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Studies on Mutagenic Effectiveness and Efficiency of Gamma Rays and Ethyl Methane Sulphonate in *Jasmine*

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Authors' contributions

This work was carried out in collaboration among all authors. Author SG designed the study, carried out the experiments, wrote the protocol and wrote the first draft of the manuscript. Authors MG and KS managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background/Aim: The present investigation was undertaken to study the mutagenic effectiveness and efficiency in M_1V_1 generation and to study effect of gamma rays on spectrum of morphological mutation in *Jasminum grandiflorum* Linn. cv. White Pitchi.

Methods: Terminal cuttings were treated with four doses of gamma rays *viz.,* 10, 15, 20 and 25 Gy and four doses of EMS *viz.,* 25, 30, 35 and 40 mM separately. Both mutagens created a high frequency as well as a wide spectrum of mutation.

Results: Totally five types of chlorophyll mutants *viz., xantha, viridis, yellow viridis, variegata* and *tigrina* were observed. The mutagenic effectiveness and efficiency were calculated based on biological damage as well as chlorophyll mutation frequency on M_1 plants. The mutagenic treatments were effective in inducing various types of morphological macro mutants, with few of them showing significant changes in plant height, flowering parameters and

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flower yield. The lower mutagen doses were associated with higher mutagenic effectiveness and efficiency.

Conclusion: The present study indicated that the physical mutagen gamma rays were more effective and efficient in causing mutations as compared to the chemical mutagen EMS.

Keywords: Jasminum; mutagen; gamma rays; EMS; effectiveness; efficiency.

1. INTRODUCTION

Among the major traditional flowers, Jasmine (*Jasminum* sp.) belonging to family Oleaceae is one of the most important crop in India. The volume of jasmine exported to the Middle East countries and the United States of America is rapidly increasing [1]. The jasmine species namely *J. grandiflorum, J. sambac, J. auriculatum* and *J. multiflorum* are commercially cultivated in Tamil Nadu, Karnataka, Andhra Pradesh, Uttar Pradesh and some parts of Bihar and West Bengal [2]. Propagation by vegetative means limits the variability in these species. Mutation breeding is one of the important tools to create variability in jasmine crop as it is vegetatively propagated.

Generally, both physical and chemical mutagens are employed in any mutation experiments. Two major factors *viz*., the rate of mutation and the mutation efficiency influence the success of mutation breeding. Mutagenic effectiveness is a measure of the frequency of mutation induced by unit dose of mutagen, whereas mutagenic efficiency gives an indication of the proportion of mutation in relation to undesirable changes like lethality. The mutation frequency and spectrum are affected by diverse factors, including radiation type, linear energy transfer, and radiation dose, as well as the plant tissue type and condition [3]. Keeping the points in view, the present investigation was undertaken to study the mutagenic effectiveness and efficiency in M_1V_1 generation and to study effect of gamma rays on spectrum of morphological mutation in *Jasminum grandiflorum* Linn. cv. White Pitchi.

2. MATERIALS AND METHODS

Terminal cuttings (13-15 cm long with 3 pairs of nodes) of cv. White Pitchi of *J. grandiflorum* Linn. were irradiated with 10, 15, 20 and 25 Gy of gamma rays at the dose rate of 5000 rad per minute in Gamma chamber - 1200 available at the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Treated cuttings were planted in polybags filled up to $3/4^{th}$ of the height with rooting medium (red soil + farm yard manure + sand) (1:1:1 ratio), and the top $1/4^{th}$ with sand, immediately after irradiation along with equal number of cuttings as the untreated control plants. Another set of cuttings were also treated with Ethyl Methane Sulphonate (EMS) at 25, 30, 35 and 40 mM. Initially, the cuttings were soaked in water (1 h) to activate the cells and also improve the uptake of the chemical mutagen. After shade drying, the cuttings were incubated at room temperature (1 h) as per the treatment schedule. After incubation, cuttings were rinsed with running tap water for 1 hour to wash out the chemical residues. Then the cuttings were planted in polybags with media mixture. The mutagenized M_1V_1 population was screened for deviation in the various traits including survival percentage in comparison with the control plants. Different kinds of chlorophyll mutants (*Xantha, chlorina* and *albino* etc.) were scored from emergence till the age of four week in M_1V_1 generation by using modified classification [4,5]. Mutation frequency was calculated as percentage of mutated M_1 progenies for both chlorophyll and morphological mutations in each treatment. The mutagenic effectiveness and efficiency and mutation rate were calculated based on the formulae suggested [6]. Mutation rate gives the information of mutations induced by a particular mutagen irrespective of dose or concentration. The data of all characters recorded in M_1 generation statistically analyzed with Statistical Analysis System software (SASs) V. 9.1 (June 2006), SAS Institute.

Mutagenic effectiveness (Gamma rays) = $Mp \times 100/ kR$

Mutagenic effectiveness (EMS) = $Mp \times 100/c \times t$

Where,

Mp = Chlorophyll or viable mutation frequency on M_1 plant basis

kR (or) Gy =Dose of gamma radiation

c = Concentration of the chemical mutagen in mM

t = Duration of treatment with chemical mutagen in hours

Mutagenic efficiency (%): Gamma rays and EMS= $Mp \times 100 / L$

Where,

Mp = Chlorophyll or viable mutation frequency on M_1 plant basis.

L = Percentage of lethality *i.e.,* percentage of reduction in survival of cuttings on 30th and 45th day in EMS and gamma rays respectively.

Mutation rate = (Sum of values of efficiency or effectiveness of particular mutagen/ Number of treatments of a particular mutagen).

3. RESULTS AND DISCUSSION

Results of the present investigation revealed that the survival percentage of *J. grandiflorum* Linn. decreased with the increase in the dosages (Table 1). It was found that the highest survival rate was recorded in control (93.67%) while the lowest (53.52%) was recorded for the plants treated with 25 Gy. The reduction in survival percentage ranged from 22.79% (25 mM) to 46.48%. In case of EMS treatments, the survival percentage ranged from 52.24% (40 mM) to 79.18% (25 mM). Reduction in survival percentage after mutagenic treatment was also reported in fenugreek [7]. The frequency of chlorophyll and viable mutants observed in M_1 generation is mainly used as a dependable measure of genetic effect in mutagen [8]. The mutation frequency showed a decrease with increase in the dose or concentration of mutagens.

3.1 Spectrum of Chlorophyll Mutants

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various pulse crops by several workers [9]. In the present study, classification of the chlorophyll mutants was done based on the colour patterns of the leaf/ whole plant [10]. Data on the frequency and spectrum of chlorophyll mutants in M_1V_1 generation of jasmine genotypes are presented in Table 2. The frequency of chlorophyll mutations varied with the mutagen dose/concentration in M_1V_1 generation.

The data revealed that among the five classes of chlorophyll mutants observed, the frequency of *xantha* was maximum with 5.17 at 20.0 Gy followed by 4.16 at 10.0 Gy, 2.00 at 25 Gy and 1.49 at 15 Gy of gamma irradiation. This clearly indicated that the maximum frequency of *xantha* is higher (12.82) than other classes of chlorophyll mutants namely, *viridis* (9.98), *yellow viridis* (8.55) and *variegata* (3.44). The total mutagenic frequency was found to be higher in 20.0 Gy of gamma rays (15.50) followed by 10 Gy (8.31), 25 Gy (8.00) and 15 Gy (2.98). In EMS treatments, the relative percentage (frequency) of *xantha* was found to be maximum at 30 mM concentration (4.41). The frequency of different classes of chlorophyll mutants in EMS treatments were 8.82 of *xantha,* 4.28 *of viridis,* 3.22 of *yellow viridis* and 1.26 of *tigrina*. The total mutagenic frequency was found to be maximum at 30 mM (8.82) followed by 25 mM (5.05).

The origin of chlorophyll deficiency is mainly due to mutations in genes, which are responsible for synthesis of photosynthetic pigments. It is reported that chlorophyll deficient mutants lack the well-defined grana structure of the chloroplasts [11]. Chimeric areas occur due to alterations in DNA of the chloroplasts [12,13].

Table 1. Effect of mutagens on survival percentage in M_1V_1 generation of *J. grandiflorum* cv. white pitchi

Treatment	Survival %	% over control	% reduction over control
Gamma rays (Gy)			
Control	93.67	100	-
10 Gy	72.33	77.21	22.79
15 Gy	67.33	71.88	28.12
20 Gy	58.33	62.27	37.73
25 Gy	50.14	53.52	46.48
EMS (mM)			
Control	94.67	100	-
25 mM	79.18	83.67	16.33
30 mM	68.25	72.09	27.91
35 mM	57.53	60.76	39.24
40 mM	52.24	55.18	44.82

Suggestions were made that, chlorophyll chimeras arise due to differential response of embryonic cells, which leads to the induction of changes that are not exhibited in the entire plant, but would acquire the form of chimeric structure. In Delphinium malabaricum (Huth) Munz. , a total of 11 types of chlorophyll mutants at varying frequencies in with various mutagenic treatments were reported [14]. Higher frequency and a wider spectrum of chlorophyll mutants with the chemical mutagen EMS have also been reported in carnation [15].

3.2 Spectrum of Morphological Mutants

Individual plants from M_1V_1 generation were observed for desirable variations *viz*., dwarfness, novelty in leaf colour variation, earliness of flowering, profuse flowering, *etc*. The data are presented in Table 3. The number of morphological mutants was observed to be high at lower doses of gamma rays. The treatment 10 Gy registered the maximum (15) number of mutants with 3 high yielding mutants, 2 leaf blight resistant mutants and 4 early flowering mutants. With respect to EMS treatments, 30 mM concentration registered maximum (13) number of morphological mutants with 4 high yielding mutants. The highest mutation frequency (20.74%) was registered in 25 Gy gamma irradiation and lowest at 15 Gy (16.00). In case of EMS, the highest mutation frequency was observed at 35 mM (19.27%) followed by 40 mM (13.18%).

3.3 Mutagenic Effectiveness, Efficiency and Mutation Rate

The data are presented in Table 4. The mutagenic effectiveness was found to be higher at 10 Gy (83.10%) followed by 20 Gy (15.50%), whereas in EMS, it was at concentration of 30 mM (29.40%) followed by 25 mM (20.20%). The mutagenic efficiency was recorded higher in 10 Gy of gamma radiation based on lethality (36.46%). In terms of effectiveness, among the two mutagens, gamma rays recorded higher mutation rate (53.11%) compared to EMS (14.82%). In terms of efficiency also, gamma rays recorded higher mutation rate (26.33%) followed by EMS (17.79%). The graphical representation (Fig. 1) also indicates that, the mutagenic effectiveness and efficiency was found to be higher at lower dosages of gamma radiation and EMS.

Mutagenic effectiveness can be considered as the frequency of gene mutations induced by a unit mutagen, while the mutagenic efficiency is a measure of the proportion of mutation in relation to undesirable changes like lethality, injury, and sterility. For obtaining high efficiency, the mutagenic effect should overcome other effects in the cells such as chromosomal aberrations and toxic effects. The determination of mutagenic effectiveness involves the mutagenic frequency and levels of doses. The results of mutagenic effectiveness of EMS in carnation are lined with

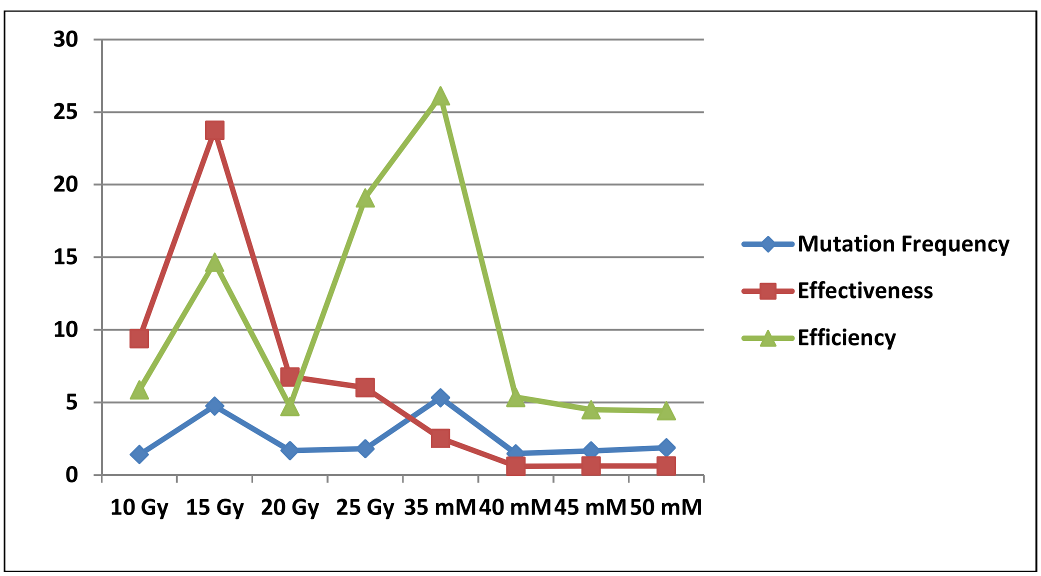

Fig. 1. Mutation frequency, effectiveness and efficiency of gamma radiation and EMS in M_1V_1 generation of *J. grandiflorum* cv. white pitchi

Table 2. Frequency and spectrum of chlorophyll mutants in the M_1V_1 generation of *J. grandiflorum* cv. white pitchi

Treatment	Number of plants observed	Spectrum of chlorophyll mutants					Total number of chlorophyll mutants	Relative percentage (frequency) of chlorophyll mutants					Total mutagenic frequency (%)
		X (*Xantha*)	V (*Viridis*)	YV (*Yellow Viridis*)	Va (*Variegata*)	T (*Tigrina*)		X (*Xantha*)	V (*Viridis*)	YV (*Yellow Viridis*)	Va (*Variegata*)	T (*Tigrina*)	
Gamma Rays (Gy)													
10 Gy	72	3	2	1	-	-	6	4.16	2.77	1.38	0.00	0.00	8.31
15 Gy	67	1	1	-	-	-	2	1.49	1.49	0.00	0.00	0.00	2.98
20 Gy	58	3	1	3	2	-	9	5.17	1.72	5.17	3.44	0.00	15.50
25 Gy	50	1	2	1	-	-	4	2.00	4.00	2.00	0.00	0.00	8.00
Total	247	8	6	5	2	-	21	12.82	9.98	8.55	3.44	0.00	34.79
EMS (mM)													
25 mM	79	2	1	-	-	1	4	2.53	1.26	0.00	0.00	1.26	5.05
30 mM	68	3	2	1	-	-	6	4.41	2.94	1.47	0.00	0.00	8.82
35 mM	57	-	-	1	-	-	1	0.00	0.00	1.75	0.00	0.00	1.75
40 mM	53	1	-	-	-	-	1	1.88	0.00	0.00	0.00	0.00	1.88
Total	257	6	3	2	-	1	12	8.82	4.20	3.22	0.00	1.26	17.50

Table 3. Spectrum of morphological mutants observed in the M_1V_1 generation of *J. grandiflorum* cv. white pitchi

Treatment	No. of plants observed	Spectrum of morphological mutants							No. of morphological mutants	Relative frequency of morphological mutants							Total mutagenic frequency (%)
		Dwarf mutant	Early flowering mutants	Altered phyllotaxy mutants	Altered stem coloured mutant	Profuse branching mutant	High yielding mutant	Leaf blight resistant mutant		Dwarf mutant	Early flowering mutants	Altered phyllotaxy mutants	Altered stem coloured mutant	Profuse branching Mutant	High yielding mutant	Leaf blight resistant mutant	
Gamma Rays (Gy)																	
10 Gy	72	2	4	1	-	3	3	2	15	2.77	5.55	1.39	0.00	4.16	4.10	2.77	20.74
15 Gy	67	1	3	-	-	3	2	1	10	1.49	4.47	0.00	0.00	4.16	2.98	1.49	14.59
20Gy	58	1	1	3	1	1	1	1	9	1.72	1.72	5.17	1.72	3.44	1.72	1.72	17.21
25 Gy	50	1	1	2	1	1	1	1	8	2.00	2.00	4.00	2.00	2.00	2.00	2.00	16.00
Total	247	5	9	6	2	8	8	5	42	7.98	13.74	10.56	3.72	13.76	10.80	7.98	68.54
EMS (mM)																	
25 mM	79	1	1	1	-	3	2	4	12	1.26	1.26	1.26	0.00	3.79	2.53	5.06	15.16
30 mM	68	1	2	-	1	2	4	3	13	1.47	2.94	0.00	1.47	2.94	5.88	4.41	19.11
35 mM	57	2	3	-	-	2	3	1	11	3.50	5.26	0.00	0.00	3.50	5.26	1.75	19.27
40 mM	53	1	1	-	-	1	2	2	7	1.88	1.88	0.00	0.00	1.88	3.77	3.77	13.18
Total	257	5	7	1	1	8	11	10	43	8.11	11.34	1.26	1.47	12.11	17.44	14.99	68.72

Table 4. Mutagenic effectiveness and efficiency based on chlorophyll mutations in the M_1V_1 generation of *J. grandiflorum* cv. white pitchi

Mutagen	% Survival reduction (L)	Mutation frequency (M)	Effectiveness	Efficiency	Mutation rate in terms of effectiveness	Mutation rate in terms of efficiency
			(M x 100) / Gy or (C x t) (%)	(M x 100) / L (%)		
Gamma rays			(Mx100) /Gy		53.11	26.33
10 Gy	22.79	8.31	83.10	36.46		
15 Gy	28.12	2.98	19.86	10.59		
20 Gy	37.73	15.50	77.50	41.08		
25 Gy	46.48	8.00	32.00	17.21		
EMS			(M x 100)/ c x t		14.82	17.79
25 mM	16.33	5.05	20.20	30.92		
30 mM	27.91	8.82	29.40	31.60		
35 mM	39.24	1.75	5.00	4.45		
40 mM	44.82	1.88	4.70	4.19		

the present study [16]. Similar results of mutation efficiency with lower dose of gamma rays were also reported in chrysanthemum [17], finger millet [18]. In another study by muutagenic effectiveness and efficiency was found to be increased with the decreased in dose or concentration [19].

In the present study, since the mutagens proved to be effective as well as efficient, the mutation rates were also calculated (Table 4). Mutation rate gives an idea about the average rate of mutation induced per mutagen. Efficient mutagenesis is the production of desirable changes with minimum undesirable effects. Generally, the mutagen that gives higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects [10]. In a mutation breeding programme, a high mutation rate accompanied by minimal deleterious effects is desirable.

4. CONCLUSION

It was observed from the above study that both gamma rays and EMS were effective in inducing morphological as well as chlorophyll variations in *Jasminum grandiflorum* Linn. Cv. White Pitchi which can be further exploited as ornamental as well as economic feature. Since chlorophyll mutants were expressed as chimeric tissue, it is recommended to undertake research and development work for management of chimera as well as to stabilize the putative mutants in future progenies to bring out the solid mutants for release as commercial variety.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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