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MUTAGENIC EFFECTS OF GAMMA RAYS AND EMS ON FREQUENCY AND SPECTRUM OF CHLOROPHYLL AND MACROMUTATIONS IN URDBEAN (*VIGNA MUNGO* L. HEPPER)

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ABSTRACT

The mutagenic effects of gamma rays (10, 20, 30 and 40 kR) and ethyl methane sulphonate (0.01, 0.02, 0.03 and 0.04M) alone or in combination (10kR + 0.02M, 20kR + 0.02M, 30kR + 0.02M and 40 kR+ 0.02M) on frequency and spectrum of chlorophyll and macromutations in two cultivars, namely, PDU1 and T9 of urdbean have been observed. Conclusively, the combination treatments have yielded the higher frequency and spectrum of chlorophyll mutations whereas the various doses of mutagenic agents have independent response towards macromutations in both the cultivars.

Key words : Gamma rays, EMS, chlorophyll, macromutations, urdbean

Mutation breeding is relatively a quicker method for improvement of crops. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants. Many physical and chemical mutagens have been used for induction of useful mutants in a number of crops. Induction of chlorophyll mutations in general is considered as a measure to assess the effectiveness of various mutagens. In the present study, the effect of gamma rays and ethyl methane sulphonate (EMS) employed singly or in combination was studied on the frequency and spectrum of chlorophyll mutations and viable macromutations in M_2 generation in urdbean.

The 300 uniform, dry and healthy seeds of two cultivars of urdbean (*Vigna mungo* L. Hepper), namely, PDU1 and T9 were exposed to ^{60}Co gamma rays each at 10, 20, 30 and 40kR doses at IARI, New Delhi. Four samples comprising 300 seeds each and presoaked in distilled water for six hours were treated with ethyl methane sulphonate (EMS) at the concentrations of 0.01, 0.02, 0.03 and 0.04M for six hours in phosphate buffer (pH 7.0). Four samples of 300 seeds each were first

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irradiated with gamma rays at 10, 20, 30 and 40kR doses and then immersed in 0.02M EMS solution in the same manner as described above for EMS treatment. After the EMS treatment, the seeds were washed in running water for one hour to eliminate the residual effect of the mutagenic chemical. A total of 26 treatments including two controls were sown in a randomized block design (RBD) with three replications. The seeds were sown at the rate of 100 seeds per plot at a spacing of 15 × 30 cm within and between rows at the University Agricultural Research Farm during the summer season of 1994. The M_2 generation was raised from individual M_1 plants following plant-to-progeny method during *Kharif*, 1994-95. The frequency and spectrum of chlorophyll mutations per 100 M_2 plants were scored at the seedling stage following the classification of Gustafsson [1]. The frequency and spectrum of different types of viable macromutants per 100 M_2 plants were scored at various developmental stages, particularly from flowering to maturity period. The spectra of chlorophyll and macromutations were scored treatmentwise to study the mutagenic effectiveness and efficiency of each treatment.

It is evident from the data (Table 1) that higher doses/concentrations of mutagens were more effective in inducing greater frequency of chlorophyll mutations in both the cultivars. Combined treatments of gamma rays and EMS were most effective in this respect. However, the effect of mutagens differed for both the cultivars as EMS was found more effective than gamma rays in cv. PDU1 and *vice versa*. The highest frequency of chlorophyll mutations (0.96%) was induced at 30kR gamma rays combined with 0.02M EMS treatment in PDU1, while 40kR gamma rays with 0.02M EMS treatment was found to induce highest frequency (0.78%) in cv. T9. Interestingly, the chlorophyll mutations were not observed at lower doses/concentrations of the mutagens in both the varieties. Marked varietal differences were present in terms of induction of chlorophyll mutations at different doses/concentrations of the mutagens. The variety T9 appeared to be more sensitive towards the mutagenic treatments as compared to PDU1.

Mutation frequency per 100 M_2 plants was highest in 40kR gamma rays (1.36%) followed by 20kR gamma rays + 0.02 M EMS (0.8%) in the variety PDU1. On the other hand, the highest macromutation frequency was observed at 30kR gamma rays (1.20%) followed by 0.02M EMS (0.94%) and 0.04M EMS (0.93%) in cv. T9 (Table 2). Most of the mutations showed independent response of the doses/concentrations as they occur at random. Gamma radiations proved to be the most effective mutagenic agent for inducing viable mutations in cv. PDU1, while EMS was most effective in cv.T9. Between the varieties under reference, T9 exhibited a higher response in terms of induction of mutations than PDU1.

Table 1. Frequency and spectrum of chlorophyll mutations per 100 M_2 seedlings in M_2 generation in urdbean cultivars PDU1 and T9

Treatment	Dose	Total plants observed	Per cent chlorophyll mutations			Total frequency
			albina	*chlorina*	*xantha*	
		cv. PDU1				
	Control	1100	-	-	-	-
Gamma rays	10kR	959	-	-	-	-
	20kR	746	-	-	-	-
	30kR	720	-	-	-	-
	40kR	654	-	-	0.30	0.30
EMS	0.01M	1067	-	-	-	-
	0.02M	924	0.10	0.32	-	0.42
	0.03M	720	-	-	-	-
	0.04M	695	-	-	-	-
Combination	10kR + 0.02M	867	-	-	-	-
	20kR + 0.02M	790	-	-	-	-
	30kR + 0.02M	618	-	0.32	0.64	0.96
	40kR + 0.02M	610	0.33	-	0.10	0.43
		Total	**0.43**	**0.64**	**1.04**	**2.11**
		cv. T9				
	Control	1100	-	-	-	-
Gamma rays	10kR	1085	-	-	-	-
	20kR	1024	0.09	-	0.19	0.28
	30kR	995	-	0.20	0.20	0.40
	40kR	820	-	-	-	-
EMS	0.01M	1013	-	-	-	-
	0.02M	945	-	-	-	-
	0.03M	904	-	0.33	0.11	**0.44**
	0.04M	805	-	-	-	-
Combination	10kR + 0.02M	910	-	-	-	-
	20kR + 0.02M	895	-	-	-	-
	30kR + 0.02M	872	0.34	0.12	-	**0.46**
	40kR + 0.02M	630	-	0.31	0.47	**0.78**
		Total	**0.43**	**0.96**	**0.97**	**2.36**

The spectrum of chlorophyll mutations observed in segregating M_2 generation of both the cultivars was analysed and their respective frequencies are given in Table 2. During the present investigation, the chlorophyll mutations, namely, *albina, chlorina* and *xantha* occurred in both the varieties. In general, the frequency of chlorophyll mutations was higher in T9 than PDU1. The frequency of different types of chlorophyll mutations revealed that the occurrence of *xantha* mutant was maximum followed by *chlorina* and *albina* in both the varieties. The variety T9 showed better response towards induction of different types of chlorophyll mutations at higher doses/concentrations of the mutagens. The spectrum of chlorophyll mutations was quite narrow as only three types, namely, *xantha, chlorina* and *albina* occurred.

The spectrum of macromutations was higher in case of T9 (seven types) as compared to PDU1 (six types) but these cultivars differed at the mutagenic specificity as revealed by the spectrum of mutations induced in both the varieties. Among all the mutagenic treatments, the mutation spectrum was much wider (four types) at 20 and 40kR doses of gamma rays followed by 10kR gamma radiation and 40kR + 0.02M EMS combination treatment (three types) whereas T9 showed four types of mutations induced at 0.04M EMS treatment followed by three types at 20 and 30kR doses of gamma rays, 0.02M EMS and 40kR + 0.02M EMS treatments. Several macromutants leading to aberrant plant type (tall, errect and bushy), plant habit (tendriller plant with slender stem), leaf (tetrafoliate in contrast to normal trifoliate), inflorescence (multiraceomose - plant bearing numerous racemes) and fertility (male sterile-flower with sterile pollen grains) could occur in both the varieties at a fair frequency in M_2.

The occurrence of chlorophyll mutations after mutagenic treatment has been reported in pigeonpea [2], lentil [3] and mungbean [4]. Chlorophyll mutation rate is a critical parameter to determine the effectiveness and efficiency of treatment of different mutagens. Chlorophyll development seems to be controlled by many genes located on several chromosomes [5] which could be adjacent to centromere and proximal segments of the chromosome [6, 7]. Mutations in these chlorophyll genes may induce chlorophyll mutations. The high incidence of chlorophyll mutations induced by EMS may be due to its specificity to affect certain regions of the chromosome [8]. Alkylating agents induce less drastic chlorophyll mutants such as *albina* in higher proportion than those induced by radiations, probably because of their apparently less drastic effect on chromosome [9].

The genetic differences in cultivars under reference for inducing spectrum and frequency of macromutants have been observed in Bengal gram [10], lentil [11] and pigeonpea [12]. Singh *et al.* [13], Verma and Singh [14], Pande and Raghuvanshi

Table 2. Spectrum and frequency of macromutations per 100 M_2 plants in urdbean cultivars PDU1 and T9 induced by mutagenic treatments in M_2 generation

Treatment	Dose	Total plants studied	Spectrum and frequency of macromutations						Total frequency
			Tendriller	Male sterile	Tall and erect	Bushy	Tetraoliate	Multi recemose	
				cv.PDU1					
	Control	1100	-	-	-	-	-	-	-
Gamma rays	10kR	759	-	0.13	0.13	-	-	-	0.26
	20kR	746	0.53	0.40	-	0.13	-	-	1.06
	30kR	720	-	-	-	-	-	-	-
	40kR	654	0.76	-	0.30	-	0.15	-	1.21
EMS	0.01M	1067	-	-	-	-	-	-	
	0.02M	924	-	-	-	-	-	-	0.10
	0.03M	720	0.41	-	-	-	-	-	0.41
	0.04M	695	-	-	-	0.28	-	-	0.28
Combination	10kR + 0.02M	867	-	-	-	-	-	-	-
	20kR + 0.02M	790	0.12	-	-	-	0.12	-	0.24
	30kR + 0.02M	618	-	-	0.32	-	-	-	0.32
	40kR + 0.02M	610	0.32	-	-	0.32	-	-	0.64
		Total	21.4	0.53	0.75	0.73	0.27	0.00	4.52

Table 2. (Cont.)

Treatment	Dose	Total plants studied	Spectrum and frequency of macromutations						Total frequency
			Tendriller	Male sterile	Tall and erect	Bushy	Tetraoliate	Multi recemose	
				cv.T9					
	Control	1100	-	-	-	-	-	-	-
Gamma rays	10kR	1085	0.46	-	-	-	-	-	0.46
	20kR	1024	-	-0.29	-	0.09	0	0.38	
	30kR	995	0.80	-	-	0.20	-	-	1.00
	40kR	820	-	0.12	-	-	-	-	0.12
EMS	0.01M	1013	-	-	0.19	-	-	-	0.19
	0.02M	945	0.74	-	-	0.10	-	-	0.84
	0.03M	904	-	0.11	-	-	-	-	0.11
	0.04M	805	-	-	0.12	-	0.24	0.37	0.73
Combination	10kR + 0.02M	910	-	-	-	0.10	-	-	0.10
	20kR + 0.02M	895	-	-	0.11	-	-	-	0.11
	30kR + 0.02M	872	0.91	-	-	-	-	0.11	1.02
	40kR + 0.02M	630	1.11	-	-	-	-	0.15	0.26
		Total	4.02	0.23	0.71	0.40	0.33	0.63	5.32

[15], Singh and Yadav [16] and Singh [17] reported mutations for plant type, branching pattern, leaf morphology, penduncle length, pod length, seed colour and boldness, etc. in mung and urd beans by radiations and chemical mutagens employed alone or in combination. The possible cause of these macromutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations [13]. Several workers have reported that these viable mutations were monogenic and recessive in nature controlled by one or more recessive gene(s) [18, 19].

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