Received: 05 Jul 2020 | Revised: 20 Sep 2020 | Accepted: 21 Sep 2020

Electronic Journal of Plant Breeding

Research Article

Mutagenic efficiency and effectiveness of gamma rays and EMS in groundnut (*Arachis hypogaea L.*)

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Abstract
An experiment was conducted to retrieve some useful chlorophyll mutants in two groundnut varieties of TMV (Gn)13 and TMV 7. The mutagenic efficiency and effectiveness were calculated by using the chlorophyll mutants. In M_1 generation, the seedling height was decreased with an increased rate of doses/concentration while lethality and pollen sterility were increased with an increased rate of doses/concentration in both the varieties on both the treatments. In the present study, the chlorophyll mutants were observed in M_2. The four different chlorophyll mutants observed were *albino, chlorina, xantha* and *striata* at different crop stages of crop growth. On perusal of data, it was inferred that *chlorina* occurred at large followed by *xantha* and *albino*. Mutagenic effectiveness were significantly higher in gamma rays for TMV(Gn)13 and TMV 7 (4.24 and 2.64) than the EMS which registered as 3.69 and 2.19 respectively. The mutagenic efficiency was increased for injury and decreased for lethality and pollen sterility with an increased rate of doses/ concentration.

Key words
Groundnut, Ethyl Methane Sulphonate (EMS), Gamma rays, mutagenic effectiveness and efficiency

INTRODUCTION

Groundnut *(Arachis hypogaea L.)* is an important oilseed and grain legume. Any improvement in the plant ideotype is required for building tolerance mechanism against various pest and disease. Moreover, being a self-pollinated crop, the infusion of genetic variability through routine conventional breeding method is not ideally suitable and efficient. Hence, innovative methods of breeding could sustain quicker and greater values for crop improvement activities. The mutation breeding is one of most potential breeding method to increase the genetic variability and yield potentiality of crop plant (Raina & Khan, 2020). The physical and chemical mutagens cause physical damage, gene mutations and chromosomal aberrations. The frequency of mutation induced by a unit dose of a mutagen is mutagenic effectiveness while the proportion of mutation in relation to other associated undesirable biological effects such as chromosomal aberration, lethality, injury caused by mutagen is mutagenic efficiency (Alemu & Habtamu, 2016). The present study was aimed to study the effect of gamma rays and ethyl methane sulphonate (EMS) on the frequency and spectrum of micro and macro mutants to evaluate the relative effectiveness and efficiency in various doses both mutagenic treatment.

MATERIALS AND METHODS

The seeds of two groundnut varieties *viz.,* TMV(Gn)13 and TMV 7 were obtained from Oilseed Research Station, Tindivanam, Tamil Nadu Agricultural University (TNAU). The research was conducted at Agricultural College and Research Institute, Killikulam. Well matured and filled seeds were selected in both the varieties for irradiation of gamma rays and EMS. The selected seeds were made to 10 different packets with 400 seeds per packet. Five packets of seeds were irradiated with the gamma rays

source Cobalt Co ^{60} with five different doses i.e,150Gy, 200Gy, 250Gy, 300Gy and 350Gy at Indhra Gandhi Centre for Atomic Research (IGCAR), Kalpakkam and another five packets were treated with EMS solution *i.e.,* 10mM, 20mM, 30mM, 40mM and 50mM at Molecular Biology Lab, Agricultural College and Research Institute, Killikulam. For EMS treatment, initially the selected seeds were soaked in water for 8 hours, after which the pre-soaked seeds were dried and then subjected to five different concentration of EMS solution *i.e.,* 10mM, 20mM, 30mM, 40mM and 50mM involving the both varieties for 6 hours. After treatment, the seeds were removed from solution and washed continuously for one hour in running water to remove the trace of any EMS in the seeds.

In M_1 generation, the biological damage was recorded in terms of injury and plant survival at 15 DAS under *in vitro* condition and pollen sterility at flowering stage. Pollen sterility was estimated through aceto-carmine in microscope. The stained pollen grains were fertile whereas unstained pollen grains were sterile. M_1 generated plants were harvested separately and forwarded into M_2 generations. In M_2 generation, chlorophyll mutants were observed and recorded in field. Chlorophyll mutant types were observed according to the Gustafsson (1940). Eight types of chlorophyll mutants were observed namely ***Albino*****:** A lethal mutant which is characterized by white coloured seedling with no chlorophyll or carotenoid is formed. ***Xantha*****:** A lethal mutant in which leaf colour of seedling changes from yellow to yellowish-white with carotenoids and no chlorophyll. ***Chlorina*****:** A lethal mutant with yellowish-green leaf colour. ***Alboviridis*****:** A viable mutant having green with white apex. ***Viridis*****:** A viable mutant with yellowish-green in early stages further it will change into green colour leaf. ***Xanthoviridis*****:** A viable mutant which characterized by the green with yellow apex. ***Sectorial chimaera*****:** A viable mutant with yellow colour present on the midrib of the leaf. ***Striata***: A viable mutant with stripped green colour around the leaves.

Mutagenic efficiency and effectiveness were calculated according to the Konzak (1965). Frequency of chlorophyll mutants were estimated as a percentage in M_2 generation progenies of segregated population.

For mutagenic effectiveness

$$\text{For Gamma rays} = \frac{Mp \times 100}{kR\ (or)\ Gy}$$

$$\text{For EMS} = \frac{Mp \times 100}{c \times t}$$

Where,

Mp – Chlorophyll or viable mutants on M_2 population,
c – Chemical concentration of mutagen in mM and
t – Duration of treatment with chemical mutagen
kR (or) Gy – Gamma radiation dose

For mutagenic efficiency

$$\text{Gamma rays and EMS} = \frac{Mp \times 100}{L}, \frac{Mp \times 100}{I} \text{ and } \frac{Mp \times 100}{S}$$

Where,
Mp –chlorophyll or viable mutants observed on M_2 population,
L – Lethality percentage *i.e.,* reduction of survival percentage on 15th days,
I – Injury percentage *i.e.,* height reduction of seedlings on 15thday and
S – Sterility percentage *i.e.,* reduction of seed fertility

RESULTS AND DISCUSSION

Mutation breeding is an effective tool for a breeder to generate variability in any crop population so as to enable the selection process more fruitful and efficient. For a self-pollinated crop like groundnut, mutation play an active role in enhances variability.

In the present studies, the biological damages were observed through injury (seedling height), lethality and pollen sterility in M_1 generations. The seedling height were gradually decreased with increased rate of doses (gamma rays) and concentration (EMS) in both the varieties whereas lethality and pollen sterility were increased with increased rate of doses (Gamma rays) and concentration (EMS) among both the varieties.

Chlorophyll mutants were the determination of mutagenic potential in creating genetic variability and also genetic changes in population (Channaoui *et al.*, 2019; Raina & Khan, 2020). Chlorophyll mutants were investigated both in M_1 and in M_2 generation at early seedling stage. Both the treatments exhibited similar mutagenic impact on the groundnut varieties choosen. In M_2 generation the four types of chlorophyll mutants were observed in both the varieties namely *albino*, *chlorina*, *xantha* and *striata* (**Fig. 1 and Table 1**). Mutation frequency increased with increasing rate of doses or concentration in both treatment and varieties. Gamma rays were more effective in creating chlorophyll mutants and frequency than EMS in both varieties. In TMV (Gn) 13, the maximum and minimum of chlorophyll mutation frequency was 1.02 to 3.72 and 1.09 to 2.70 in gamma rays and EMS respectively whereas in TMV 7, the chlorophyll mutation frequency was 0.87 to 3.17 and 0.57 to 2.83 in gamma rays and EMS respectively. TMV (Gn) 13 generated more chlorophyll mutants than the TMV 7. Hence, it could be adjudged the differential attitude of groundnut varieties to the treatments. The results are in proportionate with the findings of Burghate *et al.* (2013), Kavithamani *et al.* (2008) and Sonone *et al.* (2008).

Table 1. Frequency and Spectrum of chlorophyll mutants induced by gamma rays and EMS in M_2 generation

Treatment	Number of M_2 seedling	Chlorophyll mutant types				Chlorophyll mutated seedlings	Mutation frequency
		albina	chlorina	Xantha	Striata		
				TMV(Gn) 13			
Control	800	0	0	0	0	0	0
150Gy	683	1	3	1	2	7	1.02
200GY	712	1	4	3	2	10	1.40
250Gy	484	0	7	3	1	11	2.27
300Gy	490	1	6	4	2	13	2.65
350Gy	564	1	9	6	5	21	3.72
10mM	183	1	1	0	0	2	1.09
20mM	167	0	1	1	1	3	1.80
30mM	135	1	0	1	0	2	1.48
40mM	119	0	2	0	1	3	2.52
50mM	111	0	1	1	1	3	2.70
				TMV 7			
Control	800	0	0	0	0	0	0
150Gy	572	0	2	1	2	5	0.87
200GY	624	1	7	3	1	12	1.92
250Gy	528	1	6	2	2	11	2.08
300Gy	483	2	5	3	3	13	2.69
350Gy	442	1	9	3	1	14	3.17
10mM	175	1	0	0	0	1	0.57
20mM	153	0	1	1	0	2	1.31
30mM	127	1	1	0	0	2	1.57
40mM	118	**0**	1	1	1	3	2.54
50mM	106	**0**	2	1	0	3	2.83

Table 2. Mutagen induced chlorophyll mutation frequency and spectrum in TMV(Gn) 13 and TMV 7

Mutagen/ variety	Comparative frequency of chlorophyll mutation spectrum				Total frequency
	Albino	Chlorina	Xantha	Striata	
		TMV(Gn) 13			
Gamma rays	0.67	5.27	3.07	2.08	11.08
EMS	1.29	3.73	2.24	2.34	9.59
Average frequency	0.98	4.5	3.13	2.16	10.34
		TMV 7			
Gamma rays	0.99	5.68	2.33	1.74	10.74
EMS	1.36	4.18	2.44	0.85	8.83
Average frequency	1.16	4.93	2.36	1.30	9.79

The spectrum of chlorophyll mutations were in the order of Chlorina > xantha > striata > albino in both the varieties TMV(Gn) 13 and TMV 7 (**Table 2**). Among the chlorophyll mutants, chlorina was the highest chlorophyll mutants and frequency in both the varieties whereas albino recorded the least. In TMV (Gn) 13, the highest chlorophyll frequency was chlorina (5.27 and 3.73) in gamma rays and EMS respectively whereas least chlorophyll frequency was albino (0.67 and 1.29) in both treatments (Joshi *et al.*, 2020). Also, in TMV 7, among the scored chlorophyll mutants, chlorina was the highest as 5.68 and 4.18 in gamma rays and EMS respectively but albino was the least with values of 0.67 and 1.29 in gamma rays and EMS mutagen system.

Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of mutagen whereas mutagenic efficiency is the proportion of mutations in

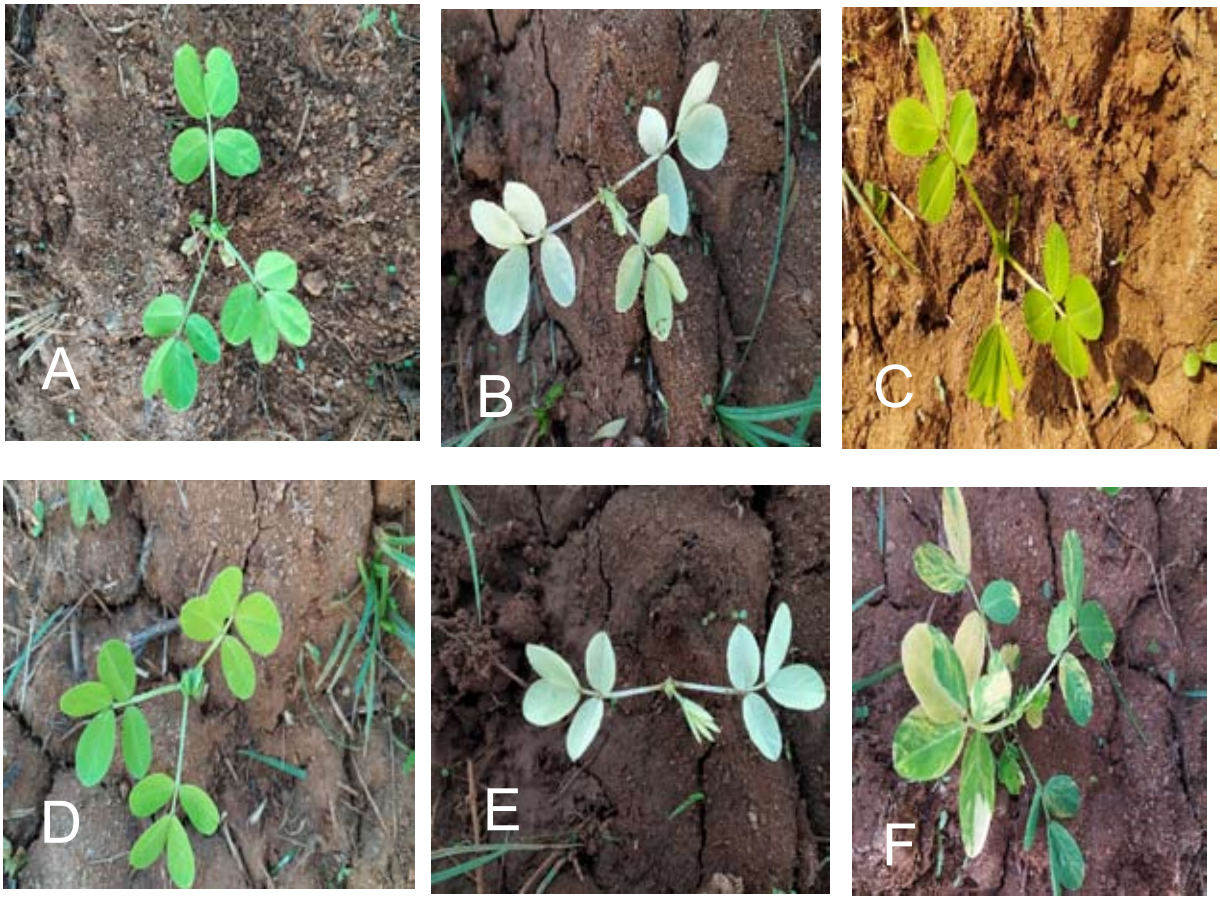

Fig. 1. Chlorophyll mutants in TMV(Gn) 13 and TMV 7,
[A: Control, B and E: Albino, C: Xantha, D: Chlorina, F: Striata]

Table 3. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation 0f TMV(Gn) 13

mutagen	Seedling height in M_1 (cm) (I)	lethality in M_1 (L)	pollen sterility in M_1 (S)	Mutation frequency (M)	Effectiveness	Efficiency		
						Injury (I)	Lethality (L)	Pollen sterility (S)
Gamma rays								
Control	29.32	0	0	0.00	0	0	0	0
150Gy	25.35	8.96	11.29	1.02	0.68	4.04	11.44	9.08
200Gy	22.83	14.16	16.92	1.40	0.70	6.15	9.92	8.30
250Gy	19.63	25.32	28.63	2.27	0.91	11.58	8.98	7.94
300Gy	16.47	33.52	37.28	2.65	0.88	16.11	7.91	7.12
350GY	13.35	51.28	52.98	3.72	1.06	27.89	7.26	7.03
Total				**11.08**	**4.24**	**65.77**	**45.51**	**39.46**
EMS								
Control	29.32	0	0	0	0	0	0	0
10mM	24.64	9.6	13.35	1.09	1.37	4.44	11.38	8.19
20mM	20.35	22.64	24.04	1.80	1.12	8.83	7.93	7.47
30mM	18.61	30.82	31.58	1.48	0.62	7.96	4.81	4.69
40mM	14.3	41.32	40.36	2.52	0.79	17.63	6.10	6.25
50mM	12.61	49.68	48.66	2.70	0.68	21.43	5.44	5.55
Total				**9.59**	**4.57**	**60.29**	**35.67**	**32.15**

relation to undesirable changes like lethality, injury and sterility (suradkar, 2019). Cell mortality due to chromosomal deformations leading aberrations and physiological disturbances can sustain reduced cell life and curtail the mutagenic effectiveness and efficiency (Amita Sharma *et al.*, 2020). The progenies of M_2 would hopefully determine the effectiveness and efficiency of mutagen.

In TMV(Gn) 13, the range of mutagenic effectiveness was from 0.68 to 1.06 in gamma rays whereas in EMS ranged from 0.68 to 1.37, the highest mutagenic effectiveness of 1.06 been at 350Gy gamma rays and 1.37 at 10mM EMS. The mutagenic effectiveness was increased with an increasing rate of doses in gamma rays whereas in EMS decreased mutagenic effectiveness with an increasing rate concentration. The range of mutagenic effectiveness was 0.58 to 0.96 in gamma rays whereas in EMS ranged from 0.66 to 0.82 in TMV 7 groundnut variety. The greater mutagenic effectiveness of 0.96 registered at 200Gy for gamma rays and 0.82 at 20mM EMS (**Table 3 and 4**). The mutagenic effectiveness were increases with increasing doses or concentration in both treatments *viz.* gamma rays and EMS. Similar reports were given by Maliata Athon Wanga *et al.* (2020), Tamilzharasi *et al.* (2019), Prasath *et al.* (2019) and Ariraman *et al.* (2015).

Table 4. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation of TMV 7

Mutagen	Seedling height in M_1 (cm)(I)	Lethality in M_1 (L)	Pollen sterility in M_1 (S)	Mutation frequency (M)	Effectiveness	Efficiency		
						Injury (I)	Lethality (L)	Pollen sterility (S)
Gamma rays								
Control	26.38	0	0	0	0	0	0	0
150Gy	24.67	6.92	12.81	0.87	0.58	3.54	12.63	6.82
200Gy	21.35	17.84	22.76	1.92	0.96	9.01	10.78	8.45
250Gy	18.95	22.46	31.11	2.08	0.83	10.99	9.28	6.70
300Gy	14.33	30.68	35.57	2.69	0.90	18.78	8.77	7.57
350Gy	12.35	45.26	46.46	3.17	0.90	25.65	7.00	6.82
Total				**10.74**	**2.64**	**67.97**	**48.46**	**36.35**
EMS								
Control	26.38	0	0	0.00	0	0	0	0
10mM	22.86	9.18	14.52	0.57	0.71	2.50	6.22	3.94
20mM	19.37	23.84	26.26	1.31	0.82	6.75	5.48	4.98
30mM	16.81	33.68	32.40	1.57	0.66	9.37	4.68	4.86
40mM	13.27	40.18	39.73	2.54	0.71	19.16	6.33	6.40
50mM	11.23	52.86	47.58	2.83	0.71	25.20	5.35	5.95
Total				**8.83**	**2.19**	**18.62**	**16.38**	**13.77**

Mutagenic efficiency was determined by the mutation frequency in relation to injury (I), lethality (L) and pollen sterility (S). For TMV (Gn) 13, the maximum mutagenic efficiency was observed at 350Gy for injury (27.89) and 150Gy for lethality (11.44) and pollen sterility (9.08) in gamma rays where as in EMS at 50mM for injury (21.43) and 10mM for lethality (11.38) and pollen sterility (8.19).
For TMV 7, the mutagenic efficiency ranged from 3.54 to 25.65 and 2.50 to 25.20 for injury in gamma rays and EMS respectively, for lethality 7.00 to 12.63 and 4.68 to 6.22 in gamma rays and EMS respectively and ranged from 6.70 to 8.45 and 3.94 to 6.40 in gamma rays and EMS respectively. The maximum mutagenic efficiency was observed at 350Gy for injury (25.65), 150Gy for lethality (12.63) and 200Gy for pollen sterility (8.45) in gamma rays whereas in EMS at 50mM for injury (25.20) and 40mM for lethality (6.33) and pollen sterility (6.40) in table 3 and 4. Same results were referred by Wani (2011), Raina and Khan (2020) and Amita Sharma *et al.* (2020). The EMS had more effectiveness than the gamma rays in the variety TMV(Gn) 13 while in TMV 7, gamma rays had more effectiveness than EMS. The mutagenic efficiency was increased with an increase in doses/concentration for injury while decreased with an increasing doses/ concentration for lethality and pollen sterility in both the treatments and in both varieties

ACKNOWLEDGMENT

Indhra Gandhi Centre for Atomic Research (IGCAR), Kalpakkam

REFERENCES

Alemu and Habtamu. 2016. Review Paper on Mutation Breeding as Applied in Groundnut (*Arachis Hypogea L.*) Improvement. *Ther.,* **1**(5): 35-40.

Amita Sharma, S. K. Singh, Rajesh Singh, Bhati, P. K., and K., a. M. M. 2020. Mutagenic Effects of Gamma Rays and EMS in M1 and M2 Generations in Rice *(Oryza sativa L.*). *Int. J. Curr. Microbiol. App. Sci,* **9**(01): 2645-2655. [Cross Ref]

Ariraman, M., Bharathi, T., and Dhanavel, D. 201). Induced mutagenic effectiveness and efficiency studies on Pigeon pea (*Cajanus cajan (L.*) Millsp). *Food Biology,* **4:** 1-4. [Cross Ref]

Burghate, S., Mishra, M., Chikhale, N., Mahalle, A., and Dhole, V. 2013. Impact of mutagens its efficiency and effectiveness in groundnut (Arachis hypogaea L.). *Scholarly Journal of Agricultural Science,* **3**(7): 284-288.

Channaoui, S., Labhilili, M., Mouhib, M., Mazouz, H., El Fechtali, M., and Nabloussi, A. 2019. Development and evaluation of diverse promising rapeseed (*Brassica napus L.*) mutants using physical and chemical mutagens. *OCL, 26*. [Cross Ref]

Joshi, P., Jadhav, M. P., Shirasawa, K., Yadawad, A., and Bhat, R. S. 2020. Foliar disease resistant and productive mutants from the introgression lines of peanut (Arachis hypogaea). *Plant Breed.* **139**(1): 148-155. [Cross Ref]

Kavithamani, D., Kalamani, A., Vanniarajan, C., and Uma, D. (2008). Mutagenic effectiveness and efficiency of gamma rays and EMS in Soybean (Glycine max (L.) Merrill). *Madras Agric. J,* **95**(7-12): 448-451.

Konzak, C. 1965. *Efficient chemical mutagenesis, in: The use of induced mutations in plant breeding.* Paper presented at the Report of the FAO/IAEA technical meeting organized by the food and agriculture organization of the United Nations and the International Atomic Energy Agency in cooperation with the European Association for Research on Plant Breeding, Rome, Italy, 25 May 1964.

Maliata Athon Wanga, Hussein Shimelis, Lydia N, Horn, and Sarsu, a. F. 2020. The Effect of Single and Combined Use of Gamma radiation and Ethylmethane Sulfonate on Early Growth Parameters in Sorghum. *Plant J.,* **9:** 827-842. [Cross Ref]

Prasath, G., Irene Vethamoni. P, Balasubramanian P, Vanniarajan C, Souframanien. J, Senthil, N., and G., a. H. 2019. Studies on mutagenic effectiveness and mutagenic efficiency of chlorophyll mutants in cluster bean (*Cyamopsis tetragonoloba (L)* Taub) Variety MDU 1. *Int. J. Chem. Stud., 7*: 3229-3233.

Raina, A., and Khan, a. S. 2020. Mutagenic effectiveness and efficiency of gamma rays and sodium azide in M2 generation of Cowpea [*Vigna unguiculata (L.)* Walp.]. *bioRxiv*. [Cross Ref]

Sonone, N., Deshmukh, S., Patil, S., Rathod, S., and More, G. (2008). Comparison of mutagenic efficiency and effectiveness of EMS and gamma rays in groundnut (*Arachis hypogaea L.*). *Crop Res.* **36**(1-3): 336-340.

suradkar, S. W. 2019. Improvement of Groundnut (*Arachis hypogaea L.*) through chemical Mutagen (EMS). *Int. e-Res. J., 110E*, 176-182.

Tamilzharasi, M., Kumaresan, D., Souframanien, J., and Jayamani, P. 2019. Study of chlorophyll deficit types through induced mutagenesis in blackgram (*Vigna mungo L.* Hepper). *Electron. J. Plant Breed.,* **10**(4): 1471-1476. [Cross Ref]

Wani, A. A. 2011. Spectrum and frequency of macromutations induced in chickpea (*Cicer arietinum* L.). *Turk. J. Biol,* **35**(2): 221-231.