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RESEARCH ARTICLE

Genetic Diversity Analysis of Indonesian Aromatic Rice Varieties (*Oryza sativa* L.) Using RAPD

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Abstract

In this research, we investigated genetic diversity of 21 rice genotypes (*Oryza sativa* L.) assessed using 38 decamer RAPD primers. A total of 405 bands were produced from 38 primers, which revealed that 84.44% was polymorphic and 15.56% was monomorphic. From the first cluster, Batang Gadis separated from 14 other genotypes. The second cluster consisted of six genotypes which all had awn in the seed except Gogo Fatuk Masin. The high number of amplified polymorphism bands showed that the markers can be used to distinguish the rice genotype well. RAPD markers can show differences in individual fingerprint patterns, since genetic variation is important for the maintenance and development of the organism's potential. The information about the genetic diversity in this study was useful for plant breeders in the selection of elders and processing of plant cultivation.

Key words : Aromatic rice, genetic diversity, polymorphism, RAPD

Introduction

Rice is the most important staple food for about 62.8% of the world's population and fulfills 20% of the caloric needs per day (Giraud 2013). In Asia, 29.3% of the population consumes rice (Timmer 2010). Increasing consumer health awareness caused by the availability of health information (Kearney 2010), trade between countries, urbanization, influence of public figures, and other factors led to change the consumption pattern (McCluskey 2015) and increase in the demands of quality and diverse products, one of which is rice. Quality improvement of consumption has an impact on the demands of quality and diverse products of rice. One of the qualities of rice that is considered by consumers is the fragrance component. The aromatic rice is an important commercial commodity. The demand of aromatic rice is higher by consumers worldwide because of its aroma and delicious taste (Patwardhan et al. 2014). The need for aromatic rice is estimated to reach 15-18% of the rice trade that produces the highest price in the world market (Giraud 2013). Indonesia is one of the countries rich in rice germplasm, including aromatic rice. However, the limited genetic diversity information of aromatic rice is an important issue of cultivation, especially for rice breeders (Ampapathi et al. 2008). Therefore, the collection, storage, classification, and characterization activities in detail are essential programs (Patwardhan et al. 2014) for further exploitation, utilization, and development. One of the challenges in the cultivation of aromatic rice to obtain superior varieties is the selection of the parent plants. Molecular analysis proved to be more accurate to obtain aromatic rice parents than conventional selection based on morphological and physiological characteristics (Jonah et al. 2011). The limitations of analysis using morphological and physiological traits cannot accurately understand individual characters of plant, because these analyses are easily influenced by environmental changes and planting treatment (Rabbani et al. 2008). The detection

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of plant characters using molecular markers had largely been done because it is useful for an individual characterization more accurately and reliably. Molecular markers can provide information about the similarities and differences of individuals (Rabbani et al. 2008) based on their genetic distance and position in phylogenetics (Hasan and Raihan 2015; Thomas et al. 2006). RAPD has been known as a nonbias and neutral marker for the genetic mapping applications (Michelmore et al. 1991) and genetics of a population (Haig et al. 1994). It does not require information about a particular sequence in the genome (Veatch-Blohm 2007). The RAPD profile helps to identify variations of the diagnostic markers on aromatic rice genotypes (Baishya et al. 2000; Kanawapee et al. 2011; Patwardhan et al. 2014), identification of rice at the level below species (Kibria et al. 2009), and effective for knowing genetic polymorphisms in different rice cultivars (Grattapaglia and Sederoff 1994). Currently, genetic tracking of aromatic rice germplas is essential for the identification and protection of natural resources. We studied about genetic characterization and authentication of the Indonesian aromatic rice based on amplification of DNA technology to analyzed the genetic diversity of aromatic rice by using random primers.

Materials and Methods

Sample collection and preparation

Twenty genotypes of aromatic rice and one genotype of non-aromatic rice as control (Situbagendit) were obtained from the Indonesian Center for Rice Research Sukamandi, Subang, West Java, and local farmers, originally from different regions of Indonesia, are showen in Table 1. The rice seeds phenotypes are shown in Fig. 1 (Suprihatno et al. 2010; Wahab et al. 2018). Seed shape observation was according to the standard evaluation system of rice (SES) by the International Rice Research Institute (IRRI 2013).

DNA extraction

Total DNA was extracted from leaves of 3-week old rice plants. Leaves (ca. 500 mg) were ground quickly in liquid nitrogen. Transfer powdered leaf tissue to 2.5 mL microtubes. The leaf powder was extracted with 500 ul extraction buffer (100 mM Tris-Cl, pH 8.0, 50 mM EDTA, 500 mM NaCl, 1.25% SDS, and 0.3% β-mercaptoethanol), and incubated at 65°C for 30 min, then 200 ul of 5 M potassium acetate was added to the solution. The mixture was incubated on ice for

Table 1. List of some aromatic rice varieties used in this study.

No.	Name of Genotype	Locations	Parental lines / Parental relations	Seed characters
1.	Rojolele Delanggu	Delanggu, Klaten、Indonesia	Local Rice	Shape : Medium, have awn, bright color
2.	Mentikwangi Banjarnegara	Banjarnegara、Indonesia	Local Rice	Shape : Medium, not have awn, bright color
3.	Pare Pulu Mandoti	Enrekang, South Sulawesi, Indonesia	Local Rice	Shape : Medium, have awn, brownish
4.	Situ Bagendit	Indonesia	Batur/S2823-7d-8-1-A//S283-7d-8-1-A	Shape : Slender, not have awn, bright color
5.	Radah Putih Karanganyar	Karanganyar, Indonesia	Local Rice	Shape : Slender, have awn, bright color
6.	Mentik Susu Karanganyar	Karanganyar, Indonesia	Local Rice	Shape : Medium, not have awn, bright color
7.	Sintanur	Indonesia	Lusi//bengawan solo	Shape : Bold, not have awn, bright color
8.	Celebes	Indonesia	Tetep/IR2415-90-4-3-2//IR19661-131 -1-2	Shape : Slender, not have awn, bright color
9.	Gilirang	Indonesia	B6672/Memberamo	Shape : Slender, not have awn, bright color
10.	Pendok	Tuban, Indonesia	Local Rice	Shape : Medium, have awn, bright color
11.	Situ Patenggang	Indonesia	Kartuna/TB47H-MR-10	Shape : Medium, not have awn, litlle brownish
12.	Mapan 05 Banjarnegara	Banjarnegara, Indonesia	Local Rice	Shape : Slender, not have awn, bright color
13.	Mentikwangi	Glempang Kecamatan Pekuncen, Indonesia	Local Rice	Shape : Medium, not have awn, bright color
14.	Pandanwangi	Cianjur, Indonesia	Local Rice	Shape : Medium, not have awn, bright color
15.	Kurik Kusut Karanganyar	Karanganyar, Indonesia	Local Rice	Shape : Slender, not have awn, brownish
16.	Inpari 7 Lanrang	Indonesia	S3054-2D-12-2/Utri Merah-2	Shape : Slender, not have awn, bright color
17.	Inpari 23 Bantul	Indonesia	B11738RS(Gilirang/ BP342F-MR-1-3// Gilirang	Shape : Medium, not have awn, bright color
18.	Batang Gadis	Indonesia	IR 64/NDR 308//IR 64	Shape : Slender, not have awn, bright color
19.	Umbuk Wangi	Kudus, Indonesia	Local Rice	Shape : Medium, not have awn, bright color
20.	Genjah Arum	Banyuwangi, Indonesia	Local Rice	Shape : Medium, have awn, bright color
21.	Gogo Fatuk Masin	Timor Timur	Local Rice	Shape : Slender, not have awn, bright color

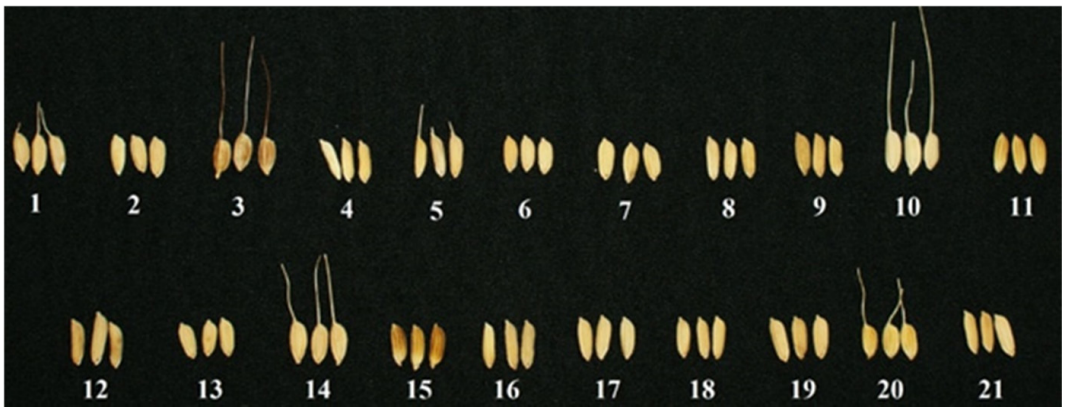

Fig. 1. The Morphology of Rice Seed. 1: Rojolele Delanggu, 2: Mentikwangi Banjarnegara, 3: Pare Pulu Mandoti, 4: Situ Bagendit, 5: Radah Putih Karanganyar, 6: Mentik Susu Karanganyar, 7: Sintanur, 8: Celebes, 9: Gilirang, 10: Pendok, 11: Situ Patenggang, 12: Mapan 05 Banjarnegara, 13: Mentikwangi, 14: Pandanwangi, 15: Kurik Kusut Karanganyar, 16: Inpari 7 Lanrang, 17: Inpari 23 Bantul, 18: Batang Gadis, 19: Umbuk Wangi, 20: Genjah Arum, 21: Gogo Fatuk Masin.

10 min, followed by centrifugation on 12,000 rpm for 10 min at room temperature. The supernatant was transferred into a new 1.5 ml tube and 625 ul isopropanol was added, shaken gently, followed by incubation at -20°C for 1 h. After incubation, the supernatant was centrifuged at 12,000 rpm for 10 min and discarded with aspirator. The DNA pellet was dissolved in 500 ul TE buffer, then 15 ul of 2 mg/mL RNAase was added, and incubated at 37°C for 30 min, followed by adding 500 ul PCI (Phenol:Chloroform:Isoamyl alcohol = 25:24:1). For further purification, the upper layer was transferred into a new 1.5 mL tube, added with an equal volume of chloroform, and then centrifuged 12,000 rpm for 10 min. The upper layer (600 ul) was transferred into a new 1.5 ml tube and precipitated by the addition 120 ul of 3 M Sodium Acetate and 480 ul Isopropanol, gently shaken until DNA was precipitated then incubated in -20°C for 1 h. Supernatant was centrifuged at 12,000 rpm for 10 min, then the supernatant was discarded, and then the DNA pellet was washed in 70% cold ethanol and centrifuged at 12,000 rpm for 10 min. After the supernatant was discarded, the pellet was dried well and dissolved in 20 ul TE buffer. The quality and quantity of total DNA was measured by Nanovue Plus (Biochrom Ltd. England).

Polymerase chain reaction amplification and gel electrophoresis

Polymerase chain reaction (PCR) was carried out in a final volume 10 ul with 2.5 ul of 200 ng DNA template, 0.5 ul of 10 pmol primers (primers listed in Table 2), 0.25 ul of 5 U *Taq* DNA polymerase, 2 ul of 10 x reaction buffer, 1 ul of 10 mM dNTP mix, and 3.75 ul miliQ. PCR amplification was performed as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 30°C for 30 s, 72°C for 1 min, and final extension at 72 ºC for 5 min. PCR products were separated by 2% agarose in 1xTAE buffer and were run in 75 V for 40 mins. Gel were stained by Ethidium Bromide and visualized under UV light using Gel Documentation System (Major Science Co. Ltd. USA).

Data analysis

The profile of DNA fragments amplified by each primer was assessed using binary code. Amplified band was scored visually for each primer. Bands were analyzed by scoring 1 to the present band and scoring 0 to the bands that did not appear to be the same size. The results of this value are further analyzed in the similarity using UPGMA online software (http://genomes.urv.cat/UPGMA/index.php) for phylogenetic tree compilation. Clustering analysis using Jaccard's similarity conducted using PAST3 software. The polymorphism of a primer commonly expressed as Gene Diversity (Nei 1973). The evaluation and the informativity level of primer was known by calculating the Gene Diversity or better known as Polymorphic Information Content (PIC) value (Shete et al. 2000). PIC value was determined using online software http://plantmolgen.iyte.edu.tr/GDdom/ (Abuzayed et al. 2017), the higher the PIC value the higher the informativity of primer. The PIC values in some dominant markers such as RAPD, ISSR, RFLP, and others are between 0-0.5, it is assumed that the same length of DNA fragments are from the appropriate locus, and represent the dominant single locus with two possible alleles (present or not) (Nagy et al. 2012). The maximum value of PIC and Heterozigocity (H) for the dominant marker is 0.5, since only two alleles per locus can be detected for this type of marker and both values are influenced by the number and frequency of alleles (Bolaric and Posselt 2005; Chesnokov and Artemyeva 2015; De Riek et al. 2001; Henry 1997).

Results

Twenty-one genotypes have been analyzed with 38 RAPD primers. RAPD primers amplified the genome ranged from 4 to 19 DNA bands (Table 2) with an average number of bands per primer being 10.66. OPA-4 and OPC-7 produced the least amount of bands while the LC-101 produced the largest number of DNA bands of different sizes. The total alleles

Table 2. Thirty-eight RAPD primers used to amplify the genomic DNA of 21 rice varieties.

No.	Primers	Primer sequence (5'-3')	A (°C)	TB	MB	Mono. (%)	PB	Poly. (%)	Size range (bp)	PIC value
1	OPA-1	CAGGCCCTTC	30	14	0	0.00	14	100.00	285–2500	0.418
2	OPA-2	TGCCGAGCTG	30	14	6	42.86	8	57.14	300–2600	0.212
3	OPA-3	AGTCAGCCAC	32	11	4	36.36	7	63.64	240–1700	0.172
4	OPA-4	AATCGGGCTG	33	4	0	0.00	4	100.00	490–1700	0.234
5	OPA-5	AGGGGTCTTG	34	8	2	25.00	6	75.00	290– 2150	0.272
6	OPA-7	GAAACGGGTG	30	10	3	30.00	7	70.00	250–2250	0.282
7	OPA-8	GTGACGPAGG	30	10	2	20.00	8	80.00	310–2750	0.253
8	OPA-9	GGGTAACGCC	34	11	3	27.27	8	72.73	280–1500	0.178
9	OPA-10	GTGATCGCAG	30	10	2	20.00	8	80.00	450–1600	0.244
10	OPA-11	CAATCGCCGT	32	15	3	20.00	12	80.00	300–2200	0.226
11	OPA-12	TCGGCGATAG	34	11	0	0.00	11	100.00	350–1500	0.314
12	OPA-13	CAGCACCCAC	32	12	3	25.00	9	75.00	300–1300	0.240
13	OPA 14	TCTGTGCTGG	32	7	2	28.57	5	71.43	200–1500	0.163
14	OPA-15	TTCCGAACCC	32	11	3	27.27	8	72.73	290–2750	0.192
15	OPA-16	AGCCAGCGAA	32	10	0	0.00	10	100.00	310–3000	0.322
16	OPA-17	GACCGCTTGT	32	11	0	0.00	11	100.00	460–3000	0.462
17	OPA-18	AGGTGACCGT	32	10	0	0.00	10	100.00	350–2350	0.225
18	OPA-19	CAAACGTCGG	32	13	0	0.00	13	100.00	290–3000	0.342
19	OPA-20	GTTGCGATCC	27	5	2	40.00	3	60.00	340–900	0.152
20	OPC-7	GTCCCGACGA	27	4	2	50.00	2	50.00	380–900	0.222
21	OPC-8	TGGACCGGTG	34	8	0	0.00	8	100.00	290–680	0.321
22	OPC-15	GACGGATCAG	27	11	3	27.27	8	72.73	400–2200	0.182
23	OPD-5	TGAGCGGACA	27	12	0	0.00	12	100.00	340–1900	0.318
24	OPD-6	ACCTGAACGG	27	15	0	0.00	15	100.00	320–3000	0.304
25	OPD-7	TTGGCACGGG	27	10	2	20.00	8	80.00	280–1250	0.366
26	OPD-8	GTGTGCCCCA	27	9	1	11.11	8	88.89	350–2100	0.258
27	OPD-10	GGTCTACACC	27	13	1	7.69	12	92.31	290–2100	0.285
28	OPJ-6	AACCCGGGAA	27	12	0	0.00	12	100.00	400–1900	0.373
29	OPJ-8	TCGTTCCGCA	27	8	0	0.00	8	100.00	300–2000	0.272
30	LC-78	CATACCGTGG	32	9	2	22.2	7	77.78	300–1500	0.236
31	LC-90	GTGATCGCAG	34	16	2	12.50	14	87.50	150–2250	0.303
32	LC-93	GTGAGGCGTC	34	16	3	18.75	13	81.25	320–3000	0.247
33	LC-95	GGACCCAACC	32	5	2	40.00	3	60.00	330–1200	0.098
34	LC-97	TGAGCGGACA	32	7	1	14.29	6	85.71	350–2100	0.262
35	LC-101	GTGTGCCCCA	34	19	1	5.26	18	94.74	340–1750	0.330
36	LC-102	GGGGTGACGA	32	11	1	9.09	10	90.91	310–1450	0.227
37	LC-106	CATCCGTGCT	32	10	1	10.00	9	90.00	330–1000	0.314
38	LC-109	GTGACATGCC	32	13	6	46.15	7	53.85	395–1450	0.170
		Total		405	63	15.56	342	84.440		
		Mean		10.66	1.66		9			0.263

A: Annealing temperature, TB: Total band, MB: Monomorphic band, PM: Polymorphic band, PIC: Polymorphic information content.

produced were 405 with the number of polymorphism alleles were 342 or 84.44% while the number of monomorphism alleles were 63 or 15.56%. Primers that produced 100% polymorphic were OPA-1, OPA-4, OPA-12, OPA-16, OPA-17, OPA-18, OPA-19, OPC-8, OPD-5, OPD-6, OPJ-6, and OPJ-8, producing 14, 4, 11, 10, 11, 10, 13, 8, 12, 15, 12, and 8 polymorphic alleles, respectively. In addition, LC-101 resulted in a percentage of polymorphism 94.74% with 18 polymorphism

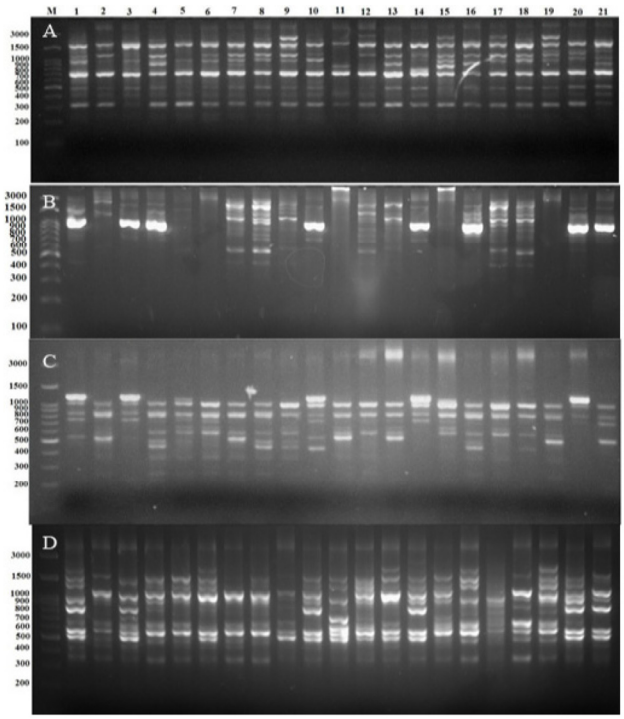

Fig. 2. PCR products profile of 21 Aromatic Rice using RAPD markers. M: 100bp leader, 1: Rojolele Delanggu, 2: Mentikwangi Banjarnegara, 3: Pare Pulu Mandoti, 4: Situ Bagendit, 5: Radah Putih Karanganyar, 6: Mentik Susu Karanganyar, 7: Sintanur, 8: Celebes, 9: Gilirang, 10: Pendok, 11: Situ Patenggang, 12: Mapan 05 Banjarnegara, 13: Mentikwangi, 14: Pandanwangi, 15: Kurik Kusut Karanganyar, 16: Inpari 7 Lanrang, 17: Inpari 23 Bantul, 18: Batang Gadis, 19: Umbuk Wangi, 20: Genjah Arum, 21: Gogo Fatuk Masin. A: OPA-11, B: OPA-17, C: OPD-7, LC-101.

bands from of 19 total bands. Primers that produced the lowest polymorphic allele were OPC-7 (50%) producing 4 bands and 2 were polymorphic. RAPD polymorphism on 21 genotypes using OPA-10, OPA-17, OPD-7, and LC-101 primers were shown in Fig. 2.

The genetic distance knowing from the simmilarity value is then used to construct the dendogram. Twenthy-one genotypes were divided into two large clusters (*I* and *II*), detailed dendogram shown in Fig. 3. The first cluster (*I*) consisted of most genotypes (15 varieties) with similarity values ranged from 0.472 between Batang Gadis and Mentik Susu Karanganyar up to 0.773 between Mentikwangi Banjarnegara and Sintanur. The second cluster (*II*) consists of six genotypes with simmilarity values ranged from 0.575 between Genjah Arum and Pare Pulu Mandoti up to 0.683 between Rojolele Delanggu and Pandanwangi.

The first cluster (*I*) was divided into two sub-clusters (*IA* and *IB*). Batang Gadis was the only genotype in first subcluster (*IA*) with the highest simmilarity value with another genotype (Gilirang) reach only 0.594. The second sub-cluster (*IB*) consisted of 14 genotypes and was divided again into two groups (IB_1 and IB_2). The IB_1 consisted of three genotypes namely Situ Patenggang, Mentikwangi and Kurik Kusut Karanganyar with similarity value from 0.655 between Mentikwangi and Situ Patenggang up to 0.672 between Mentikwangi and Kurik Kusut Karanganyar. The IB_2 consisted of eleven genotypes namely Celebes, Mentikwangi Banjarnegara, Sintanur, Gilirang, Situ Bagendit, Inpari 7 Lanrang, Mapan 05 Banjarnegara, Inpari 23 Bantul, Umbuk Wangi, Radah Putih Karanganyar, and Mentik Susu Karanganyar with similarity value from 0.564 between Mentik Susu Karanganyar and Inpari 23 Bantul up to 0.773 between Sintanur and Mentikwangi Banjarnegara. IB_2 then was divided into two groups (IB_{2a} and IB_{2b}). IB_{2a} consisted of nine genotypes namely Celebes, Mentikwangi Banjarnegara, Sintanur, Gilirang, Situ Bagendit, Inpari 7, Mapan 05 Banjarnegara, Inpari 23, and Umbuk Wangi. IB_{2b} consisted of two genetypes namely Radah Putih Karanganyar, and Mentik Susu Karanganyar.

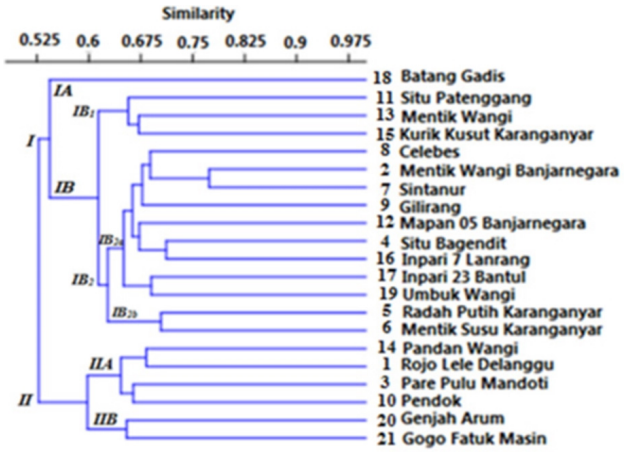

Fig. 3. Dendogram Pattern of RAPD analysis based on UPGMA.

The second cluster (*II*) consists of six varieties namely Genjah Arum, Gogo Fatuk Masin, Rojolele Delanggu, Pare Pulu Mandoti, Pendok, and Pandanwangi. All varieties in this cluster showed the same characteristics of having awn on seeds except for Gogo Fatuk Masin (Fig. 1). The similarity value in this cluster was from 0.575 between Pare Pulu Mandoti and Genjah Arum up to 0.683 between Rojolele Delanggu and Pandanwangi. This cluster is divided into two sub-clusters (*IIA* and *IIB*). *IIA* consisted of four genotypes namely Pandanwangi, Rojolele Delanggu, Pare Pulu Mandoti, and Pendok. This subcluster was divided into two, first was between Pandanwangi and Rojolele Delanggu with similarity 0.683 and the second was between Pare Pulu Mandoti and Pendok with similarity 0.664. *IIB* consisted of only two genotypes namely Genjah Arum and Gogo Fatuk Masin with similarity 0.654 (Table 3).

Discussion

The polymorphism percentages in this study were higher when compared to similar studies. Rabbani et al. (2008) studied 30 aromatic rice and reported that polymorphic DNA bands resulting from 25 RAPD primers were 186 out of 208

Table 3. Similarity Matrix based on Jaccard's Coefficient for RAPD.

	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21
01	1	0.530	0.639	0.537	0.566	0.538	0.553	0.566	0.510	0.616	0.514	0.556	0.540	0.683	0.554	0.567	0.537	0.472	0.520	0.609	0.594
02		1	0.561	0.654	0.621	0.656	0.773	0.670	0.664	0.483	0.628	0.616	0.616	0.530	0.572	0.613	0.624	0.527	0.667	0.482	0.486
03			1	0.567	0.578	0.553	0.531	0.529	0.534	0.664	0.544	0.515	0.516	0.658	0.504	0.495	0.483	0.461	0.496	0.575	0.609
04				1	0.704	0.672	0.701	0.702	0.685	0.553	0.620	0.669	0.586	0.574	0.567	0.709	0.633	0.535	0.645	0.507	0.505
05					1	0.704	0.646	0.612	0.634	0.574	0.622	0.610	0.610	0.627	0.620	0.643	0.571	0.525	0.611	0.558	0.550
06						1	0.644	0.611	0.620	0.527	0.621	0.609	0.621	0.602	0.594	0.635	0.564	0.472	0.622	0.545	0.466
07							1	0.707	0.695	0.522	0.635	0.661	0.641	0.554	0.580	0.671	0.648	0.564	0.686	0.491	0.485
08								1	0.672	0.573	0.607	0.668	0.573	0.551	0.534	0.661	0.626	0.556	0.620	0.495	0.468
09									1	0.541	0.599	0.645	0.635	0.546	0.579	0.667	0.655	0.594	0.630	0.493	0.481
10										1	0.557	0.572	0.523	0.672	0.532	0.512	0.469	0.474	0.514	0.582	0.598
11											1	0.657	0.655	0.568	0.659	0.620	0.583	0.519	0.663	0.513	0.484
12												1	0.657	0.599	0.637	0.675	0.658	0.544	0.652	0.502	0.510
13													1	0.592	0.672	0.638	0.595	0.549	0.656	0.530	0.505
14														1	0.596	0.591	0.548	0.458	0.558	0.613	0.604
15															1	0.647	0.599	0.535	0.641	0.517	0.493
16																1	0.675	0.546	0.675	0.517	0.510
17																	1	0.565	0.690	0.505	0.477
18																		1	0.576	0.514	0.494
19																			1	0.543	0.502
20																				1	0.654
21																					1

1: Rojolele Delanggu, 2: Mentikwangi Banjarnegara, 3: Pare Pulu Mandoti, 4: Situ Bagendit, 5: Radah Putih Karanganyar, 6: Mentik Susu Karanganyar, 7: Sintanur, 8: Celebes, 9: Gilirang, 10: Pendok, 11: Situ Patenggang, 12: Mapan 05 Banjarnegara, 13: Mentikwangi, 14: Pandanwangi, 15: Kurik Kusut Karanganyar, 16: Inpari 7 Lanrang, 17: Inpari 23 Bantul, 18: Batang Gadis, 19: Umbuk Wangi, 20: Genjah Arum, 21: Gogo Fatuk Masin.

bands with a percentage of 89.4%. Ray et al. (2012) in 50 aromatic rice using 12 RAPD primers resulted in 95 polymorphic DNA bands (87.15%) from a total of 109 DNA bands produced. Another study by Hasan and Raihan (2014) in 30 Bangladeshi aromatic rice reported that 50 RAPD primers produced 26 polymorphic bands (78.79%) from a total of 33 DNA bands. Another RAPD study on aromatic rice yielded polymorphism percentage of more than 50%, i.e. 80% (Tahmina et al. 2017), 93.58% (Verma et al. 1999), 95.1% (Raghunathachari et al. 2000), 96.5% (Choudhury et al. 2001), 68.94% (Kanawapee et al. 2011), 65.5% (Mathure et al. 2010), and 53.85% (Rahman et al. 2007). The resulted polymorphism presentence (94.74%) indicates that the RAPD marker can differentiate genotypes in this study. The percentage of polymorphism from RAPD study on aromatic rice above can be said that RAPD marker can be used as an analytical tool to distinguish genotype. The PIC values of 38 randomized RAPD primers ranged from 0.098 generated by LC-95 to 0.462 produced by OPA-17 primers. The average PIC value for each primer is 0.263. As well known that the PIC values generated by dominant markers such as RAPD ranged from 0 to 0.5, therefore the maximum RAPD value of 0.462 was not too low. This is assumed that the same length of DNA fragments are from the appropriate locus, and represent the dominant single locus with two possible alleles (present or not) (Nagy et al. 2012). The maximum value of PIC and Heterozigocity (H) for the dominant marker is 0.5, since only two alleles per locus can be detected for this type of marker and both values are influenced by the number and frequency of alleles (Bolaric and Posselt 2005; Chesnokov and Artemyeva 2015; De Riek et al. 2001; Henry 1997) . Although geographical distance is one of the factors affecting the genetic relationship (Wright 1943), the dendogram showed that the closeness of each genotype was not linked to the location. Some genotypes from the same region not show a high proximity relationship (see Table 1 for information of the varieties origin). In this study, there were 13 local genotypes and 8 genotypes resulted by cultivated (next known as prime varieties). All of prime varieties were grouped into the first cluster with several local varieties. Whereas all varieties in the second cluster were local varieties and have the same morphological features of awn in seeds except Gogo Fatuk Masin. In the first cluster, Batang Gadis varieties occupy their own subcluster of the other fourteen varieties, this may occur because the Batang Gadis is result of crossing beetween Indonesia local rice and International Rice (IR), and then re-crossed with IR rice so that the genetic source is mostly derived from IR resulting in a much different genetic pattern from Indonesia rice. Batang Gadis experienced very low amplification on some primers such as OPA-12 (2 of 11), OPA-18 (2 of 10), OPJ-08 (2 of 8) and not amplified at OPC-08, OPD-05, OPJ-06. Batang Gadis varieties produced

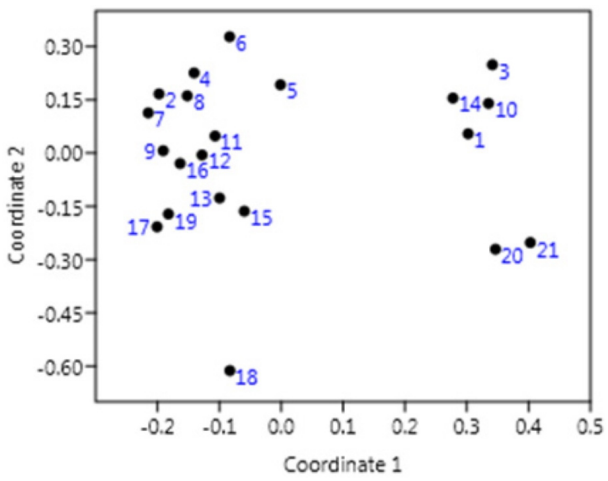

Fig. 4. Two dimensional clustering analysis of 21 rice genotype using Jaccard's similarity. 1: Rojolele Delanggu, 2: Mentikwangi Banjarnegara, 3: Pare Pulu Mandoti, 4: Situ Bagendit, 5: Radah Putih Karanganyar, 6: Mentik Susu Karanganyar, 7: Sintanur, 8: Celebes, 9: Gilirang, 10: Pendok, 11: Situ Patenggang, 12: Mapan 05 Banjarnegara, 13: Mentikwangi, 14: Pandanwangi, 15: Kurik Kusut Karanganyar, 16: Inpari 7 Lanrang, 17: Inpari 23 Bantul, 18: Batang Gadis, 19: Umbuk Wangi, 20: Genjah Arum, 21: Gogo Fatuk Masin.

181 bands of a total of 405 bands. The value of similarity of Batang Gadis varieties reached 0.594 with Gilirang varieties that produced the band as much as 219 bands from total of 405 markers. There were eight prime varieties included Situ Bagendit, Sintanur, Celebes, Gilirang, Situ Patenggang, Inpari 7, Inpari 23, and Batang Gadis. Seven varieties other than Batang Gadis were obtained from various kinds of genetic sources. The more varieties involved in crosses, the more genetic sources, for example Gilirang is crossed from B6672 and Memberamo, while Memberamo is crossed from B6555B-199-40 and Barumun, Barumun is crossed from Ptb 33/*4 IR3043. There were several varieties such as Sintanur, Celebes, and Gilirang which have genetic sources of IR rice, but have experienced some crossbreeding with other local varieties and caused the genetic pattern to be not very different with Indonesia rice. Gilirang and Inpari 23 showed the similarity value which was relatively large (0.655) because Inpari 23 is the result of a cross that one of the parent is Gilirang (see Table 1 for information on the varieties origin). The value of similarity generated in this study is between 0.458 and 0.773 which means that the varieties analyzed were not very different. Similarity values generated in other studies varied from 0.101 to 0.911 (Hasan and Raihan 2015), 0.458 and 0.773 (this study), 0.65 to 0.86 (Tahmina et al. 2017), 0.25 to 0.775 (Raghunathachari et al. 2000), 0.60 to 0.96 (Choudhury et al. 2001), 0.64 to 0.94 (Kanawapee et al. 2011), 0.50 to 0.96 (Rabbani et al. 2008), and 0.723 to 0.996 (Fukuoka et al. 2006). The value of similarity in the first cluster (0.472 to 0.773) indicates a broader vulnerability and means that the first cluster has a more diverse genotype than second cluster (0.575 to 0.683). The similarity was then used as the so conduct two dimentional clustering analysis and is shown in Fig. 4. Two dimentional clustering analysis showed the similarity between genotypes to be clearer. Batang Gadis was separated from other genotypes, but closer to the group of genotypes in the upper side (Cluster I). The six genotype that were included in the second cluster is separate from other genotypes. Cluster analysis showed that the use of 38 RAPD markers on 21 genotypes could not classify the genotypes based on aroma characters; these genotypes were grouped based on the basic genotypic equations which were drawn from the genetic patterns in the genome. Since most aromatic traits are controlled by a single random gene in chromosome 8 of *Oryza sativa* (Bradbury et al. 2005; Kumari et al. 2012), analysis using dominant markers identifies a single dominant allele (Abuzayed et al. 2017). The high number of polymorphism bands produced by some primer indicates that this marker can be used to differentiate each genotype well. The primer also grouped the variety with same morphological characteristic. Moreover, the primer used in this study differentiated the local variety from the cultivated variety. It is well known that there is a basic similarity between the cultivated rice. Detailed study on crops is essential for storage and cultivation activities, preventing errors in characterization, and to assist plant breeders and farmers. As the literature on genetic analysis of Indonesian rice is limited, this study is expected to help researchers and other parties in the future. In this study, the number of amplified DNA bands and the number of polymorphic DNA bands can be used to identify and differentiate aromatic rice genotypes. RAPD markers can show differences in individual fingerprint patterns, as it is known that genetic variation is important for the maintenance and development of an organism's potential. Information on genetic diversity in this study may be useful for plant breeders in the selection and crop cultivation process. However, there are some limitations to this study as only 21 genotypes were analyzed and only 38 primers were used. Further studies with more genotype and primer variations need to be done to provide more information.

Conclusion

Genetic similarity values determined from RAPD were also used to construct the dendogram using online software based on un-weighted pair-group method with arithmetic averages (UPGMA). Twenty-one aromatic rices were clustered into two big clusters where the first cluster has the highest members with a total of 15 genotypes.

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