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Determination of Median Lethal (LD50) and Growth Reduction (GR50) Dose of Gamma Irradiation for Induced Mutation in Wheat

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HIGHLIGHTS

- Two varieties of wheat were irradiated with six doses of γ radiation
- Seed germination, survivability and seedling length observed under laboratory conditions
- Plant height, panicle length, grains per panicle and 1000 seed weight was recorded for field studies
- LD50 for DBW 187 and K 1006 were found to be 272.71 and 278.61 respectively.
- GR50 values were 316.22 and 346.73 for DBW 187 and K 1006 respectively.

Abstract: The determination of the optimum dose of radiation through its impacts on the growth attributes of the crop is the prerequisite for successful induced mutation breeding. For evaluating the impact of different doses of gamma radiation on wheat (*Triticum aestivum*), two wheat varieties DBW 187 and K 1006 were irradiated at six different doses (200, 250, 300, 350, 400 and 450 Gy) using a Cobalt-60 source at Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India. Seed germination, survivability and seedling length of the irradiated seeds were measured at 7 days after sowing in laboratory experiments, while plant height, panicle length, grains per panicle and 1000 seed weight was recorded for field studies. It was observed that seed germination, survivability and seedling length declined with the increase in gamma radiation dose. The germination percentage showed significant differences among treatments (100 to 75%), while the survival percentage exhibited significant differences from 200 to 300 Gy in both the varieties. The LD50 for DBW 187 and K 1006 were found to be 272.71 and 278.61 respectively, while the GR50 values were 316.22 and 346.73 for DBW 187 and K 1006 respectively under laboratory conditions. The GR50 for field observations were 341.19 Gy and 339.70 Gy for DBW 187 and K 1006 respectively. Hence, the gamma radiation dose between 250 Gy and 300 Gy was found optimum to obtain desirable results. The obtained dose could be used to generate highest mutation mediated changes with least lethal effects in the subsequent generations of wheat.

Keywords: gamma radiation; *Triticum aestivum*; induced mutation; probit analysis

INTRODUCTION

Wheat (*Triticum aestivum*) is an important staple food worldwide with an annual production of 760.92 million tonnes 2020-2021 [1]. In India, wheat is cultivated over an area of 31.36 million hectares [1]. However, the annual production is severely compromised owing to an array of biotic (diseases, pests, nematodes) and abiotic (increasing temperature, drought) stresses [2]. Due to a continually growing population and rising gap between production and demand, a doubling of total wheat production is required by 2050 [3]. Hence, the development of new and robust strategies for boosting productivity by developing new varieties resistant to biotic and abiotic stresses, is the need of the hour.

However, the development of new varieties using conventional breeding methods is time-intensive as compared to other techniques (mutation breeding), due to longer crop cycles and reproductive barriers which limit the transfer of desirable traits. Mutation breeding has been a welcome step in that direction. The use of mutagens, viz. Ethyl methanesulphonate (EMS), gamma, X-rays has been widely used for increasing the genotypic and phenotypic diversity in several crops such as wheat [4], maize [5], lemongrass [6], rice [7], tomato [8], etc. The mutagens have been reported to induce alterations in the genes or chromosomal structures, leading to morphological, structural/functional changes in the irradiated plant [7]. The use of mutation breeding has been used for releasing over 3332 mutant varieties with superior and improved characters, such as tolerance to biotic and abiotic stresses, improved yield, and nutritional trait in over 240 plant species [9]. The use of gamma radiation in induced breeding has gained popularity in the recent years due to their high penetration ability into the target tissue with least damage, easy availability and lower cost as compared to other alternatives [10]. Around 264 varieties of wheat has been released worldwide using gamma irradiation, fast neutron and Ethyl methanesulphonate (EMS), of which two varieties have been released by India [11].

The impact of induced mutation has been studied under field as well as *in vitro* conditions for obtaining biotic and abiotic stress tolerant mutants [2,12,13]. *In vitro* studies have the advantage of obtaining numerous mutants with minimal resources in a smaller time frame. Beyaz [12] had developed an *in vitro* protocol for optimization and selection of mutants for abiotic stress tolerance. They cultured irradiated and unirradiated seeds in Murashige and Skoog (1962) medium supplemented with NaCl for 30 days *in vitro.* After germination, the plantlets were acclimatized by transferring to pots and growing in growth chamber for 30 days. The plantlets were irrigated with water containing 150 mM NaCl for assessing the salinity stress tolerance in plants. The surviving plants were transferred to soil and later to the field for further development. Leaf samples from 60-day-old seedlings of sainfoin were collected for a physiological analysis. This protocol has been used to assess the impact of gamma radiation on germination and seedling growth in Lathyrus [14] and Vetch [13].

The first step towards successful mutation induction is the optimization of the mutagen dose which would lead to least involuntary damage and with highest mutation frequency. For successful optimization, the parameters median lethal dose (LD50), i.e., the dose at which 50% of the population is killed, and mean growth reduction (GR50), i.e., the dose at which 50% growth reduction is observed [15]. Higher doses of irradiation of 30 to 35 krad have been previously reported to induce abnormal tillars and sterility in wheat [16]. An increase in yield attributes and plant vigour has been observed in wheat with a low dose of radiation of 0.03-0.07 kGy [4]. The present investigation was thus undertaken with an aim to quantify the effects of gamma radiation on seed germination and seedling vigour of wheat (Varieties DBW 187 and K 1006) through the identification of LD50 and GR50 under laboratory and field conditions.

MATERIAL AND METHODS

Induction of mutants

For the present study, mature, healthy and disease free seeds of two popular wheat varieties, DBW 187 and K 1006, were collected from IIWBR, Karnal. The varieties were irradiated with different doses of gamma radiation and its effects were studied. The moisture content of seeds was adjusted to 12% prior to irradiation. For gamma irradiation, the wheat seeds of both varieties were subjected to six different doses, *viz.*, 200, 250, 300, 350, 400 and 450 Gray (Gy) at the dose rate of 03.897 KGy/h emitted from ^{60}Cobalt gamma source, BRNS, Bidhan Chandra Krishi Viswavidyalaya, West Bengal. Irradiated seeds were then used for further studies.

Radio sensitivity test

Evaluation of seed germination

The radio sensitivity test was conducted at the laboratory of Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya. Standard germination test was conducted using petri plates with moistened 3 mm blotting paper. For the procedure, 20 irradiated seeds of each variety were placed on the petri plates and incubated at room temperature in the dark for seven days, with two replications (Figure 1). Seeds with >1mm coleoptiles were recorded as germinated at 7 days and the germination percentage was calculated by the formula: Germination%= (Number of seeds germinated/ Total number of seeds) x 100.

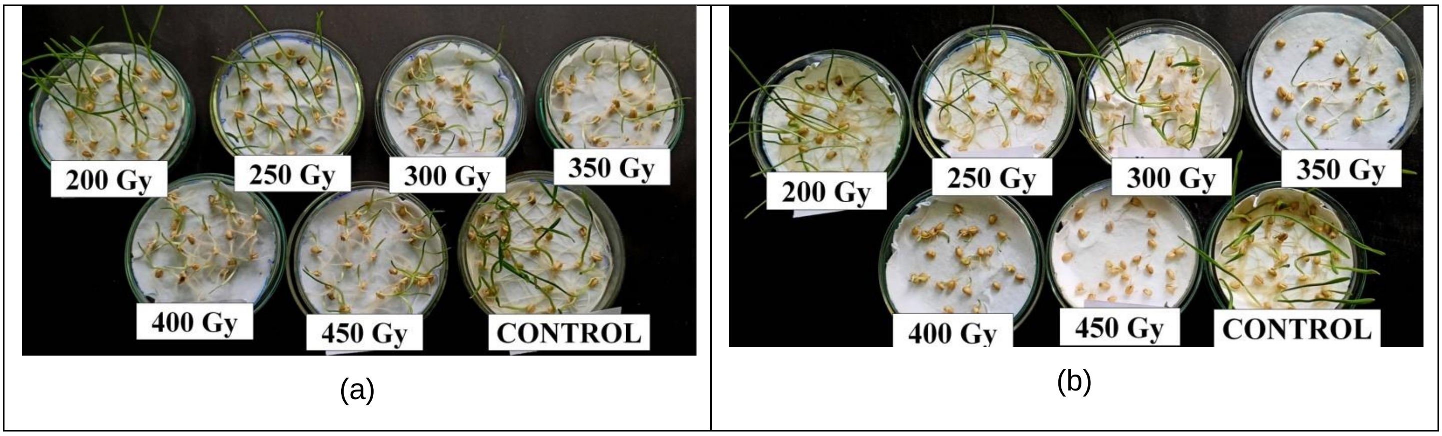

Figure 1. Germination percentage and survivability of γ-irradiated seeds at different doses in the varieties (a) DBW 187 and (b) K 1006 under controlled conditions

The survival percentage was calculated by counting viable seedlings 7 days after sowing according to the protocol given by Kumar and coauthors [17].

Growth parameters

For investigating the influence of gamma radiation on plant growth parameters, the irradiated seeds were sown in portrays and kept under natural conditions. For measuring the seedling length, the seedlings were separated carefully at the 14th day after sowing and the seedling length was recorded under *in vitro* condition (Figure 2).

Figure 2. Seedling length of γ-irradiated seeds at different doses in the varieties (a) DBW 187 and (b) K 1006 under controlled conditions.

For field studies, the irradiated wheat seeds of both the varieties were sown in experimental field, Jaguli, Mohanpur at 23.5 ºN and 89.0 ºE, at an elevation of 9.75 meter above sea level during rabi season of 2020-21 (Figure 3).

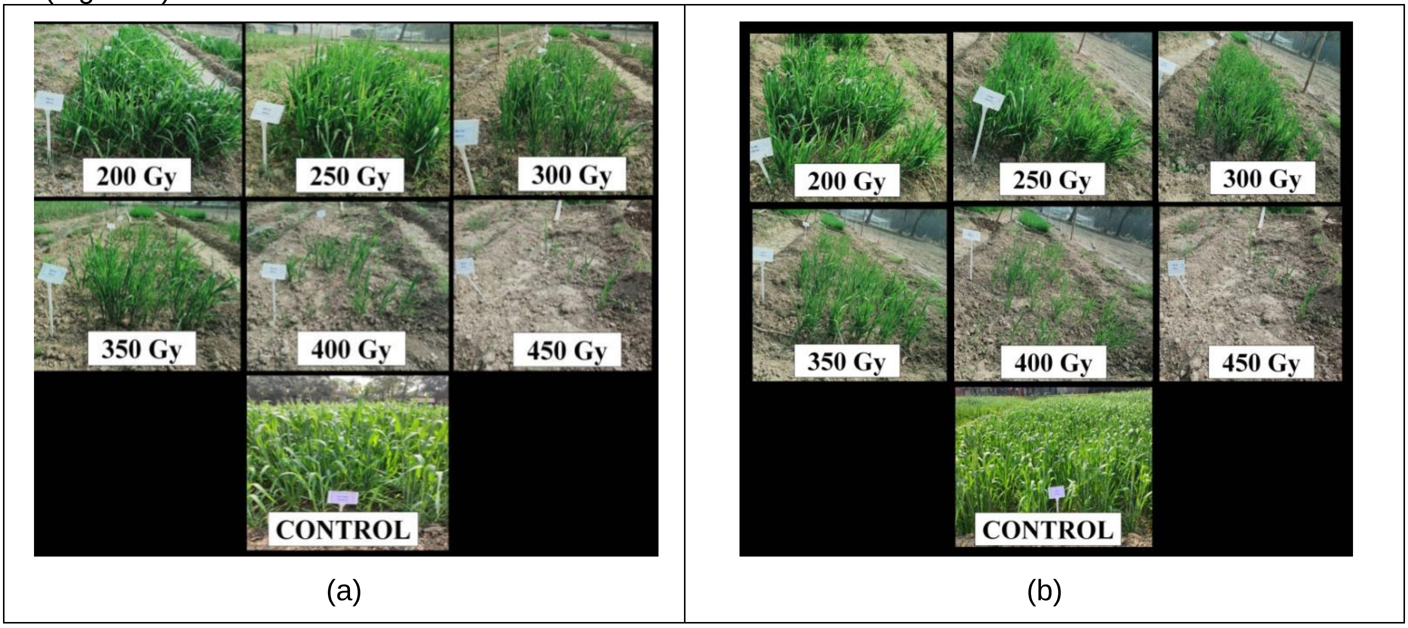

Figure 3. Field evaluation of γ-irradiated seeds at different doses in the varieties (a) DBW 187 and (b) K 1006 under field conditions

The plant height was measured from the base of the plant to the first leaf, following the protocol described by Borzouei and coauthors [18]. The panicle length, grains per panicle and 1000 seed weight were calculated after harvesting of the crop.

Statistical analysis

The observations recorded as germination percentage, survival percentage and total seedling length were used to determine the LD50 and GR50 values. For estimating the LD50 for each genotype, probit analysis was conducted on the basis of survival %, using OPSTAT software. Two way analysis of variance (ANOVA) based on germination, survival and seedling length was estimated using IBM SPSS statistics, version 26.The GR50 value for each genotype was calculated on the basis of growth parameters through linear regression analysis with the help of MS Excel, 2007. Further the treatment data was calculated and grouping of treatments was done on the basis of Tukey's Honest Significant Difference (HSD) test at P=0.05 for using IBM SPSS statistics, version 26.

RESULTS

The study was conducted to determine the influence of gamma irradiation on wheat genotypes based on germination percentage, survival percentage and growth attributes. There were significant differences in the germination, survival, and growth attributes due to the exposure to gamma radiation on the seeds under laboratory conditions and in plant height, panicle length, grains per panicle, and 1000 seed weight under field conditions (Table 1, 2).

Table 1. Analysis of variance of seed germination, survivability and seedling length due to gamma radiation under laboratory conditions

Source of variation	df	Mean sum of square		
		Germination (%)	Survivability (%)	Seedling length (cm)
Varieties	1	57.143	0.865	8.516
Treatment	6	962.434**	3501.703**	33.810**
Varieties*Treatment	6	31.217*	14.885	2.480*
Error	14	47.619	16.024	0.634

** significant at P ≤0.01, * significant at P ≤0.05

Table 2. Analysis of variance of plant height, Panicle length, grains per panicle and 1000 seed weight due to gamma radiation under field conditions

Source of variation	df	Mean sum of square			
		Seedling length (cm)	Panicle length	Grains per panicle	1000 seed weight
Varieties	1	26.514	2.332**	41.286*	119.729**
Treatment	6	189.913**	4.566**	63.988*	167.839**
Varieties*Treatment	6	2.287*	4.418*	63.869*	80.307*
Error	14	2.674	0.059	2.071	4.433

** significant at P ≤0.01, * significant at P ≤0.05

Further, a significant interaction between varieties and treatment was observed which indicated an influence of varieties on the effective doses of the treatment, which was also documented by Gowthami and coauthors [19] in rice.

Percentage of germination (%)

The germination percentage is a crucial factor for determining the viability of an irradiation study as it determines the extent of radiation mediated lethal effects. The results of gamma irradiation indicated a significant difference in different doses (treatments) among both the varieties (Figure 4). A significant decrease in germination % was observed with an increase in dose under all the treatments in both the varieties. In both DBW 187 and K 1006, the highest germination % was observed in plates treated with 200 Gy (100%), while least germination was recorded in the treatment dose of 450 Gy (60%). The decline in germination was significantly different in all the treatment doses under laboratory conditions.

Percentage of survival (%)

A decline in survival % was recorded in both the varieties with an increase in dosage of gamma radiation. Non significant differences were observed between the treatment with gamma radiation doses of 300, 350, 400 and 450 Gy in K 1006 (Figure 4).

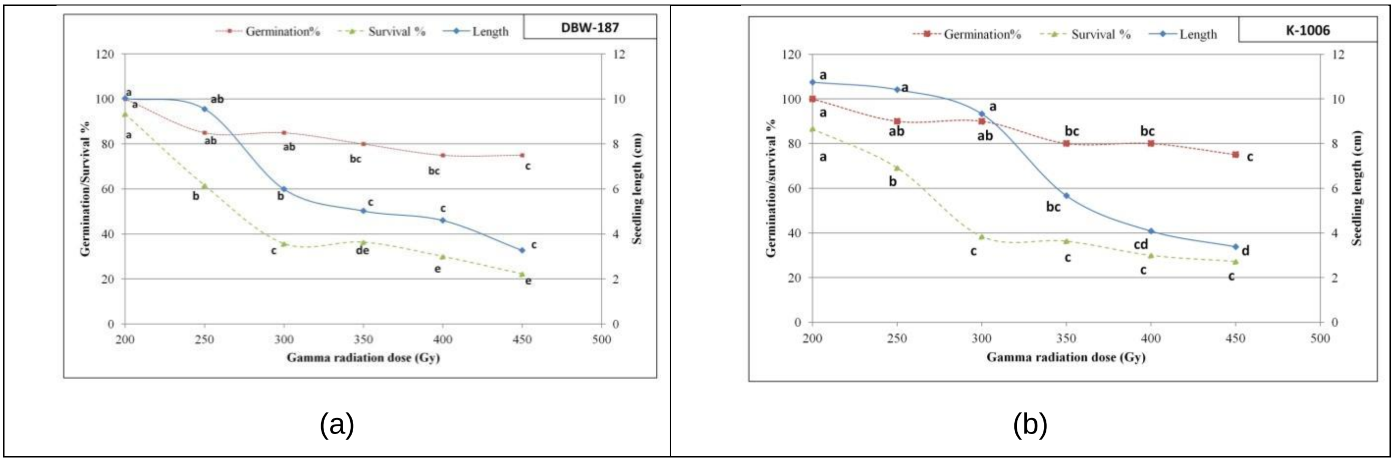

Figure 4. Germination percentage, survivability and Seedling length of γ-irradiated seeds at different doses in the variety a) DBW 187 and b) K-1006 under controlled conditions.

For K-1006, the lowest survival % was recorded at the dose of 450 Gy (27.27%) whereas the highest survival % was observed 200 Gy (86.67%). Non-significant differences were observed between the doses of 400 Gy and 450 Gy in DBW187. The lowest survival % was recorded at the dose of 450 Gy (22.22%) whereas the highest germination % was observed at 0 Gy (100%) and 200 Gy (93.33%).

Growth attributes

A pragmatic reduction in growth was observed in the treatments with an increase in dose of radiation, as compared to untreated control. Under laboratory conditions, the seedling length was significantly reduced as compared to the untreated control. The least seedling length were observed in 450 Gy, and increased gradually with a decrease in dose of radiation (Figure 4). Non-significant differences in seedling length were observed in the radiation doses from 300 to 450 Gy in K-1006, while the differences were non-significant between 400 and 450 Gy in DBW 187.

Under field conditions, non-significant differences in plant height were observed in gamma radiation doses from 300 to 400 Gy in K 1006 while significant differences were observed in DBW 187 under all doses of gamma radiation (Figure 5). The shortest plant height was recorded in K 1006 in the treatment dose of 450 Gy (15.6 cm), while the highest plant height was recorded in DBW 187 in the treatment dose of 200 Gy (44.83 cm). Among all the treatment studied, higher plant height was exhibited in DBW 187 as compared to K 1006. In general, the panicle length, grains per panicle and 1000 grain weight varied erratically with an increase in the dose of radiation in both varieties (Figure 5).

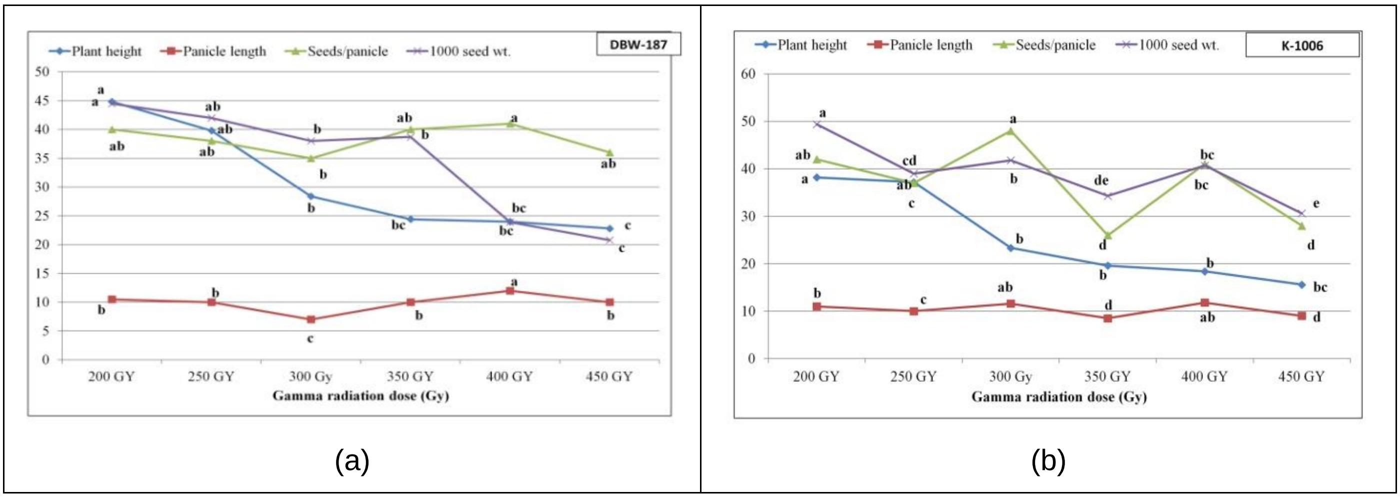

Figure 5. Plant height, panicle length, grains per panicle and 1000 seed weight of γ-irradiated seeds at different doses in the varieties a) DBW 187 b) K-1006 under field conditions

The highest panicle length was observed at the radiation dose of 400 Gy (12 cm and 11.8 cm in DBW 187 and K 1006 respectively), while the lowest panicle length was recorded in 300 Gy in DBW 187 (7 cm) and 350 Gy in K 1007 (8.5 cm). The highest seeds per panicle (41) were observed in 400 Gy in DBW 187 and K 1006, while the lowest was observed in 300 Gy in DBW 187 (35) and 350 Gy in K 1006 (26). The highest 1000 seed weight was recorded in 200 Gy in DBW 187 (44.5g) and K 1006 (49.4), while the lowest 1000 seed weight was recorded in 450 Gy in both DBW 187 (20.8 g) and K 1006 (30.6 g).

Radio sensitivity test

A linear decline was observed with the germination % and plant height upon probit analysis under laboratory conditions (Figure 6). The median lethal dose was recorded to be 272.71 Gy and 278.61 Gy in DBW 187 and K 1006 respectively. The median growth reduction was calculated to be 316.22 and 346.73 in DBW 187 and K 1006 respectively. The R^2 values for plant height and survival percentage for DBW 187 were 0.93 and 0.86, while those of K 1006 were 0.95 and 0.90 respectively.

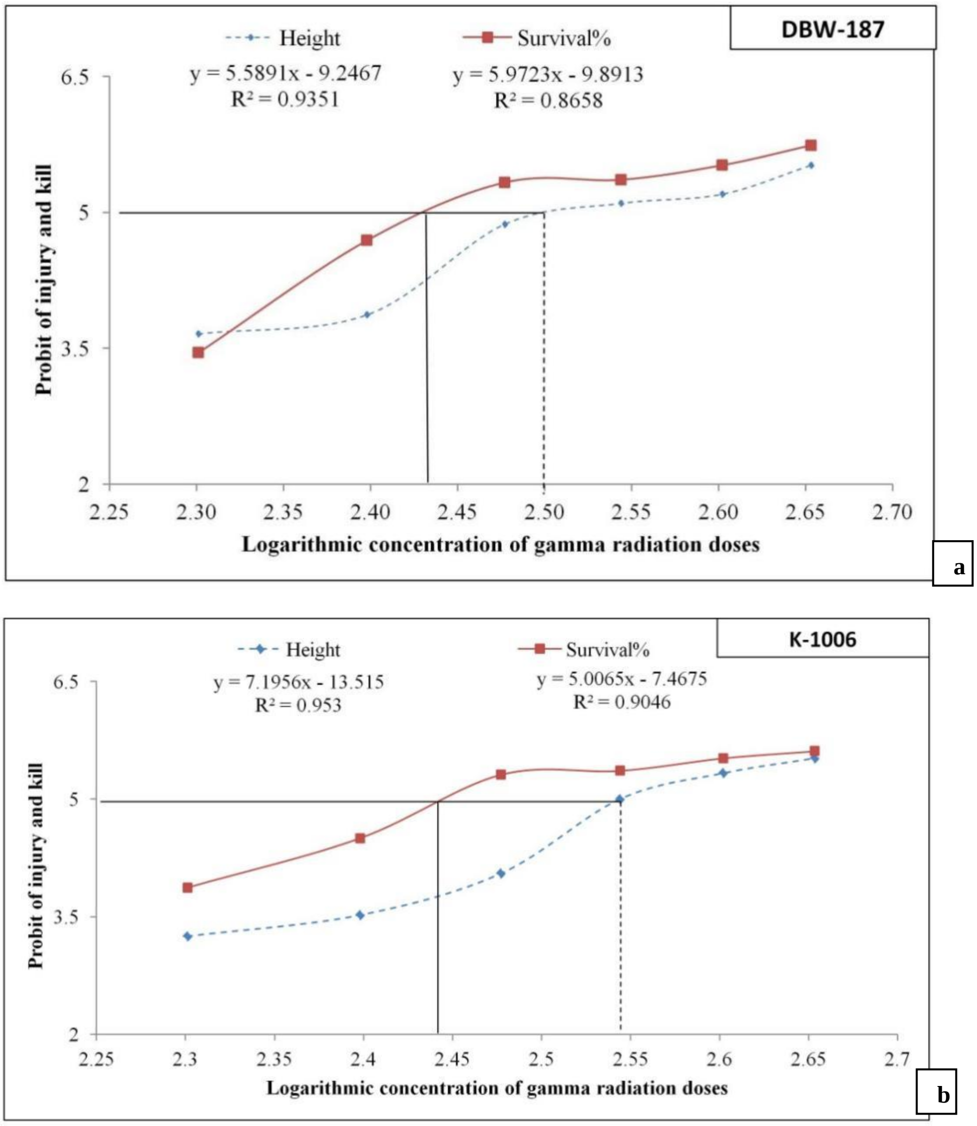

Figure 6. Probit analysis on the effects of gamma radiation on plant height and survivability in M_1 generation of the variety (a) DBW 187 and (b) K 1006 under laboratory conditions.

Under field conditions, the plant height declined linearly with an increase in dose of radiation, similar to laboratory experiment (Figure 7). The probit analysis revealed a median growth reduction dose of 341.19 Gy and 339.70 Gy for DBW 187 and K 1006 respectively. The R^2 values for plant height in DBW 187 and K 1006 were 0.92 and 0.91 respectively.

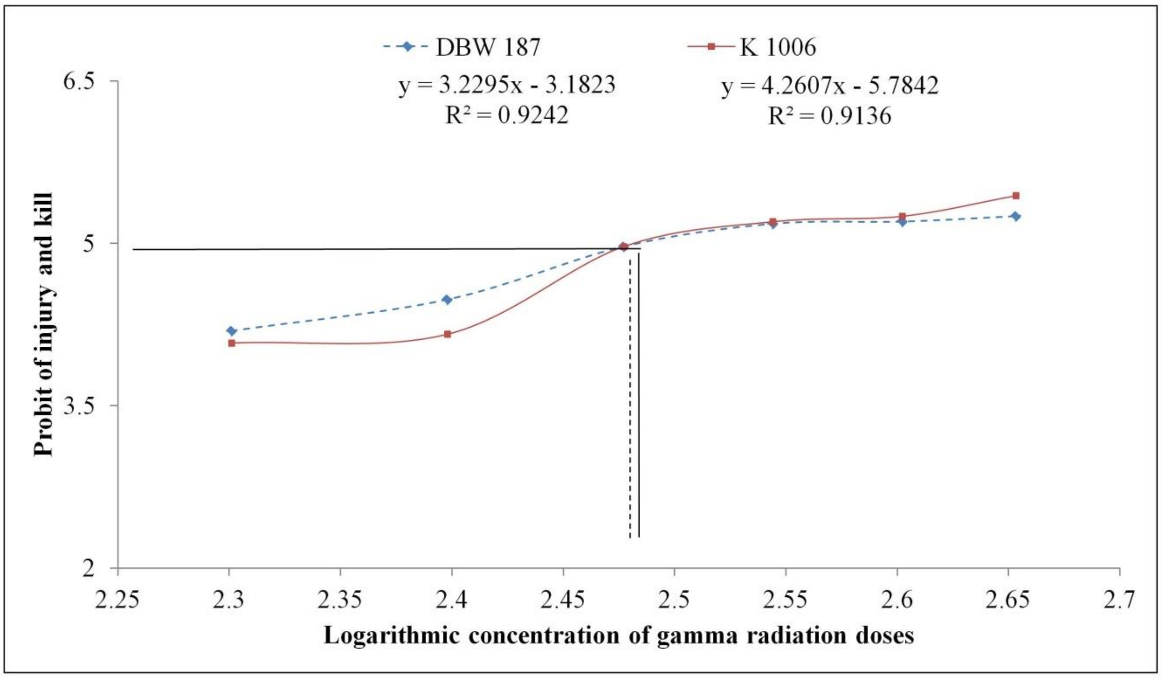

Figure 7. Probit analysis on the effects of gamma radiation on plant height in M_1 generation of the varieties DBW 187 and K 1006 under field conditions.

DISCUSSION

Induced mutation breeding is being used extensively for its high efficiency and accessibility. Previous studies have reported lethal effects of gamma radiation on seed germination and seed mortality as the dose of radiation increased [4,7,20]. The present study revealed a similar decline in seed germination, survival % and plant height in M_1 generation of DBW 187 and K 1006 with an increase in dose of gamma radiation. These findings are consistent with the results of [20] in groundnut, [21] in pigeonpea, [22] in lovegrass and [23] in linseed where increased dose of radiation resulted in a decrease in germination. The decreased germination may be attributed to an alteration in cytochrome oxidase content which might have reduced the respiration rate [24]. The increased mortality might be linked to tissue damage and/or alteration of vital cellular functions which inhibited the growth and lead to eventual death of the embryo. As observed in Cauliflower [25] and by Shimelis and coauthors [26] in wheat genotypes, the mutagen might have an inhibitory action on plumule and radical, which would have resulted in reduced seed germination as well.

The rates of survival declined significantly with an increase in the dose of radiation. Similar results were reported by Adebola and coauthors [27] in groundnut, by Ariraman and coauthors [21] in pigeon pea and by Ahumada-Flores and coauthors [11] in wheat. The decline in survival of the irradiated seeds might be linked to due to an alteration in the activities of meristematic tissues as reported by Din and coauthors [16]. As observed by Swaminathan and coauthors [24], the decline in plant survivability might also be resultant of chromosomal damage owing to increased doses of radiation.

The decline in plant height with an increase in gamma radiation dose was observed in both the varieties under both laboratory and field conditions. Similar decline was reported in Lathyrus, where a dose of above 150 Gy resulted in decline in seedling growth, seedling dry weight and chlorophyll content [14]. The decline in plant height might be due to chromosomal damage resulting in premature activation of protein synthesis as documented previously [28,29]. Previous researchers have reported the decrement in growth attributes owing to modifications in enzyme activity, enzyme degradation [30], damage to cellular constituents and/or cellular DNA [31,32].

A two way ANOVA revealed a significant interaction between the varieties and treatment, which would indicate a change in suitable gamma radiation dose with a change in variety of the crop. However, the change in germination %, survival % and plant height were found to be non-significant among varieties indicating similar morphological attributes between the varieties studied.

A major limitation of mutation is the reduction in fertility in the mutagenized plants [32]. In the present study, the yield attributes, viz., panicle length and grains per panicle exhibited erratic trend with an increase in dose. However, the 1000 seed weight was lowest in 450 Gy in both the treatments, which was in accordance with the study by Singh and Datta [4], who reported a reduction in 1000 seed weight and average grain weight about 100 Gy. They attributed the reduction in grain weight to increase in sink demand owing to an increase in tillars which rendered the photoassimilate pool insufficient.

The probit analysis revealed a linear decline in seed survivability and plant growth parameters with an increase in gamma irradiation dose under laboratory conditions. This was supported by a well-fitted R^2 coefficient, which established the reliability of the experiment. The median growth reduction was different for field conditions as compared to those observed under laboratory experiments. This might be attributed to a change in the edaphic and related factors which influenced the growth and development of the plant under field conditions.

CONCLUSION

The wheat varieties DBW 187 and K 1006 might be irradiated at gamma radiation doses between 250 Gy and 300 Gy to obtain desirable results. The same experiment can be repeated to study the impact of gamma irradiation on yield attributes and disease resistance in other popular locally cultivated varieties of wheat. The changes in M_2 generation, obtained from M_1 of the present study, with respect to growth attributes, chlorophyll content, yield attributes as well as disease resistance can be studied further to obtain biotic stress resistant superior mutants of wheat.

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Conflicts of Interest: The authors declare no conflict of interest

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