The growth and leaf chlorophyll in black rice (*Oryza sativa* L. Indica) plants induced by gamma rays as responses to the drought stress condition

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Abstract. The aims of this study to determine the growth and leaf chlorophyll content in black rice (*Oryza sativa* L. Indica) which were induced by gamma rays as responses to the drought stress conditions. This study was carried out on July–November 2020, at the experimental field (screenhouse), Medicinae Street, UNS Perum, Jati Village, Jaten District, Karanganyar Regency. Meanwhile, the chlorophyll content test was carried out at Plant Tissue Culture and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret. The study was a field trial research design without replication. The research treatments were 24 genotypes as follows: 10 genotypes of 100 gray gamma-ray irradiation with 2 control genotypes; and 10 genotypes of 200 gray gamma-irradiation with 2 control genotypes. The results showed that the plant height was lower in plants with irradiation compared to the Control-01 plant. The crown-root ratio showed irradiated plants had higher average results than control plants. The leaf chlorophyll of 100 gray irradiated plants showed higher results than control; while 200 gray irradiated plants showed similar results.

1. Introduction
The change of climate due to global warming becomes a big challenge right now and in the future. The recent increase in average temperature has decreased global food production. It was reported that an increase in temperature had decreased the world’s rice production [1]. Lack of water affects all aspects of the growth of plant including physiological, biochemical, anatomical, and morphological processes.

Lack of water causes significant decreases in crop yield, and, moreover it causes death in plants [2]. The measurement of physiological characters such as chlorophyll concentration is an approach to study the effect of the lack of water on growth and yield, because this parameter is closely related to the rate of photosynthesis [3]. In addition, another challenge in black rice cultivation is the habitus of tall plants that cause the plants to easily fall and it causes difficulties in cultivation process for farmers.

Genetic mutation is one of hybridization methods that can be done to get new superior varieties [4] which can be induced using physical mutagens such as gamma ray radiation [5]. It is considered effective and efficient ways in plant breeding programs, because the process is relatively faster than hybridization. Furthermore, it can produce new characteristic in plants [6]. Thus, applying drought stress...
and using gamma ray-induced plant were expected to give information on the growth response and chlorophyll content of black rice plants.

2. Materials and methods
This study was carried out on July-November 2020, at the experimental field (screenhouse), Medicinæ Street, UNS Perum, Jati Village, Jaten District, Karanganyar Regency. Meanwhile, the chlorophyll content test was carried out at the Plant Tissue Culture and Biotechnology Laboratory, Faculty of Agriculture, Sebelas Maret University. The study used a field trial research design without any replication. The screenhouse area is 11.1 meters long, 4 meters wide and 2.1 meters high. The cultivation was done by using polybags with a diameter of 50 cm x 50. Each genotype contained 10 plant populations for the observation, so that in total there were 240 plants.

In total the research used 24 genotypes: 10 genotypes of 100 gray gamma-ray irradiation with 2 control genotypes, and 10 genotypes of 200 gray gamma-irradiation with 2 control genotypes. In this study, drought stress treatment was carried out with 100% water requirement for controls, and 50% for all gamma irradiation treatments.

3. Results and discussion

3.1. Plant height
The plant height is a variable that could indicate the plant’s vegetative growth activities, because the growth of height plant made the plant experience cell division. The plant height growth was influenced by some factors, such as the environment, physiological conditions, and the genetics of the plant. It was measured starting from the initial growth period until before it was in the generative phase. The measurement of plant height was done from the base of the plant stem to the tip of the longest leaf.

Table 1. Black rice (Oryza sativa L.) plant height with 100 gray gamma-ray irradiation of Boyolali assessment on M4 generation with different soil and water treatments.

| Strain      | Treatment | Height Plants (cm) | Average  |
|-------------|-----------|--------------------|----------|
|             |           | Shortest | Longest    |          |
| Control-01  | 100%      | 68.90    | 73.00      | 70.3±2.33|
| M4-By-100-A | 50%       | 62.30    | 78.00      | 71.10±8.02|
| M4-By-100-B | 50%       | 64.30    | 74.40      | 70.73±5.58|
| M4-By-100-C | 50%       | 74.00    | 76.20      | 74.90±1.15|
| M4-By-100-D | 50%       | 42.10    | 64.30      | 54.13±11.21*|
| M4-By-100-E | 50%       | 66.50    | 78.00      | 70.76±6.29|

The number followed by (*) sign is significantly different from control based on the test result with α= 0.05

Table 2. Black rice (Oryza sativa L.) plant height with 100 gray gamma-ray irradiation of Bantul assessment on M4 generation with different soil and water treatments.

| Strain      | Treatment | Height Plants (cm) | Average  |
|-------------|-----------|--------------------|----------|
|             |           | Shortest | Longest    |          |
| Control-01  | 100%      | 72.20    | 81.00      | 77.06±4.47|
| M4-Bt-100-A | 50%       | 74.00    | 82.80      | 77.26±4.81|
| M4-Bt-100-B | 50%       | 64.00    | 78.90      | 72.56±7.69|
| M4-Bt-100-C | 50%       | 78.70    | 81.50      | 52.96±4.63|
| M4-Bt-100-D | 50%       | 73.90    | 87.20      | 80.76±6.66|
| M4-Bt-100-E | 50%       | 77.40    | 82.60      | 80.00±2.06|

The plant height of 100 gray Boyolali M4-By-100-D treatment showed significant average result (54.13) when compared to the plant in Control-01 (70.3). Meanwhile, the 100 gray Bantul showed (M4-Bt-100-D) showed had the highest average result (80.76). According to Suprihatno et al. [7], the significant differences of the stems are influenced by the characteristics and the nature that affects variety yields.
The number followed by (*) sign is significantly different from control based on the test result with \( \alpha = 0.05 \).

The 200 gray Boyolali treatment (M4-By-200-B) showed the lowest average result (58.40), while Control-01 showed higher average result (62.43) (Table 3). The treatment of Bantul 200 gray (M4-Bt-200-E) had the lowest average of plan height (37.00) (Table 4). The plants which were treated with gamma + drought stress 50% showed shorter plants than the ones which were not induced by gamma plant + watering 100% (control) (Table 2). From those results, it was known that by increasing dose of gamma rays, individual plants showed changes in characteristics (the genetic mutation) (Table 1). Increasing the doses would inhibit cell divisions which caused the death of cells, and it affected the plants growth process, decreased growth of cultivated plants and morphology of plants.

The induced mutation with gamma ray radiation is considered as an effective and efficient way in plant breeding program; because this process is relatively faster than the hybridization method and at the same time it can produce new characteristic for plants [6]. Thus, irradiation treatment can change the height of black rice plants to be shorter. Besides, watering plants could also affect the plants growth. The drought stress caused low rate water absorption by plant’s roots, so the plant height became less than normal. It was in line to the research conducted by Zanzibar and Sudrajat [8] about using gamma radiation in the mutation induction of cereal plants which produced mutation in plants up to 75%.

### 3.2. Crown root ratio

**Table 5.** Black rice (*Oryza sativa* L.) crown root ratio with 100 gray gamma-ray irradiation of Boyolali assessment on M4 generation with different soil water treatments.

| Strain      | Treatment | Height Plants (cm) | Average |
|-------------|-----------|--------------------|---------|
|             |           | Shortest | Longest |         |
| Control-01  | 100%      | 0.08     | 0.14    | 0.11± 0.03 |
| M4-By-100-A | 50%       | 0.32     | 0.37    | 0.35± 0.02 |
| M4-By-100-B | 50%       | 0.59     | 1.20    | 0.81± 0.33 |
| M4-By-100-C | 50%       | 0.28     | 1.00    | 0.70± 0.37 |
| M4-By-100-D | 50%       | 0.11     | 0.16    | 0.13± 0.02 |
| M4-By-100-E | 50%       | 0.25     | 0.40    | 0.31± 0.07 |

The number followed by (*) sign is significantly different from control based on the test result \( \alpha = 0.05 \).
Table 6. Black rice (*Oryza sativa* L.) crown root ratio with 100 gray gamma-ray irradiation of Bantul assessment on M4 generation with different soil water treatments.

| Strain     | Treatment | Height Plants (cm) | Average |
|------------|-----------|--------------------|---------|
|            |           | Shortest | Longest |         |
| Control-01 | 100%      | 0.36     | 1.00    | 0.58± 0.35 |
| M4-Bt-100-A | 50%      | 0.05     | 0.66    | 0.30± 0.31 |
| M4-Bt-100-B | 50%      | 0.32     | 0.43    | 0.36± 0.05 |
| M4-Bt-100-C | 50%      | 0.21     | 1.36    | 0.77± 0.57 |
| M4-Bt-100-D | 50%      | 0.08     | 0.30    | 0.18± 0.11 |
| M4-Bt-100-E | 50%      | 0.37     | 0.52    | 0.42± 0.08 |

The number followed by (*) sign is significantly different from control based on the test result $\alpha = 0.05$

Table 7. Black rice (*Oryza sativa* L.) crown root ratio with 200 gray gamma-ray irradiation of Boyolali assessment on M4 generation with different soil water treatments.

| Strain     | Treatment | Height Plants (cm) | Average |
|------------|-----------|--------------------|---------|
|            |           | Shortest | Longest |         |
| Control-01 | 100%      | 0.19     | 1.34    | 0.65± 0.60 |
| M4-By-200-A | 50%      | 0.23     | 0.38    | 0.30± 0.075 |
| M4-By-200-B | 50%      | 0.23     | 0.48    | 0.31± 0.14 |
| M4-By-200-C | 50%      | 0.23     | 0.57    | 0.42± 0.17* |
| M4-By-200-D | 50%      | 0.30     | 0.76    | 0.51± 0.23 |
| M4-By-200-E | 50%      | 0.68     | 1.40    | 1.03± 0.36 |

The number followed by (*) sign is significantly different from control based on the test result $\alpha = 0.05$

Table 8. Black rice (*Oryza sativa* L.) crown root ratio with 200 gray gamma-ray irradiation of Bantul assessment on M4 generation with different soil water treatments.

| Strain     | Treatment | Height Plants (cm) | Average |
|------------|-----------|--------------------|---------|
|            |           | Shortest | Longest |         |
| Control-01 | 100%      | 0.28     | 0.51    | 0.39± 0.11 |
| M4-Bt-200-A | 50%      | 0.38     | 1.39    | 0.78± 0.53 |
| M4-Bt-200-B | 50%      | 0.40     | 1.11    | 0.71± 0.36 |
| M4-Bt-200-C | 50%      | 0.27     | 0.62    | 0.48± 0.18 |
| M4-Bt-200-D | 50%      | 0.23     | 0.45    | 0.35± 0.11 |
| M4-Bt-200-E | 50%      | 0.05     | 5.22    | 2.36± 2.62 |

The number followed by (*) sign is significantly different from control based on the test result $\alpha = 0.05$

The Crown root ratio was obtained by dividing the dry weight of roots by dry weight of the crown. The dry weight of roots and crowns were determined after they were baked at the temperature of 70°C for about 2 x 24 hours [9]. The highest average result was on M4-By-100-C (0.81) meanwhile, the lowest average was of Control-01 (0.11) (Table 5). Based on this result, it was known that the plants with drought stress had higher weight roots than control plants (normal watering) (Table 6). The condition of stressed plants could stimulate longer root growth to get nutrients (Table 8). It was in line with the research conducted by Kakanga et al. [10] that the waterlogging suppresses root growth more than the crown growth. The characteristic can be used as an indicator of lack or excess of water in plants [11].

Bantul 100 gray plants showed the stressed plants had the lowest and highest average values. This result was in line with Torey et al. [12] which stated that the crown root ratio can be made as an indicator of lack of water in Superwin and IR 64 rice varieties, because it did not show significant results. The root ratio: the crown of Superwin, Temo, Ombong and Burungan varieties were not watered in the experimental greenhouse with soil volume 500 g, and the results were not significantly different based on Anova test [13]. Meanwhile, Bantul 200 gray (M4-By-200-C) plants had the significant result, the control plants had lower average result than the stressed plants (Table 7).
3.3. Chlorophyll content
Chlorophyll was the most pigments found in the chloroplast’s thylakoid membranes. There were two types of chlorophyll: chlorophyll a and chlorophyll b. The structure of chlorophyll consisted of four pyrrole rings that gave the color and phytol tail hydrophobic. The color of chlorophyll a is bluish-green; while the color of chlorophyll b is yellowish green [14].

![Figure 1](image1.png)

**Figure 1.** Black rice (*Oryza sativa* L.) chlorophyll content with 100 gray gamma-ray irradiation of Bantul assessment on M4 generation with different soil water treatments.

![Figure 2](image2.png)

**Figure 2.** Black rice (*Oryza sativa* L.) chlorophyll content with 100 gray and 200 gray gamma-ray irradiations of Bantul assessment on M4 generation with different soil water treatments.

Based on the results of the Figures 1 and 2, the highest result was of 100 gray (M4-Bt-C) plant (17.39). However, M4-Bt-D and M4-Bt-E had low chlorophyll contents (Figure 2). The chlorophyll content decreased in drought stress conditions. It happened due to the reduction of oxygen production in rice mutant genotypes. The lack of water can cause disruption of plant metabolic processes including the chlorophyll synthesis [15].

The drought stress from lowest until highest level was affecting the biochemistry processes that took place in the cells. The drought affected photosynthesis biochemistry reactions, so it made the rate of photosynthesis decreased. The biosynthesis of chlorophyll was one of the aspects of photosynthesis that was very sensitive to the drought stress, including a low stress condition [16]. Meanwhile 200 gray showed Boyolali and Bantul plants had different result. Boyolali control plant had the highest...
chlorophyll content; while, Bantul control plants tended to have lower chlorophyll content. The 100 and 200 gray irradiated plants showed different chlorophyll contents. It supported the statement of Harahap [17] about the abnormality of the irradiated plant populations which indicated major changes in the genome, chromosome, and DNA; so, the physiological processes in genetically controlled cells become abnormal. While according to Soeranto[18] the changes in leaves due to irradiation can occur because of an increase in the amount chlorophyll due to gamma irradiation stress. Thus, according to the result of this study, 200 gray was expected to have increased chlorophyll content due to the gamma ray irradiation.

4. Conclusion
Based on this study, the variable of the height plant showed that the irradiated plants were lower than Control-01 plant. The crown root ratio showed that the irradiation plants had a higher average result than the control plant. The chlorophyll content showed that 100 gray irradiation plants had a higher value than the control, while in 200 gray irradiated plants showed that the control plant had a higher value than irradiated plants.

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