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Research Article

Study on the effect of γ-irradiation (Co-60) on seed germination and agronomic traits in tomato plants (*Lycopersicon esculentum* L.)

Pronabananda DAS[1*], Md. Monirul ISLAM[1], Md. Humayun KABIR[1], Md. Monirul ISLAM[1], S.A.M. Shariar ISLAM[1], Md. Rafiqul ISLAM[1], Mustari T. JAHAN[1], Prodip K. ROY[3], Rathindranath HALDER[4], Protul K. ROY[2], A.N.K. MAMUN[1], Md. L. HOSSAIN[5]

[1]*Plant Biotechnology and Genetic Engineering Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka, Bangladesh; daspronab12@gmail.com (corresponding author); monirul.rubd@gmail.com; mithu_my@yahoo.com; monirgebcu@gmail.com; shariar.du12@gmail.com; mrislam_72002@yahoo.com; mustari_baec@yahoo.com; ankmamun@yahoo.com*
[2]*Molecular Radiobiology and Biodosimetry Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka, Bangladesh; protulroy2006@yahoo.com*
[3]*University of Dhaka, Department of Microbiology, Dhaka-1000, Bangladesh; pkroyprodip@yahoo.com*
[4]*Ministry of Education, Govt. Republic of Bangladesh, Bangladesh; rathindra.du@gmail.com*
[5]*Institute of Tissue Banking and Biomaterial Research, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission Dhaka, Bangladesh; liakat88ju@gmail.com*

Abstract

Mutagenesis is an important technique for creating novel mutants having improved agronomic traits. In this study, LD_{50} was determined at 807 Gy depending on the germination percentage of tomato seeds irradiated with cobalt-60, using absorbed gray (Gy) dose of 50, 100, 150, 200, 250, 500, and 1000 Gy with control. GR_{30} and GR_{50} values were 214 and 502 Gy based on the regression formula on seedling length. Though LD_{50} is important, the dose range between 214(GR_{30}) and 502(GR_{50}) is more functional to get desirable mutation as the survival of the mutants is more important than germination. The variation was observed in all of the agronomical traits among the treatments. Most of the morphological traits were found better at 250 Gy in comparison with the control and the value decreased sharply at higher doses followed. The highest weight of single fruit was 145.33 g recorded at 250 Gy while the lowest was 70.67 g noted at 500 Gy. The highest fruit yield per plant was 1270 gm obtained at 250 Gy and the lowest was 800 gm found at 500 Gy. Shelf life was found better (34 days) at the 250 Gy dose level and the lowest was 6 days got in the control treatments.

Keywords: BARI tomato-8; GR_{50}; irradiation; LD_{50}; mutation breeding; shelf life

Abbreviations: M1: Mutant 1; GR_{30}: Growth Rate; LD_{50}: Lethal Dose; BARI: Bangladesh Agricultural Research Institute; CRBD: Completely Randomized Blocked Design; CV: Coefficient of Variance; ANOVA: Analysis of Variance; DMRT: Duncan's Multiple Range Test; DAP: Days after Planting; IFRB: Institute of Food and Radiation Biology

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Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the most important worldwide cultivated vegetable crops. It belongs to the Solanaceae family having a short life cycle (Dielen *et al.*, 2001) and a simple diploid genome ($2n=2x=24$)(Kulawiec *et al.*, 2003). It is also one of the most popular crops as it is used both as a fresh and processed product. Tomato originated from Tropical America but now it is cultivated extensively all over the world including Bangladesh as it is the richest source of nutrient dietary fibers, antioxidants, and beta-carotene (Hobson and Grierson, 1993; Beecher, 1998). According to FAOSTAT (2018), total tomato production in Bangladesh was about 385308.00 tonnes upon cultivation on 28130 ha land and the yield was 136878 hg/ha (FAOSTAT, 2018). But the yield of tomato productions hampered by various biotic and abiotic stresses originating from the rapidly changing adverse climatic condition along with narrow genetic diversity of the cultivated tomatoes. Besides, shelf life is an important factor in tomato after harvesting as tomato has a very short shelf life usually 2-3 weeks at ambient conditions (Ashenafi and Tura, 2018). To overcome the tailback, introgression of the wild genome may carry well but in the other case, it may rupture the genetic background of the cultivated tomato cultivars. There is another limitation to this problem is crossing incompatibility between the wild and cultivated species. Besides, various biotechnological attempts have been made by different scientists to overcome these constraints. Several attempts were made to improve tomato transformation and regeneration in Bangladesh (Sarker *et al.*, 2009; Das *et al.*, 2015). But the major limitations with the transformation method are dipping and infiltration which have been cited as inefficient in tomato plants (Chaudhary *et al.*, 2019). In this regard, induced mutagenesis is one of the promising techniques to broaden the genetic diversity in the existing tomato genome pool which will help in crop improvement. This technique has been used in a variety number of crop species including rice (Yao *et al.*, 2018), watermelon (Tian *et al.*, 2018), and banana (Tripathi *et al.*, 2019). Mutation breeding makes use of the possibility of altering genes by exposing seeds or other plant parts to chemical or physical mutagens (Broertjes, 1978). For the improvement of tomato, induced mutagenesis, as a breeding strategy, has been explored by different scientists (Gonzalez-Cepero, 2005; Masuda and Ojiewo, 2006; Tomlekova, 2010). Among the physical mutagens, gamma-ray and fast neutrons are frequently applied for mutagenesis in tomatoes (Menda *et al.*, 2004; Matsukura *et al.*, 2007). Studies have shown that ionizing radiation like gamma-ray has a significant effect in the improvement of various agricultural traits such as reduction of post-harvest loss through suppressing sprouting and contamination, eradication of insect pests, reduction of food-borne diseases, and extensions of shelf life (Andress *et al.*, 1994; Emovon, 1996).

The shelf life of tomatoes decreases when harvested in the latter stages but it showed better storability while harvested early. On the other hand, early harvest causes yield loss in weight (Subburamu *et al.*, 1990). As a consequence, it is of great importance to increase the production and to improve the keeping quality of this plant for meeting the demand of consumers. By applying appropriate doses on tomato seeds to gamma irradiation, a desirable mutant could be found which would have significant importance to mankind providing sustainable production. The first step in the mutation breeding experiment is to optimize the dose level to get variability with the useful mutation. The lethal dose 50 (LD_{50}) and growth reduction 50 (GR_{50}) are the two most important factors to determine the high probability of producing effective mutations (Songsri *et al.*, 2011). Based on these two parameters, an optimized dose range is calculated. M1 population is then cultivated and screened based on phenotypic changes to select a variation. This study was undertaken to optimize the irradiation dose level and to observe the effect of irradiation on morphological characteristics in tomato plants (M1).

Materials and Methods

Collection of seeds

This study was carried out at the Plant Biotechnology and Genetic Engineering Division to estimate the effect of irradiation on the improvement of yield and yield attributes. Seeds of tomato, 'BARI Tomato-8', were collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur.

Irradiation of seeds

These seeds were irradiated with different doses of gamma irradiation i.e. 50, 100, 150, 200, 250, 500, and 1000 Gy at Gamma Source Division under the Institute of Food and Radiation Biology which uses Cobalt 60 as a source of Gamma. 50 seeds were used for each treatment. These treated seeds were allowed to grow in both of laboratory and field conditions along with control to estimate the effect of irradiation on seed germination. This was done using the following formula:

$$\text{Germination percentage over control} = \frac{No\ of\ seeds\ germinated\ in\ a\ specific\ dose}{No\ of\ seeds\ germinated\ in\ control} \times 100$$

Sterilization of seeds

In the laboratory test, seeds were sterilized with 70% ethanol and then washed with distilled water 3-5 times. These seeds were then plated on Petri dishes containing wet blotting paper and kept in a growth cabinet with controlled temperature. In the field, 8 pots were well prepared suitable for seed germination. Water was applied when required to maintain the soil moisture. The planted seeds were checked every day starting from the first day of germination. Germination data were recorded after the 2nd week. El-Lakany and Sziklai (1970) were followed to determine the germination criteria that the radicle had been normal and exceeded the seed length. Seedlings' height was measured with the metered rule only after the 1st leaf had stopped growing.

Determination of LD_{50} & GR_{50}

The lethal dose (LD_{50}) was assayed after 30 days counting the germination percentage. Growth Reduction (GR_{50}) was also determined using the mean seedling height affected by the irradiation which is also a very important factor to evaluate the effect of radiation on the plants. The experiment was designed to 2×2 factorial design in Completely Randomized Blocked Design (CRBD).

Data collection of M1 plant

All the morphological data i. e. plant height, number of leaves, number of branches, Internode length, number of fruits per plant, single fruit fresh weight, dry weight, fruit yield per plant, and shelf life. The shelf life was determined by keeping the fruits at room temperature from the different treatments' doses.

Statistical analysis

The data were analyzed for the estimation of variance, linear correlation, coefficient of variance (CV) at 0.05% level by analysis of variance (ANOVA). The mean differences were adjusted with Duncan's Multiple Range Test (DMRT) using the statistical computer package program. LD_{50} and GR_{50} were determined with Curve Expert 1.4.

Results

Dose optimization

The effect of treating tomato seeds with 50, 100, 150, 200, 250, 500, and 1000 Gy gamma-ray with control on the germination percentage is shown in Table 1. Germination % over control was calculated after 7 days (7 DAP) and a general decreasing tendency (Figure 1) was observed in this case with the lowest effect

(100) found at control seeds and highest radio-sensitivity was found 43.34 at a higher dose level 1000 Gy (Table 1). This lowest germination percentage revealed the injury to the treated seeds which might have prevented them from germinating well. LD_{50} was determined at 807 Gy (Figure 2).

Table 1. Effect of different doses of gamma irradiation on seed germination and seedling length after 7 DAP of BARI Tomato-08

Observation number	Irradiation doses (Gy)	Germination % over control	Mean seedling length/dose (cm)	Reduction in seedling length (%)
01	0	**100a**	13.77a	-
02	50	91.74abc	**10.73a**	22.07
03	100	88.38bc	10.44a	24.18
04	150	85.48cd	09.70ab	29.56
05	200	76.34de	09.11ab	33.84
06	250	71.33ef	08.78ab	36.24
07	500	64.73f	06.97ab	49.38
08	1000	43.59g	02.50b	81.84
CV%		3.86	27.32	

In a column, the figures with a similar letter (s) do not differ significantly by DMRT (Duncan's multiple range test) at $p<0.05$; CV: Coefficient of variation; ***= significant

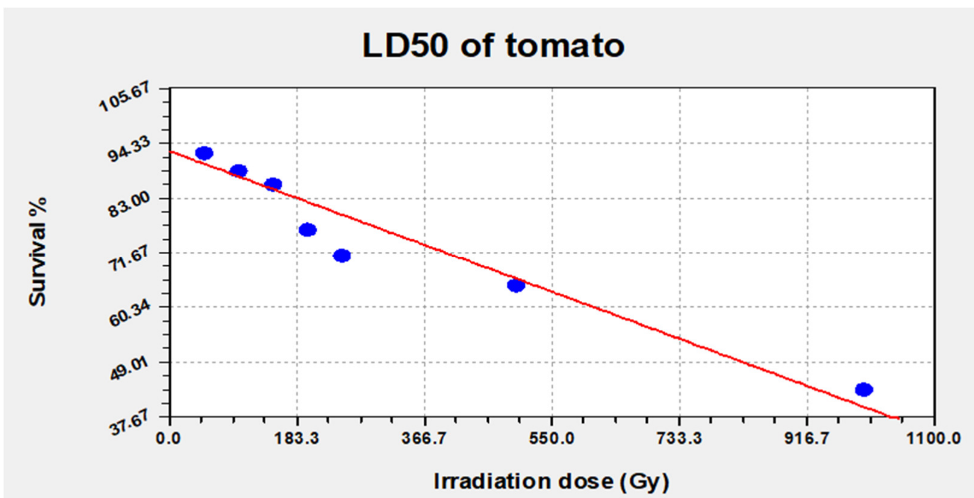

Figure 1. Determination of LD_{50} of BARI tomato-08 depending on the seedling's survival percentage on 7 DAP

At linear fit, y=a+bx; a=9.267 & b=-5.286; At 50 % survival, LD50= 807 Gy

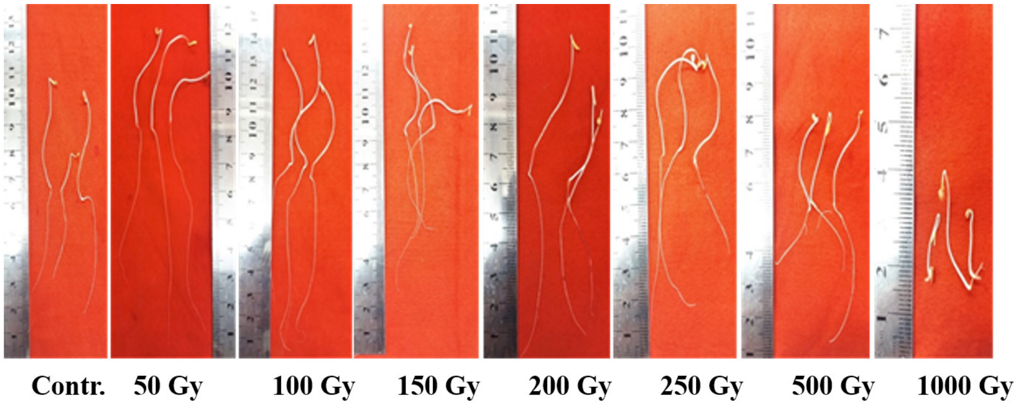

Figure 2. Effect of gamma irradiation on the seeds of BARI tomato-8 at the seedling stage

Another important criterion, growth reduction (GR_{50}) was also determined using the seedling height along with the dose of irradiation applied. GR_{50} was estimated at 509 Gy (Figure 3). Table 1 shows that there was a gradual decrease in length of seedlings as the dose level increased. But a sharp decrease was recorded 49.38% reduction at 500 Gy and 81.84% at 1000 Gy compared with the control.

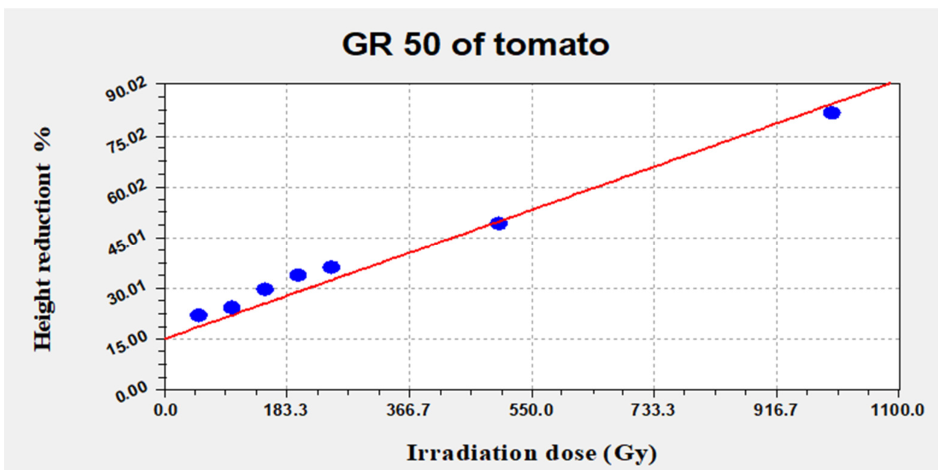

Figure 3. Estimation of seedling height reduction relating with the irradiation doses
At linear fit, y=a+bx; a=1.512 & b=6.940; At 50% Growth reduction, GR50= 502Gy; At 30 % growth reduction, GR30=214Gy

Effect of γ -Irradiation on the morphological characters of M1 plants

All treatments with gamma irradiation were found to be lethal with doses at 1000 Gy or higher. So, all remaining treatments along with control plants were transplanted in the field and assessed for the various morphological characteristics related to yield e.g., plant height, number of branches per plant, number of leaves, inter-nodal length, number of nodes per plant, number of fruits per plant, single fruit weight (dry weight and fresh weight) and shelf life.

In this experiment, through data analysis, gamma-ray applications showed to have higher variability in morphological characteristics of plants without any pattern (Table 2). Invariable changes were found with different doses of treatment. But it was found that at 250 Gy most of the traits were better compared with the control treatment and after that all the values decreased sharply.

An observation on the data revealed that the maximum plant height of 129 cm was recorded at 150 Gy and the minimum (83 cm) was in treatments irradiated with 500 Gy. The highest number of leaves (46) was found at 250 Gy comparing with other doses while the lowest was 30.67 at 500 Gy. The number of branches was 11 at the dose level of 200 & 250 Gy. Variability was also found in the intermodal length among the treatments but lower than the control plants (7.47 cm). However, all the treatments showed unique characters at the traits of fruit setting pattern. But in the case of the number of fruits per plant was variable among the different doses. Average fruit weights among the irradiated treatments were statistically higher than the control plants. The highest fruit weight (145.33 g) was recorded at the doses of 250 Gy while the lowest 70.67 g was at 500 Gy. Fruit yield was observed equal or better in the treated seeds than the control treatments except for the higher dose level of 500 Gy which had an inhibitory effect on almost all of the morphological traits.

Table 2. Effects of gamma irradiation on the morphological characteristics of M1 'BARI tomato-08' plants

Treatments	Plant height (cm)	No. of compound leaves plant^{-1}/ No of leaflet compound leaf^{-1}	No. of branches/ plant	No. of node/ plant	Internode length (cm)
0	111.00b	**50.00a/10.00**	7.00ab	18.00ab	7.47a
50 Gy	115.00b	46.00a/7.33	9.33ab	14.00bc	6.00bc
100 Gy	115.33b	42.33ab/9.67	7.000ab	18.00ab	5.13c
150 Gy	**129.00a**	32.33bc/14.33	6.33ab	21.00a	5.57bc
200 Gy	112.33b	34.67bc/13.00	11.00a	15.00bc	6.80ab
250 Gy	125.00a	**46.00a/10.33**	11.00a	16.00ab	6.67ab
500 Gy	83.00c	30.67c/7.00	6.00b	10.33c	5.20c
CV%	**2.47**	8.92/26.45	20.80	12.24	8.08

Treatments	1st fruit at internode no.	No. of fruit/ plant	Single fruit weight (gm)	% DW	Fruit yield/ plant (gm)	Shelf life (days)
0	1	12.00c	128.33ab	5.65bc	900bc	6d
50 Gy	1	17.00ab	120.67ab	5.52cd	900bc	7d
100 Gy	1	20.00a	138.33ab	5.08de	1000b	12c
150 Gy	1	11.00c	91.33ab	6.07b	856bc	12c
200 Gy	1	15.00bc	113.00ab	6.65a	1200a	28b
250 Gy	1	17.00ab	145.33a	5.20cde	1270a	34a
500 Gy	1	10.67c	70.67b	4.80e	800c	31ab
CV%		11.62	21.74	3.25	6.07	8.45

In a column, the figures with a similar letter (s) do not differ significantly by DMRT (Duncan's multiple range test) at $p<0.05$; CV: Coefficient of variation; ***= significant

The highest yield was recorded at about 1270 g per plant at dose 250 Gy followed by 200 Gy (1200 g/plant). In the case of higher doses 200, 250, and 500 Gy, the shelf life was found around 28 days or more compared with the control or lower doses (6-12 days) in M1tomato fruits (Figure 4). In the case of higher doses 200, 250, and 500 Gy, the shelf life was found around 28 days or more compared with the control or lower doses (6-12 days) in M1tomato fruits (Figure 4). It was an important finding related to the sustainable production of tomatoes.

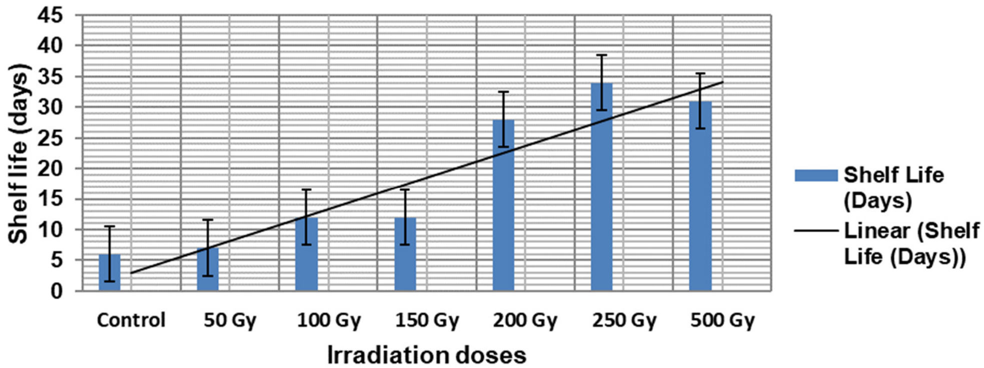

Figure 4. Effect of different doses of gamma Irradiation of BARI tomato-08 seeds on the shelf life of tomatoes

Discussion

Seeds are the most suitable material for irradiation in many mutations' induction experiments. The seed germination rate has a decreasing tendency with the increasing doses of irradiation. There are differences in mutagenic sensitivity among genotypes though this difference is much less among genotypes than species. Seeds required a higher radiation dose level than any other plant material to produce sufficient genetic mutations. The lethal dose was determined at 807 Gy. This result was not in accordance with Brunner (1995) who found the typical dose range 200-400 Gy (Brunner, 1995). It was expected that there would be differences in the germination along with the various irradiation treatments applied as many metabolic events also occur simultaneously during this process differ in their timing, both among the various organs of the particular seeds and among seeds of different species (Hegarty, 1978; Bewley and Black, 1982; Mayer and Poljakoff-Mayber, 1989). The lethal dose 50% value indicated that to get 50% germination, the seeds should be treated with 807 Gy. This data is similar to the result of Norfadzrin *et al.* (2007). GR_{50} was calculated at 509 Gy (Figure 3) which was comparable with the data of Brunner (450-600 Gy). A gradual reduction was found with the increase in dose level. Such reduction may be due to the adverse effect of irradiation in the physiological systems or growth hormone (Gunckel and Sparrow, 1961; Gaul, 1977). This significant reduction might have been caused by the plant's sensitivity to higher doses (Jamie, 2002). However, Jamie recommended that tomatoes would have an increased mutation by increasing the exposure time and the intensity of irradiation. The survived plantlets were transplanted into the experimental field to record the morphological data related to the agronomical properties, for example, height, branching, inter-nodal length (Behera *et al.*, 2012), fruit size, shape, number, etc. The maximum height (129 cm) was noted at 150 Gy and the lowest was 83 cm in 500 Gy. The overdominance type of gene action is the reason responsible for plant height (Uma and Sharma, 1997; Chodhry *et al.*, 2002). No other significant effect was found in the characteristics like number of branches/plants, number of leaves/plants, number of nodes, internodal length. Fruit weight was found maximum at 250 Gy and minimum at 500 Gy. This may be due to the genetic basis of fruit development which is controlled by many genetic loci, some with a large effect and others with a small effect as mentioned (Grandillo *et al.*, 1999; Doganlar *et al.*, 2002; Chaim *et al.*, 2006). Frary *et al.* (2000) stated that higher fruit weights might occur due to the effects of irradiation on the allele fw 2.2 (which influences fruit weight). The highest fruit yield 1270 gm/plant was recorded at 250 Gy followed by 1200 gm/plant at 200 Gy and the lowest 800 gm/plant was noticed at 500 Gy. Different biotechnological approaches have been applied that delay the ripening approaches of tomatoes by modifying metabolic pathways through genetic engineering (Smith *et al.*, 1988; Matas *et al.*, 2009; Meli *et al.*, 2010). However, there is a concern over the use of bioengineering of crops due to food and environmental safety-related questions (Boyazoglu, 2002; Qaim, 2009).

The shelf life of tomato, the most important factor, was found better from the treatments with the increase of irradiation doses applied on the seeds. At room temperature, maximum shelf life was got at 250 Gy with 34 days. The study showed that the shelf life of tomatoes ranged from 4.00-12.00 days at room temperatures collected in half ripen condition while it is 2.00-8.80 day when collected in full ripening stage (Akand *et al.*, 2015). It was an important finding related to the sustainable production of tomatoes.

Conclusions

In this study, the effective dose level for irradiation was estimated and the consequences of it were observed on the morphological characteristics of M1 plantlets. It was found that as the doses were increased, the germination percentage and seedling height decreased gradually. LD_{50} was determined at 807 Gy though it is recommended that the dose level should be in the range of 214 to 502 to get effective mutation which was determined by the estimation of GR_{30} and GR_{50}. From the investigation of the M1 population of tomatoes, 250 Gy showed the increased value in most of the traits while 500 Gy showed decreasing trends. Thus, this study it might be recommended to be useful for the future breeding program.

Authors' Contributions

Das P, carried out the study design, literature citing, creation of figures and tables, and manuscript writing. MH Kabir, MM Islam helped to conduct literature citing, table and figure formation, and manuscript writing. SAM.S Islam, MR Islam, MT Jahan contributed to manuscript reading and literature citing. RN Halder and PK Roy participated in helping edit, revise and approve the final manuscript. ANK Mamun supervised the writing of the manuscript and revised it critically for important intellectual content. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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