PLANT SCIENCE TODAY
Vol x(x): xx-xx
https://doi.org/10.14719/pst.9903

eISSN 2348-1900

RESEARCH ARTICLE

A case study on three cultivars and optimization of LD_{50} dose of gamma irradiation for inducing variability in pomegranate for horticultural traits and bacterial blight resistance

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Received: 08 June 2025; Accepted: 28 August 2025; Available online: Version 1.0: 24 September 2025

Cite this article: Munmun J, Rajesh D, Nisha S, Vishal SR, Yogesh KA, Bunty S, Meena K, Kiran B, Rajni V. A case study on three cultivars and optimization of LD_{50} dose of gamma irradiation for inducing variability in pomegranate for horticultural traits and bacterial blight resistance. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.9903

Abstract

Effective induced mutation breeding entails determining the ideal radiation dosage by examining how it influences the growth attributes of the crop. For evaluating the impact of different doses of gamma radiation on pomegranate (*Punica granatum*), three cultivars viz., Kandhari Kabuli, Bhagwa and Daru were treated with physical mutagen (gamma rays) at doses of 0, 6, 9, 12, 15, 18, 21, 24 kR to induce variability. The observations were recorded at different time intervals after sowing in the polyhouse and after transplanting in field conditions (20, 30 and 40 days after sowing and 70 days after transplanting, subsequently). The germination percentage, survival percentage and LD_{50} doses were calculated for all three cultivars. Mutant seedlings from different concentrations of gamma rays were evaluated for chlorophyll content of leaves and molecular characteristics. The LD_{50} doses for CVS. Kandhari Kabuli, Bhagwa and Daru were 15.26 kR, 15.08 kR and 13.74 kR, respectively. Statistical analysis revealed significant dose-dependent reductions in germination and survival rates ($p < 0.05$), with LD_{50} values determined to be 15.26 kR for Kandhari Kabuli, 15.08 kR for Bhagwa and 13.74 kR for Daru. These results demonstrate scientifically important variability among cultivars in their sensitivity to gamma radiation, providing critical reference points for optimizing mutagenic doses in pomegranate breeding programs.

Keywords: cultivar sensitivity; gamma radiation; LD50 dose; mutagenic variability; pomegranate; *punica granatum*

Introduction

Pomegranate is native to the region stretching from Iran to the Himalayas in northern India and it has been grown since ancient times across the Mediterranean region (1). The major pomegranate producing states in India are Maharashtra, Gujarat, Karnataka, Rajasthan and Andhra Pradesh (2). In Himachal Pradesh, the area under pomegranate cultivation is approximately 2.77 thousand ha with the production of 3.15 thousand metric tonnes. The nutritional and therapeutic benefits of the pomegranate, also known as the "fruit of paradise," are well documented (3). Each part of the pomegranate plant-including the fruit, bark, leaves and flowers-is a rich source of nutrients and diverse bioactive compounds. These components exhibit significant antibacterial activity and have demonstrated therapeutic potential in managing conditions such as hypertension, cancer and diabetes. Due to these health-promoting properties, pomegranate is often referred to as the "Elixir of Life". However, the annual production is severely compromised owing to an array of biotic and abiotic stresses. The genetic improvement of pomegranate by conventional breeding techniques has not shown much success because of a number of challenges, including a long juvenile period, polygenic characteristics and a complex genetic system (4, 5). Mutation breeding is a method used to create genetic variation and develop new, potentially beneficial plant traits. During the replication or distribution of genetic material, errors can occasionally occur, leading to sudden and heritable changes in a plant's genotype and phenotype. However, the frequency of spontaneous mutations is quite low. Therefore, attempts have been made to accelerate the rate artificially (6). Physical and chemical mutagens are both used in mutation breeding to create diversity in the genotypes that already exist. Induced mutation has emerged as an effective method in fruit breeding for improving cultivars and enhancing existing germplasm (7). The selection of mutagen depends on the type of material treated, type of mutation desired, availability of mutagen and

safety concerns. Gamma radiation treatment was used for the development of 64 % of the radiation-induced mutant varieties and radiation application has been used most frequently for mutation induction leading to direct development of mutant varieties (89 % of all mutant varieties) (8). Pomegranate production faces several challenges, including limited genetic variability, susceptibility to bacterial blight and inconsistent performance of existing cultivars under diverse agro-climatic conditions. Traditional breeding methods have had limited success in rapidly generating resistant and high-performing varieties due to the crop's long juvenile phase and narrow gene pool. To overcome these constraints, mutation breeding using physical mutagens such as gamma radiation presents a promising approach for creating novel variability in pomegranate, bacterial blight represents a major biotic stress affecting yield and fruit quality. Conventional breeding programs have identified cultivars such as 'Daru' and 'Nana' as important sources of resistance; however, their use is limited by less favourable horticultural traits. In contrast, commercial cultivars like 'Kandhari Kabuli' and 'Bhagwa' are widely cultivated due to their high fruit yield and larger fruit size but lack sufficient resistance to bacterial blight. There is an urgent need to integrate disease resistance into these commercially important genotypes to ensure sustainable production under both biotic and abiotic stress conditions. Mutation breeding using gamma irradiation provides a potential solution by creating novel genetic variability. A critical first step in this process is the determination of the median lethal dose (LD_{50}), which helps optimize mutagenic treatment by maximizing mutation frequency while minimizing population loss. The present study was designed as an initial phase in a radiation-induced mutation breeding program aimed at optimizing the LD_{50} of gamma rays for three cultivars, 'Kandhari Kabuli', 'Bhagwa' and 'Daru'. The ultimate goal is to develop improved mutant lines with enhanced horticultural performance and resistance to bacterial blight.

Materials and Methods

Collection of experimental materials

The experimental plant material comprising of 2 cultivated varieties (Kandhari Kabuli and Bhagwa i.e. F_2 selection from the cross of Ganesh x Gul-e-Shah Red) were procured from Regional Horticultural Research and Training Station, Bajaura, Kullu (HP) and a wild pomegranate genotype (Daru) from Narag, district Sirmaur (HP) for carrying out the study.

Induction of mutation by using gamma rays

The healthy fruits of pomegranate cultivars under study were collected during the 1st week of December during the year 2021. The seeds were extracted manually with the help of hands. Further, the seeds were split into three equal parts and put through the sarcotesta (juicy and edible part that surrounds each seed) extraction process. Seeds of each pomegranate cultivar were first fermented in a 10 % sugar solution for 72 hrs at room temperature. After fermentation, they were rinsed under running water for 2 min using a steel sieve, then air-dried at room temperature. The dried seeds were counted, packed and labeled according to treatment. For irradiation, 300 seeds per cultivar were placed in separate muslin cloth bags and exposed to gamma rays at doses of 0, 6, 9, 12, 15, 18, 21 and 24 kR at the Gamma Irradiation Chamber during the second week of February (Table 1). Post-irradiation, seeds were sown in pro-trays filled with a growing medium composed of sand, vermicompost and soil in equal proportions (1:1:1). Germination was monitored by recording the number of days from sowing to seedling emergence. Germination percentage was recorded between 20 and 40 days after sowing. After emergence, seedlings were transplanted into small transparent cups and maintained under glasshouse conditions for hardening. Seedling survival was assessed at 10-day intervals over a 30-day period following transplanting. The experiment was established in a Randomised Complete Block Design and with three replications.

Lethality percentage

The dose at which 50 % of the population perished following mutagen treatment is known as the Lethal Dose 50, or LD_{50}. It was calculated by using probit curve analysis. The quantile function or inverse cumulative distribution function (CDF) associated to the standard normal distribution is represented by the probit function (10).

Analysis of variance

To determine the LD_{50}, data was analysed using the analysis of variance technique using R-studio 4.2.3 software. It was estimated by using a simple linear regression model with the straight-line equation.

$$y = a + bx$$

Where, y = response variable (survival percentage), x = independent variable (mutagenic dose), a and b = slope and constant, respectively.

Morphological analysis

Foliage character Study

The observation in respect to the plant, shoot and leaf characters were recorded following UPOV descriptor (11). Plant (growth habit, intensity of grey colour of main branches), shoot (Shoot length, predominant number of leaves per node) and leaf (leaf blade length, width, leaf blade: ratio length/width, shape of apex excluding tip, Intensity of green colour) characters, petiole length, petiole anthocyanin colouration were measured.

Leaf chlorophyll content

Five fully expanded and matured leaves from each replicated bed were collected in the month of July during morning hours (12), were immediately placed in an ice box and brought to the laboratory. Each leaves samples were then finely chopped in a darkened room and 100 mg of the chopped leaves were put in vials with 7 mL of dimethyl sulphoxide. The contents of the vials were incubated at 65 °C for half an hour and then the extract was transferred to the graduated test tubes and the final volume was made to 10 mL with

Table 1. Induction of mutation by using physical mutagen (Gamma rays)

Treatment (T)	Cultivar (V)	Gamma rays (kR)
0	Kandhari Kabuli (V_1)	0 (Control)
1		6
2		9
3	Bhagwa (V_2)	12
4		15
5	Daru (V_3)	18
6		21
7		24

dimethyl sulphoxide (13). In a spectrophotometer (Thermo-Scientific Spectronic 20 D+), the extract optical density (OD) values were measured at wavelengths of 645 and 663 nm in comparison to a dimethyl sulphoxide blank. The following formula was used to determine the total chlorophyll content:

$$\text{Total chlorophyll} = \frac{20.2\ A_{645} + 8.02\ A_{663}}{A \times 1000 \times W} \times V$$

where A645 = absorbance at 645 nm, A663 = absorbance at 663 nm, V = volume of the extract generated, A = length of the light channel in the cell (typically 1 cm) and W = sample weight (g). As a result, the data were given in mg/g of fresh weight.

Inoculation of promising mutants with bacterial blight *Xanthomonas axonopodis* spv. *Punicae*

The experiment was conducted at Kiwi Block of the University. Pathogenicity tests of the isolated bacterium were conducted on various plant parts such as young and mature leaves by two methods i.e., spray inoculation and prick inoculation. The inoculated plant parts were covered with polythene for 24 hrs to maintain high humidity.

Disease severity (%)

The disease severity on leaves was recorded by using a 0-5 scale as given below after fifteen days from the inoculation. These leaves were categorised into different disease grades as per the scale and percent diseased area. The observations were recorded on 3 seedlings per treatment and averaged to workout overall disease incidence. The germplasms were categorised into different resistance categories as detailed below as per description (14).

Percent Disease Incidence (PDI) and severity on leaves was calculated by following formula:

Grade	Diseased leaf area (%)	
	Scale	Description
0	0.0	Healthy foliage
1	0.1-5	Up to 5 %of the leaf area is covered by small light brown spots that range in diameter from pinpoint to 2 mm.
2	5.1-10	Up to 5-10 % the leaf area has a yellow halo with a few dark brown dots
3	10.1-20	Spots with a yellow halo that cover up to 20 % of the leaf area and cause the afflicted leaves to start yellowing
4	20.1- 40	Spots with a yellow halo that cover up to 40 % of the leaf area, 25 % of infected leaves that have turned yellow and the beginning of leaf fall
5	60-100	Spots that cover more than 40 % of the leaf area with a yellow halo, 40 % of the leaves turned yellow and 25 % of the leaf fall

$$\text{Disease Severity (\%)} = \frac{\text{Sum of individual disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Statistical analysis

The data obtained from the present investigation were subjected to statistical analysis as per the methods suggested by Gomez and Gomez for Randomised Complete Block Design (RCBD) and Completely Randomised Design (CRD). SPASS, OPSTAT and MS Excel were used to do the statistical analysis for each character that was observed (15).

Results and Discussion

Number of days for germination

Globally, plant improvement, the productivity and the economic value of different crops have greatly increased because of mutation breeding. Results for number of days taken for germination of the pomegranate cultivars to gamma rays varied among the cultivars at different concentrations (Fig. 1). Maximum number of days taken for germination in cvs. Kandhari Kabuli, Bhagwa and Daru were recorded in 24 kR (31.67, 32.33 and 29.00 days) respectively, whereas the minimum days taken for germination was in 6 kR (26.33, 24.67 and 24.33 days), respectively. Among the progenies, the minimum days taken for germination were 6 kR in all the cultivars taken under study.

Germination percentage

The response rates of different pomegranate cultivars to γ radiation treatments were analysed and outcomes suggested that seeds and seedlings fared better in lower and moderate doses of γ radiation than in the highest doses. Gamma irradiation treatments had substantial effects on seed germination at different stages (Fig. 2). The observation for the germination percentage was taken at 20, 30 and 40 days after sowing (DAS). The data conferred in the table revealed the significant differences between the treatments and cultivars for the germination percentage. The maximum germination percentage after 20 days of sowing in cvs, Kandhari Kabuli, Bhagwa and Daru was observed in 9 kR (35.33 %, 37.67 % and 38.00 %, respectively). The maximum germination percentage 30 DAS in cvs, Kandhari Kabuli and Bhagwa was perceived in 6 kR (65.33 % and 55.33 %, sequentially), whereas, in cvs. Daru it was in 9 kR (59.33 %). The maximum germination percentage counted 40 DAS in cv. Kandhari Kabuli was observed in 6 kR (80.00 %), although in cvs. Bhagwa and Daru it was perceived in 9 kR (66.67 % and 69.67 %, respectively).

Maximum germination percentage was observed in control and lowest in treatment 24 kR in all the cultivars taken under study. The germination percentage decreased as the mutagen concentration increased. Similar results for citrus seeds (*Citrus suhuiensis*) when treated with gamma ray dosages of 50, 100, 150, 200 and 250 Gy were observed (16). *Citrus jambhiri* seeds while exposed to 40, 60, 80 and 120 Gy doses of gamma radiation, seed germination was lowest at 120 Gy and maximum at 40 Gy. Increased doses of the mutagens may create cellular disruptions at the physiological or physical level, which could account for the decline in seed germination (17). Stones of the Dusheri mango were irradiated with gamma irradiation (0, 2.5, 5, 7.5, 10, 12.5 and 15kR) and it was revealed by Karsinah et al. at Indonesian Tropical Fruits Institute, West Sumatera, Indonesia that the germination percentage was significantly highest (87.5 %) with 2.5 kR and minimum (21.7 %) in 15 kR (18).

At low doses, gamma rays can stimulate germination by enhancing enzyme activity, increasing cell membrane permeability and promoting the breakdown of stored food reserves. These mild stress signals can activate repair mechanisms and improve metabolic activity, leading to better germination. In contrast, higher doses cause damage to DNA, proteins and cellular membranes, generating oxidative stress that impairs normal cell function or leads to cell death.

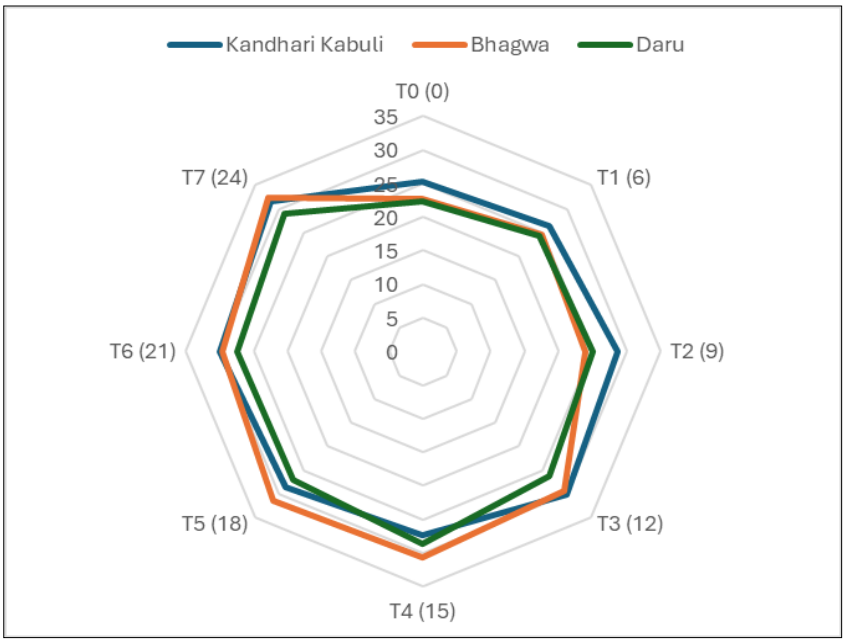

Fig. 1. Impact of different gamma-ray concentrations on the duration of germination.

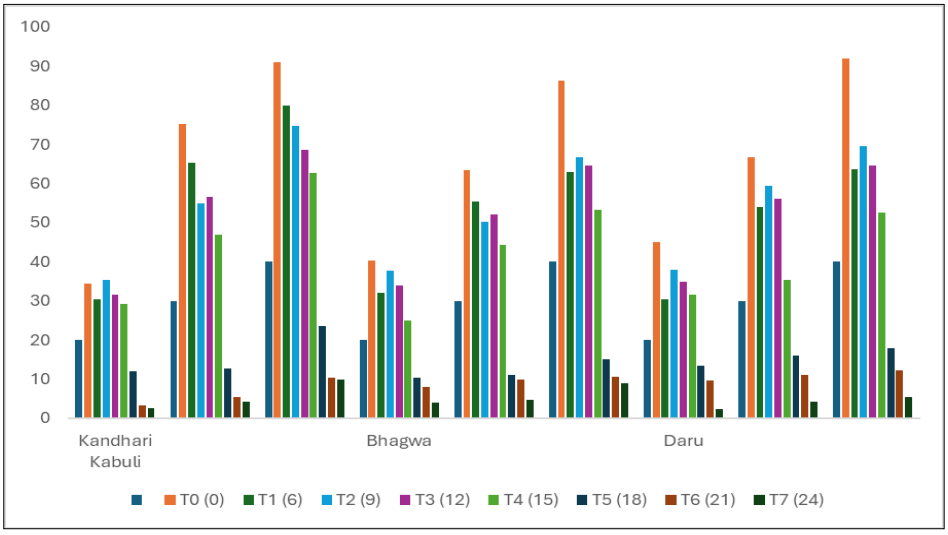

Fig. 2. Impact of different gamma-ray concentrations on germination percentage.

The study revealed that maximum germination occurred in the control group, while the lowest was recorded at 24 kR across all cultivars. A consistent decline in germination percentage was observed with increasing gamma ray doses. Similar findings were reported by previous researchers in *Citrus suhuiensis* and in *Citrus jambhiri*, where higher doses led to reduced germination. This decline is likely due to physiological and cellular disruptions caused by mutagenic stress (19). Also noted significantly lower germination at higher doses in irradiated mango stones (20).

Survival percentage

The survival of seedlings is a reliable index to evaluate the effect of mutagen. The data revealed that in general, as the dose of the mutagen increased there was a decrease in survival percentage at 40 days after sowing. Survival percentage of the treated seedlings was counted after transplanting the seedlings from portrays to small plastic cups after 40 days of sowing under the greenhouse condition (Fig. 3). The observation was undertaken 10 to 30 days after transplanting the seedlings. The maximum survival percentage in cvs. Kandhari Kabuli and Daru was discernible at 6 kR (78.67 % and 69.00 %, respectively), which was statistically equivalent to 9 kR in cv. Kandhari Kabuli and 12 kR in cv. Daru. Since, in cv. Bhagwa, it was in 9 kR (66.00 %), which was statistically at par with 12 kR (63.67 %) and 6 kR (62.33 %).

The survival percentage of seedlings is widely recognized as a reliable indicator of the physiological impact of mutagenic treatments. In the present study, a general decline in survival was observed with increasing gamma ray doses, particularly at 40 days after sowing under greenhouse conditions. Maximum survival was recorded at moderate doses (6-9 kR), which suggests a threshold beyond which radiation stress leads to irreversible cellular damage. These findings are consistent with recent reports, noted similar dose-dependent reductions in survival across various fruit and legume crops. Moderate doses may activate repair and defence mechanisms, while higher doses disrupt vital metabolic pathways, resulting in seedling mortality (21, 22).

Lethality percentage

The probit analysis for gamma rays indicated that the LD_{50}value based on survival per cent over controlfor cv. Kandhari Kabuli was recorded as 15.26 kR (Table 2) and that for cvs. Bhagwa and Daru as 15.08 kR (Table 3) and 13.74 kR (Table 4) respectively.

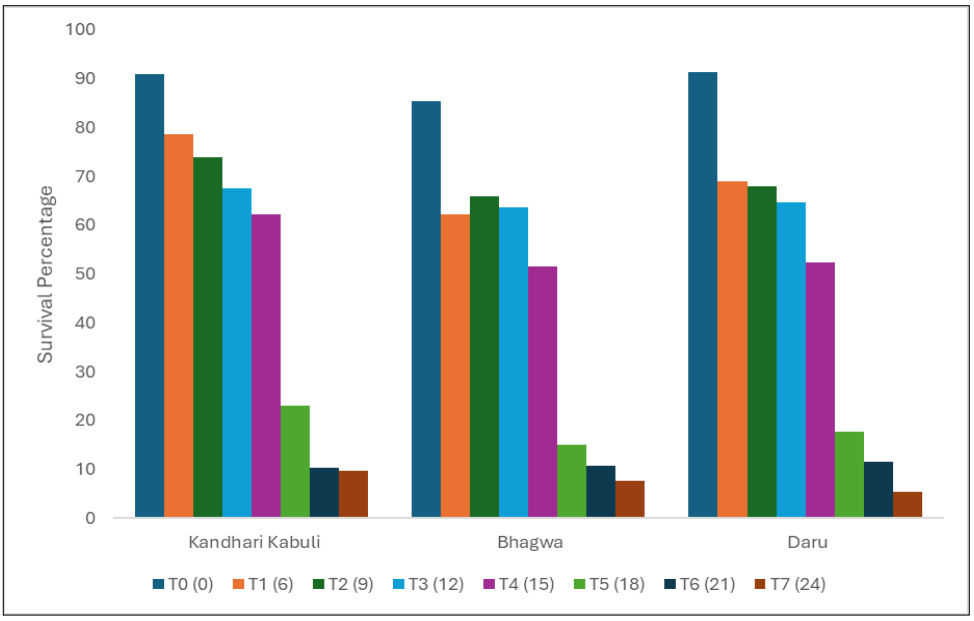

Fig. 3. Impact of different gamma ray concentrations on survival percentage.

In a view for induction of compact mutants in pears, Shin et al. irradiated dormant buds. The results indicate that the LD_{50} (dose for 50 % shoot growth reduction in comparison with controls) after gamma irradiation was 4 k rad in Hosui, Chojuno, Danbane and Deulkbae; 6 k rad in Shinshi and Niltaka and 3 krad in Hosui. Further, gamma rays were used in novel sweet cherry, local and sour cherry cultivars from Buda (23). The critical dose for most types was 20 Gy in sour cherry and 40 Gy for sweet cherry (24). Fuentes-Lorenzo et al. deliberated the LD_{50} for the Hass (27 Gy) and Duke (28 Gy) varieties of avocado. The LD_{50} for Hass (19 Gy) and Duke (25 Gy) were also established at the Güira de Melena station of the Tropical Fruit Research Institute (IIFT) Cuba (25).

The probit analysis conducted for gamma ray treatments revealed that the LD_{50} values representing the dose at which 50 % of seedlings survived compared to the control were 15.26 kR for cv. Kandhari Kabuli, 15.08 kR for cv. Bhagwa and 13.74 kR for cv. Daru. These values indicate a cultivar-specific sensitivity to gamma radiation, with cv. Daru showing higher sensitivity. Similar trends have been observed in other fruit crops; for instance, Shin et al. reported LD_{50} values ranging from 3-6 kR in pear cultivars depending on genotype. In cherry cultivars, the critical dose varied between 20 Gy (sour cherry) and 40 Gy (sweet cherry) (26). From former studies, LD_{50} values of 27-28 Gy for avocado varieties 'Hass' and 'Duke', reinforcing the use of LD_{50} as a crucial benchmark in mutation breeding to balance mutagenic effectiveness with plant survival (27).

Correspondingly, Chandra et al. reported the germination, survival percentage and the LD_{50}dose of pomegranate cv. Ganesh, according to the research, seeds were irradiated with 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 kR. On 10 DAS, seed germination ranged from 4.0 to 29.5 %. Beyond 6 kR, the seed germination was decreased and it was noted to be minimum at 30 kR, indicating that higher

Table 2. Probit Estimation for LD_{50} of cv. 'Kandhari Kabuli' as affected by gamma rays

Treatments (kR)	Log_{10} of Doses	Survival %	Percent survival overcontrol	Percent reduction over control	Empirical probit unit	LD_{50} Dose
T_0 (0)	0	91.00	100.00	-	-	
T_1 (6)	0.78	78.67	86.45	13.55	3.77	
T_2 (9)	0.95	74.00	81.31	18.69	4.01	
T_3 (12)	1.08	67.67	74.36	25.64	4.23	
T_4 (15)	1.18	62.33	68.49	74.73	4.42	
T_5 (18)	1.26	23.00	25.27	74.73	5.44	15.26 kR
T_6 (21)	1.32	10.33	11.35	88.65	5.84	
T_7 (24)	1.38	9.67	10.63	89.37	5.88	
$CD_{0.05}$		4.85				
SE±(m)		1.60				

Table 3. Probit Estimation for LD_{50} of cv. 'Bhagwa' as affected by gamma rays

Treatments (kR)	Log_{10} of Doses	Survival %	Percent survival overcontrol	Percent reduction over control	Empirical probit unit	LD_{50} dose
T_0 (0)	0	85.33	100	-	-	
T_1 (6)	0.78	62.33	73.04	26.96	4.26	
T_2 (9)	0.95	66.00	77.34	22.66	4.12	
T_3 (12)	1.08	63.67	74.61	25.39	4.19	
T_4 (15)	1.18	51.67	60.55	39.45	4.53	
T_5 (18)	1.26	15.00	17.58	82.42	5.52	15.08 kR
T_6 (21)	1.32	10.67	12.50	87.50	5.67	
T_7 (24)	1.38	7.67	8.98	91.02	5.74	
$CD_{0.05}$	-	6.30	-	-	-	
SE±(m)	-	2.08	-	-	-	

Table 4. Probit estimation for LD_{50} of cv. 'Daru as affected by gamma rays

Treatments (kR)	Log_{10} of Doses	Survival %	Percent survival overcontrol	Percent reduction over control	Empirical probit unit	LD_{50} Dose
T_0 (0)	0	91.33	100	-	-	
T_1 (6)	0.78	69.00	75.55	24.45	4.23	
T_2 (9)	0.95	68.00	74.46	25.54	4.26	
T_3 (12)	1.08	64.67	70.80	29.20	4.39	
T_4 (15)	1.18	52.33	57.29	42.71	4.72	13.74 kR
T_5 (18)	1.26	17.67	19.34	80.66	5.64	
T_6 (21)	1.32	11.67	12.78	87.22	5.81	
T_7 (24)	1.38	5.33	5.83	94.17	6.08	
$CD_{0.05}$	-	5.72	-	-	-	
SE±(m)	-	1.89	-	-	-	

doses had a negative effect on germination (28). On 26 DAS, almost all germinable seeds have germinated and no further germination was noted and the germination percent ranged from 23.00 to 67.50 in different treatments. The survival of the seedlings on 70 DAS ranged from 8.9 to 95.4 percent. From the study, it was concluded that the LD_{50} for cv. Ganesh was 9 kR and 12 kR. The LD_{50} levels were determined by Vos et al. for Citrus, Litchi, Guava, Cherimoya, Pitanga, Jaboticaba and Carambola. Experimental gamma irradiation of 20 and 30 Gy was administered to different litchi cultivars to determine the LD50 value for litchi budwood. In contrast, Carambola, Cherimoya, Pitanga and Jaboticaba were shown to have LD_{50} values of 30, 30, 45 and 70 Gy, respectively (29).

Typically, probit or logistic regression models are applied in dose-response studies due to their ability to handle binomial outcomes and provide reliable midpoint estimates. However, the absence of justification for model selection and omission of validation metrics-such as R^2 values, confidence intervals, or residual diagnostics, limits the reproducibility and statistical robustness of the LD_{50} values reported. Incorporating these elements in future studies would enhance the reliability of the dose-response relationship and support more precise recommendations for mutation breeding programs. Recent work also emphasizes the integration of model validation techniques to ensure accuracy in determining radiosensitivity parameters across crop species (30).

Morphological characterization

Foliage characters analysis

Under the polyhouse conditions, the maximum leaf blade length in cv. Kandhari Kabuli was in 6 kR and 9 kR, whereas in cv. Bhagwa and Daru it was in 24 kR and 9 kR, respectively. The leaf length varied under field conditions comparative to the polyhouse conditions. The leaf length in cv. Kandhari Kabuli, Bhagwa and Daru was 6 kR, 24 kR and 9 kR, respectively. The leaf blade width was maximum in 6 kR in cv. Kandhari Kabuli, 24 kR in cv. Bhagwa and 15 kR in cv. Daru under polyhouse conditions. In field conditions, the maximum leaf blade width in cvs. Kandhari Kabuli, Bhagwa and Daru was in 9 kR, 24 kR and 18 kR, subsequently. The maximum petiole length, under polyhouse conditions was at 18 kR in cv. Kandhari Kabuli, 24 kR in cv. Bhagwa and 9 kR in cv. Daru, while, under field conditions, the maximum petiole length in cv. Kandhari Kabuli and cv. Bhagwa was in 9 kR; although in cv. Daru it was in 9 kR and 24 kR. Data about leaf blade ratio under polyhouse and field conditions revealed that in cv. Kandhari Kabuli, it was observed moderately elongated in all the treatments including control except 9 kR (medium) under field conditions. Although in cv. Bhagwa, it was medium in control and 12 kR and remaining treatments were moderately elongated, though, in cv. Daru, medium leaf blade ratio was observed in 6 kR, 9 kR, 15 kR and 24 kR and remaining treatments were moderately elongated. The intensity of green colour of the leaf blade revealed that in cv. Kandhari Kabuli, Bhagwa and Daru, the intensity remains same (dark) in all the treatment including control under polyhouse and field conditions. Predominant number of leaves per node on the young shoot in all the treatments including control was observed more than three under polyhouse as well as field conditions. The shape of the apex, excluding the tip of the leaf blade under polyhouse conditions was moderately acute in cvs. Kandhari Kabuli and Bhagwa including control, although it was strongly acute in cv. Daru. Since, under field conditions, it was moderately obtuse excluding control (moderately acute), although it was moderately obtuse in cv. Bhagwa including control, in cv. Daru, it was strongly acute in control, 18 kR and 21 kR, whereas, moderately obtuse in all other treatments. The intensity of grey colour of the main branch of the plant was observed to be light for the cvs. Kandhari Kabuli and Bhagwa whereas, in cv. Daru it was medium under polyhouse and field conditions.The petiole anthocyanin colouration under polyhouse conditions in cv. Kandhari Kabuli remarked weak anthocyanin in 12 and 24 kR, including control, whereas medium in 6, 9 and 21 kR treatments and strong in remaining treatments. The weak (21 kR) and medium (15 kR) anthocyanin colouration was discernible in cv. Bhagwa and strong in the remaining treatments, including control, whereas in cv. Daru, weak colouration was observed in 12 kR, medium in control, 15, 21 and 24 kR and strong in 6, 9 and 18 kR treatments. Whereas, under field conditions, cv. Kandhari Kabuli remarked in 6 and 18 kR, including control, whereas strong in remaining treatments. The strong anthocyanin colouration in control was discernible in cv. Bhagwa and weak in all the treatments, whereas in cv. Daru, weak colouration was observed in 6 and 24 kR, medium in control and 21 kR and strong in 9, 12, 15 and 18 kR treatments. The maximum shoot length in cv. Kandhari Kabuli was 12 kR under polyhouse and field conditions; in cv. Bhagwa, it was 9 kR; and in cv. Daru, it was 12 kR, with the exception of cv. Daru under field conditions, where it was 12 kR.

Total chlorophyll estimation

From the statistical analysis, it was observed that the total chlorophyll content differs significantly among the cultivars (Table 5). The maximum chlorophyll in cv. Kandhari Kabuli was recorded in 6 kR (2.29 mg g^{-1}), although in cvs. Bhagwa and Daru, it was estimated in 15 kR (3.31 and 2.70 mg g^{-1}, subsequently). Cv. Daru was statistically equivalent with 18 kR (2.41 mg g^{-1}).

The observed differences in total chlorophyll content among the cultivars and gamma radiation treatments indicate a genotype-specific physiological response to mutagen exposure. Statistically significant variation (Table 5) suggests that chlorophyll biosynthesis or degradation is differentially regulated under radiation-induced stress. In cv. Kandhari Kabuli, the highest

Table 5. Effect of gamma rays on chlorophyll content (mg g^{-1}) for different pomegranate cultivars

Treatment (kR)	Kandhari Kabuli	Bhagwa	Daru
T_0 (0)	2.50	3.30	2.99
T_1 (6)	2.29	1.35	1.76
T_2 (9)	0.97	2.29	1.18
T_3 (12)	2.09	1.98	1.89
T_4 (15)	1.51	3.31	2.70
T_5 (18)	1.51	2.27	2.41
T_6 (21)	1.40	2.56	1.22
T_7 (24)	1.47	2.44	1.43
$CD_{0.05}$	0.15	0.74	0.39
SE±(m)	1.67	1.31	1.33

chlorophyll content at 6 kR (2.29 mg g^{-1}) implies that lower doses may stimulate metabolic activity or delay chlorophyll degradation. Conversely, cvs. Bhagwa and Daru showed peak chlorophyll levels at higher doses (15 kR), indicating a higher tolerance or delayed stress response. The statistical similarity between Daru's response at 15 kR and 18 kR further suggests a broader effective dose range for maintaining chlorophyll stability. These variations reflect inherent genetic differences in radiation sensitivity and adaptive responses, which are critical for selecting promising genotypes in mutation breeding.

Field tolerance to bacterial blight disease

Bacterial blight disease severity in terms of disease score was recorded by using 0 - 5 grade scale and the data are presented in Table 6. Symptoms were observed on the leaves. On the top surface of the leaves, a small, water-soaked lesion which was brown to black in colour was initially observed. Comparably, a dispersed water-soaked ring was seen surrounding the area on the bottom surface. The spots varied in form from round to angular to uneven. Over the course of the disease, these spots became larger (diameters ranging from 2.0 to 5.0 mm), coalesced and eventually extended up to the midrib, covering the majority of the leaf lamina in a week time.

The three cultivars of pomegranate were screened against the bacterial blight under different treatments with physical mutagen (gamma rays) under field conditions. At 9 kR, cv. Daru (6.57 %) recorded the minimum disease severity, ensuingly cvs. Bhagwa (9.03 %) and Kandhari Kabuli (22.97 %). The maximum disease severity incidence was documented in cv. Kandhari Kabuli (42.93 %) at 21 kR.

The data indicated that none of the cultivars showed resistant at any of the treatments but manifest the tolerance of disease. Perusal of the data indicated that in cv. Kandhari Kabuli, disease severity ranged from 22.97 to 42.93 percent; in cv. Bhagwa, it was 9.03 to 18.57 %; although in cv. Daru, the disease severity varied from 6.57 to 10.20 % and was perceived to be the lowest and most tolerant among all the cultivars taken under study.

Table 6. Response of different gamma rays treated seedlings of different pomegranate cultivars to bacterial blight

Treatmen (kR)	Kandhari Kabuli	Bhagwa	Daru
T_0 (0)	44.27	15.87	12.07
T_1 (6)	31.97	9.40	7.50
T_2 (9)	22.97	9.03	6.57
T_3 (12)	34.27	16.90	7.40
T_4 (15)	27.07	18.57	8.27
T_5 (18)	24.67	11.30	8.00
T_6 (21)	42.93	12.30	8.53
T_7 (24)	35.53	18.43	10.20
$CD_{0.05}$	8.83	2.75	1.53
SE±(m)	2.88	0.90	0.50

Benagi et al. reported that the severity of disease ranged from 0.67 to 94.80 % (31). From previous studies, surveyed for bacterial blight in major pomegranate growing areas of Karnataka and border areas of Andhra Pradesh and reported that the highest average disease severity on leaves was 26.33 %, whereas the lowest average disease severity was 22.12 %. Among the varieties, cv. Bhagwa has been perceived most susceptible with respect to disease severity (32). Similar results were concluded by previous researchers in cv. Bhagwa, where the disease severity ranged from 6.1 to 77.33 % (33).

Molecular analyses have increasingly substantiated the phenotypic observations of bacterial blight tolerance in pomegranate. Tolerant cultivars such as *Daru* have shown upregulation of key defence related genes, including pathogenesis-related (PR) proteins, WRKY transcription factors and NBS-LRR resistance gene analogues, which are commonly associated with plant innate immunity. Expression profiling using RT-qPCR has revealed activation of salicylic acid (SA) and jasmonic acid (JA) signalling pathways two major hormonal defence networks following pathogen challenge. Additionally, transcriptomic analyses and RNA-Seq studies in tolerant genotypes have indicated early and sustained expression of genes involved in oxidative burst, hypersensitive response (HR) and systemic acquired resistance (SAR). Molecular marker studies, including SSR and SNP genotyping, have also identified resistance -associated QTLs that differentiate tolerant and susceptible cultivars, providing tools for marker-assisted screening. A recent genome-wide and transcriptome exploration identified 958 resistance gene analogues across eight chromosomes including 74 differentially expressed receptor-like kinases (RLKs) and numerous NBS-LRR genes providing robust evidence for molecular defence activation in resistant cultivars (34).

The identification of bacterial blight-tolerant genotypes such as *Daru* provides a valuable genetic resource for improving susceptible commercial cultivars like *Bhagwa*. The use of tolerant donors in hybridization programs, combined with marker assisted selection (MAS), enables precise and efficient introgression of resistance genes without compromising fruit quality traits. This strategy facilitates the development of high-yielding, disease-resistant cultivars suitable for large-scale cultivation and export markets. Recent advances underscore the effectiveness of CRISPR/Cas9 in engineering disease resistance by targeting susceptibility genes and pyramiding traits for lasting resilience (35).

Moreover, enhancing resistance through genetic means reduces reliance on chemical bactericides, lowering input costs and supporting sustainable and eco-friendly pomegranate production. Long-term, the integration of genomic-assisted breeding tools, including QTL mapping and genome editing (e.g., CRISPR/Cas9), holds promise for accelerating the development of cultivars with durable and broad-spectrum resistance to bacterial blight.

Conclusion

From the results obtained, it can be concluded that LD_{50} for gamma rays was estimated to be 15.26 kR, 15.08 kR and 13.74 kR for cvs. Kandhari Kabuli, Bhagwa and Daru respectively. Seeds were treated with doses of gamma rays to induce variability, which effectively characterised molecular characters with the tolerance to bacterial blight disease in all the cultivars taken under study. In the three pomegranate cultivars screened for bacterial blight under field conditions in 9 kR, cv. Daru recorded the minimum disease severity percentage (6.57 %) over control, followed by cv. Bhagwa (9.03 %) and cv. Kandhari Kabuli (22.97 %). The maximum disease severity incidence was recorded in cv. Kandhari Kabuli (42.93 %) over control at 21 kR. Seeds were treated with doses of gamma rays for inducing variability which effectively characterised morphological, biochemical and molecular characters with the tolerance to bacterial blight disease in all the cultivars taken under study. The study suggests significant potential for integrating gamma radiation-induced variability with resistance breeding strategies in pomegranate. The tolerant cultivars, especially Daru and Bhagwa, may serve as valuable genetic resources in breeding programs aimed at improving resistance in susceptible but commercially important cultivars like Kandhari Kabuli. The combination of induced mutagenesis with molecular characterization has proven effective in identifying lines with favourable morphological, biochemical and molecular traits linked to disease tolerance. These results underscore the importance of incorporating such lines into hybridization and selection programs using marker-assisted selection (MAS) for rapid and precise trait transfer. Furthermore, the selected tolerant lines should undergo multi-location field trials to evaluate the stability and adaptability of resistance across different environments. Ultimately, this approach supports the development of high-yielding, disease-tolerant pomegranate cultivars that reduce dependence on chemical control measures and contribute to more sustainable and cost-effective disease management in the field.

Acknowledgements

The authors would like to express their sincere gratitude for the consistent support they have received from the Department of Fruit Science, YSPUHF, Nauni, Solan, Himachal Pradesh, India.

Authors' contributions

MJ carried out the research part of the paper. RD and MJ conceptualized the work. NS and YKA wrote the original draft. VSR, BS & MK carried out the corrections. NS, YKA, KB, RV and MJ reviewed and edited the manuscript. RD, MJ, YKA and NS did software analysis and figures preparation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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