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INDUCED MUTATIONS IN CHICKPEA (*CICER ARIETINUM* L.) II. FREQUENCY AND SPECTRUM OF CHLOROPHYLL MUTATIONS

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ABSTRACT

A comparative study of frequency and spectrum of chlorophyll mutations induced by two physical (gamma rays, fast neutrons) and two chemical mutagens (NMU, EMS) in relation to the effects in M_1 plants and induction of mutations in M_2 was made in four chickpea (*Cicer arietinum* L.) varieties, two *desi* (G 130 & H 214) one *Kabuli* (C 104) and one green seeded (L 345). The treatments included three doses each of gamma rays (400, 500 & 600 Gy) and fast neutrons (5, 10 & 15 Gy) and two concentrations with two different durations of two chemical mutagens, NMU [0.01% (20h), & 0.02% (8h)] and EMS [0.1% (20h) & 0.2% (8h)]. The frequencies and spectrum of three different kinds of induced chlorophyll mutations in the order albina (43.5%), chlorina (27.3%) and xantha (24.2%) were recorded. Chemical mutagens were found to be efficient in inducing chlorophyll mutations in chickpea. Highest frequency of mutations was observed in green seeded var. L 345 (83% of M_1 families and 19.9/1000 M_2 plants). *Kabuli* var. C 104 was least responsive for chlorophyll mutations.

Key words: Chickpea, *Cicer arietinum*, chlorophyll mutations, frequency & spectrum, mutagens, chlorina, albina, xantha

Chickpea is most important pulse crop of the Indian subcontinent accounting for more than 50% in area and production of all the pulse crops. In order to induce variability and utilize useful mutations for efficient plant breeding, the systematic and comparative study of induced chlorophyll mutation frequency in M_2 generation is the most dependable index for evaluating the effectiveness of mutagenic treatments. Although studies on induced mutations have been undertaken in the past in some legumes [1-14], limited reports are available on crops such as chickpea [1-4]. No systematic and comparative study of frequency and spectrum of chlorophyll mutations induced by a wide array of treatments of physical and chemical mutagens on distinctly diverse genotypes of chickpea is availabe in literature.

The present investigation was undertaken to understand the response of *desi* and *kabuli* chickpea genotypes to more than one type of mutagenic treatment with a view to identify the mutagen and treatment causing maximum chlorophyll mutations in M_2 generation.

MATERIALS AND METHODS

The material for this study comprised of two *desi* (G 130 & H 214), one *kabuli* (C-104) and one green seeded (L 345) chickpea genotype. Five hundred dry seeds with a moisture content of 10- 12% approx. were used for each treatment. Three doses each of two physical mutagens, gamma rays (400, 500 and 600 Gy) and fast neutron (5, 10 and 15 Gy) were given. Two concentrations and two durations of the radiomimetic monofunctional alkylating agents viz., N-nitroso-N-methyle urea (NMU) 0.01%(20h) & 0.02%(8h) and ethylmethane sulphonate (EMS) 0.1%(20h) & 0.2%(8h) were used. Gamma rays were secured from Gamma Cell-200 having a 2000 Curie ^{60}Co source available at Genetics Division, I.A.R.I., New Delhi. Fast neutron treatments were given at BARC, Trombay, Mumbai. NMU and EMS of Pfaltz and Bauer Inc. USA were used for preparing aqueous solutions of chemical mutagens at 5.2 pH. Treatments with chemical mutagens were given with intermittant shaking at 20 ± 2°C. Dry seeds were used as controls. The seeds treated with chemical mutagens were thoroughly washed in running water for 30 minutes to leach out the residual chemicals and then dried on blotting paper. Treated and control seeds were sown at a spacing of 15 cm in rows of 5 m long and 0.45 m apart on the same day in well prepared seed beds in the field. Each M_1 plant was harvested individually and M_2 progeny raised in separate row. The treated as well as control populations were carefully screened for lethal chlorophyll mutations from emergence till the age of four weeks after germination, whereas viable chlorophyll mutations were scored throughout the life period of the plants in the seed beds in the field. The identification and classification procedure of Gustaffson [15] was followed.

RESULTS AND DISCUSSION

The frequencies of chlorophyll mutations were recorded using the following three methods viz.,

a) mutations per cent M_1 plants
b) mutations per 100 M_1 families
c) mutations per 1000 M_2 plants

In the first two methods, mutations were recorded on the basis of segregations observed in particular progeny, while in the third method, mutants were scored from the total M_2 population. A detaliled analysis of the frequency of chlorophyll mutations variety wise, treatment-wise and mutagenic group-wise in different treatment is given in Table 1-3 and Figs. 1-2. The spectrum of chlorophyll mutations was determined by calculating the relative proportion of the diferent types of mutations (albina, chlorina and xantha) on the basis of total number of chlorophyll mutations scored and were identified in accordnace with the modified classification of Blixt [7] as albina, chlorina and xantha.

Highest frequency of chlorophyll mutations between the *desi* varieties was observed in var. H 214 and the lowest in var. G 130 (Table 1). Among the mutagens, both the chemical mutagens gave higher mutation rate than physical ones. Between the physical mutagens, gamma rays gave the highest frequency of chlorophyll mutants. Chemical mutagens behaved differently in both the *desi* varieties. No uniform trend of dose dependency could be observed in *desi* varieties.

In general, all the four mutagens induced high frequency of chlorophyll mutations whereas the untreated (control) population did not have any chlorophyll mutations. A very high overall frequency of chlorophyll mutations (1.6%) showing as many as 56.3% of M_1 families and 15.8 mutant per 1000 M_2 plants were recorded (Table 2 & Fig. 1). The recovery of such a high rate of chlorophyll mutation can be attributed to the method of treatment and efficient scoring and handling of the mutagenised population. Use of different durations and concentrations seems to have enhanced the efficacy to a great extent. Secondly, strict adherance to treatment conditions and sowing a large number of seeds to raise plant progeny in M_2 and critical screening might have resulted in recovery of higher rate of mutation frequency.

The pooled data presented (Table 3, Fig. 1 & 2) indicate clearly that the response of the two *desi* cultivars for chlorophyll mutations was similar except for some minor differences. All the treatments (exept neutrons) in *desi* var. G 130 produced all the three types of chlorophyll mutations. The frequency of albina was highest [41%] followed by chlorina [32%] and xantha [27%].

The two culinary varieties differed very much from each other. In general, the rate of mutation was much higher in green seeded var. L 345 than in *kabuli* Var. C 104. The highest frequency of mutations was also observed in EMS (0.2% 8h) in green seeded var. L 345.

Among the mutagens, NMU was the most effective mutagen, inducing chlorophyll mutations in 95.6 mutant per M_1 family and 25.67 mutant per 1000 M_2 plants and followed by gamma rays (68.0 mutant per M_1 family and 18.75 per 1000 M_2 plants. (Fig. 1b & 2b)

Table 1. Spectrum and frequency of induced chlorophyll mutations in the M_2 generation in chickpea

Mutagen	Treatment Dose/ duration	Chlorophyll mutation (%)				Population size		Frequency of		
		Chlorina	Albina	Xantha	Total	M_1 family	M_2 plants	M_1 Fam. segg.	Mut.per 100 M_1 family	Mut.per 1000 M_2 plants
				desi var. G 130						
Gamma rays	400 Gy	0.79	0.69	0.64	2.1	50	2020	32	86	21.3
	500 Gy	0.65	1.04	0.69	2.4	50	2014	42	96	23.8
	600 Gy	0.26	0.51	0.05	0.8	50	1948	18	32	8.2
Neutrons	5 Gy	0.04	-	0.08	0.1	50	2466	6	6	1.2
	10 Gy	-	0.81	0.48	1.3	50	2090	20	54	12.9
	15 Gy	0.12	0.36	0.30	0.8	50	1654	14	26	7.9
NMU	0.01% 20 (h)	0.79	0.65	0.84	2.3	50	2150	50	98	22.8
	0.02% 8 (h)	1.34	1.11	0.50	3.0	50	2604	42	154	29.6
EMS	0.1% 20 (h)	0.35	0.35	0.05	0.7	50	1990	16	30	7.5
	0.2% 8 (h)	0.05	0.36	0.26	0.7	50	1940	12	26	6.7
	Overall	**0.46**	**0.60**	**0.39**	**1.5**	**500**	**20876**	**272**	**60.8**	**14.6**
				desi var. H 214						
Gamma rays	400 Gy	0.11	1.09	0.65	1.8	50	1834	32	68	18.5
	500 Gy	0.30	0.35	-	1.3	50	1019	18	26	12.8
	600 Gy	0.35	0.35	0.59	1.3	50	850	16	22	12.9
Neutrons	5 Gy	0.46	0.08	0.15	0.7	50	2596	18	36	6.9
	10 Gy	-	0.34	0.34	0.7	50	2082	16	28	6.7
	15 Gy	0.36	0.48	-	0.8	50	2520	22	22	8.3
NMU	0.01% 20 (h)	0.58	1.58	0.84	3.0	50	1900	54	114	30.0
	0.02% 8 (h)	0.54	0.71	1.03	2.3	50	1836	38	84	22.9
EMS	0.1% 20 (h)	-	1.18	0.59	1.8	25	338	20	24	17.7
	0.2% 8 (h)	2.03	1.35	0.34	3.6	25	306	36	44	35.9
	Overall	**0.39**	**0.67**	**0.43**	**1.5**	**450**	**15281**	**242**	**50.4**	**14.9**
				kabuli var. C 104						
Neutrons	5 Gy	0.15	0.15	-	0.3	45	652	4.4	4.4	3.1
	10 Gy	0.26	-	-	0.2	45	780	4.4	4.4	2.6
	15 Gy	0.65	0.33	-	1.0	45	306	6.6	6.7	9.8
NMU	0.01% 20 (h)	-	0.23	0.58	0.8	45	866	13.	15.6	8.1
	0.02% 8 (h)	-	-	0.48	0.5	45	412	4.4	4.4	4.8
EMS	0.2% 8 (h)	-	0.76	-	0.7	20	132	5.0	5.0	7.6
	Overall	**0.16**	**0.16**	**0.22**	**0.5**	**225**	**3142**	**7.6**	**7.6**	**5.4**
				green seeded *var. L 345*						
Gamma rays	400 Gy	0.80	1.00	0.48	2.3	50	2490	38	114	22.9
	500 Gy	0.78	0.90	0.31	2.0	50	2218	46	88	19.8
	600 Gy	0.36	1.09	0.62	2.0	50	1926	34	80	20.8
Neutrons	5 Gy	0.53	0.16	-	0.7	50	1890	16	26	6.9
	10 Gy	0.35	0.40	-	0.7	50	2020	16	30	7.4
	15 Gy	0.23	1.23	-	1.5	50	2190	20	64	14.6
NMU	0.01% 20 (h)	1.19	0.98	0.51	2.7	50	2354	60	126	26.8
	0.02% 8 (h)	1.33	1.63	0.66	3.2	50	2406	58	152	31.8
EMS	0.2% 8 (h)	1.78	2.23	1.78	5.8	25	224	20	52	58.0
	Overall	**0.73**	**0.90**	**0.36**	**2.0**	**425**	**17718**	**308**	**83**	**19.9**

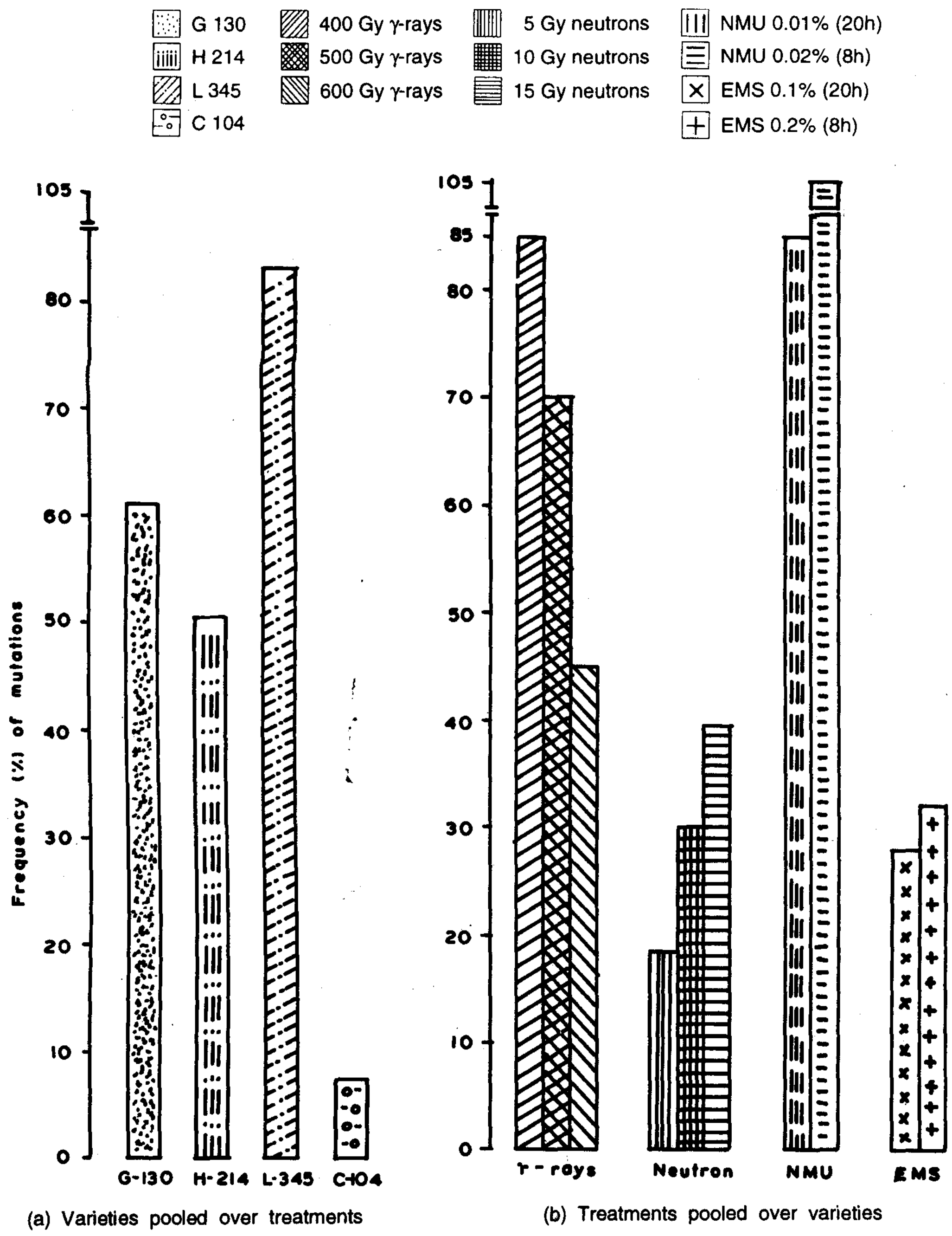


Fig. 1. Frequency of chlorophyll mutations in M_2 generation per 100 M_1 families

Based on the frequency of chlorophyll mutations induced, the order of efficiency of mutagens was:

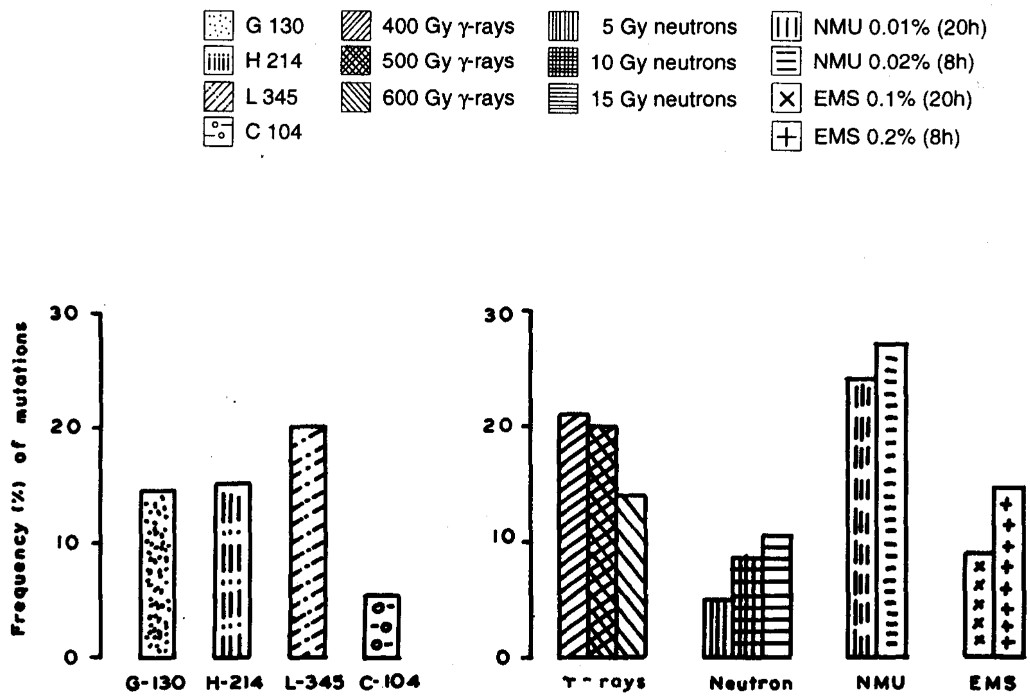
NMU (3.8%) > EMS (2.7%) > gamma rays (2.4%) > neutrons (1.2%)

The present investigation has demonstrated that the chemicals induce higher frequency of chlorophyll mutations than radiataions (gamma rays/neutrons). This is in agreement with various earlier reports in peas [5-8], lentil [9-13] and mungbean [14].

Table 2. Frequency of induced chlorophyll mutations (Total) in the M_2 generation in chickpea

Mutagen	Treatment Dose/ duration	Chlorophyll mutation (%)					Population size		Frequency	
		desi var. G130	H214	*kabuli var.* C104	Green var. L345	Total	M_1 family	M_2 plants	Mut.per 100 M_1 family	Mut.per 1000 M_2 plants
Gamma rays	400 Gy	0.68	0.54	-	0.90	2.1	150	6344	89.3	21.1
	500 Gy	0.91	0.25	-	0.84	2.0	150	5251	70.0	20.0
	600 Gy	0.34	0.23	-	0.85	1.4	150	4724	44.7	14.2
	Overall	0.66	**0.35**	-	**0.86**	**1.9**	**450**	**16319**	**68.0**	**18.7**
Neutrons	5 Gy	0.04	0.24	0.03	0.17	0.5	195	7604	18.5	4.7
	10 Gy	0.39	0.20	0.03	0.21	0.8	195	6972	29.7	8.3
	15 Gy	0.19	0.31	0.04	0.48	1.0	175	6670	39.4	10.3
	Overall	0.20	**0.25**	**0.03**	**0.28**	**0.8**	**565**	**21246**	**28.8**	**7.7**
NMU	0.01% 20 (hr)	0.67	0.78	0.10	0.86	2.4	195	7270	90.3	24.2
	0.02% 8 (hr)	1.10	0.58	0.03	1.00	2.7	195	7258	101.0	27.1
	Overall	**0.87**	**0.68**	**0.06**	**0.96**	**2.6**	**390**	**14528**	**95.6**	**25.7**
EMS	0.1% 20 (hr)	0.64	0.26	-	-	0.9	75	2328	28.0	9.0
	0.2% 8 (hr)	0.50	0.42	0.04	0.50	1.5	120	2602	31.7	14.6
	Overall	**0.57**	**0.34**	**0.02**	**0.26**	**1.2**	**195**	**4930**	**30.3**	**12.0**
	Total	**0.53**	**0.40**	**0.03**	**0.62**	**1.6**	**1600**	**57023**	**56.3**	**15.8**

Rapoport [16] for the first time referred to the nitroso compounds as "supermutagens" because of their high mutagenic effects. Superiority of NMU to induce the highest frequency of chlorophyll mutations has been demonstarted by several workers [6, 9-11]. Present observations also confirmed the supermutagenecity



(a) Varieties pooled over treatments (b) Treatments pooled over varieties

Fig. 2. Frequency of chlorophyll mutations in M_2 generation per 1000 M_2 seedlings

of NMU (about two times more mutation rate) over gamma rays, fast neutrons and EMS.

The frequency of chlorophyll mutations was the lowest with gamma rays and fast neutrons. This confirmed the results of Blixt *et. al.* [6]. Sharma & Sharma [9], and others [10, 12, 14].

In the present investigation, three types of chlorophyll mutations, albina, chlorina and xantha were isolated with all the four mutagens (Table 2 and 3). Certain chlorophyll mutations types appeared more frequently than others. The following order in their frquencies was observed:

Albina (43.5%) > chlorina (32.3%) > xantha (24.2%)

It is generally believed that ionising radiations produce a high frequency of the albina types of chlorophyll mutation and the chemical mutagens produce a high frequency of other types, such as chlorina and xantha [17]. Sharma and Sharma [9]

Table 3. Spectrum and frequencies of chlorophyll mutations induced by different kinds of mutagens

Mutagens pooled over treatments	Mutation frenquencies (%)				Population size		Frequency of		
	Chlorina	Albina	Xantha	Total	M_1 family	M_2 plants	M_1 fam. segg.	Mut.per 100 M_1 family	Mut.per 1000 M_2 plants
					***desi var.* G 130**				
Gamma rays	0.56	0.75	0.46	1.8	150	5982	30	71	17.8
Neutrons	0.05	0.39	0.29	0.7	150	6210	13	29	7.3
NMU	1.06	0.88	0.67	2.6	100	4754	46	84	26.2
EMS	0.20	0.33	0.15	0.7	100	3930	14	28	7.1
Overall	**0.44**	**0.59**	**0.39**	**1.4**	**500**	**20876**	**25**	**61**	**14.2**
					desi var. H 214				
Gamma rays	0.25	0.60	0.41	1.5	150	3703	22	39	14.7
Neutrons	0.27	0.30	0.16	0.7	150	7198	19	29	7.3
NMU	0.56	1.14	0.93	2.6	100	3736	46	66	26.4
EMS	1.01	1.26	0.46	2.7	50	644	28	34	26.8
Overall	**0.47**	**0.75**	**0.45**	**1.7**	**450**	**15281**	**27**	**47**	**17.3**
					kabuli var. C 104				
Neutrons	0.35	0.16	-	0.5	135	1738	5	5	5.1
NMU	-	0.11	0.53	0.6	90	1278	8	10	6.5
EMS	-	0.76	-	0.7	20	132	5	5	7.6
Overall	**0.18**	**0.24**	**0.18**	**0.6**	**245**	**3148**	**6**	**7**	**6.0**
					green seeded *var. L 345*				
Gamma rays	0.65	1.00	0.47	2.1	150	6634	39	94	21.2
Neutrons	0.37	0.60	-	1.0	150	6100	17	40	9.6
NMU	1.26	1.30	0.58	2.9	100	4760	59	139	29.7
EMS	1.78	2.23	1.78	5.8	25	224	20	52	58.0
Overall	**0.82**	**1.07**	**0.48**	**2.3**	**425**	**17718**	**34**	**81**	**23.2**

and Sarkar and Sharma [11] also reported these types of chlorophyll mutations using different mutagens in lentil. Blixt *et al.* [6] and Blixt [7] indicated different agents may induce different spectra, however, the difference is quantitative rather than qualitative. In general, relative differences in mutability of genes for various chlorophyll mutations with different mutagens as well as different genotypes were clearly observed.

It seems that the strong mutagens reach their saturation point even at lower doses in the highly mutable genotypes, and further increase in dose does not add to the mutation frequency. With increase in dose beyond a point, the strong mutagens become more toxic than the higher doses of relatively weaker mutagens [8].

Major differences in the mutagenic response of the four cultivars were observed in the present study. The *desi* genotypes were more sensitive towards mutagenic treatment than *kabuli* and green seeded type. Chemical mutagens were more efficient than physical in inducing chlorophyll mutations. Among the mutagens NMU was the most potent, while in the physical, gamma rays were more effective. Albina type were most frequent and xantha least frequent. Chlorina were also quite common. Among the varieties green seeded var. L 345 was most responsive for mutations and *kabuli* var. C 104 was least suitable. Out of four mutagens, NMU was the most effective and efficient in inducing a high frequency and wide spectrum of chlorophyll mutations in the M_2 followed by fast neutrons, while gamma rays showed lowest frequence and spectrum. This confirmed the results of Blixt *et al.* [6], Fillippetti *et al.* [8], Sharma and Sharma [9], and Sarkar and Sharma [11].

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