protein content and 1,000 grain weight
Taichung-65	33	$-0.094^{N.S.}$	-0.365
Tainan-3	13	-0.707^{**}	$-0.280^{N.S.}$
Taichung (Native)-1	16	$0.227^{N.S.}$	$0.163^{N.S.}$
IR-8	3	$0.806^{N.S.}$	$-0.421^{N.S.}$

** Significant at 1% level; N.S. — Not significant.

Table 3. *Scoring for protein distribution pattern in the endosperm of mutants in Taichung*-65, *Tainan*-3, *T*(*N*)-1 *and IR*-8

No.	Material	Zones 1	2	3	4
	Taichung-65				
1.	Control	****	**	**	*
2.	Dwarf-2	****	**	*	*
3.	Dwarf-3	****	**	*	*
4.	Dwarf-4	****	***	**	*
5.	Dwarf-6	****	**	*	*
6.	Dwarf-7	****	***	*	*
7.	Dwarf-8	****	**	*	*
8.	Dwarf-9	****	**	*	*
9.	Dwarf-10	****	**	*	*
10.	Dwarf-11	****	**	**	*
11.	Dwarf-13	****	**	*	*
12.	Dwarf-14	****	***	*	*
13.	Dwarf-15	****	**	*	*
14.	Dwarf-16	****	**	*	*
15.	Dwarf-17	****	**	*	*
16.	Dwarf-19	****	**	*	*
17.	Dwarf-20	****	**	*	*
18.	Indica grain-2	****	**	*	*
19.	Indica grain-3	****	***	**	*
20.	Indica grain-5	****	**	*	*
21.	Compactoid-1	****	**	*	*
22.	Compactoid-2	****	**	*	*
23.	Shattering	****	**	*	*
24.	Loose panicle	****	**	*	*
25.	Open panicle	****	**	*	*
26.	Indica plant	****	**	*	*
27.	Matching	****	**	*	*
	Tainan-3				
1.	Control	****	**	*	*
2.	Indica grain-2	****	**	*	*
3.	Indica grain-3	****	**	*	*
4.	Indica grain-5	****	**	**	*
5.	Erectoid-4	****	*	*	*
6.	Erectoid-5	****	**	*	*
7.	Bold grain	****	**	*	*
8.	Promising panicle-1	****	**	*	*
9.	Grassy clump	****	**	**	*
	Taichung (Native) 1				
1.	Control	****	**	*	*
2.	Tall	****	*	*	*
3.	Japonica grain-5	****	***	*	*
4.	Beaked-1	****	**	*	*
5.	Hooded-2	****	***	*	*
6.	Boat leaf	****	**	**	*
7.	Broad leaf	****	**	*	*
8.	Tetragonal grain	****	***	**	*
9.	Horizontal flag leaf	****	***	**	*
	IR-8				
1.	Control	****	**	*	*
2.	Awned	****	**	*	*
3.	Peculiar dwarf	****	***	*	*
4.	Fine grain-5	****	***	**	*

as grass clump and *indica* grain-5 had small protein bodies instead.

As in the *japonica* varieties, a wide variation in the distribution pattern was observed in the *indica* variety T(N)-1. The tall mutant, for instance, showed poor distribution but the tetragonal grain and hori-

Fig. 1

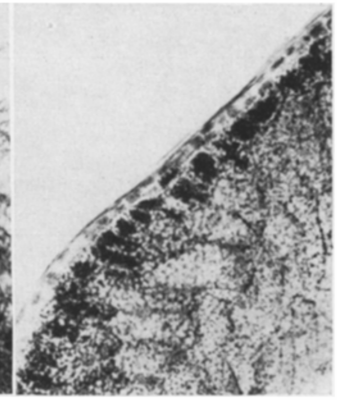

Fig. 2

Fig. 1. Transverse section of endosperm of a mutant showing no prominent protein bodies in the peripheral zone

Fig. 2. T.S. of endosperm of a mutant showing protein bodies of bigger size sparsely arranged in the peripheral zone and granule-like bodies in the subperipheral and middle zones

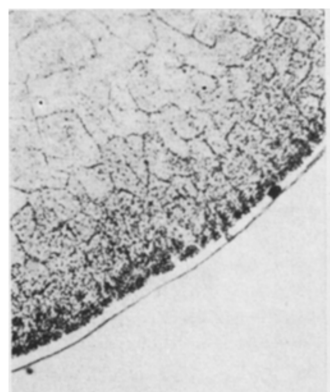

Fig. 3

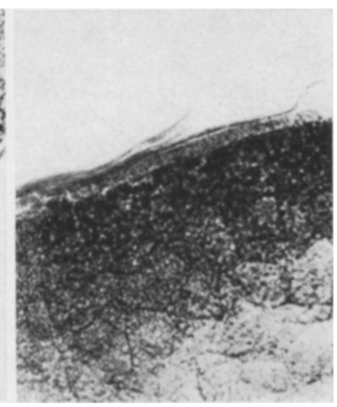

Fig. 4

Fig. 3. T.S. of endosperm of a mutant showing granule-like protein bodies scattered a little densely in the periphery and sparsely in the inner zones

Fig. 4. T.S. of endosperm of a mutant showing medium size protein bodies closely arranged in the peripheral zone and granular bodies in the inner zone

Fig. 5. T.S. of endosperm showing bigger bodies of protein coalesced in the periphery and medium to small size protein bodies in the subperipheral and mid zones. The inner zone is with sparsely scattered granule-like protein bodies

zontal flag leaf mutants had relatively uniform distribution patterns. Among the mutants of IR-8, two had medium-sized protein bodies in the sub-peripheral layer, but smaller bodies in the middle layer.

Of all the mutants and parents studied, a good protein distribution pattern overall was exhibited by the dwarf-4 and *indica* grain-3 mutants of T-65, the tetragonal grain and horizontal flag leaf mutants of T(N)-1 and a fine grain of IR-8. These mutants had large and closely-arranged protein bodies in the peripheral zone, medium-sized bodies in the sub-peripheral zone, small bodies in the middle zone and scattered granule-like particles in the inner zone.

Discussion

The importance of rice as a protein source is shown by the fact that it contributes nearly one third of the total protein in the diet. Although the quality of rice protein is superior, the narrow range of variability and low protein content have stood in the way of developing high protein rices in the past. Now there is evidence that, as well as the natural variability with genotypes having as much as 14 per cent of protein, additional variability could be generated through induced mutagenesis in superior agronomic bases (cf. Swaminathan *et al.*, 1968; Tanaka, 1969; Harn *et al.*, 1970; Haq *et al.*, 1970; and Siddiq *et al.*, 1970). Harn *et al.* (*loc. cit.*), for instance, reported that the protein in a mutagen-treated population varied between 73 and 100 per cent relative to the mother varieties. Similarly, Tanaka and Tamura (1968) induced variation in the protein content up to 185 per cent. These findings indicate that mutation breeding would be effective in improving protein characteristics. In the present study, analysis of a number of induced mutants affecting different plant parts revealed that there was a wide variation in protein content and some of the mutants had significantly more protein than their respective parents. For instance, the mutants, tetragonal grain and boat leaf, had 14.31 and 13.28 per cent of protein, respectively, compared with 10.50 per cent in the parent variety T(N)-1. These mutants, if not for direct introduction, may be valuable as additional high protein donors in the quality breeding programme.

Breeders are pessimistic about the development of high protein varieties because yield and protein content tend to be negatively correlated. However, what is important in both conventional and mutation breeding is to look for the occasional exceptions to the general trend. In other words, it would be of practical value in mutation breeding if some of the yield promoting attributes were positively associated with protein content. Harn *et al.* (1970) reported a negative correlation between protein content and test grain weight. In the present study however, no significant correlation was observed between protein content and 1000 grain weight in any of the mutant groups except that of Taichung-65, which showed a significant negative correlation, thus suggesting that there need not be any consistent association between protein content and grain weight and that such correlations might vary from variety to variety. Contrary to the generally held view that protein content is influenced by the degree of spikelet sterility, in three of the four mutant groups studied there was a positive correlation. It is interesting that in the mutants of Tainan-3 a significant negative correlation between seed sterility and protein content was observed.

The nutritive quality of rice depends equally on the extent of protein loss through milling. In this cereal, there is a considerable concentration of protein in the germ and aleurone layers of the kernel. Because of this localization a portion of the protein tends to get lost during milling and polishing. Such losses can be minimised in grain types where the protein is uniformly distributed in the middle and inner layers. Microscopic screening of a large collection of strains, as well as populations derived from mutagen treatments, by Kaul *et al.* (1969) indicated the existence of variability for the protein distribution pattern. The present study indicated a similar variation in the distribution pattern. Some of the mutants, such as dwarf-4 and *indica* grain of T-65 and fine grain of IR-8, showed a marked change in the pattern with protein bodies uniformly dispersed from the outer aleurone to the inner layers of the endosperm. It appears from the present findings that this character is easily amenable to mutagen induced genetic alterations.

A comparative study of protein content and its distribution pattern in the mutants indicated no consistent relationship between them. In the case of T-65 and IR-8, the mutants with a good distribution pattern did not show any significant increase in protein content over their respective controls. There were also cases, such as the boat leaf mutant of T(N)-1, where a higher protein content was found to be associated with a better distribution pattern. Similarly, in mutants such as grass clump, a moderate increase in the protein content was accompanied by a moderate distribution.

Although it is generally believed that increased protein content is associated with deeper deposition of protein bodies, the present study revealed that there could be exceptions to this trend. Juliano (1971) is of the view that differences in the distribution pattern of proteins may not have practical significance, since most of the endosperm remains in the milled rice fraction. If localization of protein could be manipulated favourably through induced mutations without too much effect on the protein content in a superior genotype, as demonstrated in the present study, the loss on polishing could still be minimised.

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