D-6384
[1-7]

RESEARCH ARTICLE Agricultural Science Digest

Frequency and Spectrum of Chlorophyll Mutations in Rice (*Oryza sativa* L.)

Ramasamy Muthusamy Solarasan[1], Sathya Ramalingam[1], Ambika Singaram[1],
Arulbalachandran Dhanarajan[2], Thirugnanakumar Sivagurunathan[1], Selvakumar Gurunathan[1]
10.18805/ag.D-6384

ABSTRACT

Background: This study was conducted to determine the lethal dose of the chemical mutagen ethyl-methane sulfonate (EMS) in a high-yielding rice variety, CO51. Chlorophyll mutation frequencies have been studied in the M_2 generation.
Methods: Lethal doses were determined through a pilot study, in which ten batches of rice seeds were treated with different doses of ethyl methane sulphonate (EMS) and kill curve analysis was carried out. Then, three batches of M_1 population have been generated with the EMS doses of 0.5%, 0.6% and 0.7% respectively. Subsequently, in the next cropping, three batches of M_2 population, consisting of 1030, 710 and 360 M_2 families have been subjected to chlorophyll mutation have been studied.
Result: The LD_{50} value resided between 0.5% and 0.6% EMS. A Gradual increase in the EMS dose increased the lethality in the rice in a linear fashion. The frequencies of the chlorophyll mutation were 3.59, 6.47 and 9.44 for the EMS doses of 0.5%, 0.6% and 0.7% respectively.

Key words: Chlorophyll mutant, EMS, Mutation breeding, Rice.

INTRODUCTION

Rice is one of the main staple food crops for almost half of the global human population. The generation of new variations is the first objective of the crop breeding programme for genetic improvement. Induced mutagenesis is a universal technique (Bhat and Hakeem, 2023) to create novel alleles in rice (Jadhav *et al.,* 2023) and forward (Gurunathan *et al.,* 2019) and reverse (da Luz *et al.,* 2021) genetic approaches are practiced to identify functional mutations. In rice breeding, many mutagenic methods have been applied to induce functional mutations, including ionizing radiation (*e.g.*, fast neutrons, γ rays, X rays) and chemical substances (*e.g.*, EMS, DEB, sodium azide) (Gulfishan *et al.,* 2023) to generate varieties with enhanced genetic architecture (Kumawat *et al.,* 2022). Among these, EMS is one of the majorly utilized mutagens to generate the mutant population in rice (Hameed *et al.,* 2019; Jia *et al.,* 2019) since the EMS possesses plasticity across the crop plants to generate novel genetic variations. Mutagenesis with EMS is an expensive method to induce high rates of point mutations in the crops (Gillmor and Lukowitz, 2020). Understanding on biological effects of mutagens is important for the successful plant breeding programmes (Jankowicz-Cieslak *et al.,* 2021).

Moreover, the accuracy in following the mutagenesis protocol is also important to get the appropriate mutant population in crops with high rates of mutation to achieve the targets in the breeding programmes. The best indication of mutation occurrence in a mutagenesis protocol is the presence of chlorophyll mutants in the M_2 generation. In this study, the EMS mutagen was used to create the mutant population in a rice variety CO51, recorded the different types of chlorophyll mutants and analyzed the frequency of chlorophyll mutations at different doses of EMS.

[1]Department of Genetics and Plant Breeding, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Chengalpattu-603 201, Tamil Nadu, India.
[2]Division of Crop Stress Physiology and Molecular Biology, Department of Botany, School of Life Sciences, Periyar University, Salem-636 011, Tamil Nadu, India.

Corresponding Author: Selvakumar Gurunathan, Department of Genetics and Plant Breeding, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Chengalpattu-603 201, Tamil Nadu, India. Email: selvakumaragri@gmail.com

How to cite this article: Solarasan, R.M., Ramalingam, S., Singaram, A., Dhanarajan, A., Sivagurunathan, T. and Gurunathan, S. (2025). Frequency and Spectrum of Chlorophyll Mutations in Rice (*Oryza sativa* L.). *Agricultural Science Digest*. **1-7.** doi: 10.18805/ag.D-6384.

Submitted: 05-06-2025 **Accepted:** 04-08-2025 **Online:** 03-09-2025

MATERIALS AND METHODS

Determination of lethal dose

A high-yielding variety, CO51, has been taken for the mutagenesis study. Ten batches of 100 healthy rice seeds each were pre-soaked in distilled water for 24 hours. Pre-soaked seed batches were treated with 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% of ethyl methanesulphonate (EMS) for 12 hours (Unan *et al.,* 2022). Besides, the control batch without EMS treatment was also subjected. Immediately after the EMS treatment incubation period, after decanting the EMS liquid, 5% sodium thiosulfate solution was added to the treated seed batches to neutralize the EMS. Subsequently, seeds were thoroughly washed under running tap water for 6 hours to remove the residual EMS (Sagel *et al.,* 2017). The seeds were sown in the raised bed nursery under field conditions. The observation on survival and lethality of the seedling for the different doses of EMS has been recorded at 14 DAS (Table 1). A kill curve analysis was carried out to express the lethality in a diagrammatically (Fig 1).

Generation of M_1 seeds and raising of the population

Since the LD value has resided between 0.5 to 0.6% of EMS dose, these two doses are used to generate M_1 generation along with a high dose of 0.7% of EMS. Hence, each batch containing 100 g of seeds was used to generate the M_1 generation with the aforementioned EMS doses. The seedlings were transplanted to the field 20 days after sowing. The seeds were harvested from 2462 healthy plants on a single plant basis. In these, seeds from 1253 (0.5% EMS), 842 (0.6% EMS) and 367 (0.7% EMS) plants were harvested.

Raising of M_2 families

1030, 710 and 360 M_2 families were raised from the seeds of M_1 plants harvested from the 0.5%, 0.6% and 0.7% EMS doses, respectively. The observations on chlorophyll mutants, *viz., Albina, Striata, Xantha, Viridis, Aurea* and *Chlorina*, were recorded at 10 DAS.

RESULTS AND DISCUSSION

The mutagenic efficiency depends on the dose of the mutagen and it should be determined to get the optimum number of plant recovery with a higher number of mutations (Pratap and Kumar, 2011). The germination and survival rates progressively declined with increasing EMS concentrations (Table 1), which is consistent with prior findings (Hernández-Muñoz *et al.,* 2019). Visible changes due to mutations induced by EMS could be expressed mostly in the M_2 generation (Jia *et al.,* 2019) since most of the mutations are recessive. The presence of chlorophyll mutants in the M_2 progenies is an indicator for the occurrence of mutations in the mutant population (Prasannakumari *et al.,* 2024). Our experiment generated different types of chlorophyll mutants, *viz., Albina, Striata, Aurea, Xantha and Chlorina* (Table 2; Fig 2). Lalitha *et al.* (2020) supported that the presence of mutations in the chlorophyll synthesis genes leads to the generation of different types of chlorophyll mutants.

In the 0.5 EMS dose, maximum and minimum frequencies of chlorophyll mutation were recorded as 66.6 and 3.57 in the M_2 families CO51-1973 M_1 and CO51-1285 M_1, respectively. In the 0.6% EMS dose, maximum and minimum frequency of chlorophyll mutation were recorded as 61.9 and 1.63 in the M_2 families CO51-778 M_1 and CO51-573 M_1. In the 0.7% EMS dose, maximum and minimum

Table 1: Effect of EMS doses on the survival of rice seedlings.

EMS dose	Total seed sowing	No. of germination	Survival %	Lethal% % due to EMS
Control	100	95	100	0
0.10%	100	93	97	3
0.20%	100	88	92	8
0.30%	100	87	91	9
0.40%	100	75	78	22
0.50%	100	65	68	32
0.60%	100	36	37	63
0.70%	100	28	29	71
0.80%	100	25	26	74
0.90%	100	20	21	79
1%	100	14	14	86

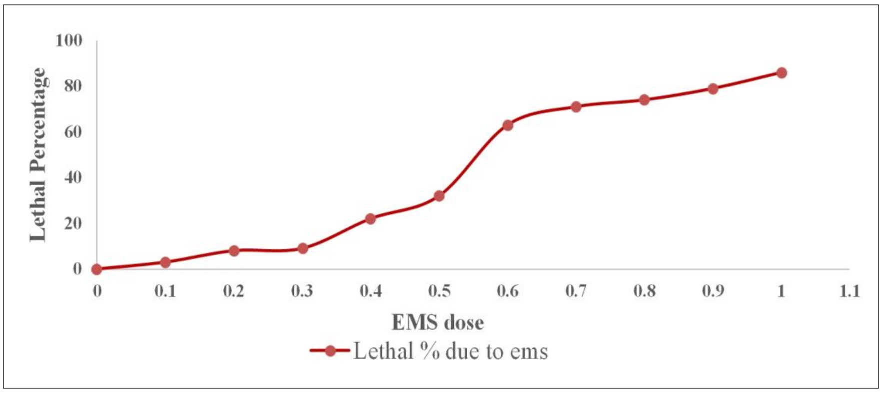

Fig 1: Kill curve of EMS doses and their effects on seedling survival.

Table 2: Details of chlorophyll mutants in the M_2 families.

	Total number of plants	No. of chlorophyll mutants	*Albina*	Striata	Xantha	Viridis	Aurea	Chlorina	Frequency
				0.50%					
CO51-1077M1	34	3	3	-	-	-	-	-	8.82
CO51-1088M1	24	1	1	-	-	-	-	-	4.17
CO51-1132M1	42	5	4	-	1	-	-	-	11.9
CO51-1139M1	25	3	-	-	3	-	-	-	12
CO51-1172M1	39	4	4	-	-	-	-	-	10.3
CO51-1192M1	46	4	-	-	4	-	-	-	8.7
CO51-1193M1	44	2	-	-	-	-	-	2	4.55
CO51-1207M1	31	4	-	1	-	-	-	3	12.9
CO51-1219M1	38	5	5	-	-	-	-	-	13.2
CO51-1285M1	28	1	1	-	-	-	-	-	3.57
CO51-1312M1	34	4	4	-	-	-	-	-	11.8
CO51-1431M1	28	2	-	-	2	-	-	-	7.14
CO51-1433M1	14	2	2	-	-	-	-	-	14.3
CO51-1467M1	55	31	18	3	5	4	-	1	56.4
CO51-1488M1	57	3	3	-	-	-	-	-	5.26
CO51-1500M1	70	10	2	-	5	3	-	-	14.3
CO51-1526M1	54	5	-	-	3	-	-	2	9.26
CO51-1533M1	31	4	4	-	-	-	-	-	12.9
CO51-1535M1	43	2	-	2	-	-	-	-	4.65
CO51-1587M1	38	4	-	4	-	-	-	-	10.5
CO51-1649M1	19	7	4	-	2	-	-	1	36.8
CO51-1677M1	33	5	5	-	-	-	-	-	15.2
CO51-1813M1	19	6	2	-	4	-	-	-	31.6
CO51-1827M1	54	6	4	-	-	-	2	-	11.1
CO51-1862M1	58	26	18	-	6	2	-	-	44.8
CO51-1864M1	44	12	12	-	-	-	-	-	27.3
CO51-1870M1	19	5	-	5	-	-	-	-	26.3
CO51-1911M1	59	7	5	-	2	-	-	-	11.9
CO51-1912M1	32	6	-	6	-	-	-	-	18.8
CO51-1930M1	34	2	2	-	-	-	-	-	5.88
CO51-1937M1	43	12	8	-	2	-	-	2	27.9
CO51-1957M1	44	14	7	-	5	-	2	-	31.8
CO51-1973M1	18	12	6	3	2	-	-	1	66.7
CO51-2011M1	54	4	4	-	-	-	-	-	7.41
CO51-2063M1	41	3	3	-	-	-	-	-	7.32
CO51-2064M1	40	6	3	-	-	-	3	-	15
CO51-2068M1	44	11	5	-	2	-	-	4	25
	1430	243	139	24	48	9	7	16	17
				0.6% EMS					
CO51-366M1	56	1	-	1	-	-	-	-	1.79
CO51-370M1	57	3	3	-	-	-	-	-	5.26
CO51-377M1	51	2	2	-	-	-	-	-	3.92
CO51-379M1	38	2	2	-	-	-	-	-	5.26
CO51-390M1	32	2	-	2	-	-	-	-	6.25
CO51-403M1	23	4	-	-	2	-	-	2	17.4
CO51-414M1	33	5	4	-	-	1	-	-	15.2
CO51-431M1	61	3	3	-	-	-	-	-	4.92
CO51-452M1	41	2	2	-	-	-	-	-	4.88

Table 2: Continue....

Table 2: Continue....

CO51-457M1	36	7	4	-	-	-	1	2	19.4
CO51-458M1	42	3	2	1	-	-	-	-	7.14
CO51-475M1	42	8	5	-	2	-	-	1	19
CO51-483M1	35	2	2	-	-	-	-	-	5.71
CO51-499M1	33	1	1	-	-	-	-	-	3.03
CO51-518M1	20	2	2	-	-	-	-	-	10
CO51-519M1	41	4	1	-	3	-	-	-	9.76
CO51-553M1	19	1	1	-	-	-	-	-	5.26
CO51-566M1	35	8	5	-	2	-	1	-	22.9
CO51-573M1	61	1	-	-	1	-	-	-	1.64
CO51-577M1	58	1	-	1	-	-	-	-	1.72
CO51-578M1	42	3	3	-	-	-	-	-	7.14
CO51-581M1	51	4	3	-	1	-	-	-	7.84
CO51-605M1	25	8	6	2	-	-	-	-	32
CO51-625M1	53	15	7	-	5	-	2	1	28.3
CO51-627M1	24	5	4	1	-	-	-	-	20.8
CO51-653M1	38	3	2	-	-	1	-	-	7.89
CO51-665M1	27	7	5	2	-	-	-	-	25.9
CO51-697M1	28	8	8	-	-	-	-	-	28.6
CO51-699M1	49	3	-	-	3	-	-	-	6.12
CO51-735M1	77	7	4	2	1	-	-	-	9.09
CO51-744M1	30	7	3	-	-	2	-	2	23.3
CO51-755M1	54	12	7	-	3	-	2	-	22.2
CO51-778M1	21	13	8	-	2	-	1	2	61.9
CO51-812M1	24	3	2	-	-	-	1	-	12.5
CO51-829M1	33	1	1	-	-	-	-	-	3.03
CO51-845M1	37	1	-	-	1	-	-	-	2.7
CO51-855M1	34	10	8	-	2	-	-	-	29.4
CO51-913M1	39	2	2	-	-	-	-	-	5.13
CO51-921M1	32	1	1	-	-	-	-	-	3.13
CO51-968M1	35	3	3	-	-	-	-	-	8.57
CO51-969M1	22	2	2	-	-	-	-	-	9.09
CO51-975M1	42	6	6	-	-	-	-	-	14.3
CO51-977M1	36	4	4	-	-	-	-	-	11.1
CO51-979M1	46	2	2	-	-	-	-	-	4.35
CO51-1024M1	30	7	4	2	1	-	-	-	23.3
CO51-1048M1	18	1	-	-	-	1	-	-	5.56
	1761	200	134	14	29	5	8	10	11.4
				0.7% EMS					
CO51-4M1	57	1	1	-	-	-	-	-	1.75
CO51-17M1	56	1	1	-	-	-	-	-	1.79
CO51-36M1	37	7	2	-	3	-	-	2	18.9
CO51-44M1	30	1	1	-	-	-	-	-	3.33
CO51-50M1	50	1	1	-	-	-	-	-	2
CO51-67M1	37	5	2	-	3	-	-	-	13.5
CO51-113M1	23	5	2	1	1	1	-	-	21.7
CO51-124M1	33	6	4	1	-	-	-	1	18.2
CO51-132M1	16	3	3	-	-	-	-	-	18.8
CO51-134M1	50	4	3	-	1	-	-	-	8
CO51-141M1	58	6	3	-	-	2	-	1	10.3
CO51-143M1	23	2	2	-	-	-	-	-	8.7

Table 2: Continue....

Table 2: Continue....

CO51-155M1	19	4	-	2	2	-	-	-	21.1
CO51-170M1	29	3	2	-	1	-	-	-	10.3
CO51-175M1	34	5	4	-	1	-	-	-	14.7
CO51-176M1	53	13	6	2	-	4	-	1	24.5
CO51-188M1	19	1	1	-	-	-	-	-	5.26
CO51-191M1	40	1	1	-	-	-	-	-	2.5
CO51-204M1	39	5	3	-	2	-	-	-	12.8
CO51-205M1	35	6	4	-	-	-	-	2	17.1
CO51-214M1	26	3	3	-	-	-	-	-	11.5
CO51-219M1	21	3	3	-	-	-	-	-	14.3
CO51-224M1	39	2	2	-	-	-	-	-	5.13
CO51-225M1	75	11	8	2	-	1	-	-	14.7
CO51-237M1	33	13	6	-	3	-	2	2	39.4
CO51-238M1	49	13	7	1	2	-	-	3	26.5
CO51-249M1	31	4	3	-	-	1	-	-	12.9
CO51-272M1	63	1	1	-	-	-	-	-	1.59
CO51-281M1	42	1	1	-	-	-	-	-	2.38
CO51-284M1	47	1	1	-	-	-	-	-	2.13
CO51-286M1	64	4	4	-	-	-	-	-	6.25
CO51-327M1	59	12	6	-	5	-	-	1	20.3
CO51-346M1	28	2	2	-	-	-	-	-	7.14
CO51-347M1	35	4	2	-	2	-	-	-	11.4
	1350	154	95	9	26	9	2	13	11.4

Fig 2: Chlorophyll mutants in M_2 families.

Table 3: Frequency of chlorophyll mutation in the M_2 population for different EMS doses based on the tested plant numbers (a) and family numbers (b).

		0.5% EMS	0.6% EMS	0.7% EMS
	a. Chlorophyll mutation frequency based on the number of plants tested			
Chlorophyll mutant	*Albina*	139	134	95
	Striata	24	14	9
	Xantha	48	29	26
	Viridis	9	5	9
	Aurea	7	8	2
	Chlorina	16	10	13
Total number of chlorophyll mutants	243	200	154	
Total number of Plants Tested	1430	1761	1350	
Mutation frequency	16.99	11.35	11.40	
	b. Chlorophyll mutation frequency based on the number of families tested			
Number of M_2 families raised and tested		1030	710	360
Number of M_2 families reported with chlorophyll mutants		37	46	34
Frequency of M_2 families with chlorophyll mutants		3.59	6.47	9.44

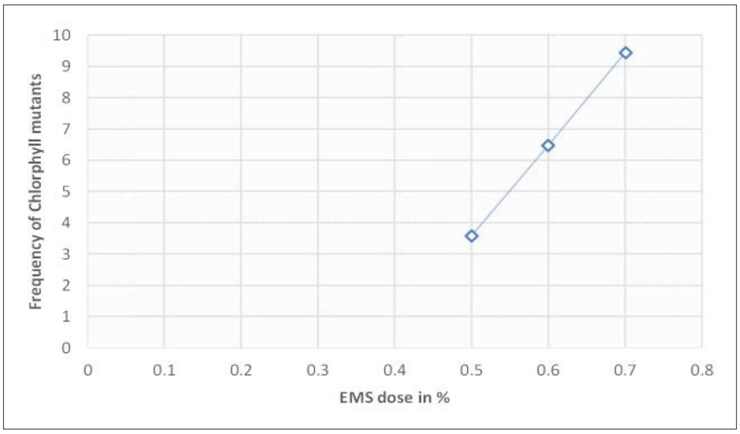

Fig 3: EMS dose on chlorophyll mutation frequency.

frequency of chlorophyll mutation were recorded as 39.39 and 1.58 in the M_2 families CO51-237M_1 and CO51-272M_1.

A total of 243, 200, 154 and chlorophyll mutants were found at the doses of 0.5% EMS, 0.6% EMS and 0.7% EMS, respectively. The chlorophyll mutation frequency from the observed plants is 16.99 (0.5% EMS), 11.35 (0.6% EMS) and 11.40 (0.7% EMS) (Table 3a). Besides, chlorophyll mutant frequency in terms of family volume numbers tested was 3.59 (0.5%), 6.47 (0.6%) and 9.44 (0.7%) (Table 3b). The increases in the EMS dose lead in increases in the chlorophyll mutation frequency in the M_2 families (Fig 3). Prasannakumari *et al.,* (2024) reported that the frequency of chlorophyll mutation is increased when increasing the EMS and Gamma doses in rice.

CONCLUSION

The present study aimed to find out the mutagenic effects of ethyl-methane-sulfonate (EMS) on rice variety CO51 to generate genetic variation for crop improvement. The germination and survival rates progressively declined with increasing EMS concentrations. The LD_{50} resided between the EMS doses of 0.5% and 0.6%, which indicates that the EMS doses between 0.5% and 0.6% could give enough plant recovery with mutations in the mutagenesis in rice. The occurrence of chlorophyll mutants in the M_2 generation confirms the accuracy level in the conduct of experiments in the mutation population generation. The frequency of mutation in the M_2 families recorded that 3.59, 6.47 and 9.44 for the EMS doses of 0.5%, 0.6% and 0.7% proves that an increase in the EMS dose leads to an increase in mutation frequency in rice. Conclusively, the mutant populations generated are highly reliable for screening for various traits of interest.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Bhat, T.A. and Hakeem, K.R. (Eds.). (2023). Biotechnologies and Genetics in Plant Mutation Breeding. Waretown, NJ, USA: *Apple Academic Press*. pp: 1.

da Luz, V.K., *Via*na, V.E., da Fonseca, G.M., Pegoraro, C., da Maia, L.C. and de Oliveira, A.C. (2021). Identification of rice mutants tolerant to cold stress at the germination stage by Tilling. In Mutation breeding, genetic diversity and crop adaptation to climate change. Wallingford UK: CABI. pp: 111-119.

Gillmor, C.S. and Lukowitz, W. (2020). EMS mutagenesis of Arabidopsis seeds. *Plant Embryogenesis: Methods and Protocols*. pp: 15-23.

Gulfishan, M., Bhat, T.A., Mir, R.A., Jahan, A., Shabir, F. and Khan, S.A. (2023). Biotechnologies and genetics in plant mutation breeding. In Revolutionizing plant biology: Plant mutagenesis-Terms and applications. CRC Press. United Kingdom.

Gurunathan, S., Ramadoss, B.R., Mudili, V., Siddaiah, C., Kalagatur, N.K., Bapu, J.R.K. and Abd_Allah, E.F. (2019). Single nucleotide polymorphisms in starch biosynthetic genes associated with increased resistant starch concentration in rice mutant. *Frontiers in Genetics*. **10:** 946.

Hameed, K., Khan, M.S., Sadaqat, H.A. and Awan, F.S. (2019). Phenotypic characterization of super basmati ethyl methane sulfonate (EMS) induced mutants. *Pakistan Journal of Agricultural Sciences*. **56(2).**

Hernández-Muñoz, S., Pedraza-Santos, M.E., López, P.A., Gómez-Sanabria, J.M. and Morales-García, J.L. (2019). Mutagenesis in the improvement of ornamental plants. *Revista Chapingo. Serie Horticultura*. **25(3):** 151-167.

Jadhav, A.H., Desai, S.S., Bhave, S.G., Mane, A.V., Sawardekar, S.V., Dalvi, V.V. and Bal, C.P. (2023). Optimization of LD_{50}, frequency and spectrum of chlorophyll mutation, efficiency and effectiveness of gamma rays in different cultivars of rice (*Oryza sativa* L.). *The Pharma Innovation Journal*. **12(6):** 4327-4333.

Jankowicz-Cieslak, J., Goessnitzer, F., Datta, S., Viljoen, A., Ingelbrecht, I. and Till, B.J. (2021). Induced mutations for generating bananas resistant to Fusarium wilt tropical race 4. In Mutation breeding, genetic diversity and crop adaptation to climate change. Wallingford UK: CABI. pp: 366-378.

Jia, Y., Wang, Z., Jia, M.H., Rutger, J.N. and Moldenhauer, K.A. (2019). Development and characterization of a large mutant population of a rice variety katy for functional genomics studies and breeding. *Crop Breeding, Genetics and Genomics*. **1(2).**

Kumawat, S., Raturi, G., Dhiman, P., Sudhakarn, S., Rajora, N., Thakral, V. and Kumar, M. (2022). Opportunity and challenges for whole genome resequencing based genotyping in plants. *Genotyping by Sequencing for Crop Improvement*. pp: 38-51.

Lalitha, R., Mothilal, A., Arunachalam, P., Vanniarajan, C., Senthil, N., Souframanien, J. and Hemalatha, G. (2020). Effectiveness and efficiency of gamma rays and electron beam in M_2 generation of Anna (R) 4 rice mutants. *Indian Journal of Agricultural Research*. **54(4):** 516-520. doi: 10.18805/IJARe.A-5334.

Prasannakumari, M., Gnanamalar, R.P., Vanniarajan, C., Souframanien, J., Anand, G., Renuka, R. and Singh, R.D. (2024). Impact of electron beam and ethyl methane sulphonate on chlorophyll mutations in rice genotypes ASD 16 and Norungan. *Journal of Phytology*. **16:** 156-162.

Pratap, A. and Kumar, J. (Eds.). (2011). Biology and Breeding of Food Legumes. CABI.

Sagel, Z., Tutluer, M.Ý., Peskýrcýoglu, H., Kantoglu, Y. and Kunter, B. (2017). Determination of effect of chemical mutagen EMS on TAEK A-3 and TAEK C-10 mutant soybean varieties in M_1 generation. *International Journal of Crop Breeding and Genetics*. **3(1):** 19-24.

Unan, R., Deligoz, I., Al-Khatib, K. and Mennan, H. (2022). Protocol for ethyl methanesulfonate (EMS) mutagenesis application in rice. *Open Research Europe*. **1:** 19.