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# Novelty, Distinctiveness, Uniformity, and Stability Testing for Three Potential Lines of Black Rice

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**Abstract**
Black rice Cempo Ireng is a local variety with a long plant life, high plant posture, and low productivity. Plant breeding by gamma irradiation was carried out to correct these weaknesses. The results of the gamma irradiation process included a selection process for each generation and produced potential line numbers such as 8, 51, and 52. The characterization was based on the NDUS (Novelty, Distinctiveness, Uniformity, and Stability) test. The purpose of this study was to characterize potential lines as one of the requirements for the release of plant varieties. The study was conducted in the paddy fields of Pakahan Village, Jogonalan District, Klaten Regency, from February to July 2022. The method used was a single-factor randomized complete block design with three replications. Data were analyzed using a T-test with a 5% level. The study results showed that the three potential lines (GH8, GH51, GH52) have morphological character differences with the Cempo Ireng and the Sembada as control varieties. Some essential characteristics, like flowering and maturing age, were faster than Cempo Ireng and Sembada, and the shortest flowering age was shown by line 52 with a difference of 11 days faster. Three potential lines also have shorter plants and can be seen as new, distinct, uniform, and stable.

**Keywords:** anthocyanin; Cempo Ireng; feature; food crop; gamma-ray irradiation

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## Introduction

Black rice Cempo Ireng is a local cultivar rich in fiber, protein, and low sugar content, so that it can be a functional food. According to (1), Cempo Ireng has weaknesses such as a relatively long harvest life (129 days after planting) and a high plant posture (±145 cm). This is because the cultivation of black padi Cempo Ireng is still limited. Plant breeding is carried out to correct the weakness of Cempo Ireng, one of which is with gamma irradiation. (2) stated that gamma irradiation causes plant mutations, increasing genetic diversity. The cells exposed to radiation will undergo genetic changes, and the conditions of the radiated plants will vary (3).

The seeds resulting from gamma radiation are then called potential lines. Cempo Ireng results from gamma irradiation in the National Nuclear Energy Agency, Jakarta, 2014, with doses of 0, 200, and 300 Gray (4, 5). Black beads resulting from gamma-ray irradiation subsequently undergo a selection process at each generation and produce potential seeds, namely the potential lines 8, 51, and 52. B. Before being registered and released into new varieties, expectancy trailers must undergo the NDUS (Novelty, Distinctiveness, Uniformity, and Stability) test.

Novelty means a variety that has never been commercialized or traded in Indonesia for up to one year. Distinctiveness is when the candidate variety shows at least one unique character difference from other varieties. Uniformity is when the characters in one population have been uniform despite varying due to different growing methods and environments on cultivated land. Stability means the character observed does not change after being replanted into the next generation.

The study aimed to identify the novelty, distinctiveness, uniformity, and stability characteristics of the three potential lines resulting from gamma irradiation. Gamma irradiation is expected to give new properties to the hope strain so it can be used as one of the conditions for releasing plant varieties.

**Materials and Methods**

The research was conducted in the rural areas of Pakahan Village, Jogonalan District, Klaten Regency, Central Java, in February-July 2022. The materials used in the study include black padi seeds of three potential lines number 8, 51, and 52, varieties Cempo Ireng as older seeds, and comparative varieties Sembada. The tools used in the research include analytical scales, vernier calipers, and NDUS plant test manuals for rice by UPOV (International Union for the Protection of New Varieties of Plants).

The study used a single-factor complete group random scheme with three replications. In each replicate, there are 5 experimental fields that in each field are planted seeds of the test. Sampling is done using the simple random sampling technique. Observation of plant characters was based on UPOV descriptors. The data was analyzed descriptively and with a T-test at a rate of 5%. Uniformity characters were tested using the coefficient of variety with low diversity values (0-25%), relatively low variability (26-50%), reasonably high diversity (51-75%), and high variability (76-100%).

**Results and Discussion**

The results of observations at the potential lines 8, 52, and 52, varieties Cempo Ireng and Sembada varieties were divided into 15 qualitative and 10 quantitative characters. Quantitative and qualitative characters based on research results are presented in Table 1.

*Pubescence of surface*

Leaf fur is one of the modified forms of leaf epidermis tissue that grows on the upper or lower surface of the leaf. Table 1 shows the difference in the surface hair of the leaves on the potential lines and Cempo Ireng. This emerging diversity is due to the presence of gamma irradiation, which causes mutations in plants. (6) argued that gamma-ray induction can affect changes in plant morphology.

*Anthocyanin coloration of auricles*

Anthocyanins are the pigments responsible for plant tissues' purple, blue, and red color. Figure 1 shows that anthocyanins were found in the ears of the leaves on the hope strain number 52. There is an anthocyanin coloring of auricles (code 9). According to (7), gamma rays cause plants to mutate and increase the presence of some bioactive compounds such as anthocyanins. Potential lines 8 and 51 did not find anthocyanins in the auricles, code 1.

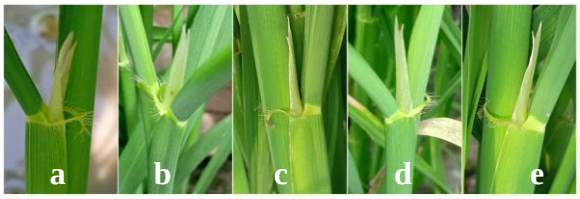


Figure 1. Auricle of Black Rice.
Description: a = GH8; b = GH51; c = GH52; d = Sembada; e = Cempo Ireng.

*Time of heading*

The time of heading or flowering is defined as the transition from the vegetative to the reproductive period or from the juvenile to the adult stage (8). Based on Table 1 shows that gamma irradiation affects the character of the flowering age. The age of the flowering of potential lines is much faster than that of Cempo Ireng and Sembada varieties. The shortest flowering age is the potential line 52, with a difference of 11 days quicker than Cempo Ireng. The presence of mutations influences the acceleration of the flowering age on the potential lines due to exposure to gamma irradiation. (9) argued that gamma-ray induction could increase genetic variation, so property selection could be done to improve plant properties.

*Attitude of flag leaf (early observation)*

The flag leaves are the last leaves that appear on the stem before the flower. According to (10), flag leaves have a higher photosynthesis capacity than others. This is because the flag leaf is positioned at the top of the headline, allowing for greater sunlight interception. The attitude of the flag leaves on the potential lines 8, 51, and 52, as well as the varieties of Cempo Ireng and Sembada, is semi-erect (code 3).

*Pubescence of lemma*

Lemma hair is a modification of the epidermal cells that grow on the surface of the lemma. According to (11), lemma hair assists in the spread of seeds and protects seed nationalization by birds and other animals. Table 1 shows that on the potential lines, number 8, number 51, and number 52, the Cempo Ireng varieties and the Sembada varieties are medium (code 5).

*Anthocyanin coloration of apex*

Apex is the top area of the lemma. The observation of the color of the anthocyanin area of the apex is carried out when the padi plant enters the flowering phase. Potential lines number 8, number 51, and number 52, as well as Cempo Ireng and Sembada varieties, did not find anthocyanins in the apex area (code 1). The apex region of the lemma at the time of initial observation was green; there was no expression of anthocyanins

*Color of stigma*

Stigma is a rice plant reproductive organ consisting of one future fruit (ovule) supporting the putik head (stigma) through two putik layers. (stylus). Based on the data from the research results of each sample on the potential lines, the Cempo Ireng varieties and the Sembada varieties show that gamma irradiation does not affect the character of stigma color. The possible lines number 8, number 51, and number 52, as well as the Cempo Ireng varieties and the Sembada varieties, are white (code 1).

*Thickness of stem*

The Stem of the rice plant has a round shape with a diameter or thickness that varies between varieties. Table 1 shows a significant difference in the thickness of the stem between the potential lines with the older and Sembada varieties. Figure 2 shows the difference in thickness of the potential lines and Cempo Ireng and Semmbada. The difference in the thickness expression of the stem is influenced by the presence of mutations resulting from the treatment of gamma irradiation. According to (12), gamma rays can cause plant mutagenesis, resulting in changes in the DNA level and triggering new properties in plants.

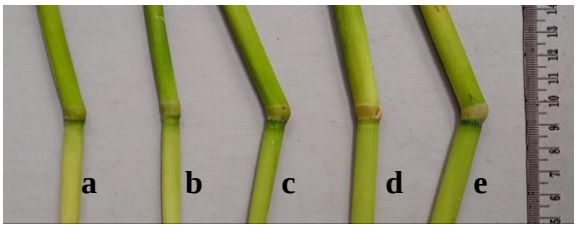


Figure 2. Stem of Black Rice
Description: a = GH8; b = GH51; c = GH52; d = Sembada; e = Cempo Ireng.

*Length of stem*

The length of the stem is related to the posture of the rice plant. Based on table 1 shows that there is a significant difference in the size of the stem between the potential lines and the Sembada varieties. Potential line number 8 differs from the older varieties Cempo Ireng and Sembada. The difference in expression is influenced by the presence of mutations resulting from gamma irradiation. According to (13), the extension of the stems is influenced by the division and prolongation of the intercalary system cells in the stem tissue. Gamma irradiation can inhibit the division and prolongation of the cells, resulting in more dwarf mutants.

*Anthocyanin coloration of nodes*

Nodes cover the streams of the rice plant. Based on the data from the research results of each sample on the potential lines, the Cempo Ireng varieties and the Sembada varieties showed that gamma irradiation did not affect the coloring of anthocyanins in the nodes (code 1).

*Distribution of awns*

Awns are organs like fur at the bottom of the keel. The potential lines number 8, number 51, and number 52, as well as the varieties of Cempo Ireng and Sembada, have the awns located at the tip only (code 9). According to (14), the existence of the awns is influenced by the cultivar of a plant. This can make the distribution character of the awns as a designer of a cultivar.

*Length of longest awns*

The existence of awns serves in plant breeding and plant defense. According to (15), long awns can protect the hail from the attack of pests, especially birds. Table 1 shows a significant difference in the length of the fur at the end of the fold between the potential lines with the varieties of Cempo Ireng. The differences that appear indicate a mutation in the rice plant. According to (16), the penetration of gamma irradiation into cells can produce new combinations of genes. The combination of these new genes gives rise to new characteristics in plants.

*Length of main axis of panicle*

The length of the central axis of the panicle is The length of the main axis of the panicle is influenced by the genotype of the padi plant. Figure 3 shows the difference in the length of the potential lines of panicle between the potential lines with the varieties of Cempo Ireng and Sembada. The differences that appear indicate a mutation in the rice plant. The same is also revealed by (17), that gamma irradiation can cause DNA mutations, thus responsible for increased or slowed growth.

Figure 3. Panicle of Black Rice
Description:
a = GH8; b = GH51; c = GH52; d = Sembada; e = Cempo Ireng.

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Figure 3. Panicle of Black Rice
Description: a = GH8; b = GH51; c = GH52; d = Sembada; e = Cempo Ireng.

*Attitude of flag leaf (late observation)*

The flag leaves are the longest ones covering the rice plant's top. Flag leaves are the primary source of assimilation during the seed-filling phase. The potential lines and the Cempo Ireng have the same behavior as the flag leaf (late observation), which is semi-erect (code 3). In contrast, the Sembada variety has the behavior of the flags (late observance) that is horizontal (code 5), which is different from the early observation (Table 1). According to (18), semi-erect leaves provide opportunities for better light distribution.

*Attitude in relation to stem*

Attitude in relation to the rice plant's stem depicts the rice padi's heritage. The potential lines number 8, number 51, and number 52, as well as the varieties of Cempo Ireng and Sembada, have a slightly drooping position (code 3). The slightly drooping position indicates that the malai grain is fine.

*Attitude branches of the panicle*

The attitude of branches is related to the development of branches that will affect the outcome of the grain. Potential lines number 8, number 51, and number 52, as well as Cempo Ireng and Sembada, have the behavior of the Malai branch, which is semi-erect (code 3). Semi-erect branches facilitate the growing space of the branches and minimize the birds' nationality.

*Exsertion*

Exsertion or appearance of panicle neck describes the ability of plants to form panicles. Table 1 shows that the potential lines and varieties of Cempo Ireng have a panicle appearance that is well exserted (code 9), different from the varieties of Sembada, which just exserted (code 5). Just exserted is marked by the presence of the panicle's neck right on the blade of the flag. The exserted appearance is marked by the panicle's neck being far from the flag leaves.

*Time of maturity*

Time of maturity or time of harvest is one of the characteristics of the agronomy of plants. (19) revealed that the physiological maturity of rice plants is characterized when 80-85% of the grain and leaves are yellow, and when the crab is pressed, it will feel loud and unbiased. Based on Table 1, there is a difference in the character of the time maturity of the black rice on the potential lines with the Cempo Ireng and Sembada. This is due to the presence of a genetic mutation that affects plant expression as a result of gamma irradiation. (3) argued that radiation causes changes in the composition of chromosomes and DNA, thus stimulating mutations that affect plant metabolism processes, including photosynthesis.

Table 1. Data Results of Research on Potential Lines, Cempo Ireng, and Sembada

| Number | Character | GH8 | GH51 | GH52 | Cempo Ireng | Sembada |
|---|---|---|---|---|---|---|
| 1. | Pubescence of surface | absent (1) | absent (1) | absent (1) | medium (5) | weak (3) |
| 2. | Anthocyanin coloration of auricles | absent (1) | absent (1) | present (9) | absent (1) | absent (1) |
| 3. | Time of heading (dap) | 69 very early (1) | 69 very early (1) | 65 very early (1) | 76 early (3) | 72 early (3) |
| 4. | Attitude of flag leaf (early) | semi-erect (3) | semi-erect (3) | semi-erect (3) | semi-erect (3) | semi-erect (3) |
| 5. | Pubescence of lemma | medium (5) | medium (5) | medium (5) | medium (5) | medium (5) |
| 6. | Anthocyanin coloration of apex | absent (1) | absent (1) | absent (1) | absent (1) | absent (1) |
| 7. | Color of stigma | white (1) | white (1) | white (1) | white (1) | white (1) |
| 8. | Thickness of stem (mm) | 4.849[2] medium (5) | 4.959[3] medium (5) | 5.280[2] medium (5) | 5.180 medium (5) | 5.676 thick (7) |
| 9. | Length of stem (cm) | 88.381[3] very short (1) | 84.5238[2] very short (1) | 85.4286[2] very short (1) | 102.5714 short (3) | 102.48 short (3) |
| 10. | Anthocyanin coloration of nodes | absent (1) | absent (1) | absent (1) | absent (1) | absent (1) |
| 11. | Distribution of awns | tip only (9) | tip only (9) | tip only (9) | tip only (9) | tip only (9) |
| 12. | Length of longest awns (mm) | 6.3333[2] short (3) | 6.7143[2] short (3) | 10.8095[3] short (5) | 7.095 short (3) | 15.86 medium (5) |
| 13. | Length of main axis of panicle (cm) | 18.3810[3] short (3) | 19.0952[2] short (3) | 21.00[3] medium (5) | 19.1905 short (3) | 17.48 short (3) |
| 14. | Attitude of flag leaf (late) | semi-erect (3) | semi-erect (3) | semi-erect (3) | semi-erect (3) | horizontal (5) |
| 15. | Attitude in relation to stem | slightly drooping (3) | slightly drooping (3) | slightly drooping (3) | slightly drooping (3) | slightly drooping (3) |
| 16. | Attitude branches of the panicle | semi-erect (3) | semi-erect (3) | semi-erect (3) | semi-erect (3) | semi-erect (3) |
| 17. | Exsertion | well exserted (9) | well exserted (9) | well exserted (9) | well exserted (9) | just exserted (5) |
| 18. | Time of maturity (dap) | 115 early (3) | 115 early (3) | 114 early (3) | 122 medium (5) | 122 medium (5) |
| 19. | Time senescence of leaf (dap) | 113 medium (5) | 113 medium (5) | 112 medium (5) | 121 late (7) | 121 late (7) |
| 20. | Sterile lemma length (mm) | 2.6190 long (7) | 2.333[1] medium (5) | 2.428 medium (5) | 2.6667 long (7) | 2.38 medium (5) |
| 21. | Weight of 1000 grains (gram) | 27.19 high (7) | 25.12 high (7) | 25.01 high (7) | 22.67 medium (5) | 22.82 medium (5) |
| 22. | Length of decorticated grain (mm) | 6.8571[2] long (7) | 7.3333[3] long (7) | 7.0000[2] long (7) | 7.0000 long (7) | 6.57 medium (5) |
| 23. | The shape of the decorticated grain | spindle-shape (4) | spindle-shape (4) | spindle-shape (4) | spindle-shape (4) | spindle-shape (4) |
| 24. | Color of decorticated grain | Black (9) | Black (9) | Black (9) | Black (9) | Black (9) |
| 25. | Plant height (cm) | 106.761[3] short (3) | 103.619[3] short (3) | 106.428[3] short (3) | 121.76 medium (5) | 119.95 medium (5) |

Description: [1] = significant difference with Cempo Ireng, [2] = significant difference with Sembada, [3] = significant difference with Cempo Ireng dan Sembada

*Time senescence of leaf*

Leaf aging is the final phase of the development of rice plants that culminates in the death of cells, tissues, and organs. Based on Table 1, both the potential lines, Cempo Ireng varieties and Sembada varieties, show that gamma irradiation affects the character time senescence of the leaf. This is because there is a variety in the character of the time senescence of leaves. The time senescence of the leaf of potential lines is shorter than that of the Cempo Ireng and Sembada plants. Potential line 52 has the fastest time senescence of leaf, 112 days after planting, a difference of 9 days with the Cempo Ireng variety. Potential lines 8 and 51 have a time senescence of leaf at 113 days after planting, which belongs to the intermediate category.

*Sterile lemma length*

A sterile lemma is a part of a glume that does not transition to a lemma. Table 1 shows a significant difference in the length of the sterile lemma between the potential lines number 51 and the Cempo Ireng variety. The sterile length of the spindle ranges between 2,3333 – 2,619 mm, while the Cempo Ireng is 2,6667 mm and the Sembada is 2.38 mm. Diversity arises as a result of mutations from gamma irradiation. (20) stated that mutations can alter or improve plants' morphological and anatomical features.

*Weight of 1000 grains*

The weight of 1000 grains is one of the components that can be used to observe the potential outcome of each possible line. Table 1 shows a significant difference in the weight of 1000 seeds between the potential lines of the Cempo Ireng and Sembada varieties. Potential line 8 has the heaviest 1,000 grains among the other test varieties, 27.19 grams, including the high category (code 7). According to (21), irradiating gamma rays with the appropriate dosage can affect the outcome of the phosphorus. There was an increase in the weight of 1000 grains on the potential lines. This increase shows that seed filling occurs at maximum.

*Length of decorticated grain*

Decorticated grain is rice produced from grain that has undergone a complex process and is only cut off on the outside (glume). Table 1 shows a significant difference in the decorticated grain between the potential lines with the Cempo Ireng and Sembada varieties. This indicates the diversity in the decorticated grain due to gamma irradiation. According to (22), the diversity emerged due to the induction treatment of mutations that caused plant genetic changes.

*The shape of a decorticated grain*

The shape of decorticated grain is one of the characteristics of the quality of seeds that influences consumers in choosing the rice to be consumed. Based on the data and research results on the potential lines, Cempo Ireng and Sembada showed that gamma irradiation did not affect this character. Potential lines number 8, number 51, and number 52, Cempo Ireng and Sembada, have a spindle-shaped rice shape (4).

*Color of decorticated grain*

The layer of peel has the content of compounds that express the color of rice. Potential lines number 8, number 51, and number 52, Cempo Ireng and Sembada, have a dark purple or black rice color. The dark purple or black color indicates the high anthocyanin content in the rice nuts. Previous research conducted by (23) revealed that the value of the anthocyanin content in the potential lines reached 75.04 ppm.

*Plant height*

Plant height is one of the agronomic characteristics that can be one of the indicators of plant growth. Table 1 showed a significantly high plant difference between the potential lines with the Cempo Ireng and Sembada varieties. Figure 4 also shows the difference in plant height of the potential lines and Cempo Ireng and Semmbada. These differences arise due to the occurrence of mutations due to gamma irradiation. According to (24), gamma irradiation can inhibit the synthesis of the hormone oxygen. The hormone auxin plays a significant role in the growth of plants. A limited amount of the hormone auxin causes plants to experience a decrease in growth.


Figure 4. Plant height.
Description: a = GH8; b = GH51; c = GH52; d = Sembada; e = Cempo Ireng

*NDUS (Novelty, Distinctiveness, Uniformity, and Stability) Test*

Potential lines 8, 51, and 52 can be said to be new in Indonesia because the general public does not know it, and this potential line plant material is still limited in Indonesia. The potential lines that have been tested have proven there are unique or distinct differences between each strain and Sembada varieties. This can be seen in the following table 2:

Table 2. Distinctiveness of Potential lines with Sembada Varietas

| Potential Lines | Number of character differences |
|---|---|
| GH8 | 1, 3, 8, 9, 12, 14, 17, 18, 19, 20, 21, 22, 25 |
| GH51 | 1, 3, 8, 9, 12, 14, 17, 18, 19, 21, 22, 25 |
| GH52 | 1, 2, 3, 8, 9, 13, 14, 17, 18, 19, 21, 22, 25 |

Description: [a] = Number of characters based on Table 1

Based on Table 2, unique features are visible, so one variety can be distinguished. Various quantitative and qualitative characteristics characterize these. The characteristics of the uniqueness in each of the potential lines are different.

Table 3 shows that the crucial characters on the potential lines that have been tested can be said to be uniform since there are no off-type characters. Based on the degree of diversity, the quantitative character of less than 25% of potential lines belongs to the low category, thus belonging to the uniform. The potential lines tested have been stable, as they have proven uniform. According to (25), a stable morphological feature can be used as a morphologic marker for identifying a variety.

Table 3. Uniformity of potential lines

| Characteristics | Diversity Coefficient and Diversity Category | | | | | |
|---|---|---|---|---|---|---|
| | GH8 | | GH51 | | GH52 | |
| Anthocyanin coloration of auricles | absent | U | absent | U | absent | U |
| Attitude of flag leaf (early) | semi-erect | U | semi-erect | U | semi-erect | U |
| Attitude of flag leaf (late) | semi-erect | U | semi-erect | U | semi-erect | U |
| Time of heading | 0% | U | 0% | U | 0% | U |
| Anthocyanin coloration of apex | absent | U | absent | U | absent | U |
| Color of stigma | white | U | white | U | white | U |
| Length of stem | 3.024% | U | 4.275% | U | 3.26% | U |
| Anthocyanin coloration of nodes | absent | U | absent | U | absent | U |
| Length of central axis of panicle | 6.076% | U | 6.175% | U | 6.023% | U |
| Distribution of awns | tip only | U | tip only | U | tip only | U |
| Pubescence of lemma | medium | U | medium | U | medium | U |
| Attitude in relation to stem | slightly drooping | U | slightly drooping | U | slightly drooping | U |
| Attitude branches of the panicle | semi-erect | U | semi-erect | U | semi-erect | U |
| Length of decorticated grain | 5.229% | U | 6.586% | U | 0% | U |
| The shape of the decorticated grain | spindle-shape | U | spindle-shape | U | spindle-shape | U |
| Color of decorticated grain | black | U | black | U | black | U |

Description: U = Uniform

## Conclusions

There are 12 differential characters on the three potential lines of black rice resulting from the irradiation of gamma rays against its parent, namely Cempo Ireng. The possible lines resulting from gamma irradiation can be said to have passed the NDUS test because it has met novelty, distinctiveness, uniformity, and stability requirements.

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