Radio-sensitivity test of gamma irradiation of local chilli pepper seeds (Capsicum annum L.)

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Abstract. Chilli pepper is a strategic horticultural commodity for its economic, nutritional, and medicinal values and is used as a spice in a variety of cuisines all over the world. Induced mutation by gamma ray’s irradiation has been used to increase chilli resistance and quality. The objective of this study was to determine the optimum dose of gamma irradiation in inducing resistance in putative mutation (M1) on chilli pepper seeds. The seeds induced were infected by Pepper Yellow Leaf Curl Virus (PYLCV) and were irradiated with 0, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, and 1000 Gy. The plants (M1) were observed for their seed germination, leaf length, leaf width, and plant height. The results indicated that the best LD50 irradiation was 288.269 Gy (y = 82.7847 + 0.0698x + 0.0002x^2 + 2.0082x^3). The results also revealed that plants irradiated with doses 150, 200, and 250 Gy were selected to be planted in the field.

1. Introduction
Chili pepper (Capsicum annum L.) is an important commodity planted in both lowland and highland areas. It can be grown an altitude 0-2000 m asl and adaptive to areas with temperatures of 24-27°C [1]. This plant also provides vitamins, protein, carbohydrates, fat, and calcium [2]. In Indonesia, there is an imbalance between product demand and the production of chili pepper. Chili consumption was increased to 1.56 kg/capita in 2018 and 1.58 kg/capita in 2019. Sadly, this rising need for chili does not go together with its production. The production was decreased year by year, 1.21 million tons in 2017, 1.18 million tons in 2018, and 1.12 million tons in 2019 [3]. This deterioration is predicted to be caused by biotic and abiotic factors such as pest and disease attack [4], the correlation between pest population and its environment, and also temperature and humidity which contribute to the pest development and population [5,6]. The attack of silverleaf whitefly (Bemisiatabaci) is one of them. This pest is a host to pepper yellow leaf curl virus, which causes deterioration in chili production, varies between 20-100% [7].

The cultivation of local varieties is one effort to increase the production of chili. The local varieties are known to be abundant and more adaptive. In North Sumatra, a local chili known as ‘cabai temper’ is preferred. This plant is characterized a purple stem, height above 1.2 m, fruit weight ranged between 931 g to 1183 g with a long life span (1.5 years) [8]. Gamma irradiation also has been used to increase plant resistance and to improve yield quality. The irradiation attributes to plant mutation, the changes in its genes, resulted in positive outcomes in plant size, flowering time, fruit ripening, fruit color, plant resistance to drought and diseases [9].
Plant response to gamma irradiation differs from each other, depends on the success of induced seeds tested. Each plant demonstrates different sensitivity, determined by a radio-sensitivity test on each plant genotype. Radio-sensitivity is distinguished by the result of LD$_{50}$ (lethal dose), where the dose causes mortality to 50% of irradiated plants [10]. Radiosensitivity can be observed by inhibition development, mortality, leaf length, leaf width, chromosome size, and somatic mutation that occurred during the seedling phase. The dose of irradiation was adjusted according to its use. High irradiation (> LD$_{50}$) is applied to produce extreme plant character [11,12]. Therefore, this research was conducted to discover a local mutant variety of chili peppers which resistant to PYLCV and also to determine the optimum dose of gamma irradiation in inducing resistance in local chili peppers obtained from Karo Regency, North Sumatra.

2. Materials and methods

2.1 Place and duration
This study was conducted from July to August 2020 in Installation for Research and Assessment of Agricultural Technology (IP2TP) in Berastagi and Indonesian Vegetable Research Institute (Balitsa) located in 1340 m asl with Andisol type soil. Gamma irradiation assay was carried out in Center for Application of Technology of Isotope and Radiation (PAIR), National Nuclear Energy Agency of Indonesia (BATAN) in South Jakarta. The materials used were infected chilli seeds, topsoil for its planting media (ratio 2:1), polybags provided with 200g sterilized soil. The tools used were gamma radiator Gammacell 220 provided by BATAN, bamboo, nets, measuring instruments, and stationery.

2.2 Gamma irradiation assay
Independent 150 local cultivar of chilli peppers (obtained from Karo Regency, North Sumatra) with moisture content 10% were irradiated with 17 different doses: 0, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 and 1000 Gy. Each dose was applied to 30 seeds planted in a row. Plant watering was done regularly to keep them moisturized using a hand sprayer.

2.3 Variables observed

2.3.1 Percentage of survived plants. After 2 weeks-1 months of application, this variable was calculated using the formula: number of plants tested/number of plants survived x 100%. The results were analyzed using Curve-Fit Analysis Software

2.3.2 Plant height. The height was measured 2-4 weeks after planting, from the highest ground point adjacent to the foliage of the species.

2.3.3 Leaf length and width. The leaf length and width were measured using a ruler. The leaf length is defined as the distance from leaf apex to leaf base, and the leaf width is defined as the maximum length of the segments perpendicular to the straight line passing through leaf apex and leaf base. The result of leaf length-width was performed using a paired sample T-test.

3. Results and discussion

3.1 Gamma irradiation effects on seed germination
The results of the Curve-Fit Analysis in Table 1 showed that the seeds induced with gamma radiation gave significant differences in pepper seed germination. This significant response could be attributed to quality differences or genetic material possessed by each seed. The results were presented in the graph (table 1). The pattern of response was described polynomial fit curve ($y = 82.7847 + 0.0698x + 0.0002x^2 + 2.0082x^3$). LD$_{50}$ was described by $y$, where if $y = 50$ (50% plants died), $x = 288.269$ Gy. This means that dose 288.269 Gy gamma irradiation has killed 50% of plants tested. The decrease in
the number of plants that survived was linked to the increase of irradiation dose, which caused physiological disorder in plants, resulted in seed damage.

Table 1. The genetic diversity of local chilli paper seed Karo by gamma-ray to percentage growth

| Character | Doses of Irradiation |
|-----------|-----------------------|
|           | 0  | 25  | 50  | 100 | 150 | 200 | 250 | 300 | 350 | 400 | 450 |
| The percentage of chilli growth at 2 weeks after the plant | 50 | 70  | 73.3| 83.33| 33.33| 70  | 23.33| 56.66| 20  | 40  |
| The percentage of chilli growth at 3 weeks after the plant | 66.66| 73.3| 76.66| 86.66| 83.33| 40  | 70  | 26.66| 56.66| 20  | 40  |
| The percentage of chilli growth at 4 weeks after the plant | 80 | 73.3| 80  | 86.66| 83.33| 40  | 70  | 26.66| 56.66| 20  | 40  |

The graph in figure 1 showed us that at dose 500 Gy the seeds still had the ability to germinate and produced leaves. However, the exposure of irradiation dose higher than 500 Gy has disabled the seeds to germinate by 100%. It indicated that the damage experienced by irradiated seeds was increased with higher irradiation doses. This damage has led to seed death. The mortality that occurred in plants after seed induced by gamma irradiation has caused deterministic effects. The seed exposed with lower radiation has a better performance compared to the seed exposed with higher radiation [13,14]. It showed that dose irradiation affects seed germination ability. The exposure of higher gamma rays caused a higher possibility of germination inability due to its DNA damage. Doses 600Gy and 800 Gy have proved to negatively affect chilli pepper development [15]. Besides, the abnormal development might cause by the inhibition of auxin production, chromosome aberrations, and assimilation decrease.

![Figure 1. Percentage of survived plants.](image.png)

3.2 Determination of plant height, leaf length, and width

Results in table 1 described the response of each irradiated plant significantly differs from each other, identified from significant leaf length and width. The application of 50-150 Gy irradiation gave...
insignificant results compared to non-irradiated plants. It might happen due to the damage caused by irradiation is dissimilar. Also, the physiological disorder that occurred in the plants caused some changes in plant tissues and plant metabolism and photosynthesis activity [16], such as cell death, abnormal cell division, induced mitosis, changes in plant development, reproduction capacity, and cell development.

| Character      | Number of survived plants treated with different doses of irradiation |
|---------------|-----------------------------------------------------------------------|
| Leaf length   | 2.671 3.09* 2.76 2.68 2.58 2.30** 2.09** 1.96** 2.350* 2.03** 2.047** |
| Leaf width    | 1.09291.075 0.96** 0.87** 0.86**0.791** 0.723* 0.652* 0.750* 0.676* 0.84** |
| Plant height  | 5.25 6.41** 5.48 6.17* 5.68 4.70 4.89 3.42** 4.46** 3.30** 2.79** |

Several changes on local pepper due to gamma irradiation treatment were shown in figure 2. The treatment has caused plants to have shorter leaf length, contributed by changes in plant tissues, chromosomes, and DNA of the plants. [17] has stated that gamma irradiation-induced changes in plant cell structure, metabolism, and photosynthesis activity. Change in mean values of plant length and leaf number characters were recorded in all irradiated populations and were significantly different control population. Irradiated plants were shorted and have fewer leaves than control plants [18].

**Figure 2.** Different length and width of pepper leaves treated with different doses of gamma irradiation.

Local chili pepper applied with 25 Gy irradiation dose exhibited non-significant leaf width compared to non-irradiated plants. However, when the plants were exposed to a higher dose (50-450 Gy), there was a decrease in its leaf width. This degradation resulted from higher irradiation exposure (figure 2) which caused cell damage and changes in its chromosomes and genes, resulted in abnormal organ development [19].

Plant height is also an important indicator of plant development. Gamma irradiation 25-150 Gy gave significant results compared to control. Gene changes were the exact cause of this phenomenon. Unfortunately, the plant height degraded as the doses increased (figure 3). This could be attributed to abnormal cell division. If the cell is damaged, cell division activity would be disrupted, resulted in a decrease in gene activity. The other factors affecting this lower height were protein synthesis disruption, causing inhibition in plant development and production of growth hormones.
Figure 3. The height of infected chilli pepper plants treated with different doses of gamma irradiation.

4. Conclusions
Gamma irradiation exhibited significant results in local chilli pepper plants obtained from Karo Regency (known as ‘temper ungu’, characterized by its purple stems) at LD50 = 288.269 Gy ($y = 82.7847 + 0.0698x + 0.0002x^2 + 2.0082x^3$). According to observation and data collection of its survived plants, leaf length, leaf width, and plant height, plants irradiated with 150, 200, and 250 Gy were selected to be cultivated in the field.

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