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## Research Article

### Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate on *Hibiscus rosa-sinensis* L. Cultivar Red Single

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#### Abstract

The present investigation was undertaken to assess the spectrum of chlorophyll mutation in *Hibiscus rosa-sinensis* L. Cultivar Red Single and also to study the mutagenic effectiveness and efficiency in  $M_1V_1$  generation of *Hibiscus rosa-sinensis* L. Cultivar Red Single. Semi-hardwood cuttings of Hibiscus were treated with three doses of gamma rays viz., 25, 30 and 35 Gy and three doses of EMS viz., 0.8% (64.43 mM), 0.9% (72.48 mM), 1.0% (80.54 mM) separately. Physical mutagens created a high frequency as well as a wide spectrum of chlorophyll mutants. Totally five types of chlorophyll mutant's viz., *albino*, *xantha*, *viridis*, *chlorina* and *xantha-viridis* were observed as a result of physical and chemical mutation. The mutagenic effectiveness and efficiency were calculated based on biological damage as well as chlorophyll mutation frequency on  $M_1V_1$  plants. Mutagenic efficiency and effectiveness were higher in gamma radiation-induced plants particularly in a lower dose of 25 Gy. The highest mutation rate in terms of effectiveness and efficiency was observed in gamma rays than the Ethyl Methane Sulphonate treatment.

#### Keywords

Hibiscus, Mutation, Gamma radiation, EMS, Mutagenic effectiveness

#### INTRODUCTION

In recent years the floriculture industry is improving by introducing many novel cultivars in the market and exploration of flower crops and their utilities raising everywhere. Many ornamental flower crops contribute beauty in landscaping particularly perennial shrubs play a prominent role in establishing gardens. Hibiscus also called as China rose regarded as a standard flowering shrub for its traditional, medicinal and commercial value. *Hibiscus rosa-sinensis* L. is a flower crop native of Asia and the Pacific Islands, which is grown all over the world. Hibiscus cultivar Red Single was found to be completely sterile and hardy. Crop improvement in asexually propagated flower crops like Hibiscus,

Jasmine, Bougainvillea, Tuberose and Gladiolus can be carried out by various breeding methods. Only a limited number of combined desirable characters with a reasonable degree of fertility could be used as parents for the Hybridization breeding programme. However, mutation breeding is a suitable option to enhance genetic variability and to improve economic traits in ornamentals. An induced mutation which leads to altered phenotypes after a permanent heritable change in the structure of the genetic material (Rego and Faria, 2001) is now established as a time-saving and inexpensive approach for flower improvement (Datta and Da Silva, 2006).

Selecting appropriate mutagen, treatment method and correct dose are essential in performing a mutation breeding programme. Different type of mutagens can be employed to induce mutagenesis in vegetatively propagated crops. Physical mutagens have been extensively used for the development of new cultivars with improved characteristics in vegetatively propagated ornamental plants (Kumar *et al.*, 2006). Gamma rays proved to be an effective physical mutagen employed in creating variation, particularly in ornamental crops. Among the alkylating chemical mutagens, Ethyl Methane Sulphonate (EMS) is a popular mutagenic and carcinogenic organic compound that generates random mutations in genetic content through nucleotide substitution. It is a popular mutagenic agent, only produces point mutations (Okagaki *et al.*, 1991). Mutagenic effectiveness and efficiency decide the utility of mutagen. Mutagenic effectiveness measures the mutations induced per unit dose of mutagen. Gustafsson (1951) defined that identifying chlorophyll mutants are the most convenient method for evaluating the genetic effect of a mutation in plants. Moreover, chlorophyll mutants occurring in hardy plants may sustain further which may add ornamental value. With these backgrounds, the present study was carried out to assess the mutation effectiveness and efficiency based on chlorophyll spectrum caused by gamma rays and Ethyl Methane Sulphonate in  $M_1V_1$  generation of *Hibiscus rosa-sinensis* L. Cultivar Red Single.

## MATERIALS AND METHODS

The experiment was conducted at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India to evaluate the effect of different doses of gamma rays and ethyl methane sulphonate in *Hibiscus rosa-sinensis* L. Cultivar Red Single. Based on the lethal dose of Red Single Cultivar, three treatments have been employed for developing  $M_1V_1$  generation. 100 semi-hardwood cuttings (15-20 cm in length with pencil thickness) of Hibiscus were used as biological material in each treatment. Unlike other annuals, perennial woody shrubs were maintained with a limited number of treatment and plants in order to get a reliable output.

Three radiation doses *viz.*, 25, 30 and 35 Gy and three different concentration of EMS *viz.*, 0.8% (64.43 mM), 0.9% (72.48 mM), 1.0% (80.54 mM) separately were used to treat the cuttings along with the untreated cuttings as control. Irradiation with gamma rays was given at Indian Institute of Horticultural Research, Bengaluru. This gamma chamber installed with consistent persuasion with BRIT (Board of Radiation and Isotope Technology) and AERB with Co-60 source capacity of 518 Terabecquerel at the dosage rate of 9 kGy/hour or 0.9 Mega Rad/hour. The exposure time in the gamma chamber was 10, 12 and 14 seconds for giving a dosage of 25, 30 and 35 Gy respectively. The treated cuttings were planted in polybags. For chemical mutation, cuttings of the desired

specification were soaked in water for 1 hour. After that, cuttings were soaked in EMS solution which is freshly prepared in phosphate buffer (pH 7.0) and incubated at room temperature for 6 hours. Finally, the cuttings are subjected to washing in tap water before planting. Treated cuttings (100 cuttings per treatment) of both gamma rays and EMS were planted in polybags which are filled with rooting media (Garden soil: Sand: Farmyard manure in the ratio of 1:1:1) in prior and placed in a mist chamber for rooting. After rooting, the plants were transferred to pots. Survival percentage was observed and recorded 45 days after planting.

The  $M_1V_1$  population was screened for a spectrum of chlorophyll mutations. The chlorophyll mutants were classified as the scheme of Gustafson (1940) and Blixt (1972). Types of mutants found in this study explained and categorized as follows:

*Albina* mutants were completely devoid of chlorophyll

*Xantha* consists of pale yellow coloured leaves due to disruption in chlorophyll.

The *viridis* were represented by light green colour in the nursery stage. This colour gradually changed to the normal green colour during the subsequent period of growth and found viable in nature.

The *chlorina* mutants were yellowish -green in colour

*Xantha-viridis* mutants were characterized by both viridine green colour and bright yellow colour occurring in the same leaf.

Mutagenic effectiveness, Mutagenic efficiency and Mutation rate were calculated based on the formulae proposed by Konzak *et al.* (1965) by incorporating the mutation frequency values recorded for each mutagenic treatment.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutagenic frequency}}{\text{Dose/Concentration of the mutagen}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutagenic frequency}}{\text{Biological damage}}$$

$$\text{Mutation rate} = \frac{\text{Sum of values of efficiency or effectiveness of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

Biological damage refers to the lethality or reduction percentage over control (survival) and sprouting percentage reduction over control in this study. Since survival and sprouting of cuttings has a primary role in the establishment of ornamentals, it contributes to the biological damage.

## RESULTS AND DISCUSSION

Chlorophyll mutants are used as markers in genetic, physiological and biochemical investigations as well as chlorophyll mutation spectrum helps in identifying the potential of a mutagen. These mutants are frequently observed and easily identified factorial mutations in  $M_1V_1$  generation of vegetatively propagated crops. The spectrum of chlorophyll mutations in  $M_1V_1$  generation of *Hibiscus rosa-sinensis* L. Cultivar Red Single was presented in **Table 1**. They are *albino*, *xantha*, *viridis*, *chlorina* and *xantha-viridis* (**Fig. 2**). The order of spectrum of chlorophyll mutants in *Hibiscus rosa-sinensis* L. Cultivar Red Single is *chlorina* (21.08) > *xantha* (19.62) > *albino* (6.89) > *viridis* (6.30) > *xantha-viridis* (3.14). More number of chlorophyll mutants found in gamma radiation plants than the EMS treated. Specifically, at the lower dosage of 25 Gy, a maximum number of 8 chlorophyll mutants have been identified followed by 35 Gy treatment of gamma radiation in which 6 chlorophyll mutants observed. Four types of chlorophyll mutants occurred in gamma radiation namely *albino*, *xantha*, *chlorina* and *xantha-viridis*. Three types of chlorophyll mutants namely *xantha*, *viridis* and *chlorina* exhibited

in EMS induced plants. *xantha-viridis* observed at lower doses of gamma irradiation (25 Gy and 30 Gy) but absent at higher dose of 35 Gy. This presence or absence of mutants in one or other dose is attributed to the polygenic control of chlorophyll formation along with high mutability of genes for chlorophyll (Gaul, 1964). Chlorophyll spectrum in *Hibiscus rosa-sinensis* L. Cultivar Red Single was more diverse with gamma irradiation than EMS application. Among the mutants recorded, *chlorina* was more predominant followed by *xantha*. *Chlorina* was present in all the doses of the mutagens and was expressed at a maximum frequency in all the mutagenic treatments (**Table 1**). These results are in concurrence with the output of Anitha *et al.* (2017) in *Bougainvillea spectabilis* Willd. (cv. Lalbagh). *Viridis* and *xantha-viridis* type of mutants were rarely induced and its frequency which also found to be low. Chlorophyll deficiency is mainly due to mutations in genes, which are responsible for the synthesis of photosynthetic pigments. It is also reported that chlorophyll deficient mutants lack the well-defined grana structure of the chloroplasts (Bendict and Ketring, 1972). Chimeric areas occur due to alterations in the DNA of the chloroplasts (Nybom, 1956).

**Table 1. Frequency and spectrum of chlorophyll mutants in  $M_1V_1$  generation of *Hibiscus rosa-sinensis* L. (Cultivar Red Single)**

Mutagenic treatments	Classes of chlorophyll mutants					Number of chlorophyll mutants	Number of plants observed	Relative percentage of chlorophyll mutants					Mutagenic frequency
	<i>Albino</i>	<i>Xantha</i>	<i>Viridis</i>	<i>Chlorina</i>	<i>Xantha-viridis</i>			<i>Albino</i>	<i>Xantha</i>	<i>Viridis</i>	<i>Chlorina</i>	<i>Xantha-viridis</i>	
<b>Gamma rays</b>													
<b>25 Gy</b>	2	2	--	3	1	8	73	2.73	2.73	0.00	4.10	1.36	10.95
<b>30 Gy</b>	1	1	--	2	1	5	56	1.78	1.78	0.00	3.57	1.78	8.92
<b>35 Gy</b>	1	3	--	2	--	6	42	2.38	7.14	0.00	4.76	0.00	14.28
<b>Ethyl Methane Sulphonate</b>													
<b>0.8%</b>	--	3	--	2	--	5	78	0.00	3.84	0.00	2.56	0.00	6.41
<b>0.9%</b>	--	1	1	2	--	4	51	0.00	1.96	1.96	3.92	0.00	7.84
<b>1.0%</b>	--	1	2	1	--	4	46	0.00	2.17	4.34	2.17	0.00	8.69
<b>Total</b>	4	11	3	12	2	32	346	6.89	19.62	6.30	21.08	3.14	

Determination of mutation frequency and manipulation of the mutation spectrum in crop improvement is a prominent step in a mutation study. In this experiment, mutation frequency recorded a maximum of 14.28 at 35 Gy gamma-ray treatment and the lowest frequency of 6.41 was noticed at 0.8% EMS. In addition, there is no linear trend formation with increase or decrease of doses in gamma radiation. Similar results was observed in the chemical mutation of rice (vinithashri *et al.*, 2020). However, an EMS treatment there is a gradual rise in mutation frequency with increased concentration (**Fig. 1**). The response of cells of higher plants to physical and chemical mutagens is influenced to a varying degree by numerous biological, environmental and chemical factors. Gaul (1970) reported that physical and chemical mutagens induce physiological damages (injury), gene

mutations (point mutation) and chromosomal mutations (chromosomal aberration) of the biological material in  $M_1$  generation. Variation caused by mutation identified and documented in  $M_1V_1$  generation itself, in case of vegetatively propagated plants. Survival and sprouting percentage of hibiscus cuttings decreased with increase in doses of gamma rays and EMS (**Table 2**). Among these two mutagens, higher diminution of survival percentage was noticed in the case of gamma irradiation than EMS (**Table 2**). These results were in agreement with Bhat *et al.* (2007), in which they proved that chemical mutagens alone produced point mutations, whereas radiations normally caused chromosomal aberrations and deletions. The gradual reduction in survival and sprouting with an increase in the dose of mutagens (**Table 2**) was also reported by Patil (2015) in chrysanthemum, Anitha *et*

al. (2017) in bougainvillea and Ghosh *et al.* (2018) in Jasmine. Reduction in survival after exposure to gamma-ray was attributed to inactivation and/or decrease in auxin content that affects cell division, resulting in poor establishment and survival (Wain and Wightman, 1956, Mahure *et al.*, 2010) or lethal effect of gamma rays caused due to chromosomal aberration (Banerji and Datta, 1990, Ditta *et al.*, 2003). At a cellular level, cell mitosis gets blocked resulting in the reduction of growth in the meristem exposed to mutagenic treatment (Arunyanart and Soontronyatara, 2002). Mutagenic effectiveness indicates the frequency of mutations induced by a unit dose of mutagen and mutagenic efficiency is a measure of the proportion of mutation in relation to lethality and sprouting percentage reduction. Biological damage is

purely dose-dependent which increased with increased concentration/dose of gamma rays and Ethyl Methane Sulphonate (Table 2). In the present study, the mutagenic effectiveness found to be highest (43.80) at a lower dose (25 Gy) of gamma radiation and lowest at a lower dose of EMS (0.8%) with the value of 9.94 (Table 3). Effectiveness was comparatively lower in EMS than Gamma radiation (Fig. 2). This is because cuttings (vegetative propagule) respond well to the physical mutagen than chemical mutagen. Mutagenic efficiency determined based on the lethality of cuttings recorded highest value (60.90) at 25 Gy gamma-ray followed by 0.8% EMS in which 51.86 was observed (Table 3). Likewise, mutagenic efficiency calculated based on sprouting reduction (Biological damage) also found in the same pattern in which the

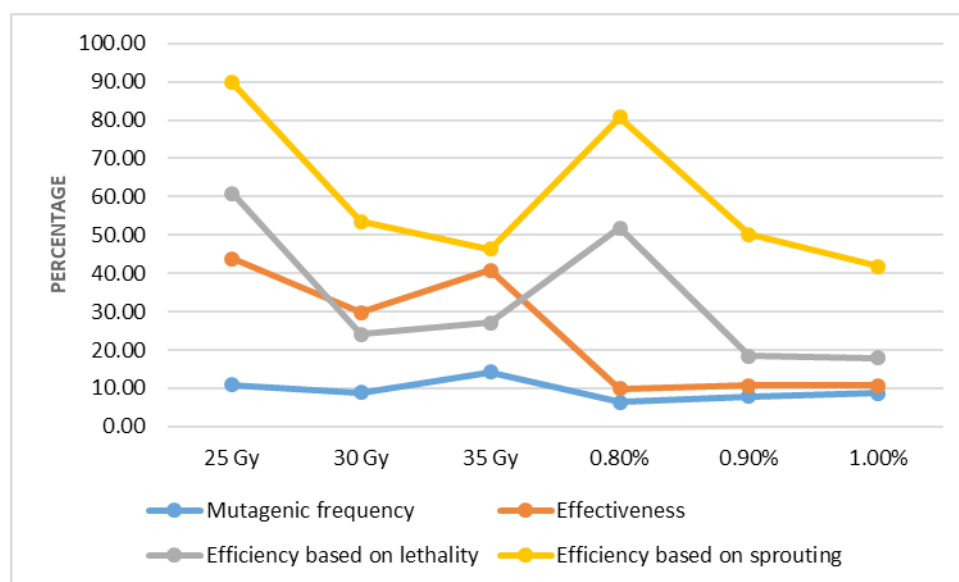


Fig. 1. Mutation frequency, effectiveness and efficiency of gamma radiation and EMS in  $M_1V_1$  generation of *Hibiscus rosa-sinensis* L. (Cultivar Red Single)

Table 2. Effect of gamma radiation on survival of *Hibiscus rosa-sinensis* L. Cultivar Red Single

Mutagens	Survival (%)	% over control	% Reduction over control	Sprouting (%)	%over control	% Reduction over control
Control	89	100.00	---	95.10	100.00	---
<b>Gamma rays</b>						
25 Gy	73	82.02	17.98	83.50	87.80	12.20
30 Gy	56	62.92	37.08	79.25	83.33	16.67
35 Gy	42	47.19	52.81	65.80	69.19	30.81
<b>Ethyl Methane Sulphonate</b>						
0.8%	78	87.64	12.36	87.56	92.07	7.93
0.9%	51	57.30	42.70	80.25	84.38	15.62
1.0%	46	51.69	48.31	75.32	79.20	20.80

mutagenic efficiency was maximum (89.75) in the lower dose of gamma radiation (25 Gy) (Table 3). Mutagenic efficiency was also found to be highest in gamma radiation treatment than EMS (Fig. 2). Similar results of mutation efficiency with the lower dose of gamma rays were also

reported by Padmadevi (2009) in chrysanthemum. In chemical mutagens, properties such as solubility, toxicity and chemical reactivity will limit their effectiveness in most of the cases (Spencer-Lopes *et al.*, 2018).



Control

*xantha-viridis* @ 25 Gy  
gamma - radiation*xantha* @ 30 Gy gamma-radiation*albino* @ 30 Gy gamma-  
radiation*xantha* @ 35 Gy  
gamma - radiation*xantha* @ 1.0% EMS*viridis*@ 0.9% EMS*chlorina* @ 0.8% EMS

Fig. 2. Chlorophyll mutants isolated in  $M_1V_1$  generation in *Hibiscus rosa-sinensis* L. (Cultivar Red Single)

**Table 3. Mutagenic effectiveness and efficiency based on chlorophyll mutations in the M<sub>1</sub>V<sub>1</sub> generation of *Hibiscus rosa-sinensis* L. (Cultivar Red Single)**

Mutagen	Biological damage		Mutation frequency	Effectiveness	Efficiency	
	Survival reduction over control (%)	Sprouting reduction over control (%)			Based on Survival reduction over control	Based on Sprouting reduction over control
<b>Gamma rays</b>						
25 Gy	17.98	12.20	10.95	43.80	60.90	89.75
30 Gy	37.08	16.67	8.92	29.73	24.05	53.50
35 Gy	52.81	30.81	14.28	40.80	27.04	46.34
<b>Ethyl Methane Sulphonate</b>						
0.8%	12.36	7.93	6.41	9.94	51.86	80.83
0.9%	42.70	15.62	7.84	10.81	18.36	50.19
1.0%	48.31	20.80	8.69	10.79	17.98	41.78

**Table 4. Mutagen Rate in Terms of Effectiveness and Efficiency in M<sub>1</sub>V<sub>1</sub> generation of *Hibiscus rosa-sinensis* L. (Cultivar Red Single)**

Mutagen	Mutation rate in terms of effectiveness	Mutation rate in terms of efficiency	
		Lethality	Sprouting reduction
<b>Gamma rays</b>	38.11	37.33	63.19
<b>Ethyl Methane Sulphonate</b>	10.51	29.40	57.60

The overall mutation rate in terms of effectiveness and efficiency was outstanding in gamma radiation treatment at a lower dose whereas, in the case of EMS, it is comparatively low (Table 4). The maximum mutation rate in terms of effectiveness (38.11) was obvious in gamma-ray mutation. At the same time, the mutation rate in terms of efficiency also reflected the same results (Table 4). Mutation rate based on lethality and sprouting were calculated as 37.33 and 63.19 respectively (Table 4). For obtaining high efficiency, the mutagenic effect should overcome other effects in the cells such as chromosomal aberrations and toxic effects. High mutation rate accompanied by minimal deleterious effects is desirable for a successful mutation programme. But generally, the mutagen that gives the higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects (Blixt *et al.*, 1964). In this experiment, both gamma rays and EMS is found to be effective and efficient. However gamma radiation is found to be effective and efficient for causing variation in vegetatively propagated crops. EMS is also a useful mutagenic agent at lower doses causing limited changes in the existing cultivar. Both physical and chemical mutagen has its role in enhancing the variation in Hibiscus.

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