Similarity of 26 New Released Rice Varieties and Rice Parental Hybrids Based on 36 SSR Markers

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ABSTRAK. Kemiripan antara 26 Varietas Padi yang Telah Dilepas dan Tetua Hibrida Atas Dasar 36 Marka SSR. *Lebih dari 200 varietas padi telah dilepas di Indonesia, namun variabilitas genetiknya diduga relatif rendah. Marka molekuler seperti SSR (Simple Sequence Repeats) merupakan alat yang dapat digunakan untuk membedakan antargenotipe, bahkan antara varietas yang memiliki kemiripan fenotipe tinggi. SSR juga dapat digunakan untuk membuktikan keautentikan suatu varietas. Penelitian ini bertujuan untuk mendapatkan data sidik jari DNA varietas-varietas terbaru dan tetua hibrida menggunakan marka SSR. Sebanyak 26 genotipe padi yang terdiri atas 3 gogo, 10 sawah irigasi, 5 rawa, serta delapan tetua hibrida digunakan dalam penelitian ini. DNA diekstrak dari daun muda, menggunakan metode CTAB yang dimodifikasi dan diaplikasikan terhadap 26 marka SSR yang dilaporkan terpaut dengan karakter-karakter penting tanaman padi dan tersebar merata di 12 kromosom padi. Penelitian dilakukan di Laboratorium Pemuliaan Tanaman Balai Besar Penelitian Tanaman Padi (BB Padi) dalam tahun 2012. Hasil penelitian menunjukkan bahwa nilai PIC (Polymorphic Information Content) dari genotipe yang diuji tergolong medium dengan rata-rata 0,4451. Analisis filogeni memperlihatkan bahwa pada batas jarak genetik 10%, genotipe yang diuji terbagi dalam 9 group, yaitu Inpago 6, Inpara 5, dan BH33d masing-masing berdiri sendiri, sedangkan group yang lain adalah (Inpara 1, Inpara 2 dan Inpara 3); (Inpari 18 dan Inpari 19), (Inpago 4, Inpago 5 dan Inpara 4); (Inpari 11, Inpari 12, Inpari 13, Inpari 14, Inpari 15, Inpari 16, Inpari 17 dan Inpari 20); (GMJ6B, B6 dan IR79156B); (PK21, BH95E, Bio9 dan R14) masing-masing dalam satu group. Hasil tersebut mengindikasikan bahwa genotipe cenderung terkelompokkan mengikuti pembagian agro ekosistem. Tetua hibrida cenderung berada pada group yang berbeda dengan varietas padi inbrida. Aplikasi marka 36 SSR mampu membedakan antar 26 genotipe yang diuji dengan destingsi sedang. Penambahan marka yang digunakan akan mampu memberikan data yang semakin lengkap dalam membedakan antar genotipe yang diuji.*

Kata kunci: Sidik jari DNA, Padi, SSR.

ABSTRACT. More than 200 rice varieties had been released in Indonesia, but the genetic variability among those released varieties was suspected to be relatively low. Molecular markers, especially SSR could be used as a tool to disect the distinctness among rice genotypes, albeit phenotipically similar varieties. The technique could also be used to prove the authenticity of a variety. This research was aimed to obtain DNA fingerprinting data of new released rice varieties and hybrid parental lines using SSR markers. A total of 26 rice genotypes consisted of three upland, ten irrigated, five swampy rice varieties, along with eight hybrid parental lines were used in this experiments. The DNA was extracted from young leaf samples using CTAB modified method and was amplified with 36 SSR markers linked to important rice traits which spread accross the 12 rice chromosomes. The experiment was conducted in Plant Breeding Laboratory of Indonesian Center for Rice Research (ICRR) during 2012. The results showed that PIC value of the genotypes were mostly at medium level of the genetic diversity with the average value of 0.4451. The phylogenetic analysis showed that at the genetic distance of 10%, the genotypes were separated into 9 groups, i.e. Inpago 6, Inpara 5, and BH33d each stood alone while (Inpara 1, Inpara 2 , and Inpara 3); (Inpari 18 and Inpari 19, Inpago 4, Inpago 5, and Inpara 4); (Inpari 11, Inpari 12, Inpari 13, Inpari 14, Inpari 15, Inpari 16, Inpari 17, and Inpari 20); (GMJ6B, B6, and IR79156B); (PK21, BH95E, Bio9, and R14) each belong to one group. The grouping of the genotypes in this study seemed to follow the adaptation type to agro ecosystems. The hybrid parental lines tended to stay in different group from the inbred varieties. The application of these 36 SSR markers was able to distinguish among 26 genotypes rather distinctly. The use of more markers should give more powerful data to distinguish among genotypes.

Keywords: DNA Fingerprint, Rice, SSR.

More than 200 of rice varieties had been released in Indonesia (Suprihatno *et al*. 2011, Sunihardi *et al*. 1999, Musaddad *et al*. 1993), consisted of varieties for the lowland, upland, and swamp land, and hybrid rice varieties. Each variety has special superiorities, such as resistance to pest and diseases, tolerance to abiotic stresses, specific amilose content, specific quality aspect such as aromatic, and high yield. Nevertheless, genetic variability among these varieties seem to be very low because some varieties have the same common ancestors (Susanto *et al*. 2003). Narrow genetic variability would give less buffering capacity to the dinamics changing of the environment. Although the natural genetic variation of rice is very big, very few were actually used in the breeding program (Vaughan and Jackson 1995). Consequently, it had narrowed the genetic variability of cultivated rice varieties and there is tendency of reduced resistance of the released varieties to major pests and disieases (Yu *et al*. 2003). Narrow genetic background would provide less genetic variation for further breeding program and their progenies would have no significant increase in the targeted traits. On the other hand, wide heterotic pools between parents would give more chance to have better hybrids.

Molecular marker technique is able to diferentiate the DNA constitution of individuals eventhough they have much similarity among genotypes. DNA fingerprinting could be used to distinguish the authenticity of a variety, and molecular marker maybe used as standard identification for germ plasm accessions (Bioversity International 2007). In relation with plant variety protection, DNA fingerprinting data could be used to prove the authenticity of a variety. Behera *et al*. (2012) reported the utilization of 36 microsatellite markers distributed over 12 chromosomes of rice to assess the genetic diversity in 33 medicinal rice genotypes. The data provides basic information on medicinal rice genotypes in India which will be useful for future reference and to protect these unique quality rices under the Intellectual Property Rights (IPR). Chuang *et al*. (2011) had tested the authentication of foreign and local rice varieties in Taiwan by applying 32 SSR markers into 36 varieties from various Taiwanesse areas and foreigner countries. Choudhury *et al*. (2001) reported that DNA finger printing could be used to identify and classify aromatic rices in India. Furthermore, DNA finger printing could be used to see genetic diversity as based for deciding on combination among the genotypes (Alvarez *et al*. 2007).

Among molecular markers, SSR is relatively cost effective, acurate, stable, and capable to distinguish multi alllele in one locus due to its codominant characteristics (Panaud *et al*. 1995, Powell *et al*. 1996). High member of Molecular marekrs uge ammount of SSR markers for rice had been available on the whole rice genome (Temnykh *et al*. 2000, McCouch *et al.* 2002, Zhang *et al*. 2007). Therefore, SSR is the most suitablne tool to do the DNA fingerprinting on rice.

DNA fingerprinting is also useful to identify specific genes which had been incorporated into the varieties used in the study. It will precisely identify the genes and will give very useful information for deciding further improvement of the variety in the further breeding activities.

This research was aimed to see the similarity of new released rice varieties and hybrid parental lines using 36 SSR markers linked to important rice traits. The information then could be used in deciding further germ plasm utilization and breeding strategy in Indonesia.

MATERIAL AND METHODS

A total of twenty six rice genotypes consisted of three upland, ten irrigated, five swampy varieties along with eight hybrid parental lines were used in this study (Table 1), and were grown during DS 2012. The seeds of release varieties were obtained from UPBS (*Unit Pengelolaan Benih Sumber*; Breeder Seed Production Unit) of Indonesian Center for Rice Research (ICRR), while the seeds of parental hybrids were obtained from Hybrid Rice Breding Group of ICRR.

SSR Markers

Thirty six SSR markers linked to important traits of rice plant and evenly spread across rice genome (Table 2) were selected from the list reported by Susan McCouch (2002).

DNA Extraction and SSR Marker Application

DNA was extracted from 25 days old leaves using modified CTAB method (without liquid N). PCR reaction then be conducted in 10 μl reaction solution consisted of 50 ng of template DNA then added with PCR ready mixed from KAPPA Bio Science. The profile of PCR reaction was 96^{o}C for 5 minutes continued by 35 cycle of denaturizing in 94^{o}C for one minutes, annealing temperature at 55^{o}C or 61^{o}C (depend on primers characteristics) for one minute, and extension at 72^{o}C for two minutes, then final product extension at 72^{o}C for five minutes. PCR product was kept in 4^{o}C until used.

Table 1. Varieties and hybrid parental lines used for DNA fingerprinting analysis, ICRR, 2012.

No	Genotypes	Suitability
1	INPAGO 4	Upland
2	INPAGO 5	Upland
3	INPAGO 6	Upland
4	INPARI 11	Lowland
5	INPARI 12	Lowland
6	INPARI 13	Lowland
7	INPARI 14 Pakuan	Lowland
8	INPARI 15 Parahyangan	Lowland
9	INPARI 16 Pasundan	Lowland
10	INPARI 17	Lowland
11	INPARI 18	Lowland
12	INPARI 19	Lowland
13	INPARI 20	Lowland
14	INPARA 1	Swampy
15	INPARA 2	Swampy
16	INPARA 3	Swampy
17	INPARA 4	Swampy
18	INPARA 5	Swampy
19	B6	Parental Hybrid, lowland
20	GMJ6B	Parental Hybrid, lowland
21	IR79156B	Parental Hybrid, lowland
22	PK21	Parental Hybrid, lowland
23	R14	Parental Hybrid, lowland
24	Bio9	Parental Hybrid, lowland
25	BH95E-Mr-15-6-2-2	Parental Hybrid, lowland
26	BH33D-Mr-57-1-2-2	Parental Hybrid, lowland

Table 2. Markers information, allele number, and PIC value of 26 rice genotypes using 36 SSR markers.

No	Marker	Chromo-some	Position (cM)	Motif	Linked Trait	Forward	Reverse	Tm	Size	Allele number*	PIC*
1	RM5341	12	65.3		Bph9	TGCATTTTCCATACAATACG	ATTTGATACATGGACGATGC	56	135	2	0.2533
2	RM234	7	88.2	CT 25	mature	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	55	156	4	0.5571
3	RM7102	12	71.85		Bph2	TAGGAGTGTTTAGAGTGCCA	TCGGTTTGCTTATACATCAG	55	168	2	0.3698
4	RM3459	8	73.8		harvest index	ATGGACTTTCGAGAATGTTG	GAGTACGAAATGAAGGCAAG	55	197	5	0.4737
5	RM247	12	32.3	CT 16	Bph1	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	55	131	3	0.5262
6	RM219	9	11.7	CT 17	Sub1	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	55	202	3	0.4683
7	RM589	6	3.2	(GT)24	Bph3	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	55	186	5	0.7042
8	RM258	10	70.8	(GA)21 (GGA)3	Tiller number	TGCTGTATGTAGCTCGCACC	TGGCCTTTAAAGCTGTCGC	55	148	3	0.5478
9	RM6308	3			Bph19	TCGACCTGGCTCTCCTCTAG	TATCAACCTGCTCCTCCTGG	60	176	2	0.0866
10	RM3701	11	45.3		pan length	GAGCTAGAGGGAGGAGGTGC	TTGACTGATAGCCGATTGGG	55	174	2	0.3613
11	RM5444	3	27.9		*Hd* 8	AACGTACCAGTCGCTGGTTC	CCCGTGATTTCCTCCGAC	60	225	2	0.3508
12	RM406	2	186.4	(GA)17	qhts2	GAGGGAGAAAGGTGGACATG	TGTGCTCCTTGGGAAGAAAG	55	146	4	0.5041
13	RM1022	3	36.9		Bph18	GGTTGAACCCAAATCTGCA	CTTTGATAGCGGCTTTGTCC	60	169	1	0
14	RM5639	3	39.8		*Hd* 8	GGAAGAACAGAGTTGCTCGG	GTGCCATTTATTTCCGTCCC	61	168	2	0.2149
15	RM3859	7	49.4		*Hd* 4	TTGCAGATCGGTTTCCACTG	GGTCCTGGATTCATGGTGTC	60	191	6	0.7345
16	RM1261	12	61.6		Harvest Index-drought	GTCCATGCCCAAGACACAAC	GTTACATCATGGGTGACCCC	60	148	4	0.6253
17	RM3571	8	108.2	G56	*Hd* 12	GAGGACCCCGAATCGATC	AACAGGGCCGGGTTAAGTAG	60	184	3	0.4316
18	RM10852	1			Salt	GAATTTCTAGGCCATGAGAGC	AACGGAGGGAGTATATGTTAGCC		171	3	0.5901
19	RM6070	8	108.2		*Hd* 12	TTGCTAGTGCTTACCACCCC	TCCCAGTCACCCTGCTACTC	60	172	4	0.5958
20	RM164	5	78.7		heading-drought	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC		246	2	0.178
21	RM6344	7	89.8		Gen Rf4	ACACGCCATGGATGATGAC	TGGCATCATCACTTCCTCAC	60	163	4	0.6932
22	RM7338	7	53		*Hd* 4	CTTATCTCTCGGCAAGCAGC	CTCACACGCATGGATCAATC	60	158	3	0.5455
23	RM443	1	122.7	(GT)10	Gen Rf3	GATGGTTTTCATCGGCTACG	AGTCCCAGAATGTCGTTTCG	55	124	4	0.6129
24	RM1287	1	58.1		QTL Saltol	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG	55	162	3	0.422
25	RM349	4	146.8	(GA)16	Xa12	TTGCCATTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG	55	136	3	0.4282
26	RM4608	6	15.8		*Hd* 3	CAGGTAATAGTCATACTCCT	GGAAACTAGATTAGCTCATA	55	135	1	0
27	RM20589	6			Xa7	CATGTATTTGTGTGCACGTACCG	ACCTTTCTTGGGCCTTTCTTGG		263	3	0.5112
28	RM464a	9			Sub1	aacgggcacattctgtcttc	tggaagacctgatcgtttcc	55	262	2	0.3648
29	RM315	1	165	(AT)4 (GT)10	Gen Rf3	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	55	133	3	0.4009
30	RM266	2	192.2	(GA)19	seed set	TAGTTTAACCAAGACTCTC	GGTTGAACCCAAATCTGCA	55	127	3	0.4802
31	RM1369	6	7.4		*Hd* 3	AACCTGAGAGTGCCAATTGG	TCCCCTAGTAAAGCGGATTC	58	176	8	0.8287
32	RM248	7	116.6	CT 25	root development	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	55	102	4	0.5815
33	RM510	6	20.8	(GA)15	Gel Consistency	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	55	122	2	0.3648
34	RM190	6	7.4	(CT)11	waxi gene	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	55	124	5	0.4907
35	RM282	3	100.6	(GA)15	grain/pan	CTGTGTCGAAAGGCTGCAC	CAGTCCTGTGTTGCAGCAAG	55	136	2	0.3249
36	RM241	4	106.2	CT 31	Plant Height	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG	55	138	3	0.1694
	Mean									3.2286	0.4451
	Minimal									1	0
	Maximal									8	0.8287
	Average									3.1944	0.4451
	Total									115	

Source: Susan McCouch (2002); * = result data of this research

Eight percent of Polyacrilamide gel electrophoresis was conducted in 1 x TBE buffer at 100volt for two to four hours depending on the product size. Ethidium bromide staining was applied for 30 minutes and DNA visualization was then conducted under UV light using Gel documentation System.

Data Analysis

Data score was indicated as 1 (present) and 0 (not present) of each SSR band in each locus. The genetic variability was measured by the value of polymorphism information content (PIC) according to the method by Anderson *et al*. (1993).

$$PICi = 1 - \sum_{j=1}^{n} Pij^2$$

Where P*ij*: the frequency of j^{th} allele at the i^{th} locus;
n: the number of allele in particular locus.

Phylogenetic analysis was calculated following Nei (1973) method for the distance and UPGMA (Unweighted Pair Group Mean Arithmatic) for the dendogram development. PIC analysis and Phylogenetic analysis were done using Power marker version 3.25 (Liu and Muse 2005).

RESULTS AND DISCUSSIONS

Level of Polymorphism

The SSR markers applied to the population have relatively high polymorphism. From 36 SSR markers applied, 34 markers (94.44%) were polymorphic which indicated that new varieties were varied among themself, possibly because some of the new released varieties were introduced from IRRI that had different parents from that of ICRR cross originated varieties. The two monomorphic markers were RM1022 and RM4608.

Allele Number

The number of allele in each locus of polymorphic markers varied from 2 to 8 with the average allele number per locus of 3.1944 and total alleles of 115 alleles. This result was lower than that reported by Lapitan *et al*. (2007) across 24 varieties using 151 SSR markers which found 5.98 alleles per locus and Ni *et al.* (2002) that attained 6.78 alleles per locus across 38 varieties using 111 SSR markers. This result was lower than 13 alleles per locus across 330 rice accessions using 30 SSR markers reported by Thomson *et al.* (2007).

This result indicated that varieties used in this study were less variable. It was because the varieties used were mostly of modern varieties, which had incorporated genes from many background, and ecotype differentiation of landraces was no longer exist in improved varieties (Khush 1997). The number of varieties used also affected the result, that the higher the population size the more allele could be found.

Polymorphism Information Content

The polymorphism information content as the reflection of genetic variability varies from 0 for the monomorphic markers and from 0.0866 (RM6308) to 0.8287 (RM1369) for the polymorphic markers, with the average of 0.4451. It was classified as reasonably informative (0.5 > PIC>0.25) by Bostein *et al*. (1980).

Base on the average of each chromosome (Table 3), the highest PIC value was found in chromosome 7 (0.6224) followed by chromosome 6 (0.5799) and chromosome 10 (0.5478). Chromosome 5 (0.1780) has the lowest PIC value.

Cluster Analysis

Phylogenetic analysis using dendogram to figure out the genetic distance among genotypes with threshold level of 10% showed that the genotypes were divided into 9 groups. They were Inpago 6, Inpara 5, and BH33d each stood by itself while Inpara 1, 2 , and 3; Inpari 18 and 19, Inpago 4, Inpago 5, and Inpara 4; Inpari 11, 12, 13,14,15, 16, 17, and 20; GMJ6B, B6, and IR79156B; PK21, BH95E, Bio9, and R14 belong to one group (Figure 1).

The hybrid parental lines tended to group together while within Inpara, Inpago, and Inpari tended to group in accordance with agroecosystem in similar grouping of the variety. It seemed that the grouping followed agro ecosystems and hybrid parental lines tended to stay in different group with inbred variety.

Table 3. Average of PIC value of each of the chromosome.

Chr	No of marker	Average PIC
1	4	0.5065
2	2	0.4922
3	5	0.2443
4	2	0.2988
5	1	0.1780
6	6	0.5799
7	5	0.6224
8	3	0.5004
9	2	0.4166
10	1	0.5478
11	1	0.3613
12	4	0.4437

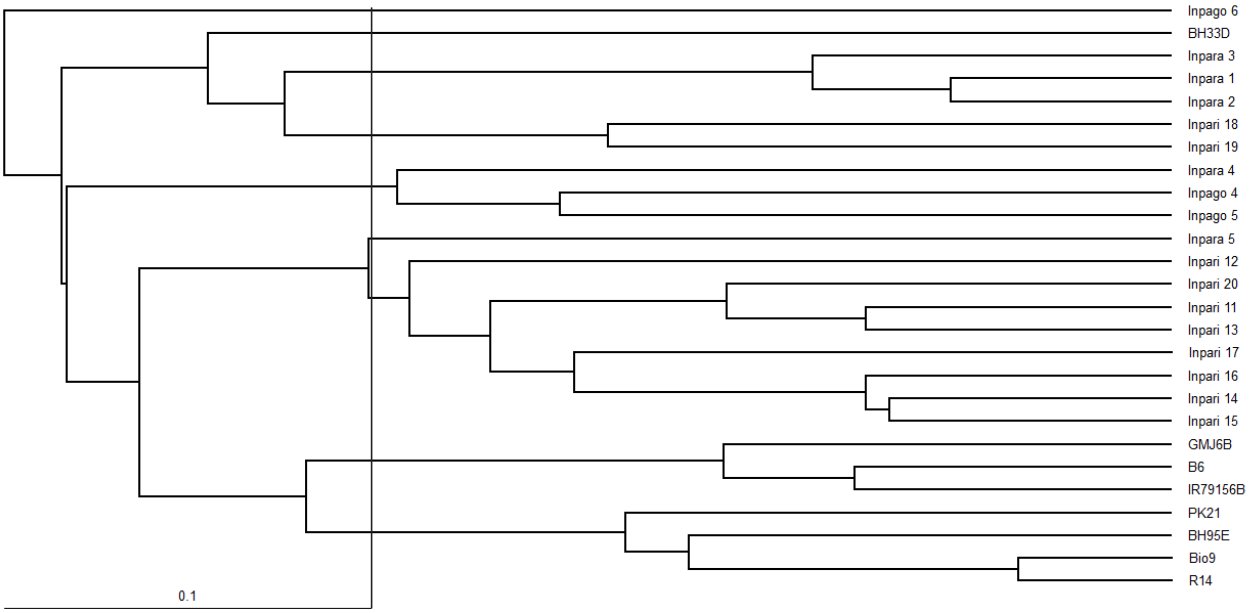

Figure 1. Dendogram of genetic distance among 36 rice varieties and hybrid parental lines using 36 SSR markers.

Based on this results, it indicated that application of 36 SSR markers was enough to distinguish the 26 genotypes. Nevertheless, application of more markers would surely give more powerful to distinguish among genotypes.

CONCLUSSIONS

PIC analysis showed that the genotypes mostly had medium level of genetic diversity with the average value of 0.4451. Phylogenetic analysis showed that at the genetic distance of 10%, the genotypes would be separated into 9 groups, i.e. Inpago 6, Inpara 5, and BH33d each stood alone while Inpara 1, 2 , and 3; Inpari 18 and 19, Inpago 4, Inpago 5, and Inpara 4; Inpari 11, 12, 13,14,15, 16, 17, and 20; GMJ6B, B6, and IR79156B; PK21, BH95E, Bio9, and R14 became each one group. It seemed that the grouping followed the agro ecosystems and hybrid parental lines tended to stay in different group with inbred variety. Application of these 36 SSR markers had been able to distinguish the 26 genotypes, however more markers would give more powerful to distinguish among genotypes.

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