CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

ISSR and SRAP for assessing genetic variability of Indonesian local rice genotypes (*Oryza sativa* L.)

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Abstract: *This study evaluated the genetic variability of 34 local rice genotypes from Simeulue Island and Sumba Island, Indonesia, using ISSR and SRAP markers. The amplification of ISSR and SRAP primers produced high percentages of polymorphism, with values of 98.17% and 99.75%, respectively. The result of the UPGMA dendrogram analysis classified the rice genotypes into three groups by ISSR and two groups by SRAP. The ISSR had a higher Resolving Power (RP = 14.66) and Marker Index (MI = 4.61) than the SRAP (RP = 9.13, MI = 3.8). However, the results of the UPGMA dendrogram corresponded to the STRUCTURAL data analysis. The ΔK of STRUCTURAL analysis performed its maximum value when K = 3 by ISSR and K = 2 by SRAP. This study pointed out that ISSR markers were more effective than SRAP markers in evaluating the degree of genetic variability of rice genotypes.*

Keywords: *ISSR, genetic variability,* Oryza sativa *L., SRAP*

INTRODUCTION

Rice (*Oryza sativa* L.) is considered one of the major cereal crops in the world (Neupane et al. 2022). This Gramineae crop species is also known to produce grains containing a variety of nutritional component, such as carbohydrates, proteins, vitamins, and minerals (Verma and Srivastav 2020). Rice is widely cultivated under different ecological and geographical conditions, and about 90% of the world's rice is produced in the Asia-Pacific Region, including Indonesia (Bandumula 2017). Rice is now consumed as a staple food by over half of the world's population (Carcea 2021) and nearly 95% of the Indonesian population (Sulistyo et al. 2016).

In Indonesia, rice germplasm is one of the national assets that can be used for research, conservation, breeding, and other important purposes. Regarding conserving and managing germplasm resources, an understanding of the diversity of local rice cultivars is greatly required. Accordingly, a genetic reservoir of interesting genes can be found in the germplasm of local rice cultivars, which can subsequently be exploited in the plant breeding program to create improved rice varieties (Villa et al. 2005).

The evaluation of genetic diversity using molecular techniques in the post-genomic era has advanced dramatically. The molecular approach to assessing genetic diversity has a number of benefits, including being easier, faster, and more precise than other methods using morphological or biochemical markers (Vijayan 2005). ISSR (Inter Simple Sequence Repeat) and SRAP (Sequenced

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Repeat Amplified Polymorphism) are dominant PCR-based markers for molecular analysis (Sun et al. 2018). ISSR markers can detect polymorphisms between two microsatellite sequences without prior knowledge of the genome sequence (Zietkiewicz et al. 1994), whereas SRAP amplifies the genome's coding region by using forward primers that target GC-rich exons and reverse primers that target AT-rich promoters, introns, and spacers (Robarts and Wolfe 2014).

In this study, ISSR and SRAP markers were used to evaluate the genetic diversity and population structure of local rice cultivars in Indonesia. The information obtained may serve as valuable data for selecting the parental lines used in the breeding technique for plant genetic enhancement.

MATERIAL AND METHODS

Plant materials

The material genotypes used were collected from local rice cultivars in Indonesia. Information on the rice genotypes, along with the coordinates of the collection sites, is presented in Table 1.

Table 1. Genotypes and geographic origin of local rice genotypes used in this study

No	Genotype code	Geographic Origin	Latitude (S)	Longitude (E)	Group*
1	M1_A(G)	Seubah Village, Simeulue Island	2°28'35.98"	96°22'33.82''	A
2	M2_A	Latitik Village, Simeulue Island	2°38'34.12"	95°59'47.52''	A
3	M3_A	Lambaya Village, Simeulue Island	2°37'59.50"	96°1'59.23''	A
4	M4_A**	Serdang Village, Bangka Island	2°53'49.19"	10°22'10.03''	A
5	M5_A	Leubang Hulu Village, Simeulue Island	2°28'5.19"	96°17'53.79''	A
6	M6_A	Leubang Hulu Village, Simeulue Island	2°28'5.19"	96°17'53.79''	A
7	M7_A	Padang Unoi Village, Simeulue Island	2°41'12.52"	95°48'41.83''	A
8	M8_A	Lambaya Village, Simeulue Island	2°37'59.5"	96°1'59.23''	A
9	M9_A(G)	Lambaya Village, Simeulue Island	2°37'59.5"	96°1'59.23''	A
10	M10_A(G)	Lafaha Village, Simeulue Island	2°44'7.37"	95°46'20.36''	A
11	M11_AI(G)	Langi Village, Simeulue Island	2°48'7.84"	95°46'41.8''	A
12	M12_AII(G)	Padang Unoi Village, Simeulue Island	2°41'12.52"	95°48'41.83''	A
13	M13_A	Leubang Hulu Village, Simeulue Island	2°28'5.19"	96°17'53.79''	A
14	M14_A	Air Pinang Village, Simeulue Island	2°33'58.67"	96°14'19.54''	A
15	M15_A	Latitik Village, Simeulue Island	2°38'34.12"	95°59'47.52''	A
16	M16_A	Leubang Hulu Village, Simeulue Island	2°28'5.19"	96°17'53.79''	A
17	M17_A(G)	Leubang Hulu Village, Simeulue Island	2°28'5.19"	96°17'53.79''	A
18	M18_A	Leubang Hulu Village, Simeulue Island	2°28'5.19"	96°17'53.79''	A
19	M19_A	Labuhan Bukti Village, Simeulue Island	2°28'5.19"	96°17'53.79''	A
20	M20_A(G)	Seubah Village, Simeulue Island	2°28'35.98"	96°22'33.82''	A
21	M21_A	Seubah Village, Simeulue Island	2°28'35.98"	96°22'33.82''	A
22	M22_A	Padang Unoi Village, Simeulue Island	2°41'13.23"	95°48'41.94''	A
23	M23_A	Latitik Village, Simeulue Island	2°38'34.12"	95°59'47.52''	A
24	M24_A(G)	Lafaha Village, Simeulue Island	2°34'59.99"	96°4'59.99''	A
25	M25_B	Praingkareha Village, Sumba Island	10°1'12.28"	120°4'4.87''	B
26	M26_B(GH)	Wudipandak Village, Sumba Island	9°58'30.86"	120°3'18.97''	B
27	M27_B	Wahang Village, Sumba Island	10°4'42.64"	120°3'17.88"	B
28	M28_B	Pinduhurani Village, Sumba Island	10°1'24.36"	120°0'31.45''	B
29	M29_B(GH)	Praing Kareha Village, Sumba Island	10°1'12.28"	120°4'4.87''	B
30	M30_B	Praing Kareha Village, Sumba Island	10°1'12.28"	120°4'4.87''	B
31	M31_B	Matawai La Pau Village, Sumba Island	10°11'46.31"	120°21'59.79"	B
32	M32_B(G)	Praing Kareha Village, Sumba Island	10°1'12.28"	120°4'4.872'	B
33	M33_B	Ramuk Village, Sumba Island	10°4'29.02"	120°8'18.038''	B
34	M34_B(G)	Wahang Village, Sumba Island	10°4'42.64"	120°3'17.88"	B

Group* is defined by A and B for rice genotypes collected from Simeulue Island and Sumba Island, respectively. MA_4** is the only rice genotype collected from Bangka Island, but it is classified in Group A due to the closer geographical distance of the collection site of this genotype to Simeulue Island than to Sumba Island.

DNA extraction

The genomic DNA of each rice genotype was extracted from leaf tissue using a commercially available kit (Genomic DNA microKit, Geneaid Biotech Ltd.) in accordance with the manufacturer's instructions. In order to accurately measure the quality of the DNA samples, the UV-Vis spectrophotometry NanoDrop 2000/c (Thermo Fisher Scientific) was used with a ratio of absorbance between 260 nm and 280 nm. The pure DNA sample particularly shows a 260/280 ratio of ~1.8.

ISSR-PCR fingerprinting

ISSR-PCR fingerprinting was prepared in a total volume of 10 µL. The PCR process started with a 5-minute denaturation step at 94 °C. This was followed by 40 cycles, each comprised of a denaturation step at 94 °C for 30 seconds, an annealing step at 94 °C for 30 seconds, and an extension step at 72 °C for 30 seconds. Furthermore, a final extension step was executed at 72 °C for 10 minutes. Amplification products were fractionated by 1.5% (w/v) TAE agarose gel electrophoresis under a constant voltage of 160 V for 90 minutes. The size of each DNA band produced was estimated using a 100-bp DNA ladder (Geneaid Biotech, Ltd). The gel electrophoresis result was then visualized and photographed.

SRAP-PCR fingerprinting

The 25 SRAP primers listed in Table 2 were used to amplify the genomic DNA of rice genotypes. The PCR reaction was performed according to the method of Li and Quiros (2001). SRAP-PCR fingerprinting was performed under the initial denaturation step for 5 minutes at 94 °C, followed by 5 cycles of 1 minute at 94 °C for the denaturation step, 1 minute at 35 °C for the annealing step, and 1 minute at 72 °C for the elongation step. The remaining 30 cycles of the PCR reaction were carried out for 1 minute at 50 °C for the annealing step and 7 minutes at 72 °C for the final elongation step (Ferriol et al. 2003). TAE agarose gel electrophoresis at 1.5% (w/v) was used to separate the PCR products for 90 minutes at 160 volts. The DNA size in each band produced was calibrated using a 100-bp DNA ladder, and the result was then photographed by the gel documentation system.

Data scoring and analysis

Across the 34 rice genotypes used in this investigation, PCR amplification products were scored based on the presence (1) or absence (0) of distinctive, independent, and reproducible DNA fragments. The binary data matrix generated from score data was used to determine the population structure and genetic diversity of the rice genotypes studied. The parameters measured included the number of total bands (NTB), the number of polymorphic bands (NPB), the percentage of polymorphism (PP), the number of monomorphic bands (NMB), polymorphic information content (PIC), resolving power (RP), and marker index (MI). NTB was calculated using the total number of clear DNA bands generated by PCR amplification. NPB was calculated based on the distinct positions of the DNA bands across the lanes. PP was calculated using the NPB/NTB ratio. NMB was determined by the number of DNA bands amplified across all genotypes. PIC value for each locus was calculated according to the formula of Serrote et al. (2020). RP is determined by the summation of band informativeness using the formula $RP = \Sigma Ib$, where Ib is given by $1 - (2 \times |0.5 - p|)$, with p representing the proportion of total genotypes containing the band. MI was derived

Table 2. List of ISSR and SRAP primers for PCR amplification

	Primers	Sequences (5´ - 3´)	Number of nucleotides
		ISSR primers	
1	UBC 807	AGA GAG AGA GAG AGA GT	17
2	UBC 808	AGA GAG AGA GAG AGA GC	17
3	UBC 809	AGA GAG AGA GAG AGA GG	17
4	UBC 810	GAG AGA GAG AGA GAG AT	17
5	UBC 813	CTC TCT CTC TCT CTC TT	17
6	UBC 814	CTC TCT CTC TCT CTC TA	17
7	UBC 815	CTC TCT CTC TCT CTC TG	17
8	UBC 817	CAC ACA CAC ACA CAC AA	17
9	UBC 819	GTG TGT GTG TGT GTG TA	17
10	UBC 820	GTG TGT GTG TGT GTG TC	17
11	UBC 823	TCT CTC TCT CTC TCT CC	17
12	UBC 825	ACA CAC ACA CAC ACA CT	17
13	UBC 826	ACA CAC ACA CAC ACA CC	17
14	UBC 862	AGC AGC AGC AGC AGC AGC	18
15	UBC 880	GGA GAG GAG AGG AGA	18
		SRAP primers	
1	Me1 (forward)	TGAGTCCAAACCGGATA	17
2	Me2 (forward)	TGAGTCCAAACCGGAGC	17
3	Me3 (forward)	TGAGTCCAAACCGGACC	17
4	Me4 (forward)	TGAGTCCAAACCGGTAG	17
5	Me5 (forward)	TGAGTCCAAACCGGTGT	17
6	Em1 (reverse)	GACTGCGTACGAATTTGC	18
7	Em2 (reverse)	GACTGCGTACGAATTGCA	18
8	Em3 (reverse)	GACTGCGTACGAATTAGC	18
9	Em4 (reverse)	GACTGCGTACGAATTTAG	18
10	Em5 (reverse)	GACTGCGTACGAATTGGT	18

from the formula MI = PIC × npi, where npi is the number of polymorphic bands, as outlined by Powell et al. (1996).

The Past4.03 software (Hammer et al. 2001) was applied to calculate similarity and distance indices between rice genotypes. The results were then used by MEGA-X (Molecular Evolutionary Genetics Analysis) software to perform clustering analysis and create an unweighted pair group method based on arithmetic averages (UPGMA) dendrogram. The population structure of rice genotypes in this study was determined by a model-based clustering method in the software STRUCTURE ver.2.3.4 (Pritchard et al. 2000). A graphical method was developed to identify the genetically distinct populations using ΔK and Ln probability data, according to Evanno et al. (2005).

RESULTS AND DISCUSSION

Polymorphism analysis

In this study, the data obtained from both molecular markers, ISSR and SRAP, showed high effectiveness in differentiating the 34 rice genotypes. The amplification of 15 ISSR primers resulted in a total of 271 high-quality, reproducible band products with an average of 18.07 bands per primer (Table 3). The highest value of the total number of bands (TNB) was generated by the UBC 826 primer, which produced 24 bands, while the lowest was 12 bands for the UBC 814 and UBC 862 primers. There were 267 polymorphic bands identified by ISSR primers, with a range of PP percentages from 87.5% to 100%. Subsequently, the amplification of 25 SRAP primers resulted in varying TNB values per primer pair, from 6 bands for the Me1-Em5 primer pair to 20 bands for the Me5-Em5 primer pair, with an average of 14.08. A total of 351 out of 352 bands produced by SRAP were found to be polymorphic, which means that all SRAP primers used to amplify the rice genotypes in this study resulted in the polymorphic bands, except the Me2-Em3 primer pair (Table 3).

It has been reported that ISSR and SRAP were extensively used to test polymorphism at the DNA level in various plant genotypes (Yan et al. 2019). ISSR and SRAP molecular markers have been found to selectively target specific regions of the genome. ISSR primers amplified the region between microsatellite sequences in the entire rice genome DNA sequence, whereas SRAP primers amplified only the targeted open reading frame (ORF) region as the functional region of the genome (Zietkiewicz et al. 1994, Li and Quiros 2001).

Other results that showed the ability of ISSR and SRAP markers to produce a high percentage of polymorphism (PP), have also been reported in other studies, such as in sorghum (Medraoui et al. 2007), faba bean (Elshafei et al. 2019), cowpea (Rini and Nuraisyah 2021), and wheat (Shaban et al. 2022). As shown in Table 3, ISSR produced more amplicons than SRAP. Both markers, ISSR and SRAP, generated high averages of PP (percentage of polymorphism), with values of 98.17% and 99.75%, respectively.

The study presented the results of PIC for both ISSR and SRAP markers (Table 3). Among all the ISSR primers tested, the UBC 808 primer generated the highest PIC value of 0.36, while the UBC 819 primer produced the lowest value of 0.18, and the average PIC value for all ISSR primers tested was 0.26. Hence, the PIC values for SRAP markers ranged from 0.17 to 0.44, with an average of 0.28. Me3-Em1 and Me3-Em4 primer pairs had the lowest PIC value among SRAP markers, whereas Me1-Em1 and Me1-Em3 primer pairs generated the highest PIC value.

Cluster analysis

The molecular genetic relationships among 34 rice genotypes were constructed by UPGMA cluster analysis based on the Euclidean distance coefficient using ISSR and SRAP markers (Figure 1). The UPGMA dendrogram grouped the rice genotypes into three distinct clusters using ISSR primers. Cluster 1 contained 23 genotypes collected mostly from Simeulue Island, and Cluster 2 consisted of a majority of the genotypes from Sumba Island (8 genotypes), with the remaining 2 genotypes in Cluster 3 (Figure 1a). The UPGMA method, using SRAP markers, divided 34 rice genotypes into two major clusters. All rice genotypes grouped in Cluster 1 were collected from Simeulue Island. The Sumba Island genotypes predominated in Cluster 2. However, genotypes from Simeulue Island were included in Cluster 2 as a sub-cluster (Figure 1b).

Genetic diversity indices were analyzed according to ISSR and SRAP on rice populations collected from Simeuleu and Sumba Islands (Table 4). Data from the ISSR analysis showed that the parameters of polymorphic loci, the number of effective alleles (Ne), Shannon's Information Index (I), and Nei's gene diversity (h) in the Simeulue population were higher

and statistically different as compared to those of the Sumba population (ρ<0.01). Also, the SRAP marker generated similar results to the ISSR markers, which informed that Ne, I, and h values in the Simuelue population were significantly higher

Table 3. Genetic diversity of rice genotypes by ISSR and SRAP primers

No	Primers	NTB	NPB	PP	PIC	RP	MI
ISSR							
1	UBC 807	18	18	100	0.23	11.71	4.14
2	UBC 808	23	23	100	0.36	24.71	8.28
3	UBC 809	15	14	93.33	0.25	17.88	3.50
4	UBC 810	16	14	87.5	0.21	16.65	2.94
5	UBC 813	15	15	100	0.32	8.76	4.80
6	UBC 814	12	12	100	0.23	6.35	2.76
7	UBC 815	21	21	100	0.30	11.00	6.30
8	UBC 817	23	23	100	0.29	21.41	6.67
9	UBC 819	16	16	100	0.18	3.94	2.88
10	UBC 820	17	17	100	0.22	8.41	3.74
11	UBC 823	14	14	100	0.25	12.18	3.50
12	UBC 825	23	23	100	0.28	16.47	6.44
13	UBC 826	24	24	100	0.22	32.59	3.74
14	UBC 862	12	11	91.67	0.20	7.18	2.20
15	UBC 880	22	22	100	0.33	20.65	7.26
	Total	271	267				
	Mean	18.07	17.8	98.17	0.26	14.66	4.61
SRAP							
1	Me1-Em1	17	17	100	0.44	14.47	7.48
2	Me1-Em2	14	14	100	0.42	12.29	5.88
3	Me1-Em3	14	14	100	0.44	12.71	6.16
4	Me1-Em4	12	12	100	0.42	9.53	5.04
5	Me1-Em5	6	6	100	0.4	5.59	2.4
6	Me2-Em1	14	14	100	0.2	4.35	2
7	Me2-Em2	10	10	100	0.26	4.47	2.6
8	Me2-Em3	16	15	94	0.17	5.76	2.38
9	Me2-Em4	19	19	100	0.25	6.53	4.25
10	Me2-Em5	11	11	100	0.2	6.35	1.8
11	Me3-Em1	14	14	100	0.17	2.82	2.38
12	Me3-Em2	16	16	100	0.21	5.12	3.36
13	Me3-Em3	17	17	100	0.24	5.65	4.08
14	Me3-Em4	19	19	100	0.17	3.82	3.23
15	Me3-Em5	19	19	100	0.3	8.29	5.7
16	Me4-Em1	15	15	100	0.22	11.53	3.3
17	Me4-Em2	14	14	100	0.33	13.35	4.62
18	Me4-Em3	7	7	100	0.33	7.24	2.31
19	Me4-Em4	13	13	100	0.23	10.00	2.99
20	Me4-Em5	11	11	100	0.3	11.71	3.3
21	Me5-Em1	16	16	100	0.35	18.65	5.6
22	Me5-Em2	13	13	100	0.3	14.24	3.9
23	Me5-Em3	11	11	100	0.23	8.29	2.53
24	Me5-Em4	14	14	100	0.26	11.59	3.64
25	Me5-Em5	20	20	100	0.2	13.88	4
	Total	352	351				
	Mean	14.08	14.04	99.75	0.2816	9.13	3.80

NTB: number of total bands, NPB: number of polymorphic bands, PP: percentage of polymorphism, NMB: number of monomorphic bands, PIC: polymorphic information content, RP: Resolving Power, MI: Marker Index.

than those of the Sumba population (ρ<0.01). The significant difference in the genetic diversity parameter between the rice populations in Simeulue and Sumba Islands pointed out that there was a strong correlation between the geographic origin and the genetic diversity of the rice populations revealed in this study. Therefore, it was suggested that the rice genotypes performed local adaptations related to different geographical and ecological conditions between Simeulue and Sumba Islands, thereby showing the genetic divergence between these populations. Furthermore, the statistical analysis of genetic diversity parameters showed that there were no significant differences in Ne, I, and h values between ISSR and SRAP markers (Table 4). These results indicated that both markers had similar results in identifying the genetic diversity of rice genotypes at the population level in both Simuelue and Sumba Islands.

The analysis of molecular variance (AMOVA) was carried out to determine the population differentiation of rice genotypes by ISSR and SRAP markers (Table 5). The AMOVA based on the ISSR markers clarified that a total of 10% of genetic variation was attributed to variation among the population, while a majority of 90% was due to variation within

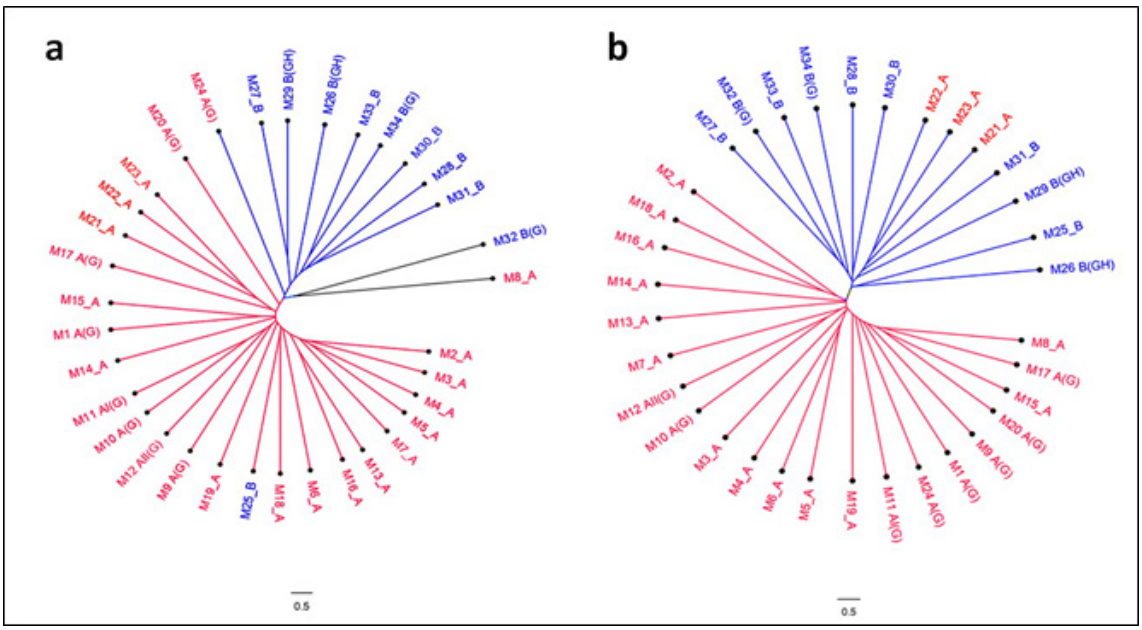

Figure 1. Circular UPGMA dendrograms using Euclidean distance coefficients of 34 rice genotypes. (a) Circular dendrogram constructed by ISSR primers. (b) Circular dendrogram constructed by SRAP primer combinations. The different branch colors indicate the different clusters. The colors of genotype labels indicate the geographic origin of the genotypes; Red for Simeulue Island (group A); Blue for Sumba Island (group B).

Table 4. Diversity parameters of local rice populations revealed by ISSR and SRAP markers

Population	Sample size	Number of loci	% polymorphic loci	Ne	I	h
ISSR marker						
Simeulue	24	225	90.59	1.340 a	0.358 a	0.222 a
Sumba	10	225	63.92	1.313 b	0.303 b	0.195 b
SRAP marker						
Simeulue	24	309	88.35	1.337 a	0.350 a	0.218 a
Sumba	10	309	73.75	1.326 b	0.310 b	0.201 b
Mean				Ne	I	h
ISSR marker				1.326 a	0.331 a	0.209 a
SRAP marker				1.332 a	0.330 a	0.210 a

Ne: number of effective alleles; I : Shannon's Information Index; h: Nei's gene diversity. Values followed by the different letters indicate significant difference between populations at ρ < 0.01

Table 5. Analysis of molecular variance (AMOVA) by ISSR and SRAP markers

Source	df	SS	MS	Est. Var.	%
ISSR marker					
Among populations	1	74.12	74.12	3.19	10
Within populations	32	928.90	29.02	29.02	90
Total	33	1003.02		32.22	100
Stat	Value	ρ			
PhiPT	0.099	0.001			
SRAP marker					
Among populations	1	99.68	99.68	4.58	12
Within populations	32	1119.25	34.97	34.97	88
Total	33	1218.94		39.56	100
Stat	Value	ρ			
PhiPT	0.116	0.001			

the population. In addition, the AMOVA by SRAP markers revealed that the variation within populations (88%) was higher than among populations (12%). According to the information used to estimate the population structure, the PhiPT values for ISSR and SRAP were 0.099 ($\rho<0.001$) and 0.116 ($\rho<0.001$), respectively, thereby indicating the statistically significant differences among rice populations by both ISSR and SRAP markers.

In this study, rice populations refer to the groups of rice genotypes collected from different islands (Table 3). The simulation produced the model without admixture by varying K from 1 to 10 over three iterations. By using the ISSR markers examined on 34 rice genotypes, the STRUCTURE output showed that ΔK performed its maximum value when K = 3 (Figure 2a). Accordingly, all the individual rice genotypes were assigned into three distinct groups, as presented in Figure 2b. Group I consisted of 21 genotypes, all of which were collected from Simeulue Island. Group II comprised 5 genotypes sampled from Simeulue Island and Sumba Island. The remaining 8 genotypes, all individuals collected from Sumba Island, were clustered into group III. Meanwhile, the results of the STRUCTURE harvester via SRAP markers have demonstrated that a distinct and sharp peak of ΔK was attained at K = 2 (Figure 2c), thereby signifying the existence of two distinct clusters. Hence, the probability value of K was used to stratify 34 rice genotypes into two clustering patterns (Figure 2d). Group I contained 14 genotypes, dominated by 10 genotypes from Sumba Island, and the remaining 4 genotypes were collected from Simeulue Island. Furthermore, group II consisted of 20 genotypes from Simeulue Island. However, the analysis of population structure by ISSR and SRAP (Figure 2) revealed similar clustering patterns to the UPGMA

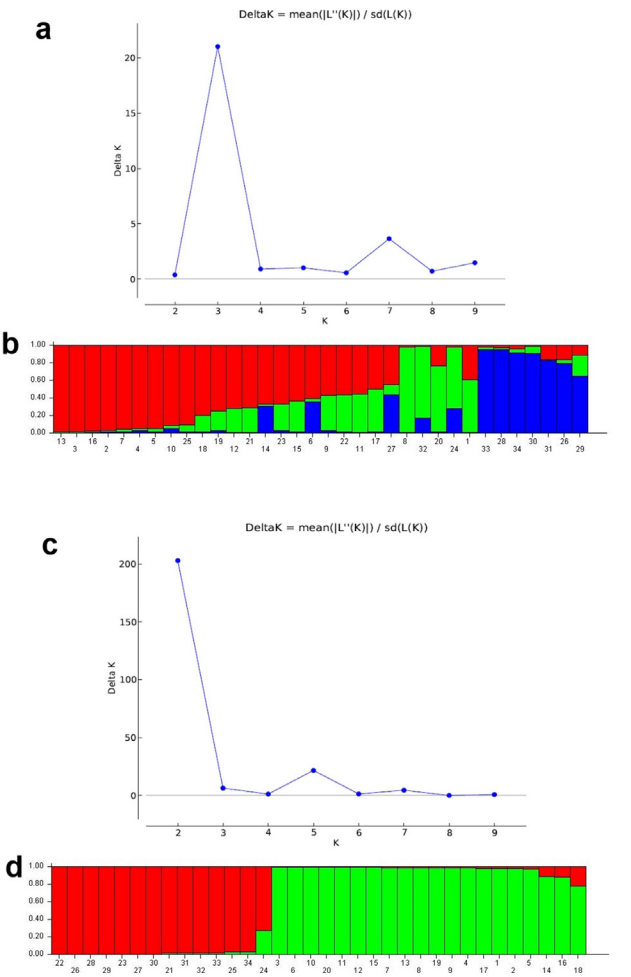

Figure 2. Population structure of rice genotypes. (a) Delta K values in the structure analysis by ISSR, (b) Bar plot at K = 3 by ISSR. (c) Delta K values in the structure analysis by SRAP, (d) Bar plot at K = 2 by SRAP.

cluster analysis (Figure 1). Therefore, it has been established that the ISSR markers employed in this study were more effective than the SRAP markers in assessing the genetic diversity of rice genotypes, based on the Resolving Power and Marker Index as the informative indices for determining marker efficacy. As presented in Table 3, ISSR exhibited higher RP and MI than SRAP.

The genetic diversity and population structure of rice germplasm assessed in our study served as the basic knowledge for rice improvement. The diversity of plant genotypes provides an opportunity for plant breeders to develop new and superior cultivars. Hybridization in plant breeding should be carried out by using genetically diverse genotypes as the parental lines to obtain a wide range of segregants. Therefore, the diversity of local rice genotypes from Indonesia revealed in our study can certainly be used as a source of genes for further breeding programs to produce commercial rice varieties.

CONCLUSION

This study showed that ISSR and SRAP were successful in identifying the population structure and variability of the local rice genotypes. ISSR markers were more robust than SRAP due to the higher value of RP and MI of ISSR than that of SRAP. The fact that rice genotype diversity was higher within populations than among populations was elegantly presented by both ISSR and SRAP markers. The results of STRUCTURE analysis, which grouped the rice genotypes based on geographic region, also supported the UPGMA clustering analysis findings.

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AUTHOR CONTRIBUTIONS

DSR obtained funding, supervised the experiment process, prepared the materials and instrument tools, designed the concept, analyzed the data, wrote the manuscript, and corresponded with the journal during the submission and review process. YB and MV carried out the experiments, analyzed the data, and wrote the manuscript. RP supervised the experiment process.

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