



RESEARCH ARTICLE

Gamma-ray radiation enhances agronomic trait diversity and °Brix content in landrace tomato (*Solanum lycopersicum* L.)

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Abstract

The objective of the present investigation was to examine the effect of gamma-ray irradiation doses on the diversity of agronomic characters and the increase in sugar content in the first mutant generation (M1) of landrace tomato. Seeds were exposed to five doses with five repetitions for each dose: Control, 125 Gray (Gy), 250 Gy, 375 Gy and 500 Gy. The research results showed that the lethal dose 50 (LD₅₀) value of the landrace tomato was 472.88 Gy. Gamma-ray irradiation significantly induced variation in fruit shape, plant height, flowering time, harvest time, number of fruits per plant, weight per plant and total soluble solid content, while stem diameter, fruit weight and fruit diameter remained unaffected. The estimated genotypic coefficient of variation (GCV) and heritability values were high for the characteristics of flowering age, number of fruits per plant, fruit weight per plant and total soluble solid content. Principal component analysis accounted for 87.20 % of the total variation. Notably, five mutant tomatoes exhibited superior total soluble solid (°Brix) characteristics. This research determined that the optimal irradiation dose to increase °Brix levels in the landrace tomato was 160.901 Gy.

Keywords: gamma irradiation; LD₅₀; mutation breeding; tomato; total soluble solids; variability

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide (1). Aside from its socio-economic importance, the tomato has become a model species for fleshy fruits because of its agronomic and genetic features and particularly as a rich plant source of carotenoids, vitamins and minerals (2, 3). Even though tomatoes are high in nutrition and are good for consumption, consumption of fresh tomatoes in Indonesia decreased by 0.40 % in 2022 (4). One of the factors causing a decrease in the consumption of fresh tomatoes is an unattractive appearance and the sour taste of the fruit (4–7). To align with consumer preferences for attractive shapes and sweeter flavours, local genetic resources like the 'Mawar' landrace require further development (8, 9). Although the 'Mawar' cultivar is heat-tolerant and possesses a unique flower-like shape, its sour taste limits consumer appeal. Therefore, an effective breeding technology is required to enhance the sweetness profile of this rose-shaped tomato.

However, the narrow genetic diversity within this landrace poses a significant constraint for conventional hybridisation.

Expanding genetic variability through the use of physical mutagens, such as gamma ray irradiation, represents a strategic and rapid approach to broaden the genetic base of the 'Mawar' landrace without significantly altering its desirable original characteristics. Mutation induction via gamma-ray irradiation is a well-established breeding technique that can be used to create a diversity of genetic resources that can be used to improve plant varieties (10). Gamma irradiation can induce genetic variations that result in agronomic yields and phenotypic traits distinct from those of the parental lines (11). The application of mutation induction using gamma rays is known to have been successful in increasing plant diversity and producing positive effects on the resulting characters.

Mutation activities in tomato plants to improve certain genetics have been widely carried out by researchers using gamma ray mutagens for the purpose of certain characters. Previous studies (12) reported that gamma-ray irradiation could cause tomato diversity where doses of 100 Gray (Gy) and 150 Gy can produce better physiological growth and flowering time. Similarly, evaluating 15 tomato genotypes resulting from gamma irradiation at doses of 495 Gy and 990 Gy yielded favourable outcomes and 3 tomato

genotypes were found that had high productivity and were resistant to fruit cracking (13). In mutant development, the effective dose in developing mutant plants is generally slightly below the lethal dose 50 (LD₅₀) value. Lethal dose 50 is the dose value that causes 50 % of the death of plants treated with mutations. A previous study (14) reported that the LD₅₀ value in tomato plants of the Kajagatota variety was 398.75 Gy, whereas another study (15) reported that the LD₅₀ value for germination of tomato plant variety first mutant generation (M1) (*Lycopersicum esculentum* cv. MT1) was 640 Gy.

Despite existing studies on LD₅₀ in tomatoes, the optimal dosage remains inconsistent across different cultivars and no specific data exists for the 'Mawar' landrace. Furthermore, there is a lack of information regarding gamma-ray required to increase total soluble solids (°Brix) in this commodity. To address these research gaps, this study aims to determine the precise LD₅₀ value for the 'Mawar' landrace and evaluate the impact of gamma-ray irradiation on enhancing its agronomic trait diversity and °Brix content, ultimately contributing to the development of superior tomato varieties that meet consumer preferences.

Materials and Methods

Study area

The research was carried out at the greenhouse of the Faculty of Animal and Agriculture Sciences, Universitas Diponegoro. Irradiation treatment was carried out at the Research and Technology Center for Isotope and Radiation Applications (PRTAIR), National Research and Innovation Agency (BRIN), using a Cobalt-60 Gammacell-220F model (Atomic Energy of Canada Limited (AECL), Canada).

Procedure

The experimental material used was landrace tomato seeds cv. (Mawar), irradiated with gamma rays using a Cobalt-60 Gammacell-220 radiation source. The study was arranged using a completely randomised design (CRD) with 5 dose treatments × 5 replications. The dose of gamma-ray irradiation given consisted of 5 levels, namely 0 Gy (Control), 125 Gy (Treatment (TM)-125), 250 Gy (TM-250), 375 Gy (TM-375) and 500 Gy (TM-500). Observations were made on qualitative characters, including fruit shape and quantitative characters, including plant height, flowering age, harvest age, number and weight of fruit per plant, diameter and weight per fruit and total soluble solid content (°Brix).

The determination of LD₅₀ is based on the percentage of plant germination 3 weeks after irradiation by making a regression graph using Curve Expert. The total soluble solid (°Brix) analysis was carried out on fruit samples for each repetition based on the total soluble solid value (°Brix) measured using a refractometer RHB-080 model from AMTAST USA Inc.

The determination of LD₅₀ is based on germination percentage at 3 weeks after planting by constructing a regression graph using Curve Expert. The germination percentage was calculated following the formula (16) given below:

$$GP (\%) = \frac{NT}{N} \times 100 \% \quad (\text{Eqn. 1})$$

Note:

GP = germination percentage; NT = proportion of the germinating seeds of each treatment; N = number of seeds used in bioassay.

Data analysis

The data obtained were analysed based on analysis of variance and continued with Tukey's Honestly Significant Difference (HSD) with a level of 5 %. An orthogonal polynomial test was performed to determine the optimal dose using Statistics Tool for Agricultural Research (STAR Version 2.1). Grouping analysis between mutants was analysed using principal component analysis (PCA) using the STAR Version 2.1. The genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated according to previously described methods (17, 18).

$$\sigma^2g = \frac{MSt - MSe}{r} \times 100 \% \quad (\text{Eqn. 2})$$

$$\sigma^2p = \sigma^2g + \sigma^2e \quad (\text{Eqn. 3})$$

Note:

σ^2g is the genotypic variance, MSt is the mean square of treatment, MSe is the mean square of error and r is the number of replications. σ^2p is the phenotypic variance, σ^2g is the genotypic variance and σ^2e is the mean square of error.

$$GCV = \frac{\sqrt{\sigma^2g}}{\text{Mean}} \times 100 \quad (\text{Eqn. 4})$$

Note:

The criteria for the relative genotypic coefficient of variation (GCV) are low (0 % ≤ x ≤ 25 %), somewhat low (25 % ≤ x ≤ 50 %), quite high (50 % ≤ x ≤ 75 %) and high (75 % ≤ x ≤ 100 %).

Results and Discussion

Germination percentage and the lethal dose 50 of Mawar cultivar

In the present study, the germination (%) of Mawar cultivar in different radiation treatments was calculated by considering control (untreated) as 100 % (Fig. 1). Overall, a substantial decrease in germination was observed among the treatments in comparison to the control. The maximum reduction in germination (%) was observed at 500 Gy treatment (48.83 % reduction over control) in Fig. 1.

Gamma ray irradiation on tomato seeds can determine the radiosensitivity of the Mawar cultivar of tomato, as indicated by the LD₅₀ values. The lethal dose (LD₅₀) is considered as a crucial factor to assess the level of radiosensitivity in plants. It quantifies a specific dose or concentration at which 50 % of the seeds are unable to germinate due to some biochemical and molecular changes (19).

The LD₅₀ value in the Mawar cultivar is shown based on the results of linear curve analysis using Curve Expert with the equation $Y = 85.51 - 0.255x + 0.0011x^2 - 1.46E^{-6}x^3$ (Fig. 2). Based on this equation, the LD₅₀ value of the Mawar cultivar is 472.88 Gy. The LD₅₀ value of this Mawar cultivar is different when compared to the LD₅₀ of other types of tomato plants. The LD₅₀ in 50 tomato genotypes ranged from 300 Gy to 450 Gy as the highest value (20). Then, based on the other research LD₅₀ in "micro-tom" tomatoes was not identified at radiation doses reaching 1000 Gy (19). Each variety has a different response to the mutation dose, so this difference has an important role in determining the effective dose for mutations for plants (21).

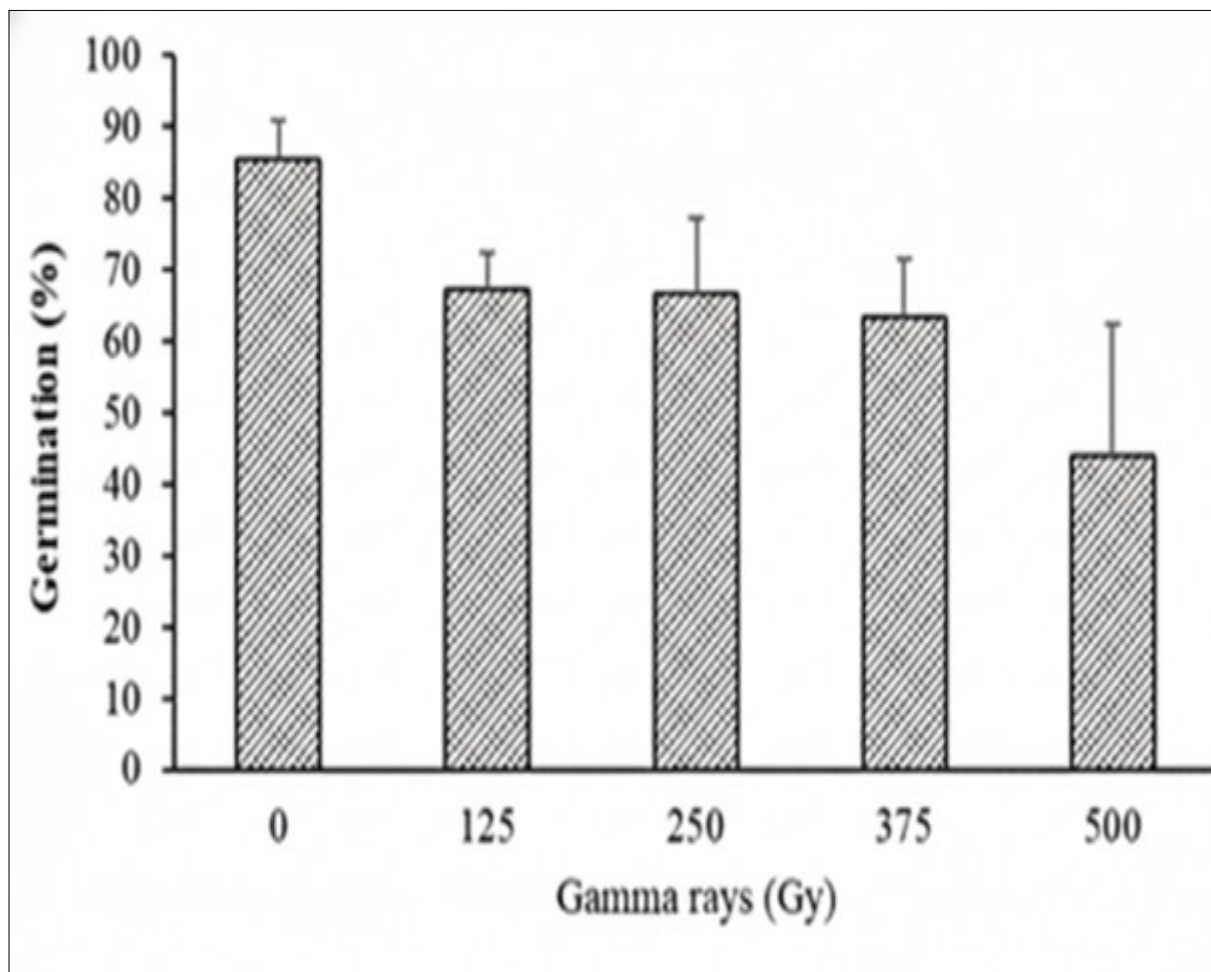


Fig. 1. Germination percentage of tomato under different gamma irradiation.

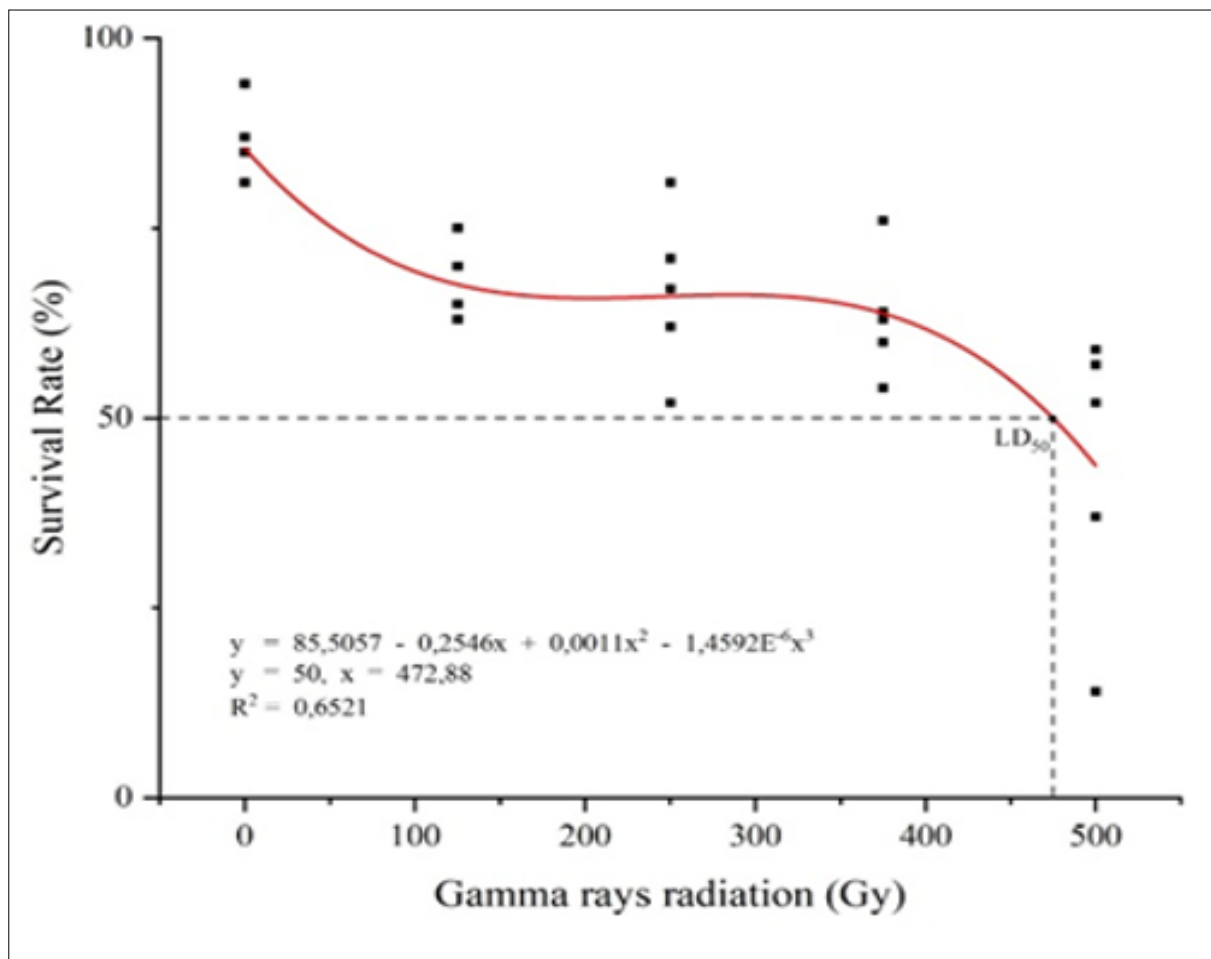


Fig. 2. Dose-response curve with polynomial fit.

Fruit shapes mutant tomato diversity

The results of observations on the characteristics of the shape of tomato fruit show that there is diversity. The diversity of the fruit shape and ribbing at the peduncle end is based on the International Union for the Protection of New Varieties of Plants (UPOV) Test Guidelines for Tomatoes. The mutation caused a change in the fruit shape of the fruit from flattened to oblate (Fig. 3) and the ribbing at the peduncle end of the fruit from strong to very strong (Fig. 4). The changes in fruit shape due to gamma-ray radiation occur due to the development of abnormal mutant cells into organ tissue that is different from the parent (22). Changes in fruit shape characteristics due to gamma-ray irradiation have been reported in previous studies. In pineapple, such irradiation has been shown to alter fruit shape, size, colour and quality compared to non-irradiated parent plants (23).

Mutants tomato quantitative traits diversity

The diversity of quantitative traits of mutant tomatoes consists of agronomy traits and total soluble content (°Brix). Gamma-ray radiation has a significant influence on several characters, namely, plant height, flowering age, harvest age, number of fruits per plant, fruit weight per plant and total soluble solid content (°Brix), while on the stem diameter characters, weight per fruit and fruit diameter did not have a significant effect (Table 1). The highest tomato plant height was recorded in the 375 Gy treatment, namely 183.8 cm, but it was not significantly different when compared with the 125 Gy and 250 Gy treatments. Plant height in tomatoes resulting from gamma-ray radiation began to decrease at treatment doses above 375 Gy, namely at a dose of 500 Gy. High doses above 375 Gy cause damage to cells, causing disruption to plant growth. Plant height resulting from gamma-ray radiation could decrease due to damage to the cell division process, which can disrupt the balance of plant growth hormones (24).

The dose of gamma-ray radiation has a significant effect on the characteristics of flowering time and harvest time. The 250 Gy dose was found to have the fastest average flowering age and was significantly different from other treatments. Mawar mutants that flower more quickly will also have a faster harvest time. This result is in accordance with the results of harvest age, where the 250 Gy treatment finds an average harvest age that is faster than other treatments. Providing gamma-ray radiation to Mawar cultivar is aimed at creating a wide variety to find superior characteristics such as a faster harvest time. Early maturing tomato plants will be of interest to farmers because they produce a faster harvest time over a longer period (25).

Gamma-ray radiation has a significant effect on the number of fruits per plant and fruit weight per plant. The highest number of fruits per plant was found by gamma ray radiation treatment with a dose of 250 Gy, which was significantly different from other treatments. The highest fruit weight per plant was also achieved by the treatment dose of 250 Gy but was not significantly different from the dose of 375 Gy. A high number and weight of fruit per plant will increase the productivity of tomatoes. Fruit yield characteristics are an important criterion in selecting plants to obtain tomato genotypes with high yields (26).

Gamma-ray radiation did not have a significant effect on fruit weight and fruit diameter. The lack of real differences is thought to be because this character is more influenced by the environment. The quantitative traits of tomato such as fruit length, fruit diameter and average fruit weight are influenced by plant interactions with the environment (27).

Total soluble solid content (°Brix) of mutant tomato

The results of the analysis of variance showed that gamma-ray irradiation had a significant effect on the total soluble solids content of fruits. The highest total soluble solid content was produced by fruit resulting from the 125 Gy treatment, but it was not significantly different from the 250 Gy treatment and the control. It was significantly different from the 375 Gy and 500 Gy treatments (Table 1). The sugar content of tomatoes increased at a dose of 125 Gy but continued to decrease at higher doses.

The quadratic regression curve resulting from the orthogonal polynomial test on the character of sugar content produces the equation $y = -0.00001243x^2 + 0.004x + 4.699$ with a value of $R^2 = 0.476$ (Fig. 5). The calculated dose points of 160.901 Gy shown as the highest total soluble solid content. However, exceeding that dose may reduce the total soluble solid content of the fruit produced. The increase and decrease in total soluble solid levels in tomatoes resulting from gamma-ray radiation are thought to be caused by the degradation of starch in the fruit. The sugar content or total soluble solids in tomatoes is produced by the degradation process of starch (carbohydrates) into simple sugars (28). Excessive doses of gamma rays are given to *Citrus limon* (L.) Burm. f. causes stress, thereby increasing phenolic and flavonoid levels to suppress the presence of reactive oxygen species (ROS) so that the energy used to hydrolyse starch into sugar decreased and cause a decrease in sugar levels in citrus fruit (29). The cells grown under any kind of stress may have to expend more metabolic energy than those grown in the absence of stress (30). Increased levels of phenolics and flavonoids are negatively correlated with sugar levels (29).

Table 1. Effect of gamma-ray radiation on quantitative traits of M1 generation tomato

Characters	Dose (Gray)				
	0	125	250	375	500
Plant height (cm)	151.8 ^b	178.4 ^a	176.8 ^a	183.8 ^a	171.8 ^{ab}
Stem diameter (cm)	1.4	1.4	1.5	1.3	1.4
Flowering days (DAP)	37.2 ^a	39.6 ^a	29 ^b	38.2 ^a	41.4 ^a
Harvesting days (DAP)	74.4 ^{ab}	76.6 ^a	67.2 ^b	74.8 ^{ab}	79.4 ^a
Fruit per plant	6.8 ^b	8.6 ^b	19.6 ^a	11.8 ^b	7.6 ^b
Fruits weight per plant (g. plant ⁻¹)	188.8 ^b	248.8 ^b	492.8 ^a	350.8 ^{ab}	188.6 ^b
Fruit weight (g. fruit ⁻¹)	29.8	29.2	25.4	29.0	25.2
Fruit diameter (cm)	4.3	4.6	4.8	4.9	4.3
Total soluble solid (°Brix)	4.6 ^{ab}	5.2 ^a	5.0 ^{ab}	4.1 ^{bc}	3.8 ^c

Foot note: Values followed by the same letter within a row are not significantly different at $p = 0.05$ (Tukey's Honestly Significant Difference); DAP: days after planting.

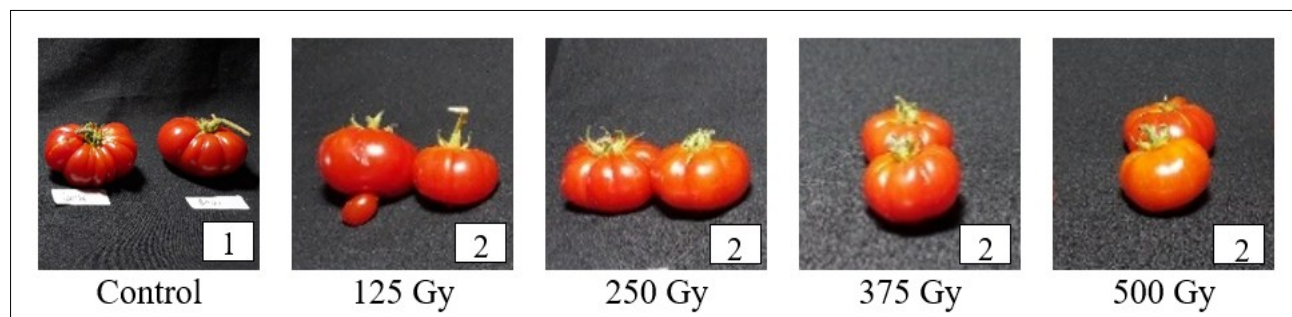


Fig. 3. Diversity of fruit shape. Description: (1) flattened; (2) oblate.

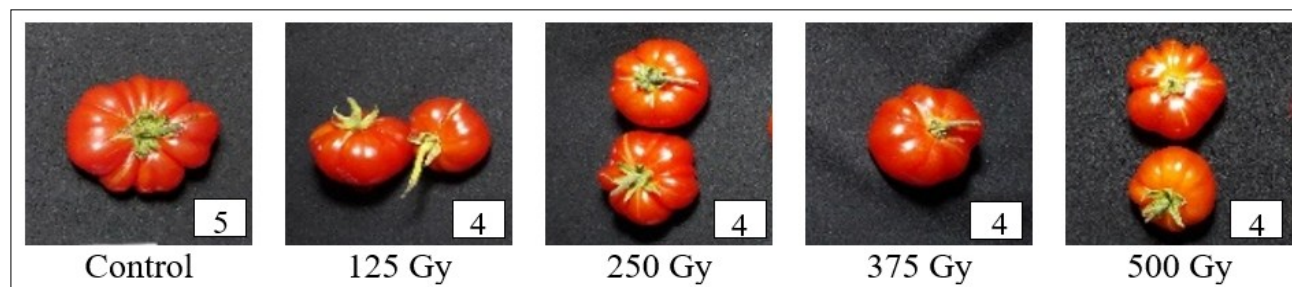


Fig. 4. Diversity of the ribbing at the peduncle end of the fruit. Description: (4) strong; (5) very strong.

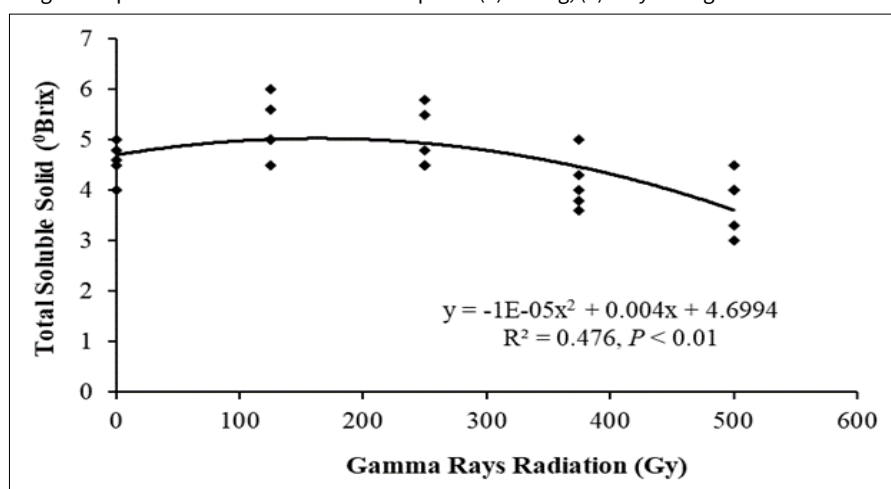


Fig. 5. The quadratic curve of the total soluble solid content of mutant tomato.

The genotypic coefficient of variation and heritability values of mutant tomato

The results of the analysis of the genotypic coefficient of variation (GCV) of nine quantitative characters of the M1 generation mutant tomato (Table 2) show that there are four characters, namely flowering age, number of fruits per plant, fruit weight per plant and total soluble solid content (°Brix) which has respective GCV that fall into the medium-high criteria.

These four characters have high diversity values, so they can be recommended as characters for the next breeding stage. Characters with high diversity have an important meaning in plant breeding because they will make new variation after the genetic segregate, which will influence the success of the breeding. In plant breeding, diversity or variability of characters in populations has an important meaning because, with wide diversity, the selection process will be more effective (31). The genotypic coefficient of variation, in a broad sense, are used to determine the genetic diversity of a character in a plant population. The GCV value can measure the presence of genetic diversity, which is used in comparing various plant characters, which can then indicate opportunities for effective improvements that can be made in plant breeding (32).

Principal component analysis

To study the similarities of all plants produced in the M1 generation of mutant tomato “Mawar cultivar” resulting from gamma-ray radiation, all observed quantitative and qualitative characters were reduced using principal component analysis. The results of the principal component analysis of 11 variables show that a total data diversity of 87.20 % was achieved in principal component IV (PC4) and the magnitude of the contribution of each character in principal components I (PC1) to IV (PC4) is presented in Table 3. Furthermore, it can be seen that using two main components, the representation of mutant tomato diversity for 11 variables reached 63.90 % (Table 3) with a grouping pattern using biplot analysis, as presented in Fig. 6.

The results of the principal component analysis form 11 main components or PCs with the first (PC1), second (PC2), third (PC3) and fourth (PC4) principal components explaining a total diversity of 87.20 % with an eigenvalue of 0.896 on PC4, 1.667 on PC3, 2.467 on PC2 and 4.562 on PC1 (Table 3). The cumulative diversity contribution of 87.20 % in PC1, PC2, PC3 and PC4 in this study can represent the entire diversity of characters of tomato plants resulting from gamma-ray radiation. Based on previous studies (33), principal component analysis has the function of

Table 2. Genotypic coefficient of variation and heritability of M1 generation mutant tomato

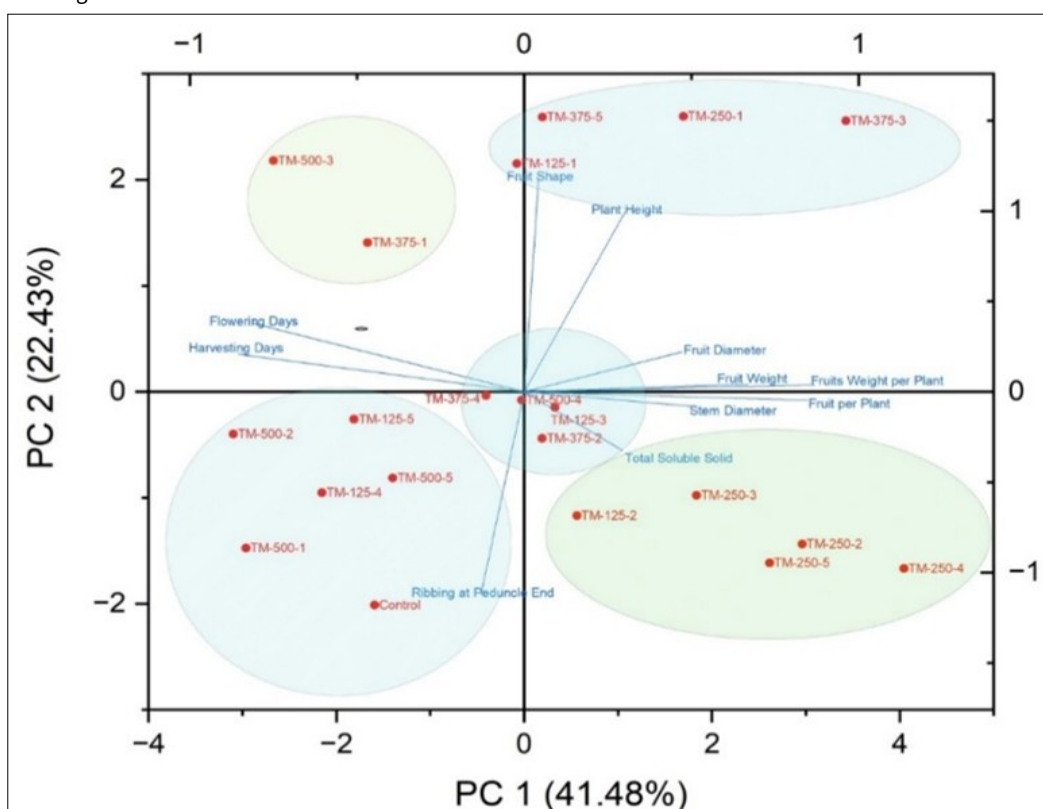
Characters	σ^2g	σ^2e	σ^2f	GCV	Criteria
Plant height (cm)	119.6	1164.4	284.0	6.34	Rather low
Stem diameter (cm)	0.001	0.02	0.02	1.97	Low
Flowering days (DAP)	20.5	11.9	32.4	12.2	High
Harvesting days (DAP)	16.1	21.9	37.9	5.4	Rather low
Fruit per plant	0.09	0.07	0.17	14.17	High
Fruits weight per plant (g. plant ⁻¹)	0.74	0.93	1.67	13.4	High
Fruit weight (g. fruit ⁻¹)	-0.01	0.086	0.074	0	Low
Fruit diameter (cm)	0.04	0.227	0.263	4.12	Rather low
Total soluble solid (°Brix)	0.31	0.3	0.606	12.17	High

Description: Absolute GCV criteria: low 0–3.54 %; rather low 3.54–7.09 %; quite high 7.09–10.63 % and high 10.63–14.17 %; GCV: genotypic coefficient of variation.

Table 3. Eigenvalue, proportion of diversity, cumulative diversity and factor loading of M1 generation mutant tomato “Mawar cultivar”

Traits	PC1	PC2	PC3	PC4
Plant height (cm)	0.151	0.499 ⁺	0.143	0.245
Stem diameter (cm)	0.256	-0.041	-0.426 ⁺	-0.400 ⁺
Flowering days (DAP)	-0.407 ⁺	0.190	0.207	0.028
Harvesting days (DAP)	-0.428 ⁺	0.104	0.041	0.171
Fruit per plant	0.424 ⁺	-0.024	-0.128	0.029
Fruits weight per plant (g. plant ⁻¹)	0.435 ⁺	0.020	0.011	0.066
Fruit weight (g. fruit ⁻¹)	0.335 ⁺	0.022	0.471 ⁺	-0.034
Fruit diameter (cm)	0.236	0.111	0.590 ⁺	-0.001
Total soluble solid (°Brix)	0.147	-0.163	-0.252	0.855 ⁺
Fruit shape	0.021	0.590 ⁺	-0.169	0.053
Ribbing at peduncle end	-0.064	-0.561 ⁺	0.273	0.096
Eigenvalue	4.562	2.467	1.667	0.896
Variability (%)	41.48 %	22.43 %	15.15 %	8.14 %
Cumulative (%)	41.48 %	63.90 %	79.06 %	87.20 %

Description: (°) contributing characters

**Fig. 6.** Biplot analysis.

reducing the number of observed characters into several main components that can represent the total diversity of the existing population. The PC1 provides a diversity contribution of 41.48 % with influential characters, namely flowering days and harvesting days, with a negative contribution and the characters fruit per plant, fruits weight per plant and fruit weight with a negative contribution. The PC2 provides a diversity contribution of 22.43 % with influential characters, namely plant height. Fruit shape with a positive contribution and the character ribbing at peduncle end

with a negative contribution. The PC3 provides a diversity contribution of 15.15 % with influential characters, namely fruit weight, fruit diameter with a positive contribution and stem diameter with a negative contribution and PC4 provides a diversity contribution of 8.14 % with influential characters, namely total soluble solid with a positive contribution and stem diameter with a negative contribution. The positive and negative contribution values for each character will play a role in grouping plants through principal component analysis. Positive contribution values to

characters will cause grouping. Whereas conversely, negative contribution values to characters will cause separation between groups in different directions (34, 35).

Biplot analysis based on the results of principal component analysis (Fig. 6) shows grouping based on character contribution to two components namely principal component 1 (PC1) and principal component 2 (PC2). Biplot analysis in principal component analysis aims to group observed genotypes by reducing the diversity of observed characters into several main components so that closeness between observed objects and correlation between characters can be obtained (36). Biplot analysis shows that plants resulting from gamma-ray radiation in the M1 generation can be grouped into 5 different groups. Group 1 consists of plants TM-375-4, TM-500-4, TM-500-4, TM-125-3 and TM-375-2 which are located far from other plants. Group 2 consists of plants TM-125-1, TM-375-5, TM-250-1 and TM-375-3 which are similar in terms of plant height and longitudinal fruit shape. Group 3 consists of TM-500-3 and TM-375-1 plants located far from other plants. Group 4 consisted of control plants, TM-500-1, TM-125-4, TM-500-5, TM-125-5 and TM-500-2, which had similar characteristics in the curve of the base of the fruit. Group 5 consists of plants TM-250-3, TM-125-2, TM-250-2, TM-250-5 and TM-250-4, which have similarities in the total soluble solid ($^{\circ}$ Brix) character.

Conclusion

The lethal dose 50 value of rose variety tomatoes is 460.23 Gray. Gamma-ray irradiation causes diversity in the quantitative and qualitative characteristics of plants. Gamma-ray irradiation induced phenotypic changes in plant height, flowering time, harvest time, number of fruits, fruit weight per plant and sugar content, so 160.901 Gy is the optimal dose to increase total soluble solid ($^{\circ}$ Brix). High genotypic coefficient of variation and heritability values were found in flowering age, number of fruits, fruit weight per plant and sugar content. The principal component analysis shows that several main components can explain 87.20 % of the data variations, with TM-250-3, TM-125-2, TM-250-2, TM-250-5 and TM-250-4 having similarity to total soluble solid ($^{\circ}$ Brix).

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Authors' contributions

PPI and FPP conceived and designed the research. PPI, MGAS and AFI performed the experiments and collected the data. SA, K and R analysed the data. FK and BH contributed reagents, materials and analysis tools. PPI wrote the original draft. FPP, SA and FK reviewed and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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