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INDUCED PROTEIN AND ISOZYME VARIATION IN *VIGNA RADIATA* VAR. PS 16

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ABSTRACT

Electrophoretic variation of protein profiles, esterase and amylase isozymes was investigated in 24 h germinated M_3 seeds of Vigna radiata var. PS 16. The patterns of induced variations suggest that there is a differential gene expression as well as gene activation due to mutagenesis. The results suggest that mutagenesis could be used for locating genetic markers for the identification of mutants and for the construction of linkage groups and gene mapping.

Following the discovery of Markert and Moller (1959) isozymes have been incresingly used in studies relating to genetics, evolution and developmental biochemistry. Various workers have employed protein and isozyme patterns as additional clue to establish phylogenetic relationships in higher plants. Isozymes are expressions almost exclusively of the genetic make up of the plant (Kiang *et al.* 1985). Protein and isozyme patterns have been used to determine the genetic affinity among the mutants (Wolff, 1980).

Information on induced isozyme variation is scarce (Endo, 1967) although the effects of ionizing radiation on the enzyme activity have been investigated. With a view to locate genetic markers which could be used in the identification of mutants from the segregating mutant lines and to understand the allelic variations produced by the mutagens, this work was undertaken. This paper reports the isozyme variation in the M_2 generation plants of *Vigna radiata* var. PS 16.

MATERIALS AND METHODS

Seeds of *Vigna radiata* (L.) Wilczek var. PS 16 (obtained from Pulse Research Laboratory. IARI, New Delhi) was used for induction of mutations. Ethyl methane sulphonate (EMS) and gamma rays were used as mutagens separately. The treatments were: 0.05%, 0.1%, 0.15% and 0.2% EMS and 5 KR, 10 KR, 15 KR and 20 KR gamma rays. EMS was dissolved in distilled water and the seeds were soaked in the aqueous solution of appropriate concentration for 10 h, after 6 h of presoaking in distilled water. The treated seeds were thoroughtly washed in running water for 12 h and the excess moiture was blotted off. The moisture content of the seed before mutagen treatment was adjusted to 10.5 - 11.0% by differential drying in an air oven (AACC, 1962) ^{60}Co was used as the source of gamma rays for the irradiation of seeds, which was carried out at the irradiation unit of Tamil Nadu Agricultural University. A control was also maintained. Complete random block design was used to raise M_1 and M_2 generation plants. Seeds of each plent of M_1 generation were harvested separately and sown in M_2 on a plant to progeny basis.

Electrophoretic variation of protein, esterase and amylase isozymes in 24 h germinated seeds was investigated in the M_3 seeds (obtained from M_2 generation plants). Poly acrylamide gel electrophoresis was used (Davis, 1964). Procedures outlined by Scandalios (1969) and Machiah and Wakil (1964) were followed to localize the enzymes.

RESULTS AND DISCUSSION

A total number of 15 bands were recorded for protein. Bands 0.13, 0.25, 0.36, 0.47, 0.54 and 0.62 were observed in most treatments. Band 0.17 was specific to 0.05% EMS treatment. 0.2% EMS and 10 KR

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Table 1. Similarity index values between pairs of treatments (based on protein profiles)

	Control	EMS				Gamma rays			
		0.05%	0.1%	0.15%	0.2%	5KR	10KR	15KR	20KR
Control	-	62	91	73	69	91	69	64	70
EMS :									
0.05%		-	57	54	64	57	53	36	54
0.1%			-	67	77	83	77	58	67
0.15%				-	62	67	50	55	50
0.2%					-	64	71	43	62
Gamma rays									
5KR						-	77	58	54
10KR							-	54	62
15KR								-	42
20KR									-

Table 2. Similarity index values between pairs of treatments (based on isozymes of esterase)

	Control	EMS				Gamma rays			
		0.05%	0.1%	0.15%	0.2%	5KR	10KR	15KR	20KR
Control	-	75	75	63	86	88	100	63	50
EMS :									
0.05%		-	56	45	63	67	75	45	35
0.1%			-	63	63	88	75	63	50
0.15%				-	72	75	63	100	83
0.2%					-	63	86	72	57
Gamma rays									
5KR						-	88	75	63
10KR							-	63	50
15KR								-	83
20KR									-

gamma rays treated genotypes showed the maximum number of bands (11) and 15 KR gamma rays treated genotypes showed the least number of bands (7). The similarity index values between pairs of treatments are given in Table 1. The maximum similarity index value (91%) was obtained in combinations involving control and 0.1% EMS, control and 5 KR gamma rays. The lowest similarity index value (36%) was obtained in combinations involving (0.05% EMS and 15 KR gamma rays).

For esterase 8 bands were recorded. Band 0.29 was specific to 0.05% EMS treatment. Maximum number of bands (7) were seen in 5 KR treated genotypes and 20 KR treated genotypes showed the least number of bands (4). The similarity index values between pairs of treatments are given in Table 2. The maximum similarity index value (100%) was seen in combinations involving control and 10 KR, and 0.15% EMS and 15 KR. The lowest similarity index values (35%) were seen in combinations involving 0.05% EMS and 20 KR.

For amylase, a total number of 4 bands were present (Table 3). 0.05% and 0.1% EMS treated genotypes showed only three bands each. All other treated genotypes had 4 bands each. The similarity index values between pairs of treatments are given in Table 4. Most

Table 3. Isozymes of amylase and their Rf values in the control and mutated genotypes.

Band Number	Rf value	Control	EMS				Gamma rays			
			0.05%	0.1%	0.15%	0.2%	5KR	10KR	15KR	20KR
A_1	0.19	+	+	+	+	+	+	+	+	+
A_2	0.36	+	+	+	+	+	+	+	+	+
A_3	0.46	+	+	+	+	+	+	+	+	+
A_4	0.52	+	-	-	+	+	+	+	+	+
Total number of bands		4	3	3	4	4	4	4	4	4

Table 4. Similarity index values between pairs of mutated genotypes (based on isozymes of amylase)

	Control	EMS				Gamma rays			
		0.05%	0.1%	0.15%	0.2%	5KR	10KR	15KR	20KR
Control	-	80	80	100	100	100	100	100	100
EMS :									
0.05%		-	100	80	80	80	80	80	80
0.1%			-	80	80	80	80	80	80
0.15%				-	100	100	100	100	100
0.2%					-	100	100	100	100
Gamma rays									
5KR						-	100	100	100
10KR							-	100	100
15KR								-	100
20KR									-

of the pairs showed maximum similarity index value (100%)

Protein and esterase profiles showed a high degree of polymorphism where as amylase profiles did not show much variation. Each isozyme band is controlled by a single gene. Alterations of genes due to mutations have been reported by many workers (Reddy *et al.*, 1986). Our studies indicate that mutagenesis is a potential tool to generate genetic markers.

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