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SEED PROTEIN VARIATIONS IN RADIATION INDUCED MUTANTS OF WHEAT

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ABSTRACT

Storage seed proteins of nine wheat mutants derived from recurrent irradiation along with parent 'SHARBATI' were analysed by Sodium dodecyl sulphate polyacryamide gel electrophoresis (SDS-PAGE). Low similarity index (58 to 75) between the mutants and parent 'Sharbati' revealed a wide variability of electrophoretic pattern of water soluble protein (glutenin). Induced mutants possessed identifiable, seedling, morphological characters and specific bands of storage protein. These mutants are important source of breeding materials, specially for improvement of protein quality of bread wheat.

Key Words : Wheat, mutants, electrophoresis, storage protein, similarity index, relative mobility, *Rht* genes.

Proteins of wheat endosperm can be separated into non-gluten forming and gluten forming groups when wheat flour is wetted and mixed with water. The term gluten refers to a viscoelastic protein, which is recovered after aqueous washing out of starch and water soluble components from wheat flour [1]. Traditionally gluten forming proteins, which represent 80-90% of the total proteins of wheat flour, have been classified into two major groups, viz., gliadin and glutenin, based on their extractability in acquous alcohol. A peculiar aspect of both groups is their high level of heterogeneity, determined by genotype. This characteristic has been used in varietal identification, detecting off types in pure seed, segregation in the advanced breeding material, gene banks and genetic resources [2]. Wheat storage proteins have been characterised by gel electrophoresis by Payne *et al.* [3] and several investigators have emphasised the importance of protein and enzyme electrophoresis for the identification of cultivars and species in wheat [4]. Polymorphism in protein banding pattern particularly glutenins, has been obtained in wheat mutants of land race 'Sharbati' at Parbhani.

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MATERIALS AND METHODS

The material for the present investigation consisted of nine wheat genotypes. Among them eight were induced mutants obtained from land race 'SHARBATI' by the recurrent irradiations [5].

Soluble protein pattern in seed : Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique of Laeminlu [6] as modified by Payne *et al.* [7] was used for analysis of seed proteins. The endosperm protein were crushed and extracted with extraction buffer in a ratio of 1 mg : 20 ul. Extraction was carried out at room temperature for one hour after which the samples were boiled in water bath for 2.5 minutes. After brief centrifugation 20 µl supernatant was loaded in each well. Coomassie blue (0.1%) stained gels were scored for presence or absence of bands for each cultivar for comparisons.

Measurement of "Rm" values : The relative mobility (Rm) of the protein bands was measured with reference to the migration of the tracking dye as follows.

Qualitative and Quantitative variation : Qualitative and quantitative differences in the banding pattern HMW glutenin subunits of the different wheat genotypes were analysed. Qualitative differences were based on the presence or absence of the specific bands in the profiles. Quantitative differences were determined on the basis of staining intensities of the bands in questions. The staining intensities were defined as : dense, medium and light.

$$Rm = \frac{\text{Distance migrated by the protein bands from top of the running gel (cm)}}{\text{Distance migrated by the tracking dye (cm)}}$$

The similarity index (SI) of mutants as compared to 'Sharbati' was calculated as per [8].

$$SI = \frac{\text{Number of similar bands}}{\text{Total number of bands for the two samples}} \times 100$$

RESULTS AND DISCUSSION

Distinguishing characters of mutants : The original parent 'Sharbati' possessed 16.91 percent seed proteins. It is interesting to note that mutants PBNS 3904 recorded protein content of 17.23 per cent. The protein content in the mutants ranged from 12.80 to 17.23 per cent, which indicated that a wide and desirable variability has been achieved through recurrent irradiation of the original parent 'Sharbati'.

The mutant PBNS-3958 recorded a protein level of 15.32 per cent, with 3.83 g per 100 grain weight. This is the only mutant which is dwarf and early with profuse

tillers. The combination of these three important traits is a rare phenomenon in wheat. The dwarfing genes *Rht*-1 and *Rht*-2 from Japanese wheat variety Norin '10' has influenced remarkably the global wheat breeding programme. However, the extensive use of these genes led to a narrow genetic base and consequent genetic vulnerability of the varieties. Therefore, exploiting presently induced mutants from Indian land race 'Sharbati' may provide wider genetic variability.

Table 1. Distinguishing characters of Sharbati and its mutants

Genotypes	Seedling vigour index	Plant height (cm)	Days to flowering	No. of tillers	Weight of 100 grain (gm)	Protein percent	Yield per plot (gm)
Sharbati	2287.52	88.60	61.00	126.00	3.24	16.91	545
PBNS-3904	2418.00	90.80	58.00	117.66	4.04	17.23	1550
PBNS-3907	2304.00	87.00	62.00	104.33	3.92	16.27	1050
PBNS-3909	2497.66	86.60	62.50	96.00	4.66	14.04	1275
PBNS-3910	2292.09	82.40	63.00	95.00	4.02	15.96	625
PBNS-3955	2407.46	106.60	58.00	105.33	4.38	13.08	1350
PBNS-3957	2274.30	90.80	70.00	151.66	2.90	12.80	800
PBNS-3958	2330.50	52.10	56.00	185.00	3.83	15.32	1075
PBNS-3959	2362.45	83.20	73.50	129.00	2.84	16.59	875
SE ±	57.03	0.87	0.77	1.79	0.037	1.05	81

High protein per cent were recorded in the genotypes PBNS-3904 followed by Sharbati, PBNS-3959, PBNS-3907. High grain yield were recorded in the genotypes PBNS-3904 followed by PBNS-3955, PBNS-3909 and PBN-4054. Highest grain number per ear was recorded in PBNS-3958 followed by Sharbati and PBNS-3955.

The highest seedling vigour index was recorded by PBNS-3960 followed by PBNS-3909 and PBN-4055.

Pattern of soluble protein bands : Electrophoretic pattern of water soluble seed protein (glutenin) of the material under study were obtained by SDS-PAGE technique. The banding patterns represented by electrophoregram is presented in Fig. 1.

The profile of the soluble proteins in the single seed indicated that bands nos 15 and 25 (Rm 0.41 and 0.64) were specific to Sharbati.

Band No. 10 (Rm (0.26) was specific to PBNS-3909, band Nos. 11 (Rm 0.27);

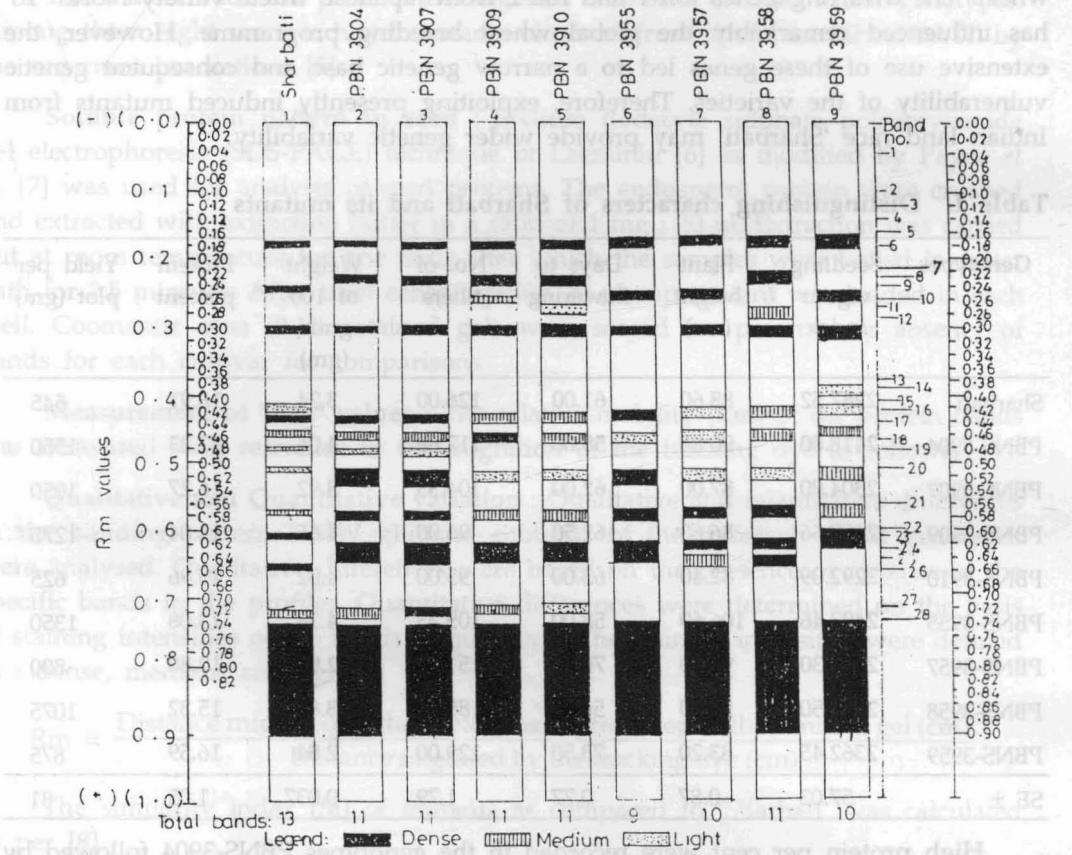

Fig. 1. Schematic diagram of the SDS PAGE profiles of seed glutenins in Sharbati and it's mutants

24 (Rm 0.63); 1, 3, 13, (Rm 0.04, 0.11, 0.38); 7 (Rm 0.21); 12 (Rm 0.34); were specific to PBNS-3910, PBNS-3957, PBNS-3960 and PBNS-3961 respectively. Band Nos. 5 (Rm 0.16), 11 (Rm 0.29) were common in Sharbati, PBNS-3904, PBNS-3907, PBNS-3958, and PBNS-3959. On the other hand band Nos. 1 and 11 (Rm 0.09, 0.27) were common in PBN-3960, PBNS-3961. The results indicated that the induced mutants from Sharbati posses high polymorphism for storage protein. Chauhan *et al.* [10] also detected polymorphism in proteins and enzymes by employing electrophoresis.

Quantitative differences were observed in band No. 17 (Rm. 0.44) present in all the 9 entries. Intensity of this band was observed to be medium in Sharbati,

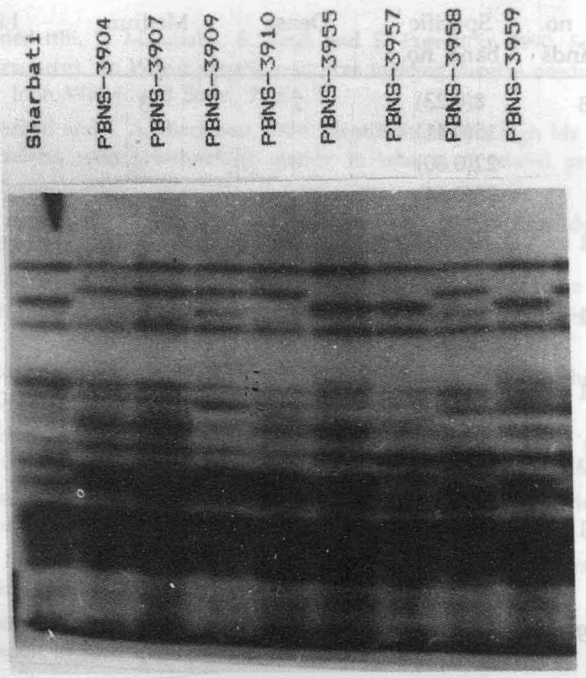

Fig. 1 (plate). Gel electrophoresis columns of land race Sharbati and it's mutants

PBNS-3904, PBNS-3910, PBNS-3907, PBNS-3958, PBNS-3959, while dense in PBNS-3909 and light in PBNS-3955.

In the present investigation potentially useful mutants for seed storage proteins have been obtained by recurrent irradiation. Variability of storage protein albumins, globulins, gliadins and glutenin have been observed in *Triticum spp. (Triticum boeoticum, Triticum urartu., T. turgidum, T. timopheevii., T. aestivum. Ae. speltoides* [10-12]. SDS PAGE electrophoresis used to determined the composition of glutenins of 106 Australian wheat cultivars has also provided key to identification of unknown samples [13].

The present studies indicated that the electrophoretic pattern of protein bands could provide quick identification of segregating breeding material. Further this can be used for characterising inbred lines and determine the extent of crossing by means of protein markers.

Table 2. Qualitative and quantitative difference among mutants and 'Sharbati'

Genotype	Qualitative difference		Quantitative difference			SI with Sharbati
	Total no. of bands	Specific band no.	Dense	Medium	Light	
Sharbati	13	8(0.23) 15(0.41) 22(0.60) 25(0.64)	7	3	3	-
PBNS-3904	11	7(0.21) 23(0.61)	8	2	1	75.0
PBNS-3907	11	7(0.21) 23(0.61)	9	1	1	75.0
PBNS-3909	11	7(0.21) 10(0.26) 11(0.27) 23(0.61)	6	4	1	66.66
PBNS-3910	11	7(0.21) 11(0.27) 23(0.61)	5	3	3	66.66
PBNS-3955	9	9(0.24) 23(0.61)	8	-	1	62.76
PBNS-3957	10	9(0.24) 23(0.61) 24(0.63)	6	1	3	61.26
PBNS-3958	11	7(0.21) 11(0.27) 23(0.61) 24(0.63)	7	3	1	58.68
PBNS-3959	10	9(0.24) 14(0.39)	5	3	2	69.56

- Figures in parenthesis are relative mobilities (Rm values); - SI = Similarity index

The results also substantiated the conclusions that the recurrent irradiation method for creating wide variability resulted in high rate of mutations affecting glutenin and glidin fraction of protein.

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