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Genetic diversity and cluster analysis of local pigmented rice from East and North Kalimantan, Indonesia based on quantitative and qualitative characters

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Abstract. *Hamidah, Sunaryo W, Rusdiansyah, Nurhasanah. 2024. Genetic diversity and cluster analysis of local pigmented rice from East and North Kalimantan, Indonesia based on quantitative and qualitative characters. Biodiversitas 25: 1938-1945.* Pigmented rice is a functional food containing antioxidants and various essential nutrients that are very beneficial for health. The substantial advantages of pigmented rice encourage the importance of research activities in exploring pigmented rice germplasms and further identifying unique and valuable traits for the development of superior rice varieties. This research aims to evaluate genetic diversity of pigmented rice based on quantitative and qualitative characters and examine their genetic relationship for future pigmented rice improvement strategies. Fifteen pigmented rice from East and North Kalimantan, Indonesia were used in this study. Nine quantitative and ten qualitative traits were observed and used to identify the genetic diversity of pigmented rice cultivars. The data then were used to group the pigmented rice based on their similarity degree using cluster analysis to form a phylogenetic tree hierarchically in a dendrogram. The results showed that there were large genetic differences based on quantitative and qualitative characters in the pigmented rice, showing a high genetic diversity either in brown or black rice cultivars. Based on the genetic relationships analysis, the fifteen pigmented rice cultivars were clustered in four groups with genetic similarity of ≥90%. The genetic diversity and relationship in this study emphasize the crucial of revealing the genetic identity of local pigmented rice from East and North Kalimantan.

Keywords: Genetic diversity, genetic relationship, Kalimantan, pigmented rice, quantitative and qualitative characters

INTRODUCTION

Pigmented rice (*Oryza sativa* L.) is a rice variety that has colored bran layers (Bhat et al. 2020), due to the accumulation of natural pigments in the seed coat, such as anthocyanins and proanthocyanidins (Limtrakul et al. 2019). These pigments not only give the rice a distinct color, but also contribute to its nutritional value. Colored rice varieties serve as a nutritious and beneficial alternative food, containing various essential nutrients such as protein, vitamins, minerals, and a favorable glycemic index (Anhar et al. 2017; Sani et al. 2018). This rice also contains bioactive phytochemicals, such as tocopherols, tocotrienols, oryzanols, and has a higher total phenolic, flavonoid and antioxidant activity content (Pengkumsri et al. 2015; Zhang et al. 2020). These properties contribute significantly to promoting human health (Limtrakul et al. 2019).

Colored rice stands out as a prominent source of diverse bioactive compounds renowned for their numerous health benefits. Enriched with potent elements, pigmented rice exhibits a spectrum of therapeutic effects, including antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity properties, along with the potential to prevent Cardio-Vascular Disease (CVD) (Yamuangmorn and Prom-u-Thai 2021). The consumption of a diet based on pigmented rice is believed to be instrumental in mitigating the risk of various health concerns. Notably, it is associated with reducing the likelihood of obesity, hepatic steatosis, hyperglycemia; and hypertension. Beyond these benefits, pigmented rice is thought to contribute against constipation, coronary heart disease, atherosclerosis, nephrological disorders, anemia, and allergy (Rathna Priya et al. 2019; Das et al. 2023). This highlights the comprehensive benefits of integrating pigmented rice into a diet, underscoring its significance as a functional food with a variety of positive effects on overall health and well-being.

Nowadays, pigmented rice is widely favored as a healthy dietary option by consumers who maintain a health-conscious lifestyle or choose it due to their health conditions (Ismoyowati et al. 2022). The increasing preference for pigmented rice underscores the significance of exploring indigenous pigmented rice becomes essential for meeting these evolving culinary preferences. This exploration not only addresses the rising demand for pigmented rice as functional food, but also holds the promise of preserving and promoting local agricultural biodiversity. Simultaneously, the development of pigmented rice varieties involves a strategic approach to breeding, aiming to enhance not only color variations but also nutritional content, yield, and adaptability to local environmental conditions.

The significant benefits associated with pigmented rice underscore the importance of study in exploring pigmented rice germplasms, and further identification of its distinctive and valuable traits that contribute to the development of superior pigmented rice varieties. East and North Kalimantan, Indonesia has high genetic resources of local rice (Nurhasanah and Sunaryo 2015; Nurhasanah et al. 2018a), including pigmented rice (Nurhasanah et al. 2018b). However, the local pigmented rice has not been well characterized. Genetic diversity analysis is a fundamental principle for the selection of desirable rice cultivars, as potential breeding material in in crop improvement breeding (Debsharma et al. 2023). Evaluating locally adapted germplasm, including indigenous pigmented rice, serves as valuable starting point as the initial stage in breeding (Nurhasanah et al. 2017; Awad-Allah et al. 2022).

The assessment of the germplasm diversity and genetic relationships among breeding materials is a crucial tool in enhancing crop improvement strategies (Gebrie and Abebe 2022). This research aims to evaluate the genetic diversity of the pigmented rice based on quantitative and qualitative characters and examine their genetic relationship for future pigmented rice improvement strategies. The assessment involves the analysis of both quantitative and qualitative traits, with the intent of investigating their genetic relationships to develop future breeding strategies for the improvement of superior pigmented rice.

MATERIALS AND METHODS

Plant materials

The pigmented rice was collected from six districts in East and North Kalimantan Provinces, Indonesia (Figure 1). Fifteen pigmented rice cultivars were collected in the exploration study of pigmented rice germplasms in East and North Kalimantan Provinces (Table 1). The pigmented rice cultivars consisted of six black and nine brown rice (Table 2).

Procedures

Field experiment

Fifteen pigmented rice cultivars were grown in a greenhouse of Faculty of Agriculture, Mulawarman University, Samarinda, Indonesia to observe their agro-morphological characteristics. Three seeds were sown in a plastic pot with holes containing 10 kg of growth media (3:1 of topsoil: organic fertilizer). After two weeks, plant thinning was conducted; only one good growing plant was maintained for the quantitative and qualitative characters observation. The experiment arrangement followed a block randomized design with three replications. The fifteen rice cultivars were grown and maintained based on the upland cultivation type. Rice seeds were directly sown in the soil. Each pot contained a single plant. Standard rice cultivation procedures were applied to maintain the plants.

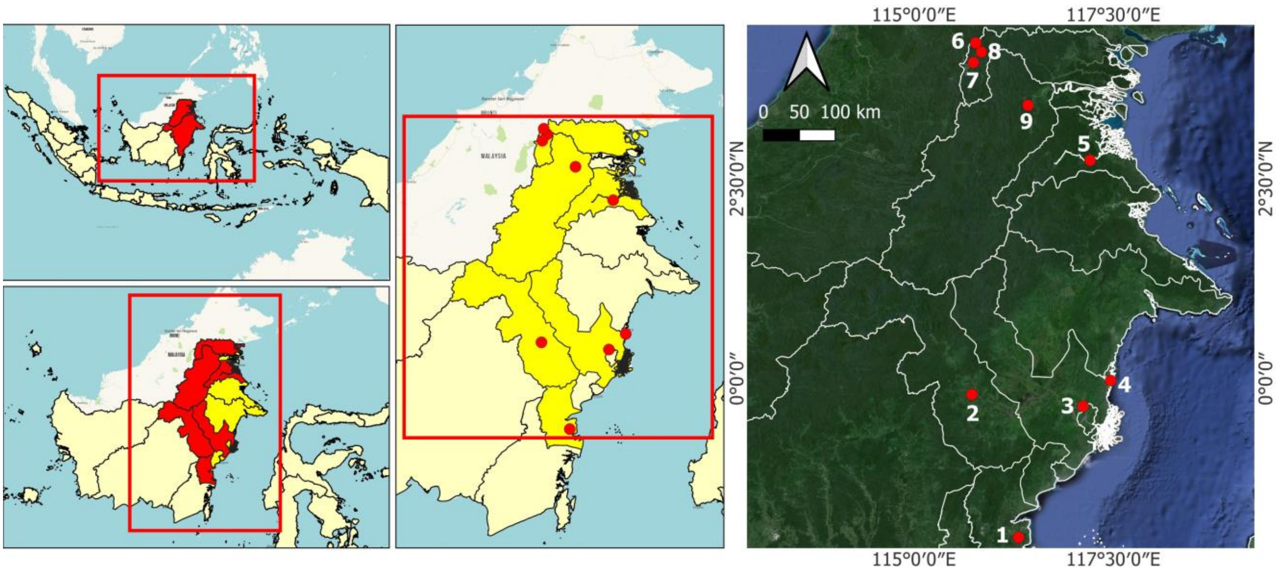

Figure 1. Pigmented rice exploration sites in East and North Kalimantan, Indonesia. East Kalimantan sites: 1. Laburan Baru; 2. Ombau Asa; 3. Bangun Rejo; 4. Santan Tengah. North Kalimantan sites: 5. Mara Satu; 6. Buduk Kinangan; 7. Lembudud; 8. Pa Payak; 9. Setulang

Table 1. The pigmented rice collection from East and North Kalimantan Province, Indonesia

Province	Districts	Villages	Number of pigmented rice cultivars
East Kalimantan	Paser	Laburan Baru	1
	Kutai Barat	Ombau Asa	2
	Kutai Kartanegara	Bangun Rejo	2
		Santan Tengah	1
North Kalimantan	Bulungan	Mara Satu	2
	Nunukan	Buduk Kinangan	2
		Lembudud	2
		Pa Payak	2
	Malinau	Setulang	1
Total			15

Table 2. Local rice genetic diversity in six districts of East and North Kalimantan, Indonesia

Districts		Paser	Kutai Barat	Kutai Kartanegara	Bulungan	Nunukan	Malinau	Total
Grain color	Black	1	0	1	1	3	0	6
	Brown	0	2	2	1	3	1	9
Cultivation type	Lowland	1	0	2	0	0	0	3
	Upland	0	2	1	2	6	1	12

Quantitative and qualitative traits observation

The quantitative traits observed in this study were plant height (cm), culm diameter (mm), leaf length (cm), leaf width (cm), ligule length (cm), grain length (mm), grain width (mm), ratio of grain length/width and weight of 100 seed (g). The qualitative characters were also examined in this study, including collar color, leaf sheath color, auricle color, ligule color and shape, leaf blade color, blade pubescence, leaf blade attitude, flag leaf attitude, and grain shape. The traits were characterized based on descriptors for rice (*Oryza sativa*) procedure by IBPGR-IRRI Rice Advisory Committee (1980) and descriptors for wild and cultivated (*Oryza* spp.) (IRRI 2007).

Data analysis

Prior to analysis all collected data were scored based on the scoring value in the rice descriptor procedure. The data then were used to group the pigmented rice based on their similarity degree using cluster analysis. Cluster analysis was carried out using hierarchical method, initially grouping two or more genotypes that shared the highest similarities (Bridges et al. 1966). This process was then iteratively applied to additional genotypes, forming a dendrogram. The clustering method involved the use of between-group linkage, and Squared Euclidean Distance was employed to determine the distances between clusters. Additionally, Pearson correlation was utilized to analyze the relationships between qualitative and quantitative traits. Data analysis was performed using IBM SPSS Statistic Data Editor Version 29.0.1.0.

RESULTS AND DISCUSSION

Pigmented rice diversity

Fifteen pigmented rice cultivars were collected in the exploration study of pigmented rice germplasms in East and North Kalimantan. The pigmented rice cultivars were observed less abundant in East Kalimantan compared to the North Kalimantan Province, in which only six pigmented rice cultivars were collected from this province (Table 1). Based on the grain color, the pigmented rice was dominated by brown rice (Table 2). Brown rice is commonly known by public than black rice. Based on a survey of food preference and acceptance assessment of pigmented rice, most of the panelists, consisting of 85 students from different cluster of knowledge in Yogyakarta, Indonesia; never encountered or see black rice (Helmyati et al. 2020). Some people have perception that black rice is black sticky/glutinous rice.

Most of the pigmented rice is cultivated as upland rice (Table 2), as the majority of the East and North Kalimantan local rice cultivation system (Nurhasanah et al. 2017; Nurhasanah et al. 2018b). Local rice cultivation methods are adapted to land types based on topography and soil characteristics. Most of the East and North Kalimantan areas are hilly and sloping; therefore, upland rice cultivation is more suitable (Nurhasanah et al. 2018b). In addition, the upland rice cultivation practices are adapted to the natural rainfall patterns, without irrigation and cultivation technology; therefore, it is more applicable for local communities that have limited resources or access to advanced agricultural technologies.

Pigmented rice is characterized by the presence of colored bran layers, attributed to natural pigments supplying many nutritional value containing antioxidants and essential nutrients that are important for human health (Pengkumsri et al. 2015; Sani et al. 2018; Limtrakul et al. 2019). The substantial advantages of pigmented rice encourage the importance of research activities in exploring pigmented rice germplasms and further identifying unique and valuable traits for the development of superior pigmented rice varieties.

Quantitative and quantitative characters

There were large differences in qualitative characters in the pigmented rice characterized in this study, either in brown or black rice cultivars (Table 3). A distinct plant performance was observed, not only in plant height which ranged from 90 to 150 cm, but also in culm diameter (7 to 14 mm), leaf length (40 to 94 cm) and leaf width (1.3 to 1.9 cm) in black rice. The same was found in the brown rice type, with plant height ranges from 109-175 cm, culm diameter of 0.53 to 1.3 cm, leaf length, 60-100 cm, and leaf width 1.4-1.9 cm. The large range of quantitative characters of the pigmented rice shows the high genetic diversity of the local pigmented rice from East and North Kalimantan.

Most of the pigmented rice have higher plant height, bigger culm, long and wide leaf, as the characteristics of local rice performance (Nurhasanah et al. 2016). These characters do not align with the ideotype of superior rice varieties which have a balance between plant height, culm strength, and leaf structure to maximize yield potential, adaptability, ease of cultivation, and efficient resource use. However, some of the local pigmented rice exhibiting an ideal individual trait, such as having low plant height (96.67 cm), big culm diameter (11.17 mm), and short leaves (46.67 cm) as shown in V_4 a black rice cultivar from Pa Payak Village, Krayan Barat Sub District, Nunukan District, North Kalimantan (Table 4).

Table 3. Minimum (min), maximum (max), mean value, and standard deviation (SD) of quantitative traits in black and brown rice cultivars

Characters	**Min.**	**Max.**	**Mean**	**SD**
Black rice				
Plant height (cm)	90.0	150.0	124.7	19.6
Culm diameter (mm)	7.0	14.0	9.9	1.9
Leaf length (cm)	40.0	94.0	60.8	14.5
Leaf width (cm)	1.3	1.9	1.5	0.2
Ligule length (cm)	1.5	3.0	2.2	0.4
Grain length (mm)	6.7	11.4	9.0	0.9
Grain width (mm)	2.0	3.1	2.5	0.3
Grain length/width	2.5	4.7	3.7	0.7
Weight of 100 seed (g)	2.2	2.9	2.5	0.3
Brown rice				
Plant height (cm)	109.0	175.0	144.0	23.1
Culm diameter (mm)	5.3	13.0	9.8	2.3
Leaf length (cm)	60.0	100.0	80.8	11.5
Leaf width (cm)	1.4	2.1	1.7	0.2
Ligule length (cm)	1.3	2.8	2.1	0.5
Grain length (mm)	7.3	11.2	9.0	0.9
Grain width (mm)	2.0	3.7	2.5	0.6
Grain length/width	2.4	5.2	3.8	0.9
Weight of 100 seed (g)	1.9	2.9	2.3	0.3

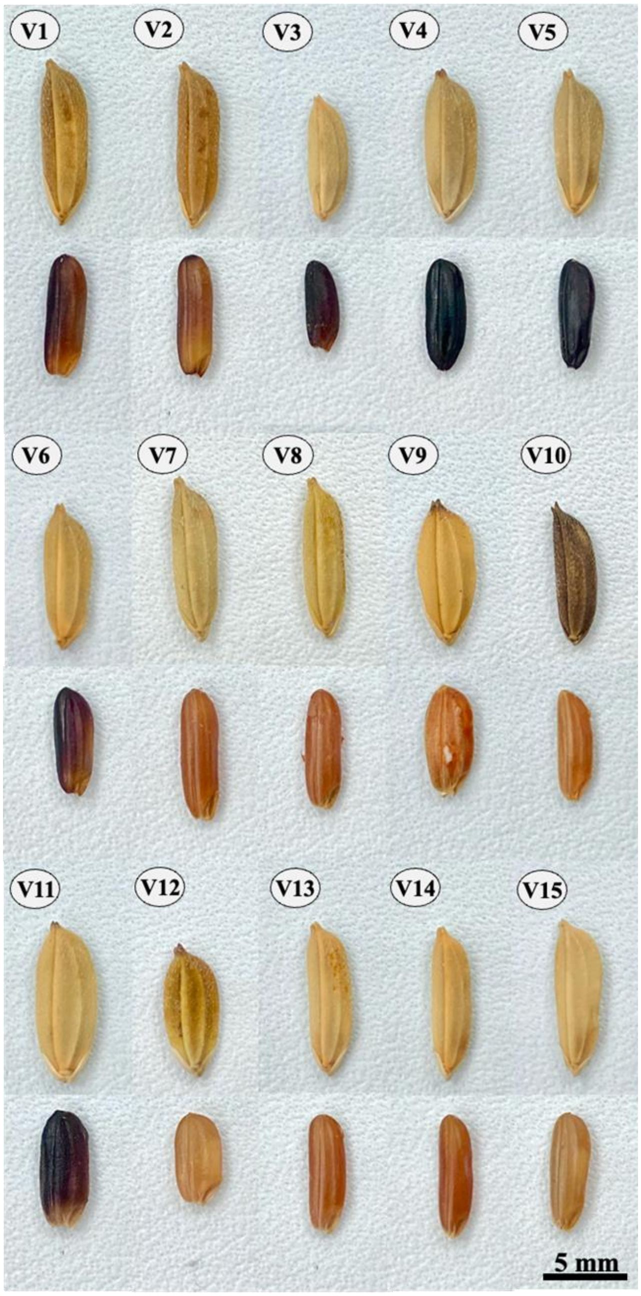

Figure 2. Variability of pigmented rice from East and North Kalimantan Province. V1-V6: Black rice; V7-V15: Brown rice

The rice ideal or model plant type is commonly associated semi dwarf stature, shorter culm length (100 cm or less) with greater culm diameter and lower relative internode elongation, high tillering capacity, and short, erect, and thick leaves (Peng et al. 2008). The ideal type is a biological model, encompasses both morphological and physiological traits, as well as their underlying genetic factors, to maximize the plant's efficiency in utilizing its functions within a specific biophysical environment under defined agricultural practices (Martre et al. 2015). It is important to identify the superior traits for selecting the ideotype of local rice cultivars to cover the breeding model plants for developing superior rice varieties (Yadi et al. 2021).

The qualitative characters of the pigmented rice observed in this study showed a distinct different among the cultivars. The similarities are only examined between V5-V6 in black rice type; between V7-V8, and V13-V14-V15 in brown rice type (Table 5). However, the grain color and shape among those cultivars are clearly diverse (Figure 2). The divergence shows not only the genetic variations among the pigmented rice but also their adaptation to various environmental conditions, since they originated and cultivated from different locations in East and North Kalimantan.

Qualitative characters are often controlled by a small number of genes, and they typically exhibit clear phenotypic variations (Sharma et al. 2023). Qualitative traits are often used in population studies to assess genetic diversity within and between populations, since these traits remain constant in expression in different environmental conditions and have the discriminative power to classify genotypes (Kachare et al. 2020). The presence or absence of specific traits in different populations can indicate the extent of genetic variation and categorize individuals into discrete groups based on their characteristics.

The Pearson correlation analysis among qualitative characters showed a very strong correlation between collar color and auricle color (1.00^{**}), as well as ligule color (0.89^{**}) (Table 6). This means that, when the coloration is presence in collar, it will also possibly be present in the auricle and ligule. The pigmented rice has colored pericarp layers due to the presence of natural pigments, such as anthocyanin (Limtrakul et al. 2019). Accumulation of anthocyanin pigments, resulting in the development of dark purple, light purple, or purple-washed leaves, as well as red, brown, black, or purple grain pericarp (Mackon et al. 2021).

Table 4. Quantitative characters of pigmented rice cultivars from East and North Kalimantan, Indonesia

Ident.	Rice type	Plant height (cm)	Culm diameter (mm)	Leaf length (cm)	Leaf width (cm)	Ligule length (cm)	Grain length (mm)	Grain width (mm)	Grain length/ width	Weight of 100 seed (g)
V1	Black rice	125.67±7.57	8.00±1.00	60.67±6.03	1.37±0.06	2.57±0.12	10.37±0.90	2.40±0.44	4.37±0.40	125.67±7.57
V2	Black rice	137.00±9.64	8.33±0.58	71.33±4.16	1.50±0.10	1.67±0.15	9.00±0.10	2.10±0.10	4.29±0.23	137.00±9.64
V3	Black rice	139.33±9.45	8.77±0.40	84.33±8.74	1.77±0.12	1.83±0.23	7.60±0.78	2.93±0.21	2.59±0.10	139.33±9.45
V4	Black rice	96.67±5.77	11.17±0.29	46.67±5.77	1.53±0.25	2.63±0.32	9.00±0.10	2.60±0.10	3.46±0.09	96.67±5.77
V5	Black rice	104.67±3.79	11.00±1.00	49.33±1.53	1.50±0.10	2.20±0.17	9.03±0.21	2.30±0.10	3.93±0.12	104.67±3.79
V6	Black rice	144.67±6.66	12.33±2.08	52.67±5.51	1.50±0.17	2.13±0.23	9.00±0.26	2.70±0.10	3.33±0.04	144.67±6.66
V7	Brown rice	127.33±5.51	8.67±0.58	77.00±3.00	1.50±0.10	2.57±0.25	10.13±1.59	2.10±0.10	4.81±0.57	127.33±5.51
V8	Brown rice	164.33±6.03	5.63±0.35	81.67±13.01	1.53±0.15	2.13±0.15	9.67±0.50	2.10±0.17	4.61±0.18	164.33±6.03
V9	Brown rice	168.33±7.64	10.93±1.10	80.00±2.00	2.00±0.10	2.33±0.15	9.00±0.10	3.50±0.20	2.58±0.17	168.33±7.64
V10	Brown rice	130.67±2.89	11.33±1.15	84.67±9.07	1.67±0.12	2.43±0.15	9.27±0.12	2.10±0.10	4.42±0.26	130.67±2.89
V11	Brown rice	144.00±5.29	6.83±0.47	88.67±3.06	1.97±0.12	1.57±0.12	8.50±0.35	3.07±0.55	2.81±0.36	144.00±5.29
V12	Brown rice	168.33±7.64	10.33±0.58	92.33±4.04	1.70±0.17	2.53±0.06	7.43±0.12	3.03±0.12	2.45±0.06	168.33±7.64
V13	Brown rice	111.33±3.21	11.53±0.50	62.67±2.52	1.67±0.06	1.40±0.10	9.20±0.10	2.20±0.10	4.19±0.14	111.33±3.21
V14	Brown rice	166.67±2.89	11.33±0.58	93.00±6.24	1.73±0.12	2.33±0.21	8.90±0.10	2.20±0.10	4.05±0.23	166.67±2.89
V15	Brown rice	115.00±2.65	12.00±1.00	67.33±8.08	1.70±0.10	1.40±0.10	9.00±0.20	2.20±0.10	4.09±0.10	115.00±2.65

Note: Data presented as Mean value±Standard deviation

Table 5. Qualitative characters of pigmented rice cultivars from East and North Kalimantan

Ident.	Rice type	Collar color	Leaf sheath color	Auricle color	Ligule color	Ligule shape	Leaf blade color	Blade pubescence	Leaf blade attitude	Flag leaf attitude	Grain shape
V1	Black rice	Pale green	Green	White	White	2-cleft	Green	Intermediate	Erect	Erect	Slender
V2	Black rice	Pale green	Purple lines	White	White	Acuminate	Purple tips	Intermediate	Erect	Erect	Slender
V3	Black rice	Pale green	Purple lines	White	White	Acuminate	Purple tips	Intermediate	Erect	Erect	Bold
V4	Black rice	Pale green	Green	White	Purple lines	Acuminate	Green	Intermediate	Erect	Erect	Slender
V5	Black rice	Pale green	Purple lines	White	Purple lines	2-cleft	Green	Intermediate	Erect	Erect	Slender
V6	Black rice	Pale green	Purple lines	White	Purple lines	2-cleft	Green	Intermediate	Erect	Intermediate	Slender
V7	Brown rice	Pale green	Purple lines	White	White	2-cleft	Green	Pubescent	Erect	Erect	Slender
V8	Brown rice	Pale green	Purple lines	White	White	2-cleft	Green	Pubescent	Erect	Erect	Slender
V9	Brown rice	Purple	Purple lines	Purple	Purple	2-cleft	Purple	Intermediate	Erect	Intermediate	Bold
V10	Brown rice	Pale green	Green	White	White	2-cleft	Purple	Pubescent	Erect	Erect	Slender
V11	Brown rice	Pale green	Green	White	Purple	2-cleft	Purple blotch	Intermediate	Erect	Erect	Bold
V12	Brown rice	Pale green	Green	White	White	2-cleft	Purple tips	Intermediate	Erect	Erect	Bold
V13	Brown rice	Pale green	Purple lines	White	White	2-cleft	Green	Intermediate	Erect	Erect	Slender
V14	Brown rice	Pale green	Purple lines	White	White	2-cleft	Green	Intermediate	Erect	Erect	Slender
V15	Brown rice	Pale green	Purple lines	White	White	2-cleft	Green	Intermediate	Erect	Erect	Slender

Table 6. Correlation analysis among agro-morphological traits in pigmented rice

	LSC	AC	LC	LS	LL	LBC	LBL	LBW	PH	CD	GL	GW	LSC
CC	0.38*	1.00**	0.89**	0.16	0.22	0.29	0.09	0.64**	0.18	0.11	-0.10	0.30*	-0.07
LSC		0.38*	0.26	-0.21	-0.48**	0.77**	0.24	0.38*	0.17	0.00	0.00	0.27	-0.19
AC			0.89**	0.16	0.22	0.29	0.09	0.64**	0.18	0.11	-0.10	0.30*	-0.07
LC				-0.06	0.30*	0.22	-0.08	0.56**	-0.02	0.15	-0.14	0.26	-0.10
LS					0.23	-0.32*	0.30*	0.16	-0.08	-0.24	0.22	0.08	0.16
LL						-0.35*	0.07	0.04	0.14	0.08	0.05	0.10	0.22
LBC							0.25	0.35*	0.26	-0.14	-0.05	0.38*	-0.11
LBL								0.09	0.60**	-0.29*	0.43**	0.30*	0.31*
LBW									0.18	-0.16	0.07	0.13	-0.07
PH										-0.26	0.25	0.17	0.18
CD											-0.10	0.05	0.11
GL												0.26	-0.10
GW													-0.03

Note: **: Correlation is significant at the 0.01 level; *: Correlation is significant at the 0.05 level; CC: Collar Color; LSC: Leaf Sheath Color; AC: Auricle Color; LC: Ligule Color; LS: Ligule Shape; LL: Ligule Length; LBC: Leaf Blade Color; LBL: Leaf Length; LBW: Leaf Width; PH: Plant Height; CD: Culm Diameter; GL: Grain Length; GW: Grain Width; WS: Weight of 100 Seed

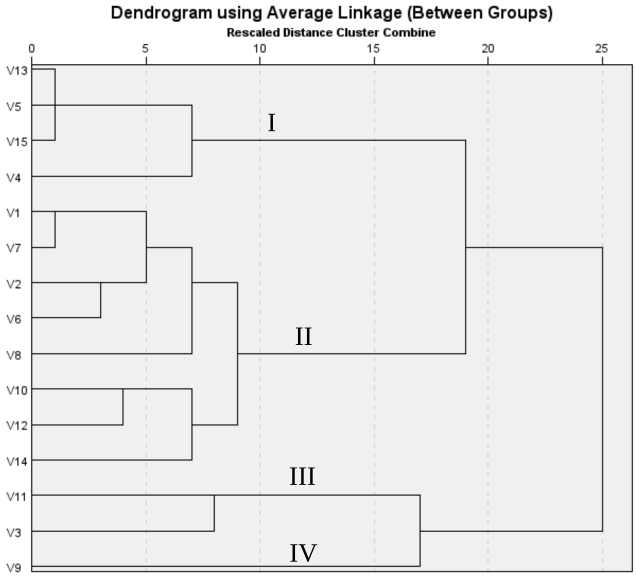

Figure 3. A dendrogram among 15 pigmented rice cultivars from East and North Kalimantan Province using hierarchical cluster analysis. V1-V6: Black rice; V7-V15: Brown rice

The presence of anthocyanins is not limited to the grains; these natural pigments can be observed in various plant organs. Purple color also occurs in leaves, leaf sheaths, internodes, and ligules with variations in coloration intensity (Sun et al. 2018). However, purple coloration appears on other organs, besides grains are rarely observed. In this study, although all bran layer shows coloration from light brown to full black (Figure 2); nevertheless, the pigment is not always present in culm, sheath, auricle, ligule, and leaf blade (Table 5). The purple color was observed most frequently in the leaf sheath, with the purple lines color. Only one cultivar (V9) exhibits almost full purple color in all plant organs, with purple collar, auricle, ligule, and leaf color. The same was also observed in four black rice varieties from West Kalimantan, in which only one cultivar (Nanga Taman) has purple color in leaf blade, auricle, and ligule (Palupi et al. 2020).

A strong positive correlation was also observed between leaf blade length and plant height (0.60^{**}), indicating that an increase in plant height corresponds to an increase in the length of the leaf blade (Table 6), as also observed in the previous study (Nurhasanah et al. 2016). Longer leaves commonly correlate with longer internodes, contributing to increased plant height (Zhang et al. 2017; Zhong et al. 2020). Plant height is not only a determining factor in the structure of a plant; it is also a significant agronomic characteristic directly associated with both harvest index and yield potential. In most of the cereal plants including rice, varieties with shorter plant height and leaves often display a more compact growth habit, which minimizes the risk of lodging, making them advantageous in densely planted fields. The compact growth habit contributes to enhanced structural stability, reducing the likelihood of lodging, particularly under the pressure of adverse weather conditions or increased competition for space and light in dense planting environments (Zhang et al. 2016).

Cluster analysis

The genetic relationships analysis among fifteen pigmented rice cultivars resulted in four groups based on 90% similarity (Figure 3). Most of the pigmented rice cultivars are clustered in group II, composed by three black rice and four brown rice cultivars. The largest genetic distance of the pigmented rice is shown by a brown rice cultivar V9 from Ombau Asa Village, Kutai Barat District, which is clustered in group IV and separated from other pigmented rice cultivars in East and North Kalimantan.

The closest genetic distance among the pigmented rice cultivars was observed between V5 (black rice), V13 (brown rice), and V15 (brown rice) in group I sharing 99% similarities. Those three pigmented rice cultivars originated from the same sub-district, Krayan Barat, in Nunukan District, North Kalimantan. The black rice cultivar V5 is considered as different genotype from V13 and V15, since it has different ligule color (purple lines) than V13 and V15 (white) (Table 5), besides a distinct bran layer color (Figure 2). On the other hand, the brown rice cultivars V13 and V15 might be the same genotypes, showing similar quantitative and qualitative characters (Tables 4 and 5). However, the grain shape showed a slight different (Figure 2). Other cultivars which also showed the closest genetic distance are V1 (black rice) and V7 (brown rice) in group II, which only showed 1% genetic difference based on the qualitative and quantitative characters observed in this study. They are indeed similar in many characters; nevertheless, the leaf sheath color and blade pubescence (Table 5), as well as the bran color (Figure 2) of the two cultivars definitely diverged. Therefore, further analysis should be conducted to distinctly identify whether similar genotypes are indeed the same or different, using molecular study approach (Sarif et al. 2020). In the previous study, the closest genetic relationships genotypes based on agro-morphological characters are actually dispersed based on molecular analysis (Susanto et al. 2015). Molecular markers can effectively distinguish among genotypes that exhibit high phenotypic similarity.

All the black rice cultivars were dispersed in different cluster and clustered in the first, second, and third group, with a genetic similarity of ≥90%. The closest genetic similarity of the black rice cultivars was observed only between V1 and V2 which shared 90% similarity. The two black rice cultivars originated from different districts in East Kalimantan, from Paser and Kutai Kartanegara District, which also showed a distinct qualitative character (Table 5) indicating that they are different genotypes.

Cluster analysis in this study was performed using combination of 19 qualitative and quantitative characters providing a more comprehensive perspective on genetic diversity (Mazal et al. 2021). Genetic characterization plays a pivotal role in the identification and selection of desirable rice cultivars, particularly as potential breeding material. Assessment of genetic diversity and the relationships among breeding materials is a critical tool for enhancing strategies in crop improvement (Gebrie and Abebe 2022; Debsharma et al. 2023)

All of the black and brown rice cultivars used in this study have no specific individual names, and are simply referred to by their color designation, as also observed in our previous research (Nurhasanah et al. 2018b). However, it is noteworthy that they exhibit genetic differences, as evidenced by the agro-morphological characters observed in this study. This underscores the importance of conducting a comprehensive identification for local pigmented rice. This identification is deemed crucial not only for understanding the distinct genetic variations among these rice varieties but also for the preservation of their genetic diversity. Acknowledging and characterizing genetic differences are fundamental steps in the broader efforts to conserve and utilize the rich genetic diversity present in pigmented rice varieties.

In conclusion, this study has revealed significant genetic variances among the 15 pigmented rice cultivars in East and North Kalimantan, as evidenced by both quantitative and qualitative traits. Through clustering analysis, four distinct groups among these cultivars were identified. These findings underscore the large genetic diversity in the local pigmented rice, which can contribute to the rice breeding program for developing superior variety supporting the development of the functional food. Such endeavors are crucial not only for elucidating the unique genetic compositions of these rice types but also for preserving their genetic diversity, which is essential for sustainable agriculture.

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