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Agromorphological and Physicochemical Characteristics of Golden Rice , Introduced Rice Line PAC Nagdong/IR36/IR64

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ABSTRACT

Golden Rice (*Oryza sativa* L.) is biofortified rice that contains β-carotene, an essential nutrient for human health. Golden Rice PAC Nagdong/IR36/IR64 was created by crossing PAC Nagdong/IR36 with IR64 rice, a native variety, to improve tolerance to Indonesia's environment. Observations were made in two stages: (a) agromorphological characterization during culture, which included plant height, tiller number, panicle length, fertility, yield potential, and grain color, and (b) physicochemical analysis to assess amylose, amylopectin, protein, and lipid levels. Golden Rice has semi-dwarf height, a good and very high number of tillers (22.60−24.40), a panicle length of 20.09−20.85 cm, grain weight per pot of 42.08−42.94 g, fertility of 67.23−81.54%, and seed color of 5Y 8/8−5Y 8/10. The physicochemical properties included amylose content of 12.41−13.37%, protein content of 4.61−5.45%, lipid content of 1.24−1.54%, swelling power of 6.72−8.08%, low gelatinization temperature, and soft gel consistency type. The results indicated Golden Rice line 302/IR-2-2(7)/2 exhibit superior characteristics, including lower amylose and protein contents, higher fertility, and soft gel consistency which identical with consumer-preferred rice quality in Indonesia. Accordingly, this line have the highest potential for commercialization, while the remaining lines depict as promising materials for further breeding improvement.

Keywords: agromorphology, Golden Rice , physicochemistry

INTRODUCTION

Paddy (*Oryza sativa* L.) is a rice-producing plant that provides sustenance for about half the world's population. Humans also require other nutrients, such as vitamin A, to function properly. Vitamin A is essential for the health of the ocular surface layers, mucosal membranes, respiratory tract, urinary tract, and intestines (Ezzati *et al*. 2004). Vitamin A requirements must be met since a lack of vitamin A can lead to a variety of health issues, including malnutrition in infants. According to Riskesdas (2018), the number of Indonesian toddlers reporting nutritional issues increased by 17.7% in 2018. Vitamin A supplementation in children can boost immunity and resilience to disease (Elvandari *et al*. 2017). Cultivating Golden Rice , a food plant rich in β-carotene or provitamin (vitamin A precursor), can help meet the need for vitamin A intake.

Transgenic rice, Golden Rice , PAC Nagdong/IR36/IR64, is the product of a cross between PAC Nagdong/IR36 rice and a local rice variety, specifically IR64. The goal of this crossing is to improve adaptability. PAC Nagdong/IR36 rice adapted to Indonesian climatic conditions (Sitaresmi *et al*. 2013). The F8 hybrid between PAC Nagdong/IR36 rice and the local IR64 rice variety produced genes with the expected properties of their parents, including genes for β-carotene production and notably resistance to blast and blight diseases in Indonesia (Mina *et al*. 2018).

Agromorphological assays are important for determining the physical properties of rice. Sutaryo (2014) stated that Golden Rice is grown in specific locations and climates. Agronomic characterization involves analyzing the agronomic properties of rice plants, such as harvest age, plant height, and number of tillers. According to Samaullah and Darajat (2009), agromorphological properties of plants served to distinguish one variety from another, including productive factors. Rice's physicochemical qualities play a vital influence in defining its cooking quality and taste. Cold rice's high amylose content promotes water release and produces a firmer texture (Febriandi *et al*. 2017). Lipids are the third greatest component, behind carbohydrates (starch) and protein. These lipids continue to be studied since they affect rice's nutritional, sensory, and functional characteristics (Yulianto 2021). Gelatinization causes amylose links to close together due to hydrogen bonding (Khunae *et al*. 2007). Swelling power was used to define the increase in volume and maximum weight of starch during swelling in water (Balagopalan *et al*. 1988). The agromorphological examination is supposed to provide

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precise information about the physical qualities of Golden Rice prior to commercialization or long-term cultivation, whereas the physicochemical analysis aims to determine the nutritional content of seeds. Both analyses would help to estimate the plant's potential.

METHODS

Agromorphological Characterization

Agromorphological was investigated on 10 IR64 variety samples 120 days after planting. All rice genotypes planted in the study were grown under greenhouse conditions at the Faculty of Agriculture, Universit of Jember. During cultivation, the plants received 25 g of NPK fertilizer every two weeks to help them flourish. Plant height, tiller number, panicle length, grain weight per pot, fertility, and seed color were among the factors observed. Except for seed color, which was assessed using Munsell Color Charts, the complete agromorphological analysis used scales and data gathering methodologies based on the IRRI Standard Evaluation System (2013).

Physicochemical Characterization

The physicochemical examination covers amylose content, protein content, lipid content, swelling power, low gelatinization temperature, and gel consistency. Except for protein and lipids, the entire study was based on the IRRI (2013) Standard Evaluation System calculation scale.

Amylose Content Analysis (Juliano 1972)

Amylose percentage was analyzed by placing a 100 g sample in a Falcon tube and adding 1 mL of 95% ethanol and 9 mL of 1 *N* NaOH, preheated for 10 min at 80−100°C. The sample was moved to another Falcon tube and filled with distilled water to a volume of 100 mL, followed by 5 mL of 2% iodine, 1 mL of 1 *N* acetic acid, and 100 mL of distilled water. Readings were collected at 620 nm with a spectrophotometer type 722N from China. The formula for calculating the amylose and amylopectin contents is as follows:

$$\%\text{Amylose} = \frac{\text{Absorbance value x Dilution factor}}{\text{Sample weight}} \times 100\%$$

The amylopectin was subsequently calculated by subtracting the amylose percentage from the total carbohydrate content, while carbohydrate was determined by the content of protein, lipid, and water itself as shown below:

$$\%\text{Amylopectin} = \%\text{Carbohydrate} - \%\text{Amylose}$$

$$\%\text{Carbohydrate} = 100\% - (\%\text{Protein} + \%\text{Lipid} + \%\text{Water content}$$

Swelling Power Measurement (Sennayake *et al.* 2013)

This was accomplished by grinding 50 mg of rice grains into flour, placing it in a tube, and adding 2 mL of distilled water to the tube. The mixture was then heated at room temperature to 90°C for 30 min, shaking every 10 min. The samples were then centrifuged at 8000 rpm for 30 min. The supernatant was dried at 130°C and calculated. The sediment (*WS*) was weighed on aluminum foil (*W*1). The score was then computed as follows:

$$\text{Swelling power (\%)} = \frac{\text{WS}}{0{,}1\ X\ (100\% - wsi)}$$

$$\text{Wsi} = \text{w1}/0.1 \times 100$$

Where:

WS : Weight of the wet sediment after centrifugation,
*W*si : Water solubility index); the percentage (%) of solids dissolved in water relative to the total sample,
*W*1 : Weight of soluble solids obtained from the supernatant after drying.

Gel Consistency

A sample of 100 g of rice flour, sieved through 100 mesh with four repetitions, was placed in a culture tube of 13 × 100 mm. Then, 0.2 mL of 95% ethanol and 0.025% thymol blue were added. The mixture was agitated until the flour was completely suspended. After that, 2 mL of 0.2 N KOH was added, vortexed for a few minutes, and heated at 100 °C with marbles on top of the culture tube, for 8 min. The bubbles should not surpass two-thirds of the tube. The tube was placed at room temperature for 5 min before being transferred to 4 °C for 15 min. Following that, the tube was rested on millimeter paper. This causes the gel to develop and harden in 35−45 min.

Gelatinization Temperature Measurement

Six grains of milled rice were placed in a container holding 10 mL of 1.7% KOH, spacing them apart so they did not contact. They were left for 23 h at 30°C. The samples were examined and the results documented using the rice efflorescence score.

Soluble-Protein Content Analysis (Bradford 1976)

A 1 g sample of finely milled flour was mixed with 3 mL of pH 7 phosphate buffer, centrifuged at 10,000 rpm for 10 min at ambient temperature (4°C). For the Bradford test, 5 µL of supernatant was taken, followed by adding 950 µL of Bradford and 45 µL of distilled water. The solution was then vortexed to homogenize it before being read with a spectrophotometer type 722N from China, which has a wavelength of 595nm.

Lipid Content Analysis (Bligh and Dyer 1959)

A 5 g sample of rice flour was placed in a test tube, and 10 mL of a chloroform:methanol (1:2) mixture was added, followed by vortexing for 10−15 min. Afterward, 3.75 mL of chloroform was added and vortexed for 1 min before adding 3.75 mL of water and centrifuging. The supernatant and sediment were collected with a Pasteur pipette. Following that, filter paper was used to filter the sample, and if a precipitate or insoluble sample material was formed, it is centrifuged with water and chloroform. The total lipid content was calculated using the following formula: ($W0$ = weight of empty container, $W2$ = weight of dry lipid residue, and $W3$ = weight of sample mixture).

$$F = \frac{(W_2 - W_0)}{W_3} \times 100\%$$

Where:
W_0 = Weight of the empty container (centrifuge tube before adding sample)
W_2 = Weight of the tube + swollen sample (residue after soaking or centrifugation)
W_3 = Weight of the dry sample used initially
F = Functional property value

Data Analysis

Data was analyzed using Analysis of Variance (ANOVA) and assessed using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Agromorphological Characteristics

Observations indicate that all rice plant varieties in the study had an average height of semidwarf (lowlands <110 cm) according to the IRRI SES (2013). Suprihatno *et al*. (2009) reported in "Description of Rice Plant Varieties" that the control plant, IR36, was 70−80 cm tall, whereas IR64 was 115−126 cm tall. Environmental factors influence plant growth and so affect a variety of plant growth traits (Cepy and Wayan 2011).

Table 1 shows that the average number of tiller in the three lines of Golden Rice (*Oryza sativa* L.) is lower than the parent. According to IRRI (2013), all rice genotypes planted in the study, including IR36 and IR64, had an average of more than 25 tillers per plant, with 31.90 and 32.30 tillers per plant, respectively. Meanwhile, the F8 302/IR-2 2(6)/2 line has an average of 26.70 tillers/plant, while the 302/IR-2-2(8)/1 can have up to 28.80. In comparison, the F8 302/IR-2-2(7)/2 line produces the fewest tillers of the other lines, 24.60 tillers/plant, indicating that it has a reasonable number of tillers (20−25 tillers/plant). The number of tillers is believed to limit rice plant productivity. This is because the initial (main) tillers have a larger photosynthetic capacity than the tillers that develop last, resulting in a flag leaf that is less susceptible to stress. Photo-oxidation reduces the activity of both the source and sink (Kariali *et al*. 2012). The final growing rice tillers likewise have lower protein and amylose levels than the initial tillers (Wang *et al*. 2007). Thus, F8 302/IR-2-2(7)/2 was identified as the best line since it produced the fewest tillers.

According to Siregar *et al.* (1998), long panicles could impact grain position more; however, if there are a lot of empty grains per panicle, the production weight per unit area is low. Suprihatno *et al*. (2009) reported that the potential yield of IR36 is 5.8 t/ha, and that of IR64 is 6.0 t/ha. The Golden Rice with the highest potential yield features was F8 302/IR-2-2(8)/1 (6.87 t/ha), whereas the strain with the lowest potential yield was F8 302/IR-2- 2(6)/2 (6.73 t/ha).

Meliala *et al*. (2016) found that the optimal rice plant produces 85−95% whole grain. Observations revealed that no Golden Rice strains met the ideal criteria, as all three strains had fertility rates below 85%. Genetic factors can impact decreased fertility, as crossover between two different genotypes results in the abortion of male and female gametes (Zichao *et al*. 1996). This process happens because of the action of genes wide compatible (WC) as a trait-controlling gene wide compatibility between two parents with distinct genetics, resulting in the abortion of male gametes (male gamete) and female gametes (female-gamet) (Zhuang *et al*. 2002). This scenario leads to an increase in seed emptiness. Although the three lines had lower fertility than their parents, there are rice lines that have Golden Rice , which has a proportion of full seeds that are near to the ideal requirements, such as F8 302/IR-2-2(7)/2, with an average fertility of 81.54%.

Morphologically, the lines produced by this cross resemble the yellow color of Golden Rice seeds

Table 1 Agromorphology characteristics of Golden Rice (PAC Nagdong/IR36/IR64)

Plant	Plant height (cm)	Number of tillers (stem)	Panicle length	Grain weight per pot (g)	Fertility	Seed color
IR36	84.00 d	31.90 a	18.87 c	40.19 b	80.01 ab	2,5Y 8/2
IR64	83.70 d	32.30 a	19.43 bc	41.52 ab	77.75 b	2,5Y 8/2
302/IR–2 2(6)/2	102.30 a	26.70 c	20.75 a	42.08 a	67.23 c	5Y 8/10
302/IR–2–2(7)/2	99.05 b	24.60 d	20.09 ab	42.68 a	81.54 a	5Y 8/8
302/IR–2–2(8)/1	97.30 c	28.80 b	20.85 a	42.94 a	79.26 ab	5Y 8/10

Remark: Values followed by the same letter in the same column are not significantly different based on DMRT at the 5% significance level (α = 0.05).

(Figure 1). The endosperm includes β-carotene, also known as provitamin A. Rice plants with high β-carotene concentration yield colorful grains. Rice grains with increased β-carotene concentration tend to be yellower (Ha *et al*. 2010).

Physicochemical Characteristics

Physicochemical testing revealed low amylose levels in all three strains. Golden Rice (12.41−13.37%) shared the features of its parent, Nagdong, which has a lower amylose level than other rice japonica varieties (Yang *et al*. 2011). The sticky (glutinous) texture of rice is less popular in Indonesia, especially in Java, where people prefer rice with a low–medium amylose content of around 18–23% (Mardiah *et al*. 2016). However, people in certain East Asian and Australian locations prefer rice with low amylose levels (Calingcion *et al*. 2014).

Assessment swelling power was calculated because it is directly related to rice's amylose content, which reflects starch's ability to absorb water under particular conditions. The value of swelling power is exactly related to rice's amylose content. According to Chavez-Murillo *et al*. (2008), higher amylose content increases the water absorption capacity, resulting in stronger swelling power. Increasing swelling power would increase the hydrophilicity of starch, which interacts directly with water (Retnowati *et al*. 2010). The F8 302/IR-2-2(7)/2 strain of Golden Rice had the maximum swelling power of 8.08%; on the other hand, the F8 302/IR-2-2(6)/2 strain had the lowest percentage, 6.72%.

Furthermore, there is a positive correlation between amylose content and gelatinization temperature, which can be used to determine the cooking time of rice. Gelatinization temperature is considered low if it is 55−60 °C, average between 70 and 74 °C, and high over 74 °C (Rayee *et al*. 2021). Rice with a low gelatinization temperature cooks quickly and has a softer texture that is simple to break apart, whereas rice with a high gelatinization temperature is often dry and less sticky (Thomas *et al*. 2013). The physicochemical tests (Table 2) reveal that the three lines of Golden Rice had lower gelatinization temperatures and amylose levels than their parents. This harmony demonstrates that one of starch's physical qualities, specifically the gelatinization temperature of each rice, is regulated by its amylose level (Alfron *et al*. 2011).

Aside from gelatinization temperature, other parameters that influence rice quality and are strongly related to amylose content include gel consistency. During the process, the water-soluble amylose fractions interact with one another to produce insoluble aggregates. At high starch content, amylose aggregates absorb water and form gel structures. Gelatinization happens when starch granules absorb water, expand, and cause irreparable damage to the crystal structure, producing a gel with a certain consistency (Jalubisma and Ardiansah 2024). Gel consistency refers to the density of the gel created by

Figure 1 Representative rice lines used in the study showing differences agromorphological traits: (a) IR36, (b) IR64, (c) 302?IR-2-2(6)/2, (d) 302/IR-2-2(7)/2, and (e) 302/IR-2-2(8)/1.

Table 2 Psychochemistry charecterictics of Golden Rice (PAC Nagdong/IR36/IR64)

Plant	Amylose	Gelatinization temperature	Swelling power	Gel consistency	Protein	Lipid
IR36	23.29 a	±4	6.11 bc	93.33 a	5.29 bc	1.94
IR64	24.43 a	±3	5.88 d	86.67 a	5.33 b	1.13
302/IR–2–2(6)/2	13.37 c	±7	6.72 b	92.33 a	5.26 c	1.45
302/IR–2–2(7)/2	12.67 d	±7	8.08 a	92.67 a	4.61 d	1.54
302/IR–2–2(8)/1	12.41 d	±7	7.38 ab	67.67 b	5.45 a	1.24

Remark: Values followed by the same letter in the same column are not significantly different based on DMRT at the 5% significance level (α = 0.05).

the interaction of component molecules with water. According to SES IRRI (2013), IR36, IR64, and the three Golden Rice strains have a soft gel consistency. The three strains of Golden Rice with a soft gel consistency demonstrate that the character soft gel consistency has the same gel consistency as its two parents, namely IR36 and IR64 (Wanchana *et al*. 2003).

Rice grains' physicochemical properties are also related to their protein and fat contents. According to Sasmitaloka *et al*. (2020), high temperatures could promote lipid breakdown, which increases the risk of lipid damage during gelatinization. Protein, on the other hand, contributes significantly to rice texture creation by establishing complex starch linkages that prevent starch granule expansion and influence the gelatinization process. In Table 2, the three lines with low gelatinization temperatures showed no significant difference in lipid or protein content from their parents with high gelatinization temperatures. Our findings revealed an average protein content of 5% in the three lines. Juliano (1999) described that rice has an average protein level of 6.3−7.1%. Aiyswaraya *et al*. (2017) divided rice protein content into three categories: high (>10.50), medium (9.01−10.50), and low (<9.01). Rice with good flavor quality contains less than 7% protein and 15.5−16.5% moisture content. Meanwhile, the lipid fraction was quite low (<2%). This is a beneficial conclusion because rice with a high lipid content spoils more quickly, likely oxidizing quickly and producing an unpleasant odor (Fitriyah *et al*. 2020).

The results of observations on the agromorphological and physicochemical characteristics of the rice lines resulting from the cross between PAC Nagdong/IR36 and IR64 rice revealed that the three lines had values that were not significantly different from their parent varieties in all parameters tested. However, lines 302/IR-2-2(7)/2 tend to perform better than the other lines. This line contains fewer tillers per panicle, less protein content, and lower gelatinization temperature, all of which are positively correlated with consumer-preferred rice quality. In contrast, this line had the highest fertility and gel consistency ratings, indicating higher quality seed and rice. Furthermore, the low amylose concentration of this line is consistent with the Indonesian rice market's predilection for soft and sticky rice textures. Based on these considerations, line 302/IR-2-2(7)/2 is thought to have the most potential for commercialization, while the other two lines can be further developed in breeding programs to produce superior rice types.

CONCLUSION

The lines emerging from the cross between PAC Nagdong/IR36 and IR64 rice were F8 lines 302/IR-2-2(6)/2, 302/IR-2-2(7)/2, and 302/IR-2-2(8)/1. These three lines produced similar agromorphological and physicochemical characteristics to their parents. However, the three lines revealed differences in values in various metrics, indicating that they may have better traits than their parents. Lower values imply more desirable characteristics in terms of the number of tillers per plant (24.60−28.80), amylose content (12.41−13.37%), and gelatinization temperature (scoring 7). Meanwhile, the grain weight per pot was relatively large, ranging between 42.08 and 42.94 g. Of the three lines, the 302/IR-2-2(7)/2 line was found to have the most excellent qualities, satisfying Indonesian customer preferences. The remaining lines can be further developed in plant breeding programs to provide IR34 rice varieties.

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