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MORPHOLOGICAL OF GENETIC RELATIONSHIPS AMONG BLACK RICE LANDRACES FROM YOGYAKARTA AND SURROUNDING AREAS

Kristamtini[1], Taryono[2], Panjisakti Basunanda[2], Rudi Hari Murti[2], Supriyanta[2], Setyorini Widyayanti[1] and Sutarno[1]
[1]Assessment Institute for Agricultural Technology (AIAT), Yogyakarta Jl. Rajawali, Demangan Baru, Yogyakarta, Indonesia
[2]Faculty of Agriculture, University of Gadjah Mada (UGM), Yogyakarta, Indonesia
E-Mail: krisniur@yahoo.co.id

ABSTRACT

Black rice was getting more popular recently and was consumed as functional food because of the anthocyanin, vitamin and mineral contents. The information on the potential of black rice was, however, very limited, and there was a need to characterize the black rice morphologically to obtain the plant description. The description was very important to know the strengths as well as the weaknesses of an organism, and to understand the potentials, which in turn might be useful to determine whether the organism can be used as the hybridization parental. The morphological characteristics was undoubtedly influenced by environment, however, it should be noted that the morphological characteristics has been proven to be useful in creating some hybrid cultivars since 1950. By this characterization the genetic relationships among cultivars or genotypes might be obtained. The current study was aimed to know the genetic relationships among 11 black rice landraces and 2 white rice based on their morphological characteristics. Results showed that the average taxonomic distance was 1, 2 in 5 groups, with group I consisting of black rice from Sragen (T) and black rice from Bantul (O); group II consisting of black rice from Banjarnegara (Y), black rice from Wonosobo (W), hairless black rice from Magelang (S) and hairy black rice from Magelang (R); group III consisting of Pari Ireng (D), black rice from NTT (E), Cempo Ireng (C) and Jlitheng (B); group IV consisting of white rice Inpari 6 (I) and Situbagendit (G); and group V consisting of one black rice cultivar Melik (A). The greater the taxonomic distance value the farther the genetic relationships between two cultivars/genotypes and the better the cultivars used as the breeding material.

Keywords: black rice, genetic relationship, morphology, Yogyakarta.

INTRODUCTION

Black rice is one kind of rice that is getting more popular recently and is consumed as functional food due to the usefulness to health. Black rice contains relatively high anthocyanin in the pericarp layer which gives the dark purple color (Ryu *et al*., 1998; Takashi *et al*., 2001). Anthocyanin pigment is effective to reduce cholesterol in the human body (Lee *et al.*, 2008). Black rice also contains higher levels of proteins, vitamins and minerals than common white rice (Suzuki *et al.*, 2004). Compared to white rice, black rice is relatively richer in the mineral contents such as Fe, Zn, Mn and P and has higher variability in mineral content depended upon varieties and soil types of the planting area (Qiu *et al.*, 1993; Liu *et al.*, 1995; Zhang, 2000).

The origin of black rice is not well understood, but it was said that black rice might be originated from many Asian countries including China (Mingwei *et al.*, 1995; Hoahua *et al.*, 1996; Gu and Xu, 1992), India (Sastry, 1978), Japan (Natsumi and Noriko, 1994), and Vietnam (Quan, 1999). Chaudary and Tran (2001) reported that black rice might be originated from Sri Lanka, the Philippines, Bangladesh, Thailand, Myanmar and Indonesia. Black rice has diversity in terms of color due to anthocyanin content and other morphological characters. Nezu *et al.* (1960) says that the morphological characters are the simplest characteristic to identify plants. Moreover, it can be used to determine the genetic relationship between cultivars and species.

The genetic relationships may be done phenotypically based on the analysis on some phenotypic appearance of an organism. The genetic relationship between two individuals or populations may be measured based on similarity of several characteristics by assuming that the different characteristics could be caused by the difference in genetic structure (Kartikaningrum *et al*., 2003). Although it is undoubtedly that the characterization measured based on the morphological characteristics may be influenced by environment, however, it should be noted that the morphological characteristics has been proven to be useful in creating some hybrid cultivars since 1950s. Baenziger *et al*., (2009) suggested that despite discussions have been emphasized on the genomic and molecular markers that for many programs are too expensive to carry out, it would be important to emphasize the importance of the phenotype and the selection. It is clear that genomic selection emphasized on the breeding values is possible only when there exists suitable phenotypic data to support the breeding values.

However, the accurate conclusion can not be drawn without support of phenotypic marker data (Pabendon *et al*., 2011). Therefore, the selection might be carried out either conventionally or unconventionally (biotechnologically). This is consistent with Azrai (2006) who stated that the selection on breeding may be speeded

up conventionally when molecular marker technology called *marker assisted selection* (MAS) is synergized. Using MAS, the selection might be more effective and efficient because the selection would be based on the plant genetic traits, and not by environmental factors.

The current study is aimed to know the genetic relationships among 11 black rice landraces and 2 white rice based on morphological character.

MATERIALS AND METHODS

Plant materials

The plants used as materials in this research were 11 black rice landraces or local cultivars from Yogyakarta and surrounding areas and 2 white rice i.e., Situbagendit and Inpari 6 (Table-1).

Table-1. Research materials.

Name of rice	Rice color	Origin of black rice	Code
1. Melik	Black	Kedon - Ganjuran - Bantul - Yogyakarta	A
2. Jlitheng	Black	Sleman - Yogyakarta	B
3. Cempo Ireng	Black	Seyegan - Sleman - Yogyakarta	C
4. Pari Ireng	Black	Padasan - Pakembinangun - Sleman - Yogyakarta	D
5. Padi Hitam NTT	Black	Alor – NTT	E
6. Situbagendit	White	Indonesian Center for Rice Research	G
7. Inpari 6	White	Indonesian Center for Rice Research	I
8. Padi hitam Bantul	Black	Njayan - Imogiri - Bantul	O
9. Padi Hitam Magelang (hairy)	Black	Sawangan - Magelang - Central Java	R
10. Padi Hitam Magelang (hairless)	Black	Sawangan - Magelang - Central Java	S
11. Padi Hitam Sragen	Black	Sragen – Central Java	T
12. Padi Hitam Wonosobo	Black	Wonosobo - Central Java	W
13. Padi Hitam Banjarnegara	Black	Banjarnegara - Central Java	Y

All materials to be used in the research were planted in plastic jars in the screen house until bearing fruit. The characterization was done during both vegetative and generative growth according to Guidelines for Characterization System and Rice Plant Evaluation (Komnas Plasma Nutfah, 2003). The purification of black rice cultivars has to be carried out before the observation of phenotypic characteristics.

Data analysis

The phenotypic observation produced data would be used to calculate the similarity to distance matrix of two cultivars. The phenotypic data must be transformed to discrete variables (Lamadji, 1998). To reduce the influence of different measurement scales and categorization of the characteristics, a standardization procedure was employed by transforming data, in which the basic principle was by subtracting the observed value of each characteristic with the average characteristic value and then dividing the result with the standard deviation of the characteristics (Beer *et al*., 1993; Autrique *et al*., 1996; Tatineni *et al*., 1996; Rohlf, 1993). The transformed data was then analyzed using Multivariate Statistical Package (MVSP) ver 3.01 by applying the Average Distance (AVERAGE) function based on DIST/taxonomic distance average coefficient (Kovach, 2007).

$$Ad_{ij} = \sqrt{\sum \frac{n \ (x_{ik} - x_{jk})^2}{n}} \quad (1)$$

Ad_{ij} = average distance
Xik = observation value of i genotype on k observation
Xjk = obersvation value of j genotype on k observation
n = number of genotype

RESULTS AND DISCUSSIONS

The observation on the morphological characteristics was categorized and scored based on the Guidelines for Characterization System and Rice Plant Evaluation (Komnas Plasma Nutfah, 2003), and was presented in Table-2. The result of the phenotypic/morphological observations of the research materials based on the scale of discrete categories is presented in Table-3. Further, the observation matrix (Table-3) was transformed and a cluster analysis was applied. The cluster analysis was carried out based on the phenotypic characteristics that have been transformed using UPGMA method in the MVSP ver 3.01 program.

Table-2. Categorized value based on rice morphological characteristics.

No.	Differentiate character	Categorial value	Character description
1	Culm: length	1	Short (irrigation: < 110 cm, upland rice :< 90 cm)
		5	Intermediate (irrigation: 110 - 130 cm, upland rice: 90-125 cm)
		9	Long (irrigation : > 130 cm, upland rice >.125 cm)
2	Childbearing	1	Very much (> 25 anakan/tanaman)
		3	much (20-25 anakan/tanaman)
		5	intermediate (10 -19)
		7	Low (5-9)
		9	Very low (< 5)
3	Culm: number	0	Low (< 5-9)
		1	Intermediate (10-19)
		2	High (> 20)
4	Maturity	0	Short (< 90 days)
		1	Intermediate (91-120 days)
		2	Long (121-140 days)
		3	Very long (> 140 days)
5	Culm: colour	0	Light green
		1	Green
		2	Dark green
		3	Purple
6	Foot: colour	0	Light green
		1	Green
		2	Yellowish green
		3	Yellow straw
		4	White with brown spot
		5	Dark green
7	Leaf blade: colour	1	Light green
		2	Green
		3	Dark green
		4	Purple at the tip
		5	Purple at the edges
		6	Mix purple and green
		7	Purple
8	Leaf blade: length	1	Very short (< 21 cm)
		2	Short (21-40 cm)
		3	Intermediate (41-60 cm)
		4	Lon (61-80 cm)
		5	Very long (> 80 cm)
9	Leaf blade: width	1	Narrow (< 1 cm)
		2	Intermediate (1, 1-1, 5 cm)
		3	Broad > 1, 5 cm)
10	Auricle: colour	1	White (not colour)
		2	Purple lines
		3	Purple
11	Ligule colour	1	White (not colour)
		2	Purple lines
		3	Purple
12	Ligule shape	1	Acute-acuminate
		2	2-cleft
		3	Truncate
13	Leaf blade: attitude	1 2 3	Erect Semi - erect / intermediate Horizontal

		4	Descending
14	Flag leaf: attitude	1	Erect
		3	Semi - erect / intermediate (45-90o)
		5	Horizontal
		7	Descending
15	Panicle: threshability	1	Difficult (< 1 %)
		3	Rather difficult (1-5 %)
		5	Intermediate (6-25 %)
		7	Rather easy (26-50 %)
		9	Easy (51-100 %)
16	Number of grain per panicle	0	Slightly (< 150)
		1	Moderate (150-200)
17	Panicle: attidute of branches	1	Compact panicle
		3	Semi-compact panicle
		5	Intermediate
		7	Semi intermediate-open panicle
		9	Open panicle
18	Panicle: secondary branching	0	Absent
		1	Sparse (~1 secondary branch per primary branch. Most spikelets borne directly on primary branches)
		2	Dense (~2-3 secondary branches per primary branch. ~50% of spikelets borne directly on primary branches)
		3	Clustered (~3-4 secondary branches per primary branch. All spikelets on secondary branches, giving a clustered appearance
		4	Dense and clustered
19	Grain: shape	0	Round (length / width = 1)
		1	Slightly rounded (length / width =1, 1 – 2)
		2	Intermediate (length / width = 2, 1 – 3)
		3	Slim / length (length / width = >3)
20	Grain: colour	1	Yellow straw
		2	Golden with straw background
		3	Yellow straw with brown spot
		4	Yellow straw with brown line
		5	Fawn (brown yellow)
		6	Redness - mauve (light purple)
		7	Purple spot
		8	Purple line
		9	Purple
		10	Black
		11	White
		12	Yellow with purple spot
		13	Yellow purple
21	Rice: shape	1	Slim (p/l = .3)
		3	Intermediate (p/l = 2, 1 -3, 0)
		5	Oval (p/l =1, 1 - 2,0)
		9	Round (p/l = < 1, 1)
22	Rice: length	1	Very long (> 7, 5 mm)
		3	Long (6, 61 - 7, 5mm)
		5	Intermediate (5, 51 - 6, 6 mm)
		7	Short (< 5, 5 mm)
23	Pericarp: colour	1	Black
		2	Black brown
		3	Brown black
		4	Clear white infinity

24	Aleuron: colour	1	Black
		2	Clear white infinity
		3	White clearly bounded
		4	White

Table 3. Matrix of morphological data.

Character \ Rice cultivar	A	B	C	D	E	G	I	O	R	S	T	Y	W
1. Culm: length	5	9	9	5	5	1	1	9	9	9	5	9	9
2. Childbearing	3	5	5	3	7	3	3	9	9	7	7	3	3
3. Culm: number	1	1	1	1	0	1	1	0	0	0	1	0	0
4. Maturity	2	2	2	2	2	1	1	2	3	3	3	3	3
5. Culm: colour	1	1	2	2	3	1	1	0	2	0	2	2	2
6. Foot colour	0	1	2	1	2	1	1	5	2	3	3	4	0
7. Leaf blade: colour	3	2	2	1	3	3	2	1	3	3	2	3	3
8. Leaf blade: length	3	3	4	3	3	2	2	2	4	4	2	5	5
9. Leaf blade: width bendera	2	3	3	2	3	2	2	3	3	3	3	3	3
10. Auricle: colour	2	2	2	2	2	1	1	1	2	2	1	2	2
11. Ligule colour	1	2	2	1	2	1	1	1	2	2	1	2	2
12. Ligule shape	1	1	2	2	1	2	2	2	2	2	2	2	2
13. Leaf blade: attitude	1	2	2	2	2	1	1	4	3	3	3	2	2
14. Flag leaf: attitude	1	3	3	3	3	1	1	7	5	3	5	3	3
15. Panicle: threshability	5	5	5	5	5	9	9	5	1	1	5	1	1
16. Number of grain per panicle	0	1	1	0	1	0	0	1	1	1	1	1	1
17. Panicle: attidute of branches	5	5	3	7	7	5	5	3	3	3	3	7	7
18. Panicle: secondary branching	1	1	1	1	1	1	1	3	3	4	3	2	2
19. Grain: shape	2	3	3	3	3	3	3	3	3	3	3	3	3
20. Grain: colour	3	8	3	7	7	1	1	12	12	13	9	4	13
21. Rice: shape	3	3	3	3	3	3	3	5	3	3	3	1	1
22. Rice: length	5	5	3	5	1	3	1	5	5	5	3	3	3
23. Pericarp: colour	2	1	1	1	1	4	4	3	2	2	2	3	2
24. Aleuron: colour	4	1	4	4	3	4	4	3	3	3	3	2	2

The taxonomic distances among the black rice cultivars/genotypes based on the morphological characteristics calculated using formulation (1) resulted in the varying values and these were presented in the matrix of taxonomic distance (Table-4). The greater of the distance value, the more distant of the relationship among the cultivars/genotypes. The closeness of the relationship among the genotypes based on 24 morphological data was presented in the dendogram form (Figure-1). The observation of rice colour (pericarp and aleuron) was presented in Figure-2.

Dendogram showed that the average taxonomic distance was 1, 2 in 5 groups, i.e., group I consisting of black rice from Sragen (T) and black rice from Bantul (O); group II consisting of black rice from Banjarnegara (Y), black rice from Wonosobo (W), hairless black rice from Magelang (S) and hairy black rice from (R); group III consisting of Pari Ireng (D), black rice from NTT (E), Cempo Ireng (C) and Jlitheng (B); group IV consisting of white rice VUB Inpari 6 (I) and Situbagendit (G); and group V consisting of one black rice cultivar Melik (A).

The implication of the genetic relationships based on the morphological characteristics was the greater of the taxonomic distance among the cultivars so the more distant genetic relationships, and then the cultivars with distant genetic relationship was suitable for breeding activities. Based on the calculation of taxonomic distance (Table-4), for the purpose of cultivar improvement of black rice, it would be better to breed black rice with white rice (such as, Situbagendit or Inpari 6) which have greater taxonomic distance (for example, between O or T with G or I, or between O and A).

The information on the taxonomic distance showed that 11 black rice landraces and white rice i.e., Situbagendit and Inpari 6, formed 2 main groups with average taxonomic distance of between 1.5 to 1.8 (average of 1.65), they are black rice group and white rice group, however Table-4 also showed that one black rice

landraces, i.e., Melik (A), is very closely related to white rice group, i.e., Situbagendit (G) and Inpari 6 (I), and that A (black rice Melik) has the farthest relationship with Bantul black rice (O), i.e., 2.085, despite the fact that both A and O are black rice. This means that there might be other characteristics that need further attention, for example, the anatomy, the biochemical content such as anthocyanin and minerals like Fe, and Zn. It was assumed that there was correlation between phenotypic/morphological properties and certain biochemical contents, such as black/purple color on rice is positively correlated with anthocyanin content, as has been reported by Yawadio *et al.* (2007), where he suggested that colored rice is potential source of anthocyanin and may be consumed as functional food source. Black rice contains relatively high anthocyanin in the pericarp layer that gives the dark purple color (Ryu *et al*., 1998; Takashi *et al*., 2001). The characterization based on morphological markers was usually influenced by micro-environment as well as macro-environment, and the plant age. Therefore, the morphological characterization needs molecular marker characterization support. According to Dwiatmini *et al*. (2003), molecular markers can provide illustration about the more accurate genetic relationship, because analysis of DNA as genetic material is not influenced by environmental conditions.

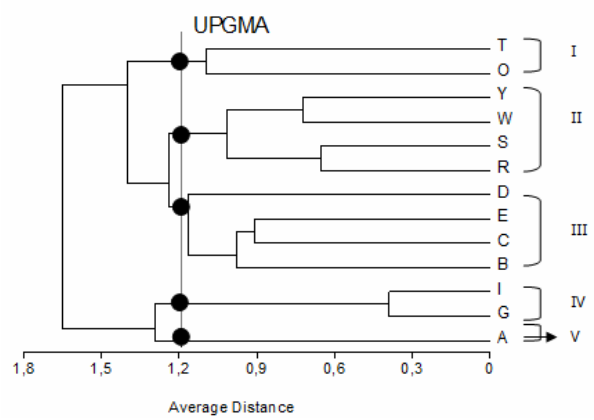

Figure-2. Rice colour observation (pericarp and aleuron).

Legend
A. Melik (A); **B.** Jlitheng; **C.** Cempo Ireng; **D.** Pari Ireng; **E.** Padi Hitam NTT; **G.** Padi Putih Situbagendit; **I.** Padi putih Inpari6; **O.** Padi Hitam Bantul; **R.** Padi Hitam Magelang (hairy); **S.** Padi Hitam Magelang (hairless); **T.** Padi Hitam Sragen; **W.** Padi Hitam Wonososbo; and **Y.** Padi Hitam Banjarnegara

Table-4. The matrix of taxonomic distance among the cultivars/genotypes of black rice and white rice matrix of distance.

	A	B	C	D	E	G	I	O	R	S	T	W	Y
A	0, 000												
B	1, 360	0, 000											
C	1, 385	0, 909	0, 000										
D	1, 305	1, 007	1, 108	0, 000									
E	1, 508	1, 048	0, 906	1, 376	0, 000								
G	1, 234	1, 595	1, 414	1, 338	1, 571	0, 000							
I	1,352	1, 644	1, 415	1, 342	1, 570	0, 388	0, 000						
O	2, 085	1, 572	1, 590	1, 685	1, 762	1, 928	1, 931	0, 000					
R	1, 737	1, 172	1, 062	1, 444	1, 130	1, 878	1, 957	1, 321	0, 000				
S	1, 736	1, 221	1, 226	1, 556	1, 405	1, 869	1, 949	1, 272	0, 652	0, 000			
T	1, 647	1, 217	1, 059	1, 225	1, 186	1, 466	1, 467	1, 096	1, 021	1, 175	0, 000		
W	1, 692	1, 255	1, 038	1, 438	1, 181	1, 730	1, 773	1, 782	1, 006	1, 082	1, 296	0, 000	
Y	1, 652	1, 151	1, 086	1, 362	1, 157	1, 801	1, 843	1, 882	0, 915	1, 054	1, 334	0, 724	0, 000
	A	B	C	D	E	G	I	O	R	S	T	W	Y

Legend
A. Melik (A); **B.** Jlitheng; **C.** Cempo Ireng; **D.** Pari Ireng; **E.** Padi Hitam NTT; **G.** Padi Putih Situbagendit; **I.** Padi putih Inpari6; **R.** Padi Hitam Magelang (hairy); **O.** Padi Hitam Bantul; **S.** Padi Hitam Magelang (hairless); **T.** Padi Hitam Sragen; **W.** Padi Hitam Wonososbo; **Y.** Padi Hitam Banjarnegara

CONCLUSIONS

Based on the characterization of the morphological properties on 11 black rice landraces and 2 white rice, it could be seen that the average taxonomic distance of 1.2 was obtained in 5 groups, i.e., group I consisting of black rice from Sragen (T) and black rice from Bantul (O); group II consisting of black rice from Banjarnegara (Y), black rice from Wonosobo (W), hairless black rice from Magelang (S) and hairy black rice from Magelang (R); group III consisting of Pari Ireng (D), black rice from NTT (E), Cempo Ireng (C) and Jlitheng (B); group IV consisting of white rice VUB Inpari 6 (I) and Situbagendit (G); and group V consisting of one black rice cultivar Melik (A). The greater of the taxonomic distance among the cultivars/genotypes so the more distant genetic relationships and the better was used as the breeding material.

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