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MUTAGENESIS THROUGH GAMMA IRRADIATION IN CHILI (*CAPSICUM ANNUUM* L. AND *CAPSICUM FRUTESCENS* L.)

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SUMMARY

Gamma-ray irradiation commonly served as a mutagenic agent to induce genetic variability in crop species. However, the appropriate gamma-ray irradiation dose requires determining before starting a mutagenesis-based breeding program. The presented study sought to determine the lethal dose (LD_{50}) and the growth reduction dose (GR_{50}) in *Capsicum annuum* cv. Kopay and *Capsicum frutescens* cv. Bara. The seeds of both cultivars sustained gamma-ray doses irradiation of 0, 200, 300, 400, and 500 Gray (Gy). This study found the seed germination percentage, survival percentage, and seedling height of the Kopay and the Bara cultivars decreased with increased gamma-ray doses compared with the control due to seed injury and impact on poor growth seedlings. The LD_{50} and GR_{50} values of the Bara were 257.64 and 246.10 Gy, respectively, while for Kopay, these were 333.56 and 318.61 Gy, respectively. The LD_{50} of the gamma ray for the Kopay was higher than the Bara, indicating the Kopay variety has less sensitivity to gamma-ray irradiation than the cultivar Bara. The study results can be beneficial for mutagenesis-based plant breeding programs in *Capsicum.*

Keywords: Chili (*C. annuum* L., *C. frutescens* L.), cultivars, acute irradiation, gamma ray, radiosensitivity

Key findings: The LD_{50} values of the chili (*C. annuum* L. and *C. frutescens* L.) cultivars Bara and Kopay were 257.64 and 333.56 Gy, respectively. The cultivar Kopay proved less sensitive to gamma-ray irradiation than the cultivar Bara. Chili breeders can use the LD_{50} values in this study to obtain mutant plants with gamma rays.

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INTRODUCTION

Chilies (*Capsicum annuum* L. and *Capsicum frutescens* L.) belong to the family Solanaceae. These species are important vegetables and spices widely cultivated in tropical and subtropical regions. In Indonesia, these two species become the choice of farmers for their cultivation because of their relatively high selling price. *Capsicum* species have many nutrients, including vitamins A, C, and E, carotenoids, capsaicin, and macro- and micronutrients, with an essential role in pharmaceuticals, such as antioxidant, anti-inflammatory, anticancer, antimicrobial, and anti-obesity effects (Olatunji and Afolayan, 2018; Azlan *et al.,* 2022; Ao *et al.,* 2022).

Genetic variability is one of the prerequisites for the success of a breeding program. However, the genetic base of *C. annuum* and *C. frutescens* has narrowed due to continuous selection in breeding activities and the use of genotypes adapted continuously. Fu (2015) and Louwaars (2018) stated a series of breeding activities affected a decrease in the genetic variability within the crop. Therefore, mutation induction is essential in both species to produce the desired genetic variation for inheritance through plant breeding. The chili breeding's extreme challenge is how to obtain high-yielding cultivars resistant to biotic and abiotic stress and improve fruit quality for specific consumer preferences or market needs.

Among various mutagenic agents, gamma rays are commonly effective for the induction of genetic diversity in numerous plants, such as chili (Gaswanto *et al.,* 2016; Aisha *et al.,* 2018; Arumingtyas and Ahyar, 2022), buckwheat (Ahmad *et al.,* 2023), chickpea (Amri-Tiliouine *et al.,* 2018), rice (Gowthami *et al.,* 2017), faba bean (Khursheed *et al.,* 2018), and watermelons (Ernest *et al.,* 2020). Gamma rays have advantages compared with other mutagens due to their simple application, high penetration power, and high frequency of gene mutations (Ernest *et al.,* 2020; Katiyar *et al.,* 2022). The genetic base, the applied dose, and the exposure duration influence the effectiveness of mutagens to induce genetic variation in plants. Therefore, the precise dose of gamma irradiation requires detection individually for each plant species to obtain highly productive plants resistant to biotic and abiotic stress with good fruit quality.

The main parameter used to determine the appropriate dose of gamma irradiation and cause mutation in the crop is the lethal dose (LD_{50}). Induction of treatment causes high phenotypic variation and produces genetics that may be essential to select individual plants with favorable traits. According to some researchers, the dose at which 50% of the total irradiated individuals die was most likely to produce new variability for genetic improvement (Sood *et al.,* 2016; Tarigan *et al.,* 2020).

Besides LD_{50}, a dose that causes a 50% reduction in growth (GR_{50}) has a strong chance of causing a successful mutant (Roy *et al.,* 2019; Álvarez-Holguín *et al.,* 2019; Ghasemi-Soloklui *et al.,* 2023). Researchers explained that the LD_{50} and GR_{50} parameters relied on the assumption that low irradiation doses have minor effects on the genome and rarely cause morphological variations. However, high irradiation doses can produce many changes in genetic material and usually cause unfavorable abnormalities. According to Álvarez-Holguín *et al.* (2019), the dose at LD_{50} or GR_{50} increases the chances of producing viable and valuable mutants for breeding programs using mutagenesis techniques. Thus, identifying LD_{50} and GR_{50} values was the initial step in mutation breeding methods, as reported by Shrivastava *et al*. (2021) and Chakraborty *et al.* (2023). Reports on the GR_{50} of gamma-ray doses on chili are insufficient and need further pursuing.

The literature review shows a wide variation in the optimal gamma-ray dose among chili cultivars (Nura *et al.,* 2015; Gaswanto *et al.,* 2016). For *C. annuum*, Nura *et al.* (2015) reported the LD_{50} values for three chili genotypes, IPB C2, IPB C10, and IPB C15, were 317.93, 591.42, and 538.88 Gy, respectively. Meanwhile, Gaswanto *et al.*

(2016) obtained the LD_{50} values for five chili cultivars (Tanjung-2, Lembang-1, Kencana, SSP, and Seloka) at 422.64, 448.84, 538.41, 614.79, and 629.68 Gy, respectively. For *C. frutescens*, Makhziah and Soedjarwo (2023) stated the LD_{50} value for the local cultivar of Tulungagung and the local cultivar of Ponorogo were 409.52 and 453.74 Gy, respectively. This variation may be in relation to the cultivars' genetic background and application dose. The improvement of chili peppers through the creation of variability using gamma rays would enable the selection of high-yielding genotypes with improved agro-morphological characteristics and increase crop productivity. The presented study sought to investigate the effect of gamma-ray irradiation on seed germination, survival percentages, and seedling height of *Capsicum annuum* var. Kopay and *Capsicum frutescens* var. Bara and determine the LD_{50} and GR_{50} in both species.

MATERIALS AND METHODS

Plant material

This study used *Capsicum annuum* L. var. Kopay and *Capsicum frutescens* L. var. Bara seeds. The seeds came from the local market in Pekanbaru, Indonesia.

Gamma-ray irradiation treatments

The seeds' treatment with gamma rays had doses of 0, 200, 300, 400, and 500 Gy at the dose rate of 15 Gy/min emitted from ^{60}Cobalt gamma as a source at the Isotope and Radiation Applied Center, National Nuclear Energy Agency (BATAN) of Indonesia. The selection of gamma-ray doses for irradiation was dependent on the report of Nura *et al.* (2015), who concluded the effectiveness of using gamma irradiation ranged from 0 to 500 Gy. Irradiated seeds were then specimens for further studies.

Radiosensitivity test

The sowing of 100 irradiated seeds for each treatment continued on the paper tissue before placing at room temperature at the Breeding and Reproduction Laboratory, Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau, Indonesia. Watering was daily to keep the moisture to ensure optimal germination. When seedlings were five days old, their transfer into polybags (15 cm × 10 cm) took place containing topsoil and manure with a ratio of 1:1, before placing them under glasshouse conditions. The experiment, laid out in a completely randomized design (CRD), received five different treatments of gamma-ray doses.

Data collection

The parameters observed included germination percentage, seedling survival, and seedling height for each gamma-irradiation treatment. The observations on germination transpired 15 days after sowing (DAS) by visually counting the number of germinated seeds for each treatment, with calculations as follows:

$$Germination\ percentage\ (\%) = \frac{Total\ number\ of\ germinated\ seeds}{Total\ number\ of\ seed\ sown}\ x\ 100\%$$

The survival percentage, as evaluated at the 15th DAS, resulted from relating the number of plants alive to the number of germinated seeds sown, with computations as follows:

$$Survival\ percentage\ (\%) = \frac{Total\ number\ of\ seedlings\ survive\ after\ germination}{Total\ number\ of\ germinated\ seed}\ x\ 100\%$$

Seedling height measurement began from above the soil surface to the tip of the primary leaf using a ruler at the 30th DAS, expressed in centimeters.

Data analysis

All the data underwent the analysis of variance (ANOVA) to determine the variation among the gamma-ray doses of chili using the general linear model procedure (PROC-GLM). Mean comparisons used the Duncan Multiple Range Test at the significant level of $P < 0.05$. Analysis employed the Statistical Analysis System (SAS) program version 9.00. Determining the LD_{50} relied on the mortality percentage, while the GR_{50} depended on seedling height. The detection of LD_{50} and GR_{50} doses further used a simple linear regression with an equation y = ax + b, where y is the response variable (mortality percentage or seedling height), x is the independent variable (gamma-irradiation dose), and a and b represent the slope and constant, respectively. Thereafter, obtaining the readings corresponding to 50% of lethal doses and growth reduction.

RESULTS AND DISCUSSION

Seed germination percentage

The germination percentage was one of the most pivotal parameters highly considered in plant mutagenesis experiments because the plant mortality rate indicates the magnitude of damage caused by gamma-ray irradiation exposure to seeds. The seed germination percentage of the chili cultivars Kopay and Bara appears in Figures 1 and 2. Treatments of gamma-ray irradiation showed significant variations ($P < 0.01$) in the seed germination percentage of the cultivar Bara but not significant in the cultivar Kopay. Gamma-ray radiation significantly decreased the seed germination percentage in the cultivar Bara compared with the control (Figure 1). Gamma-ray radiation of 200 Gy resulted in a germination percentage of 90%, then declined sharply with increasing gamma radiation dose, namely, 71% at 300 Gy, 16% at 400 Gy, and 14% at 500 Gy. A different response was visible in the cultivar Kopay (Figure 2), where various radiation doses produced a germination percentage that was similar to the control. This result shows varied cultivars respond differently to gamma radiation, and the cultivar Bara was more responsive to gamma irradiation than the cultivar Kopay.

Several researchers have also reported a decrease in the germination percentage due to gamma-ray radiation (Gaswanto *et al.,* 2016; Muhammad *et al.,* 2021; Makhziah and Soedjarwo, 2023). This decrease occurs because higher doses of gamma-ray irradiation can inhibit the functions of vital cells and ultimately cause the death of the embryo or specific cells in the seeds. Mutations in the DNA of chili seeds can make the seeds not germinate. Likewise, the decline in the percentage of seed germination observed from this study may correlate to changes in enzymatic activity, which causes reduced seed germination, or to the inhibitory action by mutagens on the plumule and radicle. These results agree with Ke *et al.* (2019) and Olaolorun *et al.* (2019). The authors revealed the mutagen treatment, particularly at higher dose levels, caused changes in enzyme action and inhibition, reducing germination percentage among the treated samples. Moreover, compounds, such as proteins, chlorophyll a, chlorophyll b, auxins, and ascorbic acid, as directly related to plant metabolism, can also suffer damage or changes due to gamma-ray irradiation, potentially inhibiting seedling germination.

This study also found some seeds exposed to higher gamma-ray irradiation germinated but died shortly. The results were in line with past studies reported by Olasupo *et al.* (2016), where seeds exposed to higher doses of mutagens may not germinate, and germinated seedlings cannot survive more than a few days after germination. Similarly, research conducted by Tan *et al.* (2023) showed that gamma-ray irradiation can inhibit plant growth due to destroyed cells and plant cell structures, hence hindering the cell division process and stopping cell division.

In the cultivar Kopay, gamma-ray radiation did not affect seed germination percentage (Figure 2). A similar result came from López-Mendoza *et al.* (2012) on chili pepper plants and Ahumada-Flores *et al.* (2020) on wheat, which showed trying various gamma-ray radiations was not significant to seed germination percentage. This study proved that the effectiveness of gamma-ray radiation depends on the genotypes used. Du *et al.* (2022) and Zafar *et al.* (2022) also declared the same results. However, our result contrasts with Samuel *et al.* (2021), who disclosed that the three cultivars of chili (*C. annuum*, *C. frutescens*, and *C. chinensis*) were not significantly different in seed germination percentage.

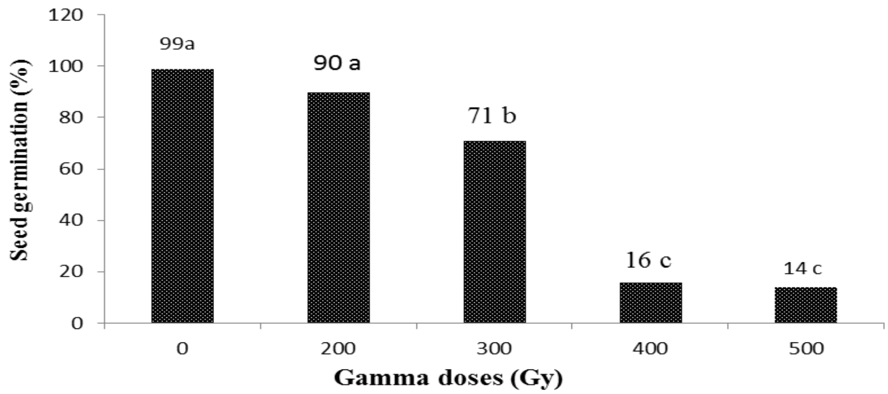

Figure 1. Seed germination percentage of *C. frutescens* var. Bara at different doses of gamma ray.

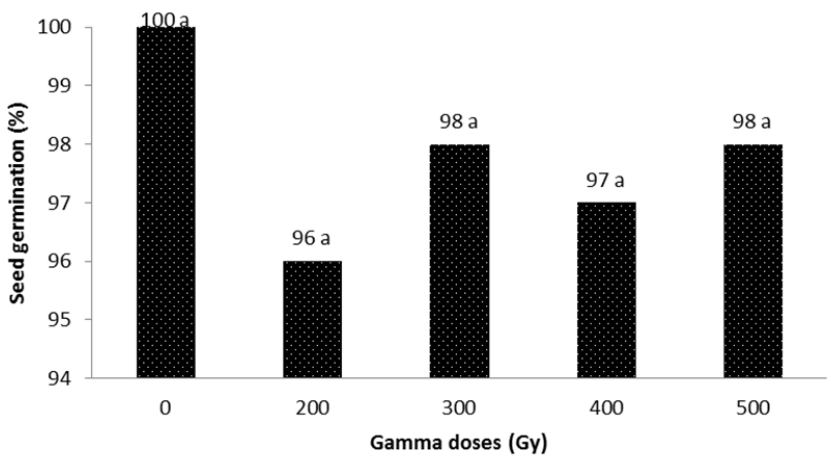

Figure 2. Seed germination percentage of *C. annuum* var. Kopay at different doses of gamma ray.

Seedling survival percentage

The seedling survival percentage of the Bara and Kopay cultivars for the gamma-ray irradiated treatments is visible in Figure 3. A highly significant difference ($P < 0.01$) resulted in the survival percentage among the gamma-ray treatments for each chili cultivar, wherein the extent of survival seedlings tended to decrease as the gamma-ray irradiation dose increased. The cultivar Bara showed a significant reduction in survival percentage at 200 Gy (84%) compared with the control (89%); increasing the radiation dose reduced the survival percentage to 4% at 400 Gy. In contrast to Kopay, gamma-ray radiation of 200 Gy has insignificantly lowered the survival percentage (96%) compared with the control (100%). The decrease in survival percentage was notable at 300 Gy to 79%. Increasing the radiation dose decreased the survival percentage to 29% at 400 Gy, and no plant survived (0%) at 500 Gy. In both cultivars tested, it can be evident that the higher the radiation dose given, the fewer plants can survive, but the cultivar Kopay was more tolerant to gamma-ray radiation; this can be visible from the seed germination rate and survival percentage of Kopay being higher than cultivar Bara. These results confirm that genotype determines the effectiveness of gamma-ray radiation. These results closely agreed with study reports from Gaswanto *et al.* (2016) and Makhziah and Soedjarwo (2023) in chilies.

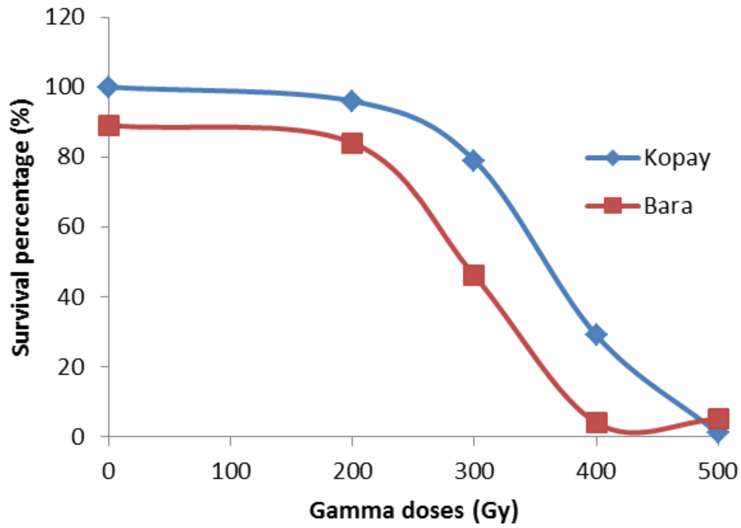

Figure 3. The survival percentage of *C. annuum* var. Kopay and Bara at different doses of gamma ray.

This study revealed a significant decrease in the survival percentage with increasing gamma-ray irradiation dose. Reports of similar results occurred in Wilman lovegrass (Álvarez-Holguín *et al.*, 2019), pigeon pea (Ariraman *et al.,* 2014), and Bambara groundnut (Muhammad *et al.,* 2021). The decrease in plant survival percentage at higher mutagen treatments could be due to the inhibitory effect of gamma irradiation on the meristematic tissues of the seeds, resulting in injury to the chromosome structure. Siahpoosh *et al.* (2020) stated that the increase in the rate of chromosome damage with increasing gamma radiation dose may be the reason for the decline in plant survival percentage.

The stimulatory effect of gamma radiation was noteworthy in low- and medium-gamma radiation treatments on two chili cultivars. The chili cultivars used in this study mainly probed plant growth and physiological development. Similar results appeared in wheat (Olaolorun *et al.,* 2019) and cauliflower (Ke *et al.,* 2019). Higher doses of mutagens caused modifications in the molecular properties of plants, which could lead to severe inhibition of plant survival percentage among the mutagenized plant materials. Similarly, their studies revealed compounds, such as proteins, chlorophyll, and plant growth promoters, directly related to plant metabolism, could incur damages or alterations by gamma irradiation treatment and potentially inhibit plant survival.

Gamma-ray irradiation effect on seedling height

Gamma radiation treatment had a significant effect ($P < 0.001$) on the height of seedlings of cultivars Kopay and Bara (Figure 4). The highest seedling height of the cultivar Kopay was 14.76 cm, observed in the control, while the lowest seedling height (6.027 cm) emerged in the 400-Gy treatment. The ultimate reduction of seedling height was 59.16% in 400 Gy compared with the control, followed by 300 Gy (34.48%). For the cultivar Bara, seedling height ranged from 1.65 to 12.10 cm, where the tallest seedling height manifested in the control treatment, and the shortest was visible in the 300-Gy treatments. The maximum reduction percentage of seedling height (84%) occurred in the 300-Gy treatment compared with the control. In the 500-Gy treatment for the cultivar Kopai and the 400-Gy and 500-Gy treatments for the cultivar Bara, all seedlings died before taking their height measurements.

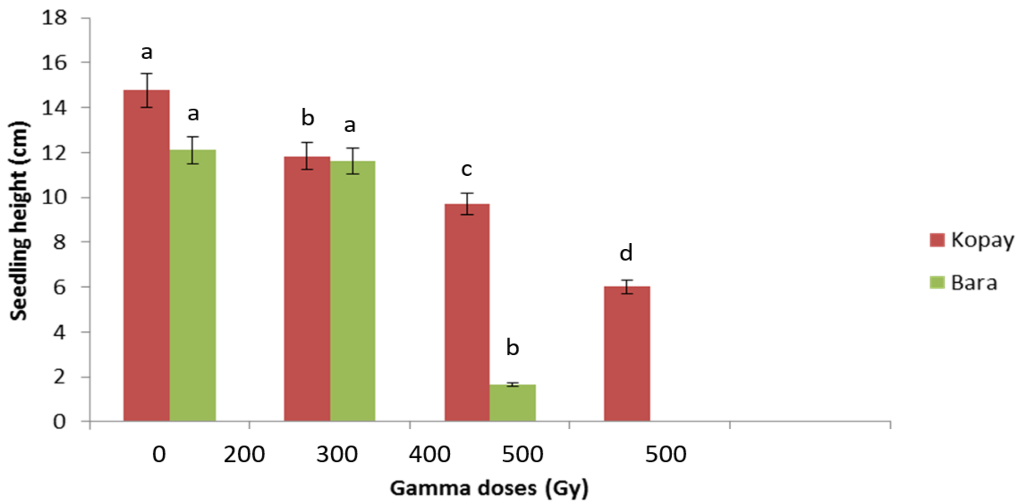

Figure 4. The seedling height of the *C. annuum* var. Kopay and *C. frutescens* var. Bara at different doses of gamma ray.

This study found that increasing the dose of gamma-ray irradiation declined the seedling height. Results were in line with those reported by Gaswanto *et al.* (2016), Sood *et al.* (2016), and Makhziah and Soedjarwo (2023) in chili; Yadav *et al.* (2019) in maize; Ernest *et al.* (2020) in watermelon; Kiani *et al.* (2022) in wheat; and Qosim *et al.* (2024) in adlay (*Coix lacryma-jobi* L.). The decrease in seedling growth may refer to the effect of mutagenic radiations that can directly cause DNA double-strand breaks and then damage to mitotic activity in the meristem tissue (Yadav *et al.,* 2019). This also gained support from Li *et al.* (2021), who explained that gamma-ray irradiation causes DNA damage in plant cells and results in various types of injuries to plant cell division, plant growth, and development processes. Sood *et al.* (2016) stated that the seeds exposed to high-dose gamma result in poor plant growth performance because mutagens can instill blockages in cellular DNA, stopping or slowing down plant growth.

Radiosensitivity

The radiosensitivity can estimate the radiation dose to cause the highest rate of mutation with minimal effect on the crop genome. The LD_{50} and GR_{50} values are standards for radiosensitivity tests. The linear regression equation of lethal dose for the cultivar Bara was Y = 0.1969X-0.7297 and R^2 = 0.8516 (Figure 5), and the LD_{50} value obtained was 257.64 Gy. For the cultivar Kopay, the linear regression equation obtained was Y = 0.2054X–18.514 and R^2 = 0.8116 (Figure 6), and the LD_{50} value obtained was 333.56 Gy. The linear regression equation of growth reduction for the cultivar Bara was Y = 0.2392X-8.8675 and R^2 = 0.7988 (Figure 7), with the acquired GR_{50} value being 246.10 Gy. For the cultivar Kopay, the linear regression equation obtained was Y = 0.1905X-10.696 and R^2 = 0.8997, and the GR_{50} value acquired was 318.61 Gy (Figure 8). These results revealed that the cultivar Kopay has less sensitivity to gamma-ray irradiation treatment when compared with the cultivar Bara. This difference indicates that varied species from the same family can differ in their sensitivity response to gamma-ray irradiation. A similar finding was a statement by Gaswanto *et al.* (2016) and Makhziah and Soedjarwo (2023) in chili, Chakraborty *et al.* (2023) in wheat, and Muhammad *et al.* (2021) in groundnut.

The LD_{50} value in this study was almost similar to previous studies declared by Makhziah and Soedjarwo (2023), who found 409.52 and 453.7 Gy for *Capsicum annuum*

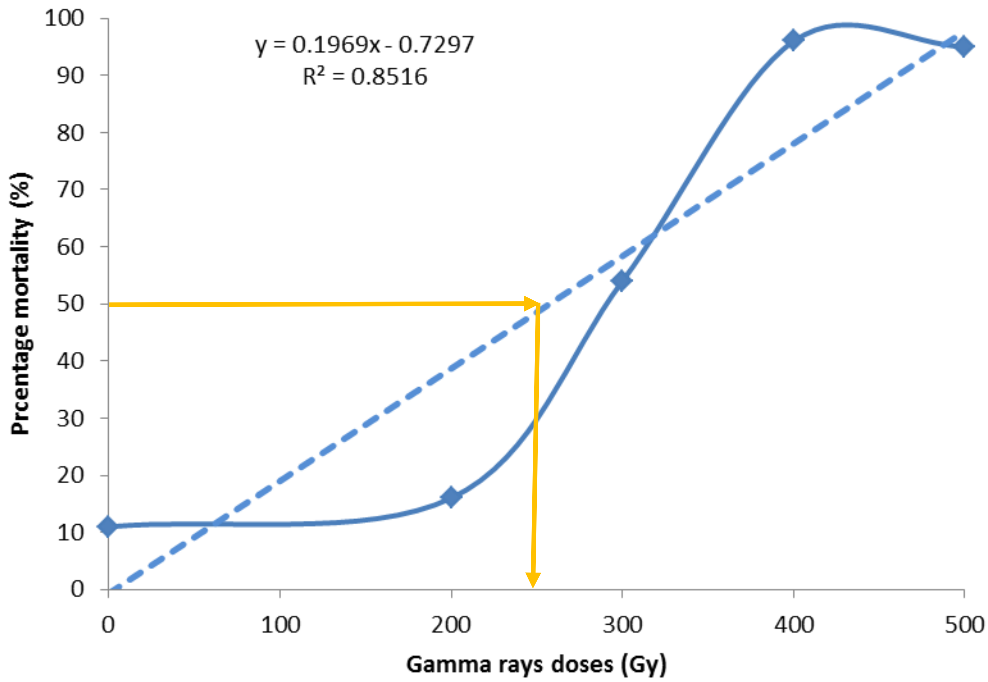

Figure 5. Analysis LD_{50} of *C. frutescens* var. Bara irradiated with different doses of gamma ray.

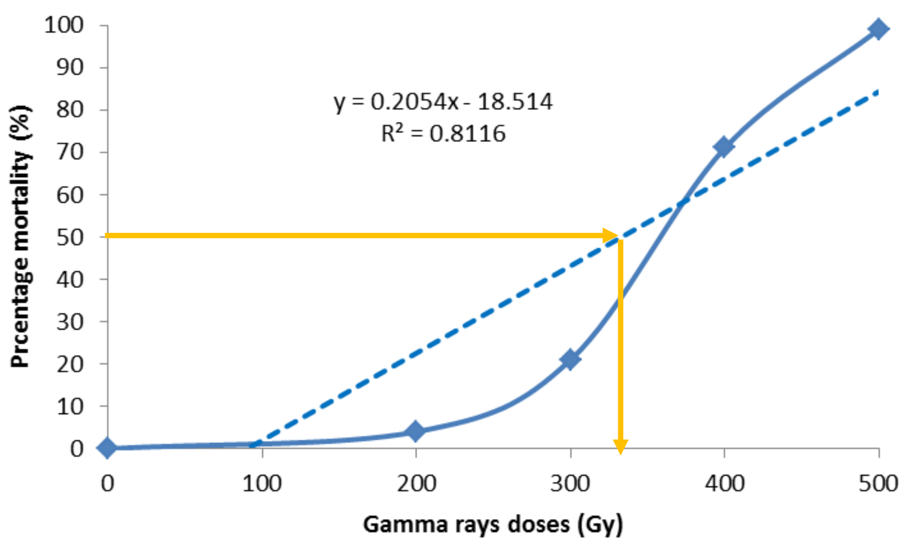

Figure 6. Analysis LD_{50} of *C. annuum* var. Kopay irradiated with different doses of gamma ray.

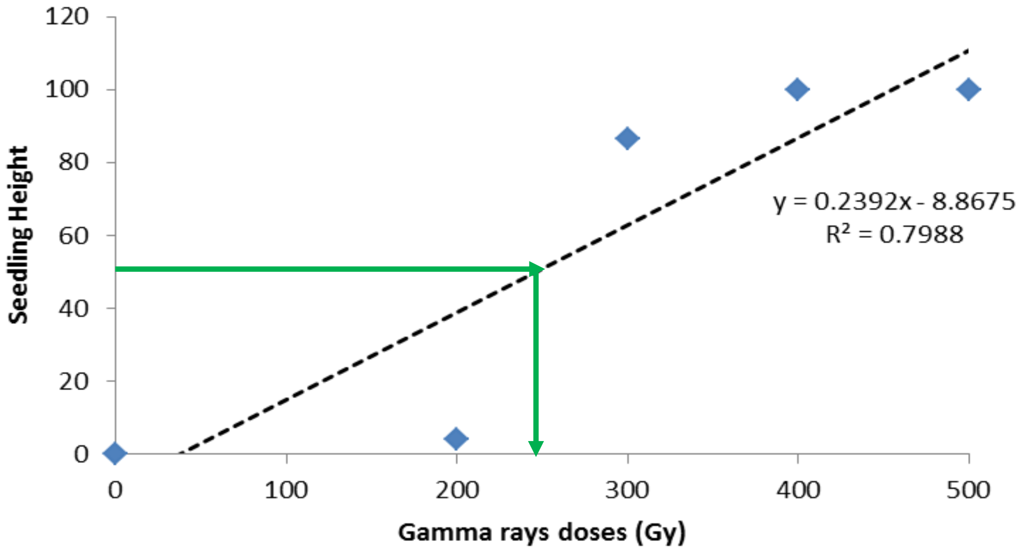

Figure 7. Effect of gamma irradiation on growth reduction (GR_{50}) of seedling height of *C. frutescens* var. Bara.

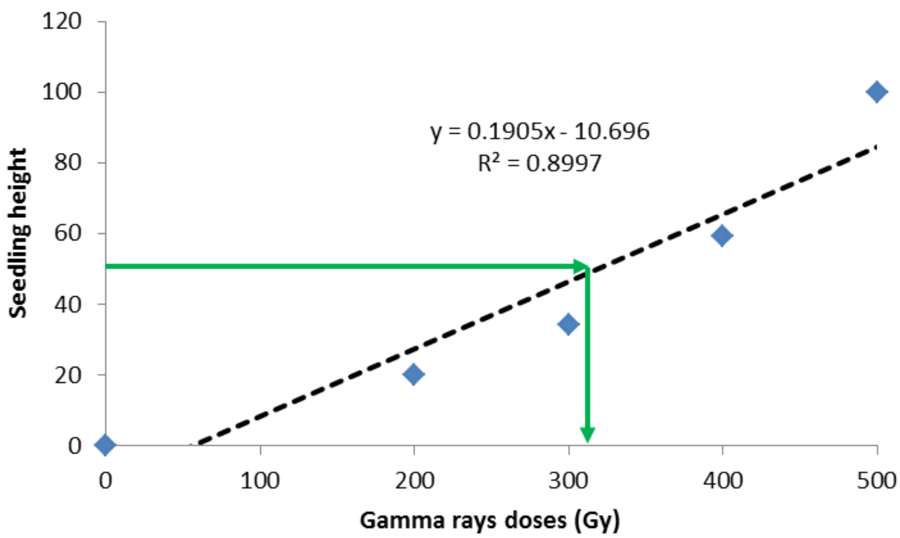

Figure 8. Effect of gamma irradiation on growth reduction (GR_{50}) of seedling height of *C. annuum* var. Kopay.

var. Tulungagung and for *Capsicum annuum* var. Ponorogo, respectively. Gaswanto *et al.* (2016) reported that the LD_{50} of the five irradiated chili genotypes ranged from 422.64 to 629.68 Gy. Aisha *et al.* (2018) obtained an LD_{50} of 310 Gy for chili Bangi3 and 447 Gy for chili Bangi5, with the LD_{50} of 572.92 Gy recommended for *C. frutescens* (Tias *et al.*, 2022). Our findings demonstrated that GR_{50} for the seedling height of the cultivars Bara and Kopay were 246 and 319 Gy, respectively. This result aligns with the findings reported by Muhammad *et al.* (2021), which obtained the GR_{50} value of 250-264 Gy in *Vigna subterranean*. Shrivastava *et al.* (2021) disclosed GR_{50} ranged from 248.7 to 374.3 Gy in safflower, and Chakraborty *et al.* (2023) obtained GR_{50} of 316.22 to 346.73 Gy for two cultivars of wheat. The magnitude variation of LD_{50} and GR_{50} parameters depends on moisture content, stage of growth development, and plant tissue (Riviello-Flores *et al.,* 2022; Álvarez-Holguín *et al.,* 2019).

The results of the radiosensitivity determination in this research showed a significant decrease in the survival rate and the seedling height with increasing gamma radiation doses in both cultivars, Bara and Kopay. The decline in the percentage of survival due to mutagenic treatments among these samples could be ascribable to the level of cell differentiation and embryo development during the mutagenic treatment. These findings were analogous to Kusmiyati *et al.* (2018) on soybeans. They reported that the sensitivity of biological samples to gamma mutagenesis treatment depends on the level of damage caused by mutagens related to growth and development processes, such as cell elongation, division, biosynthesis, and various levels of hormone pathways.

CONCLUSIONS

The lethal dose and radiosensitivity of the two cultivars of chili peppers were different, wherein the cultivar Bara had an LD_{50} of 257.64 Gy and a GR_{50} of 246.10 Gy. Meanwhile, the cultivar Kopay had an LD_{50} of 333.56 Gy and a GR_{50} of 318.61 Gy. The LD_{50} and GR_{50} in both cultivars of chili can be applicable as appropriate doses of gamma rays to obtain the most genetic variation and positive mutants for plant breeding.

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