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Pengaruh Iradiasi Sinar Gamma terhadap Hasil dan Pertumbuhan Cabai Merah (*Capsicum annuum*)

Gamma Ray Irradiation Effect Through Growth and Production of Red Chili (Capsicum annuum)

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ABSTRAK

Cabai merah (*Capsicum annuum*) merupakan tanaman hortikultura yang banyak dimanfaatkan sebagai bahan tambahan pangan yang memberikan rasa pedas khas dan memberi warna pada makanan. Menurunnya penggunaan varietas local cabai merah dalam jangka panjang dapat menyebabkan keberadaan plasma nutfah lokal semakin menurun hingga terancam punah, sehingga diperlukan upaya untuk meningkatkan produktivitas dan mutu varietas lokal agar varietas lokal kembali diminati oleh masyarakat. petani. Salah satu upaya yang dapat dilakukan untuk meningkatkan produktivitas dan kualitas tanaman cabai mentah varietas lokal adalah dengan mutasi genetik menggunakan sinar gamma. Tujuan penelitian ini adalah untuk mengetahui pengaruh iradiasi sinar gamma terhadap pertumbuhan dan hasil cabai merah varietas Tanjung-2. Hasil penelitian menunjukkan terdapat pengaruh iradiasi sinar gamma terhadap pertumbuhan dan hasil tanaman cabai merah varietas Tanjung-2. Berdasarkan pengamatan, tumbuhan kode 50.12 merupakan tumbuhan mutan tertinggi dengan tinggi 56,5 cm. Selain itu dari segi hasil, kode 150.2 mempunyai jumlah buah dan berat buah terbanyak dibandingkan seluruh tanaman sampel. Selain itu tanaman dengan perlakuan 350 Gy dapat menghasilkan cabai merah yang memiliki kadar capsaicin lebih tinggi dibandingkan tanaman tanpa perlakuan sinar gamma (0 Gy).

Kata Kunci:

Capsaicin;

Capsicum annuum;

Sinar Gamma;

Mutasi

ABSTRACT

Chili (Capsicum annuum) is a horticultural plant whose fruit is used as a food additive which gives it a distinctive spicy taste and gives color to food. The decrease in the use of local varieties in the long term can cause the presence of local germplasm to decline and be threatened with extinction, so an effort is needed to increase the productivity and quality of local varieties so that local varieties are again in demand by farmers. One of the efforts that can be done to increase the productivity and quality of local varieties of raw chili plants is by genetic mutation using gamma rays. The purpose of this study was to determine the effect of gamma-ray irradiation on the growth and yield of red chili pepper varieties Tanjung-2. The results showed that there was an effect of gamma-ray irradiation on the growth and yield of red chili plants of the Tanjung-2 variety. Based on the observations, plant code 50.12 was the tallest mutant plant with a height of 56.5 cm. In addition, in terms of yield, code 150.2 had the highest number of fruits and fruit weight compared to all sample plants. In addition, plants with 350 Gy treatment can produce red chilies which have higher capsaicin levels than plants without gamma ray treatment (0 Gy).

Keywords:

Capsaicin;

Capsicum annuum;

Gamma-Ray;

Mutations

INTRODUCTION

Red chili (*Capsicum annuum*) is one of the important plants in the horticultural group. Red chilies are generally used as food additives that provide a distinctive spicy taste and give color to food (Al-Snafi, 2015). The capsaicin content in red chilies makes red chilies classified as functional foods because the capsaicin in red chilies has a health effect when consumed (Pavón-Pérez et al., 2019). The market demand for red chili is quite high in Indonesia, causing the price of red chili in Indonesia to continue to experience price fluctuations(Fatma et al., 2020). Red chili price fluctuations are generally caused by an imbalance between supply and demand. Market demand continues to increase for red chili while the supply or stock of red chili is still quite low (Muhaimin et al., 2021). One of the reasons for the low supply or stock of red chili in the market is the low productivity of red chili plants and low fruit quality (Hervani et al., 2021).

One of the factors that affect the productivity of red chili plants is the variety used. Red chili production in Indonesia is mostly produced from hybrid varieties which are more in demand by farmers because hybrid varieties have higher productivity than local varieties (Liferdi et al., 2014). Market demand for local varieties is quite low compared to hybrid varieties because local varieties of red chili are softer or easily crushed (Basuki et al., 2016). The low productivity and quality of local varieties of red chili cause the use of local varieties to decrease. This can cause the existence of local germplasm to be threatened with extinction, so an effort is needed to increase the productivity and quality of local varieties so that local varieties are again in demand by farmers (Chaniago, 2019).

Efforts that can be made to increase the productivity and fruit quality of red chili plants are through mutation techniques. A mutation is one method that can be used to increase genetic diversity in plants. The genetic diversity obtained can produce plants with different genotypes and phenotypes from the previous plant (wildtype) (Soyam et al., 2019). Mutations in plants generally can occur naturally but natural mutations take a long time so artificial genetic mutations can be done to shorten the time. One of the genetic mutation techniques that can be used to cause mutations in plants is gamma-ray irradiation. Gamma-ray irradiation is a physical mutation treatment in plants by utilizing gamma-ray radiation (radioactive) to hydrolyze a chemical bond in plant DNA (Issa. Piri, 2011). Gamma-ray irradiation in plants will cause changes in the DNA sequence of plants which will produce mutant plants that have different properties, morphology, and biochemistry from previous plants (Thisawech et al., 2020).

Induction of gamma-ray radiation in previous studies resulted in mutant plants that had better properties than the previous plants (wild type). Gamma-ray induction with a dose of 50 Gy on red chili plants of the Shombo variety can increase the productivity of red chili plants from 12,553.5 kg/ha to 21,231.39 kg/ha. In addition, gamma-ray induction with a dose of 100 Gy can increase the number of leaves of red chili plants of the Tatase variety (Nigerian local variety) from 8.53 to 16.95 (Abu et al., 2020). Based on the description above, it is expected that gamma-ray irradiation on local varieties of red chili plants can produce mutant plants that have better productivity and fruit quality than the previous plants, so it is necessary to do research related to the effect of gamma-ray irradiation on the growth and yield of local varieties of red chili plants. (*Capsicum annuum*).

METHODOLOGY

The research was conducted in Jember, East Java. The seeds used in this study were seeds that had been irradiated by gamma rays at the Central Laboratory of Isotope and Radiation Applications at the National Nuclear Energy Agency, South Jakarta. Capsaicin analysis was carried out at the Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta. This research uses Chili varieties of Tanjung-2 as sample. The research method used in this study is the standard method (Andrew-Peter-Leon et al., 2021). There are 5 doses used in this study, namely 0 Gy, 50 Gy, 150 Gy, 250 Gy, and 350 Gy. Each dose contains 50 seeds, so a total of 250 seeds will be used. The seeds that have grown are then planted in open fields until harvesting. Data Interpretation using descriptive analysis. The number of plants planted to harvest per treatment is 50 plants so 250 plants will be used. Plants are coded to facilitate reading, for example:

- Plant code 0.1: plants without gamma-ray irradiation (0 Gy) and plant number 1
- Plant code 50.2: plants with 50 Gy gamma ray treatment and plant number 2
- Plant code 150.3: plants with 150 Gy gamma ray treatment and plant number 3

Gamma-ray Irradiation

Gamma-ray irradiation was carried out at the Central Laboratory of Isotope and Radiation Applications (National Nuclear Energy Agency), South Jakarta, Indonesia. Red chili seeds were irradiated with 4 doses of gamma rays, namely 50 Gy, 150 Gy, 250 Gy, and 350 Gy. Gamma-ray irradiation of seeds using irradiator Gamma Cell 220 AECL Upgraded. The irradiation process begins with inserting the seeds into plastic wraps or envelopes. Plastic wrap or envelope containing the seeds is then inserted into the irradiator chamber then gamma rays are irradiated to the object according to the specified dose. The duration of irradiation is determined by the gamma-ray rate, which is 3700 Gy/3600 seconds so that it is obtained:

- 50 Gy = $\frac{50}{3700}\ x\ 3600$ = 49 seconds
- 150 Gy = $\frac{150}{3700}\ x\ 3600$ = 146 seconds = 2 minutes 26 seconds
- 250 Gy = $\frac{250}{3700}\ x\ 3600$ = 243 seconds = 4 minutes 3 seconds
- 350 Gy = $\frac{350}{3700}\ x\ 3600$ = 340 seconds = 5 minutes 40 seconds

Plant Cultivation

Irradiated seeds were then seeded. After 1 month, seeds were then planted in the holes in the beds with 1 seed per hole. The seeds that have been planted are then watered and given shade to reduce evaporation.

Observation

Observations were made by measuring and observing plants according to predetermined observation variables. Variables observed in this study included plant height, leaf area, leaf chlorophyll content, number of fruits planted, fruit fresh weight per plant, fruit capsaicin content, and flower morphology.

Plant height

Plant height was measured from the base of the stem to the top of the plant crown. Plant height was measured using a cloth meter and carried out after harvesting. The unit used to measure plant height is cm.

Leaf Area

Measurement of leaf area using the help of ImageJ software which is available open source on PC. Leaf area measurement begins by placing the leaf to be measured on HVS paper and placing a ruler on the left side of the paper. The leaves that have been arranged are then photographed using a cellphone camera by including a ruler. The next step is to open the ImageJ application on the PC and click open, select the leaf photo file to be measured. Click the straight button and draw a line on the side of the ruler 1 cm long following the picture. Next is to set the image scale by clicking the analyze menu and clicking set scale. In the set scale window, fill in the known distance with the number 1 and the unit of length with cm, then check global and click the ok button. The next step is to click the process menu, click binary and click make binary. After the leaf image turns black, click the analyze menu, click tools and click ROI manager. After the ROI manager window appears, click the add button and click the leaf image whose leaf area will be analyzed using the help of the wand button. The leaf image that has been added to the ROI manager window is then analyzed for the leaf area by clicking measure and then the leaf area value will appear.

Leaf Chlorophyll index

The leaf chlorophyll index was measured using the help of a Chlorophyll meter SPAD-502. The measurement of the chlorophyll index begins by cleaning the clamp side of the SPAD and then turning on the SPAD. The SPAD that is on is then calibrated by pressing the SPAD clamp without the object in the center. Then the leaves whose chlorophyll index will be known are clamped using SPAD clamps until the SPAD value appears on the SPAD monitor. The unit of this number is the SPAD value.

Number of fruit planted

The number of fruit planted is calculated after harvesting. The number of fruits counted is the number of fruits harvested from each plant.

Weight of Fresh Fruit Planted

The fresh weight of the fruit is calculated after harvesting by weighing the entire fruit crop yield. Measuring fruit weight using digital scales.

Fruit Capsaicin Levels

Analysis of capsaicin levels in red chilies was carried out using thin layer chromatography (TLC). Determination of capsaicin levels begins with sample preparation. Fresh red chilies are cut into pieces and then dried in the oven at 45^{o}C. The dry sample was then put into a test tube and 2 ml of ethanol was added. The next step is homogenization of the sample using a vortex for 1 minute and sonication for 15 minutes. After that the samples were macerated for 24 hours at room temperature. Samples that have been macerated are then extracted by re-vortexing and centrifuged to take the supernatant. The residue was then added with 2 ml of ethanol and re-extracted. The next step is drying the supernatant using N2 gas and then adding 1 ml of ethanol. Samples that have gone through the extraction stage are then spotted on the silicagel 60 F254 plate next to the capsaicin standard. The 60 F254 silicagel plate was then put into a chamber containing saturated toluene – chloroform – acetone (45:25:30) mobile phase, then eulated to the limit and dried. The final stage is densito at a wavelength of 228 nm. Rf. 0.60.

Flower Morphology

Observation of flower morphology was carried out by observing each flower on each plant. In each treatment, one flower was selected which had a difference, especially in the number of flower petals, so that there were 5 flowers whose morphology would be observed.

RESULTS AND DISCUSSION

Mutagenesis is a change in a gene in an organism that causes a change in expression in an organism. The change in expression is caused by a change in the sequence of nucleotides in an organism that causes a change in the translated codon. Changes in these codons will produce different proteins and different expressions (Rahman et al., 2022). Mutations in plants can occur naturally and artificially. Plants can mutate naturally but it takes a long time, so artificial mutations are often used to speed up the mutation process (Yang et al., 2019). Artificial mutations generally use chemical and physical mutagens. An example of a chemical mutagen is EMS (ethyl methanesulfonate) while a physical mutagen can use gamma-ray radiation (Kamolsukyeunyong et al., 2019).

Gamma-ray irradiation caused several changes in plants, one of which is the survival rate or plant survival rate (Rifnas et al., 2020). Based on the results of the research on gamma-ray irradiation treatment on red chili plants, Tanjung-2 variety, there was a negative correlation between the dose of gamma-ray irradiation and the number of live plants. The greater the dose of gamma rays, the less the number of living plants. The number of samples per treatment was 50 plants, but the number of plants that survived was 23 plants for control plants or plants without gamma-ray irradiation treatment, 18 plants for 50 Gy gamma irradiation treatment plants, 15 plants for 150 Gy gamma irradiation treatment, 10 plants for the treatment of gamma-ray irradiation 250 Gy, and 4 plants for the treatment of gamma-ray irradiation of 350 Gy. This is in line with the research of Wanga et al., (2020) in which increasing the dose of gamma irradiation will reduce the survival percentage of sorghum plants.

Changes due to mutations can occur in plant morphology, one of which is plant height. The plant height graph is shown in Figure 1. Based on the results of research on gamma-ray irradiation on red chili plants of the Tanjung-2 variety, there were differences between control plants or plants without gamma irradiation treatment and plants that were given gamma-ray treatment. The height of control plants or plants without gamma irradiation treatment ranged from 22-46 cm, but there were plants with 50 Gy treatment that had a plant height below the control plant or plants without gamma irradiation treatment, namely plants with code 50.8 which had a plant height of 9 cm, in addition, there were 50 Gy treatment plants which had a height above control plants or plants without gamma irradiation treatment, namely plants with code 50.12 with a height of 56.5 cm. The effect seen in the results of the study shows that there is a positive influence and a negative influence. The positive effect is due to a stimulatory effect that occurs due to higher hormone activation (Warade et al., 2022), while the negative effect is caused by plant cell damage that causes abnormal organ growth (Tarigan et al., 2021).

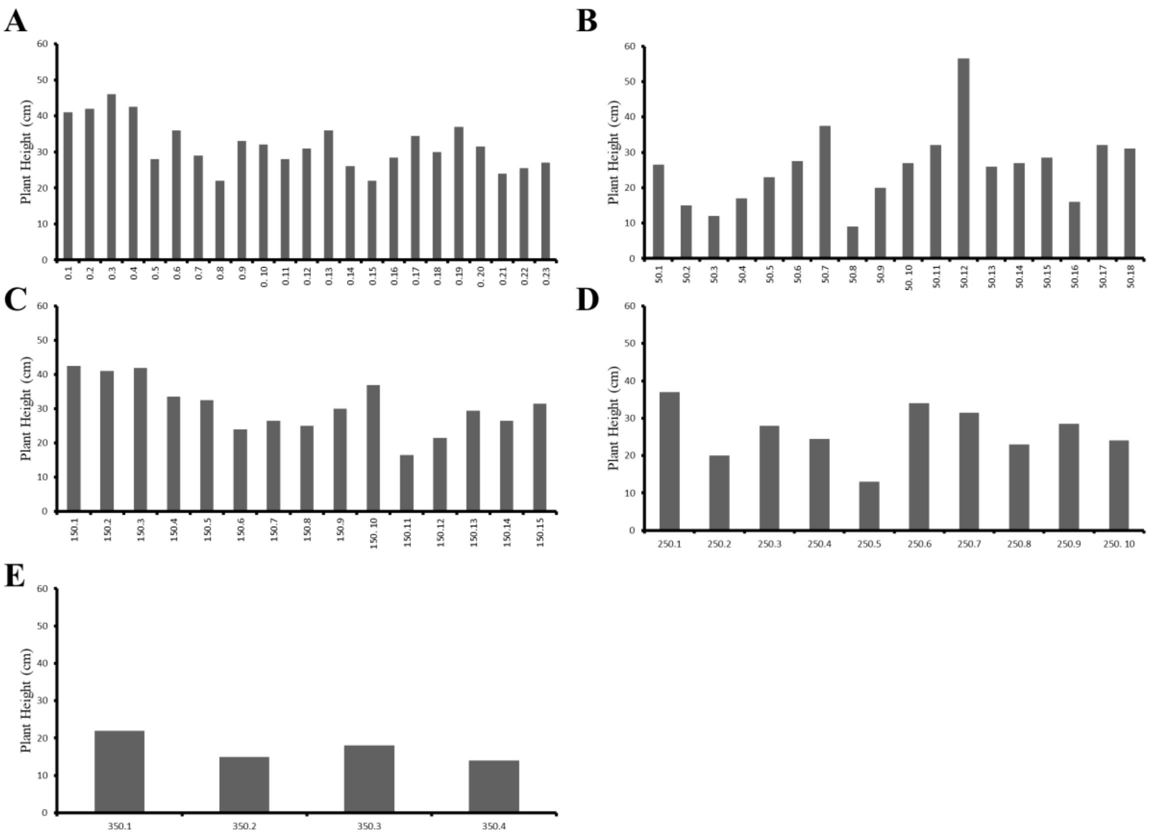

Figure 1. Plant height. The data is the value of the height measurement of each plant sample. 0 Gy (A), 50 Gy (B), 150 Gy (C), 250 Gy (D), 350 Gy (E).

The effect of mutations on plant morphology can occur in plant leaf areas. The plant height graph is shown in Figure 2. Based on the results of the study the leaf area of control plants or plants without gamma-ray irradiation treatment had a leaf area range of 3.58-9.80 cm2, while plants with gamma-ray treatment obtained plants that had higher leaf area than control plants. or plants without gamma-ray irradiation treatment, namely plants with code 150.6 with a leaf area of 11.06 ± 2.36 cm2 and plants with a code of 250.7 with a leaf area of 12.65 ± 2.16 cm2. Negative changes were found in plants with code 50.5 with a leaf area of 3 ± 0.58 cm2. Based on this, it can be seen that mutations are random. Changes that occur can be positive or negative. In this case, the change that looks significant is positive in the leaf area of chili plants in which there is an increase in plant leaf area (Zhang et al., 2018).

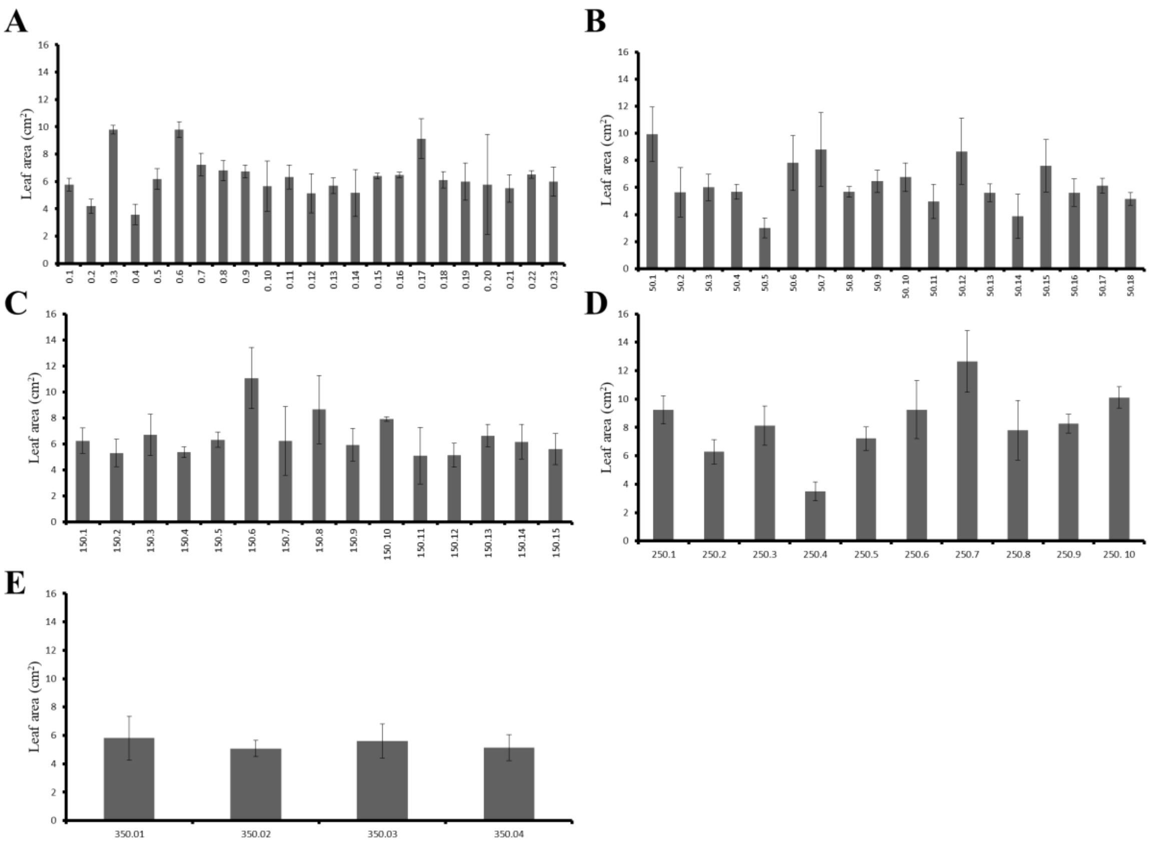

Figure 2. Leaf Area. 0 Gy (A), 50 Gy (B), 150 Gy (C), 250 Gy (D), 350 Gy (E). The data is the average leaf area per plant sample.

Observation of the effect of mutations on leaves was also carried out by measuring leaf chlorophyll levels using a Chlorophyll content meter or SPAD. Based on the results of observations of chlorophyll levels using SPAD, the SPAD value of control plants or plants without gamma-ray irradiation treatment was 55,775-79,800 and there were no gamma-irradiated plants that had SPAD values higher than the upper limit of control plants or plants without gamma irradiation treatment. Based on observations, it was found that the plants treated with gamma ray irradiation had the lowest SPAD values, namely plants with code 50.2 with SPAD values of 51.550 ± 9.07. Previous research by Deepashree et al., (2022), reported that increasing the dose of gamma rays can reduce the chlorophyll content in leaves. The decrease in the SPAD value or the chlorophyll content of the leaves of plants treated with gamma ray irradiation was caused by damage to the chloroplasts as a result of gamma-ray irradiation. Chloroplast damage in this case will result in a decrease in the accumulation of chlorophyll in the leaves (Darkwah et al., 2019). The SPAD value data for plants with gamma-ray treatment and without gamma ray treatment are shown in Figure 3.

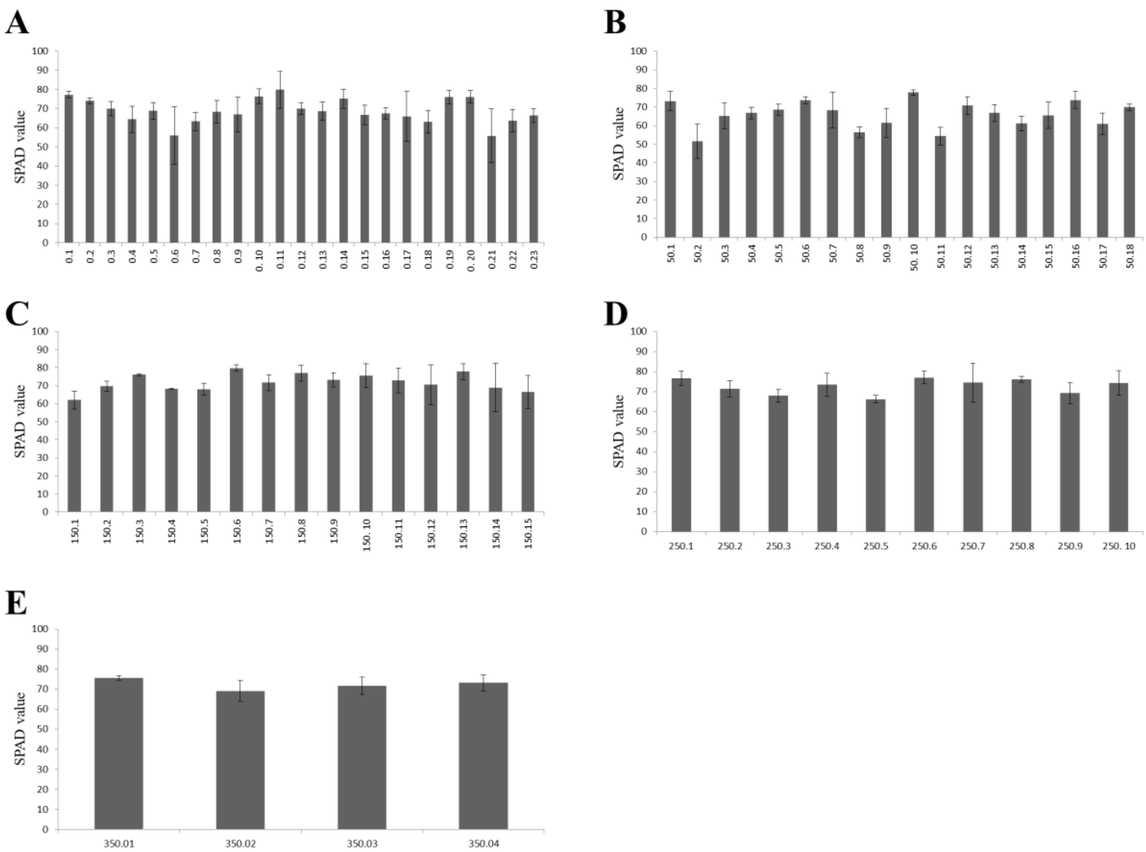

Figure 3. SPAD Value. 0 Gy (A), 50 Gy (B), 150 Gy (C), 250 Gy (D), 350 Gy (E). The data is SPAD value per plant sample.

Gamma-ray mutagen irradiation can affect crop yields, so the observation of the number of fruits was carried out to determine the effect of gamma-ray irradiation on red chili plants of the Tanjung-2 variety. Based on the observations, it is known that control plants or plants without gamma-ray irradiation treatment have fruits ranging from 14-24 fruit. There are differences in the number of fruits in the gamma-ray irradiation treatment plants as the effect of gamma-ray irradiation where the differences are negative and positive. The negative difference occurred in the code 250.5 plant which only had 1 fruit, while the positive difference occurred in the code 150.2 plant which had 32 fruits. Based on research by Mohite & Gurav, (2019), the effect of gamma-ray irradiation can be positive and negative on the number of fruits. Gamma rays as physical mutagens can change the properties of plants that have an economic effect such as the number of fruits (Shahab et al., 2018). Data on the number of fruit per tree are shown in Figure 4.

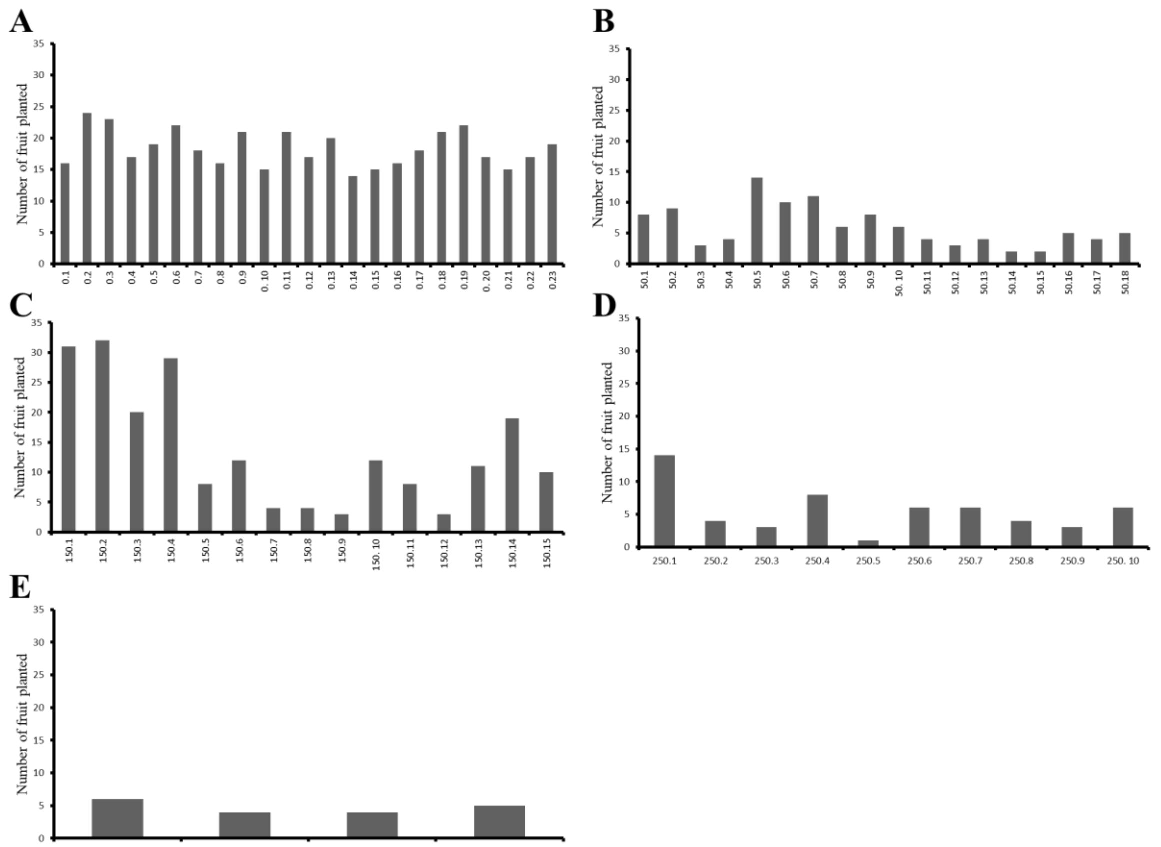

Figure 4. Number of Fruits per plant. 0 Gy (A), 50 Gy (B), 150 Gy (C), 250 Gy (D), 350 Gy (E). The data is the result of the accumulation of the number of fruits per sample plant

Observation of the effect of gamma-ray irradiation on the yield of red chili varieties Tanjung-2 was also carried out by observing the total weight of the fruit planted. Control plants or plants without gamma irradiation treatment had a total fruit weight range of 155-298 grams. Based on the observations, it was known that there were differences between control plants or plants without gamma irradiation treatment and plants with gamma irradiation treatment. The difference is positive and negative. A negative difference occurred in plants with code 250.5 which only had a fruit weight of 7 grams, while a positive difference occurred in plants with code 150.2 which had a total fruit weight of 436 grams. The positive difference is caused by the ability of gamma rays to stimulate the activity of hormones and enzymes responsible for plant growth (Saadati et al., 2022). Fruits weight per plant data for plants with gamma-ray treatment and without gamma-ray treatment are shown in Figure 5.

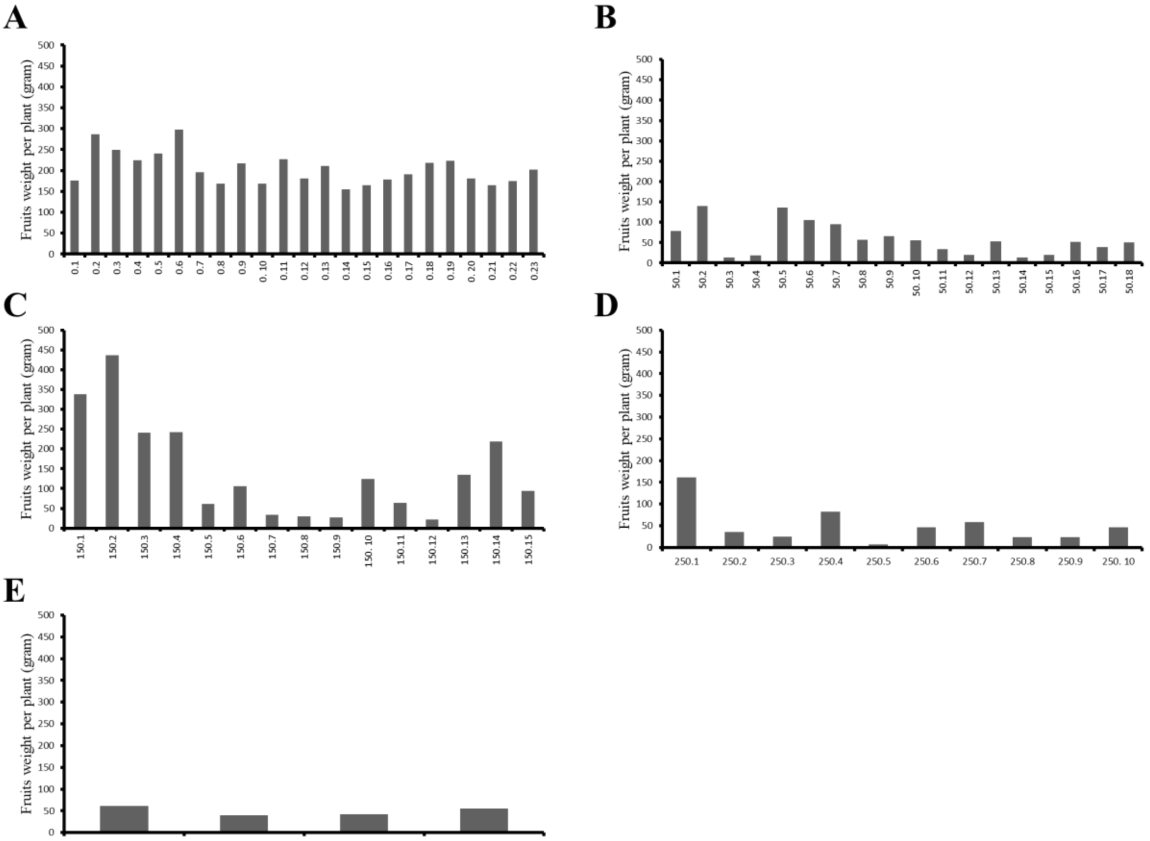

Figure 5. Fruits weight per plant. 0 Gy (A), 50 Gy (B), 150 Gy (C), 250 Gy (D), 350 Gy (E). The data is the result of the accumulation of the fruits weight per sample plant.

Measurement of the quality of red chili can be done by measuring the capsaicin content of the fruit. The measurement of capsaicin levels was carried out using the thin-layer chromatography method. Based on the results of the capsaicin content test, it is known that the capsaicin content of red chili from plants without gamma irradiation treatment (0 Gy), gamma ray treatment of 50 Gy, 150 Gy, 250 Gy, and 350 Gy respectively is 67.12 mg/Kg, 68 .37 mg/Kg, 51.02 mg/Kg, 46.96 mg/Kg, and 105.11 mg/Kg. It is known that there is an effect of gamma-ray irradiation on capsaicin levels in red chili. The effect of gamma-ray irradiation was seen to decrease and increase capsaicin levels in red chilies. The capsaicin content of plants without gamma-ray irradiation treatment (0 Gy) was 67.12 mg/Kg. The effect of gamma-ray irradiation that reduces capsaicin levels occurs in red chilies from 250 Gy gamma ray treatment plants with capsaicin levels of 46.96 mg/Kg, while the effect of gamma-ray irradiation that increases capsaicin levels occurs in red chilies from 350 Gy gamma ray treatment plants with capsaicin levels 105.11 mg/Kg. The effect of gamma-ray irradiation can decrease and increase capsaicin levels in red chili (Balakrishnan et al., 2022), this occurs due to changes in the conformation of capsaicin molecules with other compounds that cause changes in capsaicin levels in fruit (Ayob et al., 2021).

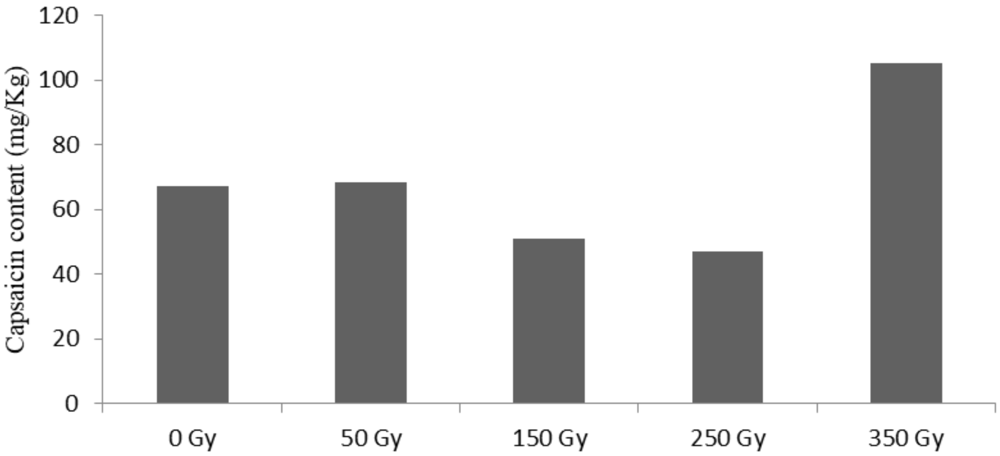

Figure 6. Capsaicin levels in control red chilies or plants without gamma irradiation treatment and gamma ray treatment.

Gamma-ray irradiation can cause changes in flower morphology, especially the number of flower crowns. Based on observations, flowers from control plants or plants without gamma irradiation treatment had 5 flower crowns, this also happened to flowers of 150 Gy, 250 Gy, and 350 Gy gamma irradiated plants. There are differences in the number of flower crowns, namely in the flowers of the 50 Gy gamma-ray irradiation treatment plant which has a total of 6 flower crowns. Research conducted by Li et al., (2022), reported that there was a change in the number of flower crowns with gamma irradiation treatment. this is due to changes in homeotic genes responsible for plant anatomy which will affect plant morphology (Akçay et al., 2021).

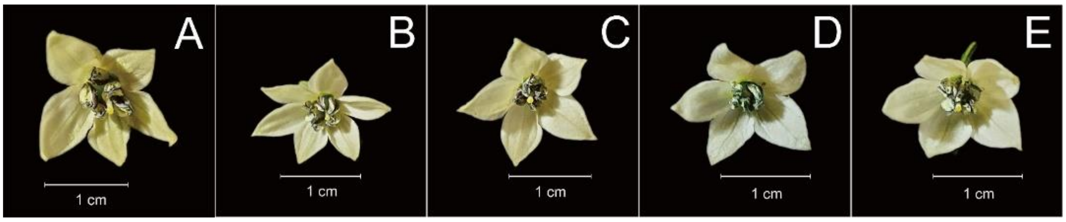

Figure 7. Flower morphology of red chili pepper varieties of Tanjung-2. 0 Gy (A), 50 Gy (B), 150 Gy (C), 250 Gy (D), 350 Gy (E).

CONCLUSION

Based on the results, it can be seen that gamma-ray irradiation on chili seeds can change plant characteristics, it can be useful for improving the quality of Tanjung-2 chili seeds as one of the local varieties in Indonesia. Improvement of the characteristics, especially the yield of chili varieties of Tanjung-2, is expected to increase farmers' interest in using chili varieties of Tanjung-2 so that these varieties do not become extinct due to the decreasing number of farmers using these varieties. In addition, the use of local varieties as planting material will help maintain the existence of local germplasm.

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