Himachal Journal of Agricultural Research 47(2): 163-168 (2021)

Effectiveness and efficiency of gamma rays and EMS (Ethyl methane sulphonate) in linseed (*Linum usitatissimum* L.)

Arjun Kumar, Satish Paul, V.K. Sood, Garima Thakur and Ronika Thakur

Department of Genetics and Plant Breeding

CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India.

*Corresponding author: negiarjun1993@gmail.com

Manuscript received: Accepted: 24.09.2021

Abstract

Mutagenic effectiveness and efficiency of gamma rays and EMS was studied based on biological injury (lethality and pollen sterility) in M_1 generation and frequency of chlorophyll mutation in M_2 generation. The results showed presence of radina, chlorina, xantha and albino type of chlorophyll chimeras in linseed and with the maximum frequency of chimeras at 60 kR (0.490 %) gamma rays and 0.6 per cent EMS (0.476 %). No dose dependent relationship was observed for both effectiveness and efficiency and varied according to cultivars of linseed. However, the gamma rays doses were found more effective and efficient then EMS.

Key words: Chemical mutagen, gamma rays, effectiveness, efficiency, linseed.

Oilseed crops occupy prestigious place in Indian agriculture due to their vital role in the sustainable economy of the country. Linseed (*Linum usitatissimum* L., 2n=30) commonly known as Alsi, is one of the important oilseed crops of India as well as in the world, cultivated for both seed and fibre. All the aerial part of linseed is utilized commercially either directly or after processing. Varying oil content of about 33 to 45 per cent which is a rich source of polyunsaturated fatty acids such as oleic (C18, 13.44-19.39%), linoleic (C18, 12.25-17.44%), and linolenic acid (C18, 39-60%) and lignans (phytoestrogens) (Singh *et al*. 2011). Omega-3 and omega-6 fatty acid present in linseed are useful to treat diseases like cardiovascular disease (CVDs), diabetes, rheumatoid arthritis and cancer (Singh and Marker 2006).

Development of high yielding varieties requires the knowledge of existing genetic variability, but due to selective breeding over the generation in linseed, conventional breeding has limited scope. Thus, mutation breeding is an alternative effective tool for creation of variability. The physical and chemical mutagen has been employed successfully in genetic improvement of many crops i.e. linseed (Green 1986; Green and Marshall 1984; Deka 2016; Jahan *et al*. 2019), Sesame (Kumari *et al*. 2016), Mustard (Julia *et al*. 2018), Cowpea (Gririja and Dhanavel 2013; Bind *et al*. 2016), peas (Dhulgande *et al*. 2011). These mutagen provides a powerful tool for creation of variation in crop plant for both qualitative and quantitative traits (Das and Misra 2005; Baisakh *et al*. 2011). Gamma and EMS are the most commonly used mutagen which are known to influence the plant growth and development by inducing cytological, genetical, biochemical and morphological changes in plants (Gunkel and Sparrow 1961; Das and Prusti 2020). However, after continuous work done over the generation, it was observed that only a few mutagenic treatments have been effective for inducing high frequency of mutation. A highly effective mutagen may not necessarily show high efficiency and vice versa. Therefore, selection of effective and efficient mutagen is very essential to recover high frequency of desirable mutations in any mutation breeding studies (Usharani and Kumar 2015; Vairam 2017). Hence the study was undertaken to assess the effect of different doses of gamma rays and EMS on the frequency of chlorophyll mutation, lethality and pollen sterility to evaluate the relative effectiveness and efficiency of different mutagenic treatments.

Material and Methods

Dry and well matured seed of three well adapted varieties of linseed i.e. Baner, Him Alsi-2 and Surbhi administered with five doses of each of Gamma rays (20 kR, 30 kR, 40 kR, 50 kR and 60 kR) and EMS (0.2, 0.4, 0.5, 0.6 and 0.8%). Gamma rays irradiation was done at Nuclear Research lab. IARI, New Delhi having Gamma chamber, an automated machine having gamma radiation source of ^{60}C with activity of 13000 curie and capacity of 5000 cc (volume). For treatment with EMS, seed were presoaked in water for 6-8 hours, blotted dry and then treated with solution of EMS for 6 hours. After treatment seeds were thoroughly washed with running water to bleach out the residual effect. To grow the M_1 generation, treated seed were sown in field with spacing of 25 × 5 cm. The survival percentage was recorded in each plot at time maturity and lethality (%) was calculated and pollen fertility was calculated based on acetocarmine attainability. The harvested seed were carried forward to M_2 generation in progeny rows. Different types of chlorophyll mutation (Xantha, Albina, Chlorina and Radina) were recorded daily and compared with respective controls (Fig.1). Frequency of chlorophyll mutation was calculated (Gaul 1960). The mutagenic effectiveness and efficiency were calculated based on formula proposed by Konzak *et al*. (1965).

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

$$\text{Effectiveness of physical mutagen} = \frac{M}{\text{Dose of mutagen in kR}}$$

$$\text{Effectiveness of the chemical mutagen} = \frac{M}{\text{conc. * time}}$$

$$\text{Efficiency of the chemical mutagen} = \frac{Mf}{\text{\% lethality or \% pollen sterility}}$$

where,

M = mutation frequency (plant basis)

Conc. = concentration of EMS in per cent

% BI = per cent biological damage

$$\text{Per cent lethality / Biological injury} = \frac{\text{Plants germinated- Plants survived at the time of maturity}}{\text{Total number of M1 sown in each treatment}} \times 100$$

Results and Discussion

In the present investigation, the lethality and pollen sterility were observed in M_1 generation (Table 1). Increases in lethality and pollen sterility were recorded in M_1 population with increase in concentration of gamma rays, EMS in all the treatment for all the varieties. The increase in lethality as pollen sterility was reported by many investigators in green gram (Das *et al*. 2006; Das and Prusti 2020; Das *et al*. 2021), blackgram (Sagade and Apparao 2011). They concluded that probable reason for increase in pollen sterility might be meiotic irregularities.

The spectrums of all type of chlorophyll mutations were observed at different concentration of gamma rays and EMS treated population in M_2 generation. In Baner cultivar, spectrum of chlorophyll mutation was found same in all the doses of mutagens i.e. one, while frequency of chlorophyll mutations was different in each treatment (Table 1). At 20 kR gamma rays and 0.5 per cent EMS, one Radina type chlorophyll mutant was observed having frequency of 0.162 and 0.212 respectively. Also one xantha type mutant with frequency 0.152 was found in 30 kR gamma rays treatment. The total maximum frequency of

Table 1. Frequency, effectiveness and efficiency of gamma rays and EMS in linseed

Treatment	Spectrum and Frequency					Per cent Lethality (%)	Per cent Pollen sterility (%)	Effectiveness %	Efficiency (%)	
	Xantha	Albino	Radina	Chlorina	Total				Based on lethality	Based on pollen
Baner										
Gamma Rays (Kr)										
20	0	0	1(0.167)	0	1(0.167)	4.40	20.40	0.833	0.038	0.008
30	1(0.156)	0	0	0	1(0.156)	10.16	22.20	0.521	0.015	0.007
40	1(0.161)	1(0.161)	0	0	2(0.322)	10.66	29.00	0.806	0.030	0.011
50	1(0.227)	0	0	1(0.227)	2(0.454)	17.67	36.80	0.909	0.026	0.012
60	1(0.243)	0	1(0.243)	0	2(0.490)	24.80	39.60	0.813	0.020	0.012
EMS (%)										
0.2	0	1(0.175)	0	1(0.175)	2(0.350)	6.91	17.80	0.351	0.051	0.020
0.4	1(0.172)	0	1(0.172)	0	2(0.344)	9.53	19.80	0.172	0.036	0.017
0.5	0	0	1(0.212)	0	1(0.212)	14.29	22.20	0.085	0.015	0.010
0.6	1(0.238)	1(0.238)	0	0	2(0.476)	18.17	26.80	0.159	0.026	0.018
0.8	0	1(0.212)	0	1(0.212)	2(0.425)	24.05	35.20	0.106	0.018	0.012
Him Alsi-2										
Gamma Rays (Kr)										
20	0	0	0	0	0	14.79	17.80	0.000	0.000	0.000
30	1(0.212)	1(0.212)	0	1(0.212)	3(0.667)	16.67	20.40	2.222	0.040	0.033
40	1(0.205)	0	1(0.205)	0	2(0.408)	24.55	35.40	1.020	0.017	0.012
50	0	0	0	0	0	32.06	39.80	0.000	0.000	0.000
60	0	0	0	0	0	36.19	40.20	0.000	0.000	0.000
EMS (%)										
0.2	0	0	0	0	0	4.03	10.00	0.000	0.000	0.000
0.4	0	0	0	1(0.238)	1(0.238)	8.78	10.50	0.119	0.027	0.023
0.5	1(0.232)	0	1(0.232)	0	2(0.465)	14.04	18.00	0.186	0.033	0.026
0.6	1(0.212)	0	1(0.212)	0	2(0.425)	20.05	24.75	0.148	0.021	0.017
0.8	0	1(0.333)	0	1(0.333)	2(0.667)	25.18	35.75	0.167	0.026	0.019
Surbhi										
Gamma Rays (Kr)										
20	0	0	0	0	0	4.40	8.25	0.000	0.000	0.000
30	0	1(0.357)	0	1(0.357)	2(0.714)	9.91	12.50	2.381	0.072	0.057
40	0	0	1(0.312)	0	1(0.312)	15.92	24.50	0.781	0.020	0.013
50	0	1(0.312)	0	0	1(0.312)	20.42	26.00	0.625	0.015	0.012
60	1(0.312)	0	0	1(0.312)	2(0.625)	25.43	35.00	1.042	0.025	0.018
EMS (%)										
0.2	1(0.400)	0	0	0	1(0.400)	10.91	42.5	0.400	0.037	0.009
0.4	0	0	1(0.400)	0	1(0.400)	25.43	49.8	0.200	0.016	0.008
0.5	1(0.400)	0	2(0.800)	0	3(1.200)	37.44	72.0	0.480	0.032	0.017

chlorophyll mutations was found in 60 kR (0.490 %) gamma rays and 0.6 per cent EMS (0.476 %). In Him Alsi-2 cultivar, at 30 kR gamma rays, xantha (1), albino (1) and chlorina (1) type of chlorophyll mutant having frequency of 0.212 was observed. In 40 kR treatment only xantha (1) and radina (1) type of mutant was observed having frequency of 0.205 per cent. At 20, 50 and 60 kR, no chlorophyll mutant was observed in Him Alsi-2. On the other hand, except 0.2 per cent EMS, all EMS treatments i.e. 0.4, 0.5, 0.6 and 0.8 per cent showed chlorophyll mutations. At 0.4 per cent EMS, only one chlorina type mutant was observed having frequency of 0.238 per cent. Among all doses of EMS and gamma rays, maximum frequency of chlorophyll mutations was observed in 30 kR (0.667 %) followed by 0.6 per cent (0.667 %) having spectrum three and two respectively. In Surbhi cultivar, except 20 kR gamma rays, all the treatment of gamma rays and EMS showed presence of chlorophyll type of mutants. Highest spectrum of chlorophyll mutants was observed in 0.5 per cent EMS i.e. radina (2) having frequency of 0.800 per cent. Among all treatment of gamma rays and EMS, maximum frequency of chlorophyll mutation was observed in 0.5 per cent EMS (1.200 %) followed by 30 kR gamma rays (0.714 %). The occurrence of chlorophyll deficient chimeras might be due to changes in genes responsible for the chlorophyll mutation (Das *et al*. 2021; Monika and Seetharaman 2017).

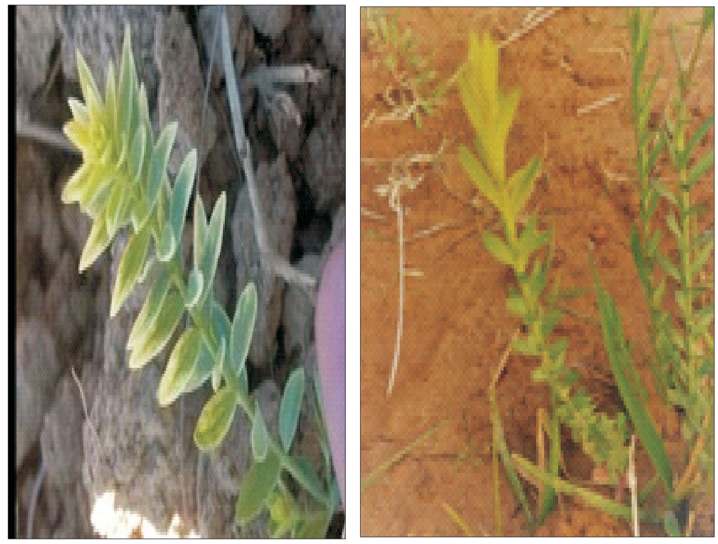
The Mutagenic effectiveness showed no dose dependant relationship for gamma rays and EMS which means mutagens effectiveness varied according to doses of mutagen and varieties of linseed (Table 1). In Baner, mutagenic effectiveness was found maximum at 50 kR (0.909) followed by 20 kR (0.833) gamma rays. On the other hand, all the doses of EMS shown less effectiveness as compare to doses of gamma rays. In EMS treatment, minimum effectiveness was observed at 0.5 per cent (0.085), while maximum was observed at 0.2 per cent (0.351). In Him Alsi-2, only two treatment of gamma rays found effective i.e. 30 kR (2.222) and 40 kR (1.020), while other treatment of gamma rays i.e 20, 50 and 60 kR found ineffective for chlorophyll mutation. Except 0.2 per cent EMS, all other (0.3, 04, 0.5 and 0.6 %) EMS treatments found effective with maximum effectiveness at 0.5 per cent (0.186). In Surbhi, except 20 kR, all other doses of gamma rays and EMS found effective for chlorophyll mutation with maximum effectiveness at 30 kR (2.381) followed by 60 kR (1.042) while minimum was observed at 0.4 per cent EMS (0.200). Similar results were obtained by Sharma *et al*. (2005), Das and Baisakh (2020), and Das and Prusti (2021).

The Mutagenic efficiency provides the best available measure to evaluate different mutagenic treatments (Konzak *et al*. 1965). It varies depending upon the concentration of mutagens, physiological and genetical characteristic of cultivars. The efficiency at lower concentration of mutagens was due to the fact that biological injury (lethality and sterility) increases with increase in doses of mutagens (Konzak *et al*. 1965; Nilan and Konzak 1961). Mutagenic efficiency based on biological injury i.e. lethality varied from 0.015 to 0.072 (Table 1). In treatment of Baner, it varies from 0.015 to 0.051 in which maximum efficiency was found in 0.2 per cent EMS (0.051) followed by 20 kR gamma rays (0.038). In Him Alsi-2, two doses of gamma rays i.e. 30 kR (0.040) and 40 kR (0.017) found efficient, and other doses i.e. 20, 50 and 60 kR found non efficient for chlorophyll mutation. Similarly, except 0.2 per cent EMS, all other (0.4, 0.5, 0.6 an d 0.8 %) found efficient. In Surbhi, similar pattern was observed as observed in Him Alsi-2. Based on pollen fertility in M_1 generation, the mutagenic efficiency of gamma rays and EMS in M_2 generation ranged from 0.07 to 0.033 and concluded that doses of gamma rays and EMS showed similar behavior of mutagenic efficiency as shown due to lethality (M_1) (Table 1). Similar result was obtained by Das *et al*. (2021).

Conclusion

The improved variety Baner showed more number of chlorophyll chimeras than other two varieties i.e. Him Alsi-2 and Surbhi. The effectiveness and efficiency based upon frequency, biological injuries (lethality and sterility) of gamma rays doses were proved to be more effective and efficient than EMS doses.

Conflict of interest: The authors declare that there is no conflict in this research paper.



Xantha type mutant **Chlorina type mutant**

Radina type mutant **Albino type mutant**

Fig. 1 Different types of morphological mutations in linseed

References

Baisakh B, Das TR and Nayak BK. 2011. Efficacy of physical and chemical mutagenic treatments in developing desire micro-mutants of urdbean. Journal of Food Legumes **24**: 106-109.

Bind D, Dwivedi VK and Singh SK. 2016. Induction of chlorophyll mutations through physical and chemical mutagenesis in cow pea (*Vigna unguiculata* (L.) Walp). International Journal of Advanced Research **4**: 49.

Das T R and Misra RC. 2005. Genetic analysis of mutagen induced variability in yield traits in greengrams (*Vigna radiate*). Environment & Ecology **23** (2): 381-384.

Das TR and Prusti AM. 2020. Mutagenic Effectiveness and Efficiency of Gamma Rays, EMS and NG in greengram (*Vigna radiate* L. Wilczek). International Journal of Current Microbiology and Applied Sciences **9**: 2336-2344.

Das TR, Baisakh B and Prusti AM. 2021. Studies on mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulfonate, nitrosoguanidine, maleic hydrazide and their combination in greengram (*Vigna radiate* L. Wilczek). International Journal of Current Microbiology and Applied Sciences **10** (01):3354-3362.

Das TR, Misra RC and Sahu PK. 2006. Efficiency of mutagenic treatments in expression of macro and micro mutations in M_2 generation in greengram and its early predictability on basis of M_1 parameters. Environment & Ecology **24** (2):283-288.

Deka SK. 2016. Studies on the mutagenic effects of Ethyl methane sulphonate (EMS) and diethyl sulphate (dES) in Linseed (*Linum usitatissimum* L.). Ph D Thesis, p 181. Department of Botany, Gauhati University, Guwahati, Assam, India

Gaul H. 1960. Critical analysis of the methods for determining the mutation frequency after seed treated with mutagens. Agriculture Genetics **12**: 297-318.

Girija M and Dhanavel D. 2013. Induced chlorophyll mutations in cowpea (*Vigna unguiculata* L. Walp).

International Journal of Current Research **2**:136-140

Green AG and Marshall DR. 1984. Isolation of induced mutants in linseed (*Linum usitatissimum*) having reduced linolenic acid content. Euphytica **33**: 321-328.

Green AG. 1986. A mutant genotype of flax (*Linum usitatissimum* L.) containing very low levels of linolenic acid in its seed oil. Canadian Journal of Plant Science **66**: 499-503.

Gunckel JE and Sparrow AH. 1961. Ionizing radiation, biochemical, physiological and morphological aspects of their effects on plants. Encyclopedia Plant Physiology (Ruhland W) Springer-verlag, Berlin **16**: 555-611.

Jahan R, Amin R, Ansari SB, Malik S and Khan S. 2019. Sodium azide affects the qualitative and quantitative traits of *Linum usitatissimum* L (var. Padmini) in M_1 generation. International Research Journal of Pharmacy **10**:45-48.

Konzak CF, Nilan RA, Wagner J and Foster RJ. 1965. Efficient chemical mutagenesis. The use of induced mutations in plant breeding (FAO / IAEA Meeting, Rome). Radiation Botany (Suppl.) **75**: 49-70.

Kumari V, Chaudhary HK, Prasad R, Kumar A, Singh A, Jambhulkar S and Sanju S. 2016. Frequency and spectrum of mutations induced by gamma radiations and ethyl methane sulphonate in Sesame (*Sesamum indicum* L.). Scientia **14**:270-278

Monika S and Seethraman N. 2017. Induced chlorophyll and viable mutation in *Lablab purpureus* (L.) sweet var. *typicus* through gamma rays and ethyl methane sulphonate. International Journal of Pharmacology and Biological Sciences **8** (2): 240 -249.

Nilan RA and Konzak CF. 1961. Increasing the efficiency of mutation induction. **In**: Mutation and Plant Breeding, NAS-NRC: 437-460.

Sagade AB and Apparao BJ. 2011. M_1 generation studies in urdbean (*Vigna mungo* (L.) Hepper). Asian Journal of Experimental Biological Sciences **2** (2): 372-375.

Sharma SK, Sood R and Pandey DP. 2005. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (*Vigna mungo* (L.) Hepper). Indian journal of Genetics and Plant Breeding **65**: 20-22.

Singh KK, Mridula D, Rehal J and Barnwal P. 2011. Flaxseed- a potential source of food, feed and fiber. Critical Reviews in Food Science and Nutrition **51**:210-222.

Singh SB and Marker S. 2006. Linseed: A plant with many uses. Agrobios Newsletter **5**: 1-3.

Usharani KS and Kumar ACR. 2015. Mutagenic efficiency and effectiveness of gamma rays and EMS and their combination in inducing chlorophyll mutations in M_2 generation of urdbean (*Vigna mungo* (L.) Hepper). Electronic Journal of Plant Breeding **6** (1): 210-217.