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Optimal Lethal Dose Determination for Gamma Rays and EMS Induced Mutagenesis in TMV7 and SVPR1 Sesame (*Sesamum indicum* L.) Varieties

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Authors' contributions

This work was carried out in collaboration among all authors. Author GP executed the research work as a part of his Master's programme and wrote the first draft of the manuscript. Authors MAP, RK and SMPK managed the analyses of the study. Author AKB conceptualized the idea, designed the study, wrote the protocol and performed statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

In the present study two sesame varieties *viz.,* TMV7 and SVPR1 were treated with varying doses of gamma rays (250, 300, 350, 400 and 450 Gy) and Ethyl Methane Sulphonate (EMS) of different concentrations *viz.,*0.20, 0.40 and 0.60%. The seed germination percentage was greatly affected by mutagenic treatment of gamma rays and EMS which showed a negative dose dependent relationship in both the varieties. The expected LD_{50} values were calculated through probit analysis. The LD_{50} values for TMV7 and SVPR1 were fixed at 416.86 Gy and 389.04 Gy for gamma rays and 0.490 % and 0.349% for EMS. The germination percentage of SVPR1 was greatly reduced (17.80 & 20.55 %) and the lethal dose to kill fifty per cent of mutated population was lower (6.68% & 28.78%)

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than that of TMV7 in both gamma ray and EMS treatment. EMS treatment exhibited significant reduction in seed germination (62.16 % & 66.67 %) than gamma irradiation (56.76 % & 54.55 %) in TMV7 and SVPR1 respectively. The study concluded that both the mutagens are effective to produce significant variations in sesame which can be further explored for mutation mapping.

Keywords: EMS; gamma rays; LD50; mortality percentage; Sesame.

1. INTRODUCTION

Sesame (*Sesamum indicum* L, 2n = 26) is one of the traditional oilseed crop popularly known as *gingelly* or *til*. Sesame is mainly grown for the seed oil purpose and it is a major ingredient in Indian food [1]. Besides, it has longer shelf life period due to the presence of antioxidants [1,2,3] and also occupies a predominant position in traditional medicines [4,5]. Sesame is widely grown as rainfed crop in the arid to semi-arid tropics during the Kharif season [6]**.**Sesame is a drought tolerant crop and widely grown in tropical and sub-tropical regions. India hold first position in terms of area (19.46 Lakh ha) and production (8.66 Lakh tonnes) but low productivity (413 Kg/ha) than other countries (535 Kg/ha) made sesame a marginal crop [7]. Low productivity of sesame is mainly due to cultivation in sub marginal lands and non-availability of elite cultivars with wider adaptability, synchronous maturity, and non-shattering types resistant to abiotic and biotic stress [8,9].

Mutation breeding helps the geneticists and breeders to create tremendous variability that cannot be achieved through selection or hybridization process. Improvement of any agronomic trait can be achieved easily through induced mutation through generation of variability and adopting selection scheme [10]. Artificial mutation can be achieved either through physical or chemical agents. Regarding the physical mutagens, gamma rays has been widely used due to its ionizing nature and high penetrating capacity [11] that produces free radicals [12] with water molecules present on the exposed biological materials which in turn disrupts the H-bond between complementary base pairs of double helix DNA. Sesame seeds are highly tolerant to radiation than any other crop [13]. In case of chemical mutagens, Ethyl Methane Sulphonate (EMS) is a common alkylating agent which causes G/C to A/T transitions leads to point mutations whereas ionizing radiations produce chromosomal anomalies and deletions [14]. However, when these two agents (gamma rays and EMS) diverge in their potential to create irreversible anomalies such as lethality and sterility, then they may be considered to differ in their mutagenic efficiency.

The choice of effective mutagen and optimum doses that induces variability is highly crucial. Therefore, it is essential to determine the appropriate dose range to develop desirable macro mutants with least biological damage. In the present investigation, we studied the lethal dose (LD_{50} values) for gamma irradiated and EMS treated sesame varieties of TMV7 and SVPR1 on the germination rate.

2. MATERIALS AND METHODS

The seeds of two sesame varieties *viz.,* TMV7 and SVPR1 for mutagenesis were obtained from Oilseeds Research Station, Tindivanam and Cotton Research Station, Srivilliputtur, Tamil Nadu Agricultural University (TNAU).

2.1 Gamma Irradiation

Two grams (per dosage) of uniform, healthy and dry seeds of two varieties of sesame *viz.,*TMV7 and SVPR1 were exposed to gamma rays at different doses (250,300, 350, 400, 450 Gy) using Cobalt-60 (^{60}Co) gamma source installed at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Chennai, Tamil Nadu. The irradiated seeds were sown in the field within 24 h of treatment.

2.2 EMS Treatment

Well filled and healthy seeds of both the varieties were pre-soaked in distilled water for 12 h prior to treatment to enhance the imbibing capacity. And then, pre-soaked seeds were immersed in varying concentration of EMS such as 0.20 %, 0.40 % and 0.60 % for 8 h followed by intermittent shaking for every 30 min. After the treatment period, seeds were washed in running water for 30 min and immediately sown.

2.3 Nursery Experiment

To study the effect of mutagens on seed germination, a total of 100 mutated seeds (M_0) of each treatment along with control were sown in

nursery beds with two replications. The experiment was conducted at Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam, TNAU during Kharif season, 2019.

2.4 Germination Percentage

Seed germination index was observed from the time of radicle emergence to fifteenth day after sowing in each treatment and expressed in percentage.

$$\text{Germination } \% = \frac{\text{No.of seeds germinated}}{\text{Total no.of seeds sown}} \text{X } 100$$

2.5 Probit Analysis for LD_{50} Fixation

The LD_{50} (lethal dose) values of gamma rays for TMV7 and SVPR1 were calculated according to the probit analysis [15]. The probit function represents the inverse cumulative distribution function or quantile function associated with the standard normal distribution. The steps for probit analysis are as follow:

1) Transformation of the dose concentration of mutagens into $\log_{10}$ values
2) Determination of the mortality % due to treatment doses

The proportions corrected for control mortality if it is more than 10% using Abbot Formula [16].

$$\text{Corrected mortality (p)} = \frac{\% \text{ responded} - \% \text{ responded in control}}{100 - \text{responded \% in control}} \text{X}100 \quad (1)$$

The corrected proportions (p) were converted to empirical probits (y) and a dose response regression curve drawn using log10 doses (x) and empirical probits (y). Empirical probits (y) values< 1 and >7 are ignored (Hayes, 2014).

$$\text{Empirical probits (y)} = 5 + \frac{(x-\mu)}{s} \quad (2)$$

From equation (2) the expected probits (Yi) were derived. Chi-square values obtained from the original mortality (Observed) and derived mortality (Expected). Non-significant Chi-square test indicates good curve fitting. The LD or LC values are derived from the curve drawn using probits and log doses. Antilog to the Log_{10} value corresponds to respective probit value and 95% fiducial confidence limits are calculated using the formula:

Fiducial Limits =Antilog (Log_{10} Dose ± 1.96 (SE))

2.6 Statistical Analysis

Probit analysis used to determine the optimal lethal dose and the empirical probit units and LD_{50} were computed in Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

3.1 Effect of Mutagen on Seed Germination

The effect of gamma rays and EMS on seed germination percentage was presented in Table 1. In Gamma irradiation germination percentage ranged from 80.0% to 10.0% for TMV7 and 72.5% to 37.5% for SVPR1. At 400 Gy and 450 Gy of SVPR1 and TMV7 observed greater than 50 % of reduction in seed germination. Per cent reduction over control ranged from 54.55 % (450 Gy) to 12.12 % (250 Gy) and from 56.76 % % (450 Gy) to 13.51 % (250 Gy) for SVPR1 and TMV7 respectively. Mean germination percentage of TMV7 (67.08 %) was greater than SVPR1 (55.14 %). In case of EMS treatment maximum percent reduction was observed at 0.60 % for TMV7 (62.16 %) and SVPR1 (66.67%). TMV7 exhibited gradual reduction whereas SVPR1 showed drastic reduction with increase in EMS concentrations. Among the mutagens, EMS treated varieties recorded maximum reduction in seed germination than gamma irradiation.

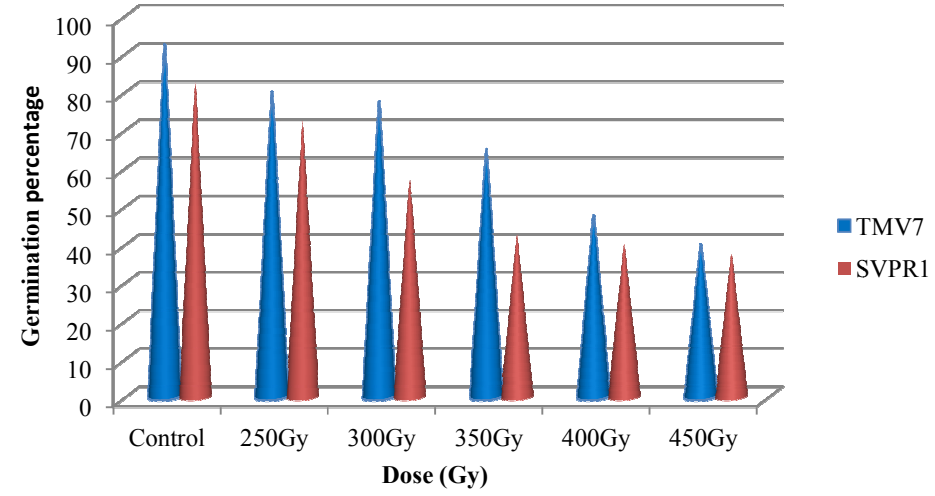
The seed germination percentage observed a negative dose dependent relationship with increase in dose or concentration in both the varieties (Fig. 1). Inhibitory effect of gamma rays attributed towards physiological injury and chromosomal damage at the cellular level of mutated seeds [17,18,19].The percentage seed germination observed gradual reduction and at higher doses maximum reduction was recorded. The above results were in agreement with [20,21] worked different sesame genotypes.

3.2 Determination of LD_{50} (Lethal Dose) Values

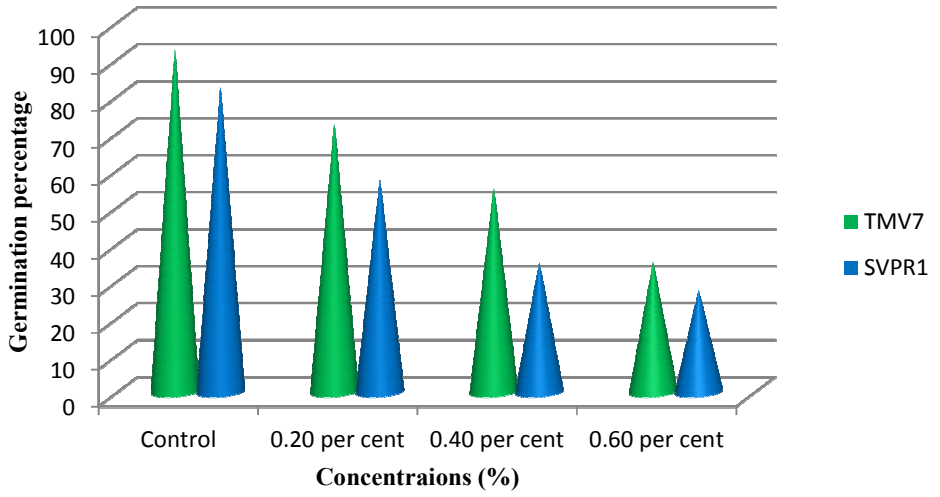
LD_{50} values for both the varieties determined through probit analysis based on seed germination. Dose response curve based on probit units were drawn and depicted in Fig. 2. The expected LD_{50} values and probit units based on mortality percentage of mutated population of TMV7 and SVPR1 presented in Table 2.

Table 1. Effect of Gamma rays and EMS on germination percentage of TMV7 and SVPR1

Mutagens		Germination percentage		Per cent above control		Per cent reduction over control	
		TMV7	SVPR1	TMV7	SVPR1	TMV7	SVPR1
Gamma rays (Gy)	Control	92.5±2.5	82.5±2.5	100.00	100.00	0.00	0.00
	250 Gy	80.0±3.7	72.5±3.5	86.49	87.88	13.51	12.12
	300 Gy	77.5±2.5	57.5±2.4	83.78	69.70	16.22	30.30
	350 Gy	65.0±4.8	42.5±2.5	70.27	51.52	29.73	48.48
	400 Gy	47.5±1.7	40.0±4.8	51.35	48.48	48.65	51.52
	450 GY	40.0±5.0	37.5±2.5	43.24	45.45	56.76	54.55
	Mean	67.08	55.14				
	SE	8.25	7.64				
EMS (%)	Control	92.5±2.5	82.5±2.5	100.00	100.00	0.00	0.00
	0.20 %	72.5±4.5	57.5±2.1	78.38	69.39	21.62	30.61
	0.40 %	55.0±0.5	35.0±1.4	59.46	42.42	40.54	57.58
	0.60 %	35.0±0.6	27.5±0.9	37.84	33.33	62.16	66.67
	Mean	63.75	50.65				
	SE	12.26	12.39				



(a)



(b)

Fig. 1. Effect of physical and chemical mutagens on germination percentage for TMV7 and SVPR1.a) gamma rays b) EMS

The lethal dose refers to the minimum concentration that cause 50% of mortality or 50% survival of mutated seeds. The LD_{50} differs between genotypes depending on the genetic background, nature of treatment and environmental conditions [22]. The LD_{50} (lethal dose) values for gamma irradiated TMV7 and SVPR1 were fixed at 416.86 Gy and 389.04 Gy respectively. For EMS treatment, the LD_{50}values were at 0.490% and 0.349% for TMV7 and SVPR1 respectively. However, earlier works in sesame reported higher dose range (600-800 Gy) causing 50% lethality of mutated seeds [23,24] and is mainly attributed due to greater genetic variability [25]. The variation in LD_{50} values of the genotypes of the two species is mainly due to their difference in size, hardness, maturity and moisture content during the period of treatment [26,27]. The difference in LD_{50}values for different genotypes within the same species were reported by earlier workers [28,29] in sesame and [10,16,30] in rice and [31] in mung bean. Similar works were done in okra [32] and stem cuttings of cassava [33] through gamma irradiation and EMS treatment.

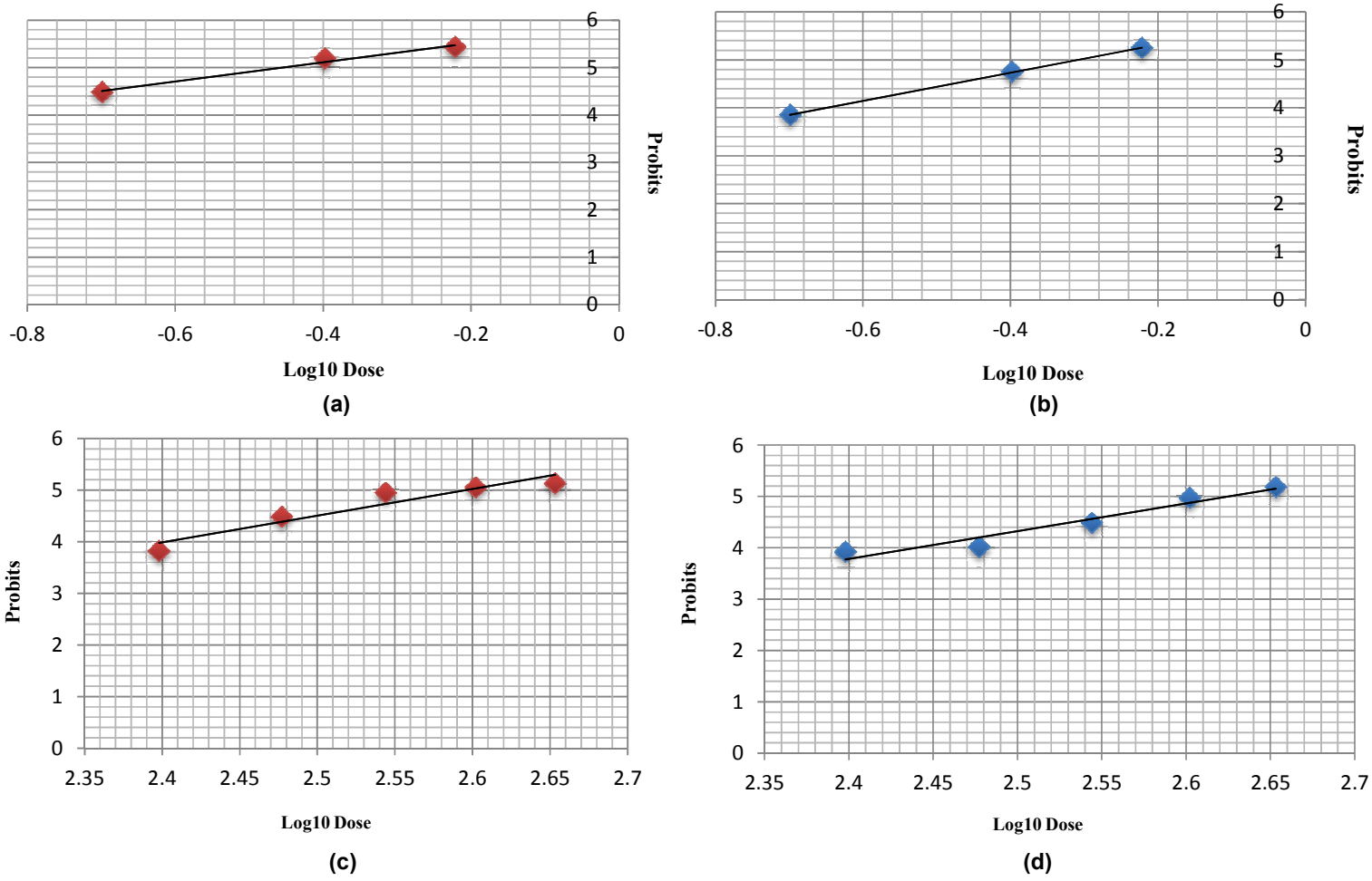


Fig. 2. Dose response curve of EMS treated (a) SVPR1,(b) TMV7 and Gamma irradiated(c) SVPR1, (d) TMV7 through probit analysis

Table 2. Probit analysis for LD_{50} calculation in TMV7 and SVPR1

Mutagens		Log10 doses	Observed mortality percentage	Corrected mortality percentage	Emprical probit units	LD_{50} value
				TMV7		
Gamma rays (Gy)	Control	-	-	-	-	416.86
	250 Gy	2.39794	20	13.51	3.92	
	300 Gy	2.477121	22.5	16.21	4.01	
	350 Gy	2.544068	35	29.72	4.48	
	400 Gy	2.60206	52.5	48.64	4.97	
	450 Gy	2.653213	60	56.75	5.18	
EMS (%)	Control	-	-	-	-	0.490
	0.20 %	-0.69897	19	12.08	3.85	
	0.40 %	-0.39794	45	40.54	4.75	
	0.60 %	-0.22185	63.5	60.16	5.25	
SVPR1						
Gamma rays (Gy)	Control	-	-	-	-	389.04
	250 Gy	2.39794	27.5	12.12	3.82	
	300 Gy	2.477121	42.5	30.30	4.48	
	350 Gy	2.544068	57.5	48.48	4.95	
	400 Gy	2.60206	60	51.51	5.05	
	450 Gy	2.653213	62.5	54.54	5.13	
EMS (%)	Control	-	-	-	-	0.349
	0.20 %	-0.69897	42.5	30.30	4.48	
	0.40 %	-0.39794	65	57.57	5.19	
	0.60 %	-0.22185	72.5	66.66	5.43	

4. CONCLUSION

From the present study, it was concluded that LD_{50} value for TMV7 was higher than SVPR1 in both the treatments, of which TMV7 recorded high germination rate. However EMS found to cause higher mortality rates than gamma irradiation in both the varieties examined. Hence it is evident that the cultivar TMV 7 is more sensitive to radiation and EMS as compared to SVPR 1. Both the mutagens are efficient to produce significant induced variations in sesame which can be further explored for developing populations and mutation mapping.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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