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Comparative study of radio-sensitivity and relative biological effectiveness of gamma rays, X-rays, electron beam and proton beam in short grain aromatic rice

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Abstract

Knowledge about the type of mutagen used and its optimized dose are of paramount importance to design and implement any plant mutation breeding programme. Present study was first time carried out to evaluate the comparative effectiveness, radio-sensitivity behavior and relative biological effectiveness of four physical mutagens *viz.,* gamma rays, X-rays, electron beam and proton beam on two short grain aromatic rice landraces *viz.,* Samundchini and Vishnubhog. The seeds of these two varieties were treated with 15 different doses of all four mutagens, ranging from 50Gy to 750Gy with an interval of 50Gy. Germination percentage and seedling growth parameters were recorded at seven and 15 days after sowing, respectively in two replications. It was observed that germination percentage, shoot and root length of the seedling gradually declined with the increase in doses of all the physical mutagens. On the basis of these observations, LD50 and GR50 doses were calculated. The present study reports the optimum range of doses for gamma ray (280 to 350 Gy); electron beam (290 to 330Gy); X-ray (200 to 250 Gy) and proton beam (150 to 200Gy). GR50 doses were observed higher than LD50 doses for all the mutagens in both landraces. However, Samundchini showed higher LD50 and GR50 doses than Vishnubhog indicating later to be more radio-sensitive. Furthermore, both the genotypes were highly radio-sensitive for proton beam and least for gamma rays. Similarly, high relative biological effectiveness was observed for proton beam followed by X-ray, electron beam and gamma rays indicating their decreasing trend of penetration capacity and lethality. Results of present study will be useful for plant breeders to use the above mutagens in an appropriate dose for mutation breeding in rice.

Key words: Rice, landraces, physical mutagens, radio-sensitivity, electron beam, proton beam, LD50, GR50

Introduction

Rice (*Oryza sativa* L.) is considered vital to the food security of over half the world's population with Asia accounting for 90 % of global rice consumption. World population will continue to grow which demands a nearly 70% increase in food production (FAOSTAT, 2020). Hence, increasing rice yield is essential to ensure the world food security and living standards of everyone. Traditional rice landraces adapted to the local environments are the valuable genetic material for developing sustainable and ideal plant types. Chhattisgarh is one of the major producer and consumer of short grain aromatic rice which are being cultivated in almost all districts in different agro-climatic zones. The aromatic short grain rice landraces enriched in cooking and eating qualities hold enormous potential to be utilized as value added products, thereby providing high market value and economic return to small and marginal farmers (Sahu et al. 2019). However, they have very tall plant stature that is prone to lodging, long maturity duration, photoperiod sensitive nature with low yield potential which makes these either marginalized or are in the process of disappearing from cultivation (Sharma et al. 2017). Such problems need to be addressed carefully in a sustainable manner. Crop improvements through recombination breeding may change the grain structure as well as grain quality. Radiation induced mutation-breeding is one of the complementary methods to generate new variations without altering its original grain structure, shape and cooking quality (Sharma et al. 2017).

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Radiation techniques have generated enormous genetic variations which are a source of phenotypic diversity and are a major driver of evolutionary diversification (Jankowicz-Cieslak et al. 2017). With the help of induced mutagenesis, mutants of different crops have been developed and mostly comprised of cereal crops (47%) with the majority (25%) being rice mutants. A total 823 rice mutant varieties have been reported from 30 countries, with 60 mutant varieties from India (FAO/IAEA MVD, 2020).

Physical mutagens, particularly gamma rays, have been used widely for inducing mutation and more than 70% of mutant varieties have been developed using physical mutagenesis (Viana et al*.* 2019). The biological effect of gamma rays is based on the interaction with cellular water to produce free radicals causing DNA damage like base modifications, strand breaks (single and double) which when left unrepaired by cellular machinery leads to mutation (Jankowicz-Cieslak et al. 2017; Oladosu et al. 2016). Cobalt-60 (Co-60) and Cesium-137 (Cs-137) are the main isotopic sources for spontaneous production of gamma rays. Similarly, X-rays are high-energy electromagnetic radiation and carry enough energy to ionize atoms and disrupt molecular bonds. X-rays are produced in an X-ray tube, electrons are accelerated in a high vacuum and then hit a tungsten target, and then they are decelerated resulting in an emission of spectrum (FAO/IAEA, 2018).

Apart from the conventional electromagnetic radiations, like X-rays and gamma rays, electron beam is now an alternative source of energy to induce mutations in plants. Electron beams have been extensively used in field of cold sterilization technology. These can be extended to other domains with evident advantages in crop improvement and food irradiation processing. The energy released by electron beam has high Linear Energy Transfer (LET) (FAO/IAEA, 2018). Recently, proton beams (H^+ or proton, He^{2+}, Li^{3+} etc.) have also attracted attention in the fields of plant breeding. Proton beams have very high LET (4-100 KeV/µm) compared to gamma, X-rays and electron beam (0.2 KeV/µ). In this type of mutagenesis, positively charged ions, accelerated at a high speed (around 20-80% of the speed of light), are used to irradiate target cells and have very different mode of interaction with genetic material expected to produce a different spectrum of mutations (Oladosu et al. 2016). In spite of its potential as a useful mutagen, there have been scanty reports on utilization of proton beam in mutation breeding in rice, due to lack of available facilities and proper standardization of doses.

Optimization of dose is the first step in radiation induced mutation breeding programme. In order to exploit induced mutagenesis for crop improvement, the radio-sensitivity test for LD50 and GR50 of a mutagen in a crop species is necessary to recover high frequency of desirable mutations (Ahloowalia et al. 2004). The dose of a mutagen that achieves the optimum mutation frequency with the least possible unintended damage is regarded as the optimal dose (Mba et al. 2010). Doses of mutagen that lead to 50% lethality (LD50) or 50% growth reduction (GR50) or a dose lower than LD50 and GR50 have often been chosen for mutation induction in crop plants (Viana et al. 2019). Different mutagens may have varied LD50 and GR50 doses based on the crop species, genotypes and material/ tissue used for treatment. Many scientists have attempted to determine the comparative effectiveness of various mutagen combinations for the induction of desirable traits in rice or other crops (Lavanya et al. 2016; Rani et al. 2016; Gawthami, et al. 2017; Veni et al. 2017; Mondal et al. 2017; Lalitha et al. 2019 and Sushmitharaj et al. 2019). However no previous study has reported to compare effectiveness and radio-sensitivity of four physical mutagens *viz.,* gamma rays, X-rays, electron beam and proton beam on aromatic short grain rices. Therefore, the present investigation was carried out for the first time to study the comparative effectiveness and radio-sensitivity behavior of above mentioned physical mutagens on two short grain aromatic rice landraces of Chhattisgarh for its efficient utilization in rice mutation breeding programmes.

Materials and methods

Mature, healthy, pure and uniformed size rice seeds of two traditional rice landraces, Samundchini and Vishnubhog were taken for the present study. Both the landraces are very popular in Chhattisgarh due to their aromatic and short grain features. Samundchini landraces has 165-170 cm plant height and 155-160 days maturity duration whereas Vishnubhog has 140-145cm plant height and 135-140 days maturity duration. Seeds of both the landraces were procured from R.H. Richharia Rice Germplasm Division of Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh.

Treatment with mutagens

Viability of seeds was tested prior to mutagen treatment and off-type seeds, if any, were removed. Around 500 seeds of Samundchini and Vishnubhog with 12% moisture content were packed in butter paper covers and labeled properly for different doses of mutagen. Non-irradiated dry seeds were taken as control. The irradiated and control seeds were used immediately for radio-sensitivity test at Department of Genetics and Plant Breeding, IGKV, Raipur during *kharif* 2019. Seeds of Samundchini and Vishnubhog landraces were treated with four physical mutagens *viz.,* gamma rays, X-rays, electron beams and proton beams with 15 different doses, ranging from 50Gy to 750Gy with an interval of 50Gy at different facility centers of the country.

Seeds were exposed to gamma irradiation using a Cobalt-60 isotope based gamma source (GC-5000, Board of Radiation Isotope Technology, India) having dose rate of-37 Gy/min at Bhabha Atomic Research Centre, Mumbai 400 085, India. Seeds were also exposed to X-ray radiation and electron beam (based on10 MeV electron linac) at 15 different doses starting from 50Gy to 750Gy with an interval of 50Gy for appropriate time at Industrial Accelerators Division, Raja Ramanna Centre of Advanced Technology, Indore, India.

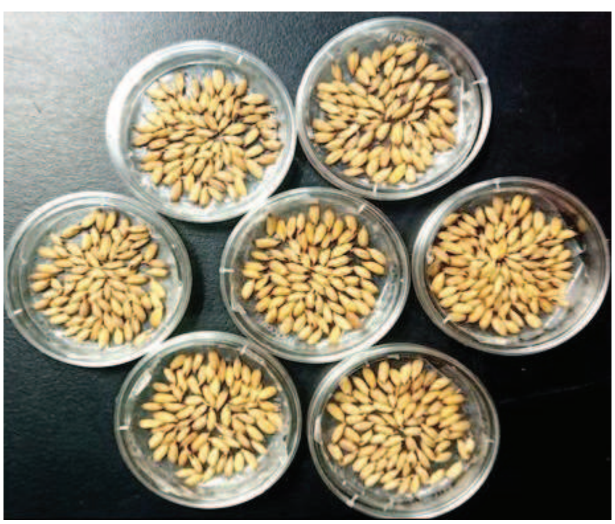
Proton beam irradiation of the rice seeds was carried out using 14 MeV BARC Pelletron Accelerator facilities at Tata Institute of Fundamental Research (TIFR), Mumbai. About 60 grains were arranged in concentric layers in each circular plastic cap with germ region of the grain facing toward the center (Fig. 1a). For irradiation, the detector setup was replaced by rice grains pasted in a specialized plastic cap. The flux incident on the grains was measured using another detector mounted at 30° in the scattering chamber. Multiple plastic caps were mounted on an irradiation wheel which can accommodate 66 samples which can be changed remotely without entering the irradiation area (Fig. 1b). After irradiation caps are taken off from the mounted wheels. The actual doses induced to rice seeds were 51,101, 146, 199, 249, 300, 347, 398, 452, 496, 547,592, 654, 691 and 750Gy.

Evaluation of seed germination and seedling growth parameters

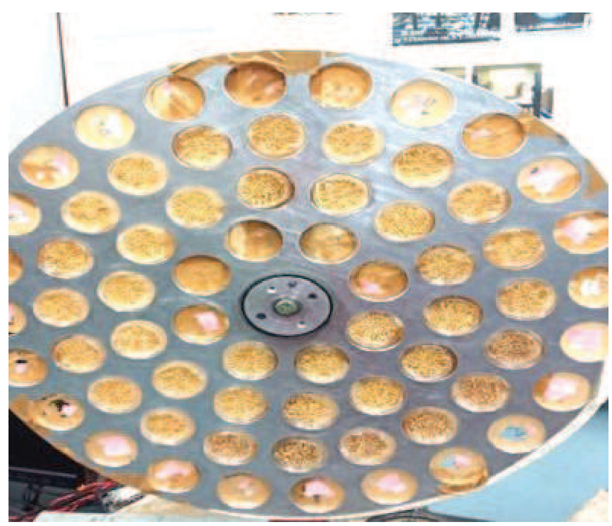
The biological effects of gamma rays, X-rays, electron beam and proton beam treatment on rice seeds and seedlings were evaluated based on radicle length (at seven days after sowing); shoot length (at 15 days after sowing) and root length (at 15 days after sowing). Experiments were conducted by following the completely randomized design (CRD) with two replications under a polyhouse.

Standard seed germination test was practiced in sterile petri-plate containing moistened 3 mm-blotting paper. Two replications of 20 seeds were placed in petri-plate and incubated at room temperature (25°C) in dark for seven days. For all the treatments, data concerning to germination was taken seven days after sowing. Seeds having >1mm long coleoptiles and radicles were considered as germinated seeds (Lee et al. 1998). Germination percentage was calculated by following formula: Germination %={(Number of seeds germinated/Total number of seeds) x 100}.

Randomly selected 20 seeds from each treatment were used to study the effect of different



(a)



(b)

Fig. 1. (a) Seeds of rice in plastic caps (b) caps mounted on circular wheel for irradiation with proton beam

mutagens on rice growth parameters *viz.*, shoot and root length through Sandwich Blotter method. Watman filter paper was cut into 15 cm x 25 cm size and folded into 3 cm width. In the trenches of folded paper, 10 small holes were made at equidistance and soaked seeds were placed near the holes. Two folded papers were used for each treatment so that 20 seeds were accommodated. The folded papers with seeds were placed in germination racks which were placed in rack stands having 3-4 cm of water in it. The rack stands were kept in plant growth chamber (Sanyo MLR-350H, Japan) with a daily cycle of a 14h photo period with a light intensity of 150 E m^{-2} s^{-1}, day/night temperatures of 25/22°C and a relative humidity of 65-75%. After 15 days, seedlings were separated carefully, wiped properly with lint free tissue paper and observations on plant growth parameters such as shoot length (cm), root length (cm), total seedling length (cm) and vigor index were taken from each treatment in two replications. Differences in root and shoot length of two rice landraces at 15 days after sowing by gamma ray irradiation is presented in Fig. 2.

(a) (b)

Fig. 2. Representation of shoot and root length of Samundchini and Vishnubhog seedlings after gamma ray treatment at 15 days after sowing

Investigations on vigor index and relative biological effectiveness

Vigor Index is the measure of the extent of damage that accumulates in seedlings till the viability declines, and the damage accumulates in seeds until the seeds are unable to germinate and eventually die. Vigor index value was computed using the following formula as suggested by Abdul-Baki and Anderson (1973) and expressed as whole number.

Vigor index = Germination (%) x Total seedling length (cm).

The Relative Biological Effectiveness (RBE) is defined as the ratio of the doses required by two radiations to cause the same level of effect on biological tissue by depositing per unit of energy. RBE was calculated for various mutagens by taking the LD50 dose of gamma ray as reference.

RBE of mutagen = (LD 50 dose of gamma rays / LD50 dose of mutagen to be compared)

Statistical analysis

The observations recorded on seed germination, root length, shoot length and total seedling length at two replications from each genotype were used to calculate the LD50 and GR50 doses for each mutagen. LD50 doses of four mutagens for each genotype were calculated through probit analysis (Finney 1971) by OPSTAT software (Sheoran 2020). The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution. GR50 doses of four physical mutagens for each genotype were calculated through linier regression analysis with the help of MS Excel, 2007. Vigor Index and RBE were calculated for all the mutagens based on the given standard formula through MS Excel, 2007. Two way analysis of variance (ANOVA) based on CRD for germination percentage and seedling growth parameters were estimated with the help of using PBTools v1.4 software (PBTools, Soft. 2014). Before performing the ANOVA, germination percentage were Arcsine transformed for normalizing the percentage values through PBTools v1.4software (PBTools, Soft. 2014).

Results and discussion

This study was undertaken to compare the radio sensitivity of rice landraces by standardizing the irradiation doses for four different physical mutagens (*viz*., gamma rays, X-rays, electron beam and proton beam) based on germination percentage and seedlings' growth parameters. Understanding the effect of these radiations on crop plants will help in determining the optimum doses for inducing mutations and increasing crop diversity. Two way ANOVA revealed significant differences in varieties (factor A) for three traits *viz*., average shoot length, average root length and average total seedling height whereas mutagens (factor B) showed highly significant differences for all the traits (Table 1). This indicated that the mutagens may have different penetrating effects on the varieties based on their different physical properties. Since, both the varieties were belongs to aromatic short grain group therefore, there is no significant differences in germination % and vigor index. However, significant differences for average shoot length, average root length and average total seedling height in both varieties may be due to their different morphological features. Moreover, the results showed a significant interaction between varieties and mutagens, indicating that any change in the mutagens is dependent upon the variety under treatment and their doses (Table 1). The result revealed that the doses of mutagens may vary according to varieties for effective mutation rate and similarly, the varieties may show different agronomic behaviors towards different mutagens as has also been observed earlier (Chauhan et al. 2019; Gowthami et al. 2017; Iqbal and Zahur 1975).

Comparison of radio-sensitivity of short grain aromatic rice landraces based on Lethal Dose 50 (LD50)

LD50 is the dose of radiation which kills 50% of viable seeds. Determination of LD50 is crucial in order to reduce the lethality level and to obtain wide range of desirable mutants. In the present investigation, the seeds of rice genotypes were treated with 15 different doses of mutagens ranging from 50Gy to 750Gy with an interval of 50Gy. Doses with an LD50 generally applied in the plant mutation breeding programmes. However, it has been observed that radiation doses lesser than LD50 gives more economically useful mutations (Oladosu et al. 2016; Mba et al. 2010). In the present study, LD50 doses for gamma ray irradiated Samundchini and Vishnubhog seeds were 304.26Gy and 293.1Gy respectively whereas in electron beam irradiated seeds it was 301.26Gy and 299.54Gy, respectively. LD50 doses for X-ray and proton beam in Samundchini and Vishnubhog was estimated 207.48Gy & 236.35Gy and 154.04Gy & 165.32Gy, respectively (Fig. 3). Differences in LD50 doses of four mutagens were observed due to different penetration ability and other attributes of the mutagens. Moreover, minor or negligible differences in LD50 doses of various mutagens on both landraces were observed. Both the landraces belongs to aromatic short grain group therefore, a major difference in LD50 doses were not observed. Iqbal and Zahur (1975) also reported that LD50 doses of various mutagens for different varietal group of any one crop may differs significantly

Table 1. Two way ANOVA based on CRD

Source of variation	DF	Mean sum of square				
		Germination percentage	Average shoot length	Average root length	Average total seedling height	Vigor index
Factor A (Varieties)	1	0.25	0.58**	0.01*	0.74**	1772.73
Factor B (Mutagens)	3	499.54**	44.05**	1.43**	60.28**	341248.07**
Interaction of A x B (Varieties x Mutagens)	3	8.64**	1.29**	0.08**	1.46**	10128.20**
Error	8	0.88	0.004	0.001	0.01	393.66
Total	15					
CD for Factor A (Varieties)	N/A	0.073	0.043	0.11	N/A	
CD for Factor B (Mutagens)	1.55	0.104	0.061	0.156	32.854	
CD value for Int. A x B (Varieties x Mutagens)	2.192	0.147	0.086	0.22	46.463	

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level based on the ontogenetic stage, exposure rate and interphase chromosome volume.

It was observed that as the dose increases, the mortality of seeds also increases which indicated that higher doses induce more lethal effect on seeds because of the increased damage to nitrogenous bases and proteins associated with DNA caused by free radicals and ions. Both the landraces are more sensitive to proton beam followed by X-rays, electron beam, whereas least sensitive for gamma rays because proton beam has relatively higher linear energy transfer (LET) capacity than other three mutagens. Therefore, proton beam showed high penetration capacity and lethal effects, as a result lower LD50 of proton beam has been observed in this study. While comparing the gamma rays with electron beam and X-rays, seeds were least sensitive to gamma rays followed by electron beam and X-rays (Fig. 3). Similar

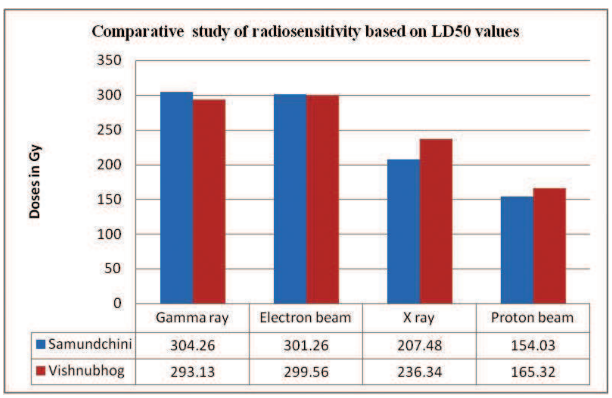


Fig. 3. Graph depicting the comparative study of radio-sensitivity of Samundchini and Vishnubhog for gamma rays, electron beam, X-ray and proton beam for LD50 estimated by Probit analysis

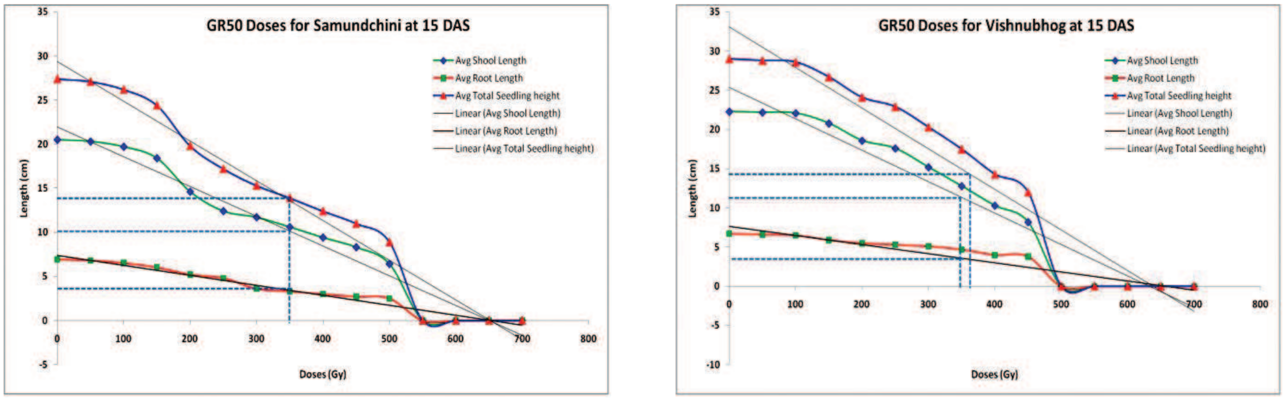
findings were also reported by (Tosri et al. 2019; Lalitha et al. 2019; Gowthami et al. 2017) while comparing radio-sensitivity of gamma rays and electron beam in rice.

Comparison of radio sensitivity of short grain aromatic rice landraces based on shoot length, root length and total seedling length

Seedlings are particularly sensitive to mutagens and provide an easy means of measuring doses effects and are widely used as an index in determining the biological effects of various physical mutagens in M_1. Seedling parameters based on dosage effect of radiations can be expressed in terms of Growth Reduction-50 (GR50), the dose of mutagen at which growth of seedlings is reduced by 50%. GR50 provides more reliable estimate of optimum mutagen dose for mutation breeding experiments (Harding et al. 2012).

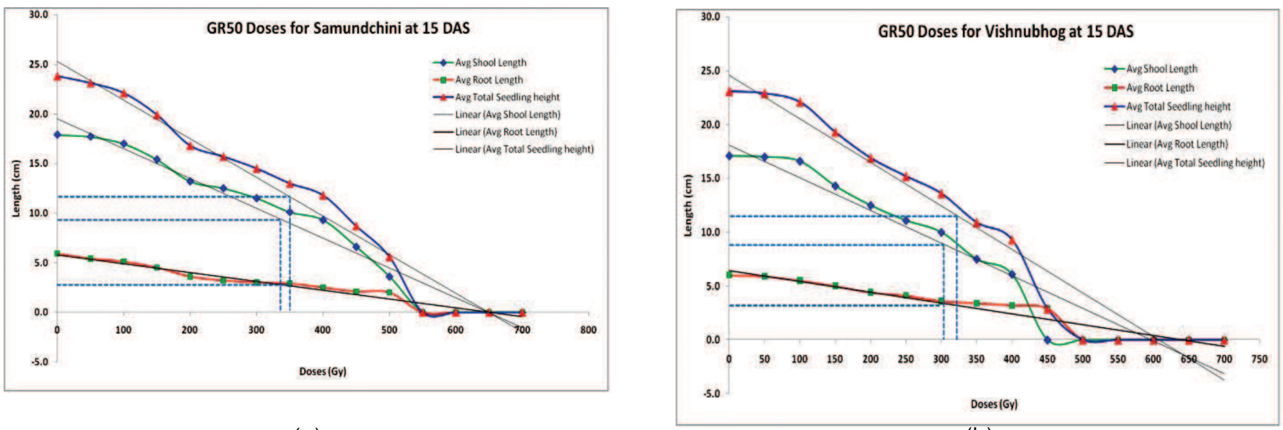
GR50 doses were calculated based on root length, shoot length and total seedling length for Samundchini and Vishnubhog. Estimation of GR50 based on shoot length is considered more reliable therefore; the further discussion is being made accordingly. GR50 doses of gamma rays based on shoot length at 15 days after sowing was 355Gy for Samundchini and 345Gy for Vishnubhog (Fig. 4). For electron beam, GR50 doses were recorded 335Gy and 320Gy for Samundchini and Vishnubhog respectively, based on shoot length (Fig. 5). In seeds treated with X-ray, GR50 value for Samundchini based on shoot length was 220Gy whereas in Vishnubhog, it was 250Gy (Fig. 6). Similarly, for proton beam irradiated seeds, GR50 values were 180Gy and 165Gy for Samundchini and Vishnubhog respectively (Fig. 7). The observations on seedling parameters of irradiated rice exhibited a linear decrease in dose responsive curve with increase in induced doses of all mutagens (Figs. 4-7). This dose dependent decrement of root length and shoot length could be due to the fact that damaging effects on genetic material are proportional to the absorbed doses of mutagens on biological tissues. Moreover, analysis of variance revealed significant differences in various doses of mutagens for root length, shoot length and total seedling length (Table 1) which indicates that doses equal to GR50 or slightly less than GR50 will be most favorable and useful for radiation induced mutation breeding in rice.

The comparative study for radio-sensitivity in both the rice genotypes for all the -physical mutagens, based on GR50 value is depicted in Fig. 8. On comparing the GR50 value in Samundchini and Vishnubhog, it is revealed that the rice genotypes are more sensitive to proton beam followed by X-rays, electron beam and least sensitive to gamma rays. This is due to high LET of proton beam due to its high energy particle nature compared to low LET nature of mutagen like gamma and X-rays. While comparing the electron beam, x-ray and gamma rays, seedlings were least sensitive to gamma rays. X-rays and electron irradiation in the current studies showed higher effectiveness due to very high dose rate and pulsed dose delivery compared to low dose rate and continuous dose delivery in case of gamma rays. Higher values of GR50 for gamma rays were also reported by (Lalitha et al. 2019: and Gowthami et al. 2017) while comparing the gamma rays and electron beam in rice which shows similarities with the results



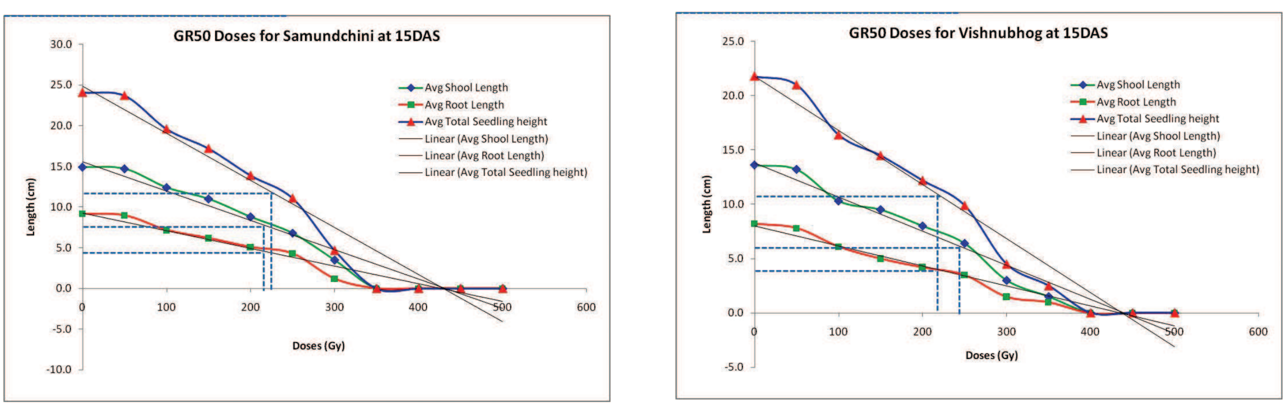
(a) (b)

Fig. 4. GR50 values of Samundchini and Vishnubhog based on shoot, root and total seedling length in gamma rays irradiated seeds



(a) (b)

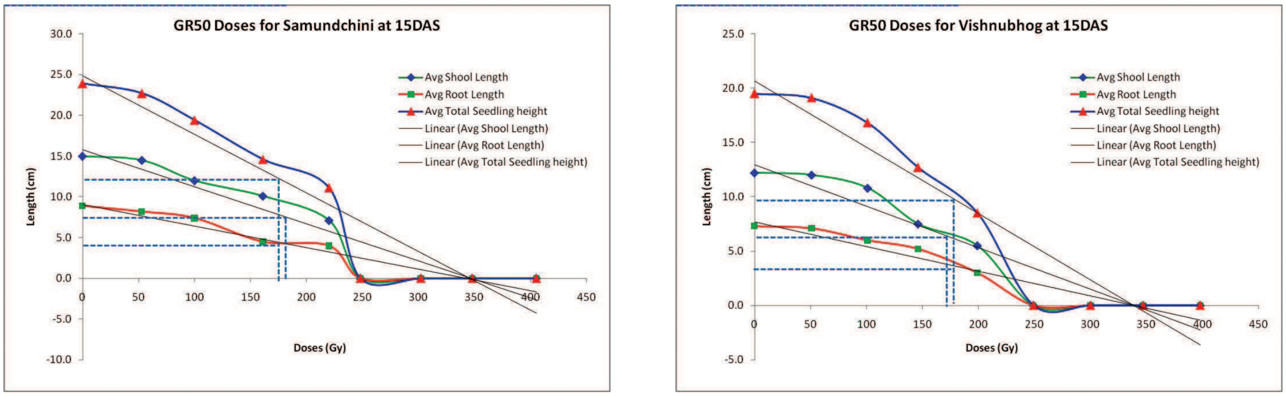
Fig. 5. GR50 values of Samundchini and Vishnubhog based on shoot, root and total seedling length in electron beam irradiated seeds



(a) (b)

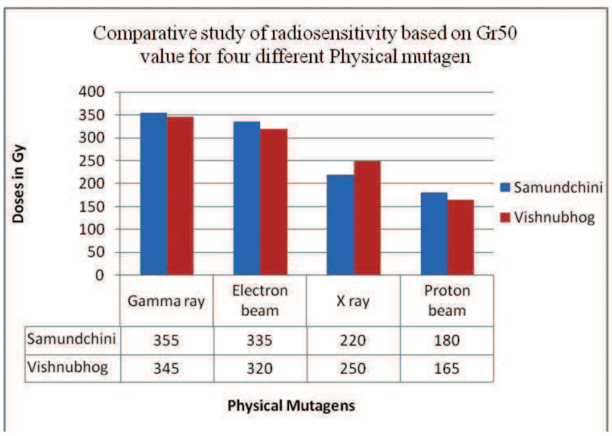
Fig. 6. GR50 values of Samundchini and Vishnubhog based on shoot, root and total seedling length in X-rays irradiated seeds

of present investigation. Based on the observations recorded for seedling parameters in Samundchini (SC) and Vishnubhog (VB), maximum physiological damages in plant tissues were recorded after 500Gy (SC) & 450Gy (VB) for Gamma rays; 350Gy (SC) & 350Gy (VB) for X-rays; 500Gy (SC) & 450Gy (VB) for electron beam and 220Gy (SC) & 200Gy (VB) for proton beam. Seedlings did not survive after these doses and hece, in results showed that Vishnubhog was more radio-sensitive than Samundchini because maximum



(a) (b)

Fig. 7. GR50 values of Samundchini and Vishnubhog based on shoot, root and total seedling length in proton beam irradiated seeds



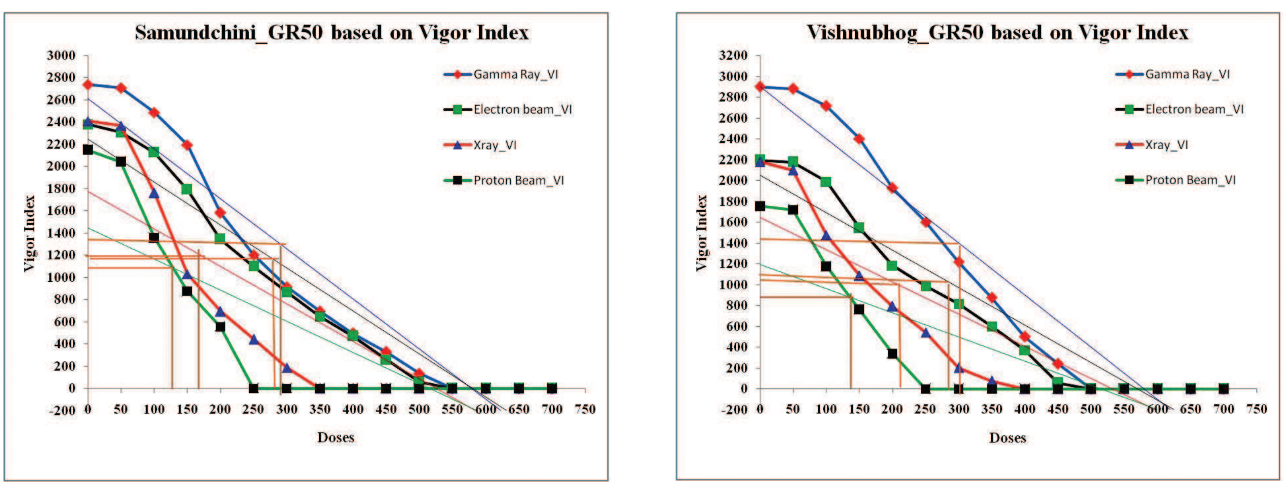
	Gamma ray	Electron beam	X ray	Proton beam
Samundchini	355	335	220	180
Vishnubhog	345	320	250	165

Fig. 8. Graph showing comparative study of radio-sensitivity of Samundchini and Vishnubhog for gamma rays, electron beam, x-ray and proton beam based on GR50 values for shoot length

physiological damages in plant tissues of Vishnubhog were recorded at slightly lower dose as compare to Samundchini.

Comparison of radio sensitivity of short grain aromatic rice landraces based on Vigor Index

GR50 doses based on vigor index were calculated for four physical mutagens in Samundchini and Vishnubhog landraces. Vigor Index is the measure of the extent of damage that accumulates in seedlings till the viability declines, and the damage accumulates in seeds until the seeds are unable to germinate and eventually die (Zhu and Hong 2008). Vigor index is considered to be more reliable parameter for estimating GR50 dose for physical mutagens in rice. Vigor index based GR50 value of gamma rays, electron beam, X-rays and proton beam for Samundchini were calculated as 300Gy, 280Gy, 220Gy and 140Gy respectively.



(a) (b)

Fig. 9. GR50 doses based on vigor index in Samundchini and Vishnubhog for gamma ray, electron beam, X-ray and proton beam

Similarly, GR50 value based on vigor index for gamma ray, electron beam, X-ray and proton beam for Vishnubhog were calculated as 290Gy, 280Gy, 175Gy and 125Gy respectively (Fig. 9). In the present investigation, the GR50 doses based on vigor index of all the mutagens for both landraces are very close or similar to their respective LD50 doses calculated based on probit values of germination percentage. These findings indicated their robustness obtained under this investigation. Chauhan et al. (2019) and Kumar et al. (2018) also reported the similar or very close GR50 dose based on vigor index and LD50 value based on probit value for proton beam irradiated rice which corroborates the present investigation.

Relative Biological Effectiveness of various mutagens in short grain aromatic rice landraces

Different types of radiations deposit energy in biological tissues in different ways, which affects the amount of cellular damage. The relative biological effectiveness (RBE) is defined as the ratio of the doses required by two radiations to cause the same level of effect on biological tissue by depositing per unit of energy (Jones, 2015). The RBE is an empirical value that varies depending on the type of radiation, the energies involved and the biological effects being considered. Predominately, gamma rays (^{60}Co) are taken as the reference radiation quality for estimating the RBE (IAEA/ICRU 2008). In a similar way, the RBE of gamma rays, X-rays, electron beam and proton beam were calculated for two landraces based on LD50 doses by considering the gamma rays as reference (Table 2). Higher RBE was recorded for proton beam for both the landraces *viz.,* Samundchini (1.97) and Vishnubhog (1.73), respectively) followed by X-rays (1.46 and 1.24) and electron beam (1.009 and 0.978). Rani et al. (2016) for X-rays in rice and Mondal et al. (2018) for electron beam in groundnut also reported more than one RBE which coincides with the findings of present study. RBE acts as a function of LET which is the energy transferred to the target material per unit length of the track. As the LET increases, RBE value also increases slowly, reaches the maximum and then falls down due to cell overkill (Willers et al. 2018). Therefore, this study reported higher RBE and LET of proton

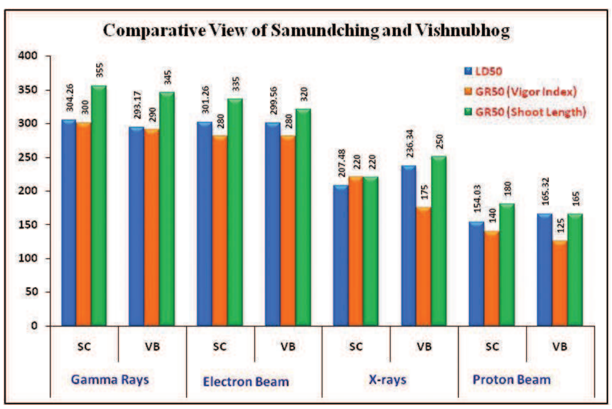


Fig. 10. Comparative view of radio-sensitivity of Samundchini and Vishnubhog for various physical mutagens

Table 2. Relative Biological Effectiveness of different physical mutagens for two rice landraces based on LD50 doses

Mutagens	Samundchini		Vishnubhog	
	LD50	RBE	LD50	RBE
Gamma ray	304.26	1	293.13	1
Electron beam	301.20	1.009	299.56	0.978
X-ray	207.48	1.46	236.34	1.240
Proton beam	154.03	1.975	165.32	1.773

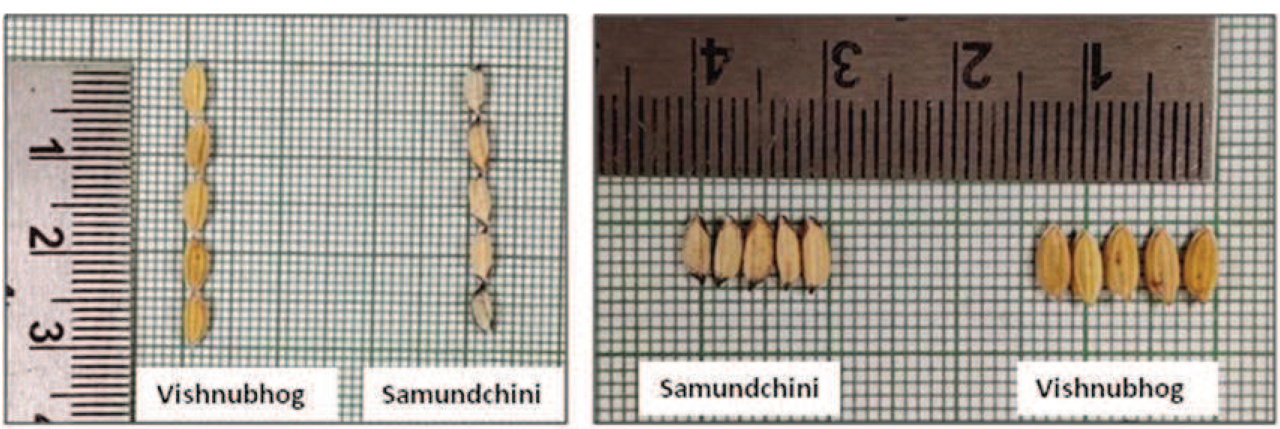


Fig. 11. Comparative view of paddy length and breadth of Samundchini and Vishnubhog

beam, indicating their deep penetration ability and lethal effects in biological tissues.

Comparison between the genotypes for radio sensitivity behaviour for various physical mutagens

On comparing the two rice genotypes *viz.,* Samundchini and Vishnubhog, the overall impact showed that the GR50 and LD50 values of all the mutagens for Samundchini were slightly higher than the Vishnubhog (Fig. 10). This indicates that Samundchini is slightly radio tolerant as compared to Vishnubhog. Variation in physical appearance of seeds, leaf characteristics and growth habits may play a vital role for creating differences in mutagen doses. The physical appearance of seeds shows Samundchini to be slightly finer (paddy width=1.7mm) and shorter (paddy length=5.70mm) than the Vishnubhog (paddy width=2.70mm & paddy length=6.40mm) which could be one of the possible reasons for its radio tolerance (Fig. 11). On observing the growth habit and leaf morphological characteristics of the genotypes, slightly narrower leaf and fast elongation growth of stem (plant height) is observed in Samundchini which may be the probable reason for its higher values of GR50 as compared to Vishnubhog. Genotype dependent variation for radio-sensitivity of mutagens was also reported by Shu et al. (1996). Zhu et al. (2008) reported that the mutagenic sensitivity of a biological material can be attributed to the level of differentiation and development of embryo at the time of treatment and also to the extent of damage to the growth processes like rate of cell division, cell elongation, various stages of hormone and biosynthetic pathways.

The optimum dose determination for potent physical mutagens would aid in utilization of these mutagens in improvement of rice through mutation breeding programs. LD50 and GR50 values obtained for two short grain aromatic rice landraces through present study will be very helpful in determining the range of optimum doses of mutagens. Based on this study we can state the optimum dose range for gamma ray is 280 to 350Gy, for electron beam is 290 to 330Gy, for X-ray is 200 to 250Gy and for proton beam is 150 to 200Gy. Doses between these ranges would be useful for irradiation of rice seeds to get the maximum number of useful mutants with minimum damage to plant survival. In summary, the present study revealed that the short aromatic rice genotypes show more radio-sensitivity for proton beam followed by X-ray, electron beam and gamma rays. Also of the two genotypes taken under study, Samundchini is slightly radio-tolerant as compared to Vishnubhog, owing to its genotypic attributes and genetic constituents. Comparison for radio-sensitivity behaviour for above mentioned four mutagens together for short slender non basmati aromatic rice makes it a unique study. In future, optimized doses for all the four types of physical mutagens in rice can be utilized for conducting mutation breeding program to compare the mutagen efficiency and mutagen effectiveness in obtaining the optimum number of mutants in field. In addition not only gamma rays, but proton beam and electron beam can also be used for creating genetic variability and novel mutants in rice.

Authors' contribution

Conceptualization of research (DS, BKD); Designing of the experiments (BKD, PKS, DS, GV); Contribution of experimental materials (DS, JPN, VCP, BKD); Execution of field/lab experiments and data collection (RS, PKS); Analysis of data and interpretation (RS, PKS, GV); Preparation of manuscript (RS, PKS).

Declaration

The authors declare no conflict of interest.

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