Received: 25 Nov 2023 | Revised: 26 Dec 2023 | Accepted: 31 Dec 2023

Electronic Journal of Plant Breeding

Research Article

Sensitivity of seedling growth, fertility and yield contributing traits in rice (*Oryza sativa* L.) to electron beam and Ethyl Methane Sulphonate

M. Prasannakumari[1], R.P. Gnanamalar[1*], J. Souframanien[2], G. Anand[3], R. Renuka[4] and R. Durai Singh[5]

[1]Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, India
[2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India
[3]ICAR - KVK, Agricultural college and Research Institute, Madurai, India
[4]Department of Biotechnology, Centre of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India
[5]Department of Agronomy, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, India
***E-Mail**: gnanamalar.rp@tnau.ac.in

Abstract
Rice (*Oryza sativa* L.) plays a pivotal role in global food security, necessitating continuous efforts to enhance yield potential and grain quality. Mutation breeding, employing physical (electron beam) and chemical (ethyl methane sulphonate, EMS) mutagens, offers a promising avenue for creating genetic variability. This study assessed the sensitivity of rice variety ASD 16 and landrace Norungan to varying doses of electron beam (100 Gy to 500 Gy) and EMS (30 mM to 70 mM). The genotypes exhibited dose-dependent responses in germination, seedling survival, shoot and root length, seedling height, pollen fertility and spikelet fertility. The LD_{50} values for electron beam under in vivo and in vitro conditions were 314.24 Gy and 348.21 Gy respectively for ASD 16 and 445.07 Gy and 461.88 Gy for Norungan whereas for EMS, the LD_{50} values for ASD 16 were 49.17 mM and 55.37 mM; 53.53 mM and 65.12 mM for Norungan under *in vivo* and *in vitro* conditions based on probit analysis. ASD 16 showed a GR_{50} value of 309.02 Gy for the electron beam and 51.60 mM for EMS while Norungan exhibited a GR_{50} value of 538.79 Gy for electron beam and 98.71 mM for EMS. The variety, ASD 16 demonstrated higher sensitivity, proved by greater growth reduction percentages and lower lethal dose 50 (LD_{50}) values compared to landrace Norungan. Electron beam treatments induce significant reductions in various growth traits, underlining the potential deleterious effects at higher doses. EMS, as a chemical mutagen, also impacted germination and growth characteristics. Both mutagens affected pollen and spikelet fertility with notable influence on yield contributing traits. This finding emphasizes the significance of precise dosage selection in mutation breeding to induce desirable traits and maintaining complete plant viability and productivity. Understanding genotype-specific responses is crucial for successful mutation breeding programmes. Overall, this study offers valuable insights into the application of electron beam and EMS mutagenesis in rice, contributing to the optimization of mutagenic protocols for crop improvement.

Keywords: Rice, mutation, Electron beam, EMS, LD_{50}, GR_{50}

INTRODUCTION

Rice (*Oryza sativa* L.) is a vital cereal crop, supplying food to over half of the global population (Tyagi *et al*., 2004). To fulfil global needs and address climate change, perpetual enhancements in food production are mandatory. Increased yield potential and desirable grain quality are the primary objectives of plant breeders which are made feasible by creating variation in the plant population. Mutation, a key source of variation in various crops particularly successful in rice using radiation and chemical mutagens (Akbar and Mansoor, 2003). The effective use of mutagens and proper handling of mutant generations are crucial for the isolation of superior mutants from the population.

The electron beam, a physical mutagen from particle accelerators, accelerates electrons to the speed close to light (about 190,000 miles per second) and is effective in inducing gene mutations by creating single-strand breaks (SSB) and double-strand breaks (DSB) in DNA. Electron beam in rice had a broader mutation spectrum, higher mutagen frequency and higher efficiency variation and less harm in M_1 than ^{60}Co rays (Baojiang *et al*., 1982). Ethyl methane sulphonate (EMS) is a frequently employed chemical mutagen that induces random point mutations when O-6-ethyl guanine aligns opposite a cytosine residue during DNA replication (Sikora *et al*., 2011). Prolonged and intense EMS treatment leads to the significant number of point mutations developing lethality and yielding high mutagenic efficiency.

The goal of inducing mutation is to create phenotypic and genotypic changes for unique traits. Numerous studies confirm that low doses of irradiation minimally affect the genome whereas high doses often lead to deleterious changes. Both electron beam and EMS were employed on the two rice genotypes, ASD 16 an indica rice with high productivity and short bold grains and Norungan, an indica landrace with drought tolerance (Amudha *et al*., 2023). Mutagens, alter the genotypes at the molecular level, manifesting the morphological traits, thereby the genuine characters of plants may be improved. Determination of Lethal Dosage 50 (LD_{50}) and Growth Reduction 50 (GR_{50}) values are essential step in mutagenesis programme. This study examines the impacts of the electron beam and EMS on the growth, fertility and biometrical traits of ASD 16 and Norungan, aiming to identify beneficial mutants.

MATERIALS AND METHODS

High yielding short duration rice variety ASD 16 and landrace Norungan were chosen for this study and seed materials were obtained from the Department of Plant breeding and Genetics, Agricultural College and Research Institute, Madurai. Five hundred well filled dried seeds with 12 per cent moisture content of ASD16 and Norungan underwent electron beam treatment at doses of 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy using a 10 MeV electron beam at Bhabha Atomic Research Center (BARC), Mumbai, India. Untreated seeds were used as controls. For Chemical mutagenesis, Liquid ethyl mesylate (boiling point 85-86 °C/10 mm Hg (lit), density 1.206 g/ml at 20°C) was used. Pre and post EMS treatment, pH was monitored and EMS mixture was prepared using phosphate buffer with neutral pH 7. After pretreatment, 300 seeds were immersed in EMS solutions of 30 mM, 40 mM, 50 mM, 60 mM and 70 mM subjected to controlled rotary shaking for six hours to ensure an even distribution of mutagen. Then, the seeds were carefully rinsed under running tap water to exclude the residual EMS and it was left to air dry for 30 minutes and sown in raised nursery beds in field along with control seeds which were pre-soaked in distilled water for 28 hours.

In vitro and *In vivo* study: In the *in vitro* study, the roll towel method was carried out in October, 2021 for the germination test. Fifty treated seeds of ASD 16 and Norungan for each dose of electron beam and EMS were placed with two replications. Germination percentage was measured on the seventh day while shoot and root length were noted on the 14^{th} day. For the *in vivo* study, 450 treated seeds were sown in raised bed nursery along with the control. On the 25th day, the seedlings from each dose were transplanted to the field at the research farm of the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai. All the agronomic practices were followed from seedling to maturity stage. Various parameters including survival percentage, seedling height on 20th day, pollen fertility during flowering and the biometrical traits *viz*., spikelet fertility, plant height, number of productive tillers per panicle, panicle length and single plant yield were observed at maturity according to the Standard Evaluation System, IRRI (2013) for rice.

Probit analysis: Probit analysis was performed to examine the dose-response relationship by fitting a straight line to data using regression, establishing the relationship between the response variable (Y) and the independent variable (X).

$$Y = a + bX + c,$$

Where a = y intercept, b= Slope of the line and c = constant

Mean data on germination percentage was expressed as percentage of the control (0 doses) and transformed into probits using the probit transformation method (Finney,1971). Regression analysis was done on the transformed data in MS Excel 2007 to determine the Y intercept and the slope (b) of the regression line. The antilog of the log 10 value corresponding to probit 5 was utilized to detect the LD_{50} (lethal dose for 50 per cent of the population) for the specific mutagen. **T**he growth reduction was calculated using a simple linear regression model, plotting the absorbed dose against the per cent reduction at 50 per cent (GR_{50}) and 75 per cent (GR_{75}).

RESULTS AND DISCUSSION

Seed germination: The mutagenic response for germination was assessed for the rice genotypes ASD

16 and Norungan which were exposed to Electron beam and EMS (**Table 2**). Both genotypes exhibited a dose dependent and negative association between dose and germination rate.

Germination percentage was decreased in both physical and chemical mutagenic treatments employed compared to control. The germination percentages of 90 and 94 were recorded in the control dose of ASD 16 and Norungan, respectively. The germination percentage for various doses of electron beam in the M_1 generation ranged from 21.02 per cent (500 Gy) to 77.47 per cent (100 Gy) for ASD 16 and from 49.04 per cent (500 Gy) to 86.64 per cent (100 Gy) for Norungan. For EMS, germination percentage ranged from 15.32 per cent in (70 mM) to 81.87 per cent in (30mM) for ASD 16 and for Norungan, 52.02 per cent in 70mM and 88.47 per cent in 30mM. Higher sensitivity was noticed in ASD 16 to electron beam and EMS compared to Norungan. Reduction in germination percentage ranged from 85.5 to 23.5 and 92.17 to 52.17 in electron beam compared to control in ASD 16 and Norungan. For EMS, the reduction in germination percentage ranged from 90.97 to 22.22 and 94.12 to 55.34 compared to control in both genotypes. The deterioration in viability can be attributed to alternations in essential cell functions such as activation of RNA or protein synthesis as reported by Abdel-Hady (2008) or caused by the inhibitory actions of mutagens on the plumule and radicle (Olaolorun *et al*., 2019).

Mutagen sensitivity: Lethal dosage 50 (LD_{50}) determination was based on the germination percentage, comparing germination in control with that of all treatment doses. From **Table 2**, the drop in the rate of germination relative to control was calculated for each dose and genotype, then fitted to probit analysis for LD_{50} estimation.

LD_{50} dose for electron beam under *in vivo* and *in vitro* conditions were 314.24 Gy and 348.21 Gy for ASD 16 and 445.07 Gy and 461.88 Gy for Norungan, respectively (**Table 1**) (**Fig. 1. and 2**). Sao *et al*. (2020) reported that the optimal dosage for an electron beam is 290 to 330 Gy for short grain aromatic rice which is similar to the LD_{50} dose of electron beam in ASD 16. In chemical mutagen EMS, the LD_{50} values for ASD 16 were 49.17 mM and 55.37 mM and 53.53 mM and 65.12 mM for Norungan under *in vivo* and *in vitro* conditions based on probit analysis (**Table 1**) (**Fig. 1. and 2**). It indicates that the ASD 16 is more sensitive to both electron beam and EMS compared to Norungan which exhibited higher LD_{50} values for both mutagens. The growth reduction parameter was estimated based on a 50 percent germination reduction value (GR_{50}) (**Table 3**). ASD 16 showed a GR_{50} value of 309.02 Gy for the electron beam and 51.60 mM for EMS while Norungan exhibited a GR_{50} value of 538.79 Gy for electron beam and 98.71 mM for EMS.

Mutagenic effect on shoot length and root length: In ASD 16 and Norungan, the electron beam resulted in a maximum shoot length of 11.45 cm and 17.21 cm at 100 Gy and 200 Gy, respectively, while for EMS, it was 10.22 cm and 15.88 cm observed at 30 mM. The lowest shoot lengths were 5.29 cm and 12.00 cm for electron beam and 5.44 cm and 10.04 cm for EMS in ASD 16 and Norungan, respectively. The percentage growth reduction of shoot length due to electron beam exceeded 25 per cent in 400 Gy and 500 Gy in Norungan, whereas for ASD 16, the reduction was started at 200 Gy (30.96 per cent). In EMS, the percent growth reduction begins at 100 Gy for ASD 16 and 200 Gy for Norungan. This showed that electron beam primarily affects cell elongation and cell division without impacting embryonic characteristics.

The maximum root length was found in 100 Gy of ASD 16 (11.15cm) and Norungan (17.25 cm) for electron beam and 30 mM of ASD16 (10.74 cm) and Norungan (18.02 cm) for EMS. A maximum root length reduction of 40 percent was observed at elevated doses of 500 Gy of electron beam and 70 mM for EMS in both the genotypes. The minimum root length was noted in 500 Gy and 70 mM of ASD 16. Both the genotypes showed maximum root length and shoot length in lower concentrations of electron beam and EMS. This result was in accordance with Ramesh *et al.* (2019). The reduction of radicle and plumule growth caused by mutagens which block mitotic activity in meristematic tissue further leads to the generation of free radicals causes growth retardation in seedlings and reduction of moisture content in seeds (Khalil *et al.,* 1986). This is also attributed to the reduction in enzyme activity and the inhibition of auxin in seeds which is responsible for root growth (Usuf and Nair,1974). The growth reduction 50 for shoot length and root length in ASD 16 were 409.51 Gy and 450.85Gy for an electron beam and 56.85 mM and 80.59 mM for EMS. In Norungan, the recorded values under electron beam were 896.05Gy and 594.53 Gy while with EMS, it was 61.49 mM and 113.12 mM.

Mutagenic effect on seedling height: Maximum seedling height was observed in ASD 16 and Norungan at 100 Gy, measuring 22.60 cm and 34.4 cm in electron beam followed by 30 mM of EMS in ASD 16 (20.96 cm) and Norungan (31.90 cm). Minimum seedling height of 11.43 cm and 21.94 cm were recorded in the electron beam of 500 Gy for ASD 16 and Norungan respectively; whereas it was 12.08 cm in ASD 16 and 19.08 cm in Norungan for 70 mM of EMS. There was a gradual reduction in root length, shoot length and seedling height for both the genotypes in response to an increase in dose that Norungan is less sensitive and ASD 16 is more sensitive to higher mutagen doses. A significant 50 percent growth reduction in seedling height was observed maximum in higher doses of mutagens for both the genotypes. The disruption in the cell division cycle at the G2/M phase, disturbance in DNA, RNA, protein and growth hormone synthesis (Solim and Rahayu, 2021; Ulukapi and Ozmen, 2018), hormonal balance and enzyme activity (Kant *et al*., 2020) were all associated with the decline in seedling height caused by

Table 1. Determination of LD_{50} for electron beam and Ethyl Methane Sulphonate (EMS) in rice variety ASD 16 and Norungan by probit analysis

Genotypes	Mutagens	Conditions	LD_{50} value
ASD 16	Electron Beam	*In vivo*	314.24 Gy
		In vitro	348.21 Gy
	EMS	*In vivo*	49.17 mM
		In vitro	55.37 mM
Norungan	Electron Beam	*In vivo*	445.07 Gy
		In vitro	461.88 Gy
	EMS	*In vivo*	53.53 mM
		In vitro	65.12 mM

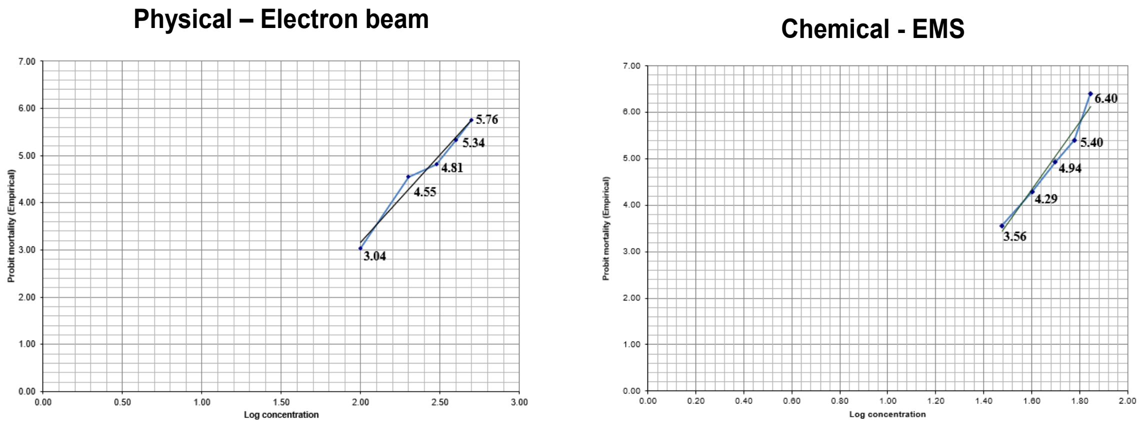

Fig. 1. Determination of LD_{50} in ASD 16 Physical – Electron beam

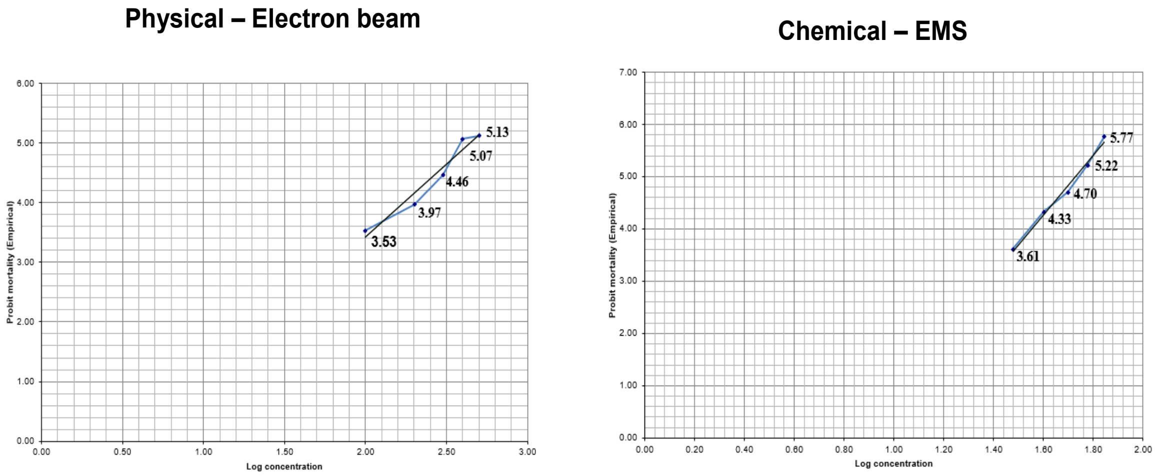

Fig. 2. Determination of LD_{50} in Norungan Physical – Electron beam

mutagens. On comparison of both the genotypes under in vitro conditions, shoot length, root length and seedling height were greater in Norungan and smaller in ASD 16.

Mutagenic effect on seedling survival : Norungan exhibited the highest survival rates at 87.67 per cent with 30 mM of EMS and 83.55 percent with 100 Gy of electron beam. ASD 16 showed the lowest survival percentage of 17.24 with 70 mM of EMS and 11.51 with 500 Gy of electron beam. These results were in accordance with Francis *et al.* (2022). The number of seedlings observed on the 14th day was significantly reduced compared to

Table 2. Effect of electron beam and Ethyl Methane Sulphonate (EMS) on seedling, fertility and yield contributing of ASD 16 and Norungan in M_1 generation

Treatments	Germination	Shoot length	Root length	Seedling height	Seedling survival	Pollen fertility	Spikelet fertility	Plant height	No. of productive tillers/plant	Panicle length	Single plant yield
Electron beam	**Mean (per cent)**	**Mean (cm)**	**Mean (cm)**	**Mean (cm)**	**Mean (per cent)**	**Mean (per cent)**	**Mean (per cent)**	**Mean (cm)**	**Mean (per cent)**	**Mean (per cent)**	**Mean (per cent)**
ASD 16											
Control	90.00	12.66	12.26	24.92	90	88.98	91.00	92.46	100	100	100
100 Gy	77.47 (13.92)	11.45 (9.56)	11.15 (9.05)	22.60 (9.31)	61.32 (31.87)	79.81 (10.31)	81.14 (10.84)	89.92 (2.74)	94.62 (5.38)	95.82 (4.18)	97.54 (2.46)
200 Gy	63.12 (29.87)	8.74 (30.96)	8.32 (32.14)	17.06 (31.54)	40.79 (54.68)	62.12 (30.19)	75.62 (16.90)	87.42 (5.45)	83.92 (16.08)	91.16 (8.84)	95.32 (4.68)
300 Gy	41.14 (54.29)	7.87 (37.84)	7.38 (39.80)	15.25 (38.80)	22.82 (74.64)	58.21 (34.58)	71.32 (21.63)	84.67 (8.42)	76.41 (23.59)	88.32 (11.68)	92 (8)
400 Gy	29.25 (67.50)	6.48 (48.82)	7.12 (41.92)	13.60 (45.43)	16.46 (81.71)	43.76 (50.82)	66.34 (27.10)	81.46 (11.89)	58.96 (41.04)	84.04 (15.96)	89.3 (10.7)
500 Gy	21.02 (76.64)	5.29 (58.21)	6.14 (49.92)	11.43 (54.13)	11.51 (87.21)	36.47 (59.01)	60.78 (33.21)	77.21 (16.49)	43.2 (56.8)	81.81 (18.19)	81.8 (18.2)
NORUNGAN											
Control	94.00	17.72	18.21	35.93	94	92.14	90.00	108.82	100	96.8	100
100 Gy	86.64 (7.83)	17.15 (3.22)	17.25 (5.27)	34.4 (4.26)	83.55 (11.12)	78.25 (15.07)	83.68 (7.02)	103.18 (5.18)	96.5 (3.50)	90.13 (3.68)	98 (2)
200 Gy	78.44 (16.55)	17.21 (2.88)	16.81 (7.69)	34.02 (5.23)	74.78 (20.45)	60.46 (34.38)	77.21 (14.21)	100.45 (7.69)	81.56 (18.44)	88.46 (5.41)	96.8 (3.2)
300 Gy	68.37 (27.27)	15.57 (12.13)	14.38 (21.03)	29.95 (16.64)	62.81 (33.18)	47.12 (48.86)	72.34 (19.62)	98.25 (9.52)	72.44 (27.56)	86.46 (7.48)	95.7 (4.3)
400 Gy	59.12 (37.11)	13.29 (25.00)	11.29 (38.00)	24.58 (31.59)	50.62 (46.15)	37.65 (59.14)	67.15 (25.39)	95.82 (11.94)	68.21 (31.79)	82.78 (11.27)	94.24 (5.76)
500 Gy	49.04 (47.83)	12.00 (32.28)	9.94 (45.41)	21.94 (38.94)	42.34 (54.96)	34.91 (62.11)	61.28 (31.91)	93.62 (13.96)	32 (68.00)	80.55 (13.58)	93.91 (6.09)
EMS											
ASD 16											
Control	90.00	12.66	12.26	24.92	90.00	88.98	91.00	92.46	100	100	100
30 mM	81.87 (9.03)	10.22 (19.27)	10.74 (12.40)	20.96 (15.89)	70.91 (21.21)	80.32 (9.73)	84.87 (6.74)	88.54 (4.23)	91.62 (8.38)	97.80 (2.20)	97.82 (2.18)
40 mM	68.94 (23.40)	7.32 (42.18)	9.81 (19.98)	17.13 (18.27)	59.47 (33.92)	69.42 (21.98)	77.98 (14.31)	83.87 (9.29)	84.86 (15.14)	94.65 (5.35)	96 (4)
50 mM	47.24 (47.51)	6.58 (48.03)	8.14 (33.61)	14.72 (14.07)	32.91 (63.43)	54.78 (38.44)	74.76 (17.85)	76.54 (17.21)	70.48 (29.52)	90.76 (9.23)	93 (7)
60 mM	26.57 (67.55)	5.88 (53.55)	7.53 (38.58)	13.41 (8.90)	28.82 (67.98)	46.13 (48.16)	68.91 (24.27)	72.72 (21.34)	57.4 (42.60)	85.93 (14.07)	90.26 (9.74)
70 mM	15.32 (77.78)	5.44 (57.03)	6.64 (45.84)	12.08 (9.92)	17.24 (80.84)	40.93 (54.00)	60.45 (33.57)	68.03 (26.44)	40.28 (59.72)	83.95 (16.04)	87.53 (12.47)
NORUNGAN											
Control	94.00	17.72	18.21	35.93	94.00	92.14	90.00	108.82	100	96.8	100
30 mM	88.47 (5.88)	15.88 (10.38)	18.02 (1.04)	31.90 (11.12)	87.67 (6.73)	83.22 (9.68)	86.95 (3.39)	101.42 (6.8)	89.45 (10.55)	90.5 (3.30)	97.5 (2.5)
40 mM	80.12 (14.77)	14.12 (20.32)	17.11 (6.04)	29.23 (18.65)	79.33 (15.61)	71.00 (22.94)	80.24 (10.84)	97.75 (10.17)	71.62 (28.38)	89.6 (4.23)	96.87 (3.13)
50 mM	73.14 (22.19)	13.92 (21.44)	14.66 (19.49)	24.58 (31.59)	68.11 (27.54)	57.23 (37.89)	75.14 (16.51)	94.74 (12.93)	60.78 (39.22)	87.43 (6.47)	95 (5)
60 mM	65.25 (30.59)	12.75 (28.05)	12.25 (32.73)	21.00 (41.55)	57.34 (39.00)	48.41 (47.46)	69.42 (22.87)	90.95 (16.42)	52.91 (47.09)	84.8 (9.19)	93.91 (6.09)
70 mM	52.02 (44.66)	10.04 (43.34)	11.04 (39.37)	19.08 (46.09)	45.57 (51.52)	42.78 (53.57)	61.32 (31.87)	86.67 (20.35)	40.36 (59.64)	81.87 (12.22)	92.75 (7.25)

*Values in the parentheses () indicates percent over reduction that recorded on the 7th day after sowing, suggesting that mutagens cause severe damage to seedlings at a later stage of development than earlier. Dosages of 500 Gy and 70 mM caused stunted growth and over 80 per cent seedling mortality in ASD 16 whereas in Norungan, the survival rate was above 50 per cent at 500 Gy and 70 mM and it was found that Norungan was comparatively less sensitive than ASD 16 to these doses. From the result, it was evident that survival reduction occurs even at the advanced growth stage mainly owing to the inhibitory action of mutagens on meristematic tissues, which causes cell injury and alterations in plant metabolism (Talebi *et al.,* 2012). Moreover, the study showed that the germination rate did not equal to the survival proportion because the seeds exposed to higher doses were germinated but many of them later perished which led to a decline in the survival rate of the seedlings.

Mutagenic effect on pollen fertility and spikelet fertility: Maximum pollen fertility of 79.81 per cent and 78.25 per cent was noticed in electron beam of 100 Gy in ASD 16 and Norungan (**Table 2**) whereas in EMS treatment, it was observed at a lower dose of 30 mM for ASD 16 (80.32 per cent) and for Norungan (83.22 per cent). This revealed the inverse correlation between pollen fertility and mutagen dose. These equivalent findings were reported by Akilan *et al*. (2019). It was observed that an electron beam was more effective than EMS particularly in the flowering stage, which was also supported by Gowthami *et al*. (2016). The highest reduction in pollen fertility due to the electron beam was 59.01 per cent for ASD 16 and 62.11 per cent for Norungan in 500 Gy, whereas in EMS, it was 54.00 per cent for ASD 16 and 53.57 per cent for Norungan at 70 mM. These findings coincided with Siddiq and Swaminathan (1968) and reported that higher chromosomal aberrations are in gamma radiation treatment compared to EMS. Sterility caused by irradiation was attributed to deletions and particular gene mutations whereas in EMS, infertility was due to chromosomal abnormalities (Sharma and Kumar, 2004). The deterioration in pollen fertility may be associated with low mitotic index, increased presence of micronuclei and abnormalities in the pollen (Kumar and Swathi, 2017; Sparrow, 1961).

Minimum spikelet fertility was 60.78 per cent at 500 Gy for electron beam and 60.45 per cent in 70 mM for EMS in ASD 16 and in Norungan and it was 61.28 per cent for electron beam and 61.32 per cent for EMS at 500 Gy and 70 mM, respectively. Pollen fertility and spikelet fertility exhibited an inverse relationship with the dose of mutagen. The GR_{50} values for pollen fertility were 408.83 Gy (electron beam) and 69.13 mM (EMS) in ASD 16, which was also accompanied by reduction in spikelet fertility at 716.33 Gy (electron beam) and 123.33 mM (EMS); mutants of Norungan exhibited 353.60 Gy and 69.58 mM, for pollen fertility whereas 773.99 Gy (electron beam) and 135.98 mM (EMS) for spikelet fertility (**Fig. 3**). Several factors, including anther growth, production of viable pollen and the presence of phytohormones determine spikelet fertility. In rice and barley, the mutation in the GAMYB (GA myeloblastosis) gene exhibits typical defects in the development of the anthers and pollen (Miller and Gubler, 2005) with mutants exhibiting typical defects in the development of exine and orbicules along with programmed cell death (PCD) of tapetal cells (Lalitha *et al*., 2019).

Mutagenic effect on yield contributing traits: ASD 16 exhibited a plant height of 92.46 cm while Norungan measured 108.82 cm in control. Exposure to 500 Gy of electron beam led to a substantial reduction in plant height which was 77.21 per cent in ASD 16 and 93.62 per cent in Norungan. Similarly, exposure to EMS at 70 mM resulted in decreased plant height in ASD 16 (68.03 per cent) and Norungan (86.67 per cent). The highest dose had a stunning effect on the M_1 population causing a decrease in plant height which may be due to the suppression of DNA synthesis, protein synthesis and enzyme activity. Growth reduction 50 values for electron beam were 1628.66 Gy for ASD 16 and 1650.16 Gy for Norungan, whereas for EMS, the recorded GR_{50} values were 150.33 mM in ASD 16 and 184.09 mM (GR_{50}) in Norungan **(Table 3)**. These finding indicates significant growth reduction at extremely high doses of both electron beam and EMS with GR 50 values beyond the doses studied.

There was a reduce in the number of productive tillers for higher doses of both the mutagens and for electron beam, the maximum fall was 56.8 per cent in 500 Gy in ASD 16 and 68.0 per cent in Norungan. For EMS, the highest drop of 59.72 per cent was observed in 70 mM in ASD 16 and it was 59.64 in Norungan which indicated a significant reduction in the number of productive tillers per plant for electron beam and EMS. Similar results were observed by Chakravarti *et al.* (2012) and El-Degwy (2013). A noticeable reduction in panicle length was detected in ASD 16 and Norungan which exposed to electron beam at 500 Gy (81.81 per cent and 80.55 per cent) and 70 mM of EMS (83.95 per cent and 81.87 per cent).

The mutants exhibited a lower grain yield than the control, which may be owing to an increase in the chaffy grains mainly by pollen sterility and reduced spikelet fertility which had an impact on single plant yield. Prabakaran (1992) observed that the increased spikelet sterility caused by physiological and biochemical alterations in the development of seeds induced by mutagens results in a significant fall in yield per plant. At 500 Gy, the single plant yield exhibited by ASD 16 and Norungan were 81.80 per cent and 93.91 per cent, respectively and EMS, it was 87.53 per cent and 92.75 per cent at 70 mM in ASD 16 and Norungan. These results underscore the adverse effects of mutagen exposure on seed yield and overall plant productivity.

This study revealed that both genotypes exhibited dose dependent reduction in germination, seedling survival,

Table 3. The effect of mutagens on ASD 16 and Norungan on seedling, fertility and yield contributing characters

Sources	Linear equation (ASD 16)	Linear equation (Norungan)	Growth and fertility reduction dose (Gy or mM)			
			ASD 16		Norungan	
			50 per cent	75 per cent	50 per cent	75 per cent
			Germination			
Electron Beam	Y= 0.1618x (R^2=0.9951)	Y = 0.0928x (R^2 = 0.9982)	309.02	463.53	538.79	808.18
EMS	Y= 0.9689 x (R^2= 0.9388)	Y = 0.5065x (R^2 = 0.9425)	51.60	77.40	98.71	148.07
			Shoot length			
Electron Beam	Y= 0.1221x (R^2 = 0.9928)	Y = 0.0558x R^2 = 0.9336	409.51	614.25	896.05	1344.08
EMS	Y = 0.8794x (R^2 = 0.987)	Y = 0.5121x R^2 = 0.9711	56.85	85.28	97.63	146.45
			Root length			
Electron Beam	Y= 0.1109x (R^2 = 0.9737)	Y = 0.0841x R^2 = 0.965	450.85	676.28	594.53	891.79
EMS	Y = 0.6204x (R^2 = 0.9855)	Y = 0.442x R^2 = 0.868	80.59	120.88	113.12	169.68
			Seedling height			
Electron Beam	Y= 0.1166x (R^2= 0.9859)	Y = 0.0702x R^2 = 0.9541	428.21	643.22	712.25	1068.37
EMS	Y= 0.752 x (R^2= 0.9923)	Y = 0.8644x R^2 = 0.9985	66.48	99.73	57.84	86.76
			Seedling survival			
Electron Beam	Y= 0.2051 x (R^2= 0.9697)	Y = 0.111x R^2 = 0.9991	243.78	365.67	450.04	675.06
EMS	Y= 1.1039 x (R^2= 0.9803)	Y = 0.6037x R^2 = 0.942	45.29	67.94	82.82	124.23
			Pollen fertility			
Electron Beam	Y= 0.1223 x (R^2= 0.9941)	Y= 0.1414x R^2 = 0.9857	408.83	613.24	353.60	530.41
EMS	Y= 0.7232x (R^2= 0.9684)	Y = 0.7185x R^2 = 0.971	69.13	103.70	69.58	104.38
			Spikelet fertility			
Electron Beam	Y= 0.0698 x (R^2= 0.9898)	Y = 0.0646x R^2 = 0.999	716.33	1074.49	773.99	1160.99
EMS	Y= 0.4054 x (R^2= 0.9711)	Y= 0.3677x R^2 = 0.9407	123.33	185	135.98	203.97
			Plant height			
Electron Beam	Y = 0.0307x R^2 = 0.9949	Y = 0.0303x R^2 = 0.9827	1628.66	3257.32	1650.16	2475.24
EMS	Y = 0.3326x R^2 = 0.9605	Y = 0.2716x R^2 = 0.9955	150.33	300.66	184.09	276.14
			No. of productive tillers per plant			
Electron Beam	Y = 0.1012x R^2 = 0.9784	Y= 0.1073x R^2 = 0.9385	494.07	741.10	465.98	698.97
EMS	Y = 0.6763x R^2 = 0.93	Y = 0.7713x R^2 = 0.976	73.93	110.89	64.82	97.23
			Panicle length			
Electron Beam	Y = 0.0385x R^2 = 0.9965	Y = 0.0273x R^2 = 0.9962	1298.70	1948.05	1831.50	2747.25
EMS	Y = 0.2006x R^2 = 0.9468	Y = 0.1481x R^2 = 0.972	249.25	373.87	337.60	506.41
			Single plant yield			
Electron Beam	Y= 0.0308x R^2 = 0.9729	Y = 0.0136x R^2 = 0.9874	1623.37	2435.06	3676.47	5514.70
EMS	Y = 0.1506x R^2 = 0.9561	Y = 0.098x R^2 = 0.9922	332.00	498.00	510.20	765.30

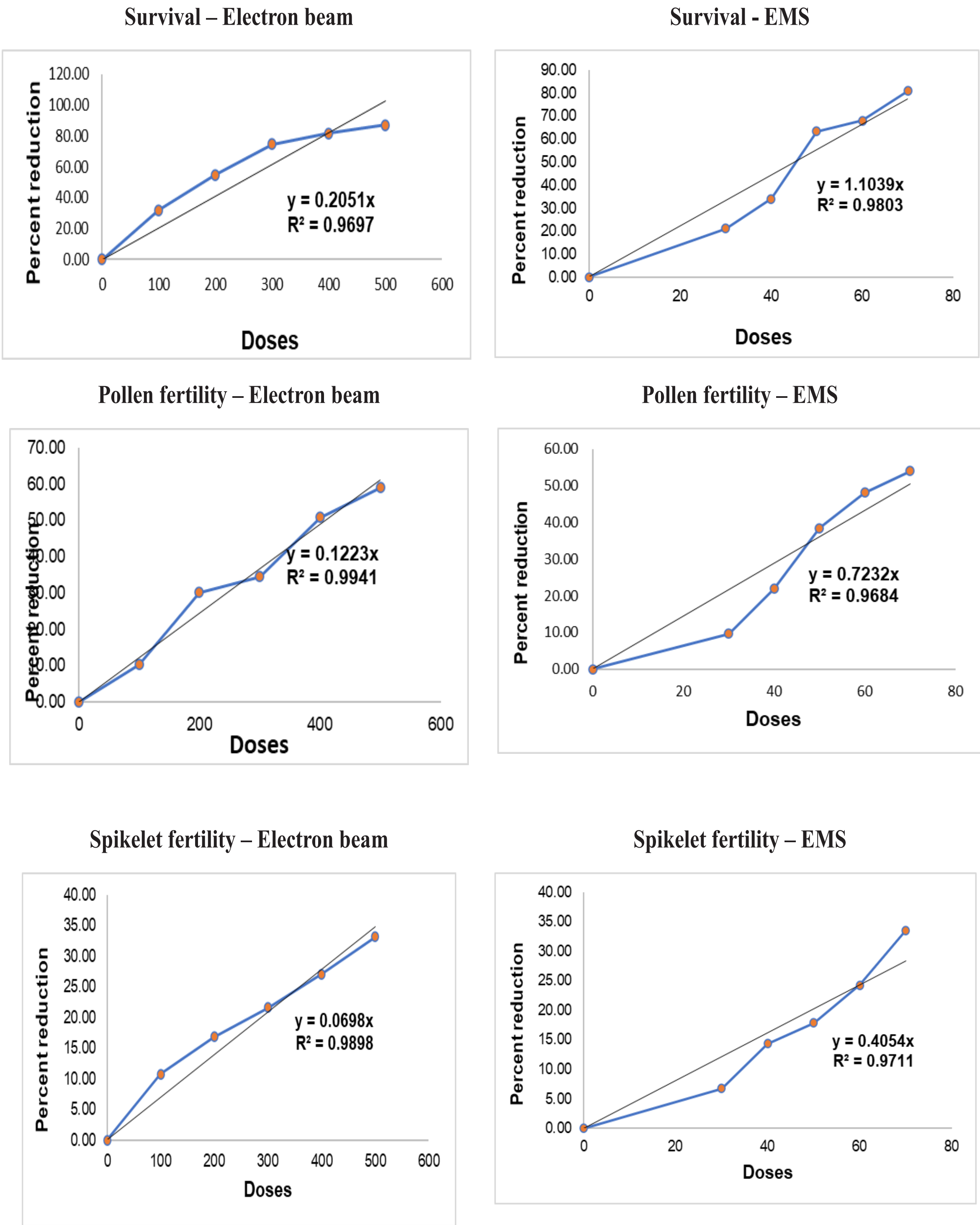

Fig. 3. The effect of radiation in ASD 16 rice on seedling survival, pollen fertility and spikelet fertility based on GR_{50}

shoot and root length, seedling height, pollen fertility and spikelet fertility when exposed to increasing doses of electron beam and EMS. ASD 16 exhibited greater sensitivity to both the mutagens compared to Norungan, as proved by higher growth reduction percentage and lower LD_{50} values. Electron beam treatments resulted in significant decreases in germination, survival and various growth traits, highlighting the potential deleterious effects of high doses. EMS, a chemical mutagen, also induced notable negative impacts on germination and growth characteristics. Electron beam and EMS treatments led to reduced spikelet and pollen fertility with higher doses. The inverse correlation between mutagen dose and fertility parameters emphasized the importance of optimal dosage for inducing desirable mutations without compromising plant reproductive capacity.

Furthermore, the mutagenic effects were noticed in yield contributing attributes such as plant height, number of productive tillers, panicle length and single plant yield. Higher doses of both electron beam and EMS resulted in significant reductions in these attributes, emphasizing the potential balance between inducing beneficial traits and compromising overall plant productivity. These findings suggest that careful consideration of mutagen dosage is key in mutation breeding programmes, aiming to strike a balance between inducing desirable traits and maintaining plant viability and productivity. ASD 16 is more sensitive than Norungan for both mutagens and the differences in sensitivity between the two rice genotypes accentuated the importance of understanding genotype-specific responses to mutagenesis. Overall, the study contributes valuable insights into the use of electron beam and EMS mutagens in rice breeding programmes, emphasizing the need for precision in dosage selection to achieve the desired mutagenic effects while minimizing detrimental impacts on plant growth, fertility and yield.

REFERENCES

Abdel-Hady, M.S., Okasha., E.M. Solimann., S.S.A. and Talat, M. 2008. Effect of gamma radiation and gibberelllic acid on germination and alkaloid production in *Atropa belladonna* L. *Australian Journal of Basic Applied Sciences.,* **2** (3): 401-405

Akbar, A. and Manzoor, B. 2003. Radio sensitivity studies in basmati rice. *Pakistan Journal of Botany.,* **35** (2): 197-207.

Akilan, M. Anand, G. Vanniarajan, C. Subramanian, E. and Anandhi, K. 2019. Study on the Impact of mutagenic treatment on pollen and spikelet fertility and its relationship in rice (*Oryza sativa* L.*). Electronic journal of Plant Breeding*, **10** (2): 525 – 534. [Cross Ref]

Amudha, K., Geetha, S., Manimekalai, M. and Ganesamurthy, K. 2023. Rice landraces of Tamil Nadu–a review. *Indian Journal of Traditional Knowledge (IJTK)*, **22**(1): 17-29. [Cross Ref]

Baojiang, G., Yuyuan, W. and Jihong, R. 1982. Studies on the mutagenic effect of 5 MeV electron irradiation on rice. - *Acta Genetica Sinica*, **9**: 461- 467

Chakravarti, S.K.R., Singh. S., Kumar. H., Lal J.P. and Vishwakarma M.K. 2013. Study of induced polygenic variability in M_1 and chlorophyll mutations in M_2 generations in aromatic rice. *The Bioscan*. **8**(1): 49-53.

El-Degwy, I.S. 2013. Mutation induced genetic variability in rice (*Oryza sativa* L.). *International Journal of Agriculture and Crop Science*, **5** (23): 2789-2794

Finney, D.J. 1971. Probit Analysis. Cambridge University Press.

Francis, N., Ravikesavan, R., Iyanar, K., Raveendran, M., Chitdeshwari, T. and Senthil, A. 2022. Dose Optimization, Mutagenic Effectiveness and Efficiency of EMS in Proso Millet (*Panicum miliaceum* L.). *Madras Agricultural Journal*, **109**: 1.

Gowthami, R. Vanniarajan, C. Souframanien, J. and Arumugam Pillai, M. 2016. Effect of gamma rays and electron beam on various quantitative traits of rice (*Oryza sativa* L.) in M_1 Generation. *Advances in life sciences*, **5** (5): 1876-1882.

IRRI, 2013. Standardization evaluation system for rice. International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines **5**: 18

Kant, A., Chakraborty, N.R. and Das, B.K. 2020. Immediate radiation effects and determination of optimal dose of gamma rays on non-basmati aromatic rice (*Oryza sativa* L.) of eastern India. *Journal of Experimental Biology and Agricultural Sciences*, **8**(5):586–604. [Cross Ref]

Khalil, S. J., Rehman, S., Afridi, K. and Jan, M. T. 1986. Damage induced by gamma irradiation in morphological and chemical characteristics of barley. *Sarhad Journal of Agriculture*., **2**: 45-54.

Kumar, G. and Swati, K. 2017. Germination and cytological aspects of *Dolichos lablab* L. exposed to gamma irradiation. *Chromosome Botany*, **12** (3): 63-71. [Cross Ref]

Lalitha, R., Arunachalam, P., Mothilal, A., Senthil, N., Hemalatha, G., Vanniarajan, C. and Souframanien, J. 2019. Radiation effect on germination and seedling traits in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*, **10** (3): 1038-1048. [Cross Ref]

Miller, A.A. and Gubler, F. 2005. The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. *The Plant Cell,* **17** (3): 705–721. [Cross Ref]

Olaolorun, B.M., Shimelis, H.A., Mathew, I. and Laing, M.D. 2019. Optimising the dosage of ethyl methane sulphonate mutagenesis in selected wheat genotypes. *South African Journal of Plant and Soil*, **36** (5): 357-366. [Cross Ref]

Prabakaran, A. J. 1992. Identification of male sterile sources through wide hybridization and induced mutagenesis in sesame (*Sesamum indicum* L.). Ph. D. Thesis, TNAU, Coimbatore

Ramesh, M., Vanniarajan, C., Ravikesavan, R., Aiyan, K. E. A. and Mahendran, P. P. 2019. Determination of lethal dose and effect of EMS and gamma ray on germination percentage and seedling parameters in barnyard millet variety Co (Kv) 2. *Electronic Journal of Plant Breeding*, **10**(2): 957-962. [Cross Ref]

Sao, R., Sahu, P.K., Sharma, D., Gautam Vishwakarma, G., Nair, J.P., Petwal, V.C. and Das, B.K. 2020. Comparative study of radiosensitivity and relative biological effectiveness of gamma rays, X-rays, electron beam and proton beam in short grain aromatic rice, *Indian Journal of Genetics and Plant Breed*ing, **80** (4): 384-394. [Cross Ref]

Sharma, V. and Kumar, G. 2004. Meiotic studies in two cultivars of *Cicer arietinum* L. after EMS treatment. *Cytologia*, **69** (3): 243-248. [Cross Ref]

Siddiq, E.A. and Swaminathan M.S. 1968. Induced mutations in relation to the breeding and phylogenetic differentiation of *Oryza sativa*. In: Rice Breeding with Induced Mutations, *International Atomic Energy Agency*, *Rice breeding with induced mutations*. *Vienna*, **86**: 25-51.

Sikora, P., Chawade, A., Larsson, M., Olsson, J. and Olsson, O. 2011. Mutagenesis as a tool in plant genetics, functional genomics and breeding. *International Journal of Plant Genomics:* **314829**: 1-3. [Cross Ref]

Solim, M.H. and Rahayu, S. 2021. Radiosensitivity of rice varieties of Mira-1 and Bestari mutants using gamma rays irradiation. In *IOP Conference Series: Earth and Environmental Science*, **911**:012014. IOP Publishing. [Cross Ref]

Sparrow, A.H., 1961. Types of ionizing radiation and their cytogenetic effects, *Mutation and Plant Breeding NAS-NRC,* **891**: 55-119.

Talebi, A.B., Talebi, A.B. and Shahrokhifar B. 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv. MR219) for lethal dose determination. *American Journal of Plant Sciences,* **3**:1661–1665. [Cross Ref]

Tyagi, A.K., Khurana, J.P., Khurana, P., Raghuvanshi, S., Gaur, A., Kapur, A., Gupta, V., Kumar, D., Ravi, V., Vij, S., Khurana, P. and Sharma, S. 2004. Structural and functional analysis of rice genome. *Journal of Genetics*, **83**: 79-99. [Cross Ref]

Ulukapi, K. and Ozmen, SF. 2018. Study of the effect of irradiation (^{60}Co) on M 1 plants of common bean (*Phaseolus vulgaris* L.) cultivars and determined of proper doses for mutation breeding. *Journal of Radiation Research and Applied Sciences,* **11**(2):157-161. [Cross Ref]

Usuf, K.K. and Nair, P.M. 1974. Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. *Radiation Botany*, **14**(4): 251-256. [Cross Ref]