www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(11): 583-588

www.thepharmajournal.com
Received: 13-08-2021
Accepted: 27-09-2021

M Durga Devi
Department of Fruit Science, HC&RI, TNAU, Periyakulam, Tamil Nadu, India

C Subesh Ranjith Kumar
Department of Fruit Science, HC&RI, TNAU, Periyakulam, Tamil Nadu, India

J Rajangam
Department of Fruit Science, HC&RI, TNAU, Periyakulam, Tamil Nadu, India

S Santha
Department of Crop Improvement, AC&RI, TNAU, Kudumiyanmalai, Tamil Nadu, India

C Sankar
Department of Fruit Science, HC&RI, TNAU, Periyakulam, Tamil Nadu, India

Corresponding Author:
M Durga Devi
Department of Fruit Science, HC&RI, TNAU, Periyakulam, Tamil Nadu, India

Determination of lethal dose (LD_{50}) and effect of physical and chemical mutagenesis in acid lime var. PKM 1

M Durga Devi, C Subesh Ranjith Kumar, J Rajangam, S Santha and C Sankar

Abstract
The study was carried out to determine the optimum dose of gamma radiation and Ethyl Methane Sulphonate (EMS) for effective mutation breeding program in acid lime var. PKM 1. Acid lime seeds were mutated by using physical mutagen *i.e*., gamma rays (^{60}Co source) at five different doses (5Gy, 10Gy, 15Gy, 20Gy and 25Gy) at IGARI, Kalpakkam, Chennai. For chemical mutagenesis, different concentrations of EMS @ 0.2%, 0.4%, 0.6%, 0.8% and 1.0% was used. The survival rate, growth potential, morphological and other physiological parameters were evaluated during the study period. Result showed that using linear regression model, The LD_{50} doses for gamma ray and EMS were observed at 7.66Gy and 0.33% respectively, and predicted as 10Gy and 0.4%EMS.Chimeral effect *viz*., minor leaf variations were observed in treated seedlings such as leaf shape, leaf length and serration and to be observed for stability. Maximum variability in seedling height, internodal length and thornless characters were observed in seeds treated with 10 Gy and 25Gy dose of gamma radiation. Mutagenic treatment significantly decreased the shoot length, leaf area, fresh weight, dry weight, and relative water content and chlorophyll content but increased root length with increasing the dose of mutagens. From the results it is observed that the variation increases with increase in the dose/concentration of the mutagens. The result of the study empharized the determination of absorbtion dose as 10Gy, 0.4% EMS respectively and produced promising variation the thornless, internodal length which has a promising impact in understanding the variation in further generation.

Keywords: Mutation breeding, gamma rays, EMS, $LD_{50,}$ Acidlime var.PKM1

1. Introduction
Acid lime (*Citrus aurantifolia* Swingle) belongs to the family Rutaceae and is commercially grown in tropical and subtropical regions of India. It is the third important citrus fruit after mandarins and sweet orange. India rank fifth among major lime producing countries in the world. In India, lime occupies an area of 0.25 million hectares with an annual production 2.78 million tonnes and productivity of 10.67 metric tonnes per hectare (Horticulture data base, 2018-19). PKM 1 is the most popular cultivar in Tamil Nadu. It is a selection from Kadayam type of Tirunelveli district of Tamil Nadu (Sundar and Gangai Selvi, 2019) [27]. Trees are small, bushy with small but sharp spines. Because of the relatively high degree of polyembryony exhibited in acid lime fruit, the seedlings are found true to type when seed propagated and seed propagation is still employed in most of the countries (Bora *et al*., 2017) [16]. Acid lime is a good source of Vitamin C (62.90 mg/100 ml), Vitamin B1, Vitamin- B2 and minerals like Calcium (90 mg /100 ml), Phosphorus (20 mg/100 ml) and Iron (0.3 mg/100 ml). It is a part of our daily salad dish as used to garnish different foods. It is also used for preparing beverages such as limeade and lime Rickey. It is a good source of edible citric acid and essential ingredient of almost all the herbal cosmetics (Abhilash *et al*., 2018) [2].
Induction of variability using physical mutagen and gamma rays has been in practice for long time by breeders (Mahadevamma *et al.*, 2012) [17]. Mutations are characterized as heritable alterations in an organism's genetic material and in turn in its characters that are not derived from genetic segregation (Van Harten, 1998) [29]. Induced mutagenesis refers to the induction of genetic variability in crop plants through apparent exposure to mutagenic agents that act on the hereditary material. It can be achieved through utilization of either physical mutagens or chemical mutagens or may be the combinations (Kumar and Pandey, 2018) [5]. Mutagenesis has been employed in fruit crops to create valuable mutants for dwarfing, earliness in flowering and fruit ripening, fruit colour, self-compatibility, self-thinning, and pathogen resistance (Janick and Moore 1996) [9].

In induced mutation, mutagens *viz*., physical (gamma rays) and chemical (Ethyl Methane Sulphonate (EMS) and Methyl Methane Sulphonate (MMS)) are most frequently used. Chemical mutagens are alkylating compounds that can be used to induce mutations. Chemical mutations are more stable and unique to a specific gene than physical mutations. Ethyl Methane Sulphonate, a chemical belonging to the alkylating agents' category, has been described as a very effective and efficient mutagen for producing somaclonal diversity in crop plants such as banana and grapes (Omar *et al.,* 1989) [20]. Chemical mutagens lead to more specific and predictable mutation, and the procedures are easier to perform without any specialized and expensive equipment. The identification of most effective mutagenic treatment and efficient mutagens is very essential to recover a high frequency and spectrum of useful mutations (Jency *et al.* 2016) [11]. Physical mutagens have been very helpful in induction of seedlessness, dwarfism and early flowering in different fruit crops (Lamo *et al.,* 2017) [15]. Lethal Dose fifty per cent is the dose which causes 50 percent reduction in seed germination in treated individuals used for a defined time. Germination percentage decreased with increase in dose or concentration of mutagen. The LD_{50} was observed at 0.5% concentration (Santhosh *et al.,* 2010) in papaya by Ge *et al.*,(2015) in their studies on establishment of an efficient protocol for *in vitro* EMS mutagenesis and the selection of 'Bingtang' sweet orange somaclones tolerant to citrus canker disease. Mutation was introduced by treating the suspension of embryogenic callus with 1.5% of EMS for 1 hr (the lethal concentration). The optimum dose was chemical (EMS) mutagenesis, in which 0.1% was the most suitable for 45DOC (day old calli), whereas 60 DOC didn't regenerate after mutagen treatment (Kumar *et al.,* 2010) [12].

Based on the above facts, the present study was framed to determine the $LD_{50}$for gamma radiation and EMS in acidlimevar. PKM 1 by subjecting the seeds of acid lime var. PKM 1 to different doses of physical and chemical mutagens and to find their effect on growth related traits and to derive desirable economic traits.

2. Materials and Methods

The present investigation was undertaken at the Department of Fruit Science, Horticultural College and Research Institute, Periyakulam, TNAU during 2019-2021.The experiment was laid out in Completely Randomized Block Design with 11 treatments and three replications. Acid lime seeds of variety PKM 1 were collected from the central farm orchard of HC&RI, Periyakulam. For mutation induction, physical (gamma rays) and chemical (EMS) mutagens were used and the work was taken out with the support from Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Chennai. The freshly extracted seeds were packed in butter paper covers and placed in the Gamma cell using the standard procedure followed at IGCAR, Kalpakkam. Seeds were exposed to gamma irradiation from the Cobalt 60 gamma source for appropriate time for each dose based on the half-life of the source like 5 Gy (22 sec), 10 Gy (45 sec), 15 Gy (68 sec), 20 Gy (91 sec), 25 Gy (114 sec), with a dose rate of 0.219 Gy S^{-1}. For chemical mutagen treatments, the seeds were treated with different concentrations of EMS (0.2%, 0.4%, 0.6%, 0.8% and 1.0%). The aqueous solution of different concentrations of EMS was prepared using Phosphate Buffer (pH 7.0). Seeds were soaked in the freshly prepared mutagenic solutions for 6 hours and kept at room temperature with intermittent shaking after pre-soaking in distilled water for 6 hours. Finally EMS treated seeds were rinse with running tap water for 1 hour to wash out the chemical residues. Non-irradiated/ treated seeds were also taken for this study and kept as control. About 150 number of treated and non-treated seeds for each treatment were sown and kept in partial shade under shade net chamber for observing the growth.

The treated seeds of gamma rays and EMS were planted in the polyethylene bags containing Red soil: FYM: Sand in the ratio of 1:1:1 and maintained for rooting. The percentage of survival, shoot length, root length, root thickness, number of secondary roots and number of leaves were measured at 120 days after planting in case of gamma radiation and EMS treated plants. The LD_{50} value was calculated based on probit analysis.

2.1. Probit analysis

Finney's approach was used to get the LD_{50} values of gamma radiation and EMS (Finney, 1971) [4]. The probit analysis was performed in MS Excel using the approach below, with some changes to the log-doses.

The percentage of seeds that died as a result of treatment dosages is calculated and rounded to the nearest whole number. The corrected mortality percentage is calculated (Abbott's formula) given below.

Corrected mortality (%) = (M observed – M control / 100 – M control) x 100

2.2. Analysis of variance

Data obtained for growth related traits were subjected to analysis of variance (ANOVA) at the significant level of 5% using SPSS software. When statistical differences were found, the least significant difference (LSD) was used to compare means at the 5% significance level. Microsoft Excel 2010 software used to calculate mean, plot curve and linear regression.

3. Results and Discussion

3.1. Determination of Lethal dose

The prime approach in mutation breeding has been to upgrade the well-adapted plant varieties by altering major agronomic traits, productivity and quality. The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half of the planting material survive) doses before massive irradiation of similar materials are accepted.

In the present study, popular and well adapted PKM1 variety of acid lime was chosen to study the effect of physical mutagen *i.e.,* Gamma rays and chemical mutagen *i.e.,* EMS on various parameters *viz.,* survival rate, shoot length, leaf length, number of leaves, root length number of secondary roots and root diameter were taken 120 days after sowing. LD_{50}values were determined with the help of probit analysis based on their survival rate of the seed after treatment with different doses/concentration of Gamma rays and EMS compared with untreated control (Table 1/ Fig1 and 2). Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. The probit curve analysis shown that the LD_{50}value for Gamma rays and EMS were 7.66Gy and 0.33% respectively (Fig. 1 and 2). The minor difference were observed in LD_{50} doses by Dhatt *et al.* (2000) [3] in kinnow seeds with gamma rays and with EMS,

Santhosh *et al.* (2010) in papaya, L.K. Sharma *et al.* (2013) [14] in *Citrus jambhiri* Lush., Ambavane *et al.,* (2015) [1] in finger millet, Bora (2017) [16], 10 to 45 mM.

3.2. Impact of Mutagenesis on survival and growth parameters

Survival percentage of acid lime var. PKM1under different dose concentrations were calculated based on the survival of seed germination after treatment and compared with control. There was an abnormal reduction in the survival of seed germination with the raise of Gamma dose (Table 1). Data analysis on germination percentage that survived showed an attendant decrease in survival significantly with applied increases in concentration of doses. Optimum seedling emergence percentages of acid lime var. PKM1 at dose rates of 15 Gy and 0.4% EMS were 64.2%, and 67.2%, respectively (Table 2). The lowest seedling emergence value, was recorded at dose rates of 25Gy and 1.0% EMS. Seedling could not survive a dose rate of 25 Gy and 1.0% EMS respectively. Reduction in germination percentage of seed due to mutagenic treatment was also reported by Saini and Gill (2009) [23] and L.K. Sharma *et al.,* (2013) [14] in Rough lemon; Kaur and Rattanpal (2010) [13] in *Citrus jambhiri* L. seedlings. Seed germination was delayed and seed germination percentage is reduced as Ethyl Methane Sulphonate dosages were increased. This was also reported by Jawaharlal *et al.* (1992) in acid lime. The majority of the adverse effects of gamma radiation treatment appeared soon after treatment and were manifested as a decrease in sprouting capability when the dose was increased (Raghami and Ghazvini 2005) [21]. In the present investigation, at higher dosage of mutagens, the seed germination and number of days for germination got delayed and the seedlings were shorter in height which subsequently died in short period (Table 2).

The shoot length in all the irradiated mutagenic population was significantly reduced as against in all EMS treated populations was significantly increased compared to respective control. The shoot length varied from 16.23cm (5Gy) to 12.67cm (25Gy) in gamma rays treatment; 22.33 (0.8%) to 17.21(0.2%) in EMS treatment. The mean of shoot length in gamma rays and EMS treated population was 14.50 cm and 20.16 cm, respectively. Data showed the lowest mean shoot length value was recorded at dose rates of 25 Gy and 0.2% EMS (12.67 and 17.21cm, respectively). Inter nodal length measured for control seedling was 3.02 cm, while the mutated seedlings measured from 1.71 to 2.42 cm for 25Gy to 5Gy and for EMS treatment decreased from 1.91 to 2.88cm in 1.0% to 0.2%. Maximum average number of leaves per seedlings in 25Gy and 1.0% EMS were 16.24 and 17.03 were observed (Table 2). Similar studies were also undertaken by Kumar and Kumar Rai (2007) [12], Kaur and Rattanpal (2010) [13], L.K. Sharma *et al.,* (2013) [14].

Regarding the root length, the mean root length recorded was significantly greater than their respective control in all the mutagens treatment. The root length ranged from 20.56 (25Gy) to 17.43 (5Gy) in gamma rays treatment. Whereas in EMS treatments, it's varied from 20.12cm (1.0%) to 17.79 cm (0.2%).In number of secondary roots, maximum average mean was observed in 25Gy (21.19) and 1.0% EMS (23.47). Significant reduction in root thickness was found with increasing dose of gamma rays. Maximum root thickness was observed in 0.6%(1.42mm) and minimum in 25Gy and EMS 0.2% (1.12 and 1.23 mm, respectively). Similar results were reported by Saini and Gill (2009) [23], Sukhjit Kaur (2015) [25] using Gamma radiation in rough lemon; L.K. Sharma *et al.,* (2013) [14] in *Citrus jambhiri.*

Thornless character and dwarfing habit was observed in seedlings treated with 10Gy and 25Gy whereas, in other treatment the seedlings were in medium height with thorns. Chimera effects such as leaf shape, leaf length, leaf colour and also stunted growth, spreading habit were observed in 5Gy, 10Gy, 25Gy, 0.4 to 0.8% EMS. Radiation treatment most likely caused modifications at the gene level, which eventually manifested in substances that trigger biochemical processes that control various aspects of growth. These substances cause chemical patterns to shift, resulting in a variety of modifications and variances in plant characteristics including as height, branching, and stem thickness, as reported by Whittwer (1971). Due to alkylation at the O6 or N7 position of guanine, which results in the replacement of cytosine with thymine base pairing, EMS causes a biased spectrum of Guanine/Cytosine-to-Adenine/Thiamine transitions. EMS also causes a low number of chromosomal breakage and lethal effects and tends to produce numerous random point mutations (Greene *et al.,* 2003) [8].

In Gamma rays and EMS treatment, leaf area (LA) reduced significantly from7.18 (5Gy) to 5.36 cm^2 (25Gy) and 7.15 (0.2%) to 4.56 cm^2 (1.0%). While untreated seedlings leaf area was in 6.52cm^2 control (Table 3). Leaf area, similar findings were reported by Kaur and Rattanpal (2010) [13], Rajib Roychowdhury and Jagatpati Tah (2011). Relative water content decreased with increasing dose of mutagens from 89.30 to 87.80% in gamma rays and In EMS decline range from 90.05 (0.2%) to 87.75% (0.4%). Fresh weight and Dry weight of acid lime seedling increased upto dose of 15 Gy and 0.8% EMS (17.67g and 8.79g; 17.65g and 8.81g, respectively). While untreated seedling FW and DW of control were 16.75g and 7.99g respectively and with further increase in dose of mutagens, the fresh and dry weight of seedlings started decreasing(Table 3). Similar result was concluded by Ulukapi and Ozmen (2017) [28] in M1 plants of common bean (*Phaselous vulgaris*) in which the low doses of gamma radiation stimulatively effected on fresh weight and dry weight of seedling. An increase in chlorophyll content significantly increased with increase in the dose of gamma radiation compared to control. Chlorophyll a, b and total chlorophyll of acid lime seedling increased upto dose (5Gy Chlorophyll a, 10Gy chlorophyll b and 10Gy total chlorophyll; 0.2% for chlorophyll a, b and total chlorophyll). Mutagenic treatment significantly decreased the shoot length, leaf area, fresh weight, dry weight, relative water content and chlorophyll content but increased root length of acid lime seedlings (Table 3). Similar findings were reported by Moussa and Abdul Jaleel (2011) [18] in *Trigonella foenum-graecum*; Ulukapi and Ozmen, (2017) [28] in Efsane and F16.

Table 1: Probit Analysis calculating LD_{50}in Gamma rays and Ethyl methane sulphonate treated populations in Acidlime var. PKM 1

Dose/Conc.	Survival (%)	% survival over control	% reduction over control	Observed mortality percentage	Corrected mortality percentage	Corrected mortality %	Log value of doses	Empirical probit unit	LD_{50} value
Control	75	99.56	-	25	-				
Gamma Rays									
T1- 5 Gy	47	62.22	37.34	53	0.38	38	0.70	4.69	7.66Gy
T2-10 Gy	58	77.33	22.23	42	0.23	23	1.00	4.20	
T3-15 Gy	52	69.33	30.23	48	0.31	31	1.18	4.50	
T4-20 Gy	26	34.67	64.89	74	0.65	65	1.30	5.39	
T5-25 Gy	23	31.11	68.45	77	0.69	69	1.40	5.50	
Ethyl methane sulphonate (EMS)									
T6-0.2%	57	76.44	23.12	43	0.24	24	2.30	4.20	0.33%
T7-0.4%	51	67.56	32.00	49	0.32	32	2.60	4.63	
T8-0.6%	41	54.22	45.34	59	0.46	46	2.78	4.90	
T9-0.8%	33	43.56	56.00	67	0.56	56	2.90	5.15	
T10-1.0%	15	20.00	79.56	85	0.80	80	3.00	5.84	
SE(m) CD(0.05)	0.64 1.82								

Table 2: Effect of mutagens on biological parameters in Acidlime var. PKM 1

Treatment	Days taken for germination	Seed germination%	Shoot length (cm)	Number of leaves	Internodal length (cm)	Root length (cm)	Thickness of primary roots (mm)	Number of secondary roots
Control	19.80	80.7	18.75	15.31	3.02	17.15	1.35	17.65
Gamma rays								
5Gy	24.47	57.1	16.23	12.26	2.42	17.43	1.21	18.53
10Gy	27.53	74.1	14.13	13.75	2.17	18.17	1.24	18.96
15Gy	23.53	64.2	15.89	14.52	1.86	19.02	1.28	20.08
20Gy	28.33	42.2	13.57	14.94	1.83	19.86	1.16	20.23
25Gy	30.13	36.9	12.67	16.24	1.71	20.56	1.12	21.19
Mean	26.80	54.9	14.50	14.16	1.10	19.01	1.20	19.80
Ethyl methane sulphonate								
0.2%	21.40	71.8	17.21	12.12	2.88	17.79	1.29	21.51
0.4%	22.17	67.2	20.18	14.62	2.67	18.54	1.38	22.26
0.6%	23.33	55.9	21.93	15.82	2.32	19.26	1.42	22.53
0.8%	26.13	49.4	22.33	16.57	2.10	19.98	1.32	23.12
1.0%	28.00	32.2	19.14	17.03	1.91	20.12	1.23	23.47
Mean	24.21	55.3	20.16	15.23	2.38	19.14	1.292	22.58
SE(d)	0.56	1.17	0.47	0.38	0.04	0.40	0.03	0.48
CD(P=5%)	1.17	2.43	0.98	0.79	0.09	0.82	0.06	1.00

Table 3: Effect of mutagens on physiological parameters in Acidlime var. PKM 1

Dose/Conc.	Leaf area (cm^2)	Relative water content (%)	Fresh weight (g)	Dry weight (g)	Chlorophyll A	Chlorophyll b	Total chlorophyll
Control	6.52	91.15	16.75	7.99	1.4005	0.5569	0.0386
Gamma rays							
5Gy	7.18	89.30	15.88	7.94	1.3954	0.5797	0.0426
10Gy	6.19	88.45	16.50	8.52	1.2942	0.5808	0.0439
15Gy	6.12	87.85	17.67	8.79	0.9798	0.4031	0.0266
20Gy	5.76	87.80	17.24	8.53	0.9644	0.4168	0.0276
25Gy	5.36	85.75	16.12	8.05	0.9249	0.4235	0.0269
Ethyl methane sulphonate							
0.2%	7.15	90.05	15.98	7.92	1.7052	1.1210	0.4005
0.4%	6.43	87.75	16.63	8.25	1.6201	0.8811	0.2508
0.6%	6.98	88.95	16.99	8.34	1.5482	0.8958	0.2606
0.8%	5.60	88.60	17.65	8.81	1.4406	0.8581	0.2312
1.0%	4.56	89.65	17.15	8.58	1.3543	0.7956	0.2164
SE(d)	0.14	NS	0.40	0.19	0.0246	0.0135	0.0051
CD(P=5%)	0.29		0.84	0.39	0.0513	0.0281	0.0106

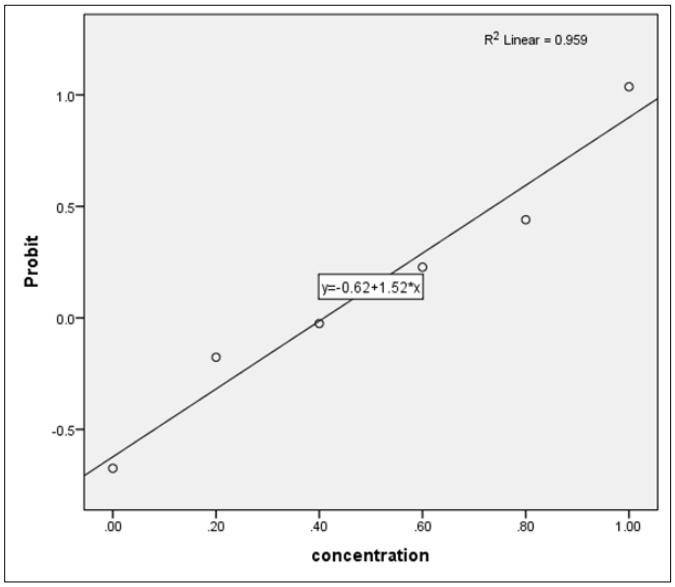

Fig 1: Probit analysis for calculation of LD_{50} for Ethyl Methane Sulphonate

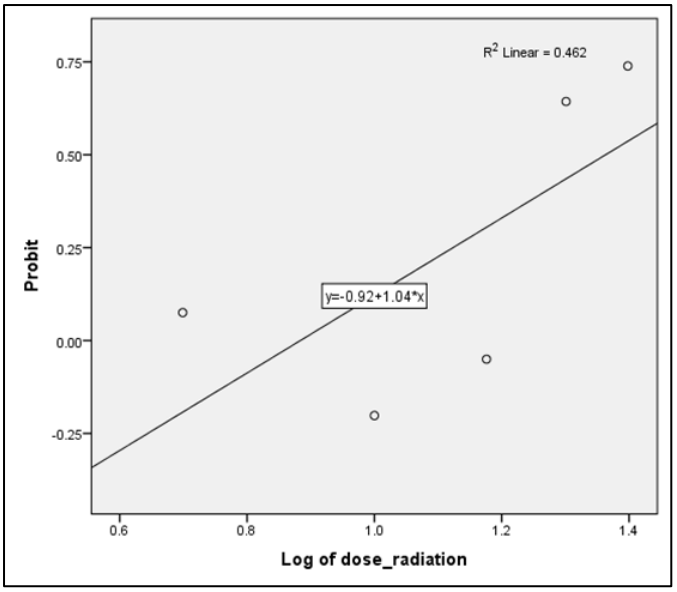

Fig 2: Probit analysis for calculation of LD_{50} for Gamma Rays

4. Conclusion

Determination of LD_{50} value for any mutagen is essential to produce maximum viable mutants with minimum damage to the plant. The LD_{50} dose based on the reduction in survival after treatment with different doses or concentration of gamma rays and EMS were significantly different as shown above. In the present study, based on the survival and growth rates, LD_{50} doses for gamma irradiation and EMS have been fixed as 7.66 Gy and 0.33%, and predicted as 10Gy and 0.4%EMS. The chimeral variable characters like thornless and shorter internode observed in their study need to be studied further for its stability in the characters for future follow up program. In acid lime the variable characters like thornless is highly preferable as an important economic traits and in the same while without manipulating the yield attributes. Likewise high density planting in acid lime is coming up and results from the studies have shown a less internodal characters and this might develop trees fit for high density planting by the future research programme to be taken up in this regard. In a nut shell study has brought promising Chimeral variation for Growth characters and optimization of absorbtion dose of chemical and physical mutagens for acid lime var. PKM 1.

5. Reference

1. Ambavane AR, Sawardekar SV, Sawantdesai SA, Gokhale NB. Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn). Journal of Radiation Research and Applied Sciences 2015;8:120-125.
2. Abhilash S, Mandal R, Dey A, Phani R, Joseph S, Chattopadhyay *et al.* Role of enhanced synoptic activity and its interaction with intra-seasonal oscillations on the lower extended range prediction skill during 2015 monsoon season. Climate Dynamics 2018.
3. Dhatt AS, Rattanpal HS, Khokhar R, Singh SN. Standardising LD_{50} dose of gamma rays and EMS in kinnow Seeds. Journal of Research Punjab Agricultural University 2000;37(3-4):194-7.
4. Finney DJ. Probit Analysis Edn 3, Cambridge University Press, London, 1971.
5. Kumar G, Pandey A. Ethyl methane sulphonate induced changes in cyto morphological and biochemical aspects of *Coriandrum sativum* L. Journal of the Saudi Society of Agricultural Sciences 2019;18:469-475.
6. Ge H, Li Y, Fu H, Long G, Luo L, Li *et al.* Production of sweet orange somaclones tolerant to citrus canker disease by *in vitro* mutagenesis with EMS. Plant Cell, Tissue and Organ Culture (PCTOC) 2015;123(1):29-38.
7. Girjesh Kumar, Prashant Kumar Rhai. EMS Induced Karyomorphological Variations in Maize (*Zea mays* L.) Inbreds. Turk Journal of Biology 2007;31:187-195.
8. Greene EA, Codomo CA, Taylor NE, Henikoff JG, Till BJ. Spectrum of chemically induced mutations from a large-scale reverse genetic screen in Arabidopsis. Genetics 2003;164:731-740.
9. Janick J and Moore JN. Fruit breeding. Tree and tropical fruits. John Wiley & Sons, New York, 1996, 1.
10. Jawharlal M, Sambandamorthy S, Irulappan I. Effect of Gamma ray and EMS on seed germination and seedling growth in acid lime (*Citrus anrantifolia* Swingle). South Indian Horticulture 1992;369:332-6.
11. Jency J, Poornima R, Ravikesavan P, Sumathi, Raveendran M. Determination of lethal dose and effect of physical mutagen on germination percentage and seedling parameters in kodo millet variety CO 3, Electronic Journal of Plant Breeding 2016;**7**(4):1122-1126.
12. Kumar K, Gill MSI, Kaur H, Choudhary OP, Gosal SS. *In Vitro* Mutagenesis and Somaclonal Variation Assisted Salt Tolerance in 'Rough Lemon' (*Citrus jambhiri* Lush.). European Journal of Horticulture Science 2010;75(6):233-238.
13. Kaur S, Rattanpal HS. Effect of mutagens on *in vitro* seed germination and growth of rough lemon (*Citrus jambhiri* Lush.) seedlings. Indian Journal of Agricultural Sciences2010;80(9):773-776.
14. Sharma LK, Manisha Kaushal, Gill MIS, Bali SK. Germination and Survival of Citrus Jambhiri Seeds and Epicotyls after Treating with Different Mutagens under *in vitro* Conditions. Middle-East Journal of Scientific Research 2013;16(2):250-255.
15. Lamo J, Tongoona P, Sie M, Semon M, Onaga G, Okori P. Upland Rice Breeding in Uganda: Initiatives and

Progress. Advances in international Rice Research 2017, 214-246.
16. Bora L, Vijayakumar RM, Ganga M. Mutagenic effect of ethyl-methyl sulphonate on *in vitro* nodal segments of acid lime cv. PKM-1. International Journal of Chemical Studies 2017;5(6):429-432.
17. Mahadevamma M, Dinesh MR, Kumari VR, Shankarappa TH. Evaluation of induced variability in papaya (*Carica papaya* L.) by physical mutagenesis. CIB Tech Journal of Biotechnology 2012;1(1):66-71.
18. Moussa HR, Abdel-Aziz SM. Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Australian Journal Crop Science 2008;1:31-36.
19. NHB. National Horticulture Board Database, Ministry of Agriculture. Government of India, 2019.
20. Omar MS, Novak FJ, Brunner H. *In vitro* action of ethyl methane sulfonate on banana shoot tips. Sciencia Horticulture 1989;40(4):283-296.
21. Raghami M, Ghazvini RF. Effects of gamma irradiation on embryogenic callus and embryogenesis from habituated calluses of lime (*Citrus aurantifolia* var. Mexican lime). Iranian Journal of Agricultural Sciences 2005;36(2):425-34.
22. Rajib Roychowdhury, Jagatpati Tah. Chemical mutagenic action on seed germination and related agro-metrical traits in M1 *Dianthus* generation. Current Botany 2011;2(8):19-23.
23. Saini HK, Gill MIS. Induction of mutation in Rough lemon (*Citrus jambhiri* Lush.) using gamma rays. Journal of Horticultural Sciences2009;4(1):41-44.
24. Santosh LC, Dinesh MR, Rekha A. Mutagenic studies in papaya (*Carica papaya* L.). Acta Horticulture 2010;851:109-112.
25. Sukhjit Kaur. Effect of mutagens on regeneration and growth of *in vitro* grown epicotyl segments of rough lemon seedlings (*Citrus jambhiri* Lush.). Journal of Applied and Natural Science 2015;7(1):459-465.
26. Kauri S, Rattanpal HS. Effect of mutagens on *in vitro* seed germination and growth of rough lemon(*Citrus jambhiri*) seedlings. Indian Journal of Agricultural Sciences 2010;80(9):773-6.
27. Sundar A, Selvi, Gangai R. An economic analysis of food grains production in Tamil Nadu ascertaining food security. International Research Journal of Agriculture Economics & Statistics2020;11(1):36-41.
28. Ulukapi, Kamile, Ozmen, Süleyman Fatih. Study of the effect of irradiation (^{60}Co) on M1 plants of common bean (*Phaseolus vulgaris* L.) cultivars and determined of proper doses for mutation breeding. Journal of Radiation Research and Applied Sciences, 2018.
29. Van Harten AM. Mutation Breeding. Theory and Practical Applications. Cambridge University Press. U.K, New York, 1998, 353.
30. Whittawer SH. Radiation induced mutation in crop plants. Outlook Agriculture 1971;6:205-17.