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Research Article

Determination of optimum dose of chemical mutagen for large scale seed treatment of white seeded sesame (*Sesamum indicum* L.) varieties

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Abstract
In the present study two white seeded sesame varieties VRI 3 and SVPR 1 were treated with seven different concentration *viz.,* 0.2 %, 0.4 %, 0.6 %, 0.8 %, 1 %, 1.2 % and 1.4 % of chemical mutagen Ethyl Methane Sulphonate (EMS). Germination percentage and germination index were tested using two different germination methods. Seed germination percentage and germination index was decreased with increased doses of EMS in both the varieties. The higher concentration of EMS completely arrested the seed germination. The optimum dose of the mutagen should be tested to maintain the require plant population in the M_1 generation and increasing the effectiveness and efficiency of the mutagen. LD 50 value was determined using probit analysis. For SVPR 1 and VRI 3, LD 50 value was found to be 0.36% and 0.44 % respectively. For fixing the lethal dose for the chemical mutagen, Pro tray method of germination test was found to be reliable than germination paper method.

Keywords
White seeded sesame, EMS, Optimum dose, Germination percentage, Germination Index.

INTRODUCTION

Sesame is an ancient oil seed crop belonging to the family Pedaliaceae. It is popularly known as 'Queen of oilseeds' because its seeds are good source of healthy fats, protein, B vitamins, minerals, fiber, antioxidants and other beneficial plant compounds (Bedigian and Harland. 1986). Compared to other vegetable oilseed crops, the productivity of sesame is relatively low. The major yield reducing factors are narrow genetic base, indeterminate flowering behaviour, lack of shattering resistance, longer days to maturity, low harvest index and prevalence of various biotic (Phytophthora blight, leaf spot and Phyllody) and abiotic stresses (Uzun *et al.*, 2013). Mutation breeding is one of the conventional breeding methods which offer scope of increasing sesame yield by modifying flowering behaviour, duration, shattering nature and resistance to stresses (FAO/IAEA 2001). Ethyl Methane Sulphonate (EMS) is a powerful chemical mutagen that produces random mutations ingenetic material by nucleotide substitution; particularly by guanine alkylation. EMS generally produces only point mutations in the genome. The higher doses of mutagen completely arrest the seed germination (Spencer-Lopes *et al.*, 2018). So, testing the effect of mutagen on seed germination is a prerequisite for mutation breeding. Mutagenic efficiency of a mutagen depends on its optimum dose. A dose which is nearer to LD 50 will be the optimum dose for that mutagen. It is the dose which would kill 50 % of the treated individuals. (Singh, 2014). Based on germination percentage, the optimum concentration of the mutagen can be fixed to maintain the appropriate plant population and maximise the chance of viable mutations. Based on literature, it is known that lethal dose of EMS varies with the seed colour in sesame. The LD50 values were determined based on probit analysis. The probit function

is the inverse Cumulative Distribution Function (CDF) or quantile function associated with the standard normal distribution (Finney, 1978). The aim of this study is to fix the optimum mutagenic dose for large-scale treatment using probit analysis by two different germination methods for white seeded sesame varieties.

MATERIALS AND METHODS

The present research was carried at Agricultural College and Research Institute, Madurai during 2019. Two white seeded varieties *viz.,* SVPR 1 and VRI 3 were used for this study. EMS stock solution was prepared by dissolving EMS into 0.1M sodium phosphate buffer at 7 PH. Well filled, health and uniform sized seeds were selected from both the varieties and soaked in distilled water for four hours. The moisture content of the seeds was removed with a blotting paper. Then the seeds were treated with 0.2 %, 0.4 %, 0.6 %, 0.8 %, 1.0 %, 1.2% and 1.4 % EMS working solution for four hours. Seeds were periodically well shaken with glass rod during the incubation period with the interval of 30 mins. After the incubation period, the seeds were rinsed with distilled water for ten times then in running tap water for 15 mins. After treatment, the toxic effect of remaining used EMS was inactivated with equal volume of 20 % sodium thiosulphate and 1 % sodium hydroxide solution for 24 hours. To study the effect of different concentrations of EMS on seed germination, about 200 seeds of each dose along with control were sown in paper towel method (ISTA, 1999) and pro tray with four and two replications, respectively. First and final count of germination was taken on third and sixth day after sowing, respectively. Germination percentage (Scott *et al*., 1984) and germination index (Bench Arnold *et al*., 1991) were calculated by using the following formula

Germination percentage =

$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Germination Index (GI)} = \frac{\text{number of germinated seeds}}{\text{days of first count}} + \frac{\text{number of germinated seeds}}{\text{days of final count}}$$

LD50 value for EMS was calculated based on probit analysis (Finney, 1978). Probit analysis was carried out in excel by following procedure

1. Mortality percentage of seeds was calculated for all the doses and the value was rounded to the nearest whole number.
2. Corrected mortality percentage was calculated using Abbott's formula

$$\text{Corrected mortality (\%)} = \frac{\text{M observed - M control}}{\text{100 - M control}} \times 100$$

3. All the corrected values were rounded to the nearest whole number.
4. Probit value was worked for the corresponding corrected mortality percentage value.
5. Probit graph was drawn using probit values on Y-axis against treatment concentration on X-axis.
6. EMS dose at corresponding probit 5 values was estimated as LD 50 for the mutagen

RESULTS AND DISCUSSION

The effect of EMS on germination and the rate of germination was calculated and depicted in **table 1**. Germination percentage and germination rate was examined under two different germination methods. Germination percentage over control was calculated for both the varieties after seven different concentration of EMS. Germination percentage and germination rate were markedly affected by the effect of EMS and it was shown in **Fig 1**. Percentage of germination and germination index varied for paper towel and pro tray seed germination methods. Seed germination decreased with increased concentration of EMS in both the varieties in both the methods. Similar results were already been published by Boranayaka *et al*. (2010), Kumari *et al.* (2014), Umavathi and Mullainathan. (2014) and Anbarasan *et al.* (2014). For VRI 3, the highest germination percentage 69.32 was observed for the concentration 0.2% and the lowest germination percentage 27.48 was observed for the EMS concentration 0.8% by paper towel germination method. By pro tray method, the highest and lowest germination percentage *viz.,* 97.22 and 37.5 was observed at 0.2% and 0.65%, respectively. For the variety SVPR 1 by paper towel method the highest and the lowest germination percentage *viz.,* 92.61 and 13.4 was observed at 0.2% and 1% concentrations. In pro tray method, 83.33 and 25 per cent are observed as the highest and lowest percentage of germination at 0.2% and 0.6% concentrations. For variety VRI 3 the germination percentage were observed was completely arrested above 0.8 % and 0.6 % concentration by paper towel and pro tray method of germination, respectively. In SVPR 1 the germination was completely arrested above 1% and 0.6% of EMS doses for paper towel and pro tray method of germination, respectively. The effect of EMS on seed germination was markedly varied between paper towel and pro tray method. Similar result was reported by Bellishree, *et al.* (2014) for plant growth promoting rhizobacteria in tomato. While, comparing with the paper towel method the highest germination percentage was observed in pro tray in VRI 3 variety and the reverse case for observed in SVPR 1. The rate of germination for control was 3.42 and 4.75 by paper towel method and 3.67 and 6 by pro tray method of germination in VRI 3 and SVPR 1, respectively. In treated samples, the highest rate of germination by paper towel (1.5 and 4.42) and pro tray

method (2.83 and 6) was observed for the minimum concentration of EMS for VRI 3 and SVPR 1 varieties, respectively. By paper towel method, the lowest rate of germination was observed as 0.5 in 0.8% EMS concentration for VRI 3 and 0.25 in 1% for SVPR 1. By pro tray method, the lowest rate of germination was observed as 0.83 in 0.6% EMS concentration for VRI 3 and 1.17 in 0.6% EMS concentration for SVPR 1.

Table 1. Effect of EMS on seed germination and germination index in VRI 3 and SVPR 1 varieties of sesame

Treatment	VRI 3				SVPR 1			
	RollTowel Method		Pro tray Method		Roll Towel Method		Pro tray Method	
	GPOC	GI	GPOC	GI	GPOC	GI	GPOC	GI
Control	100.00	3.42	100.00	3.67	100.00	4.75	100.00	6.00
0.2	69.32	1.50	97.22	2.83	92.61	4.42	83.33	3.00
0.4	62.78	1.42	66.67	1.33	80.43	3.83	43.75	2.75
0.6	28.64	0.50	37.50	0.83	79.30	2.58	25.00	1.17
0.8	27.48	0.50	0.00	0.00	56.55	1.08	0.00	0.00
1	0.00	0.00	0.00	0.00	13.40	0.25	0.00	0.00
1.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	26.89	0.56	28.77	0.71	46.04	1.74	21.73	0.99
SD	29.61	0.65	39.81	1.07	40.56	1.87	32.09	1.36
SE	11.19	0.25	15.05	0.41	15.33	0.71	12.13	0.51

GPOC: Germination Percentage Over Control GI: Germination Index

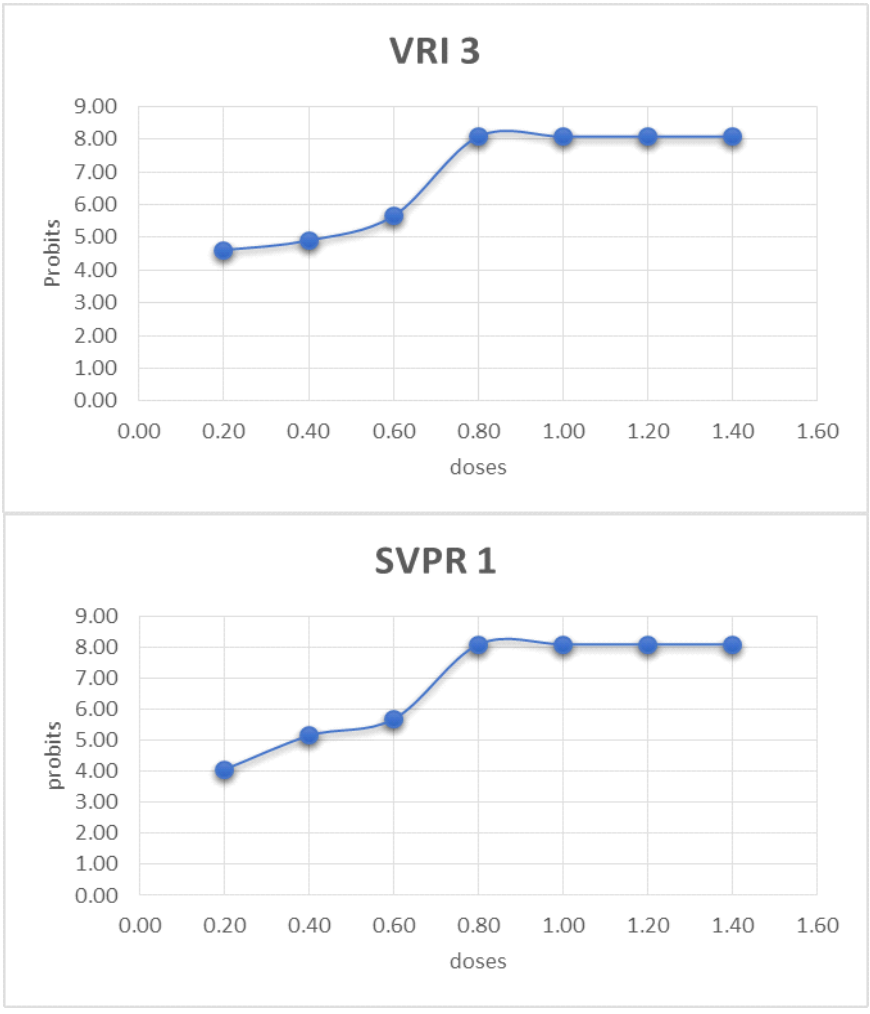
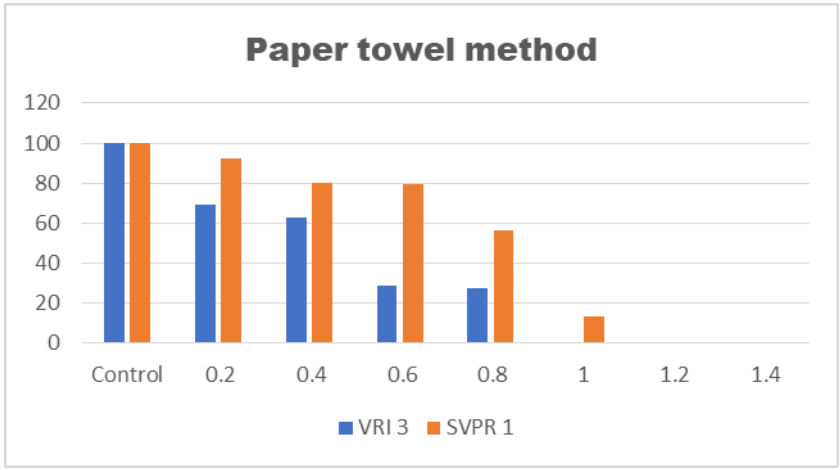


Fig. 1. Plots of Mutagenic Doses vs Probits for Calculation of LD50 of EMS in VRI 3 and SVPR 1

Table 2. Probit Analysis for Calculating LD 50 Doses

Mutagenic Doses (%)	Observed Mortality Percentage	Corrected Mortality Percentage	Probit Table Value	LD 50 Value (%)
SVPR 1				
Control	**-**	**-**	**-**	**-**
0.2	33.00	17.00	4.05	
0.4	65.00	56.00	5.15	**0.36**
0.6	80.00	75.00	5.69	
0.8	100.00	100.00	8.09	
1.0	100.00	100.00	8.09	
1.2	100.00	100.00	8.09	
1.4	100.00	100.00	8.09	
VRI 3				
Control	**-**	**-**	**-**	**-**
0.2	61.00	35.00	4.61	
0.4	80.00	47.00	4.92	**0.44**
0.6	85.00	75.00	5.67	
0.8	100.00	100.00	8.09	
1.0	100.00	100.00	8.09	
1.2	100.00	100.00	8.09	
1.4	100.00	100.00	8.09	



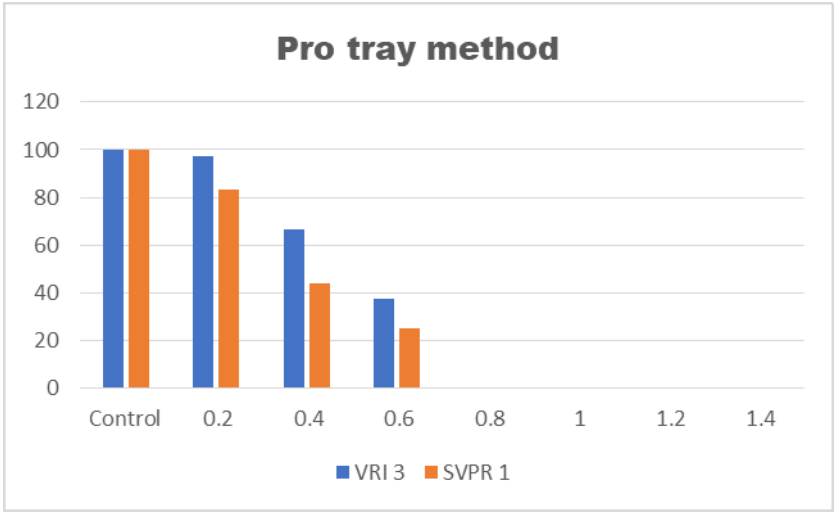


Fig. 2. Effect of EMS on Seed Germination of VRI 3 and SVPR 1

The germination index was higher in control for both the varieties and decreased with increased concentration of EMS doses. This result was in concordance with Shah *et al.* (2015). While compared to VRI 3, the germination index was higher in SVPR 1 both in control and treatments by both germination methods. The result of LD 50 value was determined by probit analysis based on mortality percentage and the probit values was depicted in **table 2**. This LD 50 value was calculated based on mortality percentage of pro tray method of germination. Graphical representation of probable LD 50 doses for VRI 3 and SVPR 1 was given in **Fig 2.** LD 50 value was calculated based on mortality percentage and probit values. Probable LD 50 value was differing in both the varieties. Calculated probability value for VRI 3 was 0.44% and for SVPR 1 was 0.36%. LD 50 value of VRI 3 was higher than the variety SVPR 1. Though VRI 3 and SVPR 1 were white seeded sesame, the response of chemical mutagen for these varieties slightly differed from each other. Different LD 50 values were observed for the different genotypes of the same crop as previously observed by Ramchander, *et al.* (2015) and Rajarajan, *et al.* (2016). From the present investigation it was concluded that the same chemical effect under same treatment procedure showed different results by different germination methods. Among the studied germination method, pro tray method of germination was more related to field condition than paper towel method. So, for fixation of LD 50 value on large scale for seed treatment germination and mortality percentage taken by pro tray method of germination should be followed.

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