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Generation of Variability in Chickpea (*Cicer arietinum* L.) through Mutagenesis

Neha Sharma, G. Katna*, Archana Joshi Saha[1] and Kamal Dev Sharma[2]

Department of Crop Improvement, CSK HPKV, Palampur-176 062, India
[1]Nuclear Agriculture and Biotechnology Division, BARC, Mumbai – 400 085, India
[2]Department of Agricultural Biotechnology, CSK HPKV, Palampur-176 062, India
**E-mail: gkatna@gmail.com*

Abstract: With the aim to create additional variability in a bold seeded *desi* chickpea variety HPG-17,its seed was treated with Ethyl Methane Sulphonate (EMS, 0.05, 0.10 and 0.15%), gamma rays (150 Gy, 200 Gy and 300 Gy) and their combination doses. The progeny plants from mutagen treated seed displayed wide range of variation in quantitative traits, however, no mutagen dose specific trend in increase or decrease of mean values of these traits was observed. All the mutagen treatments led to decrease in seed yield as well as harvest index, mutants showing early flowering, early maturity and more number of pods per plant were, however, observed in several treatments notably the 200 Gy (early flowering), 150 Gy + 0.15% EMS (early maturity) and 0.10% EMS (number of pods per plant). Maximum height and fruit bearing branches were observed at 0.05% EMS and 0.15% EMS, respectively. Only two treatments i.e. 300 Gy + 0.15% EMS and 200 Gy yielded mutants with increased 100-seed weight as compared to control. The study demonstrated that gamma rays, EMS and their combinations generated considerable variability in chickpea and that mutation breeding was an effective technique to generate additional variability in chickpea.

Keywords: EMS, Gamma rays, Variability, Induced mutations, Chickpea

Genetic variation in chickpea (*Cicer arietinum* L.) is limited primarily due to its monophyletic descendance from its wild progenitor *Cicer reticulatum*. Wild relatives of chickpea possess additional variability that can be exploited for genetic enhancement of chickpea, however, exploitation of this variability is limited by cross-incompatibility barriers and linkage drags making this pool virtually unusable for the development of new chickpea germplasm. Mutagenesis is another meanfor creation of genetic variability for exploitation in plant improvement programmes aimed at resistance to biotic and abiotic stresses or yield enhancement. Mutation breeding uses plant's own genetic resources mimicking the process of spontaneous mutations but at an enhanced rate compared to that of spontaneous mutations (10^{-5} – 10^{-8} in higher plants). Breeders exploit mutation breeding to modify well adapted plant varieties for one or two major traits that limit their productivity or quality (Kunter et al 2012). This approach has been exploited successfully to develop agronomically superior plant genotypes (Ahloowalia et al 2004, Serrat et al 2014) as well as in varieties (Anonymous 2018). The phenotypic variation following mutagenesis, particularly in self pollinated plants, is mainly due to increase in genetic components. Genetically, mutagens act either by replacing a base in the DNA or altering a base so that it specifically mispairs with another base or damages a base so that it can no longer pair with any base under normal conditions. Both physical and chemical mutagens are used to induce mutations in higher plants. Among the physical mutagens, gamma rays are preferred in higher plants due to their high penetrating capacity whereas among the chemical mutagens, ethyl methane sulphonate (EMS) is preferred as it induces high density irreversible mutations (Henikoff and Comai 2003). Use of physical and chemical mutagens together is also advocated as the combined treatments not only induce changes in DNA but may also affect fixation and recovery of potential mutants. Synergistic and antagonistic effects may, however, occur when various physical and chemical mutagens are used in combination and such affects may generate high variability than single agent. In the present study, an attempt was made to broaden the genetic base of a chickpea varietyHPG17that is cultivated widely in Himachal Pradesh, through induced mutagenesis using gamma rays, EMS and their combined treatments.

MATERIAL AND METHODS

Seeds of a well-adapted *desi* chickpea variety (HPG17) were treated with gamma-rays (150 Gy, 200 Gy, 300Gy), EMS (0.05%, 0.10% and 0.15%) and all possible combinations. For each treatment 150 seeds were taken. Gamma-irradiation was carried out in gamma chamber ^{60}Co gamma cell at Bhabha Atomic Research Centre (BARC), Mumbai. For EMS treatment, seeds were first pre-soaked in distilled water for 14 hours at room temperature and immersed in freshly prepared EMS solution followed by constant shaking for three hours. The treated seeds were washed for three hours to terminate the residual effect of the mutagenic chemical and immediate sowing was done. For combination treatments, irradiation was followed by chemical mutagen treatment. M_1 was raised and harvested in April 2014 to yield the M_2 generation. M_2 seeds were sown in October 2014 with 30 cm row to row distance and 10 cm plant to plant distance in plant to progeny rows along with control (HPG-17).

M_2 families were evaluated for various quantitative traits and observations were recorded on individual plants of M_2progeny as well as control population. The data on various traits *viz*., days to flower initiation, days to 75 per cent maturity, plant height (cm), number of primary branches, number of pods, seed yield (g), harvest index (%) and 100 seed weight (g) per plant were subjected to statistical analysis.

Relative coefficient of variation was calculated as per formula given by Montalvan and Andro 2005 which is

$$\text{Relative coefficient of variation}= \frac{CV_t}{CV_{nt}}$$

where, CV_t= Coefficient of variation of treated population, CV_{nt} = Coefficient of variation of the non treated population

Dose wise analysis based on individual plants in M_2 generation was carried out for the polygenic traits to get the mean performance of individual line as per the method of Sharma (1998).

RESULTS AND DISCUSSION

Days to flower initiation: Mutants were observed for all the traits under study. In northern region of India, medium to late flowering is considered a desirable trait as early flowers do not form pods due to frost injury. Additionally, very early flowering also compromises the vegetative growth of the plants. Considering late flowering as a desirable character, the 300 Gy + 0.05% EMS treatment was superior to others as well as control (3.60%) with 122.43 days to flower initiation (maximum mean) as compared to 118.17 days to flower initiation in control (Table 1). The mutants from treatment 200 Gy were earliest to flower with 114.25 days to flower initiation and-3.32%superiorityover control. Highest relative coefficient of variation was at 200Gy (1.69) and least at 300 Gy+0.10% EMS (0.04). Widest range in days to flowering was observed at 0.10% EMS (99-132) and least at 300 Gy+ 0.15% EMS.

Days to 75 per cent maturity: For days to 75 per cent maturity, highest mean and superiority over control (late maturity) was at 300 Gy + 0.10% EMS (185.98 and 0.13%)and minimum (early maturity) was at 150 Gy + 0.15% and 200 Gy+ 0.10% (164 and -0.01%) (Table 1).The mean value for control was 166.83 days. Maximum relative coefficient of variation was at 0.15% EMS (2.01) and minimum at 200 Gy + 0.05% EMS (0.66) while, maximum range was at 150 Gy + 0.05% EMS (165-192) and minimum at 300 Gy (188-192).

Plant height: Plants with maximum height were observed at 0.05% EMS (95.50 cm,and was superior over control) and those with minimum height and maximum inferiority over control were at 300 Gy + 0.15% EMS). The treatment 200 Gy + 0.15% EMS generated maximum variability (2.37) and plants with widest range of height (29-130 cm). The 0.15% EMS resulted in maximum number of fruit bearing branches (9.22) with maximum superiority (78.34%) over control (Table 1). .

Number of branches: The 200 Gy +0.10% EMS generated maximum relative variability in number of branches (1.45) while300 Gy + 0.10% EMS resulted in least relative variability (0.47) (Table 1). Range in number of primary branches varied from 4-15 at 0.15% EMS (maximum among all treatments).

Number of pods: The number of pods were maximum (130.30 pods plant^{-1}) at 0.10% EMS as compared to untreated control with 14.63% superiority (Table 1). In all other treatments, considerable reduction in this trait was observed.The 0.10% EMS gave widest range of mutants for number of pods (21-390) and 0.15% EMS generated maximum variability (2.33).The mutants generated with 300 Gy + 0.15% EMS had least number of pods per plant (22.81) and were inferior to control (-79.93%) with least variability (0.34) and narrow range (18-34).

Seed yield: All the treatments lead to considerable reduction in the seed yield. Minimum reduction in seed yield was at 0.10% EMS and maximum reduction at 300 Gy + 0.10% EMS as compared to control. Maximum variation was generated at 0.15% EMS (2.82) while, 0.10% EMS generated widest range of mutants for seed yield (Table 2)..

Harvest index: All the treatments lead to reduction in harvest index as compared to the control (36.00). Mutants generated with 300Gy + 0.10% EMS were most inferior (-60.86%) for harvest index. Widest range and maximum variation was observed in population generated with 200 Gy + 0.10% EMS and 200Gy + 0.15% EMS, respectively (Table 2). .

100 seed weight: The treatment 300Gy + 0.15% EMS lead to significant increase in 100 seed weight (30.01g) as compared to control (27.22 g) with 10.25% superiority (Table 2). Least superiority over control (-43.47%) was observed at 0.15% EMS with 15.36g mean 100 seed weight, however,

Table 1. Superiority over control, relative coefficient of variation (CV_t/CV_{nt}) and range for different agro morphological traits in M_2 generation of chickpea variety HPG-17

Dose	Days to flower initiation			Days to 75 per cent maturity			Plant height (cm)			Number of primary branches		
	Superiority over control	CV_t/CV_{nt}	Range (Mean)	Superiority over control	CV_t/CV_{nt}	Range (Mean)	Superiority over control	CV_t/CV_{nt}	Range (Mean)	Superiority over control	CV_t/CV_{nt}	Range (Mean)
0.05% EMS	-2.57	1.56	100-128 (115.13)	0.01	0.77	160-173 (166.73)	20.38	1.08	50-115 (95.50)	51.45	0.76	5-12 (7.83)
0.10% EMS	2.59	1.60	99-132 (121.23)	0.07	1.32	159-188 (175.91)	13.29	0.89	52-115 (89.87)	36.56	0.77	2-13 (7.06)
0.15% EMS	-0.25	0.83	111-127 (117.88)	0.05	2.01	164-185 (172.90)	12.78	0.87	73-103 (89.47)	78.34	1.33	4-15 (9.22)
150 Gy	-1.73	0.93	99-130 (116.13)	0.01	0.99	160-180 (166.00)	-9.47	0.74	53-90 (71.82)	-21.86	0.74	2-8 (4.04)
200 Gy	-3.32	1.69	90-128 (114.25)	0.00	1.84	149-178 (164.04)	-13.20	0.71	69-108 (89.80)	2.71	0.84	2-10 (5.31)
300 Gy	2.19	0.88	108-136 (120.76)	0.13	0.92	188-192 (185.72)	-3.79	1.00	34-108 (76.32)	-27.47	0.87	1-10 (3.75)
150 Gy + 0.05% EMS	-2.34	0.85	110-125 (115.40)	0.05	1.85	165-192 (173.49)	-0.63	0.90	48-98 (78.83)	-7.35	0.95	1-10 (4.79)
150 Gy + 0.10% EMS	0.14	0.93	110-129 (118.34)	0.03	1.51	160-149 (169.00)	-3.20	1.02	31-100 (76.79)	-26.89	0.87	1-7 (3.78)
150 Gy + 0.15% EMS	-1.84	0.84	110-127 (115.99)	-0.01	1.63	164-190 (164.00)	-3.59	1.00	39-99 (76.48)	-11.61	0.74	2-9 (4.57)
200 Gy + 0.05% EMS	-2.81	0.90	100-127 (114.85)	0.03	0.66	157-179 (169.63)	8.04	0.65	44-130 (85.71)	-11.80	0.52	1-10 (4.56)
200 Gy + 0.10% EMS	0.00	1.33	110-125 (116.75)	-0.01	1.91	157-188 (164.00)	-18.58	1.74	20-84 (64.59)	-39.85	1.45	1-8 (3.11)
200 Gy + 0.15% EMS	1.45	1.17	110-125 (116.46)	0.04	1.28	165-180 (171.46)	-16.69	2.37	29-130 (66.09)	-34.62	1.33	1-8 (3.38)
300 Gy + 0.05% EMS	3.60	1.51	111-133 (122.43)	0.08	1.69	166-188 (177.43)	7.44	1.30	45-106 (85.23)	-15.47	1.13	2-9 (4.37)
300 Gy + 0.10% EMS	2.71	0.04	115-129 (121.37)	0.13	1.85	180-198 (185.98)	-34.64	0.74	35-77 (51.85)	-39.65	0.47	1-5 (3.12)
300 Gy + 0.15% EMS	3.44	0.38	117-129 (122.24)	0.02	0.82	161-188 (167.35)	-38.94	1.12	30-79 (48.44)	-47.58	0.78	1-7 (2.71)
Control	0.00	1.00	115-120 (118.17)	0.00	1.00	162-166 (166.83)	0.00	1.00	74-84 (79.33)	00.00	1.00	4-6 (5.17)

EMS: Ethyl Methane Sulphonate; **Gy**: Gray; **+**: Increase in value; **-**: Decrease in value

this treatment lead to generation of maximum variability (2.62). Widest range of variation for 100 seed weight was observed at 150Gy + 0.05% EMS(19.20- 54.18g) and least (15.45- 26.76 g),at 0.15% EMS.

Wide range of variation was observed in all the quantitative traits due to mutagenesis, however, no dose specific trend in increase or decrease in mean values was observed for all the treatments. The present results are in line with those of Kozgar et al. (2011) who also reported no trend in the variation of mean of different traits with different doses of EMS. In traits such as time to flowering, early maturity, plant height and number of branches per plant; wide range of mutants with high superiority over control were obtained while, in others like number of pods per plant, seed yield and harvest index, the mutagen treatments had negative impact leading to inferiority over untreated population. Similar results were reported by Siddiqui and Singh (2010), where majority of the mutagen treatments induced negative shift in mean seed yield as compared to control. In the present study, CV of the treatments fell in both the directions of the control. Such a positive and negative shift in the CV of the treated population as compared to the control was also observed by Patil et al (2018) in cotton and Arubalachandran and Mullainathan (2009) in *Vigna mungo* L. Hepper. Results contrary to these were reported by Siddiqui and Singh (2010) in Basmati rice and Khan and Goyal (2009) in moongbean where CV increased in all the treatments vis-a-vis control. In the present study found no superiority over control for seed yield and harvest index. Begum and Dasgupta (2010)also reported EMS to be more effective in producing variation as indicated by relative coefficient of variation as compared to the gamma-rays. Wani (2011) reported intermediate doses in

Table 2. Superiority over control, relative coefficient of variation (CV_t/CV_{nt}) and range for number of pods per plant, seed yield per plant, harvest index and 100 seed weight in M_2generation of chickpea variety HPG-17

Dose	Number of pods plant^{-1}			Seed yield plant^{-1} (g)			Harvest Index			100 seed weight		
	Superiority over control	CV_t/ CV_{nt}	Range (Mean)	Superiority over control	CV_t/CV_{nt}	Range (Mean)	Superiority over control	CV_t/ CV_{nt}	Range (Mean)	Superiority over control	CV_t/ CV_{nt}	Range (Mean)
0.05% EMS	-16.69	2.28	21-316 (94.70)	-47.14	2.50	4.00-67.01 (22.69)	-36.36	1.21	20.22-44.01 (22.91)	-18.22	1.32	11.50-33.01 (22.26)
0.10% EMS	14.63	1.83	21-390 (130.30)	-17.87	2.45	3.86-94.52 (35.30)	-36.39	1.74	28.13-47.29 (22.90)	-25.06	1.57	14.80-30.22 (20.40)
0.15% EMS	-5.14	2.33	30-240 (107.83)	-44.34	2.82	4.50-61.53 (23.90)	-46.92	2.39	27.22-41.20 (19.11)	-43.57	2.62	15.45-26.76 (15.36)
150 Gy	-74.23	1.49	10-104 (29.29)	-76.11	1.65	3.67-34.88 (10.21)	-49.39	1.49	4.29-44.20 (18.22)	-14.62	0.98	18.33-36.29 (23.24)
200 Gy	-53.73	2.22	12-152 (52.59)	-39.93	0.37	3.30-50.51 (25.80)	-28.11	0.99	6.59-45.55 (25.88)	1.80	1.09	20.00-43.8 (27.71)
300 Gy	-72.44	1.49	12-102 (31.33)	-75.05	1.79	3.30-33.51 (10.67)	-54.44	1.56	2.45-45.29 (16.40)	-12.09	0.85	14.49-39.88 (23.93)
150 Gy + 0.05% EMS	-74.76	1.31	12-75 (28.69)	-85.21	2.30	2.10-21.47 (6.29)	-37.50	1.76	2.32-45.66 (22.50)	-7.75	0.77	19.20-54.18 (25.11)
150 Gy + 0.10% EMS	-54.01	1.57	12-164 (52.28)	-63.67	2.42	3.48-70.21 (15.57)	-20.64	0.84	12.69-44.11 (28.57)	-4.22	0.59	19.11-34.19 (26.07)
150 Gy + 0.15% EMS	-65.45	1.78	6-140 (39.27)	-74.35	2.45	2.18-44.14 (10.97)	-23.89	1.39	4.31-48.11 (27.40)	-5.11	0.58	18.90-34.7 (25.83)
200 Gy + 0.05% EMS	-56.22	1.21	12-194 (49.76)	-66.64	1.74	1.88-60.84 (14.29)	-50.31	1.40	1.74-46.23 (17.89)	-1.65	0.55	17.58-38.62 (26.77)
200 Gy + 0.10% EMS	-68.90	2.13	16-104 (35.35)	-83.68	2.12	3.26-19.38 (6.95)	-29.86	2.35	1.12-47.85 (25.25)	-15.69	0.97	13.07-25.11 (22.95)
200 Gy + 0.15% EMS	-79.74	2.22	10-88 (23.03)	-42.78	2.50	3.14-84.80 (24.65)	-30.22	2.05	8.2-49.01 (25.12)	-4.67	1.84	16.79-45.12 (25.95)
300 Gy + 0.05% EMS	-74.57	1.65	12-90 (28.91)	-53.71	2.27	4.23-45.59 (19.86)	-23.08	0.97	10.27-48.02 (27.69)	-2.94	0.42	12.55-36.89 (26.42)
300 Gy + 0.10% EMS	-78.01	0.74	17-66 (25.00)	-89.04	0.52	3.20-8.74 (4.64)	-60.86	0.66	6.20-36.71 (14.09)	-18.88	0.72	16.10-36.91 (22.08)
300 Gy + 0.15% EMS	-79.93	0.34	18-34 (22.81)	-62.79	2.36	3.22-42.74 (15.95)	-18.53	0.87	12.39-45.36 (29.33)	10.25	0.78	11.22-39.76 (30.01)
Control	0.00	1.00	95-132 (113.67)	0.00	1.00	37.4-49.90 (43.08)	0.00	1.00	31.10-41.36 (36.00)	0.00	1.00	24.12-29.59 (27.22)

EMS: Ethyl Methane Sulphonate; **Gy**: Gray; **+**: Increase in value; **-**: Decrease in value

combination to produce maximum variation in the mutagenized population of chickpea followed by EMS while, Sharma et al. (2018) found EMS to be more effective. These reports draw considerable support to the findings of the present investigation where EMS and combinations of EMS with gamma rays induced maximum variability in seed yield as indicated by coefficients of variation.

CONCLUSION

Wide ranges of chickpea mutants were obtained through mutagenesis. The desirable mutants i.e. with early/late flowering, early maturity, increased number of fruit bearing branches, increased number of pods, higher harvest index and higher seed yield will act as an indispensible gene pool for further crop improvement in chickpea. It can also be concluded that the gamma rays, EMS and combinations of these two mutagens generated considerable variability that can be exploited in chickpea breeding programmes.

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