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Determination of Lethal Dose (LD_{50}) and Effects of Gamma Rays and Ethyl Methane Sulphonate (EMS) Induced Mutagenesis in Linseed (*Linum usitatissimum* L.)

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A B S T R A C T

The present investigation was undertaken to study the effect of non particulate gamma radiation and EMS mutagens on linseed varieties viz., Baner, Him Alsi-2 and Surbhi. Many significant effects of EMS concentration and gamma rays dosages on seed germination were observed. The percent germination was reduced from 84, 86 and 80 to 65, 58 and 59 percent with 60 kR (kilo Roentgen) for cultivars Baner, Him Alsi-2 and Surbhi respectively. LD_{50} concentration of gamma rays varies in different varieties *viz.*, 96.63 kR (Baner), 72.94 kR (Him Alsi-2- and 53.22 kR (Surbhi). The percent germination was reduced from 87, 94 and 79 to 49, 50 and 10 percent with 0.8 percent EMS for cultivars Baner, Him Alsi-2 and Surbhi respectively. Also, lethality (LD_{50}) of EMS between varieties varies *i.e.*, Baner (0.86%), Him Alsi- 2 (0.72%) and Surbhi (0.35%) showing that lethality depends upon the characteristics of seed. Variable effects were observed in field condition for seed germination and survival in all the varieties indicating that these mutagens can be used in improvement of particular characteristics of crop by following proper selection methods.

Introduction

Linseed (*Linum usitatissimum* L., 2n=30), belongs to the genus *Linum* that comprises about 290 species (Gill 1987). *Linum usitatissimum* is the solely cultivated species and one among the vital seed and fibre crop globally. The seed contains high proportion of oil, varies from 34-47 percent in numerous varieties. The oil is additionally recognized as a good supplier of alpha omega-3 for human diet. Throughout the last decade, the interest in linseed has been fabricated in trendy societies attributes for its multiple awareness of polyunsaturated fatty acid nutrition, as linseed is one among the richest sources of polyunsaturated fatty acid (PUFA) *viz.*, linoleic (omega-6) and linolenic (omega-3. As a result of high alpha omega-3, flax oil without delay polymerizes on exposure to oxygen, creating it helpful for style of industrial product such as varnish and linoleum, whereas meal from processed seed is helpful for animal feed. Flax also additionally supplies of fibres that is employed for linen and textiles.

Assessment of genetic variability is the first step in any crop improvement programme. It can either be created through hybridization or induced mutation. Induced mutagenesis is a formidable tool for generating a lot of inherent genetic variation to develop new high yielding varieties, tolerance to biotic and abiotic stresses in unpredictable environment. Mutation can occur spontaneously or resulting from exposure to radiation or chemicals.

Mutation studies in flaxseed showed that it is quite useful for induction of variability and development of cultivars with improved traits like early maturing, lodging resistance, changed oil proportion and fatty acids (Badere and Choudhary 2004, 2007; Alka *et al.*, 2013; Mogali *et al.*, 2016; Ntiamoah and Rowland 1997; Green and Marshall 1984, and Green 1986).

Some muatgens like X-ray, gamma ray, and ultraviolet (UV) has been widely used for the development of assorted traits of crops (Khatri *et al.*, 2005; Tah 2006; Kanakamanay 2008; Pandiyan *et al.*, 2008; Songsri *et al.*, 2011; Aney 2013), but the success of mutation depends on its dose applied.

Usually, mutagen treatments scale back seed germination, rate of growth, vigour and fertility. There's substantial killing of plants throughout completely different stages of development, so significantly reduces the survival of ensuing plants. The dose needed for prime agent potency depends on properties of the mutagenic agents and material treated. Hence, an overdose may kill too many treated individuals and lesser dose can turn out fewer mutations.

The optimum dose can turn out the high frequency of mutations and cause minimum killing that varies with crop species and agent used. Therefore, determination of the LD_{50} (lethal dose), a dose that causes 50% mortality mortality to the seeds is critical. The LD_{50} is completely different between species and varieties in a species. As an example, the LD_{50} of mungbean cultivar is K-851 (54.06 kR) and Sona (53.20 kR) (Tah 2006), *Jatropha crucas* L. (600 Gray) (Songsri *et al.*, 2011), *Manihot esculenta* L. (27.5 Gray) (Kangarashu *et al.*, 2014), *Pisum sativum* L. (200 Gray) (Aney, 2013). Therefore, this study was carried out to determine the LD50 of linseed cultivar and its effect on survival of population.

Materials and Methods

A chemical mutagen, Ethyl Methane Sulphonate (EMS) and a physical mutagen, gamma rays (Co^{60}) were utilized in the current investigation to induce mutations within the hand-picked material and to realize genetic variability with fascinating characters.

500 seeds of linseed cultivars (Baner, Him Alsi-2 and Surbhi) were irradiated with gamma rays of concentration of 20, 30, 40, 50 and 60 kR. Gamma irradiation was conducted in exploitation Gamma Chamber (source Co^{60}, activity 13000 curie and capacity of 5000cc) at government agency (NRL; Nuclear Research laboratory), IARI, New Delhi. Additionally for EMS, 500 healthy and mature seeds of cultivars *viz.*, Baner, Him Alsi-2 and Surbhi soaked in water for 6 hours were treated with EMS with concentration of 0.2, 0.4, 0.5, 0.6 and 0.8 percent for 6-8 hours at room temperature followed by decanting of the EMS and rinsing with running tap water for 1-2 hour to scrub out the chemical residues. Solution of EMS was prepared in distilled water at pH 7.0 in different concentrations (0.2, 0.4, 0.5, 0.6 and 0.8%).

After treatment with Gamma radiation and EMS, 100 treated seeds of each treatment with controls of respected cultivars were placed on germination paper in petri dishes by

use of 5 ml of sterilized water. Petri dishes were placed in germination chamber for 7 days at 25 ± 2° c. Then at 3rd and 7th days after treatments, germination and survival percentage were recorded.

The LD_{50} was then determined supported the amount of survival plants at those completely different dose of gamma rays and conc. of EMS. Then, another 400 gamma rays treated and EMS treated seeds were exposed to field condition to spot the effect of the different doses in linseed.

Analysis of mean performance of germination were completed through the programme developed by Srinivasan (2004) to find LD_{50} value based on Finney's method. The percent reduction in seed germination and plant survival over control was calculated as:

$$P = \left[1 - \frac{SG\%_t}{SG\%_{nt}}\right] X\ 100$$

Where,

P = per cent population reduction over control

$SG\ \%_t$ = per cent seed germination in treatment

$SG\ \%_{nt}$ = per cent seed germination in control

$PS\ \%_t$ = per cent plant survival in treatment

$PS\ \%_{nt}$ = per cent plant survival in control

Results and Discussion

The first stage in mutation breeding is to identify the dose, since the radio sensitivity of every species, varieties and genotypes are completely different. LD50 is to be considered to be the optimum dose that causes high frequency of favorable mutation with minimum damage to the plant since doses lower than ld_{50} favours plant's recovery after treatments, while the use of higher doses increases the possibility to induce mutations either in positive or negative ways. Therefore, to avoid excessive loss of actual experimental materials, LD50 test is conducted before any mutation breeding programme.

Mean germination percentage of Baner, Him Alsi-2 and Surbhi cultivars at five completely different doses of gamma rays and EMS is presented in Table 1 and Table 2. The germination and survival was recorded on 3rd and 7th DAT. The LD_{50} was calculated on the basis of percent survival.

The results indicated that the average percent germination and survival were shriveled with increasing concentration of mutagens (EMS and gamma rays) (Fig. 1). The percent germination was reduced from 84, 86 and 80 to 65, 58 and 49 percent with increase of concentration from 20kR to 60 kR (kilo Roentgen) for cultivars Baner, Him Alsi-2 and Surbhi respectively. As per probit analysis based on survival percentage, the LD_{50} of gamma rays showed varition in different cultivars, which is 96.63 kR (Baner), 72.94 kR (Him Alsi-2) and 53.22 kR (Surbhi). The lowest LD_{50} of cultivar Surbhi may be due to different seed characters as compare to both other cultivars.

The maximum percent reduction over control in both seed germination and survival percentage was shown at 60 kR followed by 50 kR indicating a clear pattern in all the varieties studied, showing that with increase in concentration of gamma rays, percent reduction over control also increasing. This showed that higher doses were more effective.

Table.1 Effect of gamma rays and EMS on germination and survival percentage in different linseed cultivar (Lab condition)

Treatment	Per cent germination	Per cent reduction over control	Plant survival	Per cent reduction over control
Baner				
Gamma rays (kR)				
20Kr	84	14.29	82	16.33
30kR	80	18.37	78	20.41
40kR	76	22.45	75	23.47
50kR	70	28.57	66	32.65
60kR	65	33.67	64	34.69
Control	98		98	
EMS (%)				
0.2	87	11.22	86	11.34
0.4	85	13.27	84	13.4
0.5	71	27.55	70	27.84
0.6	67	31.63	60	38.14
0.8	49	50	48	50.52
Control	98		97	
Him Alsi-2				
Gamma rays (kR)				
20kR	86	12.24	83	14.43
30kR	81	17.35	78	19.59
40kR	69	29.59	65	32.99
50kR	66	32.65	62	36.08
60kR	58	40.82	56	42.27
Control	98		97	
EMS (%)				
0.2	94	5.05	91	6.19
0.4	85	14.14	76	21.65
0.5	71	28.28	65	32.99
0.6	53	46.46	52	46.39
0.8	50	49.49	42	56.7
Control	99		97	0
Surbhi				
Gamma rays (kR)				
20kR	80	17.53	78	18.75
30kR	75	22.68	74	22.92
40kR	64	34.02	60	37.5
50kR	53	45.36	51	46.88
60kR	49	49.48	45	53.13
Control	97		96	
EMS (%)				
0.2	79	17.71	71	22.83
0.4	61	36.46	56	39.13
0.5	42	56.25	40	56.52
0.6	17	82.29	12	86.96
0.8	10	89.58	7	92.39
Control	96		92	

Table.2 LD 50 of gamma rays and EMS of different cultivars

Mutagens/ Variety	Gamma rays			EMS		
	log LD_{50}	LD_{50} conc. (kR)	V(m)	log LD_{50}	LD_{50} conc. (%)	V(m)
Baner	1.96	96.63	0.012	**0.068**	0.86	0.002
Him Alsi-2	1.86	72.94	0.0040	-0.140	0.72	0.001
Surbhi	1.72	53.22	0.0020	-0.460	0.35	0.003

Table.3 Effect of gamma rays and EMS on germination and survival percentage in different linseed cultivar (field condition)

Treatment	Per cent germination	Per cent reduction over control	Plant survival percent	Per cent reduction over control
Baner				
Gamma rays (kR)				
20kR	83.00	15.31	79.60	17.94
30kR	82.60	15.71	77.80	19.79
40kR	69.00	29.59	71.00	26.80
50kR	66.80	31.84	63.20	34.85
60kR	62.40	36.33	60.40	37.73
Control	98.00		97.00	
EMS (%)				
0.2	92.25	**2.89**	90.00	5.01
0.4	90.00	**5.26**	89.50	5.54
0.5	83.50	**12.11**	82.00	13.46
0.6	80.00	**15.79**	75.25	20.58
0.8	65.00	**31.58**	64.25	32.19
CONTROL	95.00		94.75	
Him Alsi-2				
Gamma rays (kR)				
20kR	90.60	8.11	82.20	15.95
30kR	85.00	13.79	80.20	18.00
40kR	75.60	23.33	77.80	20.45
50kR	73.40	25.56	73.20	25.15
60kR	64.80	34.28	64.80	33.74
Control	98.60		97.80	
EMS (%)				
0.2	92.75	**4.13**	91.75	4.92
0.4	89.00	**8.01**	87.50	9.33
0.5	78.00	**19.38**	75.50	21.76
0.6	76.50	**20.93**	74.00	23.32
0.8	67.25	**30.49**	65.00	32.64
CONTROL	96.75		96.50	
Surbhi				
Gamma rays (kR)				
20kR	84.40	**11.72**	82.20	12.55
30kR	80.40	**15.90**	79.60	15.32
40kR	65.20	**31.80**	64.60	31.28
50kR	62.20	**34.94**	60.20	35.96
60kR	60.40	**36.82**	59.80	36.38
Control	95.60		94.00	

EMS (%)				
0.2	70.00	**7.28**	57.50	14.18
0.4	65.00	**13.91**	50.25	25.00
0.5	33.50	**55.63**	28.00	58.21
0.6	3.00	**96.03**	0.50	99.25
0.8	1.00	**98.68**	0.00	100.00
CONTROL	75.50		67.00	

Fig.1 Differences in LD50 dose of different cultivars

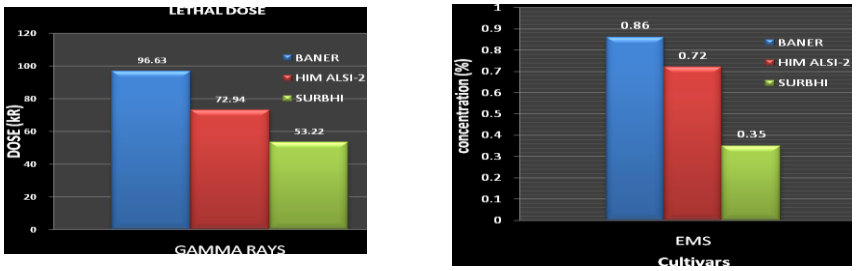
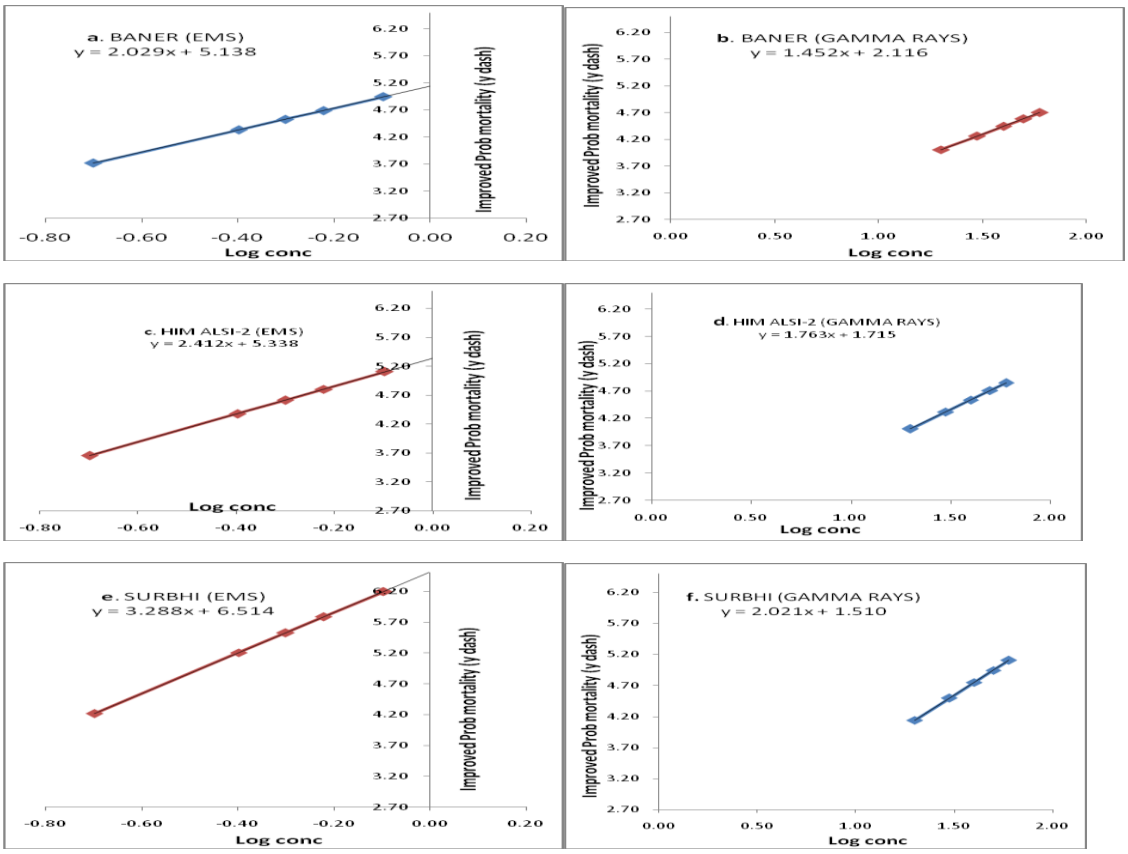


Fig.2 (a,b,c,d,e & f) Probit log graphs dose effect of EMS and Gamma rays on different linseed cultivars



Similarly, there was decrease in germination and survival percentage with increase of concentrations of EMS. The percent germination was reduced from 87, 94 and 79 to 49, 50 and 10 percent in varieties namely, Baner, Him Alsi-2 and Surbhi respectively.

Also, lethality (LD_{50}) of EMS varies between cultivars *i.e.*, Baner (0.86%, EMS), Him Alsi-2 (0.72%, EMS) and Surbhi (0.35%, extremely less as compare to LD_{50} conc. of different 2 cultivars) (Table 2). With increase in concentration of EMS, there was increase in percent reduction overcontrol. The maximum percent reduction over control for seed germination and survival were observed in Surbhi (89.58%, germination and 92.39%, survival) at 0.8 percent EMS concentration, which was far more then maximum percent reduction over control of Baner and Him Alsi-2.

Overall, LD_{50} values of different mutagen was lowest in Surbhi i.e., 53.22 kR (gamma rays) and 0.35 percent (EMS). There may be possible reasons for germination and survival inhibition as reported by different authors. Gamma irradiation generates free radicals in plant produce that may bring metabolic disorders in the seeds leading to growth retardation. It is also affects enzyme activity since higher seed vigour is related to higher germination efficiency. It is also reported that high α- amylase activity increased metabolic activity leading to enhanced seed vigour. Hence it may be infused that gamma irradiation may decrease α-amylase activity. Yellow and brown seeded linseed have different morphology and characteristics, so this may be the reason that the effect of EMS and gamma rays were observed more in Surbhi (Yellow seeded) as compare to Baner and Him Alsi-2 (both are of brown seed). The value of lethal dose was found different in all three cultivars, showing that lethal dose vary not only crop to crop but variety to variety also (Fig. 1 & Table 2).

In field condition, decrease in germination and survival percentage following proper trends was observed in both the mutagenic treatments of mutagens in all three cultivar. The minimum percent germination was found in Baner at 60kR gamma rays (62.40%) and 0.8 percent EMS (65%), Him Alsi-2 at 60kR (64.80%) and 0.8 percent (67.25%) and Surbhi at 60kR (60.40%) and 0.8 percent (1%) showing maximum percent reduction over control (Table 3). The maximum percent reduction over control was found in Surbhi at 0.8 percent EMS (98.68%) followed by 0.6 percent EMS (96.68%) depicting that these concentration of EMS were not suitable for generating survivable good mutant population and can be avoided.

Variable effect of gamma rays and EMS were observed in both lab and field condition indicating that both gamma rays and EMS can be used for generating mutants in linseed. And also LD_{50} values indicate that non effective dose can be avoided (Fig. 2).

In conclusion the determination of LD_{50} value for any mutagen is essential to produce maximum viable mutants with minimum damage to the plant. The LD_{50} dose based on the reduction in survival after treatment with different doses of Gamma rays and different concentration of EMS were different as shown above. Since there is very less literature of lethal doses in linseed crop, determination of optimum mutagen doses for the linseed could be useful while formulating linseed mutation breeding programme for improvement of specific traits in linseed. Result indicates that the LD 50 varies between varieties and depends on morpho physiological characters of seed. Due to this the effect of mutagen (gamma rays and EMS) were observed more Surbhi than Baner and Him ALsi-2. Probability of in absolute LD_{50} value for linseed cultivars *viz.*, Baner, Him Alsi- 2 and Surbhi can be used as a reference while initiating linseed mutation breeding in other species or cultivars of linseed.

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