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Genetic Diversity and Association amongst West Sumatra Brown Rice Genotype Based on Morphological and Molecular Markers

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***Abstract*— The genetic diversity of crops in West Sumatra Province is very high. One of the potentials of plant genetic resources that are well conserved is brown rice. However, the development of new rice varieties has threatened the existence of brown rice. Therefore, conservation efforts are needed to preserve West Sumatra brown-rice-landrace through several stages. The first step is exploration and collection of brown rice genotypes that are still cultivated in West Sumatra Province. The next step is to identify and characterize the brown rice to determine the character of each genotype. The purpose of this study was to explore and characterize brown rice landrace in West Sumatra. The exploration resulted in 31 brown rice genotypes. Variation in quantitative and qualitative traits was observed according to grain morphology. Analysis of relatedness base on morphological grain markers resulted in three groups of brown rice with the degree of similarity of 0.70 and with slight differences in each group. Furthermore, DNA markers categorized the relatedness of brown rice landrace into six groups with 0.70 similarities.**

***Keywords*— diversity; brown rice; genotype; morphological; DNA; marker.**

I. INTRODUCTION

West Sumatra Province is one of the Indonesian rice production centers. Unlike the majority of Indonesian people who prefer sticky-textured rice, people of West Sumatra prefer to consume in sticky-textured rice. These conditions resulted in the diversity of rice in West Sumatra differ to other provinces. Research on exploration and identification based on the character of the DNA found 50 genotypes of local rice in West Sumatra [1] and 19 genotypes of local brown rice [2]. However, exploration of local brown rice in West Sumatra needs to be continued.

Brown rice is nutritious and healthy. It contains carbohydrates (85%), fiber (7%), B vitamins (thiamin, riboflavin, vitamin B-6, folate, and niacin), magnesium, phosphorus, calcium, and potassium [3]. Anthocyanin content in brown rice is believed to prevent a variety of diseases including cancer, cholesterol, and coronary heart disease [4].

Local rice (landrace) has the flavor and aroma favored by the public. Local rice is a genetically valuable asset, and therefore must be appropriately managed [5]. Local rice has certain advantages because it has been cultivated by generations that have adapted well to various climatic conditions and specific land [6], [7]. In contrast, local rice also has some limitations, among others, long-lived, tall, not resistant to pests and diseases, as well as lower production [8]. This leads to the existence of local rice is currently being abandoned and endangered [9]. A systematic effort is needed to preserve and improve local rice to become more superior varieties [10].

Conservation of plant genetic resources consists of several stages, namely: (1) identification, (2) exploration, (3) a collection development, (4) characterization, evaluation, documentation, (5) propagation, and (6) the development of networks for utilization of genetic resources. After the exploration and collection, the next steps is characterization. Characters that can be observed are morphological characters, agronomic characters, physiological characters, markers isoenzymes and molecular markers [11]. Exploration and characterization are the earliest events that need to be done to minimize the possibility of extinction of local rice [12]. The final stage of the conservation of genetic resources is the establishment of germplasm collections aiming to guarantee the availability of collections that can be used as material improvement in future research programs. This study aimed to explore and characterize local brown rice in West Sumatra based on morphological characters, grain characters, as well as molecular characters (DNA).

II. MATERIAL AND METHODS

This experiment consisted of exploration activities in some districts in West Sumatra Province. Results of the exploration activities some brown rice genotypes obtained will be used as the material for the grain characterization activities. Furthermore, the grain was sown for leaf DNA analysis. The experiment was conducted from March to September 2016.

Exploration activities using the survey method. Exploration location determined by purposive sampling method. Locations were selected based on the information from the Department of Agriculture of West Sumatra Province, local agriculture office, community leaders, farmers, and local village leaders. Samples of brown grain rice were taken directly in farmers' fields or taken as grains that have been harvested.

Observation of brown rice grain is done for both quantitative and qualitative characters. Quantitative observations consist of long-grain, grain width, grain thickness, and length of awn grain is measured using a digital caliper and weight of 100 grains was measured with an analytical balance. Whereas qualitative observations consist of the color of grain surface, grain color, and rice shape. All quantitative data is determined by measuring the entire rice grain characters by the descriptors issued by IRRI and WARDA [13]. The quantitative data obtained were then processed with Minitab version 16:14 [14].

The diversity of germplasm is determined based on morphological data can also be determined through the analysis of RAPD (Random Amplified DNA Polymorphism). The goal is to determine the kinship between local brown rice genotypes studied. RAPD molecular marker was carried out in three stages, namely: 1) DNA extraction optimization, 2) optimization of primer and 3) RAPD analysis. DNA extraction and isolation of the rice was done following the CTAB method's Modification buffer [15]. Electrophoresis of the extracted DNAs was performed in 1.5% agarose with TAE 0.5 x. Electrophoresis was performed at a voltage of 90 V for 30 minutes using Mupid 2Plus. The gel was submerged in *ethidium bromide* (0.5 mg/l) for 20 seconds and distilled water for 15 minutes. The photographs were taken in a UV transilluminator. Amplification process was done using RAPD with 10 primers: OPE7 (5'AGATGCAGCC'3), OPE8 (5'TCACCACGGT'3), OPE15 (5'ACGCACAA CC'3), OPE20 (5'AAC GGTGACC'3), OPH1 (5'GGTCGG AGAA'3), OPH7 (5'CT GCATCGTG'3), OPH8 (5'GA AA CACCCC'3), OPM1 (5'G TTGGTGGCT'3), OPM2 (5'AC AACGCCTC'3), and OPM8 (5'TCTGTTCCCC '3). PCR was performed in a 10 µL reaction mixture containing 2 µL Buffer A, 0.2 µL MgCl2, 0.2 µL dNTP, 0.04 µL KAPA 2G, 2.56 µL sterile double distilled water, 2.5 µL of the working solution of DNA and 2.5 µL primer. Thermal cycling condition was as follows: pre-denaturation at 94^0C for 5 minutes, followed 45 cycles each of denaturation at 94^0C for 5 seconds, annealing at 32.2^0C for 30 seconds, elongation at 72^0C for 1 minute, and final elongation at 72^0C for 10 minutes.

III. RESULT AND DISCUSSION

A. Exploration of Brown Rice

The exploration of brown rice in several districts in West Sumatra Province resulted in 31 local brown rice genotypes. Brown rice genotypes were obtained from seven districts, i.e. Agam: 4 genotypes, Dharmasraya: 2 genotypes, Lima Puluh Kota: 1 genotypes, Pasaman: 7, West Pasaman: 4 genotypes, Solok: 10 genotypes, and South Solok: 3 genotypes (Table 1). More genotypes were found in Solok District than in other districts. Brown rice in Solok District, especially in the area around the Subdistrict Lembah Gumanti is still maintained for generations as it relates to the local culture.

TABLE I
GENOTYPE BROWN RICE RESULTS OF EXPLORATION IN THE PROVINCE OF WEST SUMATRA

No.	Local Name	Genotype Code	Originally District
1	Surian	BM 001	Solok
2	Travel	BM 002	Agam
3	Talang Babungo	BM 003	Solok
4	Sungai Abu	BM 004	Solok
5	Sariak Alam Tigo	BH 005	Solok
6	Solok	BH 006	Solok
7	Gunung Pasir	BM 007	Solok Selatan
8	Siarang	BM 008	Solok Selatan
9	Perbatasan	BM 009	Solok Selatan
10	Balingka	BM 010	Agam
11	Teluk Embun	BM 011	Pasaman
12	Jorong Mudiak	BM 012	Pasaman
13	Pido Manggih	BM 013	Pasaman Barat
14	Sikarujuik	BM 014	Pasaman Barat
15	Capacino	BM 015	Pasaman Barat
16	Situjuh	BM 016	Lima Puluh Kota
17	Simarosok	BM 017	Agam
18	Banuhampu	BM 018	Agam
19	Sigah	BM 019	Pasaman Barat
20	Silomlom Pulen	BM 020	Pasaman
21	Silomlom Pera	BM 021	Pasaman
22	Kotitiran	BM 022	Pasaman
23	Batu Kangkung	BM 023	Dharmasraya
24	Sitiung II	BM 024	Dharmasraya
25	Air Dingin 1	BM 025	Solok
26	Air Dingin 2	BH 026	Solok
27	Sibandung	BM 027	Pasaman
28	Ladang Dua Koto	BM 028	Pasaman
29	Bareh Hitam Talamau	BH 029	Solok
30	Bareh Merah Talamau 1	BM 030	Solok
31	Bareh Merah Talamau 2	BM 031	Solok

B. Characteristics of Grain Morphology Quantitative Characters

The observation of quantitative characters of brown rice grain can be seen in Table 2. In general, no differences in the characteristics of each brown rice genotypes. The observation of the quantitative variables showed that grain length ranged from 6.59 to 9.19 mm. Grain width ranging from 2.07 to 2.86 mm. The ratio between grain length and grain width ranging from 2.66 to 4.13 mm. Grain thickness ranged from 1.60 to 1.95 mm. Awn length ranged from 0.00 to 15.00 mm. The weight of 100-grain ranged from 3.94 to 5.12 gram.

Based on these observations, brown rice genotypes that have the most extended long grain is Travel (9.19 mm), while the shortest is Sikarujuik genotype (6.59 mm). IRRI and WARDA divide rice grain length into three classes: short (<7.5 mm), medium (7.5 to 12 mm) and long (> 12 mm). Based on the classification IRRI and WARDA, we found only one brown rice genotype that has a short length of grain and the rest had a medium-length grain [13].

TABLE II
QUANTITATIVE OBSERVATIONS OF WEST SUMATRA BROWN RICE

Genotype Code	Grain Length (mm)	Grain Width (mm)	Ratio Length-Width Grain	Grain thickness (mm)	Weight of 100 Grains (g)	Length of Awn
BM 001	8.12	2.38	3.41	1.95	4.64	0
BM 002	9.19	2.49	3.69	1.91	5.12	8
BM 003	8.47	2.48	3.42	1.83	4.79	8
BM 004	8.68	2.58	3.36	1.85	4.87	5
BH 005	8.73	2.44	3.58	1.72	4.91	0
BH 006	8.30	2.68	3.09	1.78	4.69	15
BM 007	7.61	2.86	2.66	1.82	4.38	0
BM 008	8.19	2.07	3.96	1.60	4.74	0
BM 009	8.13	2.33	3.48	1.86	4.65	0
BM 010	8.75	2.53	3.46	1.77	4.91	0
BM 011	8.09	2.59	3.12	1.76	4.60	5
BM 012	7.72	2.17	3.55	1.71	4.48	0
BM 013	8.07	2.17	3.71	1.80	4.65	0
BM 014	6.59	2.11	3.13	1.61	3.94	0
BM 015	8.33	2.36	3.53	1.84	4.74	0
BM 016	8.33	2.25	3.70	1.77	4.76	5
BM 017	8.92	2.16	4.13	1.74	5.07	0
BM 018	7.75	2.26	3.44	1.60	4.48	0
BM 019	8.98	2.25	3.99	1.74	5.07	0
BM 020	8.18	2.27	3.60	1.76	4.68	0
BM 021	8.41	2.25	3.73	1.79	4.80	0
BM 022	8.45	2.41	3.50	1.81	4.79	0
BM 023	7.79	2.72	2.86	1.86	4.46	0
BM 024	8.08	2.24	3.60	1.60	4.64	5
BM 025	8.93	2.48	3.61	1.84	5.01	5
BH 026	8.63	2.42	3.56	1.64	4.87	8
BM 027	8.58	2.21	3.88	1.70	4.89	0
BM 028	8.68	2.25	3.86	1.72	4.93	0
BH 029	9.08	2.42	3.75	1.75	4.83	15
BM 030	8.19	2.32	3.53	1.66	4.57	0
BM 031	8.41	2.26	3.73	1.65	4.94	15
Varian	0,27	0,04	0,10	0,01	0,06	0,24
Variability	Narrow	Narrow	Narrow	Narrow	Narrow	Wide

Length of awn: Awnless (0), very short (5), short (8), moderate (15).

Genotype which has the largest grain is Pasir Gunung (2.86 mm), while the shortest is the Perbatasan (2:07 mm). According to IRRI and WARDA, the width of the rice grain is classified into three criteria: narrow (<1 mm), medium (1-3 mm) and width (> 3 mm) [13]. Based on these criteria, the width of the whole grain brown rice genotypes from West Sumatra classified as medium-width.

The ratio between grain length and width can be used to determine the shape of grain. Exploration results obtained from the ratio between grain length and grain width ranged from 2.66 to 4.13. According to IRRI and WARDA, the rice grain shape can be grouped into three classes: round (≤2), moderate (2-3), and lean (> 3) [13]. Based on these criteria, obtained two brown rice genotypes classified as moderate and the rest belong to the classification of lean.

The observation of the awn length of the grain ranged from 5.00 to 15.00 mm. Awn length of the grain is classified into 4 categories: short (1-4 mm), medium (4-20 mm), long (40-60 mm), and awnless [16]. Thus, all brown rice genotypes studied have a moderate length of awn.

The variance of each observed variables can be seen in Table 2. The Table demonstrates that most of the quantitative variables observed, namely long grain, grain width, length and width ratio of grain, grain thickness, and weight of 100 seeds have narrow variability. There was only one variable that has a wide variability, which is the length of awn.

C. *Qualitative characters*

The results of qualitative observations on brown grain rice showed variations between each genotype brown rice. Grain and caryopsis of brown rice have a surface color variation and shape (Table 3 and Figure 1). The surface color of brown rice grain of 31 genotypes was as follow: straw yellow (9.68%), brown (25.81%), purple (54.84%), and black (9.68%). According to IRRI and WARDA, the color of the surface of the grain is quite diverse, which is brownish yellow, white, brown, orange-brown, light brown, red-brown and greenish brown [13].

TABLE III
QUALITATIVE OBSERVATIONS OF WEST SUMATRA BROWN RICE

Genotype Code	Lema and Palea Color	Caryopsis Color	Caryopsis Shapes
BM 001	54	2	2
BM 002	80	2	3
BM 003	80	2	3
BM 004	80	2	3
BH 005	100	3	2
BH 006	100	4	1
BM 007	20	2	3
BM 008	80	2	3
BM 009	54	2	3
BM 010	52	2	3
BM 011	80	2	3
BM 012	80	2	3
BM 013	80	2	3
BM 014	80	2	2
BM 015	54	2	3
BM 016	80	2	3
BM 017	80	2	3
BM 018	53	2	2
BM 019	80	1	3
BM 020	80	1	3
BM 021	20	1	3
BM 022	80	1	1
BM 023	20	1	2
BM 024	80	2	2
BM 025	80	1	3
BH 026	100	4	3
BM 027	80	1	3
BM 028	80	2	3
BH 029	54	4	3
BM 030	53	1	3
BM 031	54	1	3
Varian	468.880	0.729	0.359
Variability	Wide	Narrow	Narrow

Brown rice also has a seed coat (caryopsis) color variation. Most of the brown rice has a deep red color (58.06%), while the others light brown (29.03%), brown (3.23%), and black (9.68%). The difference in the color of rice is set genetically through regulation of aleurone and *endospermia* color, and starch composition in the *endospermia* [17].

Morphology of rice also showed variation within brown rice genotypes observed, namely round, semi-round, and oval. Most of the morphology of brown rice found is oval

(74.19%), followed by semi-spherical shape (19.35%), and the fewest is round shape (6.45%). Narrow variability was found based on the variety of qualitative variables observed (the color of the rice, as well as the form of rice). There is only one variable that has a wide variability, which is the color of lemma and palea.

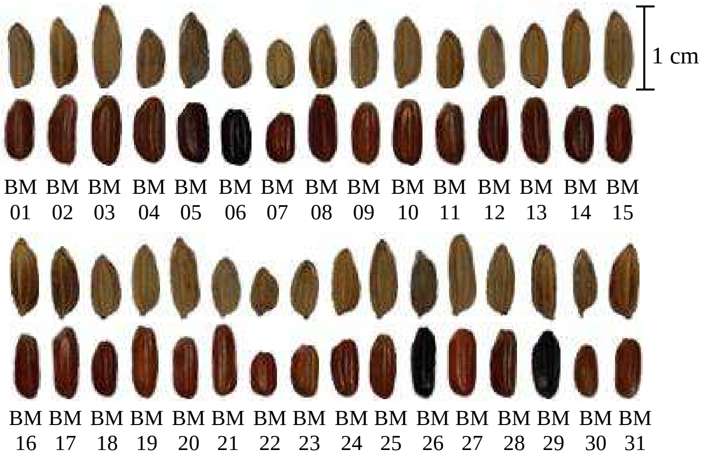


Fig. 1 Grain and caryopsis brown rice from West Sumatra

D. Cluster analysis based on grain morphology

Results of the analysis of relatedness according to grain morphological markers are presented in Figure 2. Based on the results of grouping clusters of brown rice genotypes at a rate of 70.00% similarity, brown rice genotypes were grouped into three groups. The first group consisted of three genotypes, ie 6, 29, and 31. The second group consists of eight genotypes, ie 2, 3, 4, 11, 16, 24, 25, and 26. The third group consisted of 20 genotypes, ie 1, 5, 7, 8, 9, 10, 12, 20, 13, 14, 15, 17, 18, 19, 21, 22, 23, 27, 28, and 30. High level of variability in morphological characters leads to constraints the limits for taxonomical purposes.

Figure 2 shows the degree of kinship of each brown rice genotype in the Province of West Sumatra. The size of the resemblance percentage is influenced by wide or narrow diversity (variability). The degree of variability of morphological characters will lead to difficulties in limiting taxon below the species [18]. Information on the level of kinship is needed to facilitate breeders to produce new varieties through crossbreeding. Close kinship level is used to produce varieties with the narrow level of diversity, whereas wide degree of kinship is used to produce a wide degree of diversity. The further the distance in relationships the more diverse recombinant produced is. To determine how far the kinship between plant taxa can be done by determining the similarity between taxa of plants using morphological traits because morphological traits can be used to identify and describe kinship species level [18]. The character of each cultivar may be used and developed in plant breeding activities to improve plant varieties.

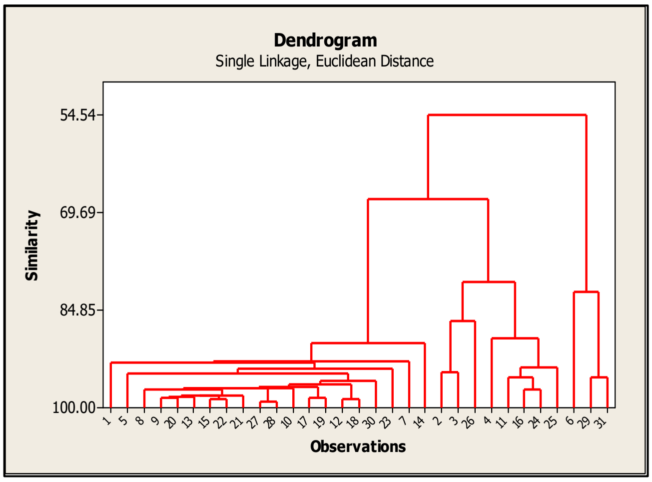


Fig. 2 Dendrogram of brown rice based on morphological grain marker

E. Correlation analysis

The ratio of length and width of seed grains is significantly correlated with the length of grain (0.59 **) and the width of grain (0.74 **). 100-seed weight significantly correlated with grain length (0.96 **) and a ratio of length: width grain (0.66 **). The thickness of the seed correlated with grain width (0.54 **). Width caryopsis correlated with grain width (0.80 **), the ratio of length: width grain (0.57 **), and the thickness of the seed (0.43 *). Caryopsis length correlated to the length of grain (0.41 *), grain width (0.39 *), ratio of length: width grain (0.61 **), and with a weight of 100 grains (0.46 **). Thick caryopsis correlated with grain length (0.43 *), grain width (0.54 **), weight of 100 grains (0.36 *), the thickness of the seed (0.50 **), and the width of caryopsis (0.52 **) (Table 4). Correlation indicates a relationship between one character to another, causing a mutual relationship between a character or characters one affect the other characters. Correlation of one character with another character on the plant shows the relationship between the characters [14].

F. Characterization Based Molecular Markers (RAPD)

Ten primers were used to amplify DNA sequences in 31 brown rice genotypes using RAPD technique. The Primer use is a primary that has proven to provide the level of total DNA polymorphism and the high of the previous research on paddy plants. Results of DNA analysis of the 31 brown rice genotypes were amplified using 10 primers (OPE7, OPE8, OPE15, OPE20, OPH1, OPH7, OPH8, OPM1, OPM2, and OPM8) can be seen in Figure 3.

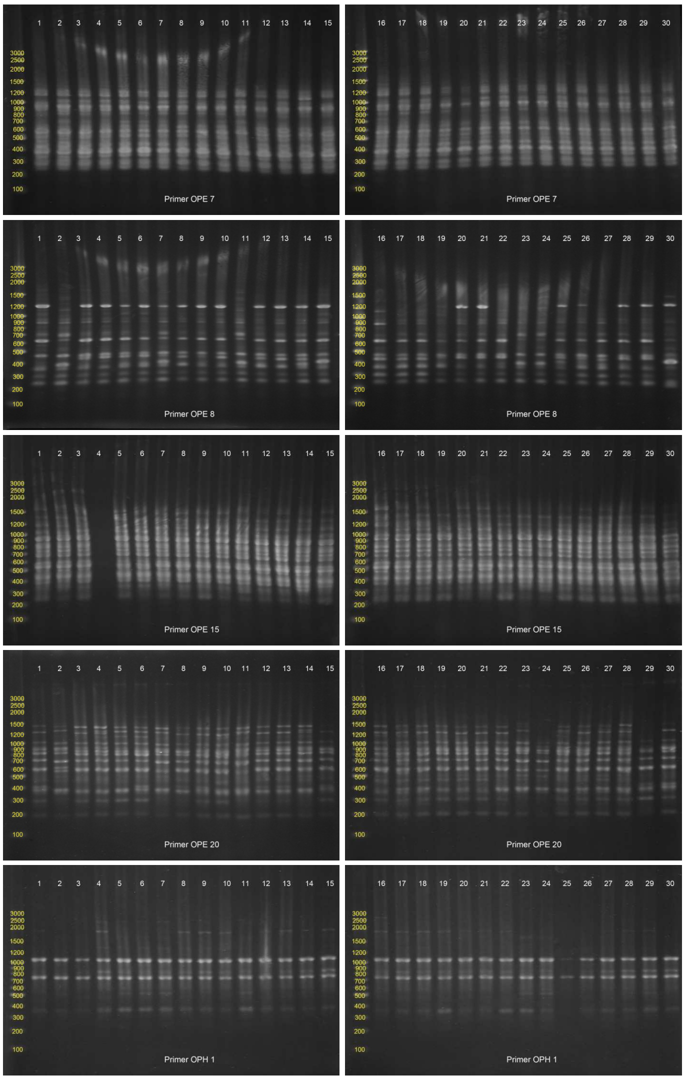
Figure 4 demonstrates that West Sumatra brown rice is divided into six major groups based on similarity coefficient of 0.70. Cultivars that have much in common would cluster into one large group. Sometimes in a large group of cultivars

TABLE IV
CORRELATION ANALYSIS BASED ON MORPHOLOGICAL OF THE GRAIN

Quantitative Characters	Grain Length (mm)		Grain Width (mm)		Ratio Length-Width Grain		Weight of 100 Grains (g)		Awn Length (mm)		Grain thickness (mm)		Caryopsis Width (mm)		Caryopsis Length (mm)	
Grain Width (mm)	0.10	ns														
Ratio Length-Width Grain	0.59	**	(0.74)	**												
Weight of 100 Grains (g)	0.96	**	(0.01)	ns	0.66	**										
Awn Length (mm)	0.32	ns	0.28	ns	(0.03)	ns	0.26	ns								
Grain thickness (mm)	0.28	ns	0.54	**	(0.26)	ns	0.22	ns	(0.01)	ns						
Caryopsis Width (mm	0.13	ns	0.80	**	(0.57)	**	0.02	ns	0.15	ns	0.43	*				
Caryopsis Length (mm)	0.41	*	(0.39)	*	0.61	**	0.46	**	(0.15)	ns	(0.24)	ns	0.12	ns		
Caryopsis Thickness (mm)	0.43	*	0.54	**	(0.16)	ns	0.36	*	0.26	ns	0.50	**	0.52	**	(0.01)	ns

*** = highly significant; * = significant; ns = non significant*

is subdivided based on the similarity coefficient is more than 0.70. The greater the coefficient values of similarity the closer the relatedness of the cultivars. Likewise, if the values are getting close to zero then the similarity coefficient of kinship among cultivars are far away, and this means that the diversity among cultivars was big enough.



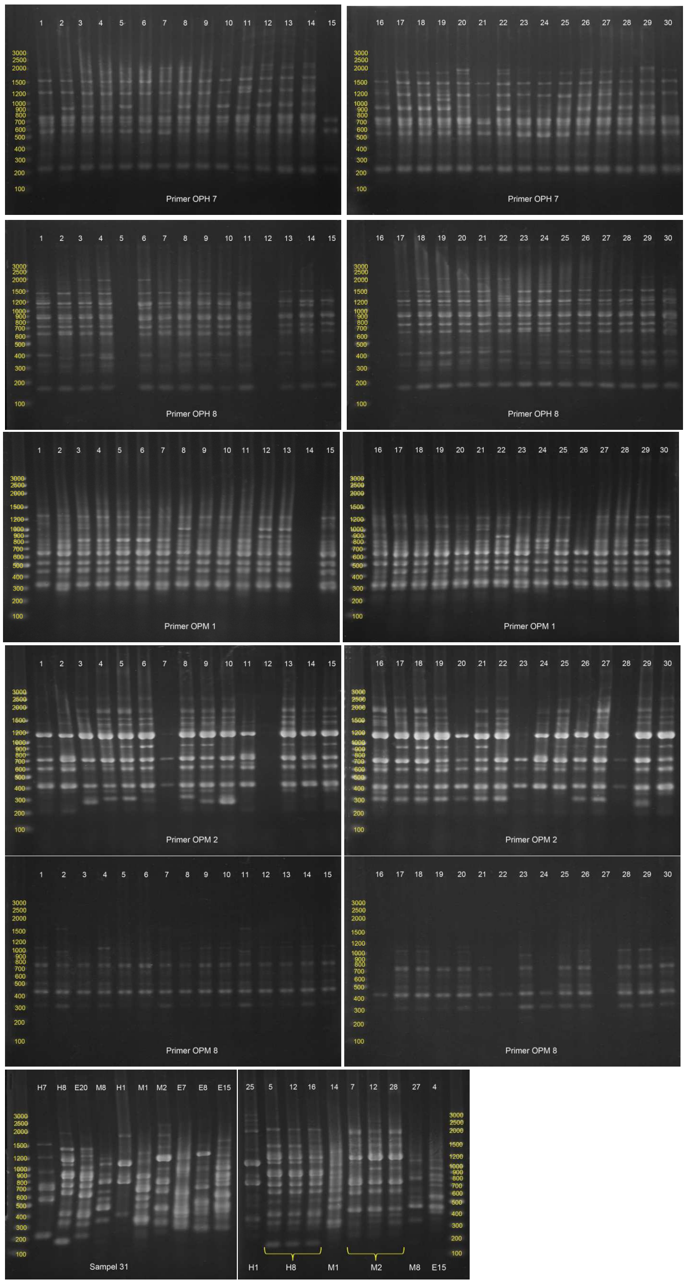


Fig. 3 DNA analysis base on RAPD with 10 primers (OPE7, OPE8, OPE15, OPE20, OPH1, OPH7, OPH8, OPM1, OPM2, and OPM8)

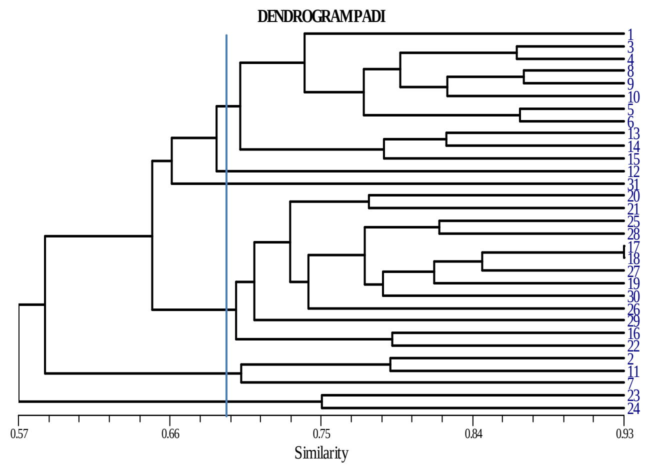


Fig. 4 Dendrogram of brown rice based on the molecular character with ten primers (OPE7, OPE8, OPE15, OPE20, OPH1, OPH7, OPH8, OPM1, OPM2, and OPM8)

The most significant similarity coefficient values indicated by genotypes BM 17 and BM 18, namely Simarosok and Banuhampu. The two genotypes derived from Agam with a similarity coefficient of about 0.93, which means both have a very close kinship. This might occur when the parents are of similar origin, but both cultivars were grown in different regions. Brown rice paddy originated from Dharmasraya District (2 genotypes) differs with brown rice from other districts. Both genotypes are joined in one group with a value of similarity coefficient is low compared to other genotypes. Both genotypes are from the areas that are geographically far apart from each other. Some genotypes of brown rice do not spread to other areas due to the agronomic traits or socio-economic aspects when people do not like the taste of particular rice which resulted in no crosses within genotypes originating from other regions.

Figure 4 shows that genotypes derived from the same district do not necessarily have a close similarity. This relates to the origin of a genotype, instead. If the same genotypes in a region are exposed to different climatic and geographic condition, the genetic traits will interact with the environmental factors. To live, each genotype has different survival mechanisms in conditions different from its original ones. The mechanism makes one or more alleles evolved which ultimately lead to new traits or different origin.

IV. Conclusions

The exploration of brown rice resulted in thirty-one brown rice genotypes in West Sumatra. One genotype found at Lima Puluh Kita District, two genotypes found at Dharmasraya. Three genotypes found in South Solok District. Four genotypes found at District Agam and four other genotypes found at West Pasaman District. Seven genotypes found at Pasaman District and ten genotypes found at Solok District. Variations in the quantitative and qualitative trait were found according to grain morphology. Analysis of relatedness base on morphological grain markers resulted in clustering brown rice of West Sumatra into three groups with a degree of similarity of 0.70% with slight differences in each group. Furthermore, relatedness according to DNA markers similarity was found in six groups with 0.70 similarity

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