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Macro-mutations induced by EMS, gamma-rays and their combined treatments in Chickpea (*Cicer arietinum* L.)

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Abstract
Treatment with EMS (0.05%, 0.10% and 0.15%), gamma-rays (150 Gy, 200 Gy, 300 Gy) and their all possible combinations were utilised to generate genetic variability in chickpea variety HPG-17. The potential of each treatment was analysed on the basis of variability generated which included range of viable and non-viable mutants. Treatment of 0.10% EMS produced maximum viable macro-mutants. Low doses of both EMS and gamma rays individually, as well as in combination failed to produce chlorophyll mutants. Highest frequency of chlorophyll mutants was observed in treatment 300 Gy + 0.15% EMS. Wide range of mutants for plant type, leaf colour, leaf shape, seed colour, seed texture and sterility was also observed.

Keywords: Macro-mutations induced by EMS treatments in chickpea mutants for plant type

Introduction
Mutagens induce macro- and micro-mutations, varying in spectrum and frequency. Macro-mutations are major indices to evaluate the mutagen effectiveness and efficiency. Further, it also helps in analyzing the amount of genetic variability produced through the treatment. Greater the variability induced, higher are the chances of genetic improvement of the crop. Various classes of physical and chemical mutagens differ in their abilities to induce mutations. Moreover, combination of two mutagens can be used to increase the spectrum and frequency of mutations, if mutation inducing processes of these mutagens is independent from each other. Therefore, it is essential to analyze the potential of a mutagen or combination of mutagens in inducing mutations in a crop variety. Chlorophyll mutations are one of the most precise indices for evaluating mutagen potential.
Ethyl methane sulfonate (EMS) has been reported to be the most effective mutagen for mutation induction in crop plants followed by gamma rays (Kul and Bhan 1977; Sikora *et al*. 2012; Sharma *et al*. 2018) [1, 2, 3]. Being an alkylating agent, EMS causes excessive cross linking of DNA, breaks PO_4^--sugar backbone and cause depurination and transitions while, gamma rays have direct ionizing effect or indirectly through reactive oxygen species [4], hence, mutation inducing processes of these two mutagens are different from each other. In the present study, we report individual and combined effects of EMS and gamma rays on spectrum and frequency of the macro-mutations produced in the M_2 population of chickpea (*Cicer arietinum* L.) which is the third most important pulse crop of the world.

Material and method
A popular variety of chickpea, HPG-17, in Himachal Pradesh, India was treated with gamma-rays (150 Gy, 200 Gy, 300Gy) and EMS (0.05%, 0.10% and 0.15%) individually and in all possible combinations. For each treatment, 150 seeds were used. Dry seeds were gamma-irradiated in gamma chamber (^{60}Co) at Bhabha Atomic Research Centre (BARC), Mumbai. Seeds were pre-soaked in distilled water for 14 hours at room temperature followed by EMS treatment for three hours with constant shaking. Freshly prepared EMS solutions were used for the experiments. Following treatment, seeds were washed for three hours to eliminate the residues of EMS followed by immediate sowing. For combined treatment with EMS and gamma rays, seeds were first irradiated and then treated with EMS. M_1 was raised and

harvested to yield the M_2 seed. M_2 seeds were sown as plant to progeny rows with row to row distance of 30 cm and plant to plant distance of 10 cm along with control (untreated HPG-17). M_2 generation plants were screened for different morphological mutants during entire growth period.

Chlorophyll mutants

The screening for chlorophyll mutations was carried out during the first fortnight after germination. The chlorophyll mutants, lethal and viable ones (Fig 1), has been characterized as below:

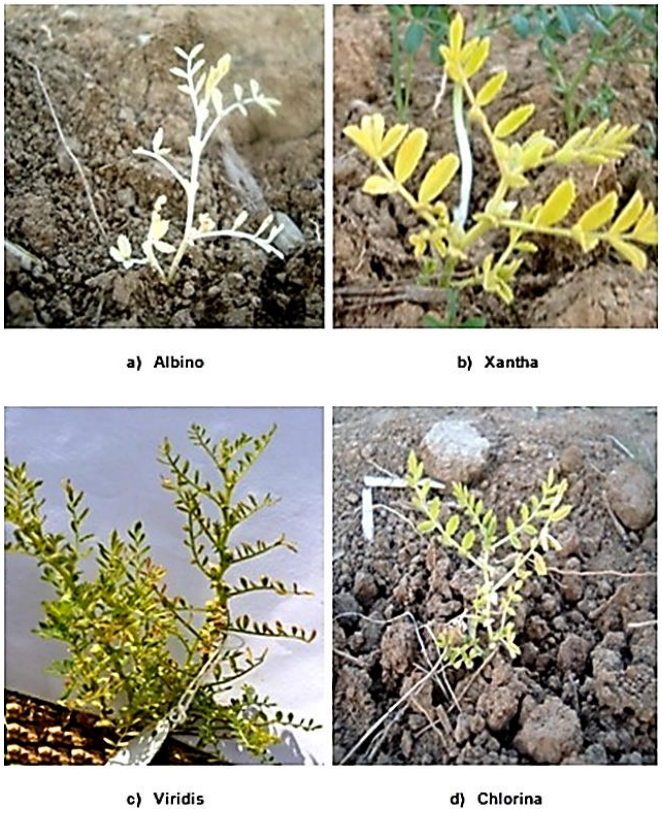

Fig 1: Types of chlorophyll mutants obtained through mutagenesis in chickpea variety HPG-17

1. Lethal mutations

a) Albino: The seedlings emerged white on germination and were dwarfer than the normal seedlings of same age. The mutants survived for about a week.

b) Xantha: The colour of young seedlings was straw yellow or pale yellow. They showed normal growth until 8-10 days. Thereafter, these started wilting and ultimately died by two week.

2. Viable mutations

a) Viridis: The mutants were light greenish in colour and remained so throughout the life cycle.

b) Chlorina: The seedlings were light yellow to yellow in colour. Some of the mutants were lethal or semi-lethal, few plants or plant parts acquired greenish tinge, later fading in colour. The plants were semi dwarf to normal in height.

Mutation frequency in M_2 generation was calculated by the formula given below:

$$\text{Mutation frequency on } M_2 \text{ plant basis } (M_f) = \frac{\text{Number of mutant plants}}{\text{Total number of } M_2 \text{ plants}} \times 100$$

Result and discussion

All types of chlorophyll mutations were scored in the M_2 generation during the seedling stage. Highest frequency (0.089%) of chlorophyll mutants was observed in treatment 300 Gy + 0.15% EMS (Table 1). Low doses of both EMS (0.05%) and gamma rays (150 Gy) individually as well as in combination (150 Gy + 0.05% EMS) did not produce chlorophyll mutants. Amongst the four types of chlorophyll mutants, chlorina had the maximum number, while viridis had the least.

Chlorophyll deficient seedlings are considered useful for measuring the spectrum of chlorophyll mutations (Karunakaran and Kiss 1971) [5]. Chlorophyll mutants in the M_2 generation of the mutagenized population are widely reported in blackgram (Rathanswamy *et al.* (1978) [6], Kharkwal (1998a) [7], Rheenen *et al.* (2003) [8], Shah *et al.* (2006) [9], Bhat *et al.* (2011) [10]. As per Wani (2009) [11], EMS was more potent in inducing chlorophyll mutations than gamma-rays and their combinations, however, no such correlation was found in the present study. Since, lower doses of EMS as well as gamma rays were used in the present study, it might be the reason for lower frequency of chlorophyll mutants in the present study or results dissimilar to Wani (2009) [11].

Table 1: Spectrum and frequency of chlorophyll mutants in M_2 generation of chickpea variety HPG-17

Treatments	Albino	Xantha	Viridis	Chlorina	Total
0.05% EMS	-	-	-	-	-
0.10% EMS	3(0.055)	-	-	-	3(0.055)
0.15% EMS	-	2(0.036)	-	-	2(0.036)
150 Gy	-	-	-	-	-
200 Gy	-	-	1(0.019)	3(0.058)	4(0.077)
300 Gy	-	1(0.003)	1(0.003)	-	2(0.006)
150 Gy + 0.05% EMS	-	-	-	-	-
150 Gy + 0.10% EMS	2(0.027)	-	-	2(0.027)	4(0.054)
150 Gy + 0.15% EMS	-	4(0.042)	-	3(0.032)	7(0.074)
200 Gy + 0.05% EMS	-	-	-	-	-
200 Gy + 0.10% EMS	-	-	-	1(0.008)	1(0.008)
200 Gy + 0.15% EMS	3(0.021)	-	-	-	3(0.021)
300 Gy + 0.05% EMS	-	2(0.031)	-	1(0.016)	3(0.047)
300 Gy + 0.10% EMS	-	-	-	-	-
300 Gy + 0.15% EMS	-	-	-	4(0.089)	4(0.089)

Note: The values outside parentheses refers to the spectrum while values in parentheses refers to the frequency.

Viable macromutations

Various frequency of viable macromutants i.e. plant type, leaf colour, narrow leaf, seed colour, seed texture and sterility were observed (Table 2). Spreading and erect mutants were observed in contrast to the semi erect nature of the HPG-17. Maximum number of the spreading mutants (15) was at 300 Gy while maximum number of the erect mutants (12) was at 150 Gy+0.015%. EMS. Short and bushy plants with distinctly different morphology as compared to the control were observed in five treatments, four of which were combination of EMS and gamma rays.

Mutants for leaf colour i.e. variants to medium green colour of the control were also observed (Table 2). Combination of 150 Gy with 0.15% EMS yielded maximum number of dark green mutants (4) while 0.10% EMS, 150 Gy+0.05% EMS and 200 Gy+0.05% and 0.10% EMS yielded light green mutants. Narrow leaved plants (leaf morphology mutants) as compared to the control were also observed in 0.10% EMS (4).

Mutants for seed coat colour with darker and lighter colours as compared to brown coloured seed coat of variety HPG-17 were also observed. Dark brown mutants for seed coat colour were observed at 0.10% EMS and 200 Gy+0.05% EMS (4 each) whereas yellow seed coat colour mutants were also observed with maximum number (6) at 0.15% EMS. Grey seed mutants (five each) were maximum at 200 Gy and 200 Gy+0.05% EMS. Not only seed coat colour, the morphology of seed was different in some mutants. Compared to smooth textured control, rough and tuberculated seed texture mutants were observed. Treatment 200 Gy gave maximum number of rough seeds (5) while 200 Gy+ 0.05% EMS gave maximum number of tuberculated seeds (5). One of the very common effects of the mutation was sterility in plants. Combination of 200 Gy with 0.10% EMS gave maximum number of sterile plants (12). No mutants for flower colour and number of flowers per node were observed in the treated population. Overall frequency of viable macro-mutants was maximum at 0.10% EMS (0.55%) followed by 0.05% EMS (0.36%) while the minimum frequency (0.052%) was at 150 Gy and 150 Gy+ 0.05% EMS. Among combinations, the maximum frequency of viable macro-mutations was observed at 300 Gy + 0.05% EMS (0.277) followed by 150 Gy + 0.15% EMS (0.263). Viable macro-mutants (distinct qualitative genetic variation) are easily identifiable and therefore can be efficiently selected for varietal development programmes or exploited through cross breeding (Mike 1988) [12]. The findings on viable macro-mutants (present study) were similar to those obtained by Rao and Jana (1976) [13], Rheenen *et al*. (1993) [14], Pathania and Sood (2006) [15], Calicius (2006) [16], Barshile (2007) [17], Bharadwaj and Sood (2008) [18] and Barshile *et al*. (2009) [19].

Table 2: Frequency of viable macro-mutants in M_2 generation in chickpea variety HPG-17

Treatment	Plant type			Leaf colour and morphology			Seed colour			Seed texture		Sterile	Total Spectrum	Total frequency
	Spreading	Erect	Short & bushy	Dark coloured	Light coloured	Narrow leaves	Dark brown	Yellow	Grey	Rough	Tuberculated			
0.05% EMS	11	3	-	-	-	-	-	2	-	-	-	2	18	0.360
0.10% EMS	13	2	-	-	1	4	4	-	3	2	-	4	33	0.550
0.15% EMS	-	-	-	-	-	-	-	6	-	-	-	-	6	0.109
150 Gy	5	-	-	-	-	-	-	1	-	-	-	-	6	0.052
200 Gy	-	-	1	-	-	-	-		5	5	2	1	14	0.269
300 Gy	15	-	-	-	-	-	-	3		-	-	-	18	0.052
150 Gy + 0.05% EMS	3	3	1	1	1	3	2	4	2	-	-	3	23	0.192
150 Gy + 0.10% EMS	3	7	-	-	-	1	-	2	2	-	-	-	15	0.200
150 Gy + 0.15% EMS	3	12	-	4	-	-	-	1	-	2	2	1	25	0.263
200 Gy + 0.05% EMS	1	1	2	2	1	-	4	3	5	4	5	3	31	0.172
200 Gy + 0.10% EMS	-	-	4	-	1	-	-	-	-	-	-	12	17	0.136
200 Gy + 0.15% EMS	10	2	-	2	-	2	1	5	-	2	1	5	30	0.207
300 Gy + 0.05% EMS	3	10	2	-	-	-	-	-	4	-	-	-	19	0.277
300 Gy + 0.10% EMS	-	4	-	-	-	2	-	-	-	-	-	-	6	0.071
300 Gy + 0.15% EMS	-	8	-	-	-	-	1	-	-	-	-	-	9	0.200
Control	Semi-erect			Medium green			Brown			Smooth				

Conclusion

In the present study EMS as well as gamma rays were utilized to generate novel mutants through their synergistic action and compared with their solo effects. EMS and gamma rays combined well to generate wide range of macro-mutants but the frequency of mutations was comparable or even lower than the individual treatments. It was further proved that EMS was better mutagen for macro-mutation generation than gamma rays or combination of EMS and gamma rays. The mutations obtained for plant type, leaf shape, seed colour and seed texture are being evaluated in subsequent generations and will be utilized in chickpea improvement.

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