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Studies on Protein Characteristics in Rice Mutants

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Summary. Twenty mutants isolated from "Latisail", "Jhingasail" and "Pankaj" varieties of rice (*Oryza sativa* L.) were screened for two aspects of nutritive quality, namely crude protein content and distribution pattern of protein in the endosperm. Observations revealed a wide variation for both characters, and while there was no consistent association between protein content and test grain weight, which varied between varieties, a positive correlation between protein content and grain sterility was noted. In a few mutants protein distribution was observed to be varied and showed a similarity to optimum milling characteristics.

Key words: Protein granule – Induced mutants – Aleurone layer – Amorphous Endosperm

Quality improvement breeding for increased protein content and uniform protein granule distribution in endosperm are extremely important objectives and there is evidence of their improvement being obtained by mutation breeding in rice (Swaminathan et al. 1970; Tanaka and Takagi 1970). In this cereal, protein concentrates in the germ cells and in the aleurone layers of the kernel and as a result the protein gets 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. With the objective of locating such grain types, a number of true breeding induced mutants of rice were screened. The protein content of each mutant line was estimated as the average of three plant values obtained by the macro-kjeldahl method. Five grains of uniform size and maturity were taken from each mutant in order to study the protein distribution pattern in the endosperm, as suggested by Vilawan and Siddiq (1973). The distribution of protein bodies was studied under light microscope. Electron microscopic studies were also conducted in order to estimate protein content and form in the induced mutants. Samples were fixed in buffer glutaraldehyde (2.5%) and famaldehyde (1.5%) for 24 hrs and then fixed with 1% aqueous osmium teroxide. After dehydration, the tissue was embedded in ultra-low viscosity resin.

The total protein content was low, ranging between 6 to 9% in most of the cultivated rice varieties. The world germplasm shows 12 to 17% protein content (Tanaka 1969). Among the mutants of "Latisail", early heading had the lowest protein content of 6% while flat

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

No.	Mutant	Percent of kernel protein content	1000 grain weight (gm)	Percent of seed sterility
	"Latisail" control	7.52	23.52	11.50
1.	Grassy	8.59	19.65	75.00
2.	Tall	8.46	25.30	10.20
3.	Round grain	11.57	23.52	26.80
4.	Abnormal panicle	9.65	19.13	86.30
5.	Semi dwarf	11.85	19.03	56.80
6.	Awned	6.66	24.23	12.60
7.	Boat leaf	12.12	19.42	67.60
8.	Extreme dwarf	8.43	20.25	70.60
9.	Early heading	6.00	22.50	19.50
10.	Late heading	7.00	19.80	34.30
11.	Scattered tiller	12.26	21.04	14.60
12.	Flat grain	16.82	32.42	94.60
	"Jhingasail" control	7.98	25.12	24.40
1.	Branch tiller	12.12	14.22	54.60
2.	Long grain	9.06	22.19	26.70
3.	Bold grain	12.12	28.42	22.60
4.	Awned	9.66	23.83	22.60
5.	Medium grain	11.88	24.28	46.60
6.	Small grain	7.59	18.10	21.10
	"Pankaj" control	6.76	25.21	15.90
1.	Small grain	10.85	21.25	12.00
2.	Round grain	15.06	17.25	30.60

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

Parentage of mutants	d.f. (N-2)	Correlation coefficient: Protein content and seed sterility	Correlation coefficient: Protein content and 1000 grain weight
"Latisail"	11	0.674*	−0.418
"Jhingasail"	5	0.662	0.063
"Pankaj"	1	0.754	−1.00**

* Significant at 5% levels, respectively
** Significant at 1% levels, respectively

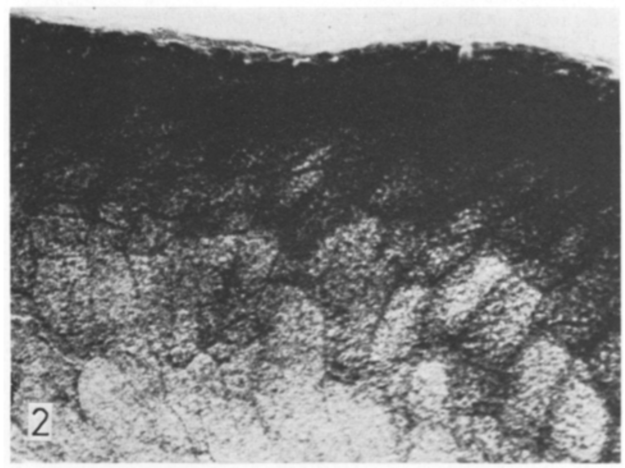
grain had 16.82% (compared with 7.52% in the control). This was similar to the situation found with the mutants of "Jhingasail" and "Pankaj" the range being 7.59 to 12.12% and 10.85–15.06%, respectively (Table 1). Most of the mutants showed higher protein content than their respective parents. These types of mutants, if unsuitable for direct introduction, may be valuable as additional high protein donors in quality breeding programmes.

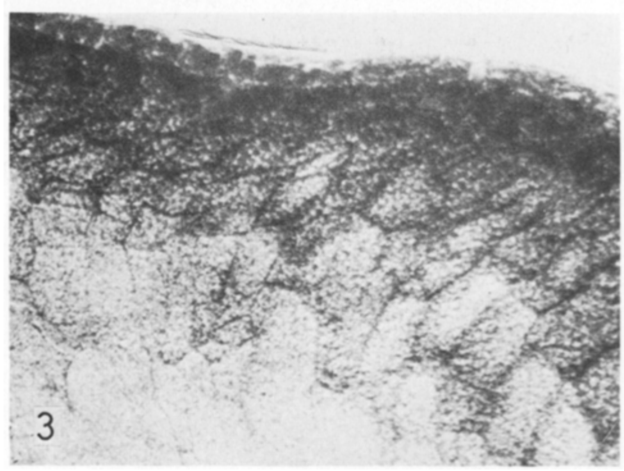
Protein lines with higher yield have not yet been reported in any crop. In the present study it has been possible to develop two high protein lines (the bolder grain of "Jhingasail" and the small grain of "Pankaj")

Table 3. Electron microscopic observation of protein content and form in the endosperm of mutant in Latisail, Jhingasail and "Pankaj"

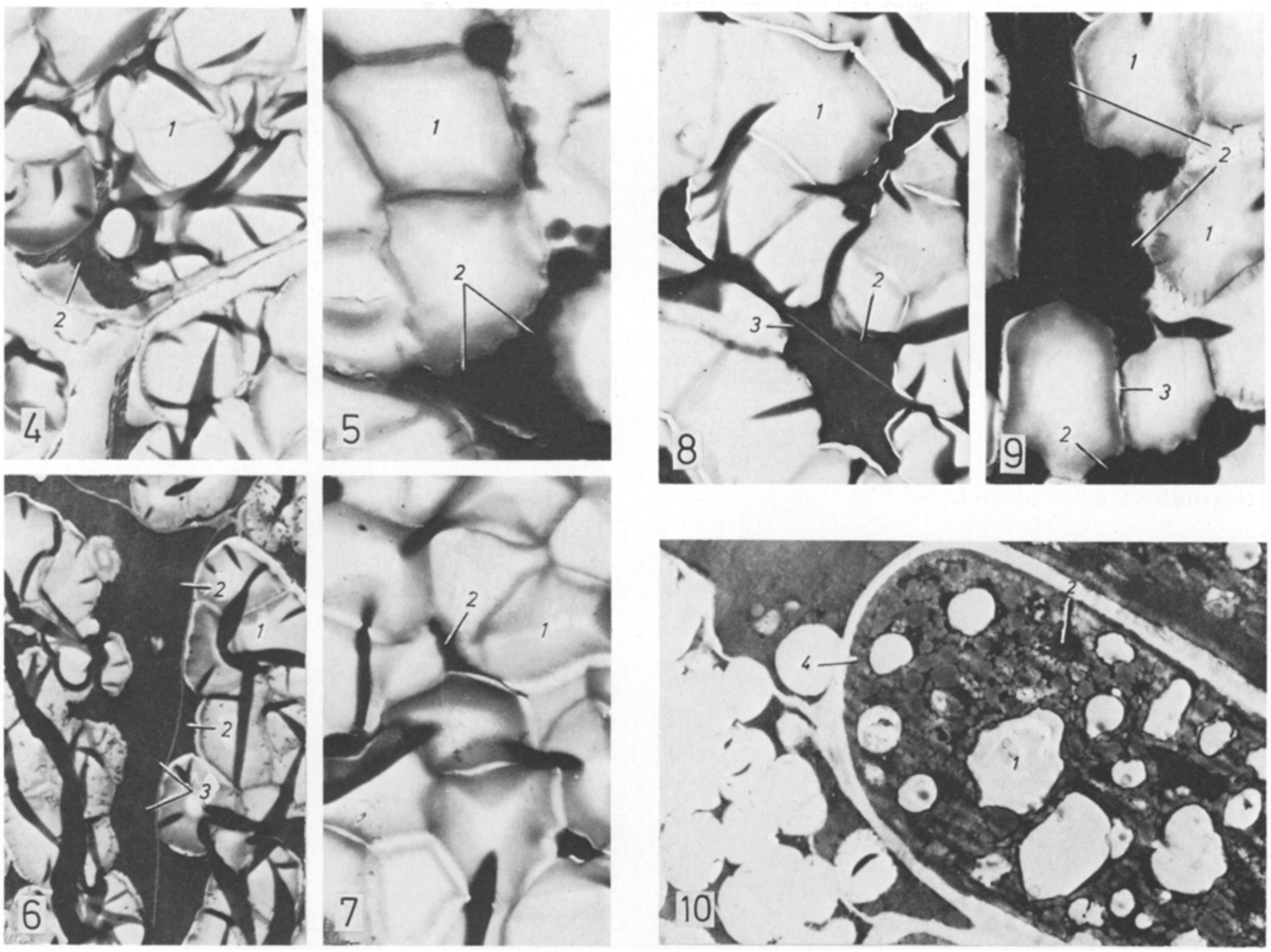
Variety	Mutant	A	B	C	D	E	F	G
"Latisail"	Control	+	–	–	–	–	–	–
	Grassy	–	+	–	–	–	–	–
	Tall	+	–	–	–	–	–	–
	Round grain	–	–	+	–	–	–	–
	Abnormal panicle	–	+	–	–	–	–	–
	Semi-dwarf	–	–	–	–	+	–	–
	Awned	–	–	–	–	–	–	+
	Boat leaf	–	–	+	–	–	–	–
	Extreme dwarf	–	–	–	+	–	–	–
	Early heading	–	–	–	–	–	–	+
	Late heading	–	–	–	+	–	–	–
	Scattered tiller	–	+	–	–	–	–	–
	Flat grain	–	–	–	–	–	+	–
"Jhingasail"	Control	–	–	–	–	–	–	+
	Branch tiller	–	–	+	–	–	–	–
	Long grain	+	–	–	–	–	–	–
	Bold grain	–	+	–	–	–	–	–
	Awned	–	–	–	–	+	–	–
	Medium grain	–	–	–	–	+	–	–
	Small grain	–	–	–	+	–	–	–
"Pankaj"	Control	–	–	–	–	–	–	+
	Small grain	–	–	–	–	+	–	–
	Round grain	–	–	–	–	–	+	–

A = Fig. 4; B = Fig. 5; C = Fig. 6; D = Fig. 7; E = Fig. 8; F = Fig. 9 and G = Fig. 10





Figs. 1–3. Transverse section of endosperm of mutant showing no prominent protein bodies in the peripheral zone; **2** Transverse section of endosperm of a mutant showing larger protein bodies coalesced in the periphery, and medium to small size protein bodies in the sub-peripheral and mid-zones; **3** Transverse section of endosperm of a mutant showing protein bodies of larger size sparsely arranged in the peripheral zone and granule-like bodies in the sub-peripheral and middle zones



Figs. 4 – 10. 4, 7 and 10 Electron micrographs showing smaller amount of amorphous protein bodies in the endosperm of different mutant grains (×6,500); **5, 6, 8 and 9** Electron micrographs showing higher amount of protein bodies in the endosperm of different mutant grains (×8300). *1* starch granules; *2* protein bodies; *3* cell wall and *4* aleurone layer

of rice with higher yields than the control. These show the yield promoting attributes as being positively associated with protein content. However, there need not be any consistent association between protein content and 1000 grain weight and such correlations might vary from variety to variety (Table 2). This result confirms the earlier findings of Vilawan and Siddiq (1973). Contrary to the generally held view that protein content is influenced by the degree of spikelet sterility, in the present study there was a positive correlation which is in confirmity with the result of Narahari and Bhatia (1975).

Microscopic examinations of transverse sections of kernels from different induced mutants, including controls, exhibited three distinct types of protein configurations in the kernels: (a) no prominent protein bodies in the peripheral zone (Fig. 1); (b) larger protein bodies coalesced in the periphery and medium to small size protein bodies in the sub-peripheral and mid zones (Fig. 2); (c) larger protein bodies sparsely arranged in the peripheral zone and granule like bodies in the sub-peripheral and middle zone (Fig. 3).

The electron microscopic structure of the kernels from different mutants revealed that there was a concentration of protein in the aleurone and sub-aleurone layers although a smaller amount of protein within the endosperm of the mutants was also noticed. The protein concentration and form of the mutants (Figs. 4, 7, 10) were amorphous whereas, in comparison, the protein bodies were more concentrated in the mutants as depicted in Figs. 5, 6, 8 and 9 (Table 3).

The mutants revealed uniform distribution of protein in the endosperm thereby indicating milling quality of the mutants. It was also noted that the protein distribution in the endosperm is not dependent on the total protein content although might be influenced by both genes and the environment (Gottschalk 1975). However, isogenic lines for uniform distribution of protein granules would be worth using in quality improvement breeding programme.

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