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Research Article

Efficiency of gamma irradiation and ethyl methane sulphonate in inducing variations in *Jasminum auriculatum* Vahl.

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Abstract
Vegetatively propagated crops like *Jasminum auriculatum* have a constraint in genetic variation due to a narrow genetic base that restricts the insights in any crop breeding programme. Mutation breeding is a potential tool that directs a way to create desirable variation effectively in vegetatively propagated crops. The optimum dose of mutagen is the one which produces the maximum frequency of mutation in turn the variation, with minimum killing. The aim of the present paper unveils the Lethal Dose (LD_{50}) and the growth reduction dose (GR_{50}) for both Gamma irradiation (GI) and Ethyl methane sulphonate (EMS) for the ecotype 'Muthu Mullai' of *Jasminum auriculatum.* These parameters of GR and EMS treated cuttings were analysed by considering the GR_{50} values of the mortality rate (57%, 48%), survival percentage (12.479Gy, 13.268mM), shoot length (18.59Gy, 18.28mM), root length (20.39Gy, 18.17mM), number of leaves (22.29Gy, 17.47mM), number of sprouts (22.97Gy, 16.17mM), vigour index (10.43Gy, 11.05mM), leaf length (21.61Gy, 19.90mM) and leaf width (19.2Gy, 16.17mM). The LD_{50} value was 12.479 Gy for GI and 13.268 mM for EMS treatment. The GR_{50} for different growth parameters ranged from 14.93 to 22.9 Gy for GI and 1.05 to 19.9 mM for EMS treatment. The mutagenic efficiency and effectiveness were 214.96 and 89.36 for GI and 48.66 and 33.77 for EMS treatment, respectively. These doses can be used for generating considerable variation, which can be put to use in future crop improvement programmes for Jasmine.

Keywords: Effectiveness, GR_{50}, LD_{50}, Mutation, Mutagenic efficiency

INTRODUCTION
Almost 95 years ago, the invention of induced mutations by H.J. Muller and L.J. Stadler in fruit fly and maize and in barley using X-rays has marked a remarkable change in plant breeding for improving the economic characteristics of a crop (Auerbach., 1976). Earlier, scientists had identified many varieties through different physical and chemical mutations. The variation in any crop depends upon the genetic constitution of the plant variety. Jasmine belongs to the family Oleaceae is one of the important traditional flowers in India. Among 200 species of Jasmine reported, *Jasminum auriculatum* (Yamaguchi *et* al., 2008) is the second most important commercial crop that has been widely used as a loose flower, in aromatherapy for its high concrete recovery and fragrance compounds, in the pharmaceutical industry for their anti-lithiatic and wound healing properties and in treating skin diseases as traditional medicine.

Improvement of a vegetatively propagated crop like *J. auriculatum* depends on the presence of genetic variation in the existing germplasm. It has been more than three decades since an improved variety in *J. auriculatum* was developed, *viz*., CO.2 Mullai, which Tamil Nadu Agricultural University developed, Coimbatore, Tamil Nadu in the year 1988(Ghosh *et al.,* 2018). Considering the breeding behaviour of jasmine, mutation breeding is an effective tool to create variation in terms of corolla tube length, fragrance, and pest and disease resistance, as well as for improving the yield. Many earlier researchers, including (Alan *et al*., 2019) and (Ghosh *et al*., 2020) have reported that the highest probability of getting effective variability is with the doses associated with 50 per cent mortality. Apart from the Lethal dose (LD_{50}), another dose that plays a major role in increasing the effectiveness of the mutagen in the Growth Reduction percentage (GR_{50}) for which the doses are fixed at 50 per cent reduction in the growth parameters of the plant by comparing with the control. Therefore, the initial step in inducing mutation is fixing the LD_{50} and GR_{50}. There are two important goals of mutation research viz., the enhancement of mutation frequency and alteration of mutation spectrum in a predictable manner. Mutagenic effectiveness can be considered as the frequency of gene mutations induced by a unit mutagen, while mutagenic efficiency is a measure of the proportion of mutation in relation to undesirable changes like lethality, injury, and sterility. The determination of mutagenic effectiveness involves the mutagenic frequency and levels of doses. Contemplating the above facts, the present experiment was conducted to induce variation by the physical mutagen Gamma irradiation (GI) and the chemical mutagen Ethyl methane sulphonate (EMS). After that, the appropriate lethal dose (LD_{50}) and growth reduction (GR_{50}) doses and the mutagenic effectiveness and efficiency for inducing variations in *J. auriculatum* were also determined.

MATERIALS AND METHODS

Planting material

A new ecotype of *J. auriculatum* called "Muthu Mullai" which is commercially cultivated in the Coimbatore district of Tamil Nadu was used for the study.

Gamma and EMS treatment

Semi-hardwood cuttings with 15 cm length of Muthu Mullai collected from a farmers' field at Vellipalayam village, Coimbatore district, Tamil Nadu, were subjected to varying doses of Gamma radiation (0, 5Gy, 10Gy, 15Gy, 20Gy, 25Gy, 30Gy and 35Gy) and Ethyl Methane Sulphonate (0, 5mM, 10mM, 15mM, 20mM and 25mM). The Gamma chamber (Model- GC 5000) with synthetic ^{60}Co gamma source available at the Indira Gandhi Centre for Atomic Research (IGCAR), Department of Atomic Energy, Kalpakkam, Tamil Nadu was used for the study. The mutated planting material was planted in a mist chamber maintained with 95% relative humidity (RH) along with the untreated control plants.

Data collection

Observations on different vegetative parameters, including survival percentage, plant height (cm), shoot length (cm), root length (cm), number of leaves, number of sprouts, leaf length (cm), leaf width (cm) and vigour Index were recorded 60 days after planting of the cuttings. The experimental design employed in the study was Completely Randomized Block design with eight doses of gamma irradiation (including control) and six doses of EMS (including control) in three replications. A hundred cuttings were used per dose for gamma rays and 50 per dose for EMS treatment.

Mutagenic effectiveness

Mutagenic effectiveness is a measure of the frequency of mutation induced by a unit dose of mutagen, whereas mutagenic efficiency gives an indication of the proportion of mutation in relation to undesirable changes like lethality. The effectiveness and efficiency of the two mutagens were worked out by using the formulae suggested by (Konzak*et al*., 1965).

Mutagenic effectiveness (%) for Gamma rays = Mp x 100 /kR (or) Gy ----(Eq. 1)

Mutagenic effectiveness (%) for EMS = Mp x 100/ c x t ….(Eq. 2)

Where,

Mp: Chlorophyll or viable mutation frequency on M1 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 x 100 / L …… Eq. 3

Where,

Mp : Chlorophyll or viable mutation frequency on M1 plant basis

L : Percentage of lethality, i.e., percentage of reduction in survival of cuttings on 60^{th} day in EMS and gamma rays respectively (Konzak *et al*., 1965).

Mutation rate

Mutation rate (MR) was calculated with the following formula.

Mutation rate = Sum of values of efficiency or effectiveness of particular mutagen / Number of treatments of a particular mutagen ……..Eq.4

This gives the information of mutations induced by a particular mutagen irrespective of dose or concentration (Ambavane *et al.*, 2015).

Statistical analysis

One-way ANOVA for all the vegetative parameters was done at 0.5% of the level of significance. The Lethal Dose (LD_{50}) was estimated by the regression method adopting Probit analysis based on mortality rates. For the analysis of GR_{50}, Simple Linear Regression on the dose-response curve has been carried out using all the vegetative parameters. The analysis was performed using the IBM-SPSS software.

RESULTS

Lethal dose (LD_{50}) for GI and EMS

In the present investigation, one-way ANOVA results for the growth parameters of the Gamma and EMS treated plants of *J. auriculatum* (Muthu Mullai) were highly significant at 5% significance level. The response of the survival percentage to the dose displayed a negative linear relationship, indicating that the increased dose of mutagen will cease the survival rate. The LD_{50} values analysed by the Probit method based on the mortality percentage for Gamma and EMS-treated Muthu Mullai plants were found to be at 12.479 Gy and 13.268 mM, respectively (Table 1).

Growth reduction (GR_{50}), mutagenic effectiveness, efficiency and mutation rate for GI and EMS

The GR_{50} values for all the growth parameters studied were highly significant at 5% level. The 50 % growth reduction for survival rate when treated with GR and EMS were 14.98Gy and 18.20mM respectively. The GR_{50} values for all the vegetative parameters were 14.98Gy and 18.20mM (Survival percentage), 18.59Gy and 18.28mM (shoot length), 20.39Gy and 18.17mM (root length), 22.29Gy and 17.47mM (no. of leaves), 21.61Gy and 19.90mM (leaf length) and 19.2Gy and 16.98mM (leaf width) for both Gamma rays and EMS treated plants respectively (Table 2 and 3).

In GI and EMS treatment, the R^2 value of 0.680 was obtained for the number of sprouts indicating that the

Table 1. Probit analysis of GI and EMS treated *J. auriculatum*

Mutagen	Linear equation	Chi-square value	LD_{50}
GI	y=4.161x -4.561	6.786	12.479
EMS	y = 2.922x-3.281	9.893	13.268

Table 2. Linear Relationship between various growth parameters and gamma irradiation dosages of *J. auriculatum*

S. No.	Parameter	GR_{50}	R^2 Value	Linear regression Equation
1.	Survival rate	14.98Gy	0.936	y = -3.114x + 96
2.	Shoot length	18.59Gy	0.827	y = -0.172x + 8.2
3.	Root length	20.39Gy	0.906	y = -0.301x + 14.15
4.	No. of leaves	22.29Gy	0.960	y = -0.209x + 9.666
5.	Leaf length	21.61Gy	0.985	y = -0.148x + 6.209
6.	Leaf width	19.2Gy	0.849	y = -0.058x + 2.62
7.	Vigour Index	10.43Gy	0.853	y = -69.66x + 2001
8.	No. of sprouts	22.97Gy	0.680	y = -0.047x + 2.583

Table 3. Linear Relationship between various growth parameters and EMS dosages of *J. auriculatum*

S. No.	Parameter	GR_{50}	R^2 Value	Linear regression equation
1.	Survival rate	18.20mM	0.997	y = -2.585x + 95.07
2.	Shoot length	18.28mM	0.902	y = -0.165x + 7.017
3.	Root length	18.17mM	0.896	y = -0.356x + 14.97
4.	No. of leaves	17.47mM	0.932	y = -0.235x + 9.107
5.	Leaf length	19.90mM	0.996	y = -0.159x + 6.425
6.	Leaf width	16.98mM	0.970	y = -0.078x + 2.750
7.	Vigour Index	11.05mM	0.892	y = -67.82x + 195
8.	No. of sprouts	16.17mM	0.778	y = -0.064x + 2.535

Table 4. Frequency and spectrum of chlorophyll mutants in *J. auriculatum* treated with GI and EMS

Treatment	No. of plants observed	Spectrum of chlorophyll mutants				Total no. of chlorophyll mutants	Relative percentage of chlorophyll mutants				Total Mutagenic frequency
		X (Xantha)	V (Viridis)	YV (Yellow Viridis)	C (Chlorina)		X (Xantha)	V (Viridis)	YV (Yellow Viridis)	C (Chlorina)	
5Gy	72	1	2	2	2	6	1.388	2.777	2.777	2.777	9.722
10Gy	59	1	2	1	2	5	1.694	3.389	1.694	3.389	10.16
15Gy	40	2	1	2	1	5	5	2.5	5	2.5	15
20Gy	22	1	-	-	1	2	4.545	0	0	4.545	9.090
25Gy	12	-	1	-	1	2	8.333	0	0	8.333	16.66
5mM	41	1	1	-	1	3	2.439	2.439	0	2.439	7.317
10mM	31	1	1	-	-	2	3.225	3.225	0	0	6.451
15mM	18	1	-	-	-	1	5.555	0	0	0	5.555
20mM	11	-	-	-	1	1	0	0	0	9.090	9.090
25mM	9	-	-	1	-	1	11.11	0	11.11	0	22.22

doses might not influence more on it. The GR_{50} for the number of sprouts is 22.97Gy and 16.17mM, respectively for gamma and EMS treated plants. The Vigour index measured by the shoot length, root length and survival percentage showed significant results and the GR_{50}value for both the Gamma and EMS treated plants observed is 10.43Gy and 11.05mM, respectively (Table 2 and 3).

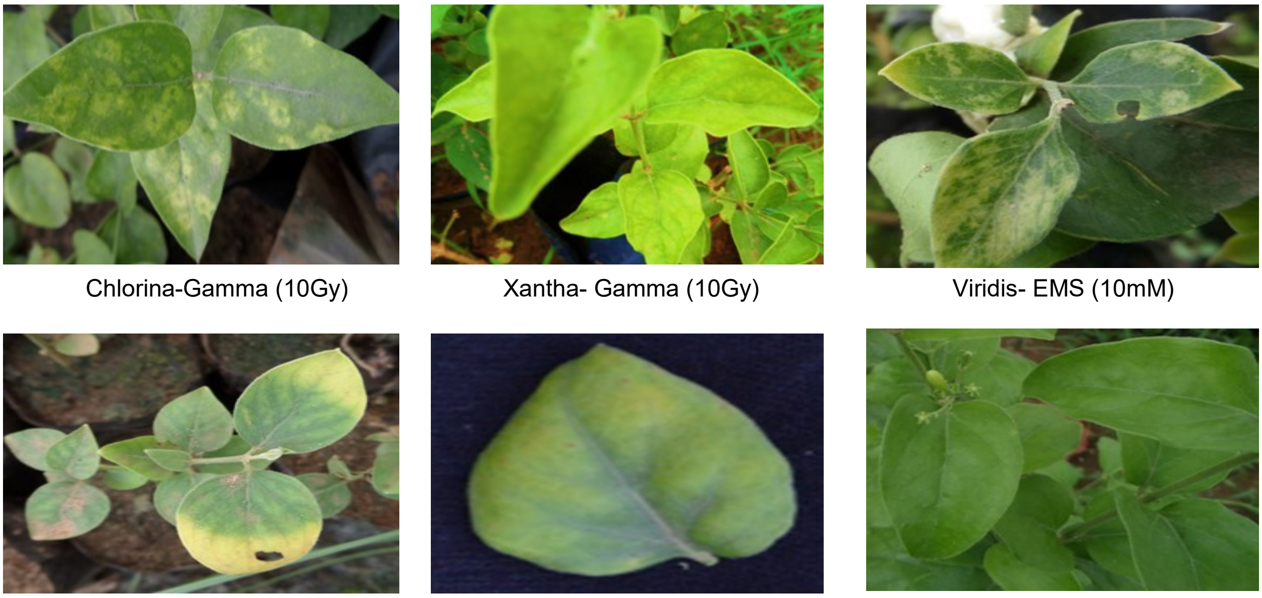
The mutagenic effectiveness was found to be higher in GI (Table 4) with 10Gy (84.74576), whereas in EMS treatment (Table 5), it was at a concentration of 5mM (73.17). The mutagenic efficiency was recorded higher in 5mM of EMS based on lethality (36.58). GI recorded a higher mutation rate (72.71) in terms of effectiveness than EMS (33.77) (Fig 1). Similarly, GR recorded a higher mutation rate of 18.12 in terms of efficiency, followed by EMS (13.25).

DISCUSSION

Many factors, such as genotype, age, chromosome number, and environment, determine the dosages required to induce mutation-induced variation (Bado *et al*., 2017). The dose necessary to trigger mutation differs considerably between vegetatively propagated and seed propagated crops, possibly due to more active metabolising tissues in the latter (Dada *et al*., 2018). Therefore, before beginning an effective mutation work, LD_{50} and GR_{50} levels are required to be standardized. The desired frequency of the doses should be assessed and standardised to enhance the possibility of causing economically useful variations and to obtain higher mutagenesis efficiency.

In the present study with Muthu Mullai, the LD_{50} values for GI and EMS treatment were 12.479 Gy and 13.268 mM, respectively. Earlier, Ghosh *et al*. (2018) fixed LD_{50}values for the cv. CO1 Mullai of *J. auriculatum* as 20.4 Gy and 44.6 mM for GI and EMS, respectively. Such variations in the doses among the variety and the ecotype of the same species might be due to genetic differences of the plant type. Datta *et al*. (2001) stated that LD_{50} is different for each type of plant depending on the growth stage, plant development, and parts of plants irradiated.

GR_{50} is another tool to achieve the anticipated mutations based on the growth reduction observed with the different doses in the GI and EMS treated plants. The GR_{50} values ranged from 14.93 Gy to 22.9Gy for and 1.05 mM to 19.9 mM for EMS, respectively. At doses lower than GR_{50}, desirable effects and variations may not be obtained. Also, higher doses might cause deleterious effects on the plants causing damage to the plant cells and the plant population per treatment was also affected (Layek *et al*., 2021). Hence, it is inferred from the present study that for getting effective genetic varia-



Chlorina-Gamma (10Gy) | Xantha- Gamma (10Gy) | Viridis- EMS (10mM)

Yellow viridis- Gamma (5Gy) | Chlorina – EMS (20mM) | Control

Fig 1. *Chlorophyll mutants obesrved with gamma irradiation and ethyl methane sulphonate treated plants of J. auriculatum (Muthu Mullai)*

Table 5. Mutagenic effectiveness, efficiency and mutation rate of GI and EMS treated *J. auriculatum*

Mutagen	% Survival reduction(L)	Mutation frequency(M)	Effectiveness	Efficiency	Mutation rate in terms of Effectiveness	Mutation rate in terms of Efficiency
			(M*100)/Gy or (C*t)	(M*100)/L (%)		
GI						
5Gy	26	4.166667	83.33333	16.02564	72.70673	18.12972
10Gy	39	8.474576	84.74576	21.72968		
15Gy	58	12.5	83.33333	21.55172		
20Gy	76	9.090909	45.45455	11.96172		
25Gy	86	16.66667	66.66667	19.37984		
EMS						
5mM	10	3.658537	73.17073	36.58537	33.77936	13.25988
10mM	30	3.225806	32.25806	10.75269		
15mM	56	2.777778	18.51852	4.960317		
20mM	70	4.545455	22.72727	6.493506		
25mM	74	5.555556	22.22222	7.507508		

tions in Muthu Mullai ecotype of *J. auriculatum,* the recommended doses for treatment of GI and EMS are 10 to 20Gy and 10mM to 20mM, respectively.

Mutagenic effectiveness and efficiency varied with doses in different genotypes. Based on the chlorophyll mutants (Table 4 and 5), the mutagenic effectiveness and efficiency were found since they are the indices for the mutability of a cultivar. Chlorina, xantha, yellow virids and viridis are the prominent chlorophyll mutants observed (Fig 1). Mutagenic effectiveness and efficiency represent the ecotype response to the increase in the dose of the mutagen. The mutagenic effectiveness was found to be higher at the 10Gy and 5mM doses for GI and EMS, indicating that biological damage at higher doses to the ecotypes is due to the mutagen effect. The mutagenic effectiveness was found to be almost lower at higher dosages of gamma radiation and EMS indicating that the higher dose of treatment will increase the lethality in the population, thereby, the effectiveness and efficiency will be affected. In *J. sambac* (Syarifah *et al*., 2005) and Bambara groundnut (Ismaila *et al*., 2021) also observed a general decrease in effectiveness with increasing doses of gamma irradiation.

Conclusion

An effective technique for improving ornamental crops is mutation breeding. *J. auriculatum* (Muthu mullai) is a vegetatively propagated crop with limited variability; mutagenesis can be used to widen the genetic variability. The LD_{50} and GR_{50} values vary with the species, variety, and the ecotype. Hence, for inducing economically useful variation in the *J. auriculatum* by GI s and EMS, the most effective and efficient mutagenic doses were 10 to 20 Gy and 10mM to 20mM for GI and EMS treatment. Based on these doses, a higher frequency of useful mutants will be observed in the successive generation and further utilized for varietal development. Hence, the study approves the applicability of EMS and GI for inducing mutation in the ecotype of *J. auriculataum.*

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Conflict of interest

The authors declare that they have no conflict of interest.

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