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Radiosensitivity of Local Chili Varieties to Gamma Rays

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ABSTRACT

Genetic variation is needed for developing new superior varieties in plant breeding. Genetic variation of chili plants could be increased by inducing mutation with gamma rays irradiation. The aim of this study was to determine radiosensitivity of two local varieties of chili pepper by calculating the lethal dose values at LD20 and LD50. Chili pepper (Capsicum frutescens L.) seeds of local varieties origin from Tulungagung and Ponorogo were irradiated with cobalt-60 gamma rays at doses of: 0; 100; 200; 300; 400; 500; 600; 700; 800; 900; 1000 Gray (Gy); each as many as 50 seeds per dose of radiation. The irradiated chili seeds were grown in small polybags containing 1:1 mixture of soil and compost. Germination parameters of chili seeds was observed 30 days after planting (DAT) to determine lethal dose of 20 (LD20) and lethal dose of 50 (LD50). The results showed that increasing doses of gamma rays caused greater damage to chili seeds therefore the seeds were not able to germinate. Local Tulungagung Variety had LD20 and LD50 of 147.62 Gy and 409.52 Gy; and the Local Ponorogo variety had LD20 and LD50 of 90.3 Gray and 453.7 Gray. Local Tulungagung Variety was more sensitive to gamma rays than Local Ponorogo Variety. LD20 and LD50 can be used to get a lot of genetic variation and obtaining the positive mutants.

1. INTRODUCTION

Chili is one of the important vegetable commodities has high economic value because it is a non-substitutional commodity that cannot be replaced with other agriculture products. During 2000-2018, chili consumption in Indonesia increased by 3.65% per year, from 696.000 tons to 1.28 million tons (PDSIP, 2020). In 2020, yield of *Capsicum frutescens* was 1.51 million tons and *Capsicum annum* was 1.25 million tons (BPS, 2021).

Increasing the potential yield of chili plants through quality seeds can be obtained by using superior varieties. Since 1980-2010, new chili varieties of 173 were released by the Agriculture Ministry, but few superior varieties were available because most of them were introducing varieties, therefore their adaptability were relatively low (Syukur *et al.*, 2013).

The development of new superior chili varieties requires the presence of high genetic variation in the basic population (gene pool) so that the chances of getting desired characters are greater. High genetic variation allows the selection to certain traits to be more effective.

Genetic variation can be increased through mutation induction by physic with cobalt-60 gamma rays and X rays. Gamma rays irradiation are more applied by plant breeders because the effectiveness and economical than other ionizing radiation due to its ease and penetration power (Marcu *et al*, 2013).

Radiation dose is very important in mutation induction because the appropriate dose would be able to induce permanent mutation in the next generation. Basically radiation causes damage to living tissue, a highly dose could causes cell or tissue death, but a very low dose would not induce mutations, therefore an optimum dose is needed. The appropriate dose will increase the frequency of mutations by 100,000 times, and the optimum radiation dose varies on plant material and varieties (Sudrajat & Zanzibar, 2009). Mutation induction with gamma rays on chili pepper at dose 100, 200 and 300 Gy raised genetic variation on qualitative characters (Rohcahyani *et al*., 2022).

Many mutations occurred in the range or below the 50% lethal dose or Lethal Dose 50 (LD_{50}), which is a dose that causes plant death by 50%. Several studies reported that the optimum radiation dose for mutation induction was obtained at around 50% lethal dose (Datta, 2019). LD_{50} serves as a parameter to determine the response of plants to radiation exposure or to measure the sensitivity of a tissue to radiation (Sudrajat & Zanzibar, 2009).

The LD_{50} dose also useful for predicting radiation doses would create of many mutants and will increase genetic variation (Anshori *et al*., 2014). The LD_{50} is different for each type of plant. The LD_{50} in soybean seeds were obtained at dose of 205.5 Gray (Warid *et al*., 2017), 532 Gray and 540 Gray in green bean seeds (Tah, 2006) and 60 Gray in black bean seeds (Thilagavati & Mullainathan, 2011). LD_{50} of three of chili genotype IPB C2, IPB C10 and IPB C15 were 317.93, 591.42 and 538.88 Gy respectively (Nura *et al*., 2015), while Gaswanto *et al*. (2016) reported LD_{50} of five chili varieties ranged from 448.84 to 629.68 Gy. This study aims to determine the LD_{20} and LD_{50} of gamma rays in local chili origin from Tulungagung and Ponorogo.

2. MATERIALS AND METHODS

The chili pepper seeds of local varieties of Tulungagung and Ponorogo were irradiated at National Nuclear Energy Agency of Indonesia (BATAN) with gamma rays or cobalt-60. Before chili seeds treatment with gamma rays, the chili seeds were germination tested based on International Seed Testing Association (ISTA) to observe viability of chili seeds. If percentage of germination ≥ 80%, then chili seeds irradiated with gamma rays.

Each local chili varieties seeds as many as 50 seeds per dose of gamma rays, were irradiated with Cobalt-60 gamma rays at doses of: 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gray using Gammacell 220 chamber. Germination of irradiated chili seeds was carried out in the germination medium consist of a mixture of compost : soil = 1:1. Germination of chili seeds was observed until the seedling were 30 days after planting.

The research parameters observed were germination parameters (germination percentage and germination rate). Lethal dose at 20 (mortality of seeds 20% or LD_{20}) and lethal dose at 50 (mortality of seeds 50% or LD_{50}) were determined using the Curve Expert version 1.3 software.

3. RESULTS AND DISCUSSION

3.1. Germination Percentage

Results showed that the increase of gamma rays dose caused decrease of the germination percentage in chili pepper Local varieties. The percentage of germination can be seen in Table 1. Germination percentage above 50% was obtained at 100-400 Gy in Local Tulungagung chili pepper, while Ponorogo Local chili pepper at 100-300 Gy.

Table 1. Chili seeds germination percentage of Local Variety of Tulungagung and Local Variety of Ponorogo due to Cobalt-60 gamma ray irradiation

Tulungagung Local chili		Ponorogo Local chili	
Gamma ray dose (Gray)	**Germination Percentage (%)**	**Gamma ray dose (Gray)**	**Germination Percentage (%)**
0	96	0	92
100	100	100	84
200	88	200	84
300	80	300	56
400	56	400	48
500	36	500	32
600	0	600	32
700	0	700	24
800	0	800	20
900	0	900	20
1000	0	1000	16

The Local variety of Tulungagung is more sensitive to gamma rays than Local Variety of Ponorogo. At doses above 500 Gy, none of the Local Variety of Tulungagung germinated, while Local Variety of Ponorogo had germinated even at dose of 1,000 Gy but their growth was severely hampered. Other researchers also reported on chili (Nura *et al*., 2015; Gaswanto *et al*. 2016) and sorghum (Nura *et al*., 2015) that the increasing of the gamma ray doses of 100-1000Gy caused a decrease in the percentage of germination.

The germination percentage of Local Variety of Tulungagung at 100 Gy was higher than without radiation (0 Gy). Similar result was reported Nura *et al*. (2015) in chili seed gemination percentage at gamma ray doses of 100-300 Gy were higher than 0 Gy. Nurrachmamila & Saputro (2017) also reported that rice seeds treated with a dose of 100 Gy gamma rays resulted higher percentage of germination than without radiation (0 Gy). Gamma radiation can stimulate RNA transcription for protein synthesis at the start of germination (Abdel-Hady *et al*., 2008).

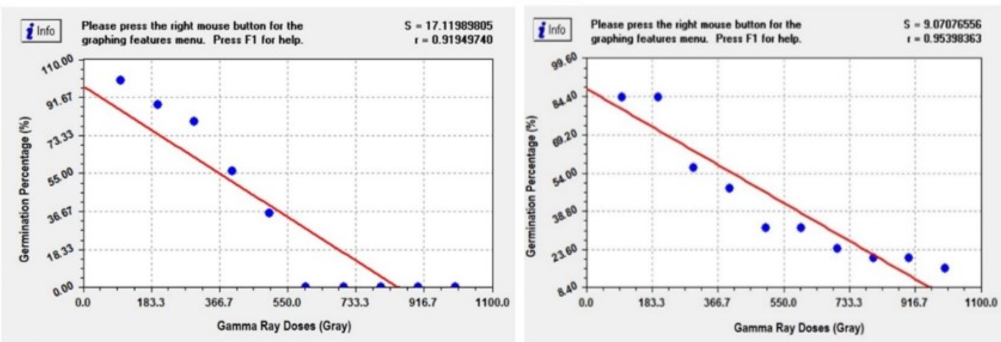
The days to germinate of chili pepper irradiated with gamma rays also increased with increasing doses of gamma rays (Table 2). Local Variety of Tulungagung germinated faster than Local Variety of Ponorogo starting at dose of 0-300 Gy. Based on two parameters of germination, percentage of germination and germination time, it looks that Local Variety of Ponorogo chili pepper is better than Local Variety of Tulungagung. This is showed that genetic factor affects the sensitivity of exposure to gamma ray radiation. Nura *et al*. (2015) also reported that three genotypes of chili pepper irradiated with gamma rays had different percentages of germination. Datta (2019) stated that genetic factor affects plant sensitivity to radiation due to physiological and biochemical differences in plants, for example fat content, vitamin C and other compounds in seeds play a role in determining radiosensitivity.

Table 2. Growth speed of chili seeds germination of Local Variety of Tulungagung and Local Variety of Ponorogo due to Cobalt-60 gamma rays irradiation

Tulungagung Local chili		Ponorogo Local chili	
Gamma ray doses (Gray)	**Days to germinate (days)**	**Gamma ray doses (Gray)**	**Days to germinate (days)**
0	8,4	0	7,9
100	7,9	100	8,5
200	8,0	200	8,4
300	8,9	300	9,6
400	10,6	400	9,8
500	12,2	500	12,3
600	0	600	13,3
700	0	700	14,4
800	0	800	15,1
900	0	900	15,7
1000	0	1000	16,4

Plant death after irradiation can occur due to deterministic effects, namely the biological effects of radiation that occur on cells cause cell death in part or all of the body (Sutapa *et al*., 2013). This deterministic effect can occur if the plant receives a radiation dose above the threshold dose, and its severity depends on the magnitude of the increase in the radiation dose at the threshold (Prabhandaru & Saputro, 2017). Exposure to high doses of gamma rays on seeds causes hampered of protein synthesis, hormone balance, gas exchange and enzyme activity. The structure, morphology and function change depending on the power and duration of gamma ray exposure (Al-Salhi *et al.,* 2004; Hameed *et al*., 2008).

Radiation causes a decrease in the cytokinin content by degrading or reducing their synthesis (Nepal *et al*., 2014), because the mitochondria and nucleus functions disrupted as a result of radiation, therefore ATP production reduced and ultimately has an impact on cytokinin synthesis (Shukla *et al*., 2016). Among of mutagens, gamma rays are the mutagens with the least damaging effects, caused point mutation or small deletions, but fast neutrons for radiation often causes large deletions, loss of chromosomes and translocations that result in the death of the individual (Zafar *et al*., 2022).



Local Tulungagung Chili

Local Ponorogo Chili

Figure 1. The relationship between gamma rays doses and the gemination percentage of chili seeds.

3.2. Lethal Dose 20 (LD_{20}) and Lethal Dose 50 (LD_{50})

The lethal dose (LD) reflects radiosensitivity of chili plants to gamma rays radiation. Radiosensitivity refers to the sensitivity of plant and animal tissues to ionizing radiation exposure associated with cell death (Datta, 2019). Figure 1 showed relationship between gamma rays radiation doses and the germination percentage of irradiated chili plants to determine LD_{20} and LD_{50}.

Scientiests agree that the opportunity of obtaining the useful mutations for plant breeding occur at radiation doses where 50% of the population dies or LD_{50} (Álvarez-Holguin *et al*., 2019). Genetic variation due to mutation induction is usually obtained at LD_{20}-LD_{50} (Soeranto, 2012).

The higher gamma rays dose needed to reach the LD_{50}, the lower the radiosensitivity. The Local of two chili plants have a different sensitivity to gamma rays radiation. Chili plants of Local variety of Tulungagung are more sensitive to gamma-ray radiation compared to Local variety of Ponorogo. The dose of gamma rays LD_{20} = 147.62 Gy and LD_{50} = 409.52 Gy for Local Variety of Tulungagung and LD_{20} = 90.31 Gy and LD_{50} = 453.74 Gy for Local Variety of Ponorogo.

Figure 2. showed the seedlings of two Local varieties of chili pepper irradiated with gamma rays. Local Variety of Tulungagung did not germinate at ≥ 600 Gray, while Local variety of Ponorogo could germinate at 600-1000 Gy but they could not grow normally at those doses. Nura *et al*. (2015) recorded that three of chili genotypes were able to grow at 700-1000 Gy but it was around 10%, and IPB C10 was more sensitive than IPB C2 and IPB 15 to gamma ray exposure. Warid *et al*. (2017) also reported on soybean plants had been irradiated with gamma rays, the Burangrang variety was more sensitive than Anjasmoro to irradiation treatment.

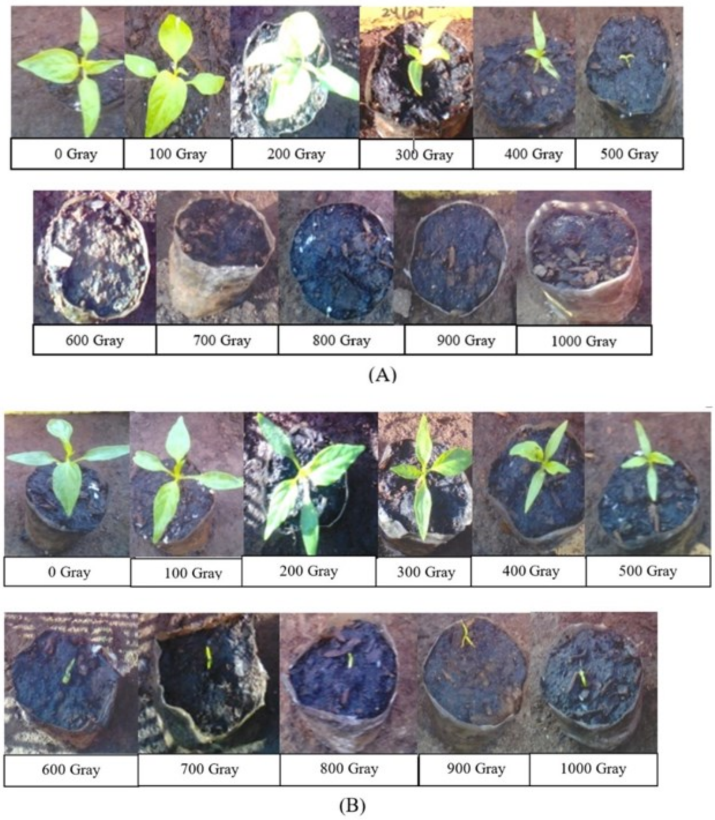


Figure 2. Chili pepper seedlings irradiated gamma rays. (A) Local Variety of Tulungagung; (B) Local Variety of Ponorogo

The work of gamma ray radiation to increase the genetic variability of a plant is also determined by the radiosensitivity level of the plant. The higher radiosensitivity of gamma radiation, the lower the seed germination, plant height, and plant root length. The decline in plant growth is due to the deterministic effect of gamma ray radiation.

The two Local varieties of chili are thought to have different physiological traits. In addition, the environment and the dose of gamma ray radiation received by two local varieties of chili plants also role played to obtain the genetic variation of chili plants. According to Barmawi *et al*. (2015) statement, the success of radiation to increase population variability was determined by the radiosensitivity of the plant. In addition, the stadia of cell development, the number of chromosomes and the age of plant tissue, oxygen, temperature, and radiation dose also affect radiosensitivity. According to Albokari *et al*. (2012) there are two factors that cause differences in radiosensitivity, biological (genetic) and environmental.

The LD_{50} is the effective dose that can cause 50% of deaths in an irradiated population. Various studies exhibited that LD_{50} is the optimum dose to induce the of mutants, and mutants formed at doses below the LD_{50}.

Generally, first generation of mutation plants (M_1) have physiological effects. The level of damage at M_1 generation plants as a result of large doses of mutagens can be measured quantitatively in various ways, such as decreased seed germination, germination rate, vigor, sterility and plant death. It can be used to determine the dose of mutagen required for mutation induction (Shu, 2013).

4. CONCLUSION

The higher gamma ray doses exposed to the chili pepper seeds caused some damage of seeds, therefore the seeds were not able to germinate. The radiosensitivity of the two Local Varieties of chili pepper were different because Local variety of Tulungagung was greater than Local Ponorogo. Local Variety of Tulungagung had LD_{20} 147.62 Gy and LD_{50} 409.52 Gy and Local variety of Ponorogo had LD_{20} 90.31 Gy and LD_{50} 453.74 Gy. LD_{20} and LD_{50} in both of local varieties of chili were able to be used as appropriate doses of gamma rays to obtain the most genetic variation caused of mutation and positive mutants that important in plant breeding.

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