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Research Article
Genetic Variability of M2 Population Obtained from Colchicine Mutation in Black Rice (*Oryza sativa* L.)

Damanhuri and Afifuddin Latif Adiredjo

Plant Breeding Laboratory, Department of Agronomy, Faculty of Agriculture, Brawijaya University, Veteran Street, Malang 65145, East Java, Indonesia

Abstract

Background and Objective: Black rice (*Oryza sativa* L.) is becoming popular for development of functional foods. However, black rice generally has a long plant life and low productivity. To obtain superior varieties, improvement of phenotype and genotype is necessary. Variety improvement programs require genetic diversity as a basic material in breeding activities. One way to increase variability on black rice is mutation breeding by colchicine. This research aimed to study about genetic variability of M2 population induced colchicine of local genotype black rice from Ungaran. **Materials and Methods:** This research was conducted in Batu city, East Java from March to August 2017. The data were collected using single plant method and analyzed using student t-test at 5% level. Planting materials were U-K0, U-K250-67, U-K250-68, U-K500-79, U-K500-83, U-K750-5, U-K750-41 local genotypes Ungaran population M2 results of colchicine induction. **Results:** The population of M2 from the colchicine mutation had low to rather high category of Genotype Coefficient of Variability (GCV) in all the character of quantitative observation. Most of the population had high value of heritability on all quantitative characters. Selected plants from this population based on quantitative character and cytological observation were U-K250-67-8, U-K250-67-129, U-K250-68-4, U-K250-68-103, U-K500-79-6, U-K500-79-144, U-K500-83-9, U-K500-83-43, U-K500-83-58, U-K750-5-8, and U-K750-41-4. **Conclusion:** M2 population of black rice had low to rather high genetic variability. Observation on cytological characters showed that there were 11 plants that had more number of chromosomes than control.

Key words: Colchicine, black rice, mutation, genetic variability, heritability

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Corresponding Author: Afifuddin Latif Adiredjo, Plant Breeding Laboratory, Department of Agronomy, Faculty of Agriculture, Brawijaya University, Veteran Street, Malang 65145, East Java, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Black rice (*Oryza sativa* L.) is becoming popular for development of functional foods. This is because the genetic variability of cultivars which causes diversity in pigmentation, phytochemical properties and nutrition value[1]. Black rice has pericarp, aleuron and red-blue to dark purple endosperm. The color indicates an accumulation of anthocyanin content[2]. Anthocyanins are phenolic compounds which include the flavonoid group and act as antioxidants. Antioxidants can preventing the aging process and can against free radicals that obtained from the body's metabolism, air pollution, food contamination and sunlight[3].

In addition to its advantages, black rice generally has a long plant life, high plant habitus and low productivity which is a constraint in its cultivation[4]. Black rice productivity is about 3 t ha^{-1} and harvest at the age 120-140 days[1]. This causes farmers not interested in cultivating black rice.

To obtain superior varieties of black rice, improvement of phenotype and genotype is necessary. Variety improvement programs require genetic diversity as a basic material in breeding activities[5]. Research about diversity in black rice is needed to find out the potential to high productivity and short plant life. One way to increase diversity is breeding through mutation. Breeding through mutation (mutation breeding) can be used as a support method in breeding programs to improve varieties. Breeding through mutation can be used to improve existing varieties, by providing a variety of genes, plant breeding can also be used as a means to assemble new varieties that are superior to pre-existing varieties. Mutation technique is able to produce plants that have superior characters that are not owned by these plants before. Mutations in black rice cempo ireng varieties were carried out by gamma ray irradiation and showed different phenotypes with controls[6]. In addition to gamma rays, mutations can also be done with chemical compounds, namely colchicine.

Colchicine cause the phenotypic and genotype diversity of a plant. Phenotype of plant caused by mutation often appear after the next generation, i.e., M2, V2 or continuation[7]. From the previous research, the selected individuals has been obtained from colchicine induction of M2 population from local genotype Ungaran. Mutation induction in local genotypic black rice has never been done and give possibilities to improve nouvel germplasm characters. This research aimed to get information about the genetic variability occurring in the M2 population of local genotype Ungaran induced by colchicine. The next selection of M3 generation will be directed at high productivity and short harvesting days.

MATERIALS AND METHODS

The research were conducted from March to August 2017 at Torongrejo village, Junrejo, Batu city with altitude 630 masl and temperature 24-28°C.

The material used in this research were the local genotype of black rice (from Ungaran, Central Java, Indonesia), i.e. U-K0, U-K250-67 (Induced 250 ppm colchicine), U-K250-68 (Induced 250 ppm colchicine), U-K500-79 (Induced 500 ppm colchicine), U-K500-83 (Induced 500 ppm colchicine), U-K750-5 (Induced 750 ppm colchicine) and U-K750-41 (Induced 750 ppm colchicine). This materials was collected from the previous research (unpublished data). The other materials were Urea (45% N), SP-36 (36% P_2O_5), KCl (60% K_2O), aquades, 8-Hydroxyquinoline, acetic acid, Hydrochloric Acid (HCl). These chemical materials were obtained from the Plant Breeding Laboratory of Brawijaya University. The tools used were analytical scales, basin, raffia, labels, nets, digital camera, microscope, waterbath, watch glasses and stationery.

This research used Single Plot Design. The data was collected using single plant method. There were 200 plants on each group of plants. The numbers of plants on this research were 1400 plants.

The observation characters were morphological and cytological characters. Morphological characters were the percentage of live plants after seedling (%), number of tillers, productive seedlings, plant height (cm), leaf length (cm), leaves number, flowering days (DAP), harvest days (DAP), number of grain per plant, percentage of filled grain (%), percentage of unfilled grain (%) and grain weight per plant (g), 100 grain weigh (g). Cytological characters were number of chromosomes.

Quantitative data were analyzed using student t-test at 5% level, then calculated the genotype coefficient of variability (GCV) and heritability value. Genotype coefficient of variability (GCV) on each character was calculated by Al-Tabbal and Al-Fraihat[8]:

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100\%$$

Where:

$\sigma^2 g$ = Genetic variant

$\bar{x}$ = Mean of characters

The criteria for variability were determined based on the genotype coefficient of variability (GCV) i.e., low ($0 \leq 25\%$), rather low ($25 \leq 50\%$), rather high ($50 \leq 75\%$) and high ($75 \leq 100\%$)[9].

Heritability value (h^2) on each character was calculated by Al-Tabbal and Al-Fraihat[8]:

$$h^2 = \frac{\sigma^2 g}{\sigma^2 ph}$$

Where:
$\sigma^2 g$ = Genotype variant
$\sigma^2 hp$ = Phenotype variant

The criteria of heritability was classified by Mendez-Natera *et al.*[10]:

$h^2 < 0.2$ = Low, $0.2 \leq h^2 \leq 0.5$ = Medium, $h^2 > 0.5$ = High

Observation of the number of chromosomes was carried out by the squashing method[11] with modification. About 4 Sample plant from each population took three root samples to observe. The root was cut at 8.15 pm and soaked in 8-Hydroxyquinoline solution for 3 h at 5°C. Then the roots were soaked in 90% acetic acid for 15 min. After being removed from the 90% acetic acid tube, the root was soaked in the acetic acid and HCl with 3:1 proportion in the waterbath. The root pieces were placed on the watch glass which has been dripped with 2% aceto-orcein solution and left for 20 min so that the solution can be absorbed by the roots. The roots were transferred to a glass preparation. The root tip was cut 2 mm, enough to taste orcein then the cover glass is installed. Then the preparation was tapped and heated for a while, then pressed using the thumb or squash 180°. The chromosomes was calculated manually using microscope with magnification 400x.

RESULTS

Based on Table 1, the percentage of live plants at 25 days showed the results that all plant populations had a higher percentage compared to control plants. The highest percentage was in the U-K250-68 plant population i.e., 97%. Observations on quantitative characters showed that all plant populations were significantly different compared to controls, except for the plant height character in colchicine-induced plant populations of 750 ppm (U-K750-5 and U-K750-41). In addition, plants that were induced by 500 ppm of colchicine (U-K500-79 and U-K750-83) also were not significantly different from the length of the leaves. On the character of the number of grains per plant in the U-K750-5 plant population is also not significantly different than the control (Table 2).

Genotype Coefficient of Variability (GCV) measurements in all plant populations showed that all plant populations included in the low criteria on characters flowering days, harvest days, grain number per plant, filled grain, unfilled grain and 100 grain weight. All plant populations in the grain weight character per plant are included in the GCV criteria rather low. The GCV value that included in rather high criteria was U-K500-79 plant population in character number of leaves (Table 3).

The measurement of heritability values in the 6 plant populations induced by colchicine showed that all plant populations were in the high heritability category in the tillers number character, productive tillers and grain weight per plant (Table 4).

Table 1: Percentage of live plant on 25 days after seedling

Population	Number of plant	Live plant	Live plant (%)
U-K0	200	116	58.00
U-K250-67	200	129	64.50
U-K250-68	200	194	97.00
U-K500-79	200	167	83.50
U-K500-83	200	165	82.50
U-K750-5	200	157	78.50
U-K750-41	200	166	83.00

UK-0: Control, UK250-67 and UK250-67: Population induced colchicine 250 ppm, UK500-79 and UK500-83: Population induced colchicine 500 ppm; UK750-5 and UK750-41: Population induced colchicine 750 ppm

Table 2: Average value of quantitative characters

	Characters											
Population	FD (DAT)	FG (%)	GNP	GWP (g)	GW (g)	HD (DAT)	LL (cm)	LN	PH (cm)	PT	TN	UFG (%)
UK-0	90	51.62	975	27.20	2.43	120.2	39.52	55.81	94.21	12.30	22.40	48.61
UK250-67	82.02**	59.17**	1060.23**	48.49**	2.46**	113.10**	43.84**	74.94**	98.93**	16.41**	27.26**	40.81**
UK250-68	82.70**	60.76**	1033.42*	50.18**	2.47**	113.10**	42.38**	66.89**	90.39**	16.11**	24.59**	39.02**
UK500-79	81.98**	59.81**	1037.43*	48.79**	2.46**	112.80**	39.77ns	88.28**	89.12**	17.05**	24.83**	39.96**
UK500-83	81.92**	58.71**	1070.32**	47.86**	2.49**	113.50**	39.58ns	79.29**	88.29**	17.78**	25.06**	41.21**
UK750-5	82.05**	58.26**	1004.21ns	43.42**	2.50**	115.20**	42.56**	80.03**	96.02ns	17.05**	26.52**	41.95**
UK750-41	81.84**	60.31**	1054.03**	54.81**	2.47**	112.90**	42.93**	67.61**	94.88ns	19.16**	28.59**	39.61**

FD: Flowering days, FG: Filled grain, GNP: Grain number per plant. GWP: Grain weight per plant, GW: 100 grain weight, HD: Harvest days, LL: Leaves length, LN: Leaves number. PH: Plant height, PT: Productive tillers. TN: Tillers number. UFG: Unfilled grain. **: Very significant, *: Significant, ns: Not significant on student t-test at 5% level

Table 3. Genotype coefficient of variability (GCV) on quantitative characters

	Characters											
Population	FD (DAT)	FG (%)	GNP	GWP (g)	GW (g)	HD (DAT)	LL (cm)	LN	PH (cm)	PT	TN	UFG (%)
UK250-67	3.10	11.70	0.60	42.20	2.00	2.10	6.00	32.20	26.10	39.50	25.80	17.10
UK250-68	3.50	10.90	0.50	43.70	5.20	2.00	10.00	20.20	7.80	34.60	22.00	17.40
UK500-79	2.40	8.80	0.50	38.70	6.80	1.30	11.20	50.20	13.00	32.60	21.00	12.40
UK500-83	2.50	6.70	0.40	36.60	3.60	2.20	10.02	25.00	11.00	33.60	25.40	9.20
UK750-5	2.30	7.60	0.80	46.20	4.80	4.00	9.70	35.40	18.40	4.50	30.80	11.20
UK750-41	0.25	7.40	0.40	37.50	5.30	2.20	7.20	19.40	4.00	36.80	33.80	11.10

FD: Flowering days, FG: Filled grain, GNP: Grain number per plant, GWP: Grain weight per plant, GW: 100 grain weight, HD: Harvest days, LL: Leaves length, LN: Leaves number, PH: Plant height, PT: Productive tillers, TN: Tillers number and UFG: Unfilled grain

Table 4: Heritability value on quantitative characters

	Characters											
Population	FD (DAT)	FG (%)	GNP	GWP (g)	GW (g)	HD (DAT)	LL (cm)	LN	PH (cm)	PT	TN	UFG (%)
UK250-67	0.80	0.74	0.63	0.79	0.42	0.46	0.41	0.55	0.90	0.63	0.65	0.73
UK250-68	0.84	0.73	0.54	0.82	0.82	0.46	0.65	0.28	0.40	0.56	0.52	0.72
UK500-79	0.72	0.62	0.57	0.76	0.88	0.23	0.66	0.74	0.60	0.56	0.51	0.57
UK500-83	0.72	0.46	0.51	0.73	0.69	0.48	0.61	0.34	0.55	0.59	0.60	0.45
UK750-5	0.69	0.54	0.84	0.79	0.81	0.76	0.63	0.59	0.19	0.67	0.71	0.55
UK750-41	0.02	0.54	0.43	0.79	0.82	0.50	0.50	0.26	0.15	0.67	0.74	0.52

FD: Flowering days, FG: Filled grain, GNP: Grain number per plant, GWP: Grain weight per plant, GW: 100 grain weight, HD: Harvest days, LL: Leaves length, LN: Leaves number, PH: Plant height, PT: Productive tillers, TN: Tillers number and UFG: Unfilled grain

Table 5: Number of Chromosomes on M2 population Ungaran induced by colchicine

Population	Number of chromosomes (2n)
U-K0 (Control)	24
U-K250-67	
U-K250-67-8	30
U-K250-67-12	24
U-K250-67-118	24
U-K250-67-129	30
U-K250-68	
U-K250-68-4	30
U-K250-68-25	24
U-K250-68-34	24
U-K250-68-103	30
U-K500-79	
U-K500-79-6	30
U-K500-79-31	24
U-K500-79-56	24
U-K500-79-144	30
U-K500-83	
U-K500-83-9	36
U-K500-83-43	30
U-K500-83-58	36
U-K500-83-122	24
U-K750-5	
U-K750-5-8	30
U-K750-5-25	24
U-K750-5-54	24
U-K750-5-121	24
U-K750-41	
U-K750-41-4	30
U-K750-41-12	24
U-K750-41-88	24
U-K750-41-125	24

Observation of the number of chromosomes carried out in plant samples showed that there were several plants that had a higher number of chromosomes than controls. Based on Table 5 and Fig. 1, some plants selected in this study were U-K250-67-8 (2n = 30), U-K250-67-129 (2n = 30), U-K250-68-4 (2n = 30), U-K250-68-103 (2n = 30), U-K500-79-6 (2n = 30), U-K500-79-144 (2n = 30), U-K500-83-9 (2n = 3x = 36), U-K500-83-43 (2n = 30), U-K500-83-58 (2n = 3x = 36), U-K750- 5-8 (2n = 30) and U-K750- 41-4 (2n = 30).

DISCUSSION

Observation on character percentage of live plants showed all percentage of live plants was under 100%. This is because long time of storage could decrease seed quality. The viability of stored seeds will decreasingly due to the deterioration of seed quality[12]. Nevertheless, the percentage of live plants induced by colchicine were higher than control. This is consistent with research on Carnation (*Dianthus caryophyllus*) given colchicine so that the percentage of living plants increases. This is because of colchicine can increase the percentage of germination[13]. But this is not in accordance with research conducted on black rice[14] that showed that black rice given colchicine had a lower percentage (44.4%) than control plant (93.6%).

The average value of quantitative characters of M2 population of black rice showed the higher of agronomist

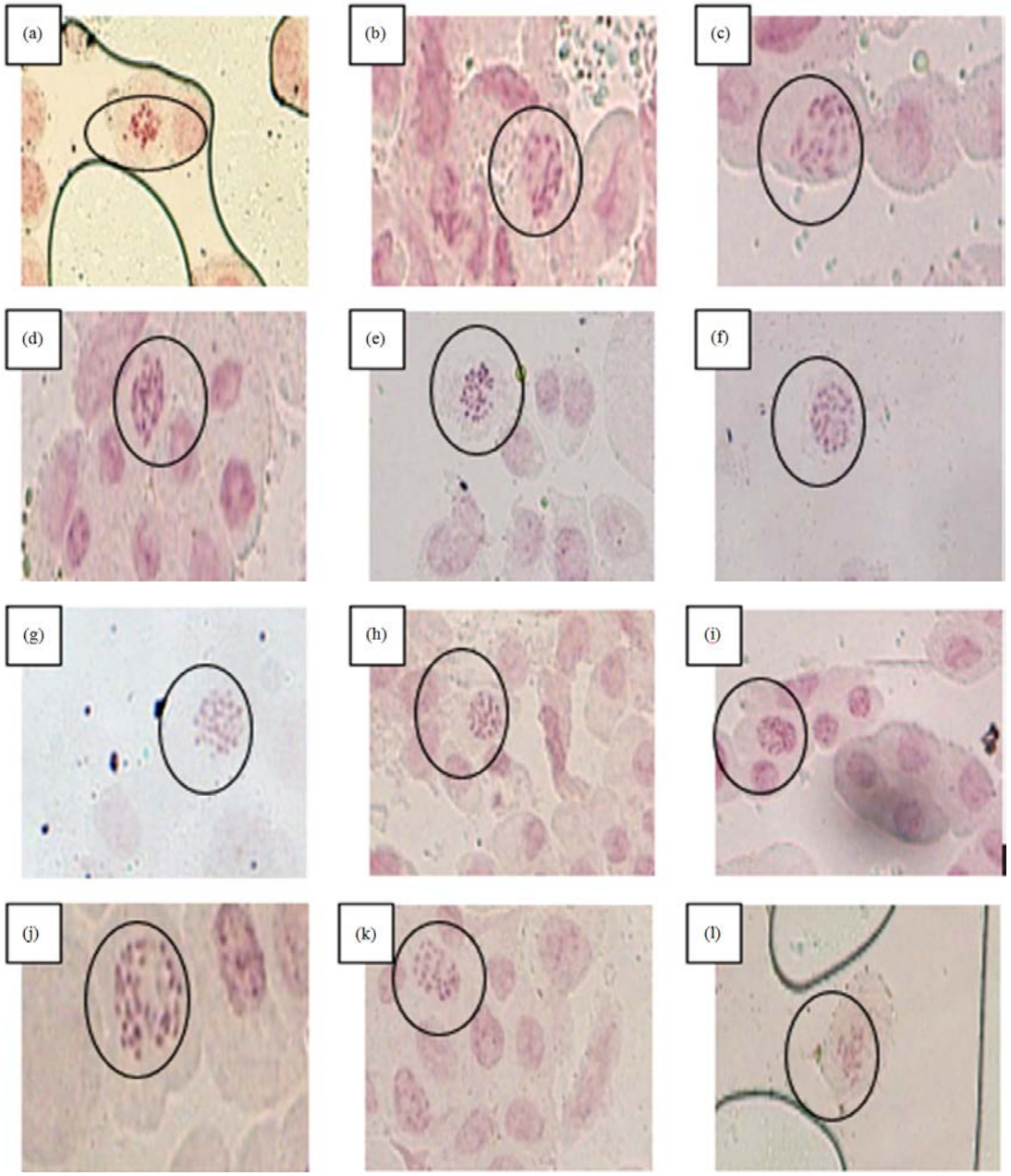

Fig. 1(a-l): Chromosome number of black rice, (a) UK0, (b) U-K0, (c) U-K250-67-129, (d) U-K250-67-8, (e) U-K250-68-103, (f) U-K250-68-4, (g) U-K500-79-144, (h) U-K500-79-6, (i) U-K500-83-9, (j) U-K500-83-58, (k) U-K750- 5-8 and (l) U-K750-41-4

characters such as filled grain, grain number, weight grain per plant, 100 grain weight, leaves length, productive tillers and tillers number. M2 population of black rice had lower average value on characters flowering days, harvest days and unfilled grain than control. Increasing on quantitative character will increasing yields of productivity. Mutation induction using colchicine can improve the quality of both qualitative and quantitative crops, especially in increasing crop productivity[15]. Colchicine induction can increase crop yields. however, when concentration reaches the optimum limit, it will reduce crop yields[16].

The result showed that the GCV on M2 population ranged from low-rather high criteria. This result is consistent with the research on mung bean[17] (*Vigna radiata* L.) induced colchicine that had low criteria of GCV on flowering day, number of pods, pod length, raw fruit and ripe fruit, number of seeds, number of branches and 100 seeds weight.

Plant breeding activities depend on genetic diversity. The next breeding activity (selection) will be more effective if implemented in populations that have high GCV values. The higher the genetic diversity makes the higher the chance to get the source of genes for the characters to be improved[18]. The success of a plant breeding program is determined by the availability of genetic varieties. The higher of genetic diversity make a higher of success for plant breeding programs.

M2 genotypes population of black rice had different values of genetic diversity and heritability i.e., low, medium and high. The highest heritability value was in the U-K250-67 population with 0.90 in the character plant height. Whereas the lowest heritability value in the U-K750-41 population is in character flowering days with 0.02. This result is not similar to research on M2 population induced colchicine of Soybean (*Glycime max* L. Merill) that showed the highest heritability on flowering days[19] with 0.95.

The higher variability in a population will increase effectiveness in a selection[20]. Observation and analysis on the quantitative character of M2 population caused by colchicine mutation showed difference of coefficient of genetic diversity value. High heritability value showed that the characters more controlled by genetic factors than environmental factors[5].

Although all quantitative characters of the M2 genotype population show diversity, the diversity caused by genetic factors is actually the main target for breeding programs. While the environment also has a major influence on the phenotypic appearance of quantitative characters, analysis is needed to show how much factor contribution genetic to the phenotypic appearance of a character. The greater the contribution of genetic factors, the more effective breeding activities will be carried out. Characters with rather low to low GCV criteria are classified as narrow variability, while characters with rather high to high GCV criteria are classified as broad variability. Breeding activities are based on the existence of genetic diversity and the genetic diversity needed is broad genetic diversity. So that the next breeding activity, namely selection, will be more effective if implemented in populations that have a high GCV value[21].

Observation on chromosomes number showed that the highest number of chromosomes was obtained at U-K500-83-9 and U-K500-83-58 with 2n = 36 chromosomes. The number of chromosomes of control plant is 2n = 24. There were 11 sample plant that had additional chromosomes from 24 total sample. Plants that did not follow the multiple of their chromosomes were caused by the addition of chromosomal genetic material and the reduction in the number of chromosomes due to the loss of chromosome segments called duplications and deletions. The deletion and duplication chromosome is evidenced by some of the results of colchicine treatments that produce plant cells with incorrect chromosome numbers with multiples of the number of haploid. There were several reasons of tetraploid plants did not formed in this study. The observation number of chromosomes in the provision of colchicines treatment were known if there were some additions to the number of chromosomes. However most of the plant cells were not increasing the number of chromosomes because a different response from each plant cell to the provision of colchicine treatments[22,23].

Observation on average of quantitative characters, GCV, heritability and number of chromosomes of M2 population of black rice induced by colchicine showed variability. Those were showed that colchicine caused genetic variability on local genotype of black rice. However, no report was previously identified for the mutation in varieties of black rice with almost all quantitative characters have high heritability.

CONCLUSION

The population of M2 from the colchicine mutation has a genotype coefficient of variability in the low to rather high category in all the character of quantitative observation. Most of the population had high value of heritability on all quantitative characters. There were 11 selected plants from this population based on quantitative character and cytology observation.

SIGNIFICANCE STATEMENT

This study discover the genetic variability in black rice due to colchicine treatments that can be beneficial for plant breeder and agronomist to develop and establish a new cultivar of black rice. Therefore, this study will help the researcher to uncover the critical areas of black rice germplasm that many researchers were not able to explore.

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