

# Genetic variability of Red Chili Peppers (*Capsicum annuum* L.) in the M2 generation resulting from gamma-ray irradiation

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## ABSTRACT

Red chili peppers (*Capsicum annuum* L.) are a horticultural commodity of high economic value, but increasing their productivity still requires the support of high-yielding varieties with adequate genetic diversity. This study analyzed phenotypic and genotypic variability, broad-sense heritability, the Coefficient of Genetic Variation (CGV), and the expected response to selection in the M2 generation of the local red chili cultivar 'Putih' derived from gamma-ray irradiation. The study was conducted in Tanjung Sari Village, Medan Selayang District, Medan City, from February to June 2025. The planting material consisted of a 0 Gy control population and M2 populations irradiated at 100 Gy and 200 Gy. Observations were made on 30 sample plants from each population. Data were analyzed using means, standard deviations, tests of mean differences relative to the control, phenotypic variance, genotypic variance, heritability, and CGV. The results showed that the 100 Gy population exhibited wide phenotypic and genetic variability in dichotomous height, plant height, fruit length, and number of fruits per plant. The 200 Gy population exhibited extensive genetic variability only in the number of fruits per plant. High heritability was found in dicot height, flowering time, leaf length, and fruit length in the 100 Gy population, as well as in flowering time and leaf length in the 200 Gy population. High CGV was found in fruit length in both irradiated populations. Based on the combination of genetic variability, heritability, and CGV, the traits of fruit length and dichotomous height in the 100 Gy population have the potential to serve as initial selection criteria for the next generation.

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## 1. INTRODUCTION

Red chili peppers (*Capsicum annuum* L.) are a strategic horticultural commodity because they are widely used as food, spices, and raw materials for the food processing industry. The dynamics of chili pepper demand and production also influence supply stability and consumer prices. Data from the Central Statistics Agency (BPS) indicate that large chili peppers are among the seasonal vegetable commodities whose production is monitored by province and crop type; therefore, the development of high-quality planting materials remains relevant to support national production stability (Badan Pusat Statistik, 2025).

Increasing chili pepper production can be achieved not only through improved cultivation techniques but also through plant breeding. One approach that can be used is mutation induction. Mutation breeding utilizes random genetic changes that can produce new variations, which are then selected to obtain the desired traits. The IAEA explains that mutation induction using physical mutagens, including gamma rays, has been widely used to increase plant genetic diversity and accelerate the development of new agronomic traits (International Atomic Energy Agency, 2026).

Gamma-ray irradiation is widely used in mutation breeding because of its high penetration capacity and ability to induce heritable genomic changes. At the cellular level, gamma rays can damage DNA directly by producing base modifications and single- or double-strand breaks, and indirectly through radiolysis-driven reactive oxygen species (ROS) that alter DNA, proteins, membranes, and mitotic activity. Mutations become fixed when DNA damage is repaired with errors, particularly through non-homologous end joining, resulting in insertions, deletions, substitutions, or chromosomal rearrangements that may modify quantitative traits such as plant architecture, flowering, fruit size, and yield components (Ma et al., 2021; Al-Sayed et al., 2025).

In plant breeding, selection efficiency is determined not only by the presence of phenotypic variation but also by the proportion of variation that is genetic and heritable. Therefore, the estimation of phenotypic variance, genotypic variance, broad-sense heritability, Coefficient of Genetic Variation (CGV), selection differential, genetic advance, and expected response to selection is essential for identifying traits that can be reliably improved in the next generation. Traits that combine wide genotypic variability, high heritability, high CGV, and high genetic advance are generally more promising because their phenotypic expression is less dominated by environmental noise and more likely to be transmitted to progenies (Rosmaina et al., 2016; Sidiq et al., 2017).

The local red chili cultivar 'Putih' from Batu Bara Regency has potential as a local genetic resource, but it still requires improvement, particularly in terms of growth characteristics and yield. Research by Hanafiah et al. (2024) indicates that gamma-ray irradiation of local chili peppers from North Sumatra can induce morphological changes. Therefore, evaluation of the M2 generation is necessary because this generation begins to exhibit observable mutant segregation for initial selection.

Previous studies have reported that gamma irradiation can alter morphological and yield-related characters in Capsicum, but the optimal dose is genotype dependent. In chili, moderate irradiation has been associated with improved morpho-physiological performance and useful genetic variation, whereas excessive irradiation may reduce growth and yield because of oxidative stress, DNA damage, and chromosomal abnormalities (Hashim et al., 2024; Karim et al., 2022). Thus, dose-specific evaluation in local germplasm remains necessary before mutant lines are advanced for selection.

This study aims to evaluate phenotypic and genotypic variability, broad-sense heritability, CGV/KKG, and the expected response to selection in M2-generation red chili cultivar 'Putih' populations irradiated at 100 Gy and 200 Gy. The central objective is to identify the most reliable selection criteria for developing superior mutant candidates in the next generation.

## 2. METHOD

### 2.1. Place and Time of Research

The study was conducted in Tanjung Sari Village, Medan Selayang Subdistrict, Medan City, from February to June 2025. The site served as a field evaluation plot for observing the growth and yield components of the M2 generation of the local red chili cultivar 'Putih' from Batu Bara Regency.

### 2.2. Tools and Materials

The planting material consisted of seeds of the local red chili cultivar 'Putih' from Lubuk Cuiik Village, Batu Bara Regency. Three populations were evaluated: non-irradiated control plants (0 Gy), M2 plants derived from 100 Gy irradiation, and M2 plants derived from 200 Gy irradiation. Fifty seeds were planted for each population; therefore, the total number of planted seeds was 150. Thirty surviving and representative plants from each population were observed for all quantitative traits, resulting in 90 plant-level observations for every character. Plants that did not survive until the observation stage or showed severe non-measurable abnormalities were excluded from quantitative measurement and should be reported separately as survival data in the field log.

The tools used in this study included a hoe, a machete, a rake, a measuring tape, scissors, an analytical balance, a sprayer, a camera, a laptop, writing materials for recording observations, and other tools necessary for conducting this study.

### 2.3. Experimental Design and Statistical Analysis

The research method used was descriptive statistical analysis, involving the planting of green and local red chili cultivar 'Putih' seeds from Batubara as controls (M0) and chili seeds irradiated with gamma rays (M2) at dose levels of 100 Gy, and 200 Gy, with 50 red chili seeds planted per dose level, resulting in a total of 150 plants.

The irradiation treatment was conducted at the National Nuclear Energy Agency's Center for Isotope and Radiation Applications (PAIR BATAN), using the Gammacell 220 irradiator machine. The gamma-ray source was artificial radioisotope, with Cobalt-60 ( $^{60}\text{Co}$ ) (dose rate 0.85 kGy/hour) with doses of 0 Gy, 100 Gy, and 200 Gy.

After irradiation, the treated seeds were planted as the M1 generation and self-pollinated to produce M2 seeds. This study evaluated the M2 generation because this generation is commonly used to screen for phenotypic variations induced by mutagenesis (Hashim et al., 2024; Saibari et al., 2023). Control seeds (0 Gy) were used as a comparison to plants that had been irradiated with gamma rays.

The data were analyzed using means, standard deviations, and tests for differences in means compared to the control. Test the difference in the average data using a paired t-test. The paired t-test is used to determine the difference in the average measurement results at 0 Gy, 100 Gy, and 200 Gy. The paired t-test is performed by comparing the levels of 0 Gy, 100 Gy, and 200 Gy separately, for example, a paired t-test of 0 Gy with 100 Gy and then 0 Gy with 200 Gy, rather than testing simultaneously at all three dose levels. The formula for the paired t-test as:

$$t = \frac{d}{S_d/\sqrt{n}} \quad (1)$$

Genetic parameters were estimated using phenotypic variance ( $\sigma^2p$ ), environmental variance ( $\sigma^2e$ ), genotypic variance ( $\sigma^2g$ ), broad-sense heritability ( $h^2$ ), and the Coefficient of Genetic Variation (CGV).

Genetic parameters were estimated for each quantitative character using population variance. The mean and standard deviation were calculated as follows:

$$\text{Mean: } \bar{x} = \sum xi / n \quad (2)$$

$$\text{Standard deviation: } SD = \sqrt{[\sum(xi - \bar{x})^2 / (n - 1)]}$$

Phenotypic variance of each irradiated M2 population was estimated as:

$$\sigma^2p = \frac{\sum_i^n = 1(x_i - \mu)^2}{n} \quad (3)$$

where  $X_i$  = observation value for plant  $i$ ,  $\mu$  = population mean,  $n$  = number of plants in the population.

Environmental variance was estimated from the non-irradiated control population as:

$$\sigma^2e = \sigma^2M0 \quad (4)$$

where  $\sigma^2M0$  = variation in the control plant population.

Genotypic variance was calculated as:

$$\sigma^2g = \sigma^2p - \sigma^2e \quad (5)$$

When  $\sigma^2g$  produced a negative value, it was considered zero for interpretation because negative genetic variance has no biological meaning.

The width of variability was determined using the two-standard-deviation criterion:

$$2SDp = 2\sqrt{\sigma^2p} \text{ and } 2SDg = 2\sqrt{\sigma^2g} \quad (6)$$

Variability was classified as wide when  $\sigma^2p$  or  $\sigma^2g$  was greater than its corresponding 2SD value, and narrow when  $\sigma^2p$  or  $\sigma^2g$  was equal to or lower than the corresponding 2SD value.

Broad-sense heritability was estimated as:

$$h^2BS = \sigma^2g / \sigma^2p. \quad (7)$$

Heritability was classified as low ( $h^2BS < 0.20$ ), moderate ( $0.20 \leq h^2BS \leq 0.50$ ), and high ( $h^2BS > 0.50$ ).

The Coefficient of Genetic Variation, also referred to as CGV, was calculated as:

$$CGV (\%) = (\sqrt{\sigma^2g} / \bar{x}) \times 100 \quad (8)$$

CGV was classified as low (<10%), moderate (10–15%), and high (>15%) (Kearsey & Pooni, 1996).

The observed parameters included dichotomous height (cm), plant height (cm), stem diameter (mm), number of branches, Flowering time (days after sowing), leaf length (cm), Harvesting time (days after sowing), fruit length (cm), weight per fruit (g), and number of fruits per plant (fruits). Unit conversion was performed because the number of fruits per plant is a count, not a weight in grams. Height to first dichotomous branching and plant height were measured in centimeters using a measuring tape. Stem diameter was measured in millimeters using a suitable measuring instrument. Leaf length and fruit length were measured in centimeters, whereas weight per fruit was measured in grams using an analytical balance. Flowering time was recorded as the number of days from sowing until the appearance of the first flower, while harvesting time was recorded as the number of days from sowing until fruit reached harvestable maturity. The number of fruits per plant was recorded as count data and was not converted into grams. Fruit weight was measured separately as weight per fruit in grams.

### 3. RESULTS AND DISCUSSION

#### 3.1. Characteristics of growth and yield

Gamma-ray irradiation affected most growth and yield traits of the M2 generation of red chili pepper (Table 1). The 100 Gy population showed higher mean values for dichotomous height, plant height, stem diameter, number of branches, leaf length, and number of fruits per plant than the control. In contrast, the 200 Gy population tended to reduce dichotomous height, stem diameter, fruit length, and fruit weight. This dose-response pattern suggests that 100 Gy generated useful variation while still maintaining plant vigor, whereas 200 Gy began to impose stronger biological injury on several quantitative traits.

**Table 1.** Means, standard deviations, and results of the mean difference test for red chili pepper traits in the 0 Gy, 100 Gy, and 200 Gy populations

Character	Population		
	I0 (0 Gy)	I1 (100 Gy)	I2 (200 Gy)
Dichotomous height (cm)	31,31 ± 2,85	33,86** ± 4,14	29,05** ± 3,20
Plant Height (cm)	62,74 ± 6,86	66,61** ± 7,20	63,27ns ± 6,92
Stem Diameter	7,44 ± 1,16	8,24** ± 1,24	6,54** ± 1,19
Number of Branches	3,93 ± 0,64	4,33** ± 0,66	4,00ns ± 0,74
Flowering time (days after sowing)	40,27 ± 1,57	37,57** ± 2,51	38,30** ± 2,28
Leaf Length (cm)	7,04 ± 0,73	7,99** ± 1,05	8,14** ± 1,23
Harvesting time (days after sowing)	89,83 ± 0,91	89,07** ± 1,01	89,07** ± 1,01
Fruit Length (cm)	13,89 ± 2,16	11,74** ± 3,38	9,24** ± 2,74
Weight per Fruit (g)	11,31 ± 2,54	9,67** ± 2,65	9,27** ± 2,69
Number of Fruits per Plant	56,67 ± 20,31	94,33** ± 23,29	86,97** ± 21,74

Note: \*\*the results of the paired t-test indicate a significant difference at  $\alpha = 0.01$  compared to the control, and ns = not significant

The increase in the number of fruits per plant in the 100 Gy and 200 Gy populations indicates that irradiation can produce individuals with higher yield potential. However, yield is not determined solely by fruit number. In this study, the increase in fruit number was accompanied by reduced fruit length and weight per fruit, particularly at 200 Gy. Therefore, selection should not focus only on fruit number but must balance fruit number, fruit size, fruit weight, and market preference.

Biologically, the better performance of the 100 Gy population can be interpreted as an intermediate-dose effect. Moderate irradiation may create enough DNA lesions to generate new allelic variation without severely disrupting cell division, photosynthetic capacity, and reproductive development. By contrast, higher irradiation can increase ROS accumulation, double-strand breaks, chromosomal bridges, fragments, micronuclei, or mitotic abnormalities, resulting in growth inhibition and reduced fruit size. This explanation is consistent with radiation hormesis and stress-injury concepts reported in plant irradiation studies, where low to moderate doses may stimulate

growth-related responses, while excessive doses suppress growth and yield (Riviello-Flores et al., 2022; Al-Sayed et al., 2025).

The reduction in certain traits at a dose of 200 Gy is one of the negative effects of mutations and radiation damage. Higher doses of gamma radiation can increase reactive oxygen species, double-strand DNA breaks, chromosome cross-links, lagging chromosomes, fragments, micronuclei, and meiosis abnormalities. These changes can reduce mitotic activity, disrupt gamete formation, decrease assimilation, and cause cytotoxic effects that ultimately manifest as shorter fruit length, lower fruit weight, and reduced stem diameter. Thus, a 200 Gy population may contain beneficial mutants, but it also carries a higher probability of deleterious mutations that require rigorous selection and progeny testing (Choi et al., 2021).

### 3.2. Phenotypic and genotypic variability

In the 100 Gy population, dichotomous height, plant height, flowering time, fruit length, fruit weight, and number of fruits per plant showed wide phenotypic variability. However, the traits with wide genotypic variability were dichotomous height, plant height, fruit length, and number of fruits per plant (Table 2). These traits are more relevant for selection because their observed variation is more strongly associated with genetic differences among M2 individuals rather than only environmental fluctuation.

**Table 2.** Phenotypic and genotypic variation and variability criteria in the 100 Gy population

Character	$\sigma^2_p$	2.SD( $\sigma^2_p$ )	Criteria	$\sigma^2_g$	2.SD( $\sigma^2_g$ )	Criteria
Dichotomous height (cm)	17,15	8,28	Wide	9,03	6,01	Wide
Plant Height (cm)	51,80	14,39	Wide	4,80	4,38	Wide
Stem Diameter	1,53	2,48	Narrow	0,18	0,86	Narrow
Number of Branches	0,44	1,32	Narrow	0,03	0,33	Narrow
Flowering time (days after sowing)	6,32	5,03	Wide	3,84	3,92	Narrow
Leaf Length (cm)	1,11	2,11	Narrow	0,57	1,52	Narrow
Harvesting time (days after sowing)	1,03	2,03	Narrow	0,20	0,89	Narrow
Fruit Length (cm)	11,45	6,77	Wide	6,77	5,20	Wide
Weight per Fruit (g)	7,00	5,29	Wide	0,56	1,49	Narrow
Number of Fruits per Plant	542,57	46,59	Wide	130,00	22,80	Wide

Notes:  $\sigma^2_p$  = phenotypic variance;  $\sigma^2_g$  = genotypic variance; 2.SD = two standard deviations of the variance.

In the 200 Gy population, wide phenotypic variability was observed in dichotomous height, plant height, flowering time, fruit length, fruit weight, and number of fruits per plant. Nevertheless, wide genotypic variability was found only for number of fruits per plant (Table 3). This indicates that several traits expressed visible phenotypic differences at 200 Gy, but most of those differences were not sufficiently supported by estimated genotypic variance.

**Table 3.** Phenotypic and genotypic variation and variability criteria in the 200 Gy population

Character	$\sigma^2_p$	2.SD( $\sigma^2_p$ )	Criteria	$\sigma^2_g$	2.SD( $\sigma^2_g$ )	Criteria
Dichotomous height (cm)	10,26	6,41	Wide	2,15	2,93	Narrow
Plant Height (cm)	47,92	13,85	Wide	0,93	1,93	Narrow
Stem Diameter	1,41	2,38	Narrow	0,06	0,50	Narrow
Number of Branches	0,55	1,49	Narrow	0,14	0,76	Narrow
Flowering time (days after sowing)	5,18	4,55	Wide	2,70	3,29	Narrow
Leaf Length (cm)	1,51	2,45	Narrow	0,97	1,97	Narrow
Harvesting time (days after sowing)	1,03	2,03	Narrow	0,20	0,89	Narrow
Fruit Length (cm)	7,50	5,48	Wide	2,83	3,36	Narrow
Weight per Fruit (g)	7,24	5,38	Wide	0,80	1,79	Narrow
Number of Fruits per Plant	472,72	43,48	Wide	60,15	15,51	Wide

Notes:  $\sigma^2_p$  = phenotypic variance;  $\sigma^2_g$  = genotypic variance; 2.SD = two standard deviations of the variance.

The wide genotypic variability in the 100 Gy population indicates that this dose was more effective for producing exploitable diversity for initial selection. In the 200 Gy population, the occurrence of wide phenotypic variability but narrow genotypic variability in many traits implies that the observed differences may have been driven by environmental variation, physiological stress, or non-heritable damage. Similar findings have been reported in irradiated chili populations, where gamma irradiation generated morphological and yield variation, but not all variations were beneficial or heritable (Hashim et al., 2024; Nabila et al., 2025). Therefore, selection in the 200 Gy population should be more conservative and should prioritize traits supported by both genotypic variability and heritability.

### 3.3. Heritability and the implications of selection

Heritability values in the 100 Gy population were high for dichotomous height, flowering time, leaf length, and fruit length (Table 4). In the 200 Gy population, high heritability was observed for flowering time and leaf length. High heritability indicates that a relatively large proportion of phenotypic variation is attributable to genetic variance; however, high heritability alone is not sufficient for selection if genetic variability and expected genetic advance are low. Thus, heritability must be interpreted together with CGV and selection response.

**Table 4.** Heritability values and criteria in the 100 Gy and 200 Gy populations

Character	h <sup>2</sup> 100 Gy	Criteria	h <sup>2</sup> 200 Gy	Criteria
Dichotomous height (cm)	0,53	High	0,21	Moderate
Plant Height (cm)	0,09	Low	0,02	Low
Stem Diameter	0,12	Low	0,04	Low
Number of Branches	0,06	Low	0,26	Moderate
Flowering time (days after sowing)	0,61	High	0,52	High
Leaf Length (cm)	0,52	High	0,64	High
Harvesting time (days after sowing)	0,19	Low	0,19	Low
Fruit Length (cm)	0,59	High	0,38	Moderate
Weight per Fruit (g)	0,08	Low	0,11	Low
Number of Fruits per Plant	0,24	Medium	0,13	Low

Note: Criteria: low <0.20; moderate 0.20–0.50; high >0.50

Plant traits with high heritability include plant height, flowering time, leaf length, and fruit length in the 100 Gy population, followed by flowering time and leaf length in the 200 Gy population. Traits with high heritability increase the likelihood of successful selection because they can be passed on to subsequent generations. Rahmawati et al. (2019) state that high heritability values indicate that the inheritance of a trait is determined by a large genetic variance. Rosmaina et al. (2016) state that plant traits with high heritability values indicate that the environmental influence on the plant's performance is small.

### 3.4. Coefficient of Genetic Variation (CGV)

The CGV value indicates the magnitude of genetic variation relative to the trait mean. In this study, high CGV values were found only for fruit length in both irradiated populations (Table 5). Moderate CGV was found for the number of fruits per plant in the 100 Gy population and for leaf length in the 200 Gy population. Traits with high CGV and high heritability, such as fruit length at 100 Gy, are expected to respond better to selection than traits with high heritability but low CGV.

**Table 5.** Coefficient of Genetic Variation (CGV) in the 100 Gy and 200 Gy populations.

Character	CGV 100 Gy (%)	Criteria	KKG 200 Gy (%)	Criteria
Dichotomous height (cm)	8,87	Low	5,04	Low
Plant Height (cm)	3,29	Low	1,52	Low
Stem Diameter	5,20	Low	3,81	Low
Number of Branches	3,83	Low	9,44	Low
Flowering time (days after sowing)	5,22	Low	4,29	Low
Leaf Length (cm)	9,49	Low	12,08	Moderate
Harvesting time (days after sowing)	0,50	Low	0,50	Low
Fruit Length (cm)	22,17	High	18,21	High
Weight per Fruit (g)	7,71	Low	9,63	Low
Number of Fruits per Plant	12,09	Moderate	8,92	Low

Note: Low <10%; moderate 10–15%; high >15%.

High CGV values in the 100 Gy and 200 Gy populations were observed only for the fruit length trait. Characteristics with high CGV values possess high genetic variation, thereby having the potential to produce high-yielding individuals in subsequent generations. High CGV for fruit length in both irradiated populations indicates that irradiation generated substantial genetic variation for this trait. Nevertheless, fruit length at 200 Gy should be interpreted carefully because the mean fruit length was lower than the control, suggesting that part of the variation may be associated with unfavorable or deleterious mutations. Therefore, selection for fruit length should prioritize individual plants that maintain or improve fruit size while also retaining acceptable fruit weight and total fruit number. For 200 Gy, the high number of fruits per plant was accompanied by lower fruit size and low heritability, indicating that the increase in fruit number may not be sufficiently stable for direct selection without progeny testing. This is consistent with Febrianto et al. (2015), who noted that the higher the coefficient of genetic variation in a population, the greater the likelihood of successful

selection in increasing the frequency or number of desired genes. Moderate CGV values for the traits of number of fruits per plant and leaf length in both the 100 Gy and 200 Gy populations still have the potential to be used as selection traits. Moderate CGV for number of fruits per plant at 100 Gy indicates that this trait still has selection potential, especially because the mean number of fruits was markedly higher than the control. However, the moderate heritability value suggests that selection should be delayed or confirmed in the next generation to improve accuracy. Hartati et al. (2012) state that a moderate genetic diversity coefficient can still be considered in the selection process.

#### 4. CONCLUSION

Gamma-ray irradiation produced different responses in M2-generation local red chili cultivar 'Putih'. The 100 Gy population exhibited wide genotypic variability in dichotomous height, plant height, fruit length, and number of fruits per plant. In contrast, the 200 Gy population exhibited wide genotypic variability only in the number of fruits per plant. High heritability in the 100 Gy population was found for dicot height, flowering time, leaf length, and fruit length, whereas in the 200 Gy population, it was found for flowering time and leaf length. High KKG was observed for fruit length in both irradiated populations. Fruit length in the 100 Gy population is the most promising selection trait because it is supported by wide genotypic variability, high heritability, and high KKG. Further selection is recommended in the M3 generation to ensure the stability of the traits resulting from irradiation.

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