Selection of M3 mutant strains from 200 gy gamma irradiation on Cempo Ireng

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Abstract. Climate change is a serious issue that can disrupt crop production and pest resistance, especially in rice crops. To tackle climate change, plant breeding can be employed to develop new characteristics that are resistant, using physical mutagenesis like gamma irradiation. Cempo Ireng is one type of rice in Indonesia with plant properties such as tall plant, long harvesting periods, and susceptibility to brown planthopper attacks. The research aims to obtain information and select M3 mutant strains with properties of shorter plants, early harvesting, and brown planthopper resistance. The study was conducted from February-July 2022 in Klaten Regency, Central Java, Indonesia. The research material consisted of 3 mutant strains of black rice M3(GH8’), M3(GH51’), and M3(GH52’), with the control variety of Cempo Ireng. The experimental design used was a Randomized Complete Block Design without replication. Data were analyzed descriptively and used t-test, at a significance level of 5%. The results showed that M3 mutant black rice had better growth and yield components than the control plant. Brown planthopper attacks of M3 mutant strains decreased to 24% compared to the control.

1 Introduction

Climate change is one of the challenges in agriculture that has a serious impact on food production. The threat of climate change is the increasing overall temperature which causes changes in the natural environment, affecting plants and animals natural patterns. The lack of adapting plants to climate change and changes in pest behavior is a catastrophic scenario for food production. The inability of plants to adapt to climate change leads to a decrease in yield due to a mismatch in plant requirements and conditions of plant growth. Also, changes in pest distribution can promote extensive pesticide application, threatening food safety[1–4].

Rice is the most widely consumed staple food commodity in Indonesia. There are several types of rice, including black rice. Black rice is usually chosen as an alternative to white rice due to superior nutritional content as anthocyanins [5]. However, Cempo Ireng black rice has not been extensively cultivated because it has some drawbacks that are considered to result...
in low yield potentials such as long harvest period, tall plant height, relatively low yield potential, and low resistance to pest attacks [6].

Plant breeding help develop plant varieties for sustainable food production by generating new plant characteristics. One of the breeding techniques is gamma-ray irradiation. Gamma-ray induction is a physical mutagen that causes changes in DNA structure resulting in the emergence of improved characteristics of plants [7]. Gamma-ray mutations change genotype structure, enhance species variation, and increase plant adaptability which is advantageous for plant breeding [8]. The breeding program for Cempo Ireng black rice by gamma-ray irradiation helps emerges new traits in plants to cope with climate change, especially pest attacks. This study aims to obtain phenotype and pest resistance in shorter plant height and early flowering of M3 black rice for individual selection.

### 2 Methodology

This study was conducted in Jogonalan, Klaten District, Central Java, Indonesia from February to July 2022. The materials are three mutant numbers of M3 rice strains with an irradiation dose of 200 Gy. Plant code of M3(GH8’) are M3(GH8’)-7, M3(GH8’)-8, M3(GH8’)-14, M3(GH8’)-25. Plant code of M3(GH51’) are M3(GH51’)-1, M3(GH51’)-4, M3(GH51’)-6, M3(GH51’)-7, M3(GH51’)-8. Plant code of M3(GH52’) are M3(GH52’)-9, M3(GH52’)-11, M3(GH52’)-14, M3(GH52’)-16, M3(GH52’)-20. Control plant variety is Cempo Ireng. The M3 plant population for each strain was 372 plants. The experimental design used was a Randomized Complete Block Design without replication. Data were analyzed descriptively and used a t-test, at significance level of 5%. The variables of observation consisted of flowering period, harvesting period (day), plant height (cm), panicle length (cm) weight of 100 seeds per clump (gram), seed weight per plant, and number of productive tillers (tiller).

### 3 Results and Discussion

#### 3.1 Phenotype of black rice

The study showed a difference in the flowering and harvesting period between M3 black rice in comparison to the control plants. Some of the individual plants of M3 have early flowering up to 7-9 days and harvesting up to 6-7 days (Table 1). The harvesting period of M3 rice showed that flowering characteristics of the black rice met the requirement of early-harvesting rice. Early harvesting rice has a flowering period ranging from 105-124 days [9]. Flowering is one of the characteristics that is affected by gamma-ray exposure. Gamma-ray exposure can cause changes in enzymes, proteins, and DNA related to flowering periods [10].

The results show that Gamma-ray irradiation causes different effects on the plant height. There is a difference in plant height in each of M3 as a result of gamma irradiation. M3(GH8’) plant has the shortest plant with a minimum height of 100 cm with an average of 129.9 cm. Meanwhile, M3(GH51’) plant has the lowest plant height of 117 cm with an average of 136.07 cm. Compared to the control plant with the lowest plant height of 115 cm with an average of 133 cm. The reduction in plant height M3(GH8’) is more visible compared to M3(GH51’) (Table 1). Gamma-ray irradiation can induce genetic mutation in plants, resulting in genetic variations. This genetic variation can be reflected in plants' physiology, one of which is plant height [11].

![Table 1: Phenotype and pest resistance in shorter plant height and early flowering of M3 black rice for individual selection.](https://example.com/table1.png)
Gamma-ray mutations change genotype and lead to genetic variation compared to the control plant. This genetic variation can be reflected in plants’ physiology, such as plant height. The study showed a difference in the flowering and harvesting period between M3 black rice. Some of the individual plants of M3 have early flowering. The results show that Gamma-ray irradiation causes different effects on the plant height. In contrast, the control plant has the shortest plant with a minimum height of 100 cm with an average of 129.9 cm.

### Table 1. Flowering time, harvesting time, plant height, and number of productive tillers of black rice.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Flowering time (day) Min-Max</th>
<th>Harvesting time (day) Min-Max</th>
<th>Plant height (cm) Mean</th>
<th>Number of productive tillers (tiller)</th>
<th>Min-M</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cempo Ireng</td>
<td>71-74</td>
<td>119</td>
<td>115-150</td>
<td>133.63 ± 8.07</td>
<td>9-29</td>
<td>16.50 ± 5.34</td>
</tr>
<tr>
<td>M3(GH8’)</td>
<td>63-71</td>
<td>112</td>
<td>100-148</td>
<td>129.90 ± 9.48</td>
<td>7-37</td>
<td>17.50 ± 4.05</td>
</tr>
<tr>
<td>M3(GH51’)</td>
<td>65-74</td>
<td>113</td>
<td>117-146</td>
<td>136.07 ± 6.89</td>
<td>7-27</td>
<td>16.93 ± 4.37</td>
</tr>
<tr>
<td>M3(GH52’)</td>
<td>65-71</td>
<td>113</td>
<td>113-149</td>
<td>133.17 ± 8.48</td>
<td>7-26</td>
<td>15.77 ± 4.59</td>
</tr>
</tbody>
</table>

Note: Numbers followed by a sign (*) are significantly different from the control as the results of T-test (α=0.05).

The number of productive tillers of M3 black rice, namely M3(GH8’) has the highest maximum value of productive tillers compared to the control plant which is 37 tillers. The average black rice productive tillers showed no significant difference. However, the highest average count of productive tillers is M3(GH8’) with 17.5 tillers (Table 1). The results of productive tillers illustrate the number of rice tillers that produce panicles. The panicles that cannot form panicles during the vegetative stage are called unproductive tillers [12].

### Table 2. Panicle length and number of seeds per panicle.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Panicle length (cm) Min-Max</th>
<th>Number of seed per panicle (seed) Min-Max</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cempo Ireng</td>
<td>23-29</td>
<td>87-236</td>
<td>155.60 ± 33.79</td>
</tr>
<tr>
<td>M3(GH8’)</td>
<td>23-28</td>
<td>72-193</td>
<td>131 ± 30.05</td>
</tr>
<tr>
<td>M3(GH51’)</td>
<td>22-29</td>
<td>67-193</td>
<td>134.23 ± 31.93</td>
</tr>
<tr>
<td>M3(GH52’)</td>
<td>23-28</td>
<td>71-191</td>
<td>129.30 ± 30.34</td>
</tr>
</tbody>
</table>

Note: Numbers followed by a sign (*) are significantly different from the control as the results of T-test (α=0.05).

The results on average panicle length showed a significant difference between M3 black rice compared to the control plant. Decreasing in panicle length of M3 black rice is due to the gamma-ray irradiation effect. The initial stages of gamma-ray irradiation affected more on quantitative plant characteristics, which lead to morphological abnormalities like plant height and panicle length [13]. Gamma-ray irradiation causes plants to repair themselves due to chromosome damage leading to inhibition of plant growth [14]. Decreasing of panicle length resulting in fewer grains formed on the plants. Irradiation causes a reduction in the number of filled grains due to alteration in cell structure related to carbohydrate translocation, indicating a decrease in carbohydrate translocation from roots to panicles [15].

The weight of 100 grains per panicle showed a significant difference between M3 black rice and the control plant. However, this result indicates a decrease in M3 grain shape compared to control plants. The weight of 100 grains represents grain shape, lower weight of 100 grains suggests a reduction in rice length. Slender grains have a heavier weight compared to round-shaped grains [16]. The grain weight per plant depends on the number of rice grains formed on productive tiller, heavier grains means more rice grains formed. The average
Gamma-ray irradiation alters compounds in rice related to cell wall thickness, such as lignin, which makes it more challenging for brown planthoppers to penetrate, effectively hindering their ability to feed from rice [21]. Thicker cell wall structure makes it more challenging for plant enzymes to break down the cell wall and increases the size of vascular tissue to make plant stems stronger and more resistant to stem-boring pests [24].

The study showed that the percentage of plants infested by brown planthoppers increased each week. The final week of observation shows that the control plant has a 52% damage percentage, 39% on M3(GH8’), and 28% on M3(GH51’) and M3(GH52’). Lower in pest infestation percentage of M3 black due to genetic changes by gamma-ray irradiation. Gamma-ray irradiation can produce generations of plants more resistant to pest attacks compared to parental plants due to genetic improvement from gamma-ray irradiation treatment [17]. Gamma-ray rice mutant improves pest resistance because gamma irradiation can help plants produce antimicrobial compounds, in rice plants by increase of phenolic acids [18].

Brown planthoppers attack rice by piercing and sucking sap from the rice stem causing direct damage and facilitating rapid transmission of plant diseases [19]. Prevention in brown planthopper feeding by increasing the hardness and thickness of the cell walls. Plant epidermis is considered to have an important role against insect attack, and determined brown planthopper feeding responses [20]. Thick cell wall structure makes it more challenging for brown planthoppers to penetrate, effectively hindering their ability to feed from rice [21]. Gamma-ray irradiation penetrates compounds in rice related to cell wall thickness, such as lignin [22]. Lignin is a phenolic polymer found in the cell walls of vascular plants and mainly

weight results show there is an increase and decrease in M3 black rice yield compared to control plants.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Weight of 100 seeds (gram)</th>
<th>Weight seed per plant (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min-Max</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Cempo Ireng</td>
<td>2.08-2.63</td>
<td>2.38 ± 0.12</td>
</tr>
<tr>
<td>M3(GH8’)</td>
<td>2.04-2.6</td>
<td>2.30 ± 0.11</td>
</tr>
<tr>
<td>M3(GH51’)</td>
<td>2.10-2.43</td>
<td>2.27 ± 0.09*</td>
</tr>
<tr>
<td>M3(GH52’)</td>
<td>1.96-2.59</td>
<td>2.28 ± 0.14</td>
</tr>
</tbody>
</table>

Note: Numbers followed by a sign (*) are significantly different from the control as the results of T-test (α=0.05).

3.2 Brown planthopper infestation

The study showed that the percentage of plants infested by brown planthoppers increased each week. The final week of observation shows that the control plant has a 52% damage percentage, 39% on M3(GH8’), and 28% on M3(GH51’) and M3(GH52’). Lower in pest infestation percentage of M3 black due to genetic changes by gamma-ray irradiation. Gamma-ray irradiation can produce generations of plants more resistant to pest attacks compared to parental plants due to genetic improvement from gamma-ray irradiation treatment [17]. Gamma-ray rice mutant improves pest resistance because gamma irradiation can help plants produce antimicrobial compounds, in rice plants by increase of phenolic acids [18].

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Fig. 1. Percentage of brown planthopper infestation on black rice.
thickened secondary walls [23]. High lignin content in rice plants enhances the thickness of
the cell wall and increases the size of vascular tissue to make plant stems stronger and more
resistant to stem-boring pests [24].

### 3.3 Individual selection

The results showed eight individual M3 black rice with shorter plant height and early
flowering that were selected for M4 generation. Selected individuals of M3 black rice met
the criteria of early harvest, short plant height, high productive tillers, and high weight per
panicle (Table 4).

<table>
<thead>
<tr>
<th>No</th>
<th>Accession number</th>
<th>Plant height (cm)</th>
<th>Harvesting period (days)</th>
<th>Number of productive tillers (tiller)</th>
<th>Panicle length (cm)</th>
<th>Weight of seed per plant (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M3(GH8')-16</td>
<td>115</td>
<td>112</td>
<td>26</td>
<td>27.5</td>
<td>56.13</td>
</tr>
<tr>
<td>2</td>
<td>M3(GH8')-17</td>
<td>100</td>
<td>112</td>
<td>27</td>
<td>25</td>
<td>54.59</td>
</tr>
<tr>
<td>3</td>
<td>M3(GH8')-19</td>
<td>125</td>
<td>112</td>
<td>25</td>
<td>28</td>
<td>62.5</td>
</tr>
<tr>
<td>4</td>
<td>M3(GH51')-7</td>
<td>120</td>
<td>113</td>
<td>22</td>
<td>25</td>
<td>46.19</td>
</tr>
<tr>
<td>5</td>
<td>M3(GH52')-1</td>
<td>124</td>
<td>113</td>
<td>24</td>
<td>27</td>
<td>53.97</td>
</tr>
<tr>
<td>6</td>
<td>M3(GH52')-9</td>
<td>130</td>
<td>113</td>
<td>17</td>
<td>26.5</td>
<td>51.33</td>
</tr>
<tr>
<td>7</td>
<td>M3(GH52')-13</td>
<td>119</td>
<td>113</td>
<td>22</td>
<td>26.5</td>
<td>50.08</td>
</tr>
<tr>
<td>8</td>
<td>M3(GH52')-15</td>
<td>130</td>
<td>113</td>
<td>13</td>
<td>25.5</td>
<td>39.08</td>
</tr>
</tbody>
</table>

### 4 Conclusion

This study showed changes in the growth components, yield components, and yield of M3
compared to a control plant. Individual selection resulted in 8 individual plants that met the
criteria of early harvest, short plant height, high productive tillers, and high weight per plant,
namely M3(GH8')-16, M3(GH8')-17, M3(GH8')-19, M3(GH51')-7, M3(GH52')-1,
M3(GH52')-9, M3(GH52')-13, M3(GH52')-15.

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