The Pharma Innovation Journal 2023; 12(11): 661-664

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(11): 661-664

www.thepharmajournal.com
Received: 16-09-2023
Accepted: 21-10-2023

Pothuraju Pradeep Kumar
Ph.D. Scholar (Fruit Science), Dr. Y. S. R Horticultural University, College of Horticulture, Venkatramannagudem, Andhra Pradesh, India

Dr. C Madhumathi
Principal Scientist (Hort.) & Head, Citrus Research Station, Petlur, Andhra Pradesh, India

Dr. M Madhavi
Associate Dean, College of Horticulture, Venkatramannagudem, Andhra Pradesh, India

Dr. B Tanuja Priya
Principal Scientist (Hort.), Horticulture Research Station, LAM, Guntur, Andhra Pradesh, India.

Dr. M Paratpara Rao
Associate Professor, College of Horticulture, Venkataramannagudem, Andhra Pradesh, India

Dr. M Kavitha
Senior Scientist, Citrus Research Station, Tirupati, Andhra Pradesh, India

Dr. EK Naik
Scientist, Citrus Research Station, Petlur, Andhra Pradesh, India

Corresponding Author:
Pothuraju Pradeep Kumar
Ph.D. Scholar (Fruit Science), Dr. Y. S. R Horticultural University, College of Horticulture, Venkatramannagudem, Andhra Pradesh, India
pothurajupradeep@gmail.com

Mutagenic impacts and LD_{50} determination in acid lime (*Citrus aurantifolia*) Cv. Petlur acid lime

Pothuraju Pradeep Kumar, Dr. C Madhumathi, Dr. M Madhavi, Dr. B Tanuja Priya, Dr. M Paratpara Rao, Dr. M Kavitha and Dr. EK Naik

Abstract
The study aimed to identify the optimal doses of gamma radiation and Ethyl Methane Sulphonate (EMS) for an effective mutation breeding program in the Acid Lime cultivar Petlur Acid Lime. Seeds of the Petlur Acid Lime cultivar were exposed to gamma irradiation using a Cobalt-60 gamma source. Five treatments, ranging from 20 Gy to 100 Gy, were applied for gamma radiation, and different concentrations of EMS (0.2%, 0.4%, 0.6%, and 0.8%) were used for chemical mutagenesis. Germination and mortality percentages were assessed throughout the study. As the dosage of both chemical and physical mutagens increased, the Petlur Acid Lime cultivar exhibited a gradual rise in mortality percentage. Higher doses were inversely related to germination percentage, resulting in a decrease. The study revealed that higher concentrations of Gamma rays and EMS proved lethal, whereas lower concentrations were safe and ideal for inducing desirable changes. The LD_{50} doses for gamma rays and EMS were observed in this study at 22.90 Gy and 0.28%, and inferred as 20 Gy and 0.2% EMS.

Keywords: Mutation breeding, gamma rays, EMS, LD_{50}, acid lime Cv. Petlur acid lime

Introduction
Acid lime (*Citrus aurantifolia*), a citrus fruit belonging to the Rutaceae family, is prized globally for its acidic juice, culinary applications, and medicinal benefits. Its adaptability to diverse soil and climatic conditions has made it a staple crop in tropical and subtropical regions. India leads the world in acid lime production, with a significant cultivation area of 248 thousand hectares and an impressive yield of 2438 metric tonnes in 2019 (NHB Data Base). Andhra Pradesh, Telangana, Maharashtra, Assam, Chhattisgarh, Karnataka, Bihar, and Gujarat are pivotal citrus-growing states in India, particularly Andhra Pradesh, boasting an expansive cultivation area of 45.8 million hectares with a production of 686.6 MT (NHB Data Base 2019) [17].

Developing acid lime cultivars with desirable traits, such as improved plant and fruit characteristics, enhanced shelf life, and resilience against biotic and abiotic stresses, is imperative. One potent method for achieving this is mutation breeding, a process involving the deliberate induction and development of mutant lines to enhance crop traits (Khan *et al*., 2017) [10]. This technique, utilizing physical or chemical mutagens or their combination (Kumar and Pandey, 2019) [13], offers a targeted approach for fortifying acid lime's natural defense mechanisms, especially against notorious diseases like canker. Developing canker-resistant varieties through mutation breeding not only curbs economic losses but also augments crop yield, reducing the reliance on chemical treatments.

The response of plants to physical and chemical mutagens is species-specific, and many species' reactions remain unexplored (Gilchrist and Haughn, 2005) [5]. Physical mutagens, including gamma rays, have demonstrated success in inducing seedlessness, dwarfism, and early flowering in various fruit crops (Lamo *et al*., 2017) [14]. Previous studies have explored mutagenesis in citrus species, leading to the development of salt-tolerant Troyer citrange (Garcia and Primo, 1995) [4], seedless and Mal Secco-resistant mutant lemons (Gulsen *et al*., 2007), and canker-tolerant mutant clones in *Citrus sinensis* (Latado *et al*., 2006) [15]. Commonly employed mutagens in citrus mutagenesis include gamma rays, EMS, and MMS (Methyl methane sulfonate) (Jain, 2005) [8]. The effectiveness of mutations induced by mutagens depends on finding the optimal dosage, and maximizing beneficial mutations while minimizing harm to the plants. The LD_{50} (lethal dose 50 percent) signifies the ideal dose inducing 50% lethality within a specific timeframe. Its determination depends on plant species, material type, state, and the assessment stage of lethality (Kumar *et al*., 2018) [12].

In light of these considerations, this study investigates the response of acid lime Cv. Petlur Acid Lime seeds to gamma radiation and EMS treatments. The research aims to pinpoint the LD_{50} for both physical and chemical mutagens.

Materials and Methods

The research study on mutagenic impacts and LD_{50} determination in acid lime Cv. Petlur Acid Lime was carried out at the Citrus Research Station, Dr. Y.S.R Horticultural University, Petlur, from 2021 to 2023. The experimental design followed a Completely Randomized Block layout comprising 10 treatments, each replicated three times. Uniform-sized, fully developed, and undamaged seeds were meticulously chosen for the experiment. Ninety seeds were treated for each dose of both physical and chemical mutagens.

Initially, freshly extracted seeds were carefully packed in butter paper covers and exposed to gamma irradiation using the standard procedure followed at IGCAR, Kalpakkam, utilizing a $Cobalt^{60}$ gamma source. The gamma radiation treatments included doses of 20 Gy, 40 Gy, 60 Gy, 80 Gy, and 100 Gy, respectively. For the EMS treatment, seeds of the Petlur Acid Lime cultivar were first soaked in distilled water for 6 hours. Subsequently, these seeds were immersed in sterilized ethyl methane sulphonate at varying concentrations: 0.2%, 0.4%, 0.6%, and 0.8% for a duration of six hours. After the treatment, the seeds were rinsed thrice with distilled water to eliminate any residual traces. Special attention was given during the application of the chemical mutagen due to its carcinogenic nature. The entire experimental procedure was conducted at a room temperature of 28±1 °C. Immediately after the completion of the treatment, the processed seeds were sown in protrays.

Additionally, a control group consisting of untreated seeds were sown in protrays filled with cocopeat, and each protray was marked with a given gamma dose.

The LD_{50} value was calculated using probit analysis, following the procedure outlined by Finney (1971) [21]. Probit analysis involves the use of the probit function, which represents the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution.

Results and Discussion

In the realm of mutation breeding, the primary goal has always been to enhance well-adapted plant varieties by altering crucial agronomic traits, productivity, and quality. The success of mutation breeding is contingent upon the mutation rate, the number of plants scrutinized, and the efficiency of these mutations. To avoid unnecessary depletion of experimental resources, conducting radio sensitivity tests to ascertain LD_{50} values (the safe dose at which half of the planting material survives) becomes imperative before subjecting similar materials to extensive irradiation.

In this study, the focus was on the widely cultivated and well-adapted Petlur Acid Lime cultivar of acid lime. Our investigation delved into the impacts of physical mutagen (Gamma rays) and chemical mutagen (EMS) on germination and mortality percentages. LD_{50} values were meticulously determined through probit analysis, utilizing mortality and germination percentages of seeds treated with varied doses and concentrations of Gamma rays and EMS, and then compared with untreated controls (Table 1 and Fig. 1). The key objective was to identify the optimum dose, one that induces the maximum mutations with minimal harm to the plant.

Determination of LD_{50} for Physical and Chemical Mutagens: The results from the probit curve analysis revealed LD_{50} values of 22.90 Gy for Gamma rays and 0.33% for EMS predicted as 20 Gy and 0.2%EMS and It's worth noting that these findings showed slight discrepancies when compared to the research outcomes of Dhatt *et al*. (2000) [2] in kinnow seeds and Devi *et al*. (2021) [22] in acid lime, both exposed to Gamma rays and EMS. Additionally, studies by Santhosh *et al*. (2010) [25] in papaya, L.K. Sharma *et al*. (2013) [23] in *Citrus jambhiri* Lush., Ambavane *et al*. (2015) [24] in finger millet, and Bora (2017) reported varied concentrations ranging from 10 to 45 mM.

Impact of Mutagenesis on Germination and Mortality Percentage

The Petlur Acid Lime cultivar exhibited a gradual increase in mortality percentage as the dosage of both chemical and physical mutagens increased. Essentially, higher doses were inversely correlated with germination percentage, leading to a decrease. Among the EMS treatments, the highest mortality percentage (69.50%) was observed in 0.8% EMS, while the lowest mortality percentage (45.00%) was noted in 0.2% EMS. In the gamma irradiated treatments, the highest mortality percentage (84.50%) was seen in 100 Gy, whereas the lowest (49.00%) was recorded in 20 Gy. Regarding germination percentage, among the EMS treatments, the maximum (55.00%) was observed in T_1-0.2% EMS, and the minimum (30.50%) was found in T_4-0.8% EMS. Among the gamma irradiated treatments, the highest germination percentage (51.00%) was noted in T_5-20 Gy, while the lowest (15.50%) was observed in T_9-100 Gy. Moreover, the untreated seeds (control) of Cv. Petlur Acid Lime exhibited the highest germination percentage (96.52%). These findings align with previous studies in citrus by Dhatt *et al*. (2000) [2], Latado *et al*. (2001) [16], and Kaur and Rattanpal (2010) [9].

The decrease in seed germination was primarily due to mutagens interfering with the metabolic activities of the seeds (Sjodin, 1962) [20]. Sinha and Godward (1972) [19] suggested that the reduction in seed germination percentage resulted from disturbances at the physiological level coupled with chromosomal damage. EMS likely caused disruptions in the formation of enzymes involved in the germination process, affecting the physiological aspects (Roychowdhury and Tah, 2011) [18]. Gamma radiation is known for causing point mutations, enzyme inhibitions, and chromosomal aberrations, leading to a decrease in respiration rate and energy production, ultimately reducing seed germination with increasing doses (Kumar and Munirajappa, 2013) [11]. It was observed that higher concentrations of Gamma rays and EMS were lethal, whereas lower concentrations were safe and optimal for inducing desirable changes.

Table 1: Determination of LD_{50} for EMS and Gamma rays in Cv. Petlur Acid lime

Dose/ concentration	Log (conc.)	Germination (%)	Mortality (%)	Probit value	LD_{50} value
EMS @ (0.2%)	3.30	55.00	45.00	4.87	0.28%
EMS @ (0.4%)	3.60	41.60	58.40	5.20	
EMS @ (0.6%)	3.77	38.80	61.20	5.28	
EMS @ (0.8%)	3.90	30.50	69.50	5.50	
GR @ (20 Gy)	1.30	51.00	49.00	4.97	22.90 Gy
GR @ (40 Gy)	1.60	36.60	63.40	5.33	
GR @ (60 Gy)	1.77	34.40	65.60	5.41	
GR @ (80 Gy)	1.90	31.10	68.90	5.50	
GR @ (100 Gy)	2.00	15.50	84.50	5.99	
Control	1.20	96.52	3.00	4.64	

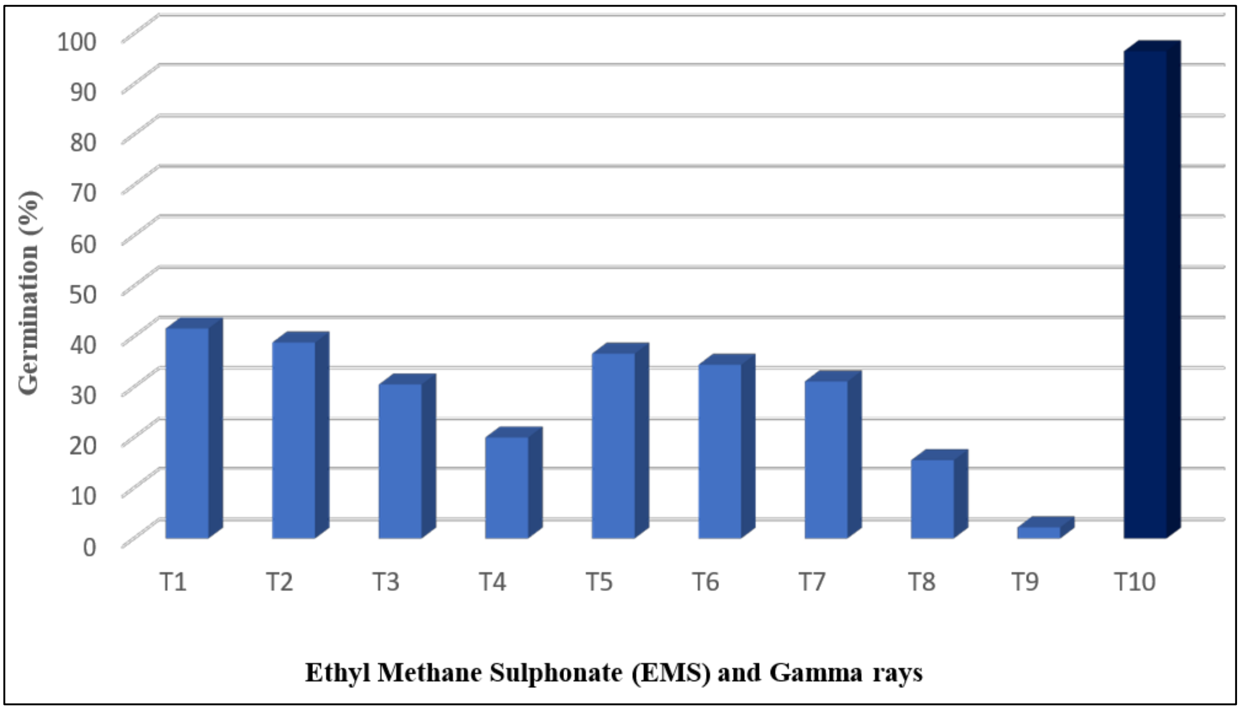

Fig 1: Effect of EMS and Gamma rays on germination (%) in Cv. Petlur Acid lime

T_1 - (0.2%) EMS, T_2 - (0.4%), T_3 - (0.6%), T_4 - (0.8%), T_5 - (20 Gy), T_6 - (40 Gy), T_7 - (60 Gy), T_8 - (80 Gy), T_9 - (100 Gy), T_{10} - (Control)

Conclusion

Determining the LD_{50} value for any mutagen is crucial to yield a maximum number of viable mutants while minimizing damage to the plant. In this study, LD_{50} doses for EMS and gamma rays were established as 0.2% and 20 Gy, respectively, based on germination and mortality percentage assessments.

Reference

1. Davies FS, Albrigo LG. Citrus. Centre for Agriculture and Bioscience International. Wallingford, U.K; c1994, p. 30-33.
2. 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):194-197.
3. Freisleben R, Lein A. Possibilities and practical implementation of mutation breeding. Plant Archives. 1944;60:211-222.
4. Garcia-Agustin P, Primo-Millo E. Selection of a NaCl-tolerant citrus plant. Plant cell reports. 1995;4(5):314-318.
5. Gilchrist EJ, Haughn GW. Tilling without a plough: a new method with applications for reverse genetics. Current opinion in plant biology. 2005;8(2):211-215.
6. Gulsen O, Uzun A, Pala H, Canihos E, Kafa G. Development of seedless and Mal Secco tolerant mutant lemons through budwood irradiation. Scientia Horticulturae. 2007;112(2):184-190.
7. Hearn CJ. Development of seedless grapefruit cultivars through budwood irradiation. Journal of the American Society for Horticultural Science. 1986;111(2):304-306.
8. Jain SM. Major mutation-assisted plant breeding programs supported by FAO/IAEA. Plant Cell Tissue and Organ Culture. 2005;82(1):113-123.
9. Kaur 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):773-776.
10. Khan MM, Yahyai RA, Said FA. The lime: botany, production and uses. 2017;978(1):845-867.
11. Kumar AHV, Munirajappa K. Evaluation of ethyl methane sulfonate (EMS) induced mutants of *Morus species* (genotype bc2-59) for nutritive and biomass productivity. International Journal of Applied and Natural Sciences. 2013;2(3):13-24.

12. Kumar A, Kumar MHD, Kumar DBM, Gurumurthy BR, Naik KAH. Estimation of LD_{50} for ethyl methane sulphonate treated population of groundnut *(Arachis hypogaea* L.). Journal of Farm Science. 2018;31(5):595-596.
13. 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(4):469-475.
14. Lamo K, Bhat DJ, Kour K, Solanki SPS. Mutation studies in fruit crops. International Journal of Current Microbiology and Applied Sciences. 2017;6(12):3620-3633.
15. Latado RR, Pompeu JJ, Figueiredo JO, Pio RM, Machado MA, Tulmann N, *et al*. Seedless and citrus canker tolerant mutant clones in sweet orange induced by gamma rays. Plant Mutation of International Atomic Energy Agency. 2006;1:10-20.
16. Latado RR, Neto AT, Ando A, Iemma AF, Pompeu JJ, Figueiredo JO, *et al*. Sweet orange 'Pera' mutants with low number of seeds obtained through mutation induction. Revista Brasileira de Fruticultura. 2001;23(2):339-344.
17. NHB, National Horticulture Board Database. Ministry of Agriculture, Government of India; c2019.
18. Roychowdhury R, Tah J. Chemical mutagenic action on seed germination and related agro-metrical traits in M1 Dianthus generation. Current Botony. 2011;2(8):19-23.
19. Sinha SS, Godward N. Radiation studies in *Lens culanaris*. Induction of mutations and type of mutants. J. Cytol. Genet; c1972. p. 131-136.
20. Sjodin J. Some observations in X1 and X2 of (*Vicia faba* L.) after treatment with different mutagens. Hereditas. 1962;48:565-586.
21. Finney DJ. Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of information in medicine. 1971 Aug;10(04):237-245.
22. Devi S, Alexandre YO, Loi JK, Gillis R, Ghazanfari N, Creed SJ, *et al*. Adrenergic regulation of the vasculature impairs leukocyte interstitial migration and suppresses immune responses. Immunity. 2021 Jun 8;54(6):1219-1230.
23. Sharma A, Kiran R. Corporate social responsibility: Driving forces and challenges. International Journal of Business Research and Development. 2013 Sep 3;2(1):18-27.
24. 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 Jan 1;8(1):120-125.
25. Khan M, Santhosh SR, Tiwari M, Rao LPV, Parida M. Assessment of *in vitro* prophylactic and therapeutic efficacy of chloroquine against Chikungunya virus in vero cells. Journal of medical virology. 2010 May;82(5):817-824.