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Resolving the acute gamma irradiation and ethyl methanesulphonate induced lethality for *Jasminum sambac* L. (Aiton) cv. Ramanathapuram Gundumalli

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Abstract

Crop improvement in *Jasminum sambac* through induced mutagenesis entails the insight on the sensitiveness of the individual plant materials of a species to different classes and nature of the mutagens. The current assessment was undertaken to analyse the mutagenic effect of acute gamma irradiation and EMS in *Jasminum sambac cv.* Ramanathapuram Gundumalli. Semi-hardwood cuttings of the plants were exposed to different dosage levels of gamma rays varying from 0 Gy (control) to 40 Gy and decapitated rooted cuttings were treated with EMS from 0 mM (control) to 90 mM. The M_1V_1 generation was evaluated by treating the cuttings with four different doses of gamma rays (15 Gy, 20 Gy, 25 Gy and 30 Gy) and three concentrations of EMS (45 mM, 60 mM, 75 mM) formulated using respective LD₅₀ doses. The lethality dosage (LD₅₀) value for the cultivar Ramanathapuram Gundumalli was determined to be 21.37 Gy and 64.57 mM for gamma irradiation and EMS, respectively applying the probit curve analysis. The maximal reduction in the survival of cuttings of 75% and 71% correspondingly in gamma and EMS treatments was discerned at higher doses in comparison to the lower doses. The plants studied in M_1V_1 generation for growth and flowering characters exhibited variable responses pertaining to differing doses or concentrations of the specific mutagenic means.

Keywords: Jasminum sambac, gamma irradiation, EMS, LD_{50} and semi-hardwood cuttings

INTRODUCTION

Jasminum sambac (L.) Aiton is one of the important species in the family Oleaceae, tribe Jasmineae and genus Jasminum commonly known as Arabian jasmine. The species is native to tropical Asia explicitly to the eastern Himalayas in Bhutan and India and distributed across humid tropical climatic South and Southeast Asian countries, islands of the Indian and Pacific Oceans, tropical America and the Caribbean (Acevedo-Rodriguez and Strong, 2012; Govaerts R, 2016). It is an evergreen perennial flowering shrub noted for its exhilarating fragrant flowers. J. sambac flowers yield classic quality flower oil nevertheless of the low concrete recovery percentage (0.12-0.19%) (Bhattacharjee and De, 2003). Significantly, Tamil Nadu holds the principal position in cultivation of *J. sambac* engrossing about 13,088.00 ha of area and 1,34,785.90 tonnes of production during 2019-2020 (Department of Horticulture and Plantation Crops, Government of Tamil Nadu).

J. sambac is a vegetatively propagated indigenous flower species embodying many cultivars, ecotypes feasibly developed in course of cultivation through the natural crossing, spontaneous mutation and autopolyploidy (Bhatnagar, 1956; Chandra, 1982) with variation in their chromosome number spanning from 2n=2x=26 to 2n=3x=39 (Raman, 1955). Among those cultivars,

Ramanathapuram Gundumalli is predominantly cultivated in Tamil Nadu owing to its better adaptability and higher yield hence, crop improvement in the cultivar has been emphasized by the floricultural community. Heterozygosity, triploid nature and negligible seed setting in the species (Srivastava and Karmakar, 1986) have constricted to adopt clonal selection, mutation and polyploidy breeding methods in the crop development.

Mutations occur constantly in nature and these spontaneous mutations determine the natural genetic variations that promote the development of new varieties or enhancement of the prevailing ones. The decelerating natural mutation rate of 1× 10⁻⁸ to 10⁻⁹ per generation in molecular organisms forces to choose induced mutations through physical and chemical agents. Totipotency nature of the plant cells employs wide variety of plant propagules i.e., seeds, tubers, stem cuttings, rhizomes, pollen, anther, embryos, microspores, in vitro cultured calli/ plantlets, whole plants in artificial random mutagenesis (Mba, 2013; Suprasanna et al., 2014; Bado et al., 2015). The optimum irradiation dose for application varies with species and genotype (Lee et al., 2014) and it must result in a mortality rate of 50% of the treated material (LD₅₀) and growth reduction of 30-60% (GR₃₀₋₆₀) in M₁ generation (Mba, 2013; Bado et al., 2015). There are about 4907 plant mutant varieties registered in the FAO/IAEA Mutant Variety Database. Tamil Nadu Agricultural University, Coimbatore has made a salient contribution of developing two high yielding varieties viz., CO.1 - Pitchi and CO.2 -Pitchi of J. grandiflorum species with gamma rays (Kumar and Gill, 1983). In J. sambac cv. Gundumalli, combination treatments of physical (gamma rays) and chemical (EMS) mutagens exhibited improved floral attributes (Kannan et al., 2002). Among diverse physical mutagens, gamma rays are commonly employed in plant mutation induction. Gamma rays are high energy, low wavelength electro-magnetic radiations discovered by Paul Villard in 1900 emitted by nuclear decay of different radioactive sources (Cobalt- 60, Caesium-137, Technetium-99m and Americium-241) while the former ones are utilized in gamma irradiation facilities for biological substances. Gamma rays cause DNA strand breaks and induce chromosomal rearrangements in treated biological material. Ethyl methanesulphonate (C₃H₂SO₃), a nontransgenic general mutagenic organic compound is ubiquitous in plant mutation breeding for typically producing single base point mutations in genetic material by substitution of nucleotides catalyzed through guanine alkylation (Sega, 1984).

The present study was taken up to assess the response of the leading *J. sambac* cultivar to different dosages of mutagenic treatments for sprouting and survival percentage and shoot parameters. Based on the reaction in the plant materials, optimum LD_{50} value was presumed.

MATERIALS AND METHODS

The experiment was conducted at the Department of Floriculture and Landscape Architecture, Coimbatore during the period from November 2019 to April 2020 and the cultivar, Ramanathapuram Gundumalli was used for mutagenic treatments. The plant materials were collected from well maintained, productive five-year-old mother plants at Polakapatti (9°56'36''N 77°53'04''E), Usilampatti taluk, Madurai district. Uniform sized semihardwood cuttings of 12-15 cm in length cuttings were subjected to acute irradiation treatments (0 Gy -control, 5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy and 40 Gy) utilizing Gamma chamber 5000 with synthetic radioactive isotope 60Co provided by Board of Radiation and Isotope Technology (BRIT) at Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Tamil Nadu. The exposure time for the absorbed dose/radiation doses of 0 Gy(control), 5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy and 40 Gy was calculated using the dosimetry value of the gamma radiation chamber on the particular day. Similarly, the three months old decapitated rooted cuttings of the cultivar were treated with EMS for six hours at different concentrations of 15mM, 30 mM, 45 mM, 60 mM, 75 mM and 90 mM and 0 mM (control) dissolved in freshly prepared phosphate buffer in neutral pH/ pH 6-7 to prevent the decomposition of the mutagen (Leitao, 2012).

The treated cuttings were planted on the following day of treatment in poly bags filled with media composed of sand, red soil and farmyard manure, as well as retained in poly tunnels. After 75 days of planting and initiation of rooting, sprouted plants of gamma ray treatments were transferred to a 50% shade net house for hardening and the survival percentage, shoot length, leaf length and width were computed. Survival percentage, the number of sprouts, leaf length and leaf width were recorded in EMS treated rooted cuttings after 45 days of treatment. To induce mutation and selection of viable mutants, an increased number of planting materials were treated with two levels of doses/concentrations higher and lower of determine LD50 value. Then these survived plants of both mutagenic treatments were transplanted in grow bags and observation of M_1V_1 generation were done.

The lethality dose value of the cultivar to gamma irradiation and EMS was deduced based on the probit regression analysis availing Finney's table (Finney, 1971). The LD_{50} was determined by fitting the straight-line equation/ simple linear regression model y=ax+b, where y is the response variable (corrected mortality percentage) and x is the independent variable (gamma irradiation dose/EMS concentration). The growth-related shoot parameters were statistically analyzed in the SPSS software package (SPPS version 16) and MS Excel spreadsheet with variance estimates and critical difference at 5% probability.

RESULTS AND DISCUSSION

The mutagen treatments resulted in a consistent reduction in survival rate of the cuttings with an increase in concentration of the mutagens. The radio-sensitivity for gamma irradiation and lethality dose for EMS was evolved from the mortality percentage of the plant materials using probit analysis (Table 1 and 2). The LD_{50} for gamma rays and EMS from the probit curve analysis was 21.37 Gy and 64.57 mM, respectively (Fig. 1 and Fig. 2). The dose of gamma radiation that brings down the survival rate to 40-60% and growth reduction rate to 30-50% over the non-irradiated control plant propagules in the gamma ray mutated plants are used to standardize the optimum dose of gamma irradiation (van Harten, 1998; Yamaguchi, 2013). The plant materials exposed to gamma rays showed 50% survival over control when exposed to 20 – 25 Gy. Gamma irradiation had a significant effect on the shoot length, the number of leaves, leaf length and leaf width (Table 3). There was a decline in the shoot length and the number of leaves of the irradiated plants to 30% in 25 Gy treatment (Fig. 3), and based on the survival percentage and growth rate, LD₅₀ dose for the cultivar can be optimized as 20-30 Gy. The radiobiological activity of ionizing gamma irradiation in plants is heterogeneous and the primary ones are meristematic damage, which

was exhibited *via* growth inhibition and plant mortality and the loss of reproductive integrity was observed through pollen sterility (Yamakawa and Sparrow, 1966; Underbrink *et al.*, 1973). The LD₅₀ value of *J. sambac cv*. Ramanathapuram Gundumalli for gamma irradiation are in accordance with the cultivars of the same genus *i.e.*, 17.8 Gy for *J. grandiflorum cv*. White Pitchi, 28 Gy for *J. multiflorum cv*. Arka Arpan, 25.1 Gy for *J. nitidum* culture line (Ghosh *et al.*, 2018) and 33 Gy for *J. officinale cv*. Samanpichcha (Nelka *et al.*, 2021). The effects of gamma rays irradiation on the hardy plant tissues are visualized in the species and genotype specific tolerability dose of the plant materials to survive, regenerate and develop promising expressive mutants.

The penetration efficiency of the chemical mutagen into the tissues of axillary and apical meristems in the vegetative propagules determines the suitability for use in mutation trials and moreover the woody natured materials are recalcitrant to any type and dose of chemical mutagens (Spencer-Lopes *et al.*, 2018). In the experiment, the survival rate of *in vivo* decapitated rooted plants soaked in EMS solutions of seven concentrations from 0(control), 15 mM, 30 mM, 45mM, 60 mM, 75mM and 90 mM revealed a diminishing proliferation pattern. The significant and

Table 1. Probit analysis for gamma irradiation in J. sambac cv. Ramana	hapuram Gundumalli
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Dose (Gy)	Log ₁₀ of doses	Survival percentage	Percent survival over control	Percent reduction over control	Observed mortality percentage	Corrected mortality percentage	Empirical probit units	LD_{50} value
0 (Control)	-	94	100.00	-	6	-	-	
5	0.70	81	86.17	13.83	19	14	3.92	
10	1.00	70	74.47	25.53	30	26	4.36	
15	1.18	68	72.34	27.66	32	28	4.42	
20	1.30	53	56.38	43.62	47	44	4.85	21.37 Gy
25	1.40	44	46.81	53.19	56	53	5.08	
30	1.48	36	38.30	61.70	64	62	5.31	
35	1.54	27	28.72	71.28	73	71	5.55	
40	1.60	24	25.53	74.47	76	74	5.64	

Table 2. Probit analysis for EMS treatments in J. sambac cv. Ramanathapuram Gundumalli

Concentration (mM)	Survival percentage	Percent survival over control	Percent reduction over control	Log ₁₀ of concentrations	Observed mortality percentage	Corrected mortality percentage	Empirical probit units	LD₅₀ value
0 (Control)	96	100.00	-	0.00	4	-	-	
15	88	91.67	8.33	1.18	12	8	3.59	
30	80	83.33	16.67	1.48	20	17	4.05	
45	68	70.83	29.17	1.65	32	29	4.45	64.57 mM
60	52	54.17	45.83	1.78	48	46	4.90	
75	44	45.83	54.17	1.89	56	54	5.10	
90	28	29.17	70.83	1.95	72	71	5.55	



Fig.1. Probit analysis for gamma radiation on survival of stem cuttings of *J. sambac cv.* Ramanathapuram Gundumalli



Fig.2. Probit analysis for EMS on survival of rooted cuttings of J. sambac cv. Ramanathapuram Gundumalli

progressive deduction in the mean values of the number of sprouts, leaf length and leaf width with an increase in concentration was noticed (**Table 4**). The mean number of sprouts (4.56), leaf length (6.40 cm) and leaf width (4.02 cm) were highest in the control plants when analogized with a mean number of sprouts (1.56), leaf length (3.10 cm) and leaf width (2.53 cm) in 90 mM of EMS treated plants. The survival rate and the number of new sprouts declined with an increase in the concentration of EMS with about 50% reduction over control non-treated plants in 60-75 mM (**Fig. 4**). Hence, the optimum dose for EMS of the cultivar can be assigned to around 60-75 mM.

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Dose (Gy)	Shoot length (cm)			No. of leaves			Leaf length (cm)			Leaf width (cm)		
	Actual	Percent over control (%)	Percent reduction over control (%)	Actual	Percent over control (%)	Percent reduction over control (%)		Percent over control (%)	Percent reduction over control (%)	Actual	Percent over control (%)	Percent reduction over control (%)
0	24.18	100.00	-	24	100.00	-	6.16	100.00	-	3.66	100.00	-
5	23.6	97.60	2.40	21.4	89.17	10.83	5.48	88.96	11.04	3.3	90.16	9.84
10	21.10	87.26	12.74	20.2	84.17	15.83	5.32	86.36	13.64	3.14	85.79	14.21
15	19.04	78.74	21.26	20	83.33	16.67	5.28	85.71	14.29	3.12	85.25	14.75
20	18.34	75.85	24.15	17.2	71.67	28.3	4.26	69.16	30.84	3.04	83.06	16.94
25	16.98	70.22	29.78	16.8	70.00	30.00	4.12	66.88	33.12	3.02	82.51	17.49
30	15.92	65.84	34.16	13.8	57.50	42.50	3.98	64.61	35.39	3.0	81.97	18.03
35	14.40	59.55	40.45	13.6	56.67	43.33	3.84	62.34	37.66	2.88	78.69	21.31
40	13.50	55.83	44.17	12.8	53.33	46.67	3.12	50.65	49.35	2.44	66.67	33.33
SEd	0.83			0.96			0.18			0.12		
CD(5%)	1.77			2.03			0.38			0.25		

Table 3. Effect of gamma rays on vegetative parameters in M_0V_0 generation



Fig. 3. Influence of gamma rays on shoot parameters of stem cuttings

These findings are analogous with the LD_{50} values for *J. grandiflorum var.* CO.1 Pitchi, White Pitchi, *J. auriculatum var.* CO.1 Mullai, *J. multiflorum var.* Arka Arpan and *J. nitidum* Ac.Jn-1 of 42.65 mM, 37.15 mM, 44.60 mM, 40.70 mM, 42.66 mM correspondingly (Ghosh *et al.*, 2018a).

Irradiation and chemical mutagen treatments of different levels had a distinct influence on vegetative and flowering characters of the jasmine cultivar in M_1V_1 generation (**Table 5 & 6**). The plant height of gamma irradiated plants ranged from 14.2 cm (30 Gy/) to 64.0 cm (15 Gy)

with the highest mean plant height of 35.99 cm in the control treatment and the least value of 27.27 cm in 30 Gy whereas, the mean plant height was highest in 45 mM EMS treated jasmine plants (34.89 cm) almost equivalent to control plants (35.05 cm) and the plant height differed from 19.5 cm (75 mM) to 51.5 cm (45 mM). The number of primary branches in gamma ray and EMS treated plant materials ranged from 2 to 9 in 20 Gy and 45 mM and 3 to 9 in 25 Gy. The increase in the number of primary branches implies a positive effect on the better framework and flower production of the plants. The mean number of

Dose		Number of s	prouts		Leaf leng	th (cm)	Leaf width (cm)			
(mM) [−]	Actual	Percent over control (%)	Percent reduction over control (%)	Actual	Percent over control (%)	Percent reduction over control (%)	Actual	Percent over control (%)	Percent reduction over control (%)	
0	4.56	100.00	-	6.40	100.00	-	4.02	100.00	-	
15	3.94	86.40	13.60	5.82	90.97	9.03	3.66	90.88	9.12	
30	3.89	85.31	14.69	5.26	82.12	17.88	3.53	87.85	12.15	
45	2.94	64.47	35.53	4.64	72.57	27.43	3.36	83.43	16.57	
60	2.67	58.55	41.45	3.97	61.98	38.02	3.13	77.90	22.10	
75	2.61	57.24	42.76	3.56	55.56	44.44	2.74	68.23	31.77	
90	1.56	34.21	65.79	3.10	48.44	51.56	2.53	62.98	37.02	
SEd	0.14			0.098			0.058			
CD (5%)	0.31			0.213			0.126			



Fig. 4. Influence of EMS treatments on survivability and growth on the rooted cuttings

secondary branches was highest for the 25 Gy gamma irradiation treatment (9.25), while the lowest was noted in 60 mM EMS treatment. The range of number of secondary branches was highest in the gamma rays treated plants of 3 to 15 and 2 to 15 in 15 Gy and 20 Gy, respectively while, the lowest range was observed in EMS treated plants in 60 mM and 75 mM (3 - 9). In case of stem girth of the mutagen treated cuttings, the mean value ranged from 5.28 mm (control) to 6.76 mm (25 Gy) for the gamma irradiation treatments and from 5.64 mm (60 mM) to 6.98 mm (45 mM) for the EMS treatments. The inter-nodal length measured ranged from 2.93 cm (30 Gy) to 3.25 cm (25 Gy) for gamma irradiation treatments and from 2.86 cm (75 mM) to 3.00 cm (control). All the vegetative characters

recorded *viz.*, plant height, the number of primary and secondary branches, stem girth and inter-nodal length were highest in ⁶⁰Co gamma rays treated plants compared to untreated plants (control). In regard to EMS treatments, plant height, the numbers of secondary branches, internodal length were observed maximum in the control plants compared to EMS treated plants in M_1V_1 generation. Thus the higher concentration of EMS has exacerbated the growth inhibition potential in the treated rooted cuttings of the jasmine plants. The lower and intermediary doses or concentrations of gamma rays and EMS have a stimulatory effect on cell replication and elongation, yielding a biopositive vegetative effect in comparison to higher ones (Chandrashekar, 2014). The dose dependent reduction in

Treatments	Plant height (cm)		Number of primary branches		Number of secondary branches		Stem girth (mm)		Inter-nodal length (cm)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
				Ga	mma Rays					
Control	35.99 ±0.57	27.2 - 52.9	5.47±0.37	3 - 7	7.0±0.61	4 - 12	5.28±0.09	3.85 - 6.61	3.11±0.11	2.0 - 4.4
15 Gy	32.29 ± 2.08	22.4 - 64.0	5.20±0.20	2 - 8	7.06±0.41	3 - 15	6.18±0.53	4.38 - 8.77	2.95±0.22	1.9 - 4.3
20 Gy	34.91±1.23	19.0 - 53.7	5.27±0.48	2 - 9	7.40±0.92	2 - 15	6.28±0.51	3.31 - 7.73	3.25±0.16	2.0 - 5.1
25 Gy	28.13±1.74	15.7 - 42.0	4.93±0.24	3 - 9	9.25±0.14	5 - 13	6.76±0.29	3.68 - 8.57	3.14±0.12	2.6 - 4.8
30 Gy	27.27±0.74	14.2 - 37.7	4.40±0.42	2 - 7	8.33±0.53	3 - 14	6.26±0.49	3.54 - 8.62	2.93±0.04	2.4 - 3.6
					EMS					
Control	35.05±1.40	26.2 - 49.5	4.87±0.24	2 - 7	8.80±0.42	4 - 15	5.96±0.60	3.76 - 8.36	3.00±0.14	1.9 - 3.8
45 mM	34.89±1.37	24.5 - 51.5	5.07±0.27	2 - 9	7.08±0.94	2 - 11	6.98±0.34	3.74 - 9.14	2.97±0.07	2.1 - 3.6
60 mM	27.06±1.01	21.5 - 39.5	4.47±0.31	3 - 8	5.33±0.46	3 - 9	5.64±0.49	3.55 - 8.13	2.91±0.14	1.8 - 3.7
75 mM	24.41±0.96	19.5 - 27.4	3.91 ±0.36	2 - 6	6.58±0.17	3 - 9	5.98±0.29	4.77 - 9.58	2.86±0.17	1.6 - 3.7

Table 5. Effects of mutagens on vegetative parameters in M_1V_1 generation

Table 6. Effects of mutagens on floral attributes in M₁V₁ generation

Treatments	Number of flowering cymes per plant		Flower buc (cm		Corolla tul (cn		Diameter of open flower (cm)		
	Mean ± SE	Range	Mean ± SE	Ra nge	Mean ± SE	Range	Mean ± SE	Range	
			G	amma Rays					
Control	5.67±0.85	2 - 10	2.36±0.03	2.0 - 2.7	1.35±0.02	1.2 - 1.6	2.72±0.08	2.4 – 3.1	
15 Gy	5.50±1.95	1 - 16	2.41±0.21	1.6 - 3.1	1.35±0.06	1.0 - 1.6	2.94±0.11	2.5 – 3.5	
20 Gy	5.83±0.22	2 - 10	2.61±0.05	2.1 - 3.0	1.49±0.01	1.2 - 1.7	3.02±0.02	2.8 -3.5	
25 Gy	4.58±0.46	2 - 11	2.54±0.11	1.9 - 2.8	1.43±0.02	1.3 - 1.5	2.96±0.09	2.4 – 3.2	
30 Gy	4.25±0.38	1 - 8	2.43±0.13	1.6 - 2.8	1.34±0.09	0.8 - 1.8	2.73±0.17	2.0 - 3.5	
				EMS					
Control	5.83±0.73	3 - 10	2.38±0.05	2.0 - 2.6	1.34±0.04	1.0 - 1.6	2.91±0.07	2.5 – 3.3	
45 mM	5.0±1.09	2 - 11	2.16±0.09	1.6 - 2.7	1.29±0.01	1.1 - 1.5	2.83±0.01	2.1 – 3.5	
60 mM	4.58±0.96	2 - 9	2.14±0.06	1.8 - 2.4	1.27±0.10	0.9 - 1.7	2.48±0.14	2.0 - 3.3	
75 mM	3.67±0.17	2 - 6	1.85±0.19	1.3 - 2.9	1.24±0.04	0.6 - 1.6	1.88±0.20	1.3 - 2.9	

morphological characters was observed in independent gamma rays and EMS, and combination treatments of gamma rays and EMS treatments in *J. sambac* cv. Gundumalli (Mekala *et al.*, 2009) and *J. grandiflorum* cv. CO.1 Pitchi, *J. auriculatum* cv. CO.1 Mullai, *J. multiflorum* cv. Arka Arpan and *J. nitidum* culture Acc- Jn-1 (Ghosh *et al.*, 2018 b).

The physical and chemical mutagen treatments boosted the existing floral quality traits of the cultivar to a certain extent in the M_1V_1 generation. The number of flowering cymes per plant ranged from 1 to 16 in 15 Gy, 2 to 11 in 25 Gy of gamma irradiation and 2 to 11 in 45 mM of EMS treatments, implying that mutagens can exert both enhanced and decreased effect on the plants. The mean number of flowering cymes was the highest in the gamma irradiation treatment of 20 Gy, while it was lowest in EMS treatments compared to the control. The maximum length of the flower buds was found in 15 Gy (3.10 cm) and 3.0 cm (20 Gy) of gamma irradiated plants and 75 mM (2.90 cm) of EMS treated plants. The corolla tube length and diameter of the open flower are interrelated, therefore increase in any one of the trait ultimately influence development of larger jasmine flowers. The corolla tube length and diameter of an open flower were observed maximum of 1.8 cm and 3.5 cm in 30 Gy gamma irradiated treatment and the mean was highest for both the parameters in 20 Gy gamma irradiated treatment. The average value of the flower characters viz., the number of flowering cymes per plant, flower bud length, corolla tube length and diameter of the open flower were relatively higher in the gamma irradiated jasmine plants compared to the control plants whereas, EMS treated rooted jasmine plants showed reduced flower attributes compared to their respective

EJPB

control plants. These responses are in accordance with the results expressed in EMS and gamma ray combination treated M_1V_1 generation of *J. sambac* cv. Gundumalli (Mekala *et al.*, 2009) and EMS treated bulbs of tuberose cultivars (Singh *et al.*, 2015).

Assessment of lethality dose for the cultivar to the mutagens thus helps in understanding the nature of responses and effects, prior to large scale mutagenic treatments. The LD_{50} value for gamma radiation and Ethyl methane sulphonate to *J. sambac cv.* Ramanathapuram Gundumalli was fixed as 21. 37 Gy and 64.57 mM and this will serve as a reference for ascertaining effective working doses in the future successful mutation breeding studies of the cultivar. The favourable mutant can be identified in M_1 generation, but to avoid choosing chimeral mutants, later generations must be examined. The selection of individual mutant plants as many as possible even with the same phenotype is must for choosing the elite variant line.

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