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Potential of Pigmented Rice Variety Cempo Ireng in Rice Breeding Program for Improving Food Sustainability

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Abstract

Cempo Ireng (*Oryza sativa* L. var. Cempo Ireng) is one of the black rice variety in Indonesia that consumed as functional food, not as a staple food because of the black rice contains anthocyanin pigment which has beneficial to human health. High concentration of anthocyanin reaching 393.93 ppm cause black color in the pericarp layer of Cempo Ireng which make this variety as an antioxidant source, low glycemic index, and free of gluten, leading to have higher price in the rice market. However, most of the farmers are not cultivate Cempo Ireng due to the longer vegetative phase up to 150 days compared to the white rice varieties which only takes 90 days, the taller phenotype of Cempo Ireng cause problems in cultivation, and also the purple color of Cempo Ireng attract birds and earhead bug (*Leptocorisa oratorius*) to feed on black rice caryopsis that cause severe yield loss. Transgenic methods, mutation technique, intensification systems, and physical treatment have been applied in Cempo Ireng to overcome those lack characteristics. In the future, Cempo Ireng also has a big potential to become a parent in cross breeding program because of the positive characters. The aim of this review is to describe the potential of Cempo Ireng in rice breeding program for improving food sustainability.

Key words: Cempo Ireng, anthocyanin, functional food, transgenic, mutation, intensification system, physical treatment

Introduction

Rice is the staple food for more than a half population world-wide which have many varieties. Besides white rice, there are pigmented rice, including black rice, brown rice, and red rice varieties. Pigmented rice varieties have been cultivated in Indonesia, since 2500 B.C. Rice grains rich in minerals and vitamins, also free of gluten, cholesterol, and sodium [1,2]. One of the well-known pigmented rice variety is Cempo Ireng (*Oryza sativa* L. var. Cempo Ireng) which originally from Sleman Regency, Yogyakarta, Central Java, Indonesia. Among the rice consumers, black rice is consumed as functional food, not as a staple food because of the black rice contains anthocyanin pigment which has beneficial to human health [3,4,5,6,7]. Cempo Ireng belongs to japonica rice sub-species which has rounded, small size, and sticky textured grains.

High concentration of anthocyanin reaching 393.93 ppm cause black color in the pericarp layer of Cempo Ireng which make this variety as an antioxidant source, leading to have higher price in the rice market [8,9,10,11]. The type of anthocyanin in the aleurone layer of Cempo Ireng are peonidin 3-O-glucoside and cyanidin 3-O-glucoside [10]. Anthocyanin content in Cempo Ireng can lower the blood cholesterol level, prevent cancer, and decrease the risk of arteriosclerosis [13,14,15,16]. However, most of the farmers are not cultivate Cempo Ireng due to the longer vegetative phase up to 150 days compared to the white rice varieties which only takes 90 days, the taller phenotype of Cempo Ireng cause problems in cultivation, and also the purple color of Cempo Ireng attract birds and earhead bug (*Leptocorisa oratorius*) to feed on black rice caryopsis that cause severe yield loss [3,17,18]. Due to the difficult cultivation, the productivity of Cempo Ireng is low. It is important to increase Cempo Ireng productivity although the day of harvesting reaching 5 months and susceptible to lodge due to tall plant height up to 154 cm. Understanding the flowering pathway of Cempo Ireng is one of the effort to shorten the flowering period in order to increase the yield. Preliminary results identified the flowering genes *Hd3a* and *RFT1* in Cempo Ireng under neutral day conditions [10,19,20].

Nowadays, black rice variety, such as Cempo Ireng has become an alternative staple food to substitute white rice due to it has higher anthocyanin content; lower carbohydrates; free of gluten; rich in amino acids, vitamin B1 and E; and more mineral contents, such as Zn, Fe, P, and Mn [10,21,22]. Methanolic extract of Cempo Ireng bran potentially suppress the growth breast cancer T47D cells [5]. Cempo Ireng also contains 315 Kkal of energy, 8 g of protein, 76.9 g of carbohydrate, and 6.1 g of fibers. Additionally, Cempo Ireng showed better physical characteristics, such as fragrant and soft texture compared to the white rice because of the low amylose content only about 22.97%. Characterization of anthocyanin content and other components in Cempo Ireng expected to accommodate plant breeders to use Cempo Ireng as parent in rice breeding programs. The bran of Cempo Ireng also become a source of macro- and micronutrients [10,23]. Production of Cempo Ireng needs to be increased by optimal the cultivation to accommodate the increasing demand from the rice consumers. The aim of this review is to describe the potential of Cempo Ireng in rice breeding program for improving food sustainability.

Major Agronomic and Quality Traits of Cempo Ireng

Cempo Ireng belongs to *Japonica* subspecies which become getting popular among the rice consumers due to the high anthocyanin content and low glycemic index. As a black rice variety, Cempo Ireng showed different major agronomic characteristics and grain quality traits compared to the white rice varieties (Table 1 and 2). Although the plant stature of Cempo Ireng is too tall, the harvest period is so long, and low productivity but this variety is resistant to bacterial blight disease and *Xanthomonas oryzae* pv. *Oryzae* (Xoo), more tolerant to drought and salinity stress [24,25,26,27]. Additionally, Cempo Ireng identified as photoperiod insensitive. After the inoculation of Xoo, Cempo Ireng expressed resistant genes *xa5*, *xa10*, *xa21*, and *RPP13-like* [25].

The physicochemical characteristics and organoleptic quality of Cempo Ireng is different from the white rice varieties. Based on the organoleptic quality test, including texture, aroma, color, and flavor of Cempo Ireng showed that this variety has softer, more fragrant aroma, and more attractive color compared to the other black rice varieties, such as Jowo Melik and Toraja [28]. Cempo Ireng also become a functional food resource because of the hypoglycemic, high anthocyanin, fiber, and protein content [17,29,30]. Among the black rice varieties in Indonesia, including Melik, Jlitheng, Pari Ireng, Beras hitam NTT, Beras hitam Bantul, Beras hitam Magelang berbulu, Beras hitam Magelang tak berbulu, Beras hitam Sragen, Beras hitam Banjarnegara berbatasan Wonosobo, Beras hitam Banjarnegara, Beras hitam Tugiyo umur panjang, Sembada hitam, Beras hitam Muharjo, Beras hitam Patalan, Beras hitam Tugiyo umur pendek, Andel hitam 1, and Beras hitam Yunianto; Cempo Ireng contains the highest anthocyanin concentration up to 428.38 mg/100g [3]. Vitamins and minerals contents such as P, Mn, Zn, and Fe of Cempo Ireng also higher than the white rice varieties [12,31].

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Table 1. Agronomic Characteristics of Cempo Ireng [24,25,26]

Agronomic Traits	Cempo Ireng
Plant height	130 – 150 cm
Total number of tillers	15
Productive tiller number	13
Category of culm length	Long (141 – 155 cm)
Anthocyanin coloration of leaf sheath	Absent
Intensity of green color of leaf blade	Light
Category of diameter at basal internode	Thick
Chlorophyll content	27 µg/cm
Relative water content	80 – 90 %
Flowering days	79 day after planted
Shoot dry weight	0.14 g
Root length	35 cm
Panicle length	22.76 cm
Category of panicle length	Long (~ 35 cm)
Total spikelet per panicle	120 – 150
Filled grain number per panicle	95.09
Productivity	5 – 7 ton/ha
Harvest age	154 day after planted
Hull color	Black
Awn length	Intermediate (~15 mm)
Bacterial leaf blight disease	Resistance
Xanthomonas oryzae pv. *Oryzae* (Xoo)	Resistance

Table 2. Grain Characteristics of Cempo Ireng [3,10,17,28,29]

Grain Quality Traits	Cempo Ireng
1000-grain weight	24.51 g
Grain shape	Rounded
Glycemic index of the grains	Low (50 – 60)
Seed color	Black
Texture	Soft
Aroma	Fragrance
Anthocyanin contents	44.80 ppm (43.2%)
Carotenoid contents	0.38 mg/g
Water contents	5.5%
Glycemic index	14.41%
Amylose contents	2.27%
Carbohydrate contents	21.6%
Protein contents	11.65%
Lipid contents	10.85%
Fiber contents	1.13%
Phosphor (P)	22.57 ppm
Ferrum (Fe)	91.46 ppm
Calcium (Ca)	38.45 ppm

Transgenic Lines of Cempo Ireng

Long harvest period is one of the lack characteristic of Cempo Ireng. Shorten the flowering period of Cempo Ireng by using molecular genetic technique can enhance the rice productivity and support national food security. Flowering period in the rice plant is controlled by day length. Basic information of flowering genes have been investigated by a semi-quantitative analysis to identify the patterns of flowering genes expression. Under a neutral day condition in a tropical environment, two flowering genes *Hd3a* (Heading date 3a) and *RFT1* (rice

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Flowering Locus T1), and also FT-like genes such as FT-L10, FT-L9, FT-L6, FT-L5, *Hd1*, and *OsCOL4* were expressed together. These flowering genes were investigated by isolated the RNA of the leaves at 48, 55, 68, 81, and 90 days after planted and cDNA synthesis for each sample. The flowering pathway of Cempo Ireng conserved the common rice flowering models [19,33].

In order to promote early flowering in Cempo Ireng, insertion flowering gene *Hd3a* can be done by transformation rolC::*Hd3a*-GFP mediated by *Agrobacterium tumefaciens* into callus of the Cempo Ireng [34,35]. According to Tamaki et al. [36], the expression of *Hd3a* in *japonica* rice sub-species was controlled by rolC promoter which accelerate the flowering period. Two different growth media, including 2N6 and MS 2,4-dichlorophenoxyacetic acid (2,4-D) were used to grow callus from scutellum. Based on the results, 2N6 media generate calli faster compared to MS 2,4-D media. Then, calli were ready for Agrobacterium-mediated transformation to insert *Hd3a* gene.

Mutation Lines of Cempo Ireng

Nowadays, the demand of Cempo Ireng has been increasing due to its health benefit, high nutritional value, low glycemic index, and free of gluten. Meanwhile, the productivity of Cempo Ireng is low because of the long harvest age and high plant stature which make the farmers are not interested to cultivate this variety. Plant mutations by gamma rays can be used to overcome these weaknesses. Induced mutation using gamma irradiation was expected to increase characters of Cempo Ireng genetically such as early flowering period, shorten the plant stature, and improve the rice productivity. The grains of Cempo Ireng irradiated with gamma-ray at a dose 100, 200, 300, 400, and 500 Gy. Results showed that mutant Cempo Ireng plant was superior compared to the control. The treatment with gamma-ray at 200 Gy showed significantly decrease the flowering age and plant heigh, and also increase the grain yield, grain weight, protein content, and anthocyanin concentration (Table 3) [6,17,29,37,38,39,40,41,42,43,44]. Mutations not only affect the morphological traits of Cempo Ireng, including plant height, grain weight, flowering age, number of grains per panicle, panicle type, apiculus color, grain color, leaf-blade color, ligule color, auricle color, leaf angle, and leaf surface but also affect the nutrition contents such as anthocyanin, protein, and amylase; and enhance the stress tolerance. In order to shorten the harvest age and to fix the plant height, and also to provide genetic variation of Cempo Ireng, plant breeding through gamma-ray irradiation was done.

Table 3. Effect of Gamma-Ray Irradiation in Cempo Ireng [6,17,29,37,38,39,40,41,42,43,44]

Doses (Gy)	Plant height (cm)	Weight of 1,000 grains (g)	Flowering age (days after sowing)	Harvest age (days after sowing)	Number of grains per panicle	Protein contents (%)	Amylase levels (%)	Anthocyanin level (ppm)
0	136.11^{a}	23.01^{a}	125.00^{a}	155.00^{a}	125.78^{a}	7.73^{a}	14.41^{a}	18.62^{a}
100	134.11^{a}	25.84^{b}	119.00^{ab}	149.00^{ab}	164.78^{b}	7.49^{a}	22.45^{b}	19.19^{a}
200	136.56^{a}	24.42^{b}	114.67^{b}	144.67^{b}	166.33^{b}	6.23^{b}	12.84^{a}	4.21^{b}
300	135.33^{a}	21.53^{a}	115.00^{b}	145.00^{b}	132.33^{a}	7.13^{a}	17.47^{ab}	11.84^{c}
400	120.11^{b}	18.69^{a}	122.33^{ab}	152.33^{ab}	117.44^{c}	10.04^{c}	8.07^{c}	4.99^{b}
500	120.56^{b}	17.23^{c}	117.33^{b}	147.33^{b}	123.56^{a}	10.09^{c}	17.91^{ab}	52.17^{d}

*Means followed with the same letter in the same column had no significant difference on mood median test level of 5%.

Gamma-ray irradiation in the Cempo Ireng causes genetic variation, including qualitative and quantitative diversity that change the morphological characters. These variations happened because the irradiation makes dramatic changes in DNA and chromosome structure due to the energy which penetrated by the gamma-ray irradiation is powerful. These changed traits are

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inherited and can be a source of variations which benefit for plant breeders to make selections to choose plants with desired characteristics. Rice seeds treated with gamma-ray irradiation can produce different responses during the vegetative and reproductive stages such as morphological characteristics of roots, leaves, stems, flowers, and grains. Alteration in DNA and chromosome arrangement by gamma-ray irradiation affected the metabolism of the plant growth, including the photosynthetic activity which also influence the seeds formation and harvest age. Mutation by gamma-ray irradiation also increase protein and proline content. Decreased amylose content in the rice grains was also affected by gamma-ray irradiation at doses of 200 Gy which influenced the rice texture becomes fluffier. Gamma-ray irradiation potentially increase anthocyanin content which reflect in the color change of rice pericarp. Productivity of Cempo Ireng also can be increased by using colchicine chemical mutagens [39,45]. The various doses of colchicine can induce polyploidies in plants, so plants increase the productivity. Based on the experiments by using four colchicine concentrations (0, 250, 500, and 750 ppm), the significant phenotypic changes happened in 750 ppm.

Intensification System and Physical Treatments in Cempo Ireng Cultivation

Application of Jajar legowo technique 4:1 row with spacing between plants 25 cm x 25 cm in Cempo Ireng cultivation can increase the rice productivity. This technique can overcome the tall phenotype of Cempo Ireng which prone to lodge [46,47]. Treatment of paclobutrazol at the dose of 100 ppm in Cempo Ireng significantly increase the rice productivity because paclobutrazol shorten the plant height up to 55.92 cm, thicken the culm, increased culm diameter, reduced internodes length, and modified the structure of parenchyma cells of culm so the culm become stronger and significantly reduce the lodge by 25% [48,49]. Paclobutrazol is a plant growth regulator with the chemical name (2RS,3RS)-1-(4-chloro-phenyl) methyl-4,4-dimethyl (1h-1,2,4-trizol-1-yl) penten-3-ol which inhibit the biosynthesis of gibberellin.

Utilization of natural organic fertilizer such as KCl from coconut husk significantly increase the rice productivity of Cempo Ireng [50,51]. The element of KCl provide phosphor and nitrogen elements in soil which stimulate the biosynthesis of sitocynin that promote the development of tiller. KCl also activate the synthesis enzymes which accelerate the photosynthetic activity, improve water use efficiency, strengthen the roots, and increase the disease resistance. The results showed that application of organic KCl fertilizer at concentration of 20 ml/L significantly promotes the morphological growth and reproductive development of Cempo Ireng, including number of tillers by 19%, plant height, number of leaves by 100%, number of stomata, leaf thickness, early flowering period, and rice productivity.

Combination of paclobutrazol and blue light treatment on seed germination of Cempo Ireng slightly decreased the germination percentage, reduced a-amylase activity, and increased nitrate reductase activity than those subjected to the sunlight. Blue light having wave length of 320 – 490 nm which have powerful energy to change the DNA and chromosome arrangement. However, the application of these combination treatments significantly increased tiller numbers, chlorophyll content, Fe content, and shorten the plant height [52,53,54,55,56,57].

Rice productivity of Cempo Ireng also can be increased by application of paclobutrazol and cytokinin [58,59]. Paclobutrazol decreased the plant height and cytokinin increase the grain filling of Cempo Ireng. Cytokinin enhanced the assimilation allocation during photosynthetic activity and improve the grain quality traits, including grain weight, the contents of sucrose, amylose, and amylopectin.

Anthocyanin content of Cempo Ireng can be increased along with the shorten plant height by application of paclobutrazol and methyl jasmonate [60,61]. Methyl jasmonate as a phytohormone that regulate the biosynthesis of secondary metabolites in plants, including anthocyanin. Dominant type of anthocyanin in the grains of Cempo Ireng which treated with paclobutrazol of 25 ppm and methyl jasmonate of 7.5 mM were delphinidin 3 O-β-D galactoside, cyanidin 3-O-β-D galactoside, and cyanidine 3-O-β-D glucoside. Chorophyll, oxalic acid concentration in leaves, tiller number, and grain yield of Cempo Ireng also increased by application of combination paclobutrazol and methyl jasmonate with appropriate doses.

The resistance of Cempo Ireng to bacterial leaf blight disease can be increased by salicylic acid treatment [21,62]. Bacterial leaf blight disease caused by *Xanthomonas oryzae* (Xoo) that reduce the rice productivity up to 60% due to the chlorophyll content in the leaves significantly reduce that make the leaves become yellow and wilt, and decrease the photosynthetic activity. After applying the salicylic acid, the gene expression of the rice resistance gene *OsNPR1* was increasing.

Rice Bran of Cempo Ireng

Cempo Ireng bran as the by-product from the rice milling process contains high concentration of anthocyanin, macro- and micronutrients [10,63]. The fermentation process of the rice bran by using *Rhyzopus oligosporus* at 30^{o}C for 72 hours has been adopted to increase the bioactivity by produce the non-volatile and volatile compounds [1,64,65]. Fermentation process in the rice bran produced new compounds that were not identified in the non-fermented rice bran, such as nicotinic acid, isorhamnetin, isorhamnetin 7-glucoside, and quercetin. Fermented rice bran also produce aroma like vanilla, caramel, sweet, pungent, cereal, acid, rancid, smokey, nutty, fatty, milky, grass, and earthy [66,67]. The results showed that a total of 72 non-volatile compounds were investigated, including carbohydrates, nucleotides, peptides, vitamins, amino acids, lipids, and secondary metabolites. Volatile compounds which detected in the fermented rice bran consist of alcohols, aldehydes, acids, ketones, phenols, esters, benzene, terpenes, furans, lactones, pyridine, pyrazine, and thiazole. These diverse compounds showed anti-hypertensive effects and lower blood cholesterol.

Conclusions

Pigmented rice variety, Cempo Ireng has a big potential to become a functional food due to the high anthocyanin content, low glycemic index, and free of gluten. Because of the health benefit from Cempo Ireng, the demand of this rice variety is increasing. Meanwhile, the cultivation of Cempo Ireng is still low among the farmers. The tall phenotype, long harvest period and low productivity of Cempo Ireng are the limited factors. Transgenic methods, mutation technique, intensification systems, and physical treatment have been applied in Cempo Ireng to overcome those lack characteristics. In the future, Cempo Ireng also has a big potential to become a parent in cross breeding program because of the positive characters, such as high anthocyanin content that potentially lower the blood cholesterol, low glycemic index which prevent diabetic, and free of gluten that good for gluten allergic consumers. The rice bran of Cempo Ireng also still have high anthocyanin content which potentially use for functional food. Parboiled of Cempo Ireng also have been developing to reduce the cooking time and also to increase the texture of Cempo Ireng.

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