Optimization of gamma ray irradiation dose on strawberry plantlets

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Abstract. As a commodity that is cultivated vegetatively, strawberries have a relatively low genetic diversity. One effort to increase the diversity of strawberries and genetic quality improvement can be made by using gamma-ray radiation. In vitro explant of strawberry cv Early Bright were radiated with Gamma-ray P3TIR BATAN facility. Starting with a radiosensitivity test to find out Lethal Dose (LD) 50 with dosage level; 5, 10, 20, 30, 40, 60, 80, 100, 125, 150, 175, and 200 Gy. LD50 observations were carried out to get the optimal dose. The treatment of several doses of gamma-ray radiation showed varied results, with the percentage of explants living decrease by increasing the dose of irradiation. Irradiation dose until 10 Gy did not affect the survival rate of explant—the treatment dose of 80 Gy and above causes 100% mortality on the final observation. The dose of 60 Gy had a significant effect on shoot growth, as indicated by curly leaves' growth. The next optimal dose recommended for radiation is set at 20 and 30 Gy to anticipate genetic changes that are not morphologically expressed and to avoid the negative effects of morphological changes.

1. Introduction
Strawberry is not native to Indonesia but can be developed in the highlands of several regions in Indonesia. The economic value of strawberries is very high, both as fresh fruit and raw material for the food processing industry. Nutritional value is also high because the various chemical compounds that are very useful are not indented [1]. Currently, several varieties have been adapted and grown by farmers in Indonesia [2]. Farmers' cultivation constraints are low productivity, fluctuations in production due to environmental fluctuations, less attractive fruit size and color, low storage capacity, and high vulnerability to pests [3].

One of the efforts to overcome cultivation constraints is by providing new superior varieties that have the expected characters. Strawberry is an octoploid plant that reproduces vegetatively. The genetic diversity is relatively low, so conventional breeding is not easy to do [4]. Mutation breeding has proven to be the most feasible approach for creating new genetic diversity in various plant species quickly without changing the overall genetic constitution [5]. Physical mutagens are more advantageous than chemical mutagens because there is no need to remove mutagens after application [6], and leaves no hazardous waste that needs special handling. Mutation induction with physical mutagens has been widely used. 90% of the mutants that exist today are acquired by physical mutations, of which 64% are
using gamma rays and 22% by X rays [7][8]. Based on data from the IAEA Mutant Database Variety, 1.665 of the 3.362 mutant varieties currently released are the result of mutations by gamma-ray irradiation.

Selection of mutagen type and the optimal dosage is important to open up plant improvement opportunities through mutation breeding [9]. The mutation dose is more important than the mutagen type. Whatever the mutagens, before application on a larger scale, the dose of the mutation must be determined correctly [10]. High-dose mutations' application opens a higher chance of acquiring mutants but often results in infertility and individual death [11]. In vitro techniques allow the induction of a large number of explant mutations and a relatively short time to separate mutated and non-mutated parts. Also, the mutants obtained are free from the disease [12]. The Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI) collects strawberry explants ready to be mutated by gamma-ray irradiation. Experiments are needed to determine the LD50 (the dose that causes 50% mortality) to get the correct dose in mutation induction (Predieri, 2001; [14]. This study aims to obtain the right dose of gamma-ray irradiation for strawberry explants as the first step for genetic improvement of strawberries in Indonesia.

2. Methods
The research was carried out at the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Brawijaya and Tissue Culture Laboratory, Indonesian Citrus and Subtropical Fruits Research Institute (ICSFRI), from May to September 2019.

The genetic material used was the Early Bright strawberry explants. The source of the explants is the result of propagation from meristem culture. The main stems obtained were cut to 500 µM and incubated to further select mutation induction materials on MS medium without hormones.

Gamma-ray irradiation activities were carried out at P3TIR National Nuclear Energy Agency of Indonesia (BATAN). Control explants were explants that were not treated with irradiation. A total of 20 explants each were irradiated at a dose of 5, 10, 20, 30 Gy, and 10 explants each were irradiated at a dose of 40, 60, 80, and 100 Gy. The irradiated explants were planted on propagation media (MS + 3% sucrose, 10% myoinositol, 0.05 mgL-1 BA and 0.025 mgL-1 NAA for recovery.

Observation of Lethal Dose (LD) 50 on irradiation was carried out for one month, and further observations were made until the plants showed significant changes in appearance. Observations included the percentage of mortality explants and the qualitative appearance of the planlets. The observations were compared and analyzed descriptively.

3. Results and Discussion
Genetic improvement by mutation is very beneficial if carried out in vegetatively reproduced plants. Once a superior genotype has been obtained, the superiority will be maintained through clonal propagation. Inducing mutations on in vitro explants of strawberries are carried out for several reasons, i.e. (1) the in vitro propagation method is relatively well established [15][16] and (2) chimeras can be isolated and can be developed into whole plants easily [17] so that the selected material will be extensive and the opportunity to get new varieties will be even greater.

The first observation of the survival percentage of explants after irradiation did not show a concomitant trend with radiation dose. Explants treated with 5 Gy irradiation decreased their vitality to about 80%, but at 10 Gy treatment, all explants were able to survive. Contamination contributed to decreased viability at the 5 Gy treatment. Explants irradiated at a dose of 60 Gy, and higher indicated high viability. The treatment that produced a percentage of 50% survives explants was a dose of 20 Gy. At doses 30 and 40, the percentage of live explants was 60% (Figure 1). Because the trend showed an unclear pattern, gamma-ray irradiation was re-treated on the new explants with five new higher doses, which are 125, 150, 175, and 200 Gy. Simultaneously with this second treatment, the treatment was also repeated with doses of 20 and 30 Gy for a higher number of explants.
In the second observation (about 4 months after irradiation), the propagated plantlets from explants treated above 60 Gy deteriorated and eventually died (Figure 1). At doses of 60 Gy and higher, there were morphological changes in the leaves, in the form of an abnormal leaf shape that became curled (Figure 2).

**Figure 1.** Percentage of survival explant (%) by different gamma ray irradiation dose

In the first gamma-ray irradiation treatment, several plantlets were obtained that could still survive, but there was a difference in their growth performance. Irradiation treatment at a dose of 60-100 Gy caused a decrease in growth performance. This shows that high doses have an impact on the decreasing quality of plantlet growth [18]. Meanwhile, the growth performance at 5-40 Gy dose of radiation treatment can still be categorized as normal (Table 1).

Treatment doses of 125, 150, 175, and 200 Gy caused a low survival rate, namely 0 - 15% and poor growth performance. The explants' growth was stagnant, the shoots were stunted and abnormal, and the number of shoots per cluster was very low. The shoot shape in treatment 175 and 200 Gy was more abnormal when compared to the results of the 150 Gy dose treatment (Figure 3).
Table 1. Growth of plantlets treated with gamma ray irradiation at different doses

| No | Treatment (Gy) | Growth performance |
|----|----------------|--------------------|
| 1  | 5              | ++++               |
| 2  | 10             | ++++               |
| 3  | 20             | ++++               |
| 4  | 30             | ++++               |
| 5  | 40             | ++++               |
| 6  | 60             | +++                |
| 7  | 80             | ++                 |
| 8  | 100            | +                  |

Figure 3. Appearance of strawberry plantlets with irradiation treatment of 100 Gy (a) 125 Gy (b) 150 Gy (c) and 175 Gy (d)

Based on the results of observations of the percentage of mortality and the growth performance of plantlets, the dosage between 20 and 30 Gy is thought to be the dosage range that results in 50% mortality, such as the results of previous studies on citrus commodities [19]. This dose will then be recommended to treat mutations with gamma rays on strawberry explants because at this dose. It is expected that genetic changes have occurred even though no morphological changes. The use of low doses is also recommended by [20], which states that low doses of gamma radiation could be used for better survival and growth in strawberries under in vitro conditions. Doses of 60 Gy and above are not recommended because they could cause too much damage. Similar research on strawberry conducted by Thanh [21] shows that doses of 60-100 Gy cause stunted plants, small and abnormal fruit.

The formation of mutated plant populations with optimal radiation doses (20 and 30 Gy) was carried out by maintaining the clusters for further separation, acclimatization, and transfer to the field. Observations of genetic diversity both morphologically and molecularly will then be carried out when the plants are mature and stable in the land.

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