Research Article

Determination of Lethal Dose for Ethyl Methane Sulphonate induced mutagenesis in Jasmine

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Abstract

Induced mutagenesis using both chemical and physical mutagens is one of the important breeding tool to enhance variability in existing germplasm. Chemical mutagens such as EMS, MMS and sodium azide have been widely used to cause a majority of practical variations in several ornamental crops. For every mutation breeding experiment, determination of mutagenic sensitivity is an important step as it will vary with every species and even among varieties. In this experiment, terminal cuttings of *Jasminum grandiflorum* cv. CO.1 Pitchi and semi hardwood cuttings of *J. auriculatum* cv. CO.1 Mullai were treated with ten different concentrations of EMS ranging from 5 mM to 50 mM.. The results revealed gradual and significant reduction in survival of cuttings, shoot length, number of leaves, leaf length and leaf width with increase in dosage of EMS. The probit curve analysis based on the survival percentage and growth rate of treated cuttings revealed the LD_{50} dosage of EMS to be 42.65mM and 44.6mM for CO.1 Pitchi and CO.1 Mullai respectively.

Keywords: Induced mutagenesis, *Jasminum*, Probit analysis, EMS

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Introduction

Jasmine is one of the important fragrant flowers used from very ancient days in India. It belongs to the family Oleaceae. The genus *Jasminum* comprises of around 200 species [1, 2] which are native to tropical and warm temperate regions of Europe, Asia and Africa. Among the species of *Jasminum*, only three species *viz., J. sambac, J. grandiflorum, J. auriculatum)* have attained commercial significance [3, 4]. Since all these species are commonly propagated by asexual means, limited variability exists in each of these species. Therefore, mutation breeding is a viable option to enhance genetic variability and to improve economic traits such as floral traits, flowering pattern, plant architecture, *etc*. Induced mutagenesis is a potential plant breeding tool which can induce the generation of allelic variants of genes that modulate the expression of traits. Mutation breeding which leads to altered phenotypes after permanent heritable change in the structure of the genetic material [5], is now established as a time-saving and inexpensive approach for flower improvement [6].

Chemical mutagens (EMS, MMS and sodium azide) and irradiation (Gamma rays, X-rays and fast neutrons) have been widely used to induce a large number of functional variations in ornamental crops. Chemicals induce mainly point mutations while ionizing radiations normally induce chromosomal rearrangements and deletions [7]. Among the alkylating chemical mutagens, Ethyl Methane Sulfonate (EMS) is the most commonly used mutagen in plants. EMS alkylates guanine bases and leads to mis-pairing of alkylated G pairs with T instead of C, resulting in primarily G/C- to -A/T transitions [8]. In order to avoid excessive loss of actual experimental materials, sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half population of the planting material survive) doses before massive exposure of similar materials. LD_{50} dose is considered as the dose at which highest frequency of mutation occurs. With this background, the present investigation was undertaken aiming to determine the optimum lethal dose (LD_{50}) for EMS in jasmine (*Jasminum* spp.).

Materials and Methods

Uniform sized healthy terminal cuttings of *J. grandiflorum* cv. CO.1 Pitchi and semi hard wood cuttings of *Jasminum auriculatum* cv. CO.1 Mullai were collected. The terminal and semi hardwood cuttings were soaked in distilled water for one hour and 6 hours respectively. Water was decanted and after shade drying, the cuttings were soaked in EMS solution (freshly prepared in phosphate buffer at pH 7.0) of different concentrations *viz.*, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mM. The soaking duration was one hour for terminal cuttings and 6 hours for semi hardwood cuttings.

After incubation at room temperature, the cuttings were thoroughly rinsed with running tap water for 1 hour to wash out the chemical residues. Thirty treated cuttings per treatment per replication were planted in nursery polythene bags filled with red soil: FYM: sand (1:1:1) along with untreated cuttings as control. Planted cuttings were placed inside polytunnels (wherein the temperature was 28–30 °C and relative humidity was 80-85%) for 25-30 days until rooting of cuttings, and then the rooted plants were shifted to 50% shade house. The percentage survival, shoot length, number of leaves, leaf length and leaf width were measured at eight weeks after planting. The experiment was laid out in Completely Randomized Design with three replicates. The LD_{50} value was calculated based on probit analysis using the survival of treated cuttings to that of control.

Probit analysis

The LD_{50} values were determined based on Probit analysis [9]. The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution.

Analysis of variance

Data obtained for growth related traits were subjected to the standard analysis of variance procedure using AGRES statistical package to identify the lethal dose (LD_{50}). The LD_{50} for each variety was estimated through the simple linear regression model by fitting the straight line equation y= a + bx; where y is the response variable (percent survival), x is the independent variable (irradiation dose), while a and b represent the slope and constant, respectively.

Results and Discussion

Determination of Lethal dose

In this present study, a gradual reduction in survival rate of cuttings with increase in dose of EMS was observed (**Table 1**). LD_{50} for EMS was fixed based on survival percentage and growth rate of cuttings in three varieties. Probit analysis was carried out based on survival rate of the stem cuttings after treatment with different doses of EMS compared with that in untreated control (**Table 2**). In the present study, LD_{50} value for EMS as assessed from the probit curve analysis and the results are depicted in **Figure 1a-b**. From the results it was found that the LD_{50}valuefor the three genotypes viz., of *J.grandiflorum* cv.CO 1 Pitchi, *Jasminum auriculatum* cv. CO.1mullai was 42.65mM and 44.65 mM respectively.

Table 1 Effect of mutagen on survival of cuttings in *Jasminum grandiflorum* (CO.1 Pitchi) and *Jasminum auriculatum* cv. CO.1 Mullai

Concentration (mM)	*Jasminum grandiflorum* cv. CO.1 Pitchi			*Jasminum auriculatum* cv. CO.1 Mullai		
	Survival percentage (%)	Per cent survival over control	Per cent reduction over control (%)	Survival percentage (%)	Per cent survival over control	Per cent reduction over control (%)
0	90	100	-	86.67	100	-
5	86.67	96.3	3.7	83.33	94.14	5.86
10	83.33	92.58	7.42	76.66	88.45	11.5
15	76.67	85.18	14.82	73.33	84.60	15.4
20	73.33	81.47	18.53	66.67	76.92	23.08
25	66.66	74.06	25.94	63.33	73.07	26.93
30	63.33	70.36	29.94	60.00	69.22	30.78
35	60.00	66.67	33.33	56.67	65.38	34.62
40	56.66	62.96	37.04	53.33	61.53	38.47
45	43.33	48.14	51.86	46.67	53.84	46.16
50	16.67	18.52	84.48	40.00	46.15	53.85
SE(d)	1.02			1.24		
CD(5%)	2.12			2.58		

Table 2 Probit analysis for calculating LD_{50} for EMS in *Jasminum grandiflorum*(CO.1 pitchi) and *Jasminum auriculatum* (CO.1Mullai)

Concentration (mM)	*Jasminum grandiflorum* (CO.1 Pitchi)				*Jasminum auriculatum* (CO.1 Mullai)			
	Observed mortality percentage	Corrected mortality percentage	Emprical probit unit	LD_{50} value	Observed mortality percentage	Corrected mortality percentage	Emprical probit unit	LD_{50} value
0	10	-	-	42.65	13	-	-	44.6
5	13	3	3.12	mM	17	5	3.35	mM
10	17	7	3.52		23	11	3.11	
15	23	14	3.92		27	16	4.01	
20	27	19	4.12		33	23	4.26	
25	33	26	4.36		37	28	4.42	
30	37	30	4.48		40	31	4.75	
35	40	33	4.56		43	34	7.82	
40	43	37	4.67		47	39	4.92	
45	57	52	5.05		53	46	4.9	
50	83	81	5.88		60	54	5.25	

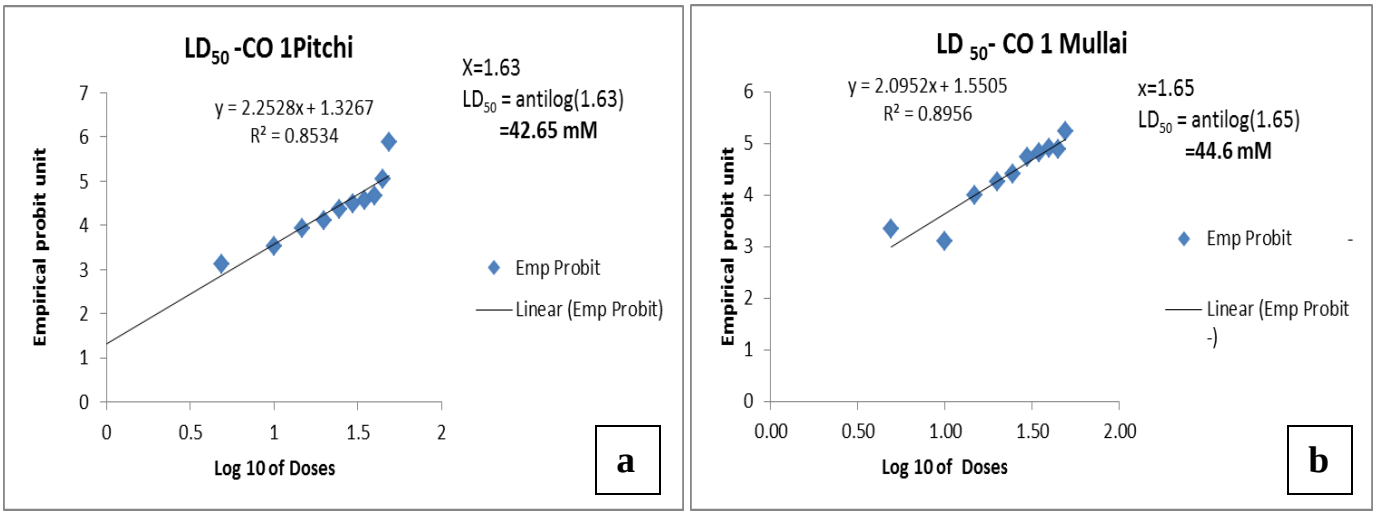

Figure 1 Probit analysis based on corrected mortality rates of (a) *J. grandiflorum* cv. CO.1 Pitchi, (b) *J. auriculatum* cv. CO.1 Mullai

Table 3 Effect of chemical mutagen on vegetative parameters of *Jasminum grandiflorum* (CO.1 Pitchi)

Concentration (mM)	Shoot length (cm)			No. of leaves			Leaf length (cm)			Leaf width (cm)		
	Actual	% over control	% reduction over control	Actual	% over control	% reduction over control	Actual	% over control	% reduction over control	Actual	% over control	% reduction over control
0	27.56	100		8.96	100		8.12	100		4.5	100	
5	25.12	91.146	8.854	7.58	84.59	15.41	7.88	97.04	2.96	4.23	94	6
10	24.63	89.36	10.64	7.25	80.91	19.09	7.15	88.05	11.95	3.96	88	12
15	24.25	87.989	12.011	6.85	76.45	23.55	6.75	83.12	16.88	3.52	78.22	21.78
20	22.48	81.56	18.44	6.53	72.87	21.13	6.31	77.7	22.3	2.85	63.33	36.67
25	20.15	73.11	26.89	6.15	68.63	31.37	6.11	75.24	24.76	2.56	56.88	43.12
30	19.85	72.02	27.98	5.45	60.82	39.18	5.69	70.07	29.93	2.43	54	46
35	17.52	63.57	36.43	5.23	58.37	41.63	5.24	64.53	35.47	2.41	53.55	46.45
40	15.69	56.93	43.07	4.56	50.89	49.11	4.88	60.09	39.91	2.3	51.11	48.89
45	12.24	44.56	55.44	4.22	47.09	52.91	3.85	47.41	52.59	2.14	47.55	52.45
50	9.42	34.17	65.83	3.34	37.27	62.73	2.79	34.35	65.65	1.22	27.11	72.89
SE(d)	0.51			0.15			0.10			0.06		
CD(5%)	1.06			0.31			0.21			0.12		

Table 4 Effect of chemical mutagen on vegetative parameters of *Jasminum auriculatum* (CO.1 Mullai)

Concentration (mM)	Shoot length (cm)			No. of leaves			Leaf length (cm)			Leaf width (cm)		
	Actual	% over control	% reduction over control	Actual	% over control	% reduction over control	Actual	% over control	% reduction over control	Actual	% over control	% reduction over control
0	20.12	100	-	24.23	100	-	5.63	100		2.75	100	
5	18.56	92.24	7.6	22.45	92.65	7.53	4.87	86.5	13.5	2.43	8.36	91.64
10	17.25	85.73	14.27	21.36	88.15	11.85	4.35	77.26	22.74	2.18	79.27	20.73
15	16.87	83.84	16.16	19.52	80.56	19.44	4.12	73.17	26.83	1.96	71.27	28.73
20	14.25	70.82	29.18	18.69	77.13	22.87	3.76	66.78	33.22	1.92	69.81	30.19
25	13.28	66	34	14.57	60.13	39.87	3.25	57.72	42.28	1.87	68	32
30	12.47	61.97	38.03	13.89	57.32	42.68	2.88	51.15	48.85	1.83	66.54	33.46
35	10.67	53.03	46.97	13.22	54.56	45.44	2.8	50.3	49.7	1.63	59.27	40.73
40	10.12	50.29	49.71	12.24	50.15	49.85	2.78	49.37	50.63	1.45	52.27	47.73
45	9.52	47.31	52.69	12.09	49.89	50.11	2.74	48.46	51.54	1.33	48.36	51.64
50	8.45	41.99	58.01	8.56	35.32	64.68	1.27	22.55	77.45	1.11	40.36	59.64
SE(d)	0.31			0.40			0.08		0.04			
CD(5%)	0.64			0.84			0.18		0.09			

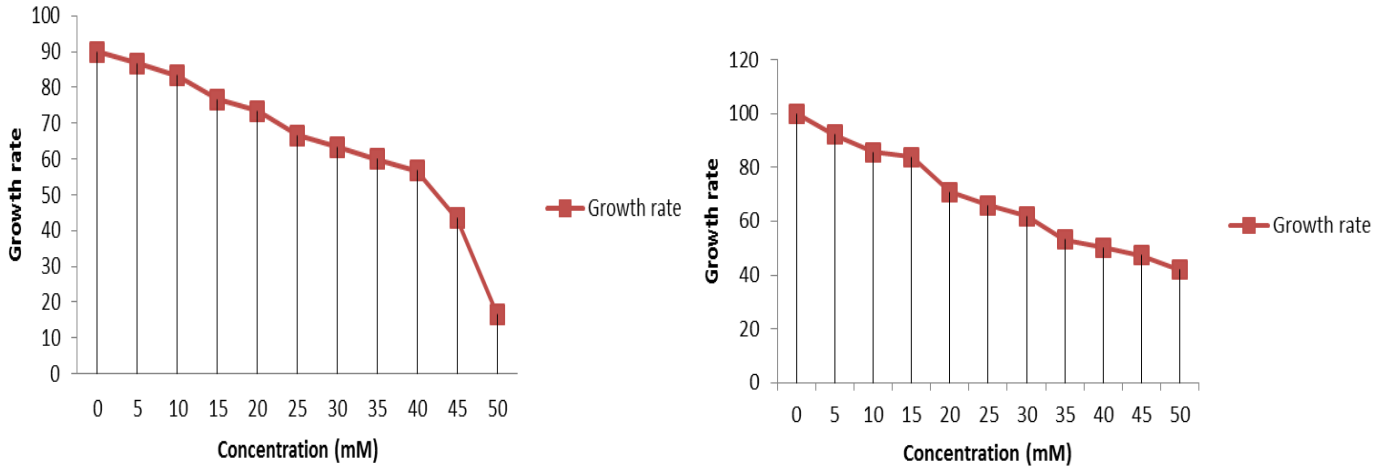

Figure 3 Mutagenic sensitivity and comparative growth rate in *J. grandiflorum* (White Pitchi)

Impact of mutagenesis on growth related traits

A declining trend with increase in EMS concentration similar to that observed for survival rate was observed for growth related traits namely, shoot length, number of leaves, leaf length and width. The least shoot length, leaf length, number of leaves, leaf width were recorded with 50 mM EMS concentration while maximum was observed in control (**Tables 3-4**).The results showed that the differences among mutation treatments considerably influence the survival, shoot length, leaf length and width and number of leaves. Moreover, with increase in the intensity of EMS concentration, the shoot length, leaf length and width shoot length proportionately decreased in a linear trend (**Figure 3**).

Among the chemical mutagens and alkylating agents, EMS has especially been demonstrated to be the most potent mutagen. Similar results of decline in the survival percentage as well as shoot length with increase in EMS concentration have been reported in Bougainvillea cultivars Roseville's Delight [10] and Mahara [11] and in cassava [12]. To identify the biological influences of different chemical mutagens, seedling height is mostly utilized as an index [13]. It has been shown that a linear dependency exists between plant height and the dosage of chemical mutagens. Observations of the present study are in agreement with the above reports.

Conclusion

The present study indicated that based on survival percentage and growth rate, LD_{50} values for EMS were 42.65 mM and 44.6mM for CO.1 Pitchi and CO.1 Mullai respectively. These optimum mutagen doses determined for the jasmine genotypes could be useful for mutation breeding studies in jasmine improvement.

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