Induction of mutations with gamma ray radiation to improve the characteristics of wheat [Triticum aestivum L.] genotype IS-Jarissa

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Abstract. Wheat [Triticum aestivum L.] is one of the strategic food crop commodities in Indonesia. Efforts to develop wheat continue to meet growing domestic needs. The aim of this study was 1) to obtain information on effective irradiation doses to obtain genetic diversity and diversity of morphological growth in wheat, 2) obtain information about the desired target mutants, which have early maturing and high-yielding wheat characters. The research began by irradiating the IS-Jarissa genotype wheat seed while looking for an effective dose of irradiation to change the genetic composition of wheat. Furthermore, planting mutants 1 [M1] and early mutant selection were conducted on M2. The results showed that: 1) IS-Jarissa wheat varieties were able to live on irradiation with a dose of 0 Gy, 100 Gy, 200 Gy, 300 Gy, and 400 Gy. 2) There were 21 mutant candidates in the M2 stage.

Keywords: mutants candidates, selection

1. Introduction

Wheat [Triticum aestivum L.] is a food commodity that is not native to Indonesia, but wheat nowadays is the second carbohydrate source after rice, which is 18 kg per capita [1]. The need for wheat in Indonesia is increasing year by year. The Chairperson of the Indonesian Flour Producers Association [APTINDO] said that the volume of Indonesia's wheat imports in 2017 recalled around 9% to 11.48 million tons from the previous year [2]. Research results [3][4][5] regarding adaptation and multi-location tests of several wheat genotypes from the Slovak Republic show that wheat can adapt well in Indonesia. Some of the genotypes, including those with high productivity, have long harvesting characteristics. Therefore, it is necessary to breed new superior wheat plants with desired characteristics, such as early maturing and high production. Plant breeding is the activity of changing the genetic makeup of individuals and plant populations for a purpose so that more useful plants can be obtained, one of which is by genetic mutations.

Plant genetic mutations can be induced by using mutagens such as gamma-ray radiation. The parts of plants that are irradiated are usually seeds or other parts of plants that can be grown. Mutation plant breeding has specific characteristics, among others, it is very effective to change a few properties in improving plant varieties [6]. Research conducted by Sulianyah showed that mutation induction was able to speed up the mutant harvest time of some local Sumatran rice [7]. The objectives of the study were to obtain information on effective irradiation doses to obtain genetic diversity and diversity of
morphological growth in wheat and to obtain information on the desired target mutants that have the characteristics of early maturing and high-yielding wheat.

2. Material and Method

In this study, the material used was the IS-Jarissa wheat genotype [which is a collection of Suliansyah, 2012]. Seed irradiated with a gamma-ray dose of 0 Gray [as a control]; 100 Gray; 200 Gray; 300 Gray; 400 Gray; 500 Gray; 600 Gray; 700 Gray; 800 Gray; 900 Gray; and 1000 Gray as much as 150 grams per dose. Seed irradiation was carried out at the National Nuclear Energy Agency [PAIR-BATAN] Isotope and Radiation Application Center, Pasar Jumat, Jakarta using gamma-ray radiation source from the Gamma Cell Co-60 Irradiator.

2.1. Phase M₁; Orientation of Effective Irradiation Doses on Genetic Improvement of IS-Jarissa Wheat Genotypes through Induction Mutations

To see the effects of various doses of irradiation on the reduction in growth rates, observations of the growth and germination rates were carried out in the seedling phase in the cold room [Agronomy Laboratory]. Germination is carried out in a seedbed containing topsoil growing media and compost in a ratio of 1:1. Seeds from each treatment of irradiated doses are planted in rows of 100 grains to observe the growth of the seeds. Planting is carried out in the seedbed for 3 weeks. The variables observed in this stage are the% of dead seeds and% of living seeds. Also, a regression analysis was performed to obtain a Lethal Dose value of 50 [LD₅₀]. Irradiation dose orientation activities by planting M₁ populations in the field [Experimental Land at the Andalas University Agricultural Technology Development Center [PATPKP UNAND] in Jorong Galagah, Alahan Panjang, Lembah Gumanti, Solok Regency, West Sumatra]. The seeds of each irradiation dose treatment were planted with a tugal-system with one seed per planting hole of 1000 grains per irradiation dose treatment. Planting is carried out on land that has been processed and has been given plastic mulch per bed with a distance of 20 cm x 25 cm. The purpose of this activity is to observe physical damage to M₁ populations as a result of irradiation treatment and to obtain seeds that will be used as material for the M₂ population. Harvesting is done by taking three main panicles from each clump to be used as M₂ lines.

2.2. M₂ Phase; Formation of Mutant Strains, Genetic Diversity and Early Mutant Selection in M₂ Populations

The seed planted is the M₁ strain seed derived from the effective irradiation dose treatment observed at the M₁ stage and the seed from the control plant as a comparison. The seeds of each strain are planted with the number of seeds that is one seed per planting hole. Planting was carried out at the Experimental Land at the Andalas University Agricultural Technology Development Center [PATPKP UNAND] in Jorong Galagah, Alahan Panjang, Lembah Gumanti, Solok Regency, West Sumatra. In this process, the seeds of each line are separated from one another. For each line, lines [labels] are given as markers and to distinguish between one line and the other lines. Crops are cultivated like wheat cultivation usually, fertilization is carried out according to the recommended dosage and time of fertilization as well as integrated weed, pest and disease control. After planting, the chlorophyll mutation of the leaf [seed sprouts] is observed. Chlorophyll mutation was observed from seed germination to age 21 days.

At this stage, an observation is made of the chlorophyll mutation form. Chlorophyll mutation is an indicator of genetic damage due to mutation treatment that causes the formation of genetic diversity. Observation of chlorophyll mutations was carried out by comparing the shape of M₂ plant leaf color to the chlorophyll mutation color indicator according to Gustafsson's method by observing the color of the plant leaves from germination to 21 days [8]. From the observation data, it can be seen the mutant frequency with the following formula:

\[
\text{Mutant Frequency} = \frac{\text{Number of Mutants}}{\text{Number of plants from all panicles germinated}} \times 100%
\]
At this stage, the target mutant selection starts. Expected target mutants are mutants that have a shorter age and more ears. Observation of early-aged mutants was carried out by observing when the first flower panicles were released on the M2 plant from planting in the field to the 50% flowering control plant. In addition to observing more rapid flowering age, vegetative growth [agronomic traits] were also observed, such as plant height, number of tillers, number of panicles per clump, number of grains per clump and age of plants.

Each selected mutant is harvested separately and labeled according to the line, and 20 plants in the line where the mutant is made as a sister plant are also harvested separately and labeled. Analysis of the genetic variables of M2 population was carried out by calculating the population mean [\( \mu \)], standard deviation, phenotype variance values [\( \sigma^2_p \)], environmental variability values [environmental variability values [\( \sigma^2_E \)], the value of genetic diversity [\( \sigma^2_G \)], heritability value [\( h^2 \)], and the value of variability in each line. Genetic diversity is said to be broad if value \( \sigma^2_G > 2 \) [\( \sigma^2_G \)] and said to be narrow if the value \( \sigma^2_G < 2 \) [\( \sigma^2_G \)]. Heritability value is calculated using the formula \( H^2 = \frac{\sigma^2_G}{\sigma^2_E} \), where the heritability is low when \( [h_{bs} \leq 0.2] \), mid \( [0.2 \leq h_{bs} \leq 0.5] \), and high \( [h_{bs} > 0.5] \). To analyze the difference in the mean values of the control plants, a statistical analysis was performed according to the T-test.

3. Result and discussion

3.1. Phase M1; Orientation of Effective Irradiation Doses on Genetic Improvement of IS-Jarissa Wheat Genotypes through Induction Mutations

3.1.1. The orientation of Effective Irradiation Doses in the Seedling Phase
Irradiation dose orientation is a screening conducted to obtain an effective irradiation dose in generating genetic diversity. The effective dosage is a dose that produces a high frequency of mutations with little physical damage. The effect of irradiation carried out can be observed from the percentage of germination that grows in the treatment of various doses of gamma-ray irradiation can be seen in Table 1.

| Doses of Gamma-Ray Irradiation [Gy] | Percentage of Sprouting [%] |
|-------------------------------------|-----------------------------|
| 0                                   | 93                          |
| 100                                 | 85                          |
| 200                                 | 82.3                        |
| 300                                 | 79.4                        |
| 400                                 | 40                          |
| 500                                 | 0                           |
| 600                                 | 0                           |
| 700                                 | 0                           |
| 800                                 | 0                           |
| 900                                 | 0                           |
| 1000                                | 0                           |

Based on the percentage of growth obtained after the plants were germinated in the seedbed, the plants that had the highest percentage of growth outside the control plants were achieved by wheat plants with a dose of gamma-ray irradiation of 100 Gy. The higher the dose given, the lower the
percentage value of wheat plant growth. Gamma rays applied to the Amorphophallus muelleri plant produce long morphological diversity, mortality and dormancy [delay in sprout growth] [11]. Exposure to ionizing radiation on a biological system activates several chemical and physical reactions starting from the initial absorption of energy until finally there is biological damage. The result of the excitation and ionization reaction results in the formation of ionized water molecules and free radicals [12]. These free radicals can damage or modify plant cell components so that they affect chemical and biological processes that may be vital to the survival of an organism [13].

**Effect of Irradiation Dose on Germination**

In this research, Gamma-ray irradiation was carried out on wheat seeds at a dose of 0-1000 Gray. Hanafiah et al. In their research stated that from many studies of mutations that utilize gamma-ray irradiation, mutations that generally result in high genetic diversity are at intervals of lethal doses of LD\(_{50}\) and below LD\(_{50}\). The optimum dose value obtained will be used as a reference to obtain genetic diversity in M\(_2\) [14]. Figure 2 shows that the response pattern of the percentage of live plants produced by wheat germ is a Linear response: \( y = a + bx \), with \( a = 91.36 \) and \( b = -0.113 \). The LD\(_{50}\) value obtained is equal to 28.42 Gray.

3.1.2. Orientation Doses of Irradiation in the Field as well as Planting Population M\(_1\)

In planting M\(_1\) in the field, wheat plants that grow in the field only at doses of 0-400 Gy, while wheat plants with a treatment dose of 500-1000 Gy nothing grows can be seen in Figure 2. M\(_1\) mutant plants that can grow in the field, in general, there is no difference between growth and harvest time of mutant plants and their native plants [control plants]. However, some plants from the M\(_1\) population grow differently from control plants in Figure 3. The difference between control plants and mutant plants is probably the result of physiological damage to gamma-ray irradiation which causes the growth of several different plants with control plants. Induction of physical mutations with gamma-ray irradiation influences changes in plant morphology. Irradiation in plants can cause different leaf shapes including growth inhibition [dwarf], leaf fusion, and mosaic [discoloration] [15].

One thing that is desirable in induced mutations is large genetic damage and small physiological damage. This is a very valuable factor in producing high genetic variability. For the induction of genetic diversity, it is expected that the induction of mutations can cause very few chromosomal aberrations, physical damage, and sterility, and at the same time can be controlled to produce the desired mutations [16]. In this study, the LD\(_{50}\) was obtained at a dose of 300-400 Gy. Guided by the results of the study [17], then in this study with LD\(_{50}\) between doses of 300-400 Gy, it is very possible the optimum irradiation dose at 200-400 Gy.
3.2. \textit{M}_2 \textit{Phase; Formation of Mutant Strains, Genetic Diversity and Early Mutant Selection in M}_2 \textit{Populations}

3.2.1. \textit{Formation of Mutant Strains and Analysis of Genetic Diversity}

This phase of M\textsubscript{2} aims to form mutant lines, observe genetic diversity formed due to the induction mutation treatment and selection of early age characters [early mutants]. At this stage, forms of morphological abnormalities began to emerge as indicators of genetic diversity caused by the treatment of induced mutations. Morphological abnormalities have emerged at the time the sprouts emerge to the ground such as mutations in M\textsubscript{2} germination which are commonly called chlorophyll mutations. Gamma-ray irradiation treatment with a dose of 200 Gy, 300 Gy and 400 Gy cause chlorophyll mutations and several types of mutations that appear on M\textsubscript{2} germination. The types of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Wheat beds of gamma irradiation treatment with a dose of 500 Gray, 600 Gray, 700 Gray, 800 Gray, 900 Gray and 1000 Gray that do not grow.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Difference between control plants and mutant plants; [a] difference in control plant height and mutant plant height; [b] differences in panicle control plants and mutant plants.}
\end{figure}
chlorophyll mutants that appear in M\textsubscript{2} germination such as Albina, Tigrina, Xantha, Viridis, Marginata, Spotting leaf can be seen in Figure 4.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Mutant} & \textbf{Alb} & \textbf{Tig} & \textbf{Vir} & \textbf{Mar} & \textbf{Xan} & \textbf{Spot} \\
\hline
Total & 3 & 6 & 31 & 2 & 9 & 13 \\
\hline
\hline
\textbf{Mutant Frequency} & 0.026 & 0.052 & 0.267 & 0.017 & 0.078 & 0.112 \\
\hline
\textbf{Control} & - & - & - & - & - & - \\
\hline
\end{tabular}
\caption{Chlorophyll Mutation Types, Number of Mutants, and Frequency of Mutants in M\textsubscript{2} Phase with Irradiation Doses of 200 Gy, 300 Gy, and 400 Gy.}
\end{table}

\textbf{Figure 4}. Chlorophyll mutations that occur in M\textsubscript{2} generation.

M\textsubscript{2} sprouts grow diverse and there is a morphological difference from the control plants. Based on research [18], the abnormality of the population being treated for irradiation shows that there is a big change in the level of genomes, chromosomes, and DNA so that the physiological processes in genetically controlled cells become abnormal. In this M\textsubscript{2} study, observations on the type of mutation, number of mutations, mutation frequency, and mutant frequency found in M\textsubscript{2} strains with irradiation doses of 200 Gy, 300 Gy, and 400 Gy can be seen in Table 2.

Table 2 shows that irradiation doses of 200 Gy, 300 Gy, and 400 Gy have produced a total of 6 mutations of chlorophyll, namely Albina, Tigrina, Viridis, Marginata, Xantha and Spotting leaf. Of the
six types of chlorophyll mutants, the type of mutation of *Chlorophyll viridis* is more common than other types of chlorophyll mutants with a mutant frequency of 0.267%. Then followed by the chlorophyll xantha mutant with a mutant frequency of 0.017% and the least found was the chlorophyll marginata mutant with a mutant frequency of 0.02%.

In this study mutations that occur in chlorophyll are not yet known whether caused by mutations that occur in chromosomal [chromosomal genomes] or mutations that occur in extrachromosomal [cytoplasmic genome]. To find out the chlorophyll mutation that occurs in this mutant strain, genomic analysis needs to be done to determine whether the type of mutation that formed in leaf chlorophyll occurs due to mutations that occur on chromosomal or occur on extrachromosomal.

Chlorophyll seems to be controlled by many genes located on several chromosomes that can be adjacent to the centromere and proximal segments of the chromosome. With mutations due to physical mutagens, deletion or DNA band deficiency on chromosomes is suspected. The effect of this deficiency can damage the function of chlorophyll so that the leaf color is not formed [slightly formed] [19].

### 3.2.2. Mutant Selection in M₂ Population

Planting M₂ of each M₁ family is used as the basis for the formation of lines for the next stage of the breeding process. From the planted lines it is expected to contain the diversity of characters that are the target of selection and the emergence of target mutants has a great opportunity. Data on genetic parameters observed through plant height [cm], number of tillers [clumps], number of panicles [clumps], number of pithy grains [grain] and age of harvest [days] can be seen in Table 3.

| Character            | µ   | σ²p | σ²e | σ²g | h²   | Category | 2.Sd | Variability |
|----------------------|-----|-----|-----|-----|------|----------|------|-------------|
| Plant height [cm]    | 69.57 * ± 12.74 | 162.42 | 29.71 | 132.71 | 0.82 | High     | 25.49 | Large       |
| Number of tillers    | 13.03 * ± 8.19 | 67.04 | 29.42 | 37.61 | 0.56 | High     | 16.38 | Large       |
| Number of panicles   | 11.69 * ± 7.69 | 59.11 | 25.88 | 33.23 | 0.56 | High     | 15.38 | Large       |
| Number of pithy grains [grain] | 123.09 * ± 107.43 | 11,541.33 | 7,807.73 | 3,733.60 | 0.32 | Mid      | 214.86 | Large       |
| Harvest age [day]    | 110.69 * ± 4.59 | 21.11 | 0    | 21.11 | 1.00 | High     | 9.19  | Large       |

Note: *] significantly different at the 0.05 level according to the t-test, ] not significantly different.

In Table 3 generally seen that the estimated value of heritability in all characters is in the high category. However, the estimated heritability value that is classified as moderate is found in the character of the number of pithy grain. The high estimated heritability indicates that the character is more dominantly influenced by plant genetic factors than by environmental factors. This shows that genetic factors make an important contribution to the next process. The value of heritability illustrates how the proportion of a gene can be passed on to future generations.

High or low heritability can be caused by genetic evaluation methods. If the evaluation is based on individual plants, the heritability is relatively lower. In contrast, the heritability is relatively high if evaluated based on plant population. On increasing genetic capacity through plant breeding, certain traits such as yield are usually measured based on plots rather than on individual plants [20].
Selection activities for target mutants [early mutants] begin at this M$_2$ stage. The selection activity is carried out by selecting individual mutant plants that have a faster harvest age compared to the harvest age of the original plants. The results of the individual selection of early mutants can be seen in Table 4.

**Table 4. Results of Early Mutant Selection that Appears in M$_2$ Populations as a result of Gamma-Ray Induction**

| Irradiation Dose [Gy] | Age [day] | Total Mutants | Total Population | Mutant Frequency |
|-----------------------|-----------|---------------|------------------|------------------|
| 200                   | - 5 -     | 5             | 6608             | 0.08             |
| 300                   | - 9 -     | 9             | 3000             | 0.30             |
| 400                   | - 7 -     | 7             | 2000             | 0.35             |
| [Control]             | TF 50     | 945           | -                |                  |

Note: TP 50 [Time Flowering control plant 50%]

In Table 4 it can be seen that the results of individual selection of the early adult mutant candidates [harvest age 91-100 days] at a dose of 200 Gy were 5 plants, at a dose of 300 Gray were 9 plants and at a dose of 400 Gray were 7 plants. While the age of harvest of native plants [control] is in groups above 101 days. Early mutants that appear in the M$_2$ generation can be seen in Figure 5.

![Figure 5. Early-age mutants that appear in the M$_2$ generation.](image)

In general, phenotypically early candidate mutants have a lower posture [semi-dwarf] when compared to the original plant phenotype. The effect of induction mutations in this study produced early-aged mutants and had a short posture from the original plant [control]. At the dose of gamma-ray irradiation with a dose of 200 Gy, 300 Gy and 400 Gy can give rise to mutant properties as a result of genetic changes due to mutations in genes that control plant age. Radiation mutations provide wider genetic diversity so breeders have more choice in making selections. Radiation mutations given affect the flowering age to be faster, and the age of harvest is faster than the parent varieties. Radiation mutations are reported to influence the age of flowering and harvest age so that it becomes more early [21][22]. This is caused by genetic changes due to the influence of gamma-ray radiation. This genetic change is permanent and passed down to the next generation, so it is a new individual who has different characteristics from its parent [23].

**4. Conclusions**

An effective dose of irradiation in producing high genetic diversity and low physical damage is at a dose of 200 Gy-400 Gy. 21 candidates for early mutants have been obtained with a harvest age range of 91-100 days. At a dose of 200 Gy were 5 plants, at a dose of 300 Gray were 9 plants and at a dose...
of 400 Gray were 7 plants. From the results of this study, it can be suggested that further research needs to be carried out such as purification of mutants 3 [M3] and mutant sister plants, DNA analysis, nutritional quality testing of sediment lines and testing stages M4 and M5 for efforts to release new superior varieties as genetic improvement results of IS-Jarissa wheat varieties that have better potential.

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