Morphological characterization of 3 potential lines Cempo Ireng black rice result of Gamma-Ray irradiation

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Abstract. Gamma-ray irradiation on Cempo Ireng can lead to the variation of morphological characteristics; hence, morphological characterization is necessary to determine changes in potential lines. This research aimed to characterize the potential lines as one of the requirements for proposing plant variety release and add black rice genetic diversity information. The research was carried out in the rice fields of Pakahan Village, Jogolalan, Klaten in January-June 2020 with the arrangement of potential lines 8, 13, and 44 of M6 in a row. In total 30 plants were selected randomly for each potential line and observed 19 morphological characters. Data analysis was carried out descriptively and qualitatively with the Chi-Square test. The results showed that gamma-ray irradiation affects the characters of leaf angle, auricle color, ligule color, leaf-blade color, leaf surface, panicle type, grain color, apiculus color, and rice length. Potential line 8 had different characteristics compared to non-irradiated Cempo Ireng in the characters of auricle color, leaf-blade color, leaf width, panicle length, grain width, rice length, and rice width, while on potential lines 13 and 44 occur in the characters of the leaf surface and panicle length.

1. Introduction
Oryza sativa L. ‘Cempo Ireng’ is one of the local black rice cultivars which originate from Seyegan, Sleman, Yogyakarta which is still rarely cultivated by non-local farmers. Farmers are reluctant to cultivate it because of the high habitus, which ranges from 200-230 cm, and the long life span which can reach 174 days; on the other hand, the Cempo Ireng black rice potentially become one of the functional food sources with anthocyanin content reach 428.38 mg/100 g [1]. Black rice potentially become functional food source in addition to anthocyanin content; also, compared to red and white rice, the black rice has higher fiber content, higher protein content and lower sugar content [2].

Plant breeding through gamma-ray irradiation was done to fix the high habitus and long life span condition and add genetic variation in the variety. Gamma-ray irradiation can bring up qualitative and quantitative diversity, one of them in black rice morphology characters [3]. Gamma-ray exposure on plant parts especially seeds can make mutagenic changes in living cells. Irradiation can make a severe change in cells and tissue because the energy penetrated is very large. These changes occur due to...
disruption of the seeds’ DNA of the plant which can bring about changes in the inherited traits. Changes in DNA from seeds exposed to gamma-ray irradiation can be a source of potential variation [4].

Rice treated with gamma-ray irradiation can produce different growth responses. This depends on the nature characters of each variety that is irradiated, so it is necessary to make selections to get plants with characters that are suitable for the breeding purposes that have been applied by plant breeders. Characterization is also needed to determine the changes that arise due to the given gamma-ray irradiation treatment [5].

Morphological characterization is an attempt to determine the morphological characteristics of a plant, which is the physical form of a plant. The morphological characterization of rice was carried out by observing the physical form of rice during the vegetative and generative periods. The characterization was carried out on the morphological characteristics of stems, leaves, flowers, and fruit or grain [6]. This study aims to obtain a description of the varieties of the potential lines as one of the requirements for submitting the release of plant varieties and to add information on the genetic diversity of black rice.

2. Materials and method
The research was carried out in January-June 2020 in the rice fields of Pakahan Village, Jogonalan District, Klaten Regency, Central Java. Plant materials used in this study included Cempo Ireng black rice seeds M6 of potential lines 8, 13, 44, and non-irradiated seeds as controls. All materials were planted in a row with the Jajar Legowo planting pattern with a spacing of 20 x 20 cm with a legowo of 40 cm and a distance between potential lines of 50 cm. Plant samples were randomly selected as many as 30 plants in each potential line and control. Characterization was carried out in the vegetative and generative growth phases according to the Panduan Sistem Karakterisasi dan Evaluasi Tanaman Padi [7]. There were 19 morphological characteristics observed; including leaf angle, flag leaf angle, auricle color, ligule color, leaf-blade color, leaf surface, leaf length, leaf width, leg color, culm color, type panicle, panicle length, grain length, grain width, grain (lemma palea) color, grain tip (apiculus) color, rice length, rice width, and brown rice (aleurone) color. Observational data was then converted into categorical data based on the categorization in Table 1 and analyzed descriptively. The test was carried out using the Chi-Square test with a critical limit of 0.05 and the table Chi-Square value at a significance of 5%, but if it did not qualify the Chi-Square test, an alternative Fisher Exact test was carried out using IBM SPSS Statistics 25 software. Tests were carried out to determine whether gamma-ray irradiation had an affected on the morphological characteristics of the potential lines.

Table 1. Description and Classification of Variable Values of Nineteen Characteristics of Rice Morphology

| Characters          | Code                      |
|---------------------|---------------------------|
| Leaf angle          | 1. Erect (<45°),          |
|                     | 2. Intermediate (45-90°)   |
|                     | 3. Horizontal (90°)       |
|                     | 4. Descending (>90°)      |
| Flag leaf angle     | 1. Erect                  |
|                     | 2. Intermediate (+45°)    |
|                     | 3. Horizontal             |
|                     | 4. Descending             |
| Auricle color       | 1. White (colorless)      |
|                     | 2. Purple lines           |
|                     | 3. Purple                |
| Ligule color        | 1. White                  |
|                     | 2. Purple lines           |
|                     | 3. Purple                |
| Leaf-blade color    | 1. Light green            |
|                     | 2. Green                 |
3. Dark green
4. Purple tips
5. Purple margins
6. Purple blotch (purple mixed with green)
7. Purple

Leaf surface
1. Glabrous
2. Intermediate
3. Pubescent

Leaf length
1. Very short (<21 cm)
2. Short (21-40 cm)
3. Medium (41-60 cm)
4. Long (61-80 cm)
5. Extra long (>80 cm)

Leaf width
Enter the actual measurements in centimeters (cm)

Leg color
1. Green
2. Light gold
3. Purple lines
4. Purple

Culm color
1. Green
2. Light gold
3. Purple lines
4. Purple

Panicle type
1. Compact
3. Between compact and intermediate
5. Intermediate
7. Between intermediate and open
9. Open

Panicle length
Enter the actual measurements in centimeters (cm)

Grain length
1. Extra long (>7.50 mm)
3. Long (6.61-7.50 mm)
5. Medium (5.51-6.60 mm)
9. Short (<5.51 mm)

Grain width
Enter the actual measurements in millimeters (mm)

Grain color
0. Straw
1. Gold and gold furrows on straw background
2. Brown spots on straw
3. Brown furrows on straw
4. Brown (tawny)
5. Reddish to light purple
6. Purple spots on straw
7. Purple furrows on straw
8. Purple
9. Black
10. White

Apiculus color
1. White
2. Straw
3. Brown (tawny)
4. Red
5. Apex is red
6. Purple
7. Apex is purple
Rice length
1. Extra long (>7.5 mm)
3. Long (6.61-7.5 mm)
5. Medium (5.51-6.6 mm)
7. Short (<5.5 mm)

Rice width
Measurements were made on rice after peeling and before grinding

Aleurone color
1. White
2. Light brown
3. Speckled brown
4. Brown
5. Red
6. Variable purple
7. Purple

Source: Panduan Sistem Karakterisasi dan Evaluasi Tanaman Padi from Komnas Plasma Nutfah in 2003

3. Results and discussion

3.1. Leaf angle
Leaf angle was one of the important characters because it was related to crop architecture which was related to sunlight acceptance and competition. Gamma-ray irradiation affected the leaf angle character, but there was no difference in the leaf angle character in the potential lines compared to the control (Table 2). All samples in all potential lines showed character of an erect leaf angle, while in the control there were 17 samples with erect leaf angle and 13 samples with an intermediate leaf angle. This difference in leaf angle was influenced by the collar tissue where if the collar tissue was longer, the leaf angle formed would be larger or the leaf-blade would grow away from the leaf vagina that grew vertically [8]. The erect leaf angles in all these potential lines would be very beneficial when rice was cultivated with dense plant density conditions because the leaves would not shade each other [9].

3.2. Flag leaf angle
Flag leaf angle was one of the important morphological characteristics to observe, not only because it affected light reception for photosynthesis, but also affected plant architecture. Based on the Chi-Square test that had been carried out, it showed that the p-value cannot appear (Table 2). This was due to the uniformity of the flag leaf angle characters in all samples of the potential lines and control. The flag leaf angles of all potential lines and control samples were classified as erect leaf angles. This erect flag leaf angle provides several advantages, including reducing photoinhibition and making it possible to cultivate rice with high density [10].

3.3. Auricle color
Auricle was one part of the leaf that distinguished rice plants from grasses. Gamma-ray irradiation affected the auricle color character. The control had the same white auricle color as the potential lines 13 and 44, while the potential line 8 had purple lines auricle color (Table 2). Diversity of auricle color with purple lines appeared in all potential lines which were not originally found in control, although in different amounts. This showed that gamma-ray irradiation can bring up new diversity or variations of rice plants caused by gene mutations from the original plant [11].

3.4. Ligule color
Ligule was one part of the leaf which was located on the border between the vagina and the leaf blade. The ligule served as a barrier to water entering between the vagina and the stem. Gamma-ray irradiation affected the color of the ligule, although the color of the ligule in both the potential lines and control was classified as purple lines (Table 2). The ligule color of black and red rice accessions 92.5% were white, while the remaining 7.5% were purple [12]. The condition where the ligule color initially had a
purple line was what allowed the appearance of the ligule color overall purple caused by gamma-ray irradiation.

3.5. Leaf-blade color
The leaf-blade color on rice can show how the nitrogen intake received by the rice and affected the absorption of solar radiation. Gamma-ray irradiation affected the leaf-blade color character, where the leaf-blade color in the control was classified as purple tips, this was the same as the leaf-blade color in the potential lines 13 and 44, while the potential line 8 had green leaf-blade color (Table 2). Gamma-ray irradiation at low doses can affect gene expression in rice leaves both morphologically and seen from the DNA structure since germination [13]. This caused the appearance of leaf-blade color that was different from the leaf color in control.

3.6. Leaf surface
Leaf surface was one of the important morphological characteristics to observe. This can be related in addition to being a characteristic of a rice variety, it can also relate to the resistance of a rice variety to certain pests and diseases. Gamma-ray irradiation affected the leaf surface character, as indicated by the control, and the potential line 8 had pubescent leaf surfaces, while the potential line 13 was intermediate, and the potential line 44 was intermediate-pubescent (Table 2). The condition of the pubescent and intermediate-pubescent rice leaf surface is advantageous because it had good resistance to bacterial leaf blight. This was because the number of hairs on the leaf surface affects the activity of pests and microorganisms that colonize the leaf surface [14].

3.7. Leaf length
The character of leaf length was important to know the leaf size, related to light reception, and plant architecture. Gamma-ray irradiation did not affect leaf length, where the leaf lengths of both control and potential lines were medium (Table 2). Gamma-ray irradiation may not affect a plant character due to resistance to irradiation caused by physiological mechanisms, DNA repairability, and many other factors such as the life history of the plant itself [15].

3.8. Leaf width
Leaf width was one of the observed morphological characters because it can affect leaf size, light reception, and plant architecture. The leaf width character was not tested by Chi-Square because the data must be presented according to the results of field observations without statistical tests. the average leaf width of the control samples, the potential lines 13 and 44 had the same width of 2.1 cm, while the potential line 8 had a larger leaf width of 2.3 cm. Leaf width is not only influenced by genetic factors that can change due to irradiation treatment, it was also influenced by the environment where the plant grows. Drought stress can affect leaf size and stomata index in rice. This was a form of adaptation to certain environmental conditions to survive [16].

3.9. Leg color
The rice leg was the lowest part of the canopy above the roots. The results of the Chi-Square test show that the p-value of the leg color character did not appear, because all uniform data were considered as constants (Table 2). This was because all the samples in both the control and potential lines had the same leg color, which was green. The color of the rice leg is generally green or purple. The green color comes from chlorophyll, while the purple color comes from anthocyanins [17]. Gamma-ray irradiation can not affect a character because the effect of irradiation was random.

3.10. Culm color
The rice culm is the part that is above the rice leg. The results of the Chi-Square test show that the p-value on the culm color characters did not appear, because all uniform data were considered as constants (Table 2). This was because all the sample plants in both the control and potential lines had the same
stem color, which was green. The color of rice culm was generally green, but it was possible to found purple and purple tinges depending on the type of rice [18]. Gamma-ray irradiation did not affect the character of the culm color because of the random effect of irradiation so it cannot be estimated on the character of whether irradiation can affect.

3.11. Panicle type
Panicle type was a model of branching, main branch angle, and grain density in panicles. Gamma-ray irradiation affected the panicle type character, although all of the potential lines and control had a compact panicle type (Table 2). The number of panicles that were classified as compact in the potential lines was more than the control. This is different from the results of previous studies which stated that the panicle types of black rice varieties commonly found were between compact and medium, medium, and between medium and open [19]. This was caused by gamma-ray irradiation which improved the panicle type condition from the previous condition. The compact panicle type had the potential to produce more rice grains, thus potentially increasing yields.

3.12. Panicle length
Panicle length was one of the important morphological characters to know because it was related to the number of seeds. The panicle length was measured from the neck of the panicle to the tip of the panicle. There was no categorization on the panicle length variable so that the Chi-Square test was not carried out. The average panicle length between the potential lines was different. The longest average panicle length was owned by the control, while the shortest panicle length was owned by the potential line 8 (Table 2). Each variety had different potency for panicle length. This can be caused by the arrangement of chromosomes and DNA in each variety [20]. The decrease in panicle length that occurs in the potential line can be influenced by gamma-ray irradiation. Gamma-ray irradiation causes random mutations that had the possibility of mutations occurring in genes that regulate panicle length expression.

3.13. Grain length
Grain length was one of the morphological characters related to grain size. Comparison of grain length and width can be used in determining grain size and can be used as an indicator in classifying rice subspecies. The results of the Chi-Square test showed that the p-value on the grain length character did not appear, because all uniform data were considered as constants (Table 2). The potential lines and control all had extra long grain lengths. Grain length is controlled in a coordinated manner by cell proliferation and cell expansion in the husk which regulated grain storage capacity and limits grain filling. The very long grain length indicated overexpression of the gene responsible for regulating the expression of grain length [21].

3.14. Grain width
Grain width was an important morphological character to know related to grain size. The grain width character was not tested using the Chi-Square test because it did not have categorization. The average width of the control grain was longer than the potential lines. The average grain width in the control was 2.9 mm, while the potential lines were 2.8 mm. Grain width can be influenced by genetic and environmental factors. Genetic factors regulated the ability to form protein and starch as the main constituents of rice seeds, while the environment provides the necessary materials for the formation of protein and starch [22]. Based on this, the decrease in the average grain width was not only influenced by gamma-ray irradiation but also environmental factors that were less supportive of producing more sink, thus affecting the width of the grain.

3.15. Grain color
Grain color was the color of the lemma and palea that covers the grain. Gamma-ray irradiation affected the grain color character, although both control and potential lines had straw grain color (Table 2). Gold and gold furrows on straw background, brown furrows on straw, and brown were also found in some of
the potential lines and control in small amounts. This was according to the results of previous studies which stated that the colors of grain found in Indonesia were straw, gold and gold furrows on straw background, brown spots on straw, brown furrows on straw, brown, and black [23].

**Table 2.** Recapitulation of p-value Chi Kuadrat Test Results and Character Description of Potential Lines

| No. | Characters               | p-value | Control      | Potential lines |
|-----|-------------------------|---------|--------------|-----------------|
|     |                         |         | 8            | 13              | 44              |
| 1.  | Leaf angle              | .000    | Erect        | Erect           | Erect           | Erect           | 44               |
| 2.  | Flag leaf angle        |         | Erect        | Erect           | Erect           | Erect           | 44               |
| 3.  | Auricle color          | .000    | White        | Purple lines    | Purple lines    | Purple lines    | 44               |
| 4.  | Ligule color           | .030    | Purple lines | Purple lines    | Purple lines    | Purple lines    | 44               |
| 5.  | Leaf-blade color       | .000    | Purple tips  | Purple lines    | Purple lines    | Purple lines    | 44               |
| 6.  | Leaf surface           | .000    | Pubescent    | Intermediate-Pubescent | Intermediate-Pubescent | 44               |
| 7.  | Leaf length            | .213    | Medium       | Medium          | Medium          | Medium          | 44               |
| 8.  | Leaf width (cm)        | Not tested | 2.1          | 2.3             | 2.1             | 2.1             | 44               |
| 9.  | Leg color              |         | Green        | Green           | Green           | Green           | 44               |
| 10. | Culm color             |         | Green        | Green           | Green           | Green           | 44               |
| 11. | Panicle type           | .000    | Compact      | Compact         | Compact         | Compact         | 44               |
| 12. | Panicle length (cm)    | Not tested | 24.6             | 20             | 22.1             | 23.8             | 44               |
| 13. | Grain length           |         | Extra-long   | Extra-long      | Extra-long      | Extra-long      | 44               |
| 14. | Grain width (mm)       | Not tested | 2.9          | 2.8             | 2.8             | 2.8             | 44               |
| 15. | Grain color            | .002    | Straw        | Straw           | Straw           | Straw           | 44               |
| 16. | Apiculus color         | .015    | Straw        | Straw           | Straw           | Straw           | 44               |
| 17. | Rice length            | .000    | Long         | Medium          | Long            | Long            | 44               |
| 18. | Rice width (mm)        | Not tested | 2.2          | 2.1             | 2.2             | 2.2             | 44               |
| 19. | Aleurone color         | .187    | Variable     | Variable purple | Variable purple | Variable purple | 44               |

Note: p-value is tested with the Chi-Square value table at the significance of 5% to see the effect of irradiation on a character

3.16. **Apiculus color**

Apiculus color was one of the important morphological characters to know because it was one of the characteristics of a rice variety. Gamma-ray irradiation affected the apiculus color character, although the potential lines and control had a straw apiculus color (Table 2). The apiculus color on potential line 44 and control, apart from straw, there was also a small amount of brown. The majority of apiculus colors in many rice accessions were straw and yellowish-brown [24]. Judging from the data that had been collected, gamma-ray irradiation can reduce diversity, this can be seen from the absence of brown apiculus color in the potential lines 8 and 13.

3.17. **Rice length**

This character along with rice width can be used as a reference in determining the size and shape of rice. Gamma-ray irradiation affected the rice length (Table 2). The control, potential line 13, and potential line 44 had long rice length, while potential line 8 had medium rice length. Rice length character was
influenced by genetic factors, agroecosystem, and soil fertility [25]. This genetic factor regulated the ability of rice to fill its grain. Gamma-ray irradiation can affect genes that regulated grain filling and rice formation. This caused differences in the rice length in each variety.

3.18. Rice width

Rice width was one of the characteristics of grain quality which together with the rice length determines the rice size. Rice width character cannot be tested by Chi-Square test because there was no categorization. The average rice width of the potential lines 13, 44, and control was the same, which was 2.2 mm, while the average rice width of the potential line 8 was 2.1 mm. Rice width decrease in potential line 8 can be influenced by genetic and environmental factors. Genetic factors regulate the filling of grain, while environmental factors play a role in providing the needs for grain filling. Differences in rice width can be easily observed, this can be used as a reference in distinguishing certain varieties to maintain purity [26].

3.19. Aleurone color

Aleurone color was the color of the epidermis of the rice and was not the color of the endosperm. Gamma-ray irradiation did not affect the aleurone color character and it was indicated by the majority of the sampled rice color in both the control and the potential lines was variation purple (Table 2). The aleurone color in black rice contains a high anthocyanin content which was more influenced by genetic factors than environmental factors [27]. Gamma-ray irradiation which did not affect the character of the aleurone color can be caused by the random nature of the mutation so that the affected character was also random and cannot be determined. This indicated that the gene that regulates the aleurone color did not occur mutations.

4. Conclusion

Gamma-ray irradiation affected the characters of leaf angle, auricle color, ligule color, leaf-blade color, leaf surface, panicle type, grain color, apiculus color, and rice length. The differences in the morphological characters of the potential line 8 compared to the control occurred in the auricle color, leaf-blade color, leaf width, panicle length, grain width, rice length, and rice width, while in the potential lines 13 and 44 occurred on the leaf surface and panicle length.

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