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Research Article

Comparative efficiency of irradiation and its recurrent mutation on induced variability and population characteristics in rice (*Oryza sativa* L.)

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Abstract

The present study was carried out to elucidate the mutagenic efficiency of gamma rays (100 Gy, 150 Gy), electron beam (100 Gy, 150 Gy) used alone and in combination with EMS (30mM) as a recurrent treatment in the subsequent generation of Anna (R) 4 rice cultivar. The study was aimed to analyse the induced genetic variability, mean performance as well as population characteristics for various quantitative traits in M₂ generation. The mutant lines were selected based on earliness, grain shape and yield. In general, bidirectional shift without any uniform trend was observed for all the traits except productive tillers per plant and grain weight. Recurrent EMS approach had no significant effect on earliness, however, it had significant effect on reduction of plant height (8.19 %) and productive tillers per plant (25.27 %) in EB 150Gy + EMS 30mM. Whereas 150 Gy gamma irradiation had increased effect on grains per panicle (21.99 %), panicle weight (34.73 %) and grain yield per plant (9.77 %). Though selection was exercised for desirable traits, co-efficient of variation was still higher for productive tillers per plant (31.90 %), grains per panicle (30.20 %), panicle weight (30.87 %) and grain yield per plant (31.55 %). From the mean performance and population characteristics, it is concluded that recurrent EMS mutagenic approach was less efficient than irradiation for induction of desirable changes in earliness, grain shape, grain yield and other quantitative traits.

Key words

Rice, recurrent mutation, Gamma rays, electron beam, Ethyl Methane Sulphonate.

Introduction

Genetic variability is the central base line for any successful crop improvement programme. In fact, plant breeders use the variability created through natural (spontaneous) or artificial methods. Mutation breeding has evolved as an efficient tool for plant breeders especially in case of traits where available variability is either inadequate or lacking. Various physical (y-rays, X- rays, fast neutrons, thermal neutron) and chemical mutagens [Ethyl Methane Sulphonate (EMS), Sodium Azide (SA)] have been widely applied in plant breeding programme to induce mutations. Recurrent irradiation in subsequent generation was yet another technique to create additional variability over a single irradiation (Briggs and Constantin, 1977), to disallow the crop from escape on strong DNA repair mechanisms. The effects of repeated irradiation (X rays and Gamma rays) have been studied in several crops such as oats (Caldecott and North, 1961), durum wheat (Mugnozza and Monti, 1966), barley (Vasti and Jensen, 1984), peanut (Emery, 1972), chrysanthemum (Micke et al., 1990), banana (Mishra et al., 2007), wild tomato (Nunoo et al., 2014), rice (Manikandan, 2016) and black gram (Kuralarasan, 2016) . In most of the

cases it was concluded that repeated mutagenic treatment gives rise to increase in mutagenic sensitivity (Abrams and Frey, 1964).

Different kinds of radiation have varying energy transfer patterns. Linear energy transfer (LET) is defined as the energy deposited to the target plant material when ionizing radiations pass through it. The mutagenic effect of radiation is known to be a function of LET and usually expressed in kilo electron volt per micrometre (KeV/ µm). Gamma rays are the most energetic form of electromagnetic radiation. Nowadays electron beam is used as an alternative source of energy to induce the mutation as a replacement of commercial electromagnetic radiation. Both γ -rays and electron beams have only low LETs of around 0.2 KeV/ µm (Magori et al., 2010). However, electron beam is administered as a short impulse while gamma irradiation is continuous, due to the fact that electron beam has a higher dose rate compared to y-rays (Souframanien et al., 2016). In India till now only few published reports are available on use of electron beam in rice, Gowthami (2016) reported that electron beam was more effective in rice as it generates a greater



number of valuable trait variation than gamma rays. Efficiency of mutagenesis decides the frequency of occurrence of desirable changes in mutant population. In rice, the potentiality of ionizing radiation and chemical mutagens had different ability to induce mutation which varies significantly from genotype to genotype (Singh and Sharma2013).

Anna (R) 4 is a short duration (100-105 days) and drought tolerant rice variety derived from Pantdhan 10 x IET 9911 suitable mainly for rainfed tracts of Tamil Nadu. Even though this variety having high yield and drought resistant, had less consumer preference and market value due to its long slender grain type and breakage during milling. Thus, the present study was conducted to determine the comparative efficiency of electron beam and gamma rays and its recurrent doses of EMS as well as to induce variation and to correct undesirable traits associated with Anna (R) 4.

Materials and Methods

The present study was carried out during *kharif* 2018 at Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai. The M₁ seeds of Anna (R) 4 treated with gamma rays (GR) [100Gy, 150Gy] and electron beam (EB) [100Gy, 150Gy] were obtained from Department of Plant Breeding and Genetics.

A part of M₁ seeds derived from gamma rays (100Gy, 150Gy) and electron beam (100Gy, 150Gy) irradiation were treated again with 30 mM EMS as recurrent treatment. For EMS treatment, seeds were pre-soaked in distilled water for 12 hr at room temperature to ensure complete hydration of the seeds. Such pre-soaked seeds after removing excess moisture from the seed surface were treated with freshly prepared EMS solution of 30 mM for 6 hr at room temperature with intermittent shaking. The volume of the mutagenic solution used was three times as that of the seed volume so as to facilitate uniform conditions. The treated seeds were washed thoroughly in running tap water for one hour to remove the residual chemical from the seeds (Aravindgosh, 2017). Immediately after EMS treatments, seeds of recurrent treatments [(GR 100Gy + EMS 30mM, GR 150Gy+ EMS 30mM), (EB 100Gy + EMS 30mM, EB 150Gy + EMS 30 mM)], irradiation [GR (100Gy, 150Gy) and EB (100Gy, 150Gy)] and untreated Anna (R) 4 were raised in nursery. Twenty-one days old seedling of eight different mutant (M2) populations were transplanted into the main field with the spacing of 25 cm x 15 cm and the recommended package of practices were performed to maintain a good plant stand.

The observations on M_2 population were recorded for the traits namely days to flowering, plant height (cm), productive tillers per plant (no.), panicle length (cm), panicle weight (g), grains per panicle (no.), grain weight (g) and grain yield per plant yield (g). The data for these traits were recorded on control and 150 selected plants based on earliness, grain shape and yield characters for each population. The data was statistically analysed using MS Excel -2016. Irradiation and recurrent EMS treated mutant populations mean were compared against the control by t test at 5 % and 1% level of significance.

Results and Discussion

Single and recurrent mutations had significant (P >0.05) effect on days to flowering, resulted in early flowering of five to seven days from Anna (R) 4 rice cultivar. Maximum reduction in flowering of seven days was observed in 150 Gy electron beam irradiation (Table 1). Though the significant difference in mean performance of days to flowering was noticed, but the days to maturity of mutant lines were almost similar to control indicating that recurrent EMS approach had no significant effect on earliness in Anna (R) 4 rice cultivar, similar observation was reported by Nunoo et al. (2014) in wild tomato for days to 50 % flowering. Further in the previous studies high frequency of late flowering was noticed than early flowering in Anna (R) 4 rice induced with different concentration of EMS (Aravindgosh, 2017). Therefore, irradiation alone will be sufficient to induce the earliness in rice, Singh and Sharma, (2013) reported that the doses are invariably genotype depended. Many early lines have been developed in rice using gamma irradiation (Shadakshari et al., 2001). Sharma Deepak et al. (2017) observed 6 to 21 days earliness in long duration Dubraj rice variety at 300 Gy gamma irradiation.

Changes in the plant height is widely used as an index to determine the biological effect of any mutagen. Reduction in plant height was more in recurrent EMS approach when compared to single irradiation. Recurrent mutagenic approach drags the mean towards the negative direction and maximum reduction (8.19 %) was found in 150Gy electron beam + 30 mM EMS (Table 1). This is due to the fact that repeated action of mutagens, may interfere with the process of cell division and cell elongation (Iqbal, 1969) resulting in the plant height reduction (Manikandan, 2016; Nunoo et al., 2014; Vasti et al., 1984). But irradiation alone resulted in increased plant height (9.45 %) at 150 Gy electron beam. Similarly, Gowthami (2016) also reported an increase in plant height up to 9.96



per cent in ADT 37 at 200 Gy electron beam irradiation.

The productive tillers per plant, panicle length, panicle weight, grains per panicle and grain weight plays significant role in raising the grain yield per unit area in rice. Both single radiation as well as recurrent EMS approaches had mutagenic influence on all the yield attributing characters. When compared with irradiation, recurrent approach had more mutagenic sensitivity, for productive tillers per plant (Table 2) led to shift the mean towards the negative direction as maximum as 25.27 per cent (150 Gy EB + 30 mM EMS). In panicle length, shift in mean towards negative direction was observed except 100 Gy EB and 150 Gy EB + 30 mM EMS (Table 2). This result was in contrast with the results obtained by Manikandan (2016) in rice, where the recurrent irradiation (200 Gy EB + 100 Gy GR) caused a drift in the mean towards positive side for productive tillers per plant and panicle length. For seed weight both approaches led to switch the mean towards positive direction as maximum as 6.97 per cent in 100 Gy of gamma rays.

Irradiation treatment had increasing effect on grains per panicle (21.99 %), panicle weight (34.73 %) and grain yield per plant (9.77 %) as maximum as 150 Gy gamma ray treatment (Table 3, 4) and led to the mean towards the positive direction. Whereas, radiation combined with EMS as recurrent during subsequent generation resulted in increased sensitivity and lower grain fertility possibly due to aborted pollen grains or embryo and reduced accumulation of food in developing seeds (Sparrow, 1961). It is evident by the observed shifts in the mean towards the negative side due to recurrent mutation for above characters except in 150 Gy gamma ray + 30 mM treatment. The marked reduction in grain yield per plant (Table 4) could be possibly attributed by the reduction in seed fertility, as an outcome of physiological and biochemical disturbances in the development of seeds (Prabakaran, 1992). Reduction in seed fertility is indirectly reflected as reduction in grains per panicle, panicle weight and grain weight in turn reduce the grain yield per plant. It clearly evident that yield related traits drastically affected with repeated mutagenic treatments (Emery, 1972)

In the present investigation, despite the fact that selection was exercised mainly based on earliness, grain shape and grain yield in M_2 generation, coefficient of variation was still higher for number of productive tillers per plant (31.90 %), number of grains per panicle (30.20 %), panicle weight (30.87 %) and grain yield per plant (31.55 %). Among the mutagenic approaches irradiation alone has been

reported to provide wider range of variability for productive tillers per plant and panicle weight (Muduli and Misra, 2007). However recurrent EMS approach provides an even greater range of variability than single irradiation for grains per panicle and grain yield per plant (Khadr and Frey, 1965). This indicates that irradiation alone and combined with EMS induce mutagenic effect in growth and yield characters, which led to more variability in the M_2 population studied.

The population characteristics of selected mutant lines of gamma ray and electron beam irradiated alone also combined with EMS as recurrent treatment are presented in the Table 5. While an attempt was made to know the behaviour of different quantitative characters under particular selection pressure in mutant population. It was observed that, irrespective of mutagenic approach, difference in mean performance was noticed in mutant population for all the traits compared to Anna (R) 4 rice. However, no significant difference was observed between irradiation and recurrent EMS approach, except for the traits such as plant height, grains per panicle and grain yield per plant. The population characteristics of these three traits were depicted in the figure 2.

Efficient selection was made for earliness, which was evident from positive skewness for all the mutant population. Relatively flat peakedness (lepto) of the curve infers that both mutagenic approaches had only little effect on induction of earliness. As a result of recurrent approach plant height (Fig. 2a) showed high peakedness (lepto) of curve at the positive skewed area, evident that the mean plant height was drastically reduced by the repeated mutagenic treatment. Whereas irradiation had only moderate effect as indicated by the negative skewness. Among the irradiation treatment, electron beam was able to cause increase in the plant height. Further increase in the plant height was positively correlated with panicle length, panicle weight and productive tillers per plant (Gowthami, 2016; Immanuel et al., 2011) also evident from significant increase in grain yield per plant of electron beam irradiated population.

Second important selection criteria used in the present investigation was grain shape with an aim to isolate medium slender and bold grain mutants. Both mutagenic approaches had effect on alteration of grain shape, which was evident from the significant increase in mean performance of seed weight as compared to control. The frequency distribution of seed weight in all mutant population shows that positive skewness coupled with high peakedness of the curve (lepto).The grain characteristics of the selected mutants were differed



from long slender Anna (R) 4 rice by means of decreased grain length (long to medium) and increased grain width (slender to bold). The similar result was reported by Moeljopawiro (2002) and Xie *et al.* (2013), mainly due to existence of negative association between grain length and grain width.

Selection was excised to select the increased panicle length and a greater number of productive tillers per plant. Selection on increased panicle length was reflected from negative skewness with lepto kurtic nature of the curve. Increase in panicle length had direct effect on panicle weight, number of grains per panicle and hundred grain weight (Immanuel et al., 2011). Unlike the panicle length, panicle weight, number of grains per panicle (Fig. 2b) and grain yield per plant (Fig. 2c) were positively skewed in all the population but peakedness of the curve varied, which infers that these traits were more sensitive to mutagens. In case of grains per panicle irradiation had positive effect, whereas recurrent approach had a negative effect, which was evident from the varying level of peakedness of the curve.

The present study revealed a unidirectional shift towards reduced number of productive tillers per plant and increased grain weight as result of mutagenic effect of irradiation and recurrent EMS approaches in rice. For all other traits bidirectional shift was observed but no uniform trend was noticed between two approaches. Population characteristics as well as mean performance of quantitative traits indicated that recurrent EMS in an already irradiated material had no significant effect on earliness, alteration of grain shape and yield attributing traits. In general, recurrent treatment of Ethyl Methane Sulphonate resulted in more mutagenic sensitivity led to drastic reduction in plant height and grains per panicle. In practical point of view the recurrent mutation able to expand genetic variability for grains per panicle and grain yield per plant. These results conclude that irradiation alone has more efficient in induction of desirable changes in polygenic traits but the doses have to be standardised depending upon the crop species as well as the genotype. In future recurrent mutation studies, either by increasing the radiation dose above 150 Gy / concentration of EMS beyond 30 mM can be used to create additional variability for various quantitative traits in rice.

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Table 1. Mean, range, shift in mean and co-efficient of va	ariation (CV) for days to flowering and	plant height of Anna (R) 4 rice in M ₂ generation
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	Days to floweringPlant height (cm)									$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$
Treatment	Maan SE	Dongo	Shift in	mean	CM(0/)	Maan / SE	Damaa	Shift in	CV(0)	
Treatment	Mean + SE	Kange	Days	%	- $CV(%)$	Mean + SE	Kange	cm	%	UV (%)
Control	82.50±0.96	78-87	-	100	3.67	99.17±1.60	92-104	-	100	3.95
Gamma rays (Gy	r)									
100	80.56±1.72**	73-89	-1.94	97.64	6.39	99.97±0.70*	80-115	0.80	100.8	5.03
150	81.02±1.46*	77-86	-1.48	98.21	4.42	102.56±0.79**	90-125	3.39	103.42	6.07
Electron beam (C	Gy)									
100	81.81±1.63	72-95	-0.69	99.16	7.99	108.24±0.71**	89-128	9.07	109.15	6.88
150	78.31±1.62**	71-90	-4.19	94.92	7.49	108.54±0.72**	81-127	9.37	109.45	8.69
Gamma rays (Gy	y)+EMS (mM)									
100+30	80.71±1.57**	76-87	-1.79	97.83	4.74	94.63±0.71	81-107	-4.54	95.42	5.90
150+30	83.10±1.31	77-89	0.6	100.73	5.14	103.44±0.76**	93-114	4.27	104.31	5.16
Electron beam (C	Gy)+EMS (mM)									
100+30	81.57±1.29	77-86	-0.93	98.87	4.18	91.05±0.59	74-103	-8.12	91.81	5.68
150+30	80.71±2.15**	73-87	-1.79	97.83	7.03	91.11±0.63	81-103	-8.06	91.87	5.21
			CD: P (0.05) = 1.08; P (0	0.01) = 1.14			CD: P (0.0	05) = 0.68; P (0	0.01) = 0.89

Table 2. Mean, range, shift in mean and co-efficient of variation (CV) for prod. tillers per plant and panicle length of Anna (R) 4 rice in M₂ generation

		Productive tille	rs per plant (No			Panicle 1	length (cm)			
Traatmont	Moon + SE	Domas	Shift in mean		CV(0)	Moon + SE	Danga	Shift in	CV(0)	
Treatment	Mean ± SE	Kalige	numbers	%	C V (%)	Mean ± SE	Kange	cm	%	CV (%)
Control	15.67±0.95	15-19	-	100	8.52	25.17±1.38	20.6-27.2	-	100	6.29
Gamma rays (Gy)										
100	14.57±0.54	4-23	-1.1	92.98	33.38	24.92±0.26	16.0-29.3	-0.25	99.01	8.79
150	13.98±0.45	5-23	-1.69	89.21	29.25	24.61±0.24	17.0-29.5	-0.56	97.78	9.02
Electron beam (Gy	()									
100	14.75±0.38	6-26	-0.92	94.13	30.98	25.90±0.28**	19.9-32.1	0.73	102.9	11.51
150	13.53±0.33	9-24	-2.14	86.34	29.64	25.13±0.22	17.4-33.3	-0.04	99.84	10.71
Gamma rays (Gy)	+EMS (mM)									
100+30	13.51±0.42	8-23	-2.1	86.21	23.09	24.35±0.27	19.5-29.4	-0.82	96.74	8.71
150+30	11.72±0.40	5-18	-3.95	74.79	24.06	24.26±0.32	18.0-28.7	-0.91	96.38	9.40
Electron beam (Gy	y)+EMS (mM)									
100+30	12.52±0.49	10-22	-3.15	79.9	21.29	24.69±0.31	15.1-32.3	-0.48	98.09	10.69
150+30	11.71±0.36	6-19	-3.96	74.73	22.97	25.46±0.33**	20.0-35.1	0.29	102.15	9.70
			CD: P (0.05	5) = 0.30; P ((0.01) = 0.39			CD: P (0.0	(5) = 0.20; P(0)	(0.01) = 0.26



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Table 3. Mean, range, shift in mean and co-efficient of variation (CV) for panicle weight and grains per panicle of Anna (R) 4 rice in M₂ generation

		Panicle	weight (g)				Grains			
Treatment	Maan SE	Damaa	Shift i	n mean	CW(0)	Marra / CE	D	Shift in	CV(0)	
Treatment	Weall ± SE	Kange	g	%	Cv (%)	Mean ± SE	Kange	numbers	%	CV (%)
Control	2.62±0.15	2.1-3.2	-	100	6.48	125.17±4.05	113-139	-	100	7.93
Gamma rays (Gy))									
100	3.21±0.10**	1.4-5.4	0.59	122.51	26.47	137.29±5.03**	62-200	12	109.68	24.58
150	3.53±0.12**	1.2-5.7	0.91	134.73	30.87	152.69±4.67**	63-262	27.52	121.99	25.01
Electron beam (G	y)									
100	3.17±0.08**	1.4-54	0.55	120.99	25.24	132.42±8.36**	71-237	7.25	105.79	27.43
150	3.27±0.07**	1.3-5.6	0.65	124.81	25.3	149.65±4.56**	82-232	24.48	119.56	27.43
Gamma rays (Gy))+EMS (mM)									
100+30	2.58±0.09	1.3-4.6	-0.04	98.47	26.85	116.97±4.91	71-170	-8.2	93.45	24.11
150+30	2.89±0.13**	1.1-5.1	0.27	110.31	30.79	129.22±6.90*	63-198	4.05	103.24	30.2
Electron beam (G	y)+EMS (mM)									
100+30	2.48±0.13	1.2-4.2	-0.14	94.66	22.18	109.91±4.12	68-160	-15.26	87.81	21.87
150+30	2.58±0.11	1.24.4	-0.04	98.47	28.68	103.39±5.62	65-164	-21.78	82.60	26.91
			CD: P (0.0	05) =0.07; P (0	(0.01) = 0.09			CD: P (0	0.05) =4.02; P	(0.01) = 5.30

Table 4. Mean, range, shift in mean and co-efficient of variation (CV) for grain weight and grain yield per plant of Anna (R) 4 rice in M₂ generation

		Grain v	weight (g)				Grain yie	eld per plant (g)	
Treatment	Meen + SE	Danga	Shift i	Shift in mean		$M_{oon} + SE$	Danga	Shift i	CV (%)	
Treatment	Weat - 5E	Kange	g	%	CV (/0)	Weat + 5L	Range	g	%	C V (70)
Control	2.44 ± 0.01	2.4-2.6	-	100	2.08	27.53±0.65	25.2-32.3	-	100	9.92
Gamma rays (O	Gy)									
100	2.61±0.03**	1.9-3.7	0.17	106.97	10.16	27.80±0.02	9.80-48.1	0.27	100.98	29.85
150	2.52±0.03**	2.0-3.3	0.08	103.28	9.44	30.22±0.91**	12.4-48.5	2.69	109.77	29.94
Electron beam	(Gy)									
100	2.50±0.02**	2.0-3.2	0.06	102.46	9.31	27.68±0.77	13.2-49.7	0.15	100.54	29.3
150	2.51±0.02**	1.8-3.3	0.07	102.86	11.51	29.64±0.71**	7.01-49.5	2.11	107.66	29.52
Gamma rays (O	Gy)+EMS (mM)									
100+30	2.46±0.02*	2.1-3.0	0.02	100.81	6.54	27.30±0.73	11.9-37.5	-0.23	99.16	26.14
150+30	2.45 ± 0.04	1.3-3.1	0.01	100.41	10.49	28.28±0.79*	13.2-35.1	0.75	102.72	23.02
Electron beam	(Gy)+EMS (mM)									
100+30	2.45±0.02	2.1-2.8	0.01	100.41	7.18	26.57±0.64	6.70-35.8	-0.96	96.51	25.54
150+30	2.48±0.05**	2.0-3.2	0.04	101.64	8.02	28.33±0.90*	10.5-42.0	-0.80	102.91	31.55
			CD: P (0.0	5) = 0.01; P (0	(.01) = 0.03			CD: P ((0.05) = 0.62; P	(0.01) = 0.82



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Table 5. Population characteristics of different quantitative traits in M₂ generation of Anna (R) 4 rice variety

Traits	Mean					Skewness				Kurtosis			
	Anna(R)4	GT	ET	RGT	RET	GT	ET	RGT	RET	GT	ET	RGT	RET
Days to flowering	82.5	80.73**	80.24**	82	81.14*	0.2	0.61	0.14	-0.38	-0.67	-0.45	-1.19	-1.13
Plant height (cm)	99.17	101.39**	104.98**	98.5	91.08	0.64	-0.04	-0.06	-0.52	1.29	-0.1	1.15	1.22
Prod. tillers per plant (nos.)	15.67	13.69	13.19	13.29	12.18	0.14	0.28	0.48	0.26	-0.58	-0.29	0.26	0.55
Panicle length (cm)	25.17	24.75	25.56**	24.31	25.02	-0.76	-0.07	-0.11	0.07	1.64	0.22	-0.18	2.59
Panicle weight (g)	2.62	3.39**	3.23**	2.71*	2.52	0.12	0.32	0.55	0.57	-0.45	0.1	0.06	0.5
Grains per panicle (nos.)	125.17	146.50**	145.47**	123	109	0.32	-0.07	0.17	0.23	0.87	0.08	-0.58	-0.38
Grain weight (g)	2.44	2.49**	2.51**	2.46*	2.49**	1.09	0.27	0.25	0.27	4.13	0.91	2.34	1.58
Grain yield per plant (g)	27.53	29.13**	28.81**	23.64	21.46	0.06	0.31	0.26	0.42	-0.37	-0.52	-0.73	0.29



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CONTROL GT1 GT2 ET1 ET2 RGT1 RGT2 RET1 RET2



GT1 – 100 Gy gamma rays GT2 - 150 Gy gamma rays ET1 - 100 Gy electron beam ET2 - 150 Gy electron beam RGT1 - 100 Gy gamma rays + 30 mM EMS RGT2 - 150 Gy gamma rays + 30 mM EMS RET1 - 100 Gy electron beam + 30 mM EMS RET2 - 150 Gy electron beam + 30 mM EMS

Fig. 1. Mean performance of different quantitative traits in M₂ generation of Anna (R) 4 rice cultivar





Fig. 2a

Fig. 2b

Fig. 2c

* GT – Gamma rays treatment; ET – Electron beam treatment; RGT - Gamma rays + EMS treatment; RET - Electron beam + EMS treatment.

Fig. 2. Population distribution of different quantitative traits in M₂ generation of Anna (R) 4 rice variety



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