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IMMEDIATE RADIATION EFFECTS AND DETERMINATION OF OPTIMAL DOSE OF GAMMA RAYS ON NON-BASMATI AROMATIC RICE (*Oryza Sativa* L.) OF EASTERN INDIA

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ABSTRACT

Non-basmati aromatic rice is very popular for its excellent grain quality with an inbuilt aroma. But these cultivars have been ignored in the mainstream industrial agriculture due to low yielder. It is a serious matter that most of these cultivars are fast disappearing from cultivation in the locality. Therefore, there is an urgent need to improve these cultivars. Mutation breeding is one of the options to improve/rectify of specific traits of these cultivars within a very short period without altering the in-built quality traits viz. aroma. The prime objectives of the experiment were to determine radiation effects and to estimate the optimal dose which was necessary for any mutagenesis-based breeding programme. Popular cultivars namely Badshahbhog, Bahadurbhog and Blackjoha were taken for gamma irradiation ranging from 200Gy to 400Gy with an interval of 50Gy and unexposed treatment used as control. The experiment was designed at *in vitro* and *in vivo* condition in a randomized block design with three replications. Data were analyzed through Duncan's test and regression analysis. Most of the traits of each cultivar were exhibited a drastic reduction with increasing doses of gamma rays. The optimal dose of gamma rays based on the weighted mean of LD₅₀ and GR₃₀/GR₅₀ with 40% and 10% weighted, respectively under *in vitro* and *in vivo* conditions were estimated at 358.37Gy & 346.10Gy in Badshahbhog; 331.19Gy & 319.17Gy in Bahadurbhog; 314.55Gy & 314.05Gy in Blackjoha. This optimum dose of gamma rays can be used for obtaining desirable mutants of these cultivars with minimal damages. Blackjoha had the highest radio-sensitivity while Badshahbhog showed relatively tolerant.

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1 Introduction

Aromatic rice is a small and special group of rice which is known for its fragrance and best in quality. It is more popular or demandable in Middle-East, European countries, and the United States. The dominant aromatic rice in world trade is 'Basmati' which grown in north-western India e.g. Punjab, Haryana, Uttarakhand, Himachal Pradesh, Delhi, Western U.P., and Pakistan (Hameed et al., 2020). Basmati rice production is not satisfactory in eastern India like West Bengal, eastern U.P., Jharkhand and Odisha due to non- retention of its original aroma, difficulty in milling because of very poor head rice recovery and also high susceptibility to insects-pests (Chakraborty & Kole, 2014). However, some aromatic landraces such as Badshabhog, Gobindabhog, Blackjoha, Bahadurbhog, Radhunipagol, Seetabhog, Tulaipanja etc., are very popular in eastern India due to its excellent grain quality, aroma and specific cooking quality features but they are not recognized as 'Basmati' rice due to their small to medium grain size (Chakraborty & Kole, 2014). These cultivars have some undesirable traits such as lodging susceptibility due to higher plant height (156-174 cm.) along with the less sturdy stem, high maturity duration (150-178 days) and blast susceptibility in the fluctuating environment caused yield reduction (Chakravarti et al., 2012). Therefore, there is an urgent need to improve the yield potential of such types of non-basmati aromatic rice without disrupting its original grain quality and cooking quality features. Genetic improvement in specific traits like the height of the plant, duration of maturity, yield and its component characters through hybridization and recombination often becomes difficult due to break-down of aroma and cooking quality characters. Mutation breeding is one of the options to improve/rectify specific traits of these cultivars within a very short period without break-down of in-built quality traits.

Mutation induction offers for generating genetic variability, which may allow to identification of new desired attributes that either cannot be found in nature or have been lost during evolution (Oladosu et al., 2016). The most important aspects of mutation breeding have been the induction of polygenic mutation which can increase yield as well as other important agronomic characters (Abdelnour-Esquivel et al., 2020; Mamun et al., 2020). Globally, the 3,329 crop mutant cultivars including 513 mutant rice varieties obtaining by gamma irradiation were officially registered in FAO/IAEA mutant variety database (MVD, 2020). Since the 1960s, gamma rays have been most frequently used by the breeders in plant mutation breeding programme. Because gamma rays are considered as the most penetrating electromagnetic radiation and its operation is simpler from the other physical mutagens (Kovacs & Keresztes, 2002; Li et al., 2019).

Generally, Radio-sensitivity of the crops is varied and depending on the species, the varieties, plant part of the mutation and the

water content of the material (Spencer-Lopes et al., 2018). Free radicals, can modify or damage important components of plant cells, are produced on the interaction with molecules in the plant cells, particularly water (Gowthami et al., 2017). One group of researchers agrees that the maximum probability to generate effective mutations founds at a dose where 50% of the irradiated individuals die (Ángeles-Espino et al., 2013). While other scientists have pointed out that another dose with the highest probability of producing beneficial mutations, abreast of LD₅₀, is where 30% or 50% of a growth reduction (GR₃₀/GR₅₀) finds (Khalil et al., 2014).

The optimal radiation dose for crop improvement programs is determined by using the LD₅₀ and the GR₃₀/GR₅₀ to induce effective mutations with minimal impact on the genome, while with high doses, the genome may bear multiple impacts that regularly produce aberrations or negative changes (Thole et al., 2012). Furthermore, low irradiation doses may induce cell division and enzymatic activity or increase the number of photosynthetic pigments (Aparna et al., 2013), whereas, high irradiation doses may hamper enzyme and hormonal activities that may cause physical damage to the organelles or chemical changes in the cells (Alvarez-Holguin et al., 2019). These types of response were not related to genetic modifications, they will not acquire. In this perception, low and high irradiation doses may cause plant phenotypic changes that may be misguided for genetic changes (Alvarez-Holguin et al., 2019). Therefore, the optimal radiation dose must be determined before starting a mutagenesis-based genetic improvement programme; this will increase the possibility of finding effective mutations that help to avoid excessive losses in terms of time, cost of manpower, field area and experimental materials. The present investigation was undertaken to conduct a gamma rays sensitivity test for biological and quantitative traits and determination of optimal radiation dose in non-basmati aromatic rice Badshabhog, Bahadurbhog, and Blackjoha.

2 Materials and Methods

2.1 Plant materials and mutagen treatment

Three non-basmati aromatic cultivars of rice namely Badshabhog (whitish creamy, short-medium cylinder seeds, maturity 150-160days), Bahadurbhog (brownish, short cylinder grain, maturity 155-165 days), Blackjoha (blackish, medium cylinder grain with an awn, maturity 140-150 days) were collected from Rice Research Station Chinsurah, Hooghly, West Bengal, India for radiation treatment. Genetically uniform, disease free, healthy seeds with 12 percent moisture of three cultivars each weighing 100g were taken in six packets for the experiment. Five such packets of each cultivar were used for gamma irradiations. The seeds were placed inside the gamma radiation chamber to get the seeds irradiated with five different doses of gamma rays viz. 200Gy, 250Gy,

300Gy, 350Gy, and 400Gy from the Cobalt 60 gamma rays source for the appropriate time for each dose based on the half-life of the source at BARC, Trombay, Mumbai in India. The dose rate was of the order of 17Gy/min in the radiation chamber. Sixth unexposed seed packet was used as control.

2.2 Experimental design, Growing plants, Data collection, and Statistical analysis

The experiments were carried out at the Department of Genetics and Plant Breeding (*in vitro* experiment) and Agriculture farm (*in vivo* experiment) of Palli Shikha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, West Bengal, India. Both experiments were organized in a completely randomized block design with three replications. Under *in vitro* condition, 50 irradiated seeds (Spencer-Lopes et al., 2018) of each dose of three cultivars along with their control were grown on vertically stand blotting papers.

Another experiment was carried out under *in vivo* conditions. The remaining seeds of each dose with their control were sown in raised nursery beds in the field at the same time. All necessary nursery practices were applied from time to time. Twenty-five days old seedlings of all three cultivars of each dose along with control were transplanted into the main field by adopting intra-row spacing of 15cm with a single seedling per hill and 20cm row to row spacing in completely randomized block design with three replications. The recommended dose of N P K fertilizer and standard cultural practices were carried out for better crop growth.

The number of germinated seeds when both plumule and radicle were fully emerged counted for a period at 7th DAS (days after sowing) under both *in vitro* and *in vivo* condition (Lalitha et al., 2019) and expressed in percentage. Shoot length and root length were measured at 14th DAS when the first true leaves in control seedlings of each cultivar have been stopped growing as well as the second leaf just to initiate. The measurement was taken on randomly selected seedling in centimeters from the root-shoot junction (under *in vitro*) or soil level (under *in vivo*) up to the tip of the first leaf for the shoot. Seedling height (cm) under *in vivo* was taken on randomly selected seedlings (completed three leaves stage) in centimeters at 25th DAS (just before transplanting into the main field). Seedling survival percent as counted at 14th and 25th DAS (just before transplanting into the main field) under both *in vitro* and *in vivo* condition respectively and expressed in percentage. Data on various quantitative traits were also recorded on ten randomly selected plants in the main field.

Data were subjected to the standard analysis both *in vitro* and *in vivo* conditions and determination of LD₅₀ as well as GR₃₀/GR₅₀ doses of gamma rays using Microsoft excel 2016. The comparison between treatment means was tested for biological and quantitative

traits with Duncan's test (Duncan, 1955) by using IBM SPSS Statistics 25.

Determination of the lethal dose (LD₅₀) of gamma rays for germination of seeds and seedling survival under *in vitro* as well as *in vivo* conditions was carried out through Probit analysis (Finney, 1971; Gowthami et al., 2017). Probit mortality values for each genotype were plotted on the y-axis in a graph against to the Log10 doses on the x-axis through linear regression line by fitting linear regression equation, $y = mx + c$ (where y is response variable, x is the irradiated Log dose) was used to estimate the Log10 dose associated with a probit of 5. Antilog of the Log10 value corresponding to the probit 5 was taken to find out the LD₅₀ value of gamma rays for the particular trait under study.

Percentage over control {(Mean of irradiated treatment/mean of control treatment) × 100} and percentage reduction (100-percentage over control)(Spencer-Lopes et al., 2018) were applied to determine the percent growth reduction (% GR) of the seedling growth parameters to gamma rays. Straight line equation $y = mx + c$ (where y is percentage reduction values, x is the irradiated dose) were used to estimate the GR₃₀ and GR₅₀ dose of gamma rays associated with 30% and 50% growth reduction, respectively in traits under study.

Under *in vitro* condition, a weighted mean for each cultivar was calculated based on the results of the LD₅₀ obtained for germination (%) and seedling survival (%); GR₃₀ and GR₅₀ were obtained from shoot length and root length, respectively. Whereas under *in vivo* condition, a weighted mean for each cultivar was estimated based on the results of LD₅₀ obtained for germination (%) and seedling survival (%); GR₃₀ obtained for shoot length and seedling height. The LD₅₀ was weighted with 40% for germination (%) and seedling survival (%), and the GR₃₀/GR₅₀, with 10% for remaining traits (shoot length, root length, and seedling height). The germination (%) and seedling survival (%) were the variables to which the highest weighting (40% for each) was assigned because the death of the individual indicates the highest damage that gamma radiation can produce. A single value under *in vitro* and *in vivo* condition was thus obtained, whereby the optimal radiation dose of gamma radiation for inducing mutagenesis in Badshabhog, Bahadurbhog, and Blackjoharice were established.

3 Results

3.1 Effects of gamma rays on various growth traits

Table 1 showed significant differences at the 0.01 level of probability due to gamma irradiation in all three cultivars on various growth traits. Under *in vitro* and *in vivo* conditions at the period of 7th DAS, germination percentage decreased drastically in all three cultivars with increase gamma doses. All treatments mean were

Table 1 Gamma rays sensitivity for various biological traits

Treatments	Germination (%)		Shoot length (cm.)		Root length (cm.)	Seedling survival (%)		Seedling height (cm.)
	<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>	(<i>In vitro</i>)	<i>In vitro</i>	<i>In vivo</i>	(<i>Invivo</i>)
Badshabhog								
Control	97a	93.14a	7.97a	13.4a	16.02a	95a	91.00a	21.01a
200Gy	91b	83.79b	6.93ab	11.8b	9.44b	85b	80.21b	19.36a
250Gy	83c	73.86c	6.29bc	9.61c	9.03b	78c	71.00c	18.73ab
300Gy	80c	68.50c	5.58bc	8.51cd	8.17b	69d	64.93d	16.52bc
350Gy	59d	48.36d	4.91cd	7.68d	5.38c	49e	44.79e	14.97c
400Gy	39e	36.00e	3.61d	4.54e	4.33c	37f	30.93f	10.79d
Mean	74.83	67.27	5.88	9.26	8.73	68.83	63.81	16.90
S.Em	1.83	1.81	0.46	0.38	0.79	1.33	1.84	0.75
CV	3.47	3.80	10.95	5.82	12.87	2.73	4.07	6.30
Bahadurbhog								
Control	97a	92.21a	8.32a	10.94a	15.07a	94a	90.43a	23.43a
200Gy	84b	80.21b	6.81b	9.94ab	11.66b	81b	77.64b	20.31b
250Gy	75c	64.36c	6.21bc	9.01b	8.68c	71c	61.00c	19.01bc
300Gy	59d	50.21d	5.69c	8.85b	8.02cd	53d	45.93d	18.66c
350Gy	47e	39.29e	5.43c	7.06c	5.80de	41e	34.29e	17.65c
400Gy	37f	33.93e	3.85d	4.96d	4.51e	35f	28.93f	10.87d
Mean	66.50	60.04	6.05	8.46	8.96	62.50	56.37	18.32
S.Em	1.76	1.73	0.25	0.38	0.70	1.33	1.68	0.42
CV	3.74	4.06	5.83	6.35	11.12	3.01	4.20	3.22
Blackjoha								
Control	93a	89.14a	9.09a	12.53a	19.24a	91a	85.93a	24.27a
200Gy	81b	73.57b	7.89ab	10.39b	9.82b	78b	70.71b	20.81b
250Gy	71c	60.79c	7.24bc	9.5b	8.85b	64c	56.50c	20.33b
300Gy	52d	49.29d	6.11cd	7.63c	8.40b	50d	46.79d	19.99b
350Gy	42e	34.43e	5.96cd	6.72c	6.33c	37e	29.79e	17.74c
400Gy	32f	32.07e	4.95d	4.94d	5.21c	32e	26.64e	15.32d
Mean	61.83	56.55	6.87	8.62	9.64	58.67	52.73	19.74
S.Em	2.14	1.50	0.41	0.52	0.54	1.86	1.46	0.57
CV	4.89	3.74	8.47	8.54	7.85	4.49	3.91	4.07

All biological traits under studied were exhibited significant differences (F- Test) at the 0.01 level of probability; S.Em: Standard error due to mean; CV: Coefficient of variation.

exhibited a linear trend as per Duncan's test, except irradiation at 250Gy & 300Gy under both conditions in Badshabhog @ 350Gy & 400Gy under *in vivo* condition in Bahadurbhog and Blackjoha where the response was statistically at par.

Shoot length under *in vitro* and *in vivo* condition and root length under *in vivo* condition of all three cultivars at 14th DAS were reduced significantly at a higher dose of gamma rays.

While under *in vivo* condition, the response of gamma irradiation for shoot length at 250Gy & 300Gy in Bahadurbhog and 200Gy & 250Gy and 300Gy & 350Gy in Blackjoha were exhibited statistically non-significant between each other (Figure 1). No significant differences could be found among 200Gy, 250Gy & 300Gy in Badshabhog as well as in Blackjoha and also 350Gy & 400Gy in all three germplasm for root length (Figure 1).

Figure 1 Gamma rays sensitivity on shoot and root length (cm.) at 14th DAS under *in vitro* condition of all three non-basmati aromatic cultivars

Figure 2a Gamma rays sensitivity on seedling survival at 14th DAS under *in vitro* condition of Badshahbhog

Figure 2b Gamma rays sensitivity on seedling survival at 14th DAS under *in vitro* condition of Bahadurbhog

Figure 2c Gamma rays sensitivity on seedling survival at 14th DAS under *in vitro* condition of Blackjoha

Figure 3 Gamma rays sensitivity on seedling survival at 25th DAS under in vivo condition of all three non-basmati aromatic cultivars

Drastically reduction in seedling survival (%) at 14th DAS under *in vitro* (Fig. 2a, b, c) and 25th DAS under *in vivo* condition (Figure 3) was observed in each germplasm at 400Gy, which was significantly differed from control, 200Gy, 250Gy, 300Gy & 350Gy except in Blackjoha where response at 350Gy & 400Gy was noticed statistically nonsignificance under both *in vitro* as well as *in vivo* condition.

Experimental results on the seedling height in all three cultivars at 25th DAS were significantly reduced under *in vivo* condition at 400Gy which was significantly differed from control and other doses. Non significant effect of irradiated treatments could be observed in Badshabhog at 0Gy (control) & 200Gy while in the case of Bahadurbhog and Blackjoha this was reported at 300Gy & 350Gy and 200Gy, 250Gy & 300Gy respectively (Figure 4a, b, c).

3.2 Determination of doses

Effective doses were determined by using percent germination, percent seedling survival, and seedling growth parameters which were considered as an indication for immediate mutagenic effects. Estimation of the optimum dose was done by determining weight mean based on LD₅₀ and GR₃₀/GR₅₀ doses of gamma rays (Table 2). The LD₅₀ values were estimated from germination percentage and seedling survival percentage both under *in vitro* and *in vivo* conditions. The GR₃₀/GR₅₀ values were determined from the seedling growth characters.

Probit analysis was done to determine the LD₅₀ value for gamma irradiation. Figure 5 showed that germination percentage under *in vitro* condition LD₅₀ has arrived at 389.60Gy, 346.78Gy & 332.02Gy for Badshabhog, Bahadurbhog, and Blackjoha,

respectively. Under *in vivo* condition, LD₅₀ was determined 363.95Gy, 327.04Gy & 319.35Gy in Badshabhog, Bahadurbhog and Blackjoha respectively (Figure 6). In case of seedling survival (%) under *in vitro* condition, LD₅₀ was estimated at 364.82Gy, 333.99Gy & 323.35Gy in Badshabhog, Bahadurbhog, and Blackjoha, respectively (Figure 7). Under *in vivo* condition maximum LD₅₀ dose of gamma rays has arrived at 350.36Gy in Badshabhog followed by 312.13Gy in Bahadurbhog and 307.18Gy in Blackjoha (Figure 8).

By using linear regression equation, GR₃₀ dose of gamma rays was determined and this was reported 292.76Gy & 271.11Gy for shoot length in Badshabhog; 283.09Gy & 312.90Gy in Bahadurbhog and 304.73Gy & 265.87Gy in Blackjoha under *in vitro* and *in vivo* conditions, respectively (Figure 9, 10). In the case of root length characteristics, a drastic reduction was reported among the various seedling parameters. GR₅₀ dose of gamma rays for root length was determined 273.30Gy, 305.77Gy & 219Gy in Badshabhog, Bahadurbhog and Blackjoha respectively (Figure 11). In the case of seedling height under *in vivo* condition at 25th DAS, GR₃₀ dose of gamma rays arrived at 332.64Gy, 322.16Gy & 368.24Gy in the case of Badshabhog, Bahadurbhog, and Blackjoha, respectively (Figure 12).

Table 2 represented the *in vitro* and *in vivo* optimal radiation dose for selected rice cultivars; this was based on the weighted mean of LD₅₀ and GR₃₀/GR₅₀ resulting from percent germination, percent seedling survival, and seedling growth parameters. The results on the optimal dose of gamma irradiation under *in vitro* and *in vivo* conditions were determined at 358.37 Gy & 346.10Gy for Badshabhog; 331.19Gy & 319.17Gy for Bahadurbhog and 314.55Gy & 314.05Gy for Blackjoha, respectively.

Badshabhog

Figure 4a Gamma rays sensitivity on seedling height (cm.) at 25th DAS under *in vivo* condition of Badshabhog

Bahadurbhog

Figure 4b Gamma rays sensitivity on seedling height (cm.) at 25th DAS under *in vivo* condition of Bahadurbhog

Blackjoha

Figure 4c Gamma rays sensitivity on seedling height (cm.) at 25th DAS under *in vivo* condition of Blackjoha

Table 2 Determination of optimal radiation dose

Experiments	Doses (Gy)	Parameters	Weighting factors (w)	Badshabhog		Bahadurbhog		Blackjoha	
				x	wx	x	wx	x	wx
<i>In vitro</i>	LD ₅₀	Germination %	40	389.6	15584	346.78	13871.2	332.02	13280.8
		Seedling survival %	40	364.82	14592.8	333.99	13359.6	323.35	12934
	GR ₅₀	Shoot length	10	292.76	2927.6	283.09	2830.9	304.73	3047.3
		Root length	10	273.3	2733	305.77	3057.7	219.24	2192.4
		SUM (Σ)	100		35837.4		33119.4		31454.5
	Optimal radiation	Weighed mean ($\Sigma wx / \Sigma w$)			358.374		331.194		314.545
<i>In vivo</i>	LD ₅₀	Germination %	40	363.95	14558	327.04	13081.6	319.42	12776.8
		Seedling survival %	40	350.36	14014.4	312.13	12485.2	307.18	12287.2
	GR ₅₀	Shoot length	10	271.11	2711.1	312.9	3129	265.87	2658.7
		Seedling height	10	332.64	3326.4	322.16	3221.6	368.24	3682.4
		SUM (Σ)	100		34609.9		31917.4		31405.1
	Optimal radiation	Weighed mean ($\Sigma wx / \Sigma w$)			346.099		319.174		314.051

Where, wx is the corresponding weighting factor; x is the LD₅₀ and GR₅₀/GR₅₀ values of respective parameters; wx is the multiplication of w and x .

Figure 5 Determination of LD₅₀ dose of gamma rays for seeds germination at 7th DAS under *in vitro* condition in all three non-basmati aromatic cultivars

Figure 6 Determination of LD₅₀ dose of gamma rays for seeds germination at 7th DAS under *in vivo* condition in all three non-basmati aromatic cultivars

Figure 7 Determination of LD₅₀ dose of gamma rays for seedlings survival at 14th DAS under *in vitro* condition in all three non-basmati aromatic cultivars Figure 8 Determination of LD₅₀ dose of gamma rays for seedlings survival at 25th DAS under *in vivo* condition in all three non-basmati aromatic cultivars Figure 9 Determination of GR₃₀ dose of gamma rays for shoot length at 14th DAS under *in vitro* condition in all three non-basmati aromatic cultivars Figure 10 Determination of GR₃₀ dose of gamma rays for shoot length at 14th DAS under *in vivo* condition in all three non-basmati aromatic cultivars

Figure 11 Determination of GR_{50} dose of gamma rays for root length at 14th DAS under *in vitro* condition in all three non-basmati aromatic cultivars

Figure 12 Determination of GR_{30} dose of gamma rays for seedling height at 25th DAS under *in vivo* condition in all three non-basmati aromatic cultivars

3.3 Effects of gamma rays on quantitative traits

The effects of gamma rays on plant height, number of effective tillers, panicle length, panicle exertion, number of filled grains, percent spikelet fertility, and grain yield were represented in Table 3.

3.3.1 Plant height

Short height plant was obtained at a higher dose in all three genotypes. Decreasing of plant height was not significant with increasing dose but a sharp reduction was observed in higher doses in all three genotypes. However, the plant height was not significantly reduced in all doses of gamma-ray received. Plant height of gamma-irradiated plants and control plants (un-irradiated) differed significantly in all three genotypes except between 250Gy & 350Gy in Badshabhog as well as Bahadurbhog; and 350Gy & 400Gy in Blackjoha.

3.3.2 Number of effective tillers

Effective tiller or the production of lateral branches is an important agronomic trait that regulates shoot framing and grain production in rice. Based on the findings, the number of effective tillers

produced per plant was significantly influenced by the dose applied. A significant increase in the number of effective tillers/hill was recorded at 250Gy & 350Gy dose in Bahadurbhog, while in the case of Badshabhoga significant reduction was observed at 300Gy, 350Gy & 400Gy doses. No significant effect of radiation treatment could be found in Blackjoha.

3.3.3 Panicle length

Panicle length is one aspect of panicle architecture and is usually measured as an important yield-related trait that strongly affects yield components such as grain number, grain density, etc. In Badshabhog, significant short panicle length was observed at a higher dose. No significant effect of radiation treatment could be observed in the rest two genotypes.

3.3.4 Panicle exertion

Panicle exertion seems to be related to many other traits in rice such as plant height, panicle length, and biomass production. Plant stature and panicle exertion are associated with each other. A significant reduction in panicle exertion was recorded at 200Gy dose onwards in Bahadurbhog and Blackjoha while in Badshabhog effects of all five doses were statistically at par with each other.

Table 3 Gamma rays sensitivity for various quantitative traits

Treatments	Plant height (cm)	Panicle length (cm)	Panicle exertion (cm)	Number of effective tillers/hill	Number of filled grains/panicle	Spikelet fertility %	Grain yield (g/plant)
Badshabhog							
Control	156.15a	27.56a	16.88a	11.24a	194.20a	74.16a	22.43a
200Gy	153.53ab	27.16a	15.36ab	11.22a	146.78b	61.53b	16.73b
250Gy	151.49b	26.51ab	13.27b	11.13a	127.36bc	49.69bc	14.99b
300Gy	150.17b	25.31bc	13.68b	9.73b	103.00c	41.44c	11.90c
350Gy	145.98c	25.78bc	13.95b	8.42c	100.73c	41.03c	9.14c
400Gy	141.61d	25.08c	13.95b	8.76bc	94.73c	37.33c	9.16c
Mean	149.82	26.23	14.52	10.09	127.80	50.86	14.06
S.Em	1.04	0.40	0.65	0.34	10.89	4.03	0.97
CV	1.20	2.62	7.77	5.82	14.75	13.71	11.95
F –Test	**	**	*	**	**	**	**
Bahadurbhog							
Control	162.70a	24.45	18.10a	10.67bc	137.58a	81.50a	14.22a
200Gy	158.33ab	23.91	15.64b	10.20c	124.33b	72.94b	9.78b
250Gy	153.70bc	24.58	15.25bc	11.13ab	109.71c	65.16c	8.81b
300Gy	153.93bc	25.54	14.93bc	10.80bc	97.42d	51.12d	7.41c
350Gy	149.19cd	24.82	14.39cd	11.53a	71.78e	41.11e	6.36cd
400Gy	144.81d	25.84	13.52d	10.53bc	63.51e	34.18f	5.56d
Mean	153.78	24.86	15.30	10.81	100.72	57.67	8.69
S.Em	1.66	0.70	0.27	0.20	3.30	0.57	0.43
CV	1.87	4.88	3.04	3.16	5.67	1.71	8.51
F –Test	**	NS	**	*	**	**	**
Blackjoha							
Control	162.14a	22.31	16.39a	11.13	90.02a	75.09a	12.43a
200Gy	157.87b	22.42	15.28b	11.53	79.60b	63.84b	9.94b
250Gy	154.58c	23.02	13.66c	10.20	77.02b	55.90c	8.40c
300Gy	148.43d	22.93	12.13d	10.53	67.91c	49.23d	6.09d
350Gy	144.73e	22.05	11.35e	10.33	46.96d	41.40e	4.22e
400Gy	142.77e	22.44	9.75f	10.40	49.07d	38.37f	3.38f
Mean	151.75	22.53	13.09	10.69	68.43	53.97	7.41
S.Em	0.95	0.40	0.23	0.43	2.76	0.84	0.26
CV	1.08	3.07	3.08	6.95	6.97	2.69	6.10
F –Test	**	NS	**	NS	**	**	**

*, ** and NS: significant at the 0.05, 0.01 level of probability and not significant, respectively; S.Em: standard error due to mean; CV: coefficient of variation; F –Test: significance test.

Table 4 Reduction percentage on various quantitative traits

Treatments	Plant height (cm)		Panicle length (cm)		Panicle exertion (cm)		Number of effective tillers/hill		Number of filled grains/panicle		Spikelet fertility %		Grain yield (g/plant)	
	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction	% over control	% reduction
Badshabhog														
Control	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
200Gy	98.32	1.68	98.54	1.46	90.97	9.03	99.80	0.20	75.58	24.42	82.96	17.04	74.58	25.42
250Gy	97.02	2.98	96.17	3.83	78.60	21.40	99.01	0.99	65.58	34.42	67.00	33.00	66.81	33.19
300Gy	96.17	3.83	91.82	8.18	81.05	18.95	86.56	13.44	53.04	46.96	55.87	44.13	53.07	46.93
350Gy	93.49	6.51	93.53	6.47	82.61	17.39	74.90	25.10	51.87	48.13	55.32	44.68	40.74	59.26
400Gy	90.69	9.31	90.99	9.01	82.64	17.36	77.87	22.13	48.78	51.22	50.33	49.67	40.84	59.16
Bahadurbhog														
Control	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
200Gy	97.31	2.69	97.82	2.18	86.41	13.59	95.63	4.37	90.37	9.63	89.50	10.50	68.76	31.24
250Gy	94.47	5.53	100.55	-	84.24	15.76	104.38	-	79.74	20.26	79.96	20.04	61.93	38.07
300Gy	94.61	5.39	104.49	-	82.50	17.50	101.25	-	70.81	29.19	62.73	37.27	52.11	47.89
350Gy	91.69	8.31	101.53	-	79.48	20.52	108.13	-	52.17	47.83	50.45	49.55	44.75	55.25
400Gy	89.01	10.99	105.70	-	74.70	25.30	98.75	1.25	46.16	53.84	41.94	58.06	39.10	60.90
Blackjoha														
Control	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
200Gy	97.37	2.63	100.48	-	93.25	6.75	103.59	-	88.42	11.58	85.03	14.97	79.98	20.02
250Gy	95.34	4.66	103.19	-	83.36	16.64	91.62	8.38	85.56	14.44	74.44	25.56	67.63	32.37
300Gy	91.54	8.46	102.79	-	74.00	26.00	94.61	5.39	75.44	24.56	65.56	34.44	49.00	51.00
350Gy	89.26	10.74	98.81	1.19	69.28	30.72	92.81	7.19	52.16	47.84	55.14	44.86	33.93	66.07
400Gy	88.05	11.95	100.57	-	59.48	40.52	93.41	6.59	54.51	45.49	51.10	48.90	27.21	72.79

3.3.5 Number of filled grains

Rice grain filling mainly depends upon standard agronomic practices. The tillering capacity is most important because late formed tillers fail to produce filled grains. From the study, the number of filled grain was reduced at increasing dose. In Badshabhog effect of 250Gy, 300Gy, 350Gy, and 400Gy; in Bahadurbhog 350Gy and 400Gy and Blackjoha effect of 200Gy & 250Gy and 350Gy & 400Gy were statistically not significant with each other.

3.3.6 Percent Spikelet fertility

Spikelet fertility (%) in rice depends on important physiological processes e.g. anther dehiscence, pollen germination, pollination, and fertilization. Spikelet fertility percent was significantly reduced at a higher dose of gamma irradiation in linear trends except for 300Gy, 350Gy & 400Gy in Badshabhog where the response was statistically at par.

3.3.7 Grain yield

Grain yield is one of the most important and complex traits in rice. Yield is determined by indirect traits like plant height, tillering ability, panicle length, grains/panicle, etc. A significant reduction in grain yield per plant from control was observed at all five doses in all three genotypes. The doses 200Gy & 250Gy and 300Gy, 350Gy & 400Gy in Badshabhog; 200Gy & 250Gy in Bahadurbhog were statistically no different.

3.3.8 Percentage Reduction

Table 4 displayed a reduction (%) of quantitative traits. Drastic reduction (%) was observed in the number of filed grain, spikelet fertility (%), and grain yield per plant. The highest reduction in the number of filled grains and spikelet fertility was observed at 53.84% and 58.06%, respectively at higher irradiation (400Gy) of gamma rays in Bahadurbhog followed by Badshbhag (51.22% and 49.67%) and Blackjoha (45.49% and 48.90%). Whereas

maximum reductions for grain yield (72.79%) were noticed at higher irradiation (400Gy) in the case of Blackjoha followed by Bahadurbhog (60.90%) and Badshabhog (59.16%).

4 Discussion

4.1 Effects of gamma rays on biological traits

Knowledge of immediate effects on radiation and fixation of optimal radiation dose is very important to execute genetic improvement programme on these non-basmati aromatic cultivars namely Badshabhog, Bahadurbhog, and Blackjoha through gamma rays induced mutations.

The present investigation revealed that the gradual inhibition in germination (%) was observed at a higher dose (400Gy) of gamma irradiation in Blackjoha followed by Bahadurbhog and Badshabhog. Such a dose-dependent decrease in germination percent was also reported by Vasline (2013) for gamma rays. Similar kinds of results are reports by Lalitha et al. (2019), Wijesena et al. (2019), and Yasmine et al. (2019). Reduced germination with high radiation might be due to higher physiological damage in seeds resulting from inhibition of auxin synthesis (Gordon, 1957), catalase, peroxidase, and cytochrome oxidase (Kleinhofs et al., 1974), cells death in the embryo part of seed (Olasupo et al., 2016).

The seedling survival percentage in all three cultivars was decreased progressively with the increasing dose of gamma irradiation due to inhibition of plant growth via alteration of physiological as well as morphological traits. The similar kind of findings was reported by Gowthami et al. (2017) and Lalitha et al. (2019).

Gaul (1977) reported that the damage to the biological material will be reflected in the germination and seedling survival. It may be considered as an indication for the mutagenic effects. The stimulating effect of radiation on germination may be inhibited to the activation of RNA or protein synthesis and it may be founded during the starting of germination (Chandrashekar, 2014).

Various seedlings parameters (shoot length, root length, and seedling height) through ionizing radiation offer a more valuable estimate for mutation breeding programme. Severe reduction in seedling parameters was caused by the highest dose (400Gy), while the highest expression for these characters was noticed through the lowest dose (200Gy). Similar results were reported by Gowthami et al. (2017). Different factors such as disturbances of balance between promoters, inhibitors of growth regulators, and changes in metabolic activity of cells have attributed to more sensitivity at higher doses of mutagens (Olasupo et al., 2016; Kumar & Swati, 2017). The sensitivity of root growth to mutagens may be due to the simultaneous effect of mitotic arrest and the preponderance of cells

in the G₂ phase in the meristems because such phase of the cell cycle is more sensitive as compared to the G₁ phase of the cell cycle to irradiation (Oney-Birol & Balkan, 2019). Various explanations have been offered for the reduction in seedling growth due to higher activity of the antioxidants (Jan et al., 2012), alteration in ascorbic acid content and biochemical and physiological interference (Ussuf & Nair, 1974), inhibit mitotic division in meristematic tissues (Khalil et al., 1986) and disturbs the hormone balance, synthesis of protein, water exchange, leaf gas-exchange and enzyme activity which may be the possible causes of adverse effects of gamma rays on plant height (Khah & Verma, 2015).

The optimal radiation dose is established through the lethal dose (LD₅₀) or growth reduction (GR₃₀/GR₅₀) (Khalil et al., 2014). The LD₅₀ was estimated from germination percent and seedling survival percent values. The growth reduction (GR) is one of the important factors to predict the radiosensitivity level in plants (Alvarez-Holguin et al., 2019; Kumar et al., 2018). It quantifies a dose where 50% of seeds can germinate, survive, and expose to their normal growth. In this study, in the case of shoot length (*in vitro* and *in vivo* condition) and seedling height (*in vivo* condition), the GR₃₀ value was calculated instead of GR₅₀. The reason behind that irradiation dose was more than 400Gy of gamma irradiation, were required to reduce 50% growth for all three cultivars, which crosses the limit of the irradiation dose. This indicates that irradiated doses in this study were safe for the parameters. However, the values of LD₅₀ and GR₃₀/GR₅₀ in all variables under *in vitro* and *in vivo* conditions were ranged from 273.3 to 389.6 Gy and 271.11 to 363.95Gy for Badshabhog; 283.09 to 346.78Gy and 312.13 to 327.04Gy for Bahadurbhog; 219.24 to 332.02Gy and 265.87 to 368.24Gy for Blackjoha, respectively. Considering the wide range, a weighted mean was appraised to establish the optimal radiation dose of the gamma radiation in the seeds of these three cultivars. The optimal radiation dose for Badshabhog, Bahadurbhog, and Blackjoha are found to be 358.37Gy & 346.09Gy, 331.19Gy & 319.17Gy and 314.54Gy & 314.05Gy under *in vitro* and *in vivo* conditions, respectively. Both values in each cultivar are very close to each other. This indicates gamma rays dose of 350-360Gy for Badshabhog, 320-330Gy for Bahadurbhog, and 315Gy for Blackjoha may be an optimum dose for irradiation. Whereas, Blackjoha was more sensitive compare with the other two cultivars used in this experiment. According to Rutger (1992), the effective dosage of gamma rays generally varied from 150 to 300Gy which permits 60 percent survival of seedling in rice. Many workers determined LD₅₀ values from their experiments. The LD₅₀ value of 238Gy, 232Gy & 223Gy for Basmati 37, Basmati Pak and Super Basmati, respectively by Cheema & Atta (2003); ranged between 345 to 423Gy for 13 rice varieties by Harding et al. (2012); 230Gy for "Taromahalli" landrace by Hallajian et al. (2014); 288 & 354Gy for two rice cultivars by Ramchander et al. (2015); 353 Gy for Binadhan-14 and 241 Gy for Ashfal by Rani et

al. (2016); 376.57Gy for Anna (R) 4 rice variety by Lalitha et al. (2019) were reported earlier. The GR₅₀ value ranged between 350 to 500Gy for seedling height by Spencer-Lopes et al. (2018); 403.26Gy for shoot length and 246.31Gy for root length by Lalitha et al. (2019) were recorded previously.

4.2 Effects on quantitative traits due to gamma rays

Most of all quantitative traits under study were exhibited significant variation due to gamma irradiation in all three non-basmati aromatic rice cultivars. A similar type of significant differences due to gamma irradiation for yield and yield associate traits was reported by Ramchander et al. (2015).

Chakraborty & Kole (2008) reported that plant height decreased with an increase in the dose of gamma radiation. The results are in agreement with Sasikala & Kalaiyarasi (2010). Various explanations have been offered for growth inhibition due to mutagenic treatments like auxin destruction (Joshi & Gour, 1974) or inhibition of auxin synthesis, disbalance in the maintenance of nutritional level, failure of assimilatory mechanisms, inhibition of mitosis, interruption in DNA synthesis, and chromosomal damage with associated physiological changes (Riley, 1953).

The number of effective tillers plays a vital role in determining grain yield (Oladosu et al., 2014). The number of effective tillers is influenced by the cultivar and radiation doses. Badshahbhog, Bahadurbhog, and Blackjoha variety possess 9 to 11 effective tillers per hill. However, a significant response in effective tillers/hill was recorded in Badshahbhog and Bahadurbhog to radiation doses and Blackjoha where the response was statistically at par. Similar result was reported by Haris et al. (2013), in which gamma-ray radiation affects significantly the number of first-generation productive tillers (M₁). Purwanto et al. (2019) also reported that gamma irradiation produced an insignificant difference in the character of the tiller number in M₁ generation. An increase in effective tillers/hill might have resulted from the stimulatory effect of mutagen.

Panicle length and panicle exertion were not affected much by radiation doses. Similarly, Sarawgi & Soni (1993) reported that panicle length was not affected much by mutagenic treatment in the M₁ generation. But the shift of mean value in some mutated progenies in the negative direction could be attributed to the deleterious effect of radiation.

Some stimulatory effects of gamma rays were observed on the number of filled grains/panicle. The higher reduction of spikelet fertility (%) may be due to higher pollen sterility. Akilan et al. (2019) reported a higher percentage of spikelet sterility in gamma-ray induced rice mutants. The results revealed that the grain yield of the M₁ plant was drastically decreased with an increasing dose

of gamma rays in Blackjoha followed by Bahadurbhog and Badshahbhog. These results are in agreement with the findings of Ramchander et al. (2015) and Lalitha et al., (2019). Reduction in grain yield per plant might be due to a large number of sterile spikelets and negative effects of other yield contributing traits.

Conclusions

The overall results indicated that, in general, all the biological traits and quantitative traits were decreased with an increase in the dose of gamma rays at a variable rate due to the negative effect of the mutation. However, Germination percent, Seedling survival, shoot length, root length, and seedling height parameters were affected by high doses of gamma radiations, which made it possible to determine the lethal dose (LD₅₀) and the growth reduction (GR₃₀/GR₅₀). To attain the genetic improvement of specific traits for Badshahbhog, Bahadurbhog, and Blackjoha rice cultivars, it recommends using the optimal radiation dose which causes a maximum of mutation with the minimum of damage to the plant, obtained through the weighting of both parameters (350-360Gy for Badshahbhog, 320-330Gy for Bahadurbhog and 315Gy for Blackjoha). According to the results, Blackjoha cultivar was highly sensitive to gamma rays followed by Bahadurbhog and Badshahbhog. Moreover, the number of effective tillers/hill was increased in some of the irradiated population over control, which might be due to the stimulatory effect of mutagen while panicle length remained almost unaffected. Gamma rays also offered polygenic mutation which can help to improve important agronomic characters. Knowledge of this experiment will contribute to the development of new genetic material that can be utilized for the commercial cultivation of popular non-basmati aromatic rice in eastern India.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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