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Influence of gamma radiation and EMS on chlorophyll and macro-mutation in mungbean (*Vigna radiata*)

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ABSTRACT

Present study was carried during rainy (*kharif)* season of 2021 (M_1 generation), summer 2022 (M_2 generation) and rainy (*kharif)* seasons of 2022 (M_3 generation) at College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan for creating genetic variability in mungbean [*Vigna radiata* (L.) R. Wilczek]. The experiment was conducted with 26 treatments and 2 popular archaic varieties of mungbean (Ganga-1 and GM-4), solitary application of gamma radiation (200, 400 and 600 Gy), and ethyl methane sulphonate (EMS) (0.1 and 0.2%) as well as their combinations. A wide spectrum of chlorophyll mutants (*Chlorina, Xantha, Albina, Viridis* and complex type) and morphological mutants (plant type, flower colour, pod type, leaf variation and maturity period) were identified at various crop growth stages. All mutagen treatments including solitary application of gamma radiation and EMS as well as their combinations were found effective for creating genetic variability. The lower concentration of EMS (0.1%) was found to be relatively more effective as compared to 0.2%. Similarly, the lower dose of gamma radiation (200 Gy) was also more effective than 400 and 600 Gy. Among all the treatment combinations, combined application of 200 Gy + 0.1% EMS was found most effective in generating chlorophyll and morphological mutants. Among two varieties of mungbean, Ganga-1 was found to be more sensitive to mutagens as compared to GM-4. Numerous mutants identified in the mutagenized population of both varieties exhibited stability and superiority in terms of agro-morphological traits over Ganga-1 and GM-4. A mutant (S-92 of GM-4) identified during M_1 in mutagen treatment 0.1% EMS was very deep lobbed which altered the plant morphology and appearance. This novel unique mutant identified in M_1 generation maintained its stability in M_2 and M_3 generations also. The leaf colour of this mutant was darker than the original leaf colour of GM-4 and found relatively more tolerant to soil moisture-deficit stress.

Keywords: Chlorophyll, EMS, Gamma radiation, Mungbean, Mutation breeding, Morphological mutants

Mungbean [*Vigna radiata* (L.) R. Wilczek] stands out as a crucial short-duration pulse crop (Kumar *et al.* 2021). Within the human diet, it serves as an outstanding source of high-quality food protein and is extensively embraced by health-conscious individuals globally (Auti 2012). Originating in India and central Asia (Dewanjee and Sarkar 2018), it holds the position of the third most significant pulse crop in India, following chickpea and pigeonpea (Singh *et al.* 2015). Cultivation of mungbean has been practised in India since 2200 BC (Das *et al.* 2021). Due to its self-pollinated nature, this crop exhibits limited genetic variability (Kumar 2014). Belonging to the family Fabaceae and subfamily Papilionaceae, it possesses a chromosome number of $2n = 2x = 22$, with a genome size of 579 Mb (Kang *et al.* 2014). The restricted genetic variability in key traits acts as a constraint to its improvement (Grover 2011). The mutation breeding approach stands out as a comparatively swift method in crop improvement programmes (Kumar *et al*. 2022). In India, mutation breeding has yielded substantial dividends, contributing to the understanding of various mutagenesis processes relevant to crop improvement and resulting in over 345 improved mutant varieties across 57 crop species (Kumar *et al.* 2021). Induced mutations have demonstrated the ability to enhance yield and other quantitative traits in plants (Sarkar and Kundagrami 2018). A variety of physical and chemical mutagens have been employed to induce beneficial mutants in numerous crops (Khattak *et al.* 2008). Physical mutagens, in particular, have been utilized in developing over 70% of mutant crop varieties (IAEA 2021). Recognizing the significance of mutation breeding, especially in self-pollinated crops, an investigation was conducted using both physical and chemical mutagens to generate genetic variability for agro-economic traits in two popular ancient varieties of mungbean.

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MATERIALS AND METHODS

A Field experiment was conducted during rainy (*kharif*) season of 2021 (M_1 generation), summer 2022

(M_2 generation) and rainy (*kharif*) seasons of 2022 (M_3 generation) at College of Agriculture, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan. Two mungbean varieties namely Ganga-1 and GM-4 were exposed to varying doses of gamma radiation (200, 400 and 600 Gy); and ethyl methane sulphonate (EMS) (0.1 and 0.2%), and their combinations. Healthy dry seeds of both varieties were irradiated with gamma radiation at Bhabha Atomic Research Centre, Trombay (Mumbai) and for chemical mutagen treatment, firstly seeds were uniformly soaked in distilled water for 4 h at room temperature and after that treated with freshly prepared 0.1% and 0.2% ethyl methane sulphonate (EMS) in phosphate buffer solution for 6 h at room temperature. A total of 26 treatment combinations (13 treatments of each variety) along with control (seeds soaked in distilled water for 4 h) and absolute control (normal dry seeds) were sown in the M_1 generation using randomized block design (RBD) during *kharif* 2021. Standard package and practices were followed for crop cultivation. The experiment was critically observed at various crop growth stages to identify variants in all plots of mutagenized population. Chlorophyll and macro-mutations identified at various crop growth stages were tagged and harvested separately at maturity. The individual plant progeny of selected plants was grown in paired rows maintaining plant geometry of 30 cm × 15 cm during summer 2022 and *kharif* 2022 using augmented design.

The spectrum of chlorophyll mutants was studied in both M_2 and M_3 generations and classified as *Chlorina, Xantha, Albina, Viridis* and complex types according to Gandhi *et al*. (2014). The mutagenic frequency, effectiveness and efficiency of chlorophyll and morphological mutations were calculated based on the formula suggested by Konzak *et al*. (1965).

RESULTS AND DISCUSSION

Frequency of chlorophyll and morphological mutants in M_2 and M_3 generations: Frequency of chlorophyll mutants in M_2 generation was observed 0.522 and 0.479% for mungbean varieties Ganga-1 and GM-4, respectively (Fig. 1). Various types of chlorophyll mutants, such as *Chlorina, Xantha, Albina, Viridis* and complex types, were documented under different treatment combinations of gamma radiation and EMS. The frequency of chlorophyll mutants exhibited an upward trend with the escalating doses of both physical and chemical mutagens, ranging from 0.144% (0.1% EMS) to 0.981% (600 Gy + 0.2% EMS) in variety Ganga-1 and 0.139% (0.1% EMS) to 0.955% (600 Gy + 0.2% EMS) in GM-4. The overall frequency of chlorophyll mutants was relatively higher in variety Ganga-1 (0.522%) compared to GM-4 (0.479%). The frequency of chlorophyll mutants in the M_3 generation was consistent at 0.229% for both varieties. Chlorophyll mutation frequencies ranged from 0.306% (GM-4) with a mutagen treatment of 0.1% EMS to 0.985% (GM-4) with a mutagen treatment of 600 Gy + 0.2% EMS. Only three types of chlorophyll mutants, namely *Chlorina, Xantha*, and complex types, were recorded with low frequency in the M_3 generation. The frequency of chlorophyll mutants progressively increased with the rise in mutagen dose. No chlorophyll mutant was detected in the mutagen treatments of 400 Gy, 600 Gy, 0.1% EMS, 0.2% EMS, 200 Gy + 0.1% EMS, and 200 Gy + 0.2% EMS doses in variety Ganga-1; and 200 Gy, 600 Gy, 0.1 EMS, 400 Gy + 0.1% EMS, 400 Gy + 0.2% EMS, and 600 Gy + 0.1% EMS in GM-4 (Table 1 and 2). Similarly, Gandhi *et al.* (2014), reported analogous increases in chlorophyll mutation frequency with the augmentation of mutagen doses.

The mutagenic frequency for morphological mutations increased with the increase in mutagen dose and it was found relatively higher for combined application of gamma radiation and EMS as compared to their solitary doses (Fig. 1 and 2). The overall frequency of morphological mutants in the M_2 generation was nearly equivalent for both Ganga-1 and GM-4. However, under various treatment combinations, this frequency ranged from 2.17% (0.1% EMS) to 8.346% (600 Gy + 0.2% EMS) in variety Ganga-1 and from 1.985% (200 Gy) to 8.439% (600 Gy + 0.2% EMS) in GM-4. The frequency of morphological mutants in both mungbean varieties increased with the combined application of higher doses of gamma radiation and EMS. In the M_3 generation, the frequency of morphological mutants was consistent at 1.820% for both varieties.

At various mutagenesis doses, the frequency of morphological mutants varied highly. The chlorophyll mutation frequencies varied from 0.689% (Ganga-1) to 5.911% (GM-4) in 200 Gy + 0.1% EMS and 600 Gy + 0.2% EMS. These results are in agreement with the earlier findings of Auti (2012), Vairam *et al.* (2017) and Sarkar and Kundagrami (2018).

A mutant (Selection-92 of GM-4) identified during M_1 in mutagen treatment 0.1% EMS was very deep lobbed, which altered the plant morphology and appearance. This mutant identified in M_1 generation maintained its stability in M_2 and M_3 generations also. The leaf colour of this mutant

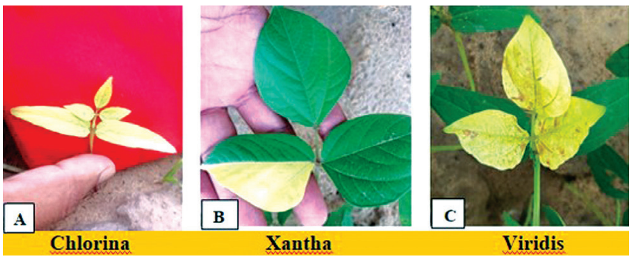

Fig. 1 Chlorophyll mutants.
A, Chlorina; B, Xantha; C: Viridis.

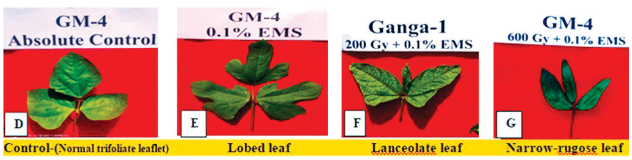

Fig. 2 Leafy mutants.
D, Control; E, Lobed leaf; F, Lanceolate leaf; G, Narrow-rugose leaf.

Table 1 Frequency of chlorophyll and morphological mutants in M_2 generation for Ganga-1 and GM-4 cv. of mungbean

Mutagens	Dose	Variety	Total number of M_2 plants	Chlorophyll mutant		Morphological mutants								
				Total	M_2 population basis mutation frequency (%)	Plant height mutants (Tall, Dwarf and Exterem dwarf)	Growth habit mutants (Spreading type, Bushy type, Ereact type, Prostrate type and Indeterminate)	Leaf mutants (Bifoliate, Tetra foliate, Penta foliate, Narrow leaflet, Round-cuneat leaflet, Lanceolate leaflet, Wrinkled leaflet, Dark green leaflet and Yellow green leaflet)	Maturity type mutants (Early mature and Late mature)	Flower type mutants (White and Dark yellow)	Pod type mutants (Long pod, Shourt pod, lobbed pod, Brown and Highly pubsecnse pod)	Pleiotropic changes (Extreme dwarf and sterile and dwaf and late flowering)	Total	M_2 population basis mutation frequency (%)
Gamma radiation	200 Gy	Ganga-1	684	2	0.29	1	3	4	1	1	7	2	19	2.777
		GM-4	705	1	0.14	1	3	3	1	1	4	1	14	1.985
	400 Gy	Ganga-1	671	3	0.45	3	4	9	1	1	3	1	22	3.278
		GM-4	691	3	0.43	1	3	9	1	0	5	2	21	3.039
	600 Gy	Ganga-1	653	5	0.77	3	5	16	1	2	9	4	40	6.125
		GM-4	663	4	0.60	7	8	18	4	1	12	2	52	7.843
EMS	0.1% EMS	Ganga-1	691	1	0.14	2	1	4	3	0	3	2	15	2.17
		GM-4	715	1	0.14	1	3	6	2	0	6	4	22	3.076
	0.2% EMS	Ganga-1	687	2	0.29	0	2	6	0	2	6	0	16	2.328
		GM-4	708	2	0.28	0	2	4	3	0	3	3	15	2.118
Combination (Gamma radiation and EMS)	200 Gy + 0.1% EMS	Ganga-1	681	3	0.44	0	1	9	4	0	4	3	21	3.083
		GM-4	701	2	0.29	1	4	6	1	0	5	1	18	2.567
	200 Gy + 0.2% EMS	Ganga-1	678	3	0.44	2	2	5	3	0	5	5	22	3.244
		GM-4	695	3	0.43	1	3	5	1	2	4	1	17	2.446
	400 Gy + 0.1% EMS	Ganga-1	654	4	0.61	1	1	11	1	1	4	3	22	3.363
		GM-4	684	4	0.58	2	2	7	4	1	4	1	21	3.07
	400 Gy + 0.2% EMS	Ganga-1	643	4	0.62	3	2	6	4	0	7	2	24	3.73
		GM-4	676	5	0.74	2	5	13	1	0	7	1	29	4.289
	600 Gy + 0.1% EMS	Ganga-1	620	5	0.81	4	5	15	0	3	10	5	42	6.774
		GM-4	644	5	0.78	4	5	16	3	0	9	4	41	6.366
	600 Gy + 0.2% EMS	Ganga-1	611	6	0.98	4	7	19	2	4	11	4	51	8.346
		GM-4	628	6	0.96	5	8	19	3	2	11	5	53	8.439
Total		Ganga-1	7273	38	0.52	23	33	104	20	14	69	31	294	4.042
		GM-4	7510	36	0.48	25	46	106	24	7	70	25	303	4.034

Gy, Gray; EMS, Ethyl Methane Sulphonate.

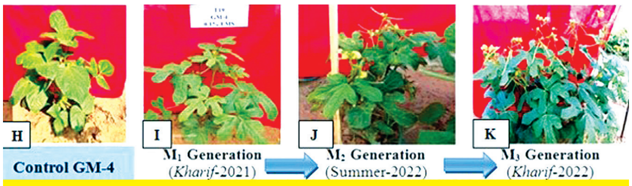

Fig. 3 A noval deep lobed leaf mutant (Selection-92). H, Control; I, Deep lobed leaf mutant in M_1; J, Deep lobed leaf mutant in M_2; K, Deep lobed leaf mutant in M_3.

was darker than the original leaf colour of GM-4 and was found relatively more moisture stress tolerant (Fig. 3).

Mutagen effectiveness and efficiency for chlorophyll and morphological mutants in M_2 generation: The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also on its efficiency. In both varieties Ganga-1 and GM-4, the effectiveness and efficiency of mutagens reduced gradually as the dose of mutagen increased for solitary mutagens (gamma rays and EMS) and as well as in combinations (gamma rays + EMS). The average effectiveness and efficiency of mutagens was found to be higher in variety Ganga-1 as compared to variety GM-4 (Table 3). These results are in agreement with the findings of Dhasarathan *et al.* (2017), Rani (2020), Das and Baisakh (2020) and Das *et al.* (2021) in mungbean.

Table 2 Frequency of chlorophyll and morphological mutants in M_3 generation for Ganga-1 and GM-4

Mutagens	Dose	Variety	Number of M_3 palnts	Total chlorophyll mutants (Chlorina, Xantha, Albina, Viridis and complex types)	M_2 progeny/ M_3 population basis mutation frequency (%)	Morphological mutants (Tall, Dwarf, Spreading type, Bushy type, Ereact type, Prostrate type, Indeterminate, Deep Lobed leaflet, dark green and yellow-green leaflet, early and late mature, white flower, brown pod colour, Highly pubsecnse pod)	Morphological mutants (Pleiotropic changes i.e. Dark green and Deep Lobed leaflet, Higly pubescence pods and Intermediate pod position)	Total	M_3 population basis mutation frequency (%)
Gamma radiation	200 Gy	Ganga-1	306	1	0.326	3	0	3	0.980
		GM-4	328	0	0.000	5	0	5	1.524
	400 Gy	Ganga-1	278	0	0.000	4	0	4	1.430
		GM-4	293	1	0.341	3	0	3	1.020
	600 Gy	Ganga-1	244	1	0.409	7	0	7	2.868
		GM-4	261	0	0.000	7	0	7	2.681
EMS	0.1% EMS	Ganga-1	318	0	0.000	3	0	3	0.940
		GM-4	334	0	0.000	9	2	11	3.293
	0.2% EMS	Ganga-1	311	0	0.000	3	0	3	0.964
		GM-4	326	1	0.306	3	0	3	0.920
Combination (Gamma radiation and EMS)	200 Gy + 0.1% EMS	Ganga-1	290	0	0.000	2	0	2	0.689
		GM-4	302	1	0.326	3	0	3	0.993
	200 Gy + 0.2% EMS	Ganga-1	285	0	0.000	3	0	3	1.052
		GM-4	280	1	0.357	4	0	4	1.428
	400 Gy + 0.1% EMS	Ganga-1	256	1	0.390	3	0	3	1.171
		GM-4	274	0	0.000	3	0	3	1.094
	400 Gy + 0.2% EMS	Ganga-1	266	1	0.375	3	0	3	1.127
		GM-4	270	0	0.000	4	0	4	1.481
	600 Gy + 0.1% EMS	Ganga-1	231	2	0.865	7	0	7	3.030
		GM-4	224	0	0.000	9	0	9	4.017
	600 Gy + 0.2% EMS	Ganga-1	217	2	0.921	9	0	9	4.147
		GM-4	203	2	0.985	12	0	12	5.911
Total			6097	14	0.229	109	2	111	1.820

Gy, Gray; EMS, Ethyl Methane Sulphonate.

Table 3 Mutagenic effectiveness and efficiency based on the chlorophyll and morphological mutants in M_2 generation for Ganga-1 AND GM-4 cv. of mungbean

Dose	Lethality (%)		Mutation frequency				Effectiveness MF/kR* (or) C × T				Efficiency MF/L			
			Chlorophyll		Morphological		Chlorophyll		Morphological		Chlorophyll		Morphological	
	Ganga-1	GM-4	Ganga-1	GM-4	Ganga-1	GM-4	Ganga-1	GM-4	Ganga-1	GM-4	Ganga-1	GM-4	Ganga-1	GM-4
200 Gy	21.67	15.33	0.29	0.14	2.78	1.99	0.01	0.01	0.14	0.10	0.01	0.01	0.13	0.13
400 Gy	48.33	38.33	0.45	0.43	3.28	3.04	0.01	0.01	0.08	0.08	0.01	0.01	0.07	0.08
600 Gy	54.67	48.67	0.77	0.60	6.13	7.84	0.01	0.01	0.10	0.13	0.01	0.01	0.11	0.16
0.1% EMS	6.67	13.33	0.14	0.14	2.17	3.08	0.23	0.23	3.62	5.13	0.02	0.01	0.33	0.23
0.2% EMS	18.33	16.33	0.29	0.28	2.33	2.12	0.24	0.23	1.94	1.77	0.02	0.02	0.13	0.13
200 Gy + 0.1% EMS	24.67	18.67	0.44	0.29	3.08	2.57	0.04	0.02	0.26	0.21	0.02	0.02	0.13	0.14
200 Gy + 0.2% EMS	30.67	28.00	0.44	0.43	3.24	2.45	0.02	0.02	0.14	0.10	0.01	0.02	0.11	0.09
400 Gy + 0.1% EMS	49.67	39.33	0.61	0.58	3.36	3.07	0.03	0.02	0.14	0.13	0.01	0.02	0.07	0.08
400 Gy + 0.2% EMS	54.67	48.00	0.62	0.74	3.73	4.29	0.01	0.02	0.08	0.09	0.01	0.02	0.07	0.09
600 Gy + 0.1% EMS	57.67	54.33	0.81	0.78	6.77	6.37	0.02	0.02	0.19	0.18	0.01	0.01	0.12	0.12
600 Gy + 0.2% EMS	62.00	58.33	0.98	0.96	8.35	8.44	0.01	0.01	0.12	0.12	0.02	0.02	0.14	0.15
Average	39.00	34.42	0.53	0.49	4.11	4.11	0.06	0.06	0.62	0.73	0.02	0.01	0.13	0.13

MF, Mutation frequency; 10 Gy, 1KR; C, Concentration; T, Time; and L, Lethality.

The value of mutagenic effectiveness and efficiency for morphological mutants in both the varieties of mungbean were found to decrease with the gradual increase in dose (except for higher doses i.e. 600 Gy + 0.2% EMS). In Ganga-1 and GM-4, respectively, the effectiveness was greater at the mutagenic dosages of 0.1% EMS, 3.616% and 5.133%. The effectiveness and efficiency of mutagens were found relatively higher in Ganga-1 at lower doses of mutagens, however looking at the dose of gamma radiation and EMS; the effectiveness and efficiency was higher in variety GM-4 as compared to Ganga-1. These results are in agreement with the findings of Dhasarathan *et al.* (2018), Rani (2020), Das and Baisakh (2021), and Das *et al*. (2021) in mungbean.

It is concluded that solitary application of gamma radiation and EMS as well as their combinations are effective mutagen for creating genetic variability in mungbean. The lower dose of EMS (0.1%) was found to be relatively more effective as compared to 0.2%. Similarly, the lower dose of gamma radiation (200 Gy) was also more effective than 400 and 600 Gy. However, among all treatment combinations, a combined application of 200 Gy + 0.1% EMS was found most effective in generating chlorophyll and morphological mutants in mungbean. Between the two varieties of mungbean, Ganga-1 was found to be more sensitive to mutagen as compared to GM-4. Numerous mutants identified in the mutagenized population of both varieties exhibited stability and superiority in terms of agro-morphological traits over Ganga-1 and GM-4. A mutant (Selection-92 of GM-4) identified during M_1 in mutagen treatment 0.1% EMS was very deep lobbed, which absolutely altered the plant morphology and appearance. This mutant identified in M_1 generation maintained its stability in M_2 and M_3 generation also.

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