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Research Article

Morphoagronomical Evaluation of Several Indonesian Pigmented Rice (*Oryza sativa* L.) Accessions from East Java and Central Java, Indonesia

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Abstract Pigm ented rice is reported have high levels of bioactive compounds, it suitable for functional food. Additionally, it has been reported that pigmented rice extract has the potential as an anti-obesity, anti-inflammatory, antioxidant, and anti-cancer agent. In Indonesia, pigmented rice is less favored by local farmers due to low public demand (limited awareness) and cultivation difficulties (prone to falling and susceptible to pests), which could lead to the extinction of the plant. Qualitative and quantitative characterization can serve as the basis for plant breeding. This study aims to analyze the morpho-agronomic traits of several pigmented rice accessions from Central Java and East Java, thus providing a source of information for local farmers in the pigmented rice breeding process. Morpho-agronomic traits observed include qualitative and quantitative characters. The grouping and its relationship between 22 rice accessions were analyzed using Principal Component Analysis (PCA)-biplots and dendrogram clustering analysis based on Unweighted Pair Group Method Using Arithmetic Averages (UPGMA). The results showed the characteristics influencing the variation of Indonesian pigmented rice, especially from East Java and Central Java, out of 22 accessions, such as leaf color, shoot color, and tiller number. The grouping based on their morpho-agronomic characters showed that there were two main clusters. Twenty out of 22 rice accession grouping in cluster 1 with diverse pigmented rice pigment type. Meanwhile, cluster 2 consists of IR Ngawi Hitam and Wojaloka. These two accessions are classified as black rice. Our findings are valuable for breeding programs, especially in rice.

Key words Characterization, Indonesian pigmented rice, Morpho-agronomy, Phylogeny

Introduction

Indonesia is one of the countries with high biodiversity. One of the plants that is widely found in Indonesia is rice. Rice (*Oryza sativa* L.) is a species of the genus *Oryza* and the family Poaceae. Currently, rice is a staple crop in Indonesia (Fatchiyah et al. 2020). The use of rice as a staple crop is not only in Indonesia but almost half of the world's population and is centered in Asia (Brar et al. 2018). In Indonesia, white rice is more commonly chosen because it is easier to process and has a softer texture compared to pigmented rice (Sirisoontaralak et al. 2020). However, in recent years, many researchers studying pigmented rice have stated that it contains high levels of bioactive compounds, thus making it suitable for functional food (Kumar et al. 2018).

Pigmented rice refers to rice that contains proanthocyanidins and anthocyanins in the fruit skin, seed coat, and aleurone layer (Agustin et al. 2021). There are four groups of pigmented rice namely black rice, red rice, brown rice, and purple rice. These pigmented rice accessions are reported to be rich in beneficial phytochemical

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compounds and amino acids, contributing to their nutritional value (Kumar et al. 2018). Currently, pigmented rice has become increased in the agro-industrial field because of its nutritional value. Some researchers have reported that pigmented rice extracts contain bioactive compounds identified as antidiabetic (Bello et al. 2018), anti-inflammatory, antioxidant (Pradipta et al. 2023), and anti-cancer agents (Nafisah et al. 2023). Furthermore, pigmented rice also has higher concentrations of micronutrients and vitamins compared to white rice (Kang et al. 2022).

Despite its numerous nutritional compositions and health benefits, pigmented rice faces limited popularity within communities, particularly in Indonesia. The public demand for pigmented rice is notably low, prompting local farmers to prefer cultivating white rice instead. This preference is influenced by factors such as low awareness among consumers. Additionally, there are practical challenges for farmers, including susceptibility to pests and issues related to yield stability (Shinta et al. 2014). With this reality, pigmented rice will become extinct if people continue to focus on white rice only. Not many people know about the pigmented rice accession because there is still little information about the characteristics of the rice accession.

Characterization of pigmented rice needs to be carried out to increase public awareness and local farmers to increase its production. Characterization is a process of seeding specific characteristics in plants that are used to differentiate individuals within plant species. This characterization can be done either on the phenotypic or morphological character of the plant and using molecular markers. Morphological characterization needs to be done as basic information for the development and utilization of pigmented rice. With information related to the morpho-agronomy of pigmented rice, the community has become more familiar with and utilizes pigmented rice to meet consumption needs and also as a genetic source of plant breeding which aims to increase the potential of superior pigmented rice.

Research related to the morpho-agronomy of native pigmented rice from Indonesia has not been done much. The existing descriptions and publications only include Aek Sibundong brown rice (Shinta et al. 2014), Cempo Ireng black rice (Kurniasih et al. 2019), and highland rice (Sary et al. 2022). Therefore, this study was carried out to characterization and grouping 22 pigmented rice accessions from Central Java and East Java, Indonesia based on their morpho-agronomical characters. This study can support the sustainability of pigmented rice as valuable germplasm for further utilization and breeding program, especially in rice.

Materials and Methods

Materials

A total of 22 accessions of pigmented rice (*Oryza sativa* L.) obtained from Central Java and East Java available at the Research Center of Smart Molecule of Natural Genetics Resources, Universitas Brawijaya, Indonesia was selected (Table 1). The 22 rice accessions were planted with four replicates for each accession. Before planting, the rice seeds were soaked in distilled water at temperature of 25℃ overnight. After soaking, the soaked seeds were then planted in a germination tray containing a mixture of soil and compost (3:1; w/w). Two-weeks-old seedlings were planted in 20 L polybags containing soil: compost (3:1; w/w) and in each polybag contain three individuals with a distance of 20 cm between plants. Fertilization is carried out using NPK 16:16:16 fertilizer and applied in the first week after planting (WAP). In addition, liquid organic fertilizer was given after eight WAP. Rice plant maintenance such as pest control is done by applying the insecticide Profenofos 500 g/l from Syngenta and the Mancozeb fungicide 80% from Dow Agro Science Indonesia.

Table 1. The accession source of Indonesian pigmented rice.

No	Accession	Pigmen category	Origin
1	Wojaloka	Black	East Java
2	Jeliteng	Black	Central Java
3	IR Ngawi hitam	Black	East Java
4	Melik	Black	East Java
5	Mentik Ngawi	Red	Central Java
6	Arumba	Red	East Java
7	Blambangan A2	Red	East Java
8	Blambangan A3	Red	East Java
9	N790 merah	Red	Central Java
10	Inpari 24	Red	East Java
11	Inpari 32	Red	East Java
12	IR Ngawi merah	Red	East Java
13	Berlian	Brown	Central Java
14	N790 coklat	Brown	East Java
15	Sigunca 02	Brown	East Java
16	Sertani 13	Brown	East Java
17	Sigupai	Brown	East Java
18	HMS 700	Brown	East Java
19	Rojolele	Brown	East Java
20	Mr 308	White	East Java
21	Siam-siam	White	East Java
22	Kabir 07	White	East Java

Morphoagronomical observation of pigmented rice

Morpho-agronomic characteristics observed based on the Rice Plant Characterization and Analysis System published by the Indonesia Agency for Agriculture Research and Development, Ministry of Indonesia Agriculture (Silitonga et al. 2003) include qualitative and quantitative traits. The characterization protocol refers to the Descriptor for Wild and Cultivated Rice (*Oryza* spp.) published by IBPGR-IRRI. The quantitative traits measured in this study such as plant height, number of tillers, flag leaf length, panicle emergence, harvest age, panicle length, number of panicles, length, and width of grain, length and width of dehulled grain, awn length, weight of 100 grain (gr), percentage of total filled and unfilled grains or panicle. Meanwhile, the qualitative traits measured such as the color of the leaf, tiller, ligule, and rice grain, type of panicle and axis, then the axis of the flag leaf.

Statistical analysis

Data from the characteristics of morpho-agronomic is processed in the Microsoft Excel 2016 program. The statistical analysis using R studio software version 1.3.959. Principal Component Analysis (PCA) and biplot were conducted using the factoextra program to evaluate the characteristics of accessions. Additionally, dendrogram construction using the PAST program and the Unweight Pair Group Method Using the Arithmetic Method (UPGMA) and Pearson's Correlation Analysis.

Results

Qualitative traits of Indonesian pigmented rice from East Java and Central Java Indonesia

The results of the qualitative data obtained on rice plants include the plant parts color, then type and shape of the panicles and leaves (Table 2). The results obtained from the qualitative character analysis of Indonesian pigmented rice were found that there was no significant difference between all accessions, except for the color character of the rice grain (Table 2). The color of this rice grain is the main qualitative character because it is a differentiator between accessions. Rice accession with black grain consisted of Wojaloka, Jeliteng, IR Ngawi Hitam, and Melik. Accessions with red rice grains are Mentik Ngawi, Arumba, Blambangan A2, Blambangan A3, N790 Merah, Inpari 24, Inpari 32, and IR Ngawi Merah. Brown rice grains consist of Sigupai and HMS 700. Based on this characterization, several grains of white rice were obtained consisting of Berlian, N790 Brown, Sigunca 02, Sertani 13, Rojolele, Mr 308, Siam-Siam, and Kabir 07. These results show that there is a difference between the pigmented rice category in the specimen and the results obtained. The cause of difference in the color of rice grains in this rice accession can be caused by genetic factors. The morphology of pigmented rice accession from East Java and Central Java Indonesia can be observed in Fig. 1. There are differences in the morphological appearance of rice grain color after the dehulling process.

Table 2. Qualitative data of 22 accessions of pigmented rice from East Java and Central Java, Indonesia.

Accession	Color				Panicle		Flag leaf axis
	Leaf	Tiller	Ligule	Rice grain	Type	Axis	
Wojaloka	Dark Green	Black Light	Green	Black	Compact	Intermediate	Intermediate
Jeliteng	Green	Green	Green	Black	Intermediate	Intermediate	Intermediate
IR Ngawi Hitam	Dark Green	Black Light	Green	Black	Compact	Intermediate	Erect
Melik	Green	Green	White	Black	Intermediate	Intermediate	Intermediate
Mentik Ngawi	Green	Red Light	White	Red	Intermediate	Intermediate	Erect
Arumba	Green	Green	White	Red	Intermediate	Intermediate	Intermediate
Blambangan A2	Green	Green	White	Red	Intermediate	Intermediate	Erect
Blambangan A3	Green	Green	White	Red	Intermediate	Intermediate	Erect
N790 Merah	Green	Green	White	Red	Intermediate	Intermediate	Intermediate
Inpari 24	Green	Green	White	Red	Intermediate	Intermediate	Intermediate

Table 2. Continued.

Accession	Color				Panicle		Flag leaf axis
	Leaf	Tiller	Ligule	Rice grain	Type	Axis	
Inpari 32	Green	Green	White	Red	Compact	Intermediate	Intermediate
IR Ngawi Merah	Green	Green	Green	Red	Intermediate	Intermediate	Intermediate
Berlian	Green	Green	White	White	Intermediate	Droopy	Intermediate
N790 Coklat	Green	Green	White	White	Intermediate	Intermediate	Intermediate
Sigunca 02	Green	Green	Green	White	Intermediate	Intermediate	Intermediate
Sertani 13	Green	Green	Green	White	Intermediate	Intermediate	Intermediate
Sigupai	Green	Green	White	Brown	Intermediate	Intermediate	Intermediate
HMS 700	Green	Green	White	Brown	Compact	Droopy	Intermediate
Rojolele	Green	Green	White	White	Intermediate	Intermediate	Intermediate
Mr 308	Green	Green	White	White	Intermediate	Intermediate	Intermediate
Siam-siam	Green	Green	White	White	Intermediate	Intermediate	Intermediate
Kabir 07	Green	Green	White	White	Intermediate	Intermediate	Intermediate

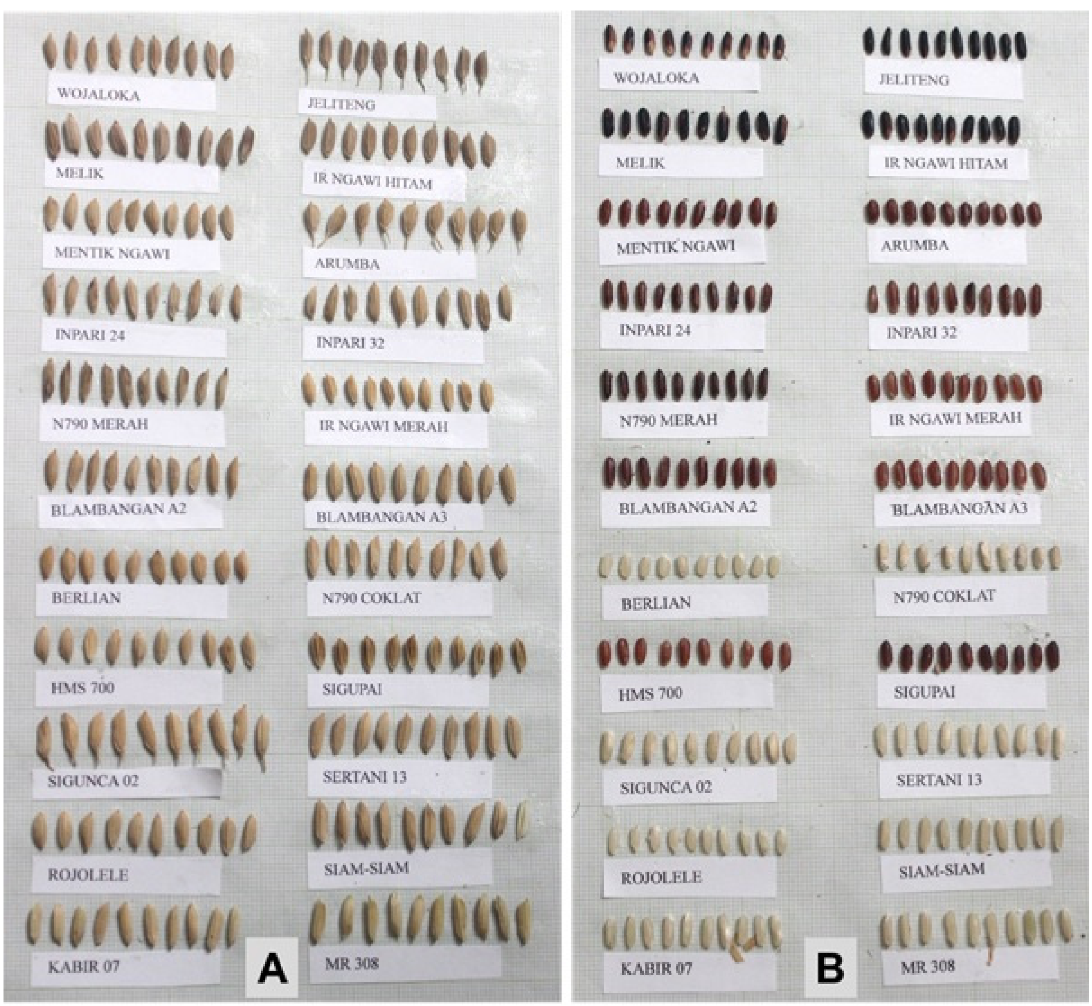

Fig. 1. Morphological grain of each accession of Indonesian pigmented rice (*Oryza sativa* L.) from East Java and Central Java, Indonesia Grain (A), and dehulled grain (B).

White rice is obtained through the process of dehulling and polishing using machinery to separate the hull and bran. In this study, several rice accessions categorized as brown pigmented, such as Berlian, N790 Coklat, Sigunca 02, Sertani 13, and Rojolele exhibit white grain color after a single dehulling process (Fig. 1). There was no difference in color among other accessions after a single dehulling process. Therefore, the four pigmented rice accessions (Berlian, N790 Coklat, Sigunca 02, Sertani 13, and Rojolele) can be referred to as brown rice with a single dehulling process. In general, rice grains come in various colors such as black, red, brown, and white.

Quantitative traits and reproductive traits of Indonesian pigmented rice (*Oryza sativa* L.) from East Java and Central Java, Indonesia

The quantitative characters of 22 pigmented rice varieties can be seen in Tables 3 and 4. Several accessions show quite varied characters. The quantitative characters showed varied among Indonesian pigmented rice obtained. Based on Table 3, the height of the plant has a range of 99-132 cm. The rice accession with the smallest plant height was Sertani 13 with (99 cm), while the accession with the highest plant height was HMS 700 (132 cm). Based on the height of the rice plant, most farmers have a shorter type of rice than the tall one. This is because taller rice is easier to collapse than short rice. The of tiller number in 22 accessions was 10-38 (Table 3). Rojolele produces the highest tiller number (38), followed by IR Ngawi Hitam (32), IR Ngawi Merah (31), and Inpari 32 (30). Meanwhile, HMS 700 produces the lowest tiller number (10). The flag leaf length ranged from 15.20-27.60 cm (Table 3). Rojolele (27.60 cm) showed the longest flag leaf, while N790 Merah (15.20 cm) showed the shortest flag leaf. The fastest panicle emergence age was measured from Siam-siam (50 days after planting/DAP), followed by Kabir 07 (52 DAP). Meanwhile, Mr 308 takes the longest time of panicle emergence (82 DAP), followed by HMS 700 (80 DAP). There were 22 accessions with varying harvest age of 98-129 DAP (Table 3). The Sigupai kind has the quickest harvest age (98 DAP), while Berlian accession has the longest harvest age (129 DAP).

Table 3. Quantitative traits of Indonesian pigmented rice (*Oryza sativa* L.) from East Java and Central Java, Indonesia.

Accession	Plant height (cm)	Total tiller number	Flag leaf length (cm)	Panicle emergence age (DAP)	Harvest age (DAP)
Wojaloka	107±2.96	24±2.34	20.60±1.02	65±0.01	116±0.04
Jeliteng	105±3.05	26±1.26	24.60±1.85	72±0.03	115±0.02
IR Ngawi Hitam	103±2.88	32±1.78	21.20±1.72	62±0.01	118±0.01
Melik	119±1.58	16±3.14	20.80±1.72	69±0.02	123±0.02
Mentik Ngawi	110±1.92	29±1.81	18.40±1.02	68±0.01	118±0.01
Arumba	102±1.64	18±1.24	19.80±2.31	72±0.04	123±0.05
Blambangan A2	106±2.49	14±1.72	15.80±1.93	60±0.01	114±0.02
Blambangan A3	107±1.48	16±3.66	16.10±0.89	61±0.02	121±0.06
N790 Merah	102±1.30	12±3.08	15.20±4.79	68±0.04	125±0.07
Inpari 24	103±1.58	15±2.53	19.60±1.49	76±0.03	119±0.02
Inpari 32	106±2.55	30±2.52	21.80±1.32	69±0.02	119±0.03
IR Ngawi Merah	122±2.55	31±1.83	24.20±2.78	71±0.01	111±0.02
Berlian	112±1.94	15±2.00	21.20±1.71	67±0.01	129±0.01
N790 Coklat	105±2.58	13±2.87	17.10±1.41	71±0.02	117±0.02
Sigunca 02	104±1.51	28±4.22	19.40±2.41	77±0.01	125±0.01

Table 3. Continued.

Accession	Plant height (cm)	Total tiller number	Flag leaf length (cm)	Panicle emergence age (DAP)	Harvest age (DAP)
Sertani 13	99±2.40	20±3.72	21.20±1.72	62±0.02	128±0.01
Sigupai	103±3.67	19±3.74	20.60±1.02	67±0.03	98±0.02
HMS 700	132±2.30	10±2.50	19.40±1.01	80±0.05	122±0.02
Rojolele	124±2.28	38±2.60	27.60±1.49	70±0.02	122±0.04
Mr 308	101±3.83	25±4.06	18.20±1.41	82±0.01	119±0.02
Putih Siam-siam	114±2.55	24±2.90	16.60±1.62	50±0.01	120±0.06
Putih Kabir 07	114±2.07	18±2.71	18.60±2.05	52±0.06	122±0.06

Table 4. Reproductive traits of Indonesian pigmented rice (*Oryza sativa* L.) from East Java and Central Java, Indonesia.

Accession	Panicle length (cm)	Panicle number/ clump	Grain		Dehulled grain		Awn length (cm)	Total grain number/ panicle	Percentage of total filled grain/panicle (%)	Percentage of total unfilled grains/ panicle (%)	100-grain weight (gram)
			Length (cm)	Width (cm)	Length (cm)	Width (cm)					
Wojaloka	24.50±1.83	23±1.58	0.51±0.03	0.28±0.03	0.45±0.04	0.23±0.01	0	51±0.84	78.95±0.04	21.05±0.21	2.34±0.37
Jeliteng	22.83±2.12	20±3.56	0.40±0.02	0.28±0.02	0.34±0.00	0.21±0.03	0.24±0.07	45±1.48	86.36±0.01	13.64±0.15	1.94±0.04
IR Ngawi Hitam	22.58±1.31	13±2.55	0.49±0.02	0.28±0.02	0.42±0.05	0.22±0.02	0	54±1.47	54.55±0.14	45.45±2.11	1.98±0.01
Melik	31.16±2.24	14±1.94	0.43±0.03	0.31±0.04	0.40±0.05	0.28±0.03	0	72±1.30	78.57±0.05	21.43±2.23	2.08±0.05
Mentik Ngawi	24.25±1.42	24±1.30	0.39±0.02	0.29±0.03	0.33±0.06	0.27±0.02	0	62±1.58	86.67±0.08	13.33±1.98	2.35±0.03
Arumba	21.83±1.40	15±1.92	0.52±0.03	0.32±0.02	0.45±0.03	0.24±0.03	0.31±0.04	67±2.07	84.62±1.12	15.38±1.12	1.86±0.01
Blambangan A2	21.41±1.97	10±1.14	0.84±0.06	0.28±0.04	0.75±0.05	0.25±0.02	0	39±2.08	73.73±1.15	26.67±1.14	2.08±0.11
Blambangan A3	22.25±1.65	13±2.58	0.84±0.03	0.30±0.02	0.64±0.04	0.23±0.02	0	60±1.67	83.33±0.03	16.67±1.03	2.16±0.04
N790 Merah	20.41±1.72	11±0.83	0.79±0.27	0.24±0.02	0.71±0.05	0.21±0.03	0	24±1.14	64.29±0.02	35.71±2.10	2.13±0.02
Inpari 24	14.91±1.16	12±2.00	0.87±0.06	0.26±0.03	0.66±0.05	0.23±0.03	0	30±1.87	83.83±1.17	16.67±1.17	1.84±0.02
Inpari 32	22.91±1.92	20±1.00	0.89±0.05	0.24±0.03	0.73±0.04	0.19±0.02	0	63±0.89	88.89±0.03	11.11±1.02	2.46±0.03
IR Ngawi merah	21.41±1.44	14±1.30	0.80±0.04	0.24±0.02	0.61±0.03	0.22±0.01	0	69±2.59	85.71±0.05	14.29±1.08	2.23±0.02
Berlian	22.50±1.24	12±1.94	0.73±0.03	0.31±0.02	0.57±0.03	0.25±0.02	0	36±1.92	45.55±1.15	54.45±1.13	2.32±0.02
N790 Coklat	20.58±1.67	11±1.58	0.82±0.04	0.27±0.02	0.62±0.09	0.22±0.03	0	29±1.14	85.71±0.02	14.29±1.15	2.32±0.03
Sigunca 02	21.16±1.94	15±2.40	0.87±0.05	0.33±0.02	0.70±0.07	0.24±0.03	0.34±0.05	48±1.16	80.00±1.12	20.00±1.25	2.23±0.01
Sertani 13	22.16±0.93	17±1.92	0.87±0.05	0.29±0.02	0.68±0.05	0.23±0.02	0	55±1.14	64.71±1.14	35.29±1.07	2.16±0.02
Sigupai	19.10±1.75	17±1.67	0.80±0.06	0.30±0.03	0.61±0.05	0.213±0.030	0	62±1.15	73.68±1.16	26.32±1.13	2.24±0.02
HMS 700	19.08±1.37	9±2.12	0.75±0.04	0.32±0.02	0.70±0.05	0.243±0.028	0	80±0.89	78.57±1.11	21.43±1.14	2.03±0.01
Rojolele	23.75±1.21	23±5.02	0.79±0.03	0.36±0.03	0.55±0.03	0.228±0.029	0	74±1.15	69.57±0.03	30.43±1.15	1.75±0.03
Mr 308	21.58±2.27	19±1.94	0.86±0.06	0.26±0.09	0.77±0.04	0.231±0.032	0	36±1.48	70.00±1.17	30.00±0.16	2.34±0.43
Siam-siam	20.91±1.92	21±1.87	0.80±0.04	0.29±0.03	0.71±0.04	0.219±0.014	0	45±1.00	73.68±0.02	26.32±0.05	2.15±0.03
Kabir 07	21.58±2.81	12±1.58	0.80±0.04	0.30±0.03	0.71±0.05	0.227±0.015	0	26.0±0.84	80.95±1.14	19.05±0.03	2.14±0.02

Our results also showed that the height of 22 pigmented rice accession ranged between 99-132 cm. Sertani 13 showed the shortest (99 cm), while HMS 700 had the highest (132 cm). There were only three accessions that have a height more than 120 cm, namely HMS 700, Rojolele, and IR Ngawi Merah. As taller plants are more prone to collapse than shorter ones, farmers often wish their crops were not too tall. Based on the our result showed that there variation among 22 rice accessions with ranged 98-129 DAP. Sigupai has the fastest harvest age (98 DAP), while Berlian has the longest harvest age (129 DAP).

The growth of rice is divided into three stages, such as vegetative, reproductive, and maturation stages. The reproductive stage is characterized by an increase in the length of several upper nodes of the plant stem, the emergence of flag leaves, and flowering. In this study, the observed reproductive characters are panicle length, number of panicles, length and width of grain, length, and width of dehulled grain, awn length, the weight of 100 grain, and percentage of total filled and unfilled grains or panicle. Based on Table 4 shows that panicle length ranged from 14.91-31.16 cm, while the number of panicles also varied between 9-24 (Table 4). This study shows that Melik has the longest of the length of panicle (31.16 cm), followed by Wojaloka (24.50 cm), and Mentik Ngawi (24.25 cm).

The length of the grain ranged from 0.39-0.89 cm and the width of the grain ranged from 0.26-0.36 cm (Table 4). Out of 22 accessions, 16 accessions had the longest length of grain. The longest cultivar was Inpari 32 (0.89 cm), while, the widest grain was Rojolele (0.36 cm). The length and width of the grain are important characteristics because they can determine the size of the grain which will improve the quality of rice yields (Kurniasih et al. 2019). Length of dehulled grain ranged from 0.33-0.77 cm and width of dehulled grain ranged from 0.19-0.28 cm (Table 4). This study shows that Mr 308 has the longest length of dehulled grain (0.77 cm) and Melik has the widest width of dehulled grain (0.28 cm).

Out of 22 accessions, only 3 accessions had an awn on the grains, there were Jeliteng, Arumba, and Sigunca 02 with the lengths 0.24; 0.31; and 0.34 cm, respectively (Table 4). The total number of grains per panicle ranged from 24-74. The highest total number of grains per panicle were Rojolele and Melik. The percentage of filled and unfilled grains per panicle ranged from 11-88%. The highest percentage of filled and unfilled grains were Inpari 32 (88.89%) and Berlian (54.45%). Range of grain weight 100 gram from 1.75-2.35 gram. The heaviest grains were Mentik Ngawi (2.35 grams), followed by Wojaloka.

Clustering and correlation analysis of morpho-agronomic characters of Indonesian pigmented rice

The diversity of pigmented rice from East Java and Central Java, Indonesia was analyzed using PCA analysis based on morpho-agronomic characters. Table 5 showed that the eigenvalue as a percentage of the total variation among observed traits in the 22 pigmented rice accessions. In addition, based on Table 5 showed that the cumulative principal component was 35.93% (PC1=15.01%; PC2=35.93%). According to Table 6, the traits that significantly influence and have a positive contribution value on the first component (PC1) were tiller color, leaf color, and total tiller number. The traits that significantly influence and have a positive contribution value on the second component (PC2) are the percentage of total filled grains per panicle, leaf color, and tiller color. The graph illustrates the distribution of morpho-agronomic traits among 22 accessions from East Java and Central Java, Indonesia as shown in Fig. 2.

Table 5. The eigenvalues and morpho-agronomic trait variations of the three principal components from 22 accessions.

	PC1	PC2
Eigenvalue	4.18	3.00
Variability (%)	20.91	15.01
Cumulative %	20.91	35.93

Table 6. Eigenvalues of 20 morpho-agronomic traits from three principal component of 22 accessions.

No	Characteristics	F1	F2
1	Plant height (cm)	0.01	-0.09
2	Flag leaf length (cm)	0.22	-0.19
3	Panicle emergence age (days)	-0.01	-0.21
4	Harvest age (days)	-0.11	0.15
5	Panicle length (cm)	0.24	-0.01
6	Panicle number/clump	0.24	-0.17
7	grain length	-0.33	0.00
8	Awn length (cm)	0.03	-0.26
9	Total grain number/panicle	0.22	-0.20
10	Percentage of total filled grains/panicle (%)	0.04	-0.47
11	Percentage of total unfilled grains/panicle (%)	-0.04	0.47
12	100-grain weight (gram)	-0.00	0.09
13	Leaf color	0.36	0.30
14	Ligula color	0.28	0.01
15	Rice grain panicle	0.26	-0.02
16	Type panicle	-0.26	-0.17
17	Axis panicle	-0.13	0.20
18	Flag leaf axis	-0.14	-0.19
19	Tiller color	0.39	0.25
20	Total tiller number	0.31	-0.14

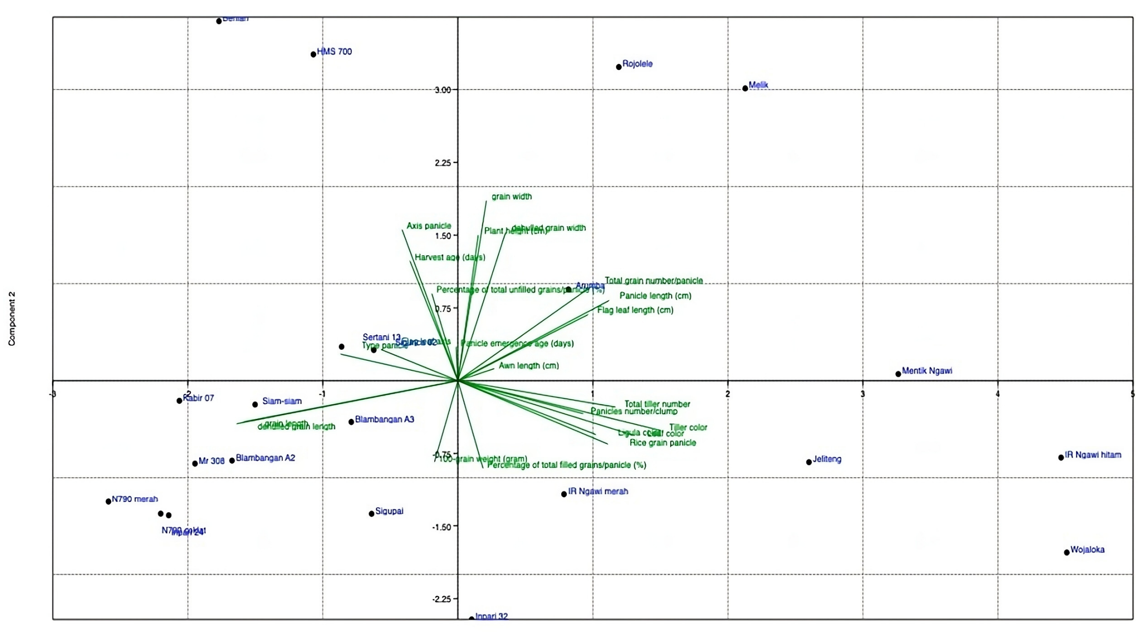

Fig. 2. Morpho-agronomic characteristic of 22 accessions based on PCA and Biplot analysis. Blue font color: name of accession. Green line: characters.

According to the PCA biplot analysis (Fig. 2) showed four quadrants where morpho-agronomic traits are distributed among the 22 accessions. The first quadrant consisting of Melik, Mentik Ngawi, Arumba, and Rojolele, exhibits strong relationships and similarities in traits such as panicle emergence age, grain width, plant height, dehulled grain width, total grain number per panicle, panicle length, flag leaf length, and awn length. The second quadrant consisting of Berlian, Sigunca 02, Sertani 13, and HMS 700, shows strong relationships and similarities in traits such as panicle emergence age, percentage of total unfilled grains per panicle, harvest age, panicle axis, and type of panicle. The third quadrant consisting of Blambangan A2, Blambangan A3, N790 Merah, Inpari 24, N790 Coklat, Sigupai, Mr 308, Siam-siam, and Kabir 07, demonstrates strong relationships and similarities in traits such as grain length, dehulled grain length, and 100-grain weight (grams). Meanwhile, the fourth quadrant comprising Wojaloka, Jeliteng, IR Ngawi Hitam, Inpari 32, and IR Ngawi Merah and shows strong relationships and similarities in traits such as percentage of total filled grains per panicle, number of tillers, number of panicles, color of tillers, leaf color, ligule color, and number of filled grains.

The correlation between morphoagronomic characters can be shown in the plot of univariate correlation results with Pearson's Correlation Analysis which can be seen in Fig. 3.

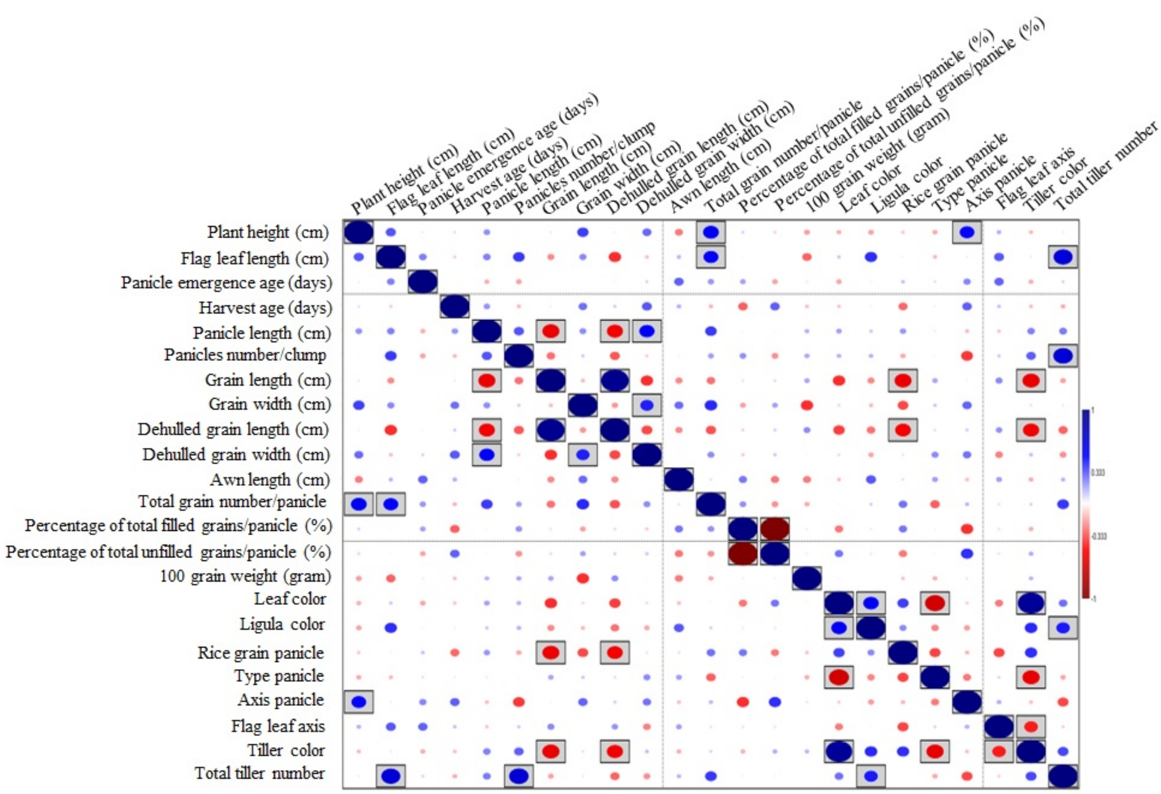

Fig. 3. Univariate correlation with Pearson's Correlation Analysis (*p*<0.5=significant). Blue boxes: positive correlation; Red boxes: negative correlation.

Based on Fig. 3, there was a positive correlation between total grain number with plant height and flag leaf length (*r*=0.526), panicle axis with plant height (*r*=0.04), and number of tillers with flag leaf length (*r*=0.636). This analysis supports the PCA biplot analysis that these morpho-agronomic characters have axes that were located close to each other. In contrast, grain length has a negative correlation with panicle length (r=-0.539). This can be seen in the PCA biplot analysis image which shows the location of the opposite axis. Analyzing the characteristics of the 22 pigmented rice accessions, further analysis can be done to classify the relationship between various groups.

Based on Fig. 4, shows the results of a highly diverse of 22 pigmented rice accessions with Euclidean distances from 0-8. The grouping based on their morpho-agronomic characters showed that there were two main clusters. Twenty out of 22 rice accession grouping in cluster 1 with diverse pigmented rice pigment type. The cluster 1 include Melik, Mentik Ngawi, Blambangan A2, Blambangan A3, N790 Merah, N790 Coklat, Mr 308, Inpari 24, Sertani 13, Siam-siam, Kabir 7, Sigupai, Inpari 32, IR Ngawi Merah, Sigunca 02, jeliteng, Arumba, Rojolele, Berlian, and HMS 700. Meanwhile, the cluster 2 consist of IR Ngawi Hitam and Wojaloka. These two accession are classified as black rice. Accessions group with Euclidean distance 2-3 were Siam-siam, Kabir 07, Blambangan A2, and Blambangan A3. Accessions group with Euclidean distance 3-4 were N790 Coklat and Mr 308. Accessions group with Euclidean distance 4-5 were N70 Merah, Inpari 24, Sertani 13, Inpari 32, IR Ngawi Merah, Jeliteng, and Arumba. Accessions group with Euclidean distance 5-6 were Melik, Mentik Ngawi, Sigupai, Sigunca 02, IR Ngawi Hitam, and Wojaloka. Accessions group with distances 6-7 were Berlian and HMS 700. Accessions group with distance 7-8 were Rojolele.In this study, the accession with the lowest Euclidean value was Siam-siam, Kabir 07, Blambangan A2, and Blambangan A3. Based on their Euclidean value indicated that these four accession have closer relationship between them.

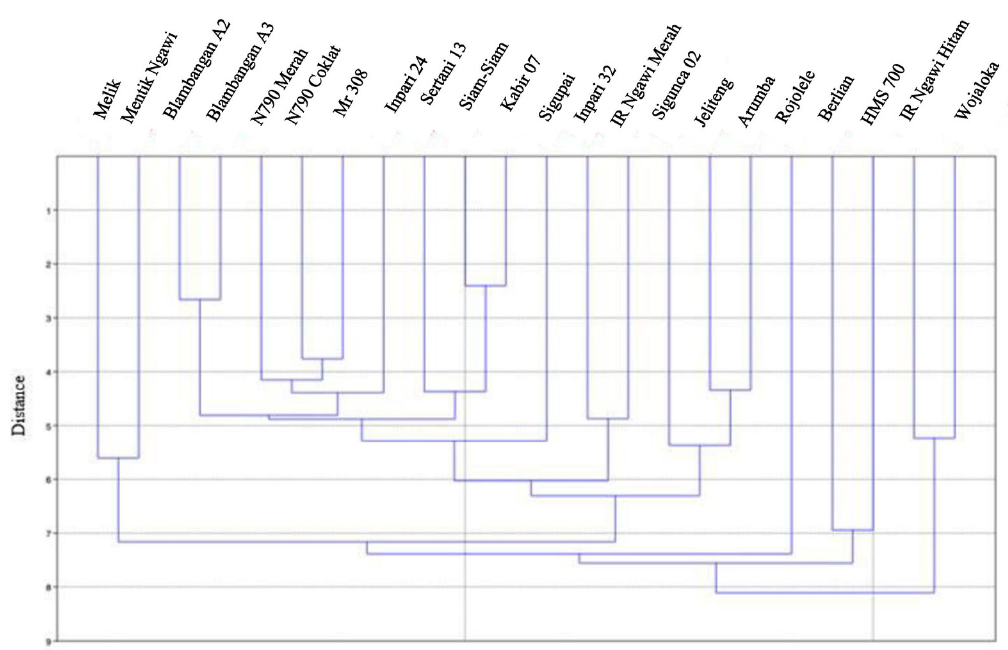

Fig. 4. Grouping of 22 Indonesian pigmented rice accessions based on their morpho-agronomical characters.

Discussion

Qualitative and quantitative traits of Indonesian pigmented rice from East Java and Central Java Indonesia

This study was to analyze the genetic variability of 22 Indonesian pigmented rice accessions from East and Central Java (Table 1). The analysis was conducted by morpho-agronomic characters that include qualitative and quantitative characters of plants. Based on the observation of qualitative characters (Table 2), there were no significant differences between accessions, except for the color characters on rice grains. Rice with black grain color includes Wojaloka, Jeliteng, IR Ngawi Hitam, and Melik. Rice with red grain color include Mentik Ngawi, Arumba, Blambangan A2, Blambangan A3, N790 Merah, Inpari 24, Inpari 32, and IR Ngawi Merah. Rice with brown grain color include Sigupai and HMS 700. Then, rice with white grain color includes Berlian, N790 Coklat, Sigunca 02, Sertani 13, Rojolele, Mr 308, Siam-siam, and Kabir 07. The difference in the color of rice grains in Indonesian pigmented rice is caused by several factors, one of which is genetic factors. Rahmawati et al. (2021) and Ham et al. (2015) explained that genetic factors can determine parental traits to produce grain suitable for rice accession.

In addition, there were morpho-agronomical characters in our study showed different results with previous studies. Based on previous information some accessions have a brown grain color (Berlian, N790 Coklat, Sigunca 02, and Sertani 13) but in this study showed all brown accessions have white grain color. This change in the color of the rice grains is caused by the milling process (Fig. 1). Therefore, these four pigmented rice accessions can be referred to as brown rice with a single dehulling process. The milling process has an important role in determining the quality of rice in terms of appearance, one of which is the color of the grain. The rice milling process has several stages, namely the first milling stage that produces the initial rice color in this study. The next grinding stage is the seed coat destruction process, the smoothing of the aleurone layer, and the last stage, namely the endosperm polishing (Ren et al. 2021). According to Mohidem et al. (2022), the color grouping of white or brown rice grains can be affected by the milling process carried out. Generally, rice grains come in various colors such as black, red, brown, and white. These differences are attributed to the aleurone pigments present in the endosperm (Kurniasih et al. 2019).

Based on the quantitative characters of Indonesian pigmented rice showed varied among accession. In general, the height of the plant has the same range except for the Sertani and HMS 700 accessions because they have the lowest and highest heights, respectively. These data indicated that rice plants with too low and high height are not in demand by farmers. Farmers prefer rice plants with plant height that tends to be low but not too low such as the Sertani 13 accession. This is because farmers think that plants that are too tall or low are prone to collapse. Stem stiffness, panicle dry weight, and plant height are factors that affect the level of rice resistance from collapse (Shinta et al. 2014). There is a correlation between the level of resistance of rice plants in dry cultivation to collapse with quantitative characteristics of plants such as internode basal length, plant height, and balance between internode basal and top of the panicle (Wu et al. 2023). Another quantitative characteristic observed in this study is the number of tillers. The increase in harvest yield is influenced by several factors, one of which is the number of tillers (Solís et al. 2015). The number of tillers in rice is one of the agronomic traits that determines the number of panicles and grain yields. The greater the number of tillers, the more pigmented rice production. In addition, in this study, the character of flag leaf length was also observed where there are rice accessions with long leaf flags but there are also accessions with short flag leaves. The short length of the flag leaf of this rice plant can be caused by several factors such as nutrient supply, growing environment, and genetics. The lowest flag leaf can be affected by a lack of nutrients and the increase of photosynthetic area, which supports the accumulation of carbohydrates in a panicle (Solís et al. 2015). Leaf size is one of the main determinants of plant architecture and the potential yield in rice to carry out photosynthesis. The size of the leaf the capacity for photosynthesis and its relationship with yield-related traits are influenced by genetic factors (Tang et al. 2018).

In this study found that the panicle length has wide range among 22 pigmented rice accessions. Panicle length and several panicles are preferred because they will produce a lot of grain, thereby increasing crop yields (Oktaviani et al. 2022). The length and width of dehulled grain can be used to analyze the content of amylose and antioxidant activity (Oktaviani et al. 2022). This study showed that Melik has the longest panicle (31.16 cm), followed by Wojaloka (24.50 cm), and Mentik Ngawi (24.25 cm). Based on this study, the Indonesian pigmented rice accessions, especially black-type, have long and high numbers of panicles). Length panicles and several panicles are preferred because they produce a lot of grain, increasing crop yields (Oktaviani et al. 2022). This indicates that black rice has a higher level of productivity than white rice. Based on our results, the length and width of rice grains varied among 22 rice accessions. Rice accessions with the longest grains are owned by accessions from the brown, brown, and white rice groups. This is also similar to the width of the rice grain. In general, the grain with the widest size is owned by rice of the brown and white accessions. Characterization was also carried out on dehulled grain where it was found that rice from the white rice accession, namely Mr. 308, had the longest dehulled grain, and Melik from the black rice accession had the widest dehulled grain size. The length and width of the grain are important characteristics because they can determine the size of the grain which will improve the quality of rice yields (Kurniasih et al. 2019). The length and width of dehulled grain can be used to analyze the content of amylose and antioxidant activity (Oktaviani et al. 2022). The trait in this reproductive character is a character that can determine the level of reproduction of rice plants. Based on the traits obtained, it can be a reference for farmers to choose the accessions of rice they plant and a reference for the community in choosing rice for consumption. Panicle is an important architectural trait for breeders and agronomists and is associated with the number of panicles to determine the final yield of rice production (Embate et al. 2021). The presence of an awn on the grain can protect grain from animals such as birds and usually the shortest length of an awn cultivar is more widely cultivated because easy to harvest (Kurniasih et al. 2019). The total number of grains per panicle ranged from 24- 74. The highest total number of grains per panicle are Rojolele and Melik. The percentage of filled and unfilled grains per panicle ranged from 11-88%. The highest percentage of filled and unfilled grains are Inpari 32 (88.89%) and Berlian (54.45%). Range of grain weight 100 (grams) from 1.75-2.35 gram. The heaviest grains are Mentik Ngawi (2.35 grams), followed by Wojaloka. The number of grains per panicle, the Total of filled and unfilled grains per panicle, and the grain weight of 100 gr determine the quality and quantity of the rice yields (Oktaviani et al. 2022).

Clustering and correlation analysis of morpho-agronomic characteristics of pigmented rice

The genetic variability of pigmented rice was analyzed using PCA based on 20 morpho-agronomic characters. This analysis aims to determine the characteristics that affect the genetic diversity of 22 pigmented rice accessions. Based on the eigenvalue and the results of the main component, it shows that the main component of PC1 has the largest percentage value, which was 20.91% (Table 5) given by the characters of tiller color, leaf color, and total tiller number with a positive contribution value (Table 6). Positive values on characters indicate that there will be clustering, while negative values will result in separation between groups.

Based on the biplot graph (Fig. 2), it shows that the distribution of 22 pigmented rice accessions showed quite wide, it can be seen in the groups of accessions in the four quadrants. This study also analyzed the relationship between characters that influence the diversity of Indonesia pigmented rice accession from East Java and Central Java, Indonesia (Fig. 3). Between total grain number with plant height and flag leaf length, panicle axis with plant height, and number of tillers with flag leaf length there was a positive correlation. In contrast, grain length has a negative correlation with panicle length. The character that determines the grouping of rice accessions is one of the pieces of information needed by farmers and the community to have rice accessions that suit their needs. The accession in quadrant one is a rice accession with superior agro morphological characteristics in the form of a wide grain size along with the number of panicles and length. This rice accession is suitable for farmers who focus on large quantities and sizes but the grains are not too long. As for rice accessions that have the opposite character, namely rice accessions with long grains are in the third quadrant where overall dominated by red and white accessions.

The results of the genetic diversity analysis of 22 accessions based on morpho-agronomic characters using cluster analysis were obtained from the dendrogram (Fig. 4). Based on dendrogram showed that the lowest Euclidean value is the accession Siam-siam, Kabir 07, Blambangan A2, and Blambangan A3. A dendrogram is a phylogenetic tree constructed based on clustering results. The purpose of phylogenetic tree construction is to determine the group of accessions and the diversity between accessions. In the dendrogram, the Euclidean value is a value used to determine the distance or extent of the relationship between accessions. Based on our results also showed that the kinship of Indonesian pigmented rice accessions was very random. Rice accessions are found in other clusters along with rice of other accessions. In this case, based on qualitative characters, it was found that there were two accessions of brown rice, namely Sigupai and HMS 700. However, in the clustering analysis, those two rice accessions were located in different clusters. Characterization based on phenotypic character is different from genetic characterization. Phenotypic is generally associated with genetic changes in an organism and can change when mutations occur. In addition, differences in results can also be caused by the interaction between genes and the environment so it has a phenotypic effect (Orgogozo et al. 2015). The interaction between genes and the environment and between genes needs to be analyzed to evaluate traits in Indonesian pigmented rice.

Conclusion

The 22 Indonesian pigmented rice from East Java and Central Java used in this study showed varied among accession based on morpho-agronomical characters. The grouping based on their morpho-agronomic characters showed that there were two main clusters. Twenty out of 22 rice accession grouping in cluster 1 with diverse pigmented rice pigment type. The cluster 1 include Melik, Mentik Ngawi, Blambangan A2, Blambangan A3, N790 Merah, N790 Coklat, Mr 308, Inpari 24, Sertani 13, Siam-siam, Kabir 7, Sigupai, Inpari 32, IR Ngawi Merah, Sigunca 02, jeliteng, Arumba, Rojolele, Berlian, and HMS 700. Meanwhile, the cluster 2 consist of IR Ngawi Hitam and Wojaloka.

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