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Research Article

Comparison of radiosensitivity of two rice (*Oryza sativa* L.) varieties to gamma rays and electron beam in M_1 generation

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Abstract
The present study was carried out to compare effect of gamma rays and electron beam on two rice varieties *viz.*, ADT-37 and ADT(R) 45. Dried seeds of both the varieties were treated with gamma rays and electron beam with different doses *viz*., 100Gy, 200Gy, 300Gy, 400Gy, 500Gy for gamma rays and 200Gy, 300Gy, 400Gy, 500Gy, 600Gy for electron beam along with the control. The lethal dose was determined by probit analysis. LD_{50} dose for ADT-37 and ADT(R) 45 was 300.03Gy and 300.00Gy for gamma rays and 286.45Gy and 275.05Gy for electron beam respectively. Results showed that germination, shoot length, root length, seedling height on 20th day, pollen fertility and spikelet fertility in M_1 generation reduced steadily with the increasing doses of both mutagens. Based on the overall consideration of M_1 effects, the variety ADT(R) 45 was more sensitive to mutagens than ADT-37 and a prominent reduction was noticed in electron beam compared to gamma rays in both the varieties.

Key words
Electron beam, Gamma rays, LD_{50} value, and germination.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops, which supplies food for more than half of the world's population (Tyagi *et al.,*2004). A considerable improvement has already been made by exploiting the natural variation through conventional breeding. However, for changing the plant type, mutation breeding is effectively employed as an alternative source. Mutation has been successfully employed in breeding of several food crop varieties, ornamentals and export crops.

Radiation breeding induce plant mutation; by X-ray, γ-ray, ion beam, laser beam, neutron and electron beam, which result in gene mutation and chromosome aberration, and then gain new variety. Gamma rays are the most energetic form of electromagnetic radiation, can be useful for the alteration of physiological characters. The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose. The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas exchange, water exchange and enzyme activity.

Electron beam is a physical mutagen. Electron beams are produced from particle accelerators capable of accelerating electrons to near thespeed of light (~190,000 miles/second). This electron beam generator uses commercial electricity as an energy source and can be simply switched on or off. The absorbed dose rate of electron beam on biomaterials may reach 10^{10} Gy.s^{-1}, which is much higher than that of γ-rays (usually under 60 Gy.s^{-1}) and those of other radiation methods (Zhu *et al*., 2008). Compared with gamma rays or X-rays, the electron beam is limited to treating relatively thin packages because of the low penetrating power (< 2 inches) of electrons. Electron beam radiation has little influence on the function of plasma membrane and protein, while it results in gene mutation through inducing much DNA damage of single strand breaks (SSB) and double strand breaks (DSB). The *G*-value for DSB formation of electron beam radiation in aqueous solution was 5.7 times higher than that caused by ^{60}Co-gamma rays. Electron beam radiation has higher efficiency variation, low cost, safety and smaller radiation damage. Electron beam irradiation proved to be stronger mutagenic than gamma irradiation if one considers their effects at the same dose and for the same cell type. 5 MeV electrons and ^{60}Co-gamma-radiation were used to irradiate dry seeds of rice. The results showed that electron beam possess lower damage in M_1 and higher mutagen frequency, and wider mutation spectrum than ^{60}Co γ-rays in rice (Baojiang *et al*., 1982). In rice, mutation work has not been carried out so far by using Electron beam in India.

The success of mutation using the radiations depends on its dose, rate of mutation, the number of screened plants and the mutation efficiency. Low dose cannot cause mutation and therefore there are no changes in mutated seeds, but higher doses inevitably bring about mortality, high pollen

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and seed sterility and deleterious mutations. To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50}. Plants differ in radiosensitivity, in cereals, radiosensitivity is genotypic dependence. It is highly desirable to establish a radiosensitive curve and determine LD_{50} of mutagen for treating seeds, for the induction of mutations in cereals and others. LD_{50} of the crops is varied depending on the species, the varieties, part of the mutation and the water content of the material. Thus, we must first determine the LD_{50} (lethal dose), a dose that causes 50% mortality to the seeds or a safe dose where 50% of the seeds can survive. Hence the present investigation was undertaken to fix Lethal dose and to compare effect of gamma rays and electron beam on rice in M_1 generation.

Materials and Methods

Plant material: The seeds of two varieties namely ADT-37 and ADT (R) 45 for the induction of mutation treatment were obtained from Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai, India.

Mutagen treatment: Gamma rays: Around well filled 500 seeds of ADT-37 and ADT(R) 45 with the moisture content of 12 per cent were packed in butter paper covers and placed in the Gamma chamber, it was exposed to gamma irradiation from the Cobalt 60 gamma source for appropriate time for each dose based on the half-life of the source in the gamma chamber installed at the Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Non-irradiated dry seeds were taken as control. The seeds of ADT-37 and ADT (R) 45 were irradiated at five different doses starting from 100 Gy to 500 Gy with an interval of 100 Gy. The irradiated seeds were sown on the same day in raised bed nursery established at Agricultural College and Research Institute, Madurai during summer season of 2014.

Electron beam: Around well filled 500 seeds of ADT-37 and ADT (R) 45 with the moisture content of 12 per cent were treated with 10 MeV electron beam from electron accelerator facility at Electron Beam Centre, Bhabha Atomic Research Centre, Kharghar, Navi, Mumbai, India. The seeds of ADT-37 and ADT(R) 45 were irradiated at five different doses starting from 200 Gy to 600 Gy with an interval of 100 Gy. The irradiated seeds were sown in raised bed nursery established at Agricultural College and Research Institute, Madurai during summer season of 2014.

Treated seeds were sown separately in germination paper by roll towel method, paper cups under *in vitro* condition and in nursery with the two replications. Another set of treatment were carried out and the seeds were sown in raised beds (*in vivo*) immediately after the treatment along with the control seeds. Under *in vitro* condition, germination percentage, shoot length and root length were measured on seventh day. Survival percentage (20 DAS), seedling height on 20^{th} day, pollen fertility during flowering and spikelet fertility after harvest was observed from the plants raised in the field. Germination percentage, shoot length and root length were observed three days and 14 days after treatment (DAT). Twenty one days old seedlings were transplanted to main field by adopting 20 x 10cm spacing in randomized block design with two replications.

Probit analysis: The LD_{50} values of gamma radiation and Electron beam for both the genotypes were determined based on the probit analysis (Finney, 1971). The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution. The procedure for determination of LD_{50} using probit analysis is as follows.

1) Mutagen dose values were transformed into $\log_{10}$ value.
2) Mortality percentage of seeds due to treatment doses were worked out and rounded to the nearest whole number.
3) Corrected mortality percentage was calculated using Abbott's formula given below and rounded to the nearest whole number.
4) The corrected values were converted to the probit transformation.
5) Probit values were graphed (Y-axis) against Log_{10}concentration (X-axis) and a straight line passing through most of the plotted points were drawn to estimate the Log_{10} concentration associated with a probit of 5.
6) Antilog to the Log_{10} value corresponding to the probit 5 was calculated to find out the LD_{50} for the particular mutagen under study.

Results and Discussion

Determination of lethal dose: Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. LD_{50} was noticed for the variety ADT-37 at 300.03 Gy of gamma rays and 286.45 Gy of Electron beam. The gamma irradiation of 300.00 Gy and 275.05 Gy of electron beam were recorded as lethal dose for the variety ADT (R) 45 (Table 1). This clearly revealed that, the LD_{50} is fixed more or less same in both varieties under both conditions. Similar observations were recorded in the rice treated with gamma rays. Therefore, LD_{50} dose observed in this study is the optimum dosage for mutagenizing the rice seeds of different varieties to induce mutations to produce viable mutants and maintenance of population for mutation breeding.

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Effect of mutagens on germination and survival: The response of varieties ADT-37 and ADT(R) 45 for gamma radiation and electron beam treatments in relation to germination was studied and represented in Table 2 and Table 3. Both the varieties displayed a dose dependent negative linear relationship between dose and germination percentage. In case of ADT-37 reduction in germination ranged from 14.36 per cent (100 Gy) to 53.75 per cent (500 Gy) for gamma rays and 22.96 per cent (200 Gy) to 71.68 per cent (600 Gy) for electron beam. Similarly, in M_1 generation of ADT(R) 45, germination percentage reduction ranged from 13.68 per cent (100 Gy) to 51.54 per cent (500 Gy) for gamma ray treatments and 25.77 per cent (200 Gy) to 70.54 per cent (600 Gy) for electron beam. This shows significant influence of mutagen on germination.

A drastic reduction in survival of plants at 20th day after planting was observed due to gamma ray as well as electron beam treatment. Higher reduction in survival percentage was noticed in higher dose in both gamma rays and electron beam when compared to the lower dose. The decrease in the percentage survival with increase in gamma radiation is in agreement with results of Cheema and Atta (2003), who worked on Basmati rice varieties and reported decrease in percentage field survival of M_1 plants with increase in radiation dose. In both the varieties of ADT-37 and ADT(R) 45, dose dependant negative linear relationship between dose of mutagen and survival of plants were noticed. Similar kind of results was also reported by Ramachander *et al.* (2015) for gamma rays. Gaul (1970) reported that the damage to the biological material will be reflected in the above parameters and may be considered as an indication for the mutagenic effects. In this investigation, the germination percentage and plant survival were found to be greatly affected by mutagenic treatments in both the varieties of ADT-37 and ADT(R) 45 (Fig 3).

The percentage of germination reduced progressively with the increasing dose or concentration of mutagen and a greater reduction was observed at higher dose in both varieties. Such a dose dependent decrease in germination was reported previously by Vasline (2013) for gamma rays. The stimulating effect of gamma ray on germination may be attributed to the activation of RNA or protein synthesis and it may be occurred during the early stage of germination after the seeds were irradiated. The decrease in germination at higher doses of the mutagens may also be attributed to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both. The rhythm of seed hydration during germination may also play a role in determining the degree of radiosensitivity reported in peas. Kiong *et al*., (2008) found that radiation increases plant sensitivity to gamma rays and this may be caused by the reduced amount of endogenous growth regulators, especially the cytokines, as a result of breakdown, or lack of synthesis, due to radiation.

Effect of mutagens on shoot length, root length and seedling height: In the present study, the shoot length of ADT-37 and ADT(R) 45 after treatment with gamma radiation and electron beam follows the linear relationship between increase in dose and shoot length reduction. In ADT-37, higher reduction in shoot length was noticed in higher dose 500 Gy (63.15 %) of gamma rays and 600 Gy (70.83 %) of electron beam. Whereas in ADT(R) 45 higher reduction was observed in 500 Gy (65.37%) of gamma radiation and 600 Gy (72.51%) of electron beam. Dehpour *et al*. (2011) also reported that, the maximum decrease in shoot length was observed when rice genotypes were exposed by gamma ray dose higher than 200 Gy.

The decrease of root length was confirmed by the increased doses in both gamma irradiated and electron beam treated ADT-37 and ADT(R) 45 (Fig 3). Cheema and Atta (2003) reported that, reduction in root length with each corresponding increase in gamma ray dose that is in line with present findings. Higher reduction of root length was observed at higher dose of gamma rays and electron beam of 500 Gy and 600 Gy. These results were in accordance with Ramchander *et al.* (2015) for gamma rays.

Seedling height is widely used as an index in determining the biological effects of various physical mutagens in M_1. The present study exhibited that the seedling height measured at 20 DAS was decreased with the proportion of increase in dose in both the varieties of ADT-37 and ADT(R) 45 (Fig 1 and 2). Similar results for gamma rays have been reported by Vasline (2013). Khalil *et al.*1986 attributed decreased shoot and root lengths at higher doses of gamma rays to reduced mitotic activity in meristematic tissues and reduced moisture contents in seeds respectively. The reduction in seedling height may be attributed to damage induced during cell division and elongation following mutagenic treatment. Reduced growth has been attributed to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances (Usuf and Nair, 1974).The high-dose of gamma ray irradiation caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome. The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity which may the possible causes of adverse effects of gamma radiations on plant height (Khah and Verma, 2015).Carbon partitioning is altered by increasing the radiation dose because of damage to radiosensitive cells responsible for the transport of carbohydrates in the phloem.

Growth reduction in M_1 plants caused by the physical damage of the mutagen should be enough to get a probability of obtaining high frequencies of useful mutations in the M_2 generation.

Effect of mutagens on pollen fertility and spikelet fertility: Effect of mutagens on pollen fertility of ADT-37 and ADT(R) 45 in M_1 generation was studied and presented in Table 2 and Table 3. Per cent reduction in pollen fertility of ADT-37 ranged from 10.56 per cent (100 Gy) to 42.65 per cent (500 Gy) for gamma rays and 13.61 per cent (200 Gy) to 46.82 per cent (600 Gy) for electron beam, whereas in ADT(R) 45 it was ranged from 10.95 (100 Gy) to 46.02 per cent (500 Gy) for gamma rays and 13.27 per cent (200 Gy) to 49.97 per cent (600 Gy) for electron beam. Mutagens have been effective in decreasing the mitotic index or increasing micronuclei number and pollen abnormalities. One of the factors responsible for poor seed setting was due to sterility of pollen grains caused by gamma irradiation. In this study, induced pollen sterility increased with increasing doses. Higher reduction in pollen sterility was noticed in 500 Gy of gamma rays and 600 Gy of electron beam in both the varieties. The pollen fertility in Samba Mahsuri (BPT 5204) after the gamma radiation treatment showed quite lower percentage in a dose 0.05 kGy (Kumar *et al*., 2013). The decrease in pollen fertility of rice after irradiation is considered to be due to chromosomal aberrations.

The overall study on the effect of gamma radiation and electron beam germination, survival, shoot length, root length, seedling height at 20th day, pollen fertility and spikelet fertility in M_1 generation of two rice varieties concluded that, ADT(R) 45 was highly sensitive to gamma rays and electron beam than ADT-37. The present results (Table 2) revealed that the per cent reduction in germination percentage, survival percentage, shoot length, root length, seedling height at 20 DAS, pollen fertility and spikelet fertility was higher in electron beam than gamma rays in most of the cases.

Conclusion

Efficient mutagens and their treatments are obligatory for the cost effective use of the mutagen as a tool for the induction of mutations and their direct/ indirect utilization in successful breeding programme. Optimum dose is the dose that causes maximum of mutation with minimum damage to the plant and knowledge on LD50 is vital importance to maintain the mutant population for further improvement through mutation breeding. In the present study, LD_{50} dose for gamma rays was 300.03 and 300.00 Gy for ADT-37 and ADT(R) 45 varieties respectively, whereas, LD_{50} dose for electron beam on ADT-37 and ADT(R) 45 was 286.45 Gy and 275.05 Gy respectively. These optimum mutagen doses determined for the rice genotype could be useful while formulating rice mutation breeding programme for improvement of specific traits in rice. According to the results in the present study, the germination percentage, survival percentage, shoot length, root length, seedling height, pollen fertility and spikelet was gradually decreased with increase in dose of gamma rays and electron beam and a prominent reduction was noticed in electron beam compared to gamma rays in both the varieties and concluded that, ADT(R) 45 variety was highly sensitive to gamma rays and electron beam than ADT-37.

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Table 1. Determination of lethal doses by gamma rays and electron beam in ADT-37and ADT(R) 45 - Probit analysis

ADT-37						ADT(R) 45					
Treatment	**Log_{10} of doses**	**Observed mortality percentage**	**Corrected mortality percentage**	**Empirical probit unit**	**LD 50 value**	**Treatment**	**Log_{10} of doses**	**Observed mortality percentage**	**Corrected mortality percentage**	**Empirical probit unit**	**LD 50 value**
Gamma rays (Gy)						Gamma rays (Gy)					
Control	-	-	-	-		Control	-	-	-	-	
100 Gy	2.00	23.08	14.53	3.94		100 Gy	2.00	24.71	14.44	3.94	
200 Gy	2.30	37.59	30.66	4.49	300.03 Gy	200 Gy	2.30	41.20	33.18	4.57	
300 Gy	2.48	66.12	62.36	5.31		300 Gy	2.48	67.87	63.49	5.34	300.00 Gy
400 Gy	2.60	67.45	63.83	5.35		400 Gy	2.60	68.10	63.75	5.35	
500 Gy	2.70	70.17	66.86	5.44		500 Gy	2.70	69.06	64.84	5.38	
Electron beam (Gy)						Electron beam (Gy)					
Control	-	-	-	-		Control	-	-	-	-	
200 Gy	2.30	32.68	25.20	4.33		200 Gy	2.30	38.31	29.90	4.47	
300 Gy	2.48	67.52	63.91	5.36	286.45 Gy	300 Gy	2.48	68.15	63.81	5.35	
400 Gy	2.60	68.73	65.26	5.39		400 Gy	2.60	69.18	64.98	5.38	275.05 Gy
500 Gy	2.70	74.89	72.10	5.59		500 Gy	2.70	71.40	67.50	5.45	
600 Gy	2.78	88.00	86.67	6.11		600 Gy	2.78	87.68	86.00	6.08	

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Table 2.Effect of gamma rays and electron beam on biological parameters of ADT-37 and ADT(R) 45 in M_1 generation

Treatments	Germination percentage		Shoot length		Root length		Seedling height		Survival percentage		Pollen fertility		Spikelet fertility	
	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction
ADT-37														
Gamma rays (Gy)														
Control	100.00		100.00		100.00		100.00		100.00		100.00		100.00	
100 Gy	85.64	14.36	88.43	11.57	88.21	11.79	92.67	7.33	84.16	15.84	89.44	10.56	93.76	6.24
200 Gy	72.92	27.08	71.20	28.80	70.92	29.08	90.42	9.58	63.15	36.85	78.50	21.50	89.06	10.94
300 Gy	49.74	50.26	50.56	49.44	51.79	48.21	88.04	11.96	49.39	50.61	68.51	31.49	83.44	16.56
400 Gy	48.61	51.39	43.24	56.76	44.54	55.46	86.79	13.21	47.09	52.91	66.01	33.99	81.04	18.96
500 Gy	46.25	53.75	36.85	63.15	33.19	66.81	84.47	15.53	43.19	56.81	57.35	42.65	76.78	23.22
Electron beam (Gy)														
Control	100.00		100.00		100.00		100.00		100.00		100.00		100.00	
200 Gy	77.04	22.96	67.50	32.50	69.52	30.48	89.83	10.17	68.31	31.69	86.39	13.61	88.50	11.50
300 Gy	48.54	51.46	48.52	51.48	46.55	53.45	86.31	13.69	49.04	50.96	75.34	24.66	82.85	17.15
400 Gy	47.51	52.49	40.28	59.72	39.04	60.96	83.54	16.46	48.33	51.67	63.93	36.07	76.61	23.39
500 Gy	42.01	57.99	34.81	65.19	28.38	71.62	81.85	18.15	40.32	59.68	62.91	37.09	73.95	26.05
600 Gy	28.32	71.68	29.17	70.83	22.97	77.03	79.94	20.06	13.74	86.26	53.18	46.82	70.79	29.21
ADT(R) 45														
Gamma rays (Gy)														
Control	100.00		100.00		100.00		100.00		100.00		100.00		100.00	
100 Gy	86.32	13.68	86.96	13.04	87.54	12.46	91.27	8.73	80.62	19.38	89.05	10.95	92.05	7.95
200 Gy	71.81	28.19	70.13	29.87	70.39	29.61	90.55	9.45	67.06	32.94	75.23	24.77	89.87	10.13
300 Gy	49.52	50.48	49.69	50.31	49.92	50.08	88.44	11.56	46.25	53.75	65.12	34.88	87.01	12.99
400 Gy	49.30	50.70	38.33	61.67	37.76	62.24	85.96	14.04	46.05	53.95	62.04	37.96	81.95	18.05
500 Gy	48.46	51.54	34.63	65.37	35.27	64.73	84.00	16.00	45.26	54.74	53.98	46.02	74.23	25.77
Electron beam (Gy)														
Control	100.00		100.00		100.00		100.00		100.00		100.00		100.00	
200 Gy	74.23	25.77	67.31	32.69	69.11	30.89	84.07	15.93	65.66	34.34	86.73	13.27	89.75	10.25
300 Gy	49.28	50.72	47.75	52.25	48.72	51.28	77.27	22.73	46.02	53.98	71.12	28.88	87.93	12.07
400 Gy	48.36	51.64	38.33	61.67	33.84	66.16	73.24	26.76	45.15	54.85	60.02	39.98	77.91	22.09
500 Gy	46.36	53.64	33.22	66.78	29.76	70.24	68.62	31.38	39.15	60.85	54.25	45.75	64.97	35.03
600 Gy	29.46	70.54	27.49	72.51	24.17	75.83	59.49	40.51	10.89	89.11	50.03	49.97	60.08	39.92

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Fig 1. Effect of different doses of gamma rays and electron beam on germination, shoot length, root length, survival percentage, seedling height at 20th day, pollen fertility and spikelet fertility of ADT-37

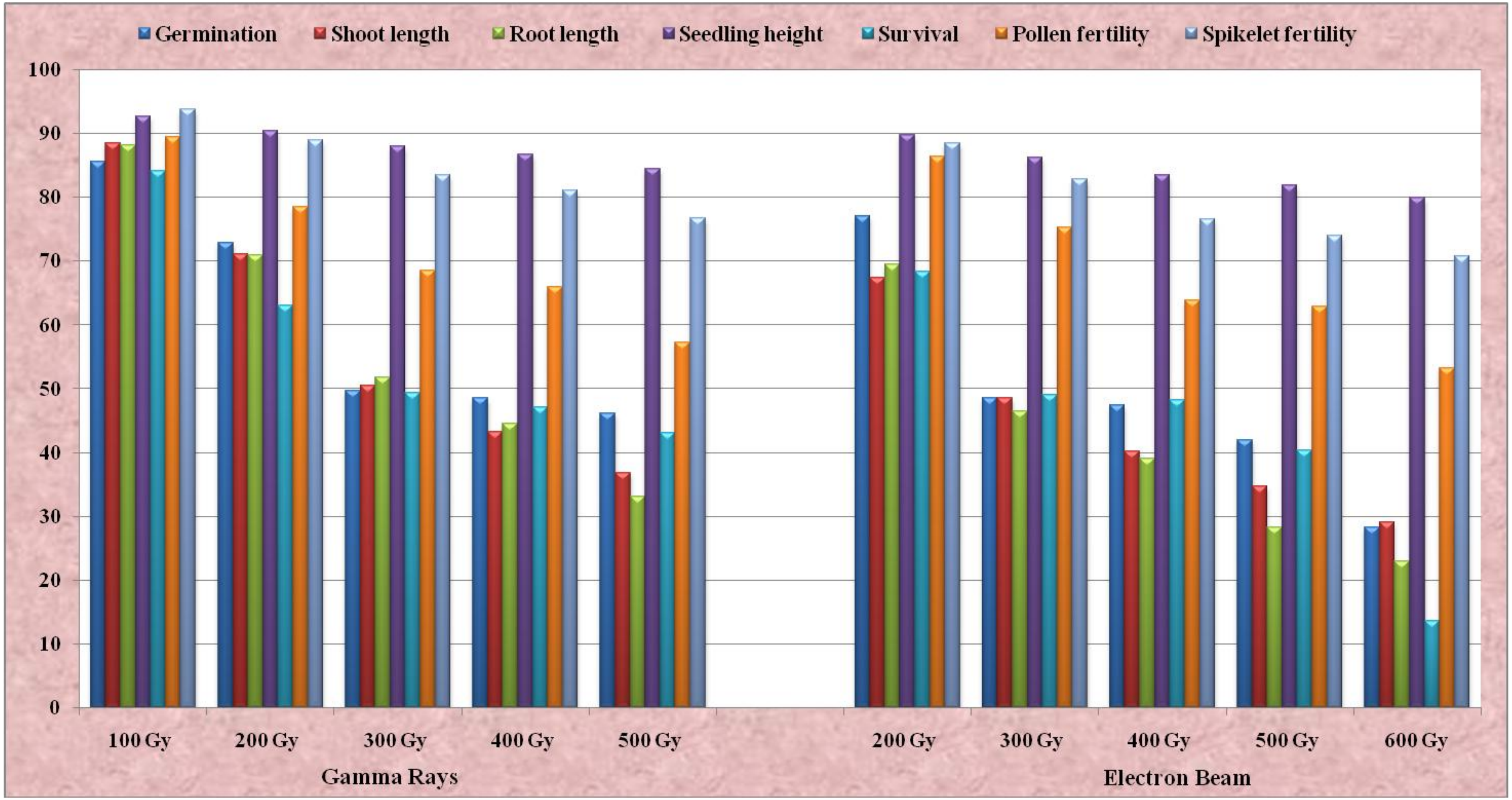

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Fig 2. Effect of different doses of gamma rays and electron beam on germination, shoot length, root length, survival percentage, seedling height at 20th day, pollen fertility and spikelet fertility of ADT(R) 45

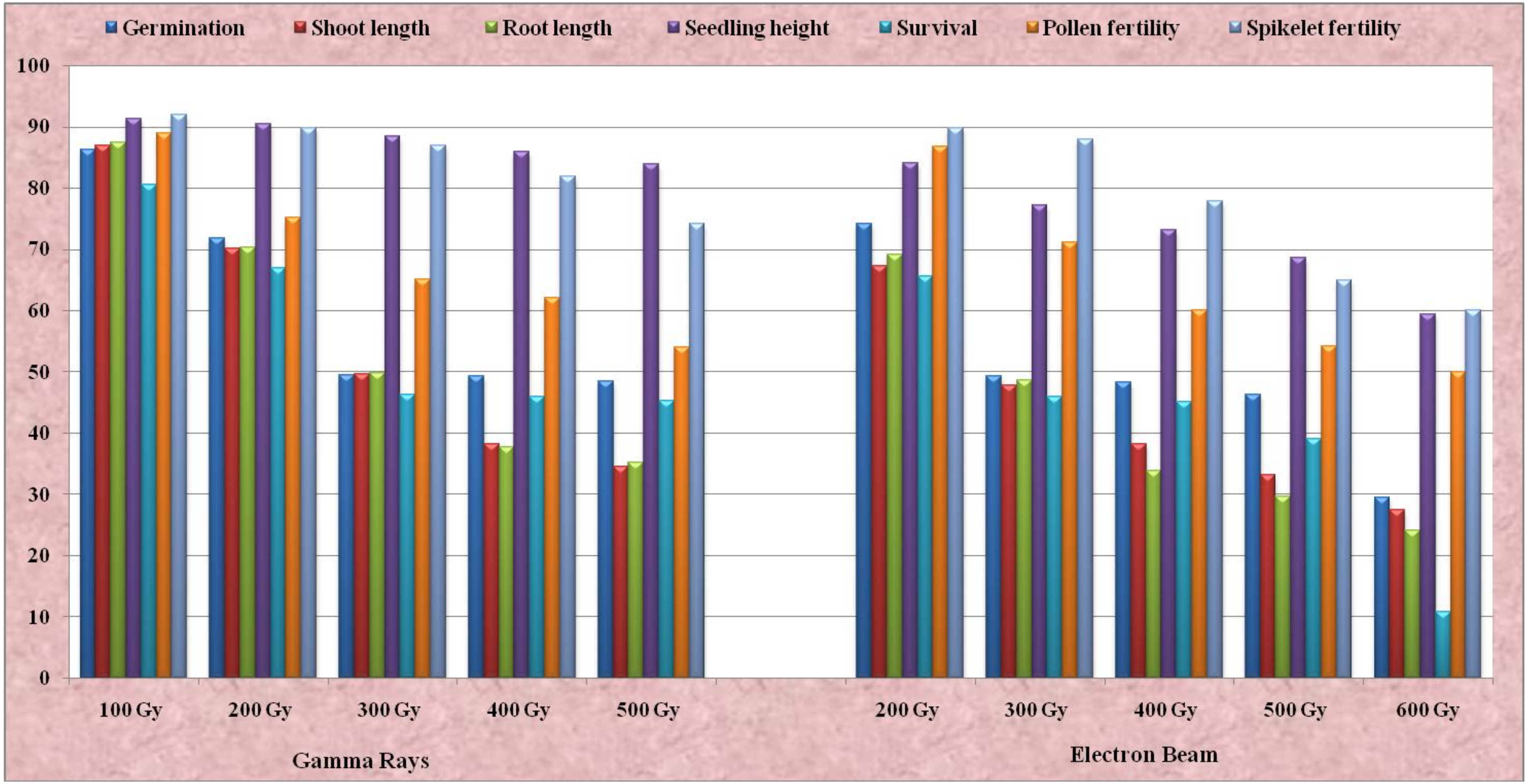

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Fig 3. Comparision of germination percentage and survival percentage reduction over control at different doses of gamma rays and electron beam in ADT-37 and ADT(R) 45