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ORIGINAL RESEARCH

Genetic diversity of rice germplasm (*Oryza sativa* L.) of java island, Indonesia

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Abstract

The role of genetic diversity in crop germplasm is an important concept within genetic conservation. In this research, 43 accessions were analyzed at the agro-morphological and genetic levels. Clustering based on the agro-morphological resulted in four sub-groups. Analysis at the genetic level was conducted using 22 microsatellites, which revealed a total number of alleles to be 203, with a range per allele between 2 and 17 and an average of 9.2 alleles per locus. The highest and lowest Polymorphic Information Content (PIC) values were found in RM431 and RM11, which were 0.95 and 0.67, respectively. The genetic diversity value ranged from 0.71 to 0.95. The genetic similarity among accessions ranged from 0.00 to 0.90. Clustering based on the genetic relatedness divided the Java rice samples into two major groups. The classification created through this research many inform future breeding programs aimed at improving the quality and quantity of yield production.

Keywords Diversity · Agro-morphological · SSR · Rice · Germplasm

Introduction

Rice (*Oryza sativa* L.) is the main staple food rich in carbohydrates and the largest ex-situ germplasm in the world. It is the second most important crop in the world after wheat (Rajamoorthy et al. 2015). Rice is one plant that requires plenty of water and heat also well suited to grow in a tropical climate like Indonesia. According to the FAO database (FAOSTAT: https://www.fao.org/faostat/en/#home), Indonesian ranks the third-largest rice production (70 million tons) in 2014 after China (208 million tons) and India (155 million tons). In Indonesia, five provinces produce most rice which are South Sumatra, West Java, Central Java, East Java, and South Sulawesi. Based on BPS (2013), in 2012, Java Island harvested area of paddy field is 6,165,079 Ha equivalent to 46.89% from the total Indonesian harvested area. Moreover, every harvested area produces rice as many as 34,404,557 tons equivalent to 52.32% from the national paddy of Indonesia.

Java has six provinces such as Banten, DKI Jakarta, West Java, Central Java, Yogyakarta, and East Java of around 138,793.6 km^2 with a density of 1317 people/km^2. Java was formed from a series of volcanoes running west to east. More volcano mountains help to split the interior into a series of relatively isolated regions suitable for rice cultivation. Volcanoes and an abundant supply of water resources, these factors make fertile agricultural land in addition to increasing the potential for genetic diversity. As such, the island of Java was chosen as a focus area for this study because of its rich germplasm which may be used to generate new and superior varieties supply (BPS 2015).

The genotypes of germplasm were focused on for this analysis so that information could be collected from every accession about their properties and important agronomic characteristics (Ahnert et al. 2017). Individual accession

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was important because different germplasm has different methods of adaptation to their local environments based on the climate condition which formed several rice diversities (Hue et al. 2018). It can be used to plant breeding program which has target traits that need to investigated (Nachimuthu et al. 2015).

Characterization of genetic diversity is used by breeders as a tool to reveal information about the genetic resources of closely-related accessions. In any crop improvement program, genetic diversity is the main source of variability for adjusting complexes like quantitative traits, yield, and quality. Thus, knowledge about the relationship between genotypes is important for predicting the effects and results of various breeding and conservation strategies (Gasim et al. 2019).

DNA-based molecular markers, especially have been used extensively for the study of genetic diversity, over morphological, pedigree, heterosis, and biochemical data (Yadav et al. 2013). Compared with agro-morphological markers, they are not influenced by environmental factors and are generally more sensitive to differences among genotypes at the DNA level, thus increasing their detection efficiency and fast (Ming et al. 2010). Currently, simple sequence repeats (SSRs) or microsatellites are the molecular tools used for diversity evaluation and detecting relationships among different crop species, populations, or individual rice accessions (Pachauri et al. 2013). According to Allhgolipour et al. (2014), SSRs markers suitable for evaluating genetic diversity among closely related rice accessions. Previous studies of Wang et al. (2014) have identified that microsatellite loci or knowns as simple sequence repeats (SSRs) may be used to detect genetic variation and genetic relationships within rice through genome studies as well as allelic diversity analysis.

This research set out to investigate the genetic diversity of 54 rice accessions based on the 22 SSR markers distributed from chromosome one through 12. The aims of this study were to: (1) analyze the genetic diversity of rice germplasm from the island of Java; (2) collect information from every rice germplasm regarding the genetic similarity between them.

Material and methods

Plant material

In this present study, 54 accessions were analyzed including 43 different accessions (Table 1) of rice germplasm were collected from provinces on Java island including Banten, West Java, Middle Java, East Java, and Yogyakarta (Fig. 1). In this research, there is five control from the *Indica* group and six control from the *Japonica* group. Control *Indica* such as IR64, IR66, IR46, IR36, and Taichung Native 1 (Roy et al. 2016). While control *Japonica* which is Nagdong, Ilpum, Cuchong, Hwayeong, Dongjin, and Nipponbare.

Agro-morphological characterization

Agro-morphological traits were measured including days to flowering, plant height, leaf length, leaf width, flag leaf length, flag leaf width, culm length, culm internode length, grain length, grain width, grain length to the width, 1000 grain weight, panicle length, panicle per plant, and ligule length.

Genotyping characterization

Fresh and young green leaf samples were collected from 28-day seedlings during the vegetative phase. In this study, DNA from leaves tissue was extracted according to the SDS protocol with minor modification (Dellaporta et al. 1983). NanoDrop spectrophotometer (Thermo Scientific NanoDrop™ 1000 Spectrophotometer) was used to quantify DNA. A total of 22 SSR primer pairs (markers) were used for genetic diversity analysis for the 12 chromosomes available in rice plant cells. PCR reactions of 10 µl were carried out using a Programmable Thermal Cycler (MJ Research Inc. USA) that consisted of 1 µl DNA template, 5 µl PCR Nexpro master mix reaction, 0.5 µl of each primer forward and reverse and 3 µl DDH2O water. The temperature cycles were programmed for 94 °C for 5 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, for 35 cycles and additional temperature of 72 °C for 5 min for the final extension. The quality of DNA was visualized by running it on 1.7% agarose gel with 1 × TAE buffer (Tris-acetate-EDTA) at 100 V for 30 min and observed by UV Reader. The only gels which were scored were those on which unambiguous bands appeared (Saini et al. 2004; Wong et al. 2009).

Statistical and genetic diversity analysis

The number of alleles per locus, major allele frequency, gene diversity, and Polymorphism Information Content (PIC) values was calculated using Power Marker Version-3.25 (Liu and Muse 2005). The presence and absence of the SSR bands were scored for all 43 genotypes with 11 controls from *Indica* and *Japonica*. Then, the data were exported to the binary format which is one for presence allele and zero for absence allele. Nevertheless, the UPGMA (unweighted pair group method with arithmetic means) dendrogram was constructed using PAST (Hammer et al. 2001) and Jaccard's coefficient to show the similarity index interrelationship among accessions.

Table 1 List of accessions in this study

No	Genotype	Origin	Pedigree	Grain type
1	Kewal Bulu Hideung	Banten	Local rice	Slender
2	Tambleng	Banten	Local rice	Slender
3	Kewal Gudril	Banten	Local rice	Slender
4	Kambang	Banten	Local rice	Medium
5	Pare Mas	Banten	Local rice	Medium
6	Kewal Sampai Putih	Banten	Local rice	Medium
7	Kewal Benur	Banten	Local rice	Medium
8	Seksek	Banten	Local rice	Medium
9	Care Ware	Banten	Local rice	Slender
10	Care Lintang	Banten	Local rice	Medium
11	Tungguh Hideung	Banten	Local rice	Medium
12	Kewal Bulu Putih	Banten	Local rice	Medium
13	Jalawara	Banten	Local rice	Medium
14	Kuriak Kusuik	Banten	Local rice	Medium
15	Care Merah	Banten	Local rice	Medium
16	Ketan Bayong	Banten	Local rice	Medium
17	Inpago	West Java	Batutegi/cigeulis/ciherang	Slender
18	Mota	West Java	Local Rice	Medium
19	Ketan Hitam II	West Java	Local Rice	Medium
20	Beureum Taleus	West Java	Local Rice	Medium
21	Segon Geulis	West Java	Local Rice	Slender
22	Inpari 14	West Java	Cipeundeuy C/Carreon//Wae Apo Buru///IR64	Slender
23	Pandan Wangi II	West Java	Local rice	Medium
24	Cibogo	West Java	S487B-75/2*IR19661-131-3-1//2*IR64	Slender
25	Conde	West Java	IR64*6/IRBB7	Slender
26	Situ Patenggang	West Java	Kartuna/TB47H-MR-10	Medium
27	Towuti	West Java	S499B-28/Carreon//2*IR64	Medium
28	Martapura	West Java	Siam Unus/Dodokan	Medium
29	Mendawak	West Java	Mahsuri/Kelara	Medium
30	Siak Raya	West Java	Batang Ombilin/Kelara	Medium
31	Banyuasin	West Java	Cisadane/Kelara	Medium
32	Dendang	West Java	Osok/IR5657-33-2	Medium
33	Indragiri	West Java	B6256-MR-3-5P/Barumun//Rojolele/IR68	Medium
34	Sertani	West Java	Local rice	Slender
35	Ketan Unggul	West Java	Local rice	Medium
36	Anak Doro Magelang	Middle Java	Local rice	Slender
37	Merah Saleman	Yogyakarta	Local rice	Medium
38	Situbagendit	East Java	Local rice	Medium
39	Susu Putih	East Java	Local rice	Slender
40	Mentik	East Java	Local rice	Slender
41	Pandan Wangi	East Java	Pandanwangi cianjur 1596	Medium
42	Lamongan I	East Java	Local rice	Medium
43	Jember I	East Java	Local rice	Slender

Results

Agro-morphological characterization and principal component analysis

In this research, data were collected for 15 agronomic traits (DtF, Ht, LL, LW, FLL, FLW, CL, CmIL, GrL, GrW, GrLW, TGW, PL, PnP, and LgL). This data was analyzed across the five provinces used for this research (Table 2). The period of days to flowering differed across regions. West Java had the highest average with 74.50 ± 5.89 compared with another region East Java (73.93 ± 8.85)

Fig. 1 Map location of the accessions collection sites in the five provinces in Java Island

and Middle Java (73.00 ± 7.94). The mean for days to flowering found in samples from Banten province with 71.71 ± 6.55 as the overall average, with the *Japonica* control at 83.22 ± 8.46 as the highest value and the *Indica* control as the lowest (70.13 ± 7.38). Regarding grain length, Middle Java again showed the highest mean with minimum and maximum values of 6.50 ± 0.41, 6.23, and 6.98, respectively. Middle Java was observed to show the lowest mean with min and max compared to control *Japonica* (6.37 ± 0.22; 5.00 and 7.97) and control *Indica* (6.04 ± 0.53; 5.51 and 6.52). This showed that Middle Java had longer grain length 0.13 cm compared to control *Japonica* and also longer grain length 0.46 cm compared to control *Indica*. Among the agro-morphological traits, there were some highly correlated phenotypic characters, such as LW and GrL (0.68) and TGW and LgL (0.67). These values indicate a positive correlation between them.

PCA was used to analyze the forty-three accessions with eleven controls accessions for all fifteen agro-morphological traits (Fig. 2). The high positive loading in the PCA analysis contained Ht, CL, LL, and CmIL. PC1 showed the high positive loading to be Plant Height (Ht) with a value of 0.88 while PC2 showed the high positive loading to be Culm Length (CL) with 0.74. PC3 showed the positive loading is Leaf Length (LL) with 0.67. Moreover, The highest positive loading by Culm Internode Length (CmIL) is showed PC4 with 0.98. PCA results showed that four principal components (Eigenvalue > 1) cumulatively accounted for 63.2% of the total phenotypic variance. The principal component analysis performed using 15 agro-morphological traits revealed no clears groups and no specific clusters based on region. Almost of accessions from West Java and Banten on the left position in PCA biplot, while almost of accessions of Middle Java, Yogyakarta and East Java on the right position in PCA biplot.

Agro-morphological clustering

Agro-morphological characters were clustered based on Ward's Hierarchical Cluster, revealing two categories similar to the results from PCA (Fig. 3). Group I accounted for 14 genotypes with one control variety (IR 66). Two sub-clusters were formed which labeled as IA that consist of 6 accessions from West Java, while group IB consists of nine accessions from variation region from the variation region (Banten, West Java, Middle Java, and East Java). *Indica* group controls IR 36, IR 64, TN1, and IR 46 were found in group IIA. Two sub-clusters were found within IIA consisting of 18 accessions, and IIB contained 11 accessions.

Statistical and genetic diversity analysis

The Polymorphism Information Content (PIC) value reflects allelic diversity and frequency among molecular markers. The PIC value of each marker can be evaluated based on alleles as well as by comparing it to other SSR loci. The highest PIC value was that of RM431 at 0.95, followed by RM 452 at 0.94 (Table 3). The lowest PIC was found at RM11, at 0.67. The highest genetic diversity value was 0.97 at RM431 followed by RM452 (0.96), and RM408 (0.95). The lowest genetic diversity value was found at RM11 0.69 followed by RM154 (0.70) and RM536 (0.74). With a range of 2–17 in the number of alleles per locus, and an average of 9.2.

Genotypic clustering

Cluster analysis based on the UPGMA method using Jaccard's coefficient grouped the 54 accessions into two larger clusters at similarity coefficient ranged from 0.00 to 0.90 of and divided into Group I (IA, IB, and IC) and Group II (Fig. 4). Cluster IA had the highest number of accessions with 23 accessions, mostly from Banten (9) and West java (8) origin except for one accession (Jember I). Group IA was assumed closeness to *Indica* group. Cluster IB had 13 accessions, mostly from West java (7), East Java (2), and Middle Java (1), while cluster IC had 15 accessions, mostly from Banten (7), Yogyakarta (1) and East Java (3). Three accessions (Inpari 14, Pandan Wangi, and Cibogo) were formed in cluster II.

Discussion

Genetic diversity is the source of the potential for improvements in rice breeding programs. Essentially, genetic diversity offers breeders selections that may produce superior varieties of rice. Rice germplasm is a gene donor that informs plant characters that have superior traits such as

Table 2 Agro-morphological characters analysis

Traits		Origin						
		Banten	West Java	Middle Java	East Java	Yogyakarta	Control Japonica	Control Indica
DtF	Mean ± SD	71.71 ± 6.55	74.50 ± 5.89	73.00 ± 7.94	73.93 ± 8.85	71.00 ± 6.00	83.22 ± 8.46	70.13 ± 7.38
	Min	61.00	62.00	64.00	60.00	65.00	69.00	60.00
	Max	84.00	88.00	79.00	83.00	77.00	93.00	87.00
	CV%	9.14	7.91	10.87	11.97	8.45	10.17	10.52
Ht	Mean ± SD	99.76 ± 13.77	110.50 ± 11.54	155.33 ± 9.50	155.47 ± 11.27	122.33 ± 2.89	71.83 ± 9.26	94.08 ± 13.72
	Min	42.00	55.00	146.00	47.50	119.00	47.00	80.75
	Max	210.00	190.00	165.00	170.00	124.00	100.00	107.00
	CV%	5.68	10.44	6.12	9.76	2.36	12.89	14.58
LL	Mean ± SD	45.94 ± 8.20	31.44 ± 5.14	54.00 ± 13.53	33.70 ± 4.26	64.67 ± 4.04	44.59 ± 0.26	22.20 ± 3.09
	Min	20.00	14.00	40.00	14.00	60.00	15.90	19.13
	Max	82.50	70.00	67.00	47.00	67.00	82.50	24.88
	CV%	17.85	16.36	25.05	12.65	6.25	2.340	13.90
LW	Mean ± SD	1.32 ± 0.18	1.07 ± 0.19	1.53 ± 0.13	1.18 ± 0.08	1.10 ± 0.17	1.56 ± 0.12	1.02 ± 0.13
	Min	0.50	0.50	1.40	1.00	1.00	0.50	0.88
	Max	2.20	1.50	1.65	1.53	1.30	2.40	1.12
	CV%	13.86	18.14	8.19	7.10	15.75	7.97	12.76
FLL	Mean ± SD	36.43 ± 10.25	29.94 ± 5.93	65.00 ± 10.00	22.29 ± 2.82	37.97 ± 2.61	24.02 ± 3.86	21.51 ± 4.37
	Min	13.00	11.00	55.00	11.00	35.00	12.00	17.40
	Max	74.00	85.00	75.00	30.00	39.90	40.00	25.48
	CV%	28.15	19.79	15.38	12.65	6.87	16.06	20.32
FLW	Mean ± SD	1.50 ± 0.17	1.28 ± 0.16	1.20 ± 0.05	1.01 ± 0.09	1.10 ± 0.10	1.13 ± 0.12	1.09 ± 0.10
	Min	0.90	0.70	1.20	0.90	1.00	1.00	1.00
	Max	2.60	2.20	1.30	1.20	1.20	1.50	1.18
	CV%	11.23	12.89	4.00	8.81	9.09	10.97	8.88
CL	Mean ± SD	89.01 ± 11.90	79.29 ± 5.94	133.33 ± 15.27	94.20 ± 4.80	51.33 ± 4.04	64.43 ± 6.06	68.81 ± 4.62
	Min	40.00	30.00	120	40.00	47.00	50.00	65.00
	Max	160.00	129	150	128.00	55.00	89.00	73.75
	CV%	13.37	7.48	11.45	5.09	7.87	9.41	6.71
CmIL	Mean ± SD	9.22 ± 2.02	12.80 ± 5.01	13.63 ± 1.66	12.11 ± 2.18	9.50 ± 0.87	7.63 ± 1.76	7.25 ± 1.28
	Min	3.50	3.00	12.10	5.00	8.50	4.40	6.18
	Max	21.50	87.30	15.40	23.00	10.00	14.00	8.55
	CV%	21.90	39.16	12.19	17.97	9.12	23.04	17.70
GrL	Mean ± SD	6.24 ± 0.36	6.26 ± 0.47	6.50 ± 0.41	6.11 ± 0.23	6.21 ± 0.21	6.37 ± 0.22	6.04 ± 0.53
	Min	5.00	5.00	6.23	4.50	6.05	5.00	5.51
	Max	7.45	7.87	6.98	4.50	6.45	7.97	6.52
	CV%	5.72	7.49	6.37	3.76	3.44	3.49	8.79
GrW	Mean ± SD	2.07 ± 0.34	1.98 ± 0.31	2.03 ± 0.25	2.30 ± 0.37	2.50 ± 0.26	2.38 ± 0.29	1.74 ± 0.36
	Min	1.00	0.80	1.80	1.20	2.20	2.00	1.39
	Max	2.80	3.00	2.30	2.90	2.70	2.80	2.05
	CV%	16.56	15.55	12.38	16.03	10.58	12.14	20.67
GrLW	Mean ± SD	3.18 ± 0.58	3.44 ± 0.59	3.22 ± 0.22	2.72 ± 0.41	2.50 ± 0.25	2.70 ± 0.33	3.65 ± 0.84
	Min	2.26	2.00	3.03	2.34	2.33	2.40	2.64
	Max	6.30	7.50	3.46	3.14	2.78	3.02	5.50
	CV%	18.34	17.00	6.86	15.05	9.87	12.20	22.94
TGW	Mean ± SD	24.32 ± 4.32	24.28 ± 2.20	21.20 ± 3.90	26.56 ± 1.24	19.16 ± 0.31	19.72 ± 1.86	20.16 ± 2.57
	Min	12.34	19.76	16.70	24.09	18.80	15.45	17.78
	Max	35.34	30.78	23.45	30.56	19.34	30.89	22.52
	CV%	17.75	9.06	18.38	4.69	1.63	9.42	12.77

Table 2 (continued)

Traits		Origin						
		Banten	West Java	Middle Java	East Java	Yogyakarta	Control Japonica	Control Indica
PL	Mean ± SD	23.5 ± 4.71	20.25 ± 3.24	30.33 ± 5.13	16.40 ± 2.13	17.23 ± 1.93	26.31 ± 3.09	15.95 ± 3.15
	Min	12.00	10	26	12.00	15.00	15.00	12.71
	Max	48.30	43	36	25.00	18.50	38.00	18.64
	CV%	20.03	15.98	16.91	13.01	11.25	11.73	19.74
PnP	Mean ± SD	18.56 ± 4.82	17.86 ± 3.57	10.67 ± 3.79	23.47 ± 3.57	26.00 ± 3.61	17.43 ± 2.32	16.58 ± 2.92
	Min	7.00	6.00	8.00	13.00	22.00	6.00	13.50
	Max	32.00	45.00	15.00	38.00	29.00	28.00	19.00
	CV%	25.99	19.96	35.49	15.21	13.87	13.31	17.59
LgL	Mean ± SD	1.61 ± 0.44	1.66 ± 0.26	2.29 ± 0.77	1.35 ± 0.20	0.90 ± 0.26	1.08 ± 0.18	1.69 ± 0.39
	Min	0.60	0.50	1.40	0.70	0.60	0.30	1.35
	Max	3.54	2.80	2.80	2.00	1.10	1.80	2.08
	CV%	27.19	15.75	33.78	14.63	29.40	16.62	23.11

DtF days to flowering, *Ht* plant height, *LL* leaf length, *LW* leaf width, *FLL* flag leaf length, *FLW* flag leaf width, *CL* culm length, *CmIL* culm internode length, *GrL* grain length, *GrW* grain width, *GrLW* grain length to width, *TGW* thousand grain weight, *PL* panicle length, *PnP* panicle per plant, *LgL* ligule length

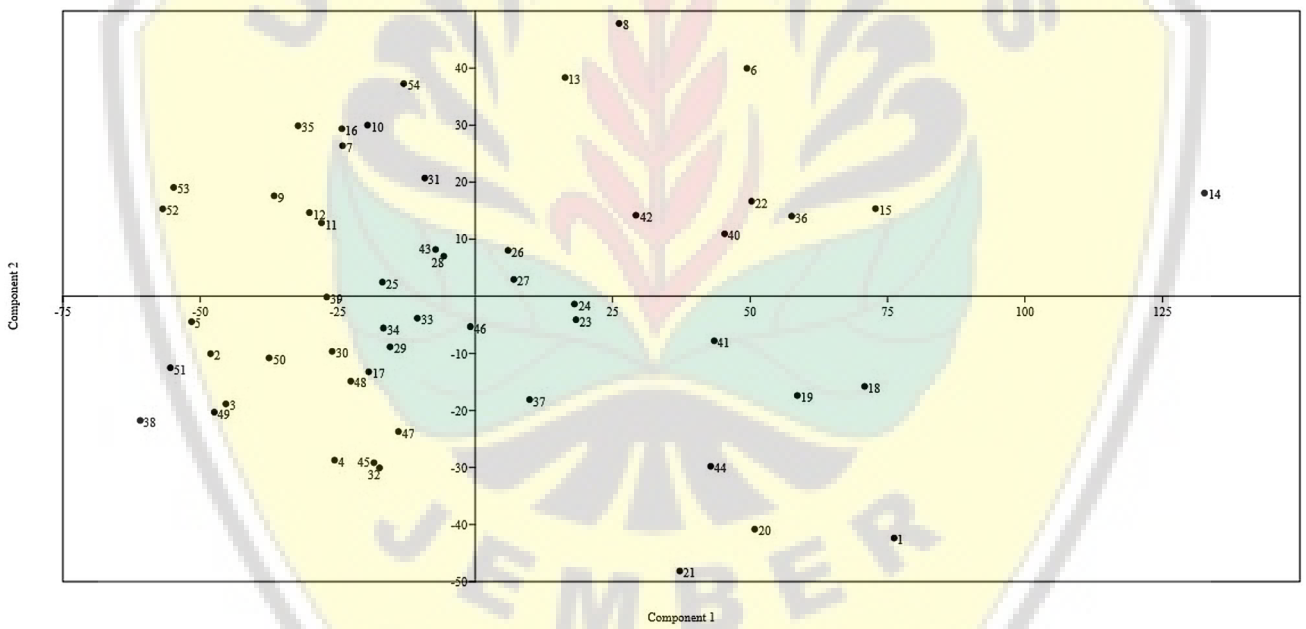

Fig. 2 Principal Component Analysis of 54 accessions (1) Kewel Bulu Hideung (2) Tambleng (3) Kewel Gudril (4) Kambang (5) Pare Mas (6) Kewel Sampai Putih (7) Kewel Benur (8) Seksek (9) Care Ware (10) Care Lintang (11) Tungguh Hideung (12) Kewel Bulu Putih (13) Jalawara (14) Kuriak Kusuik (15) Care Merah 16.Ketan Bayong (17) Inpago (18) Mota (19) Ketan Hitam II (20) Beureum Taleus (21) Segon Geulis (22) Inpari 14 (23) Pandan Wangi II (24) Cibogo (25) Conde (26) Situ Patenggang (27) Towuti (28) Martapura (29) Mendawak (30) Siak Raya (31) Banyuasin (32) Dendang (33) Indragiri (34) Sertani (35) Ketan Unggul (36) Anak Doro Magelang (37) Merah Saleman (38) Situbagendit (39) Susu Putik (40) Mentik (41) Pandan Wangi (42) Lamongan I (43) Jember I (44) IR66 (45) IR36 (46) IR46 (47) IR64 (48) Nagdong (49) Ilpum (50) Cucheong (51) Dongjin (52) Nipponbare (54) Hwayeong

resistant to biotic or abiotic that are targeted for crop improvement (Singh et al. 2016). In this study, 54 accessions were analyzed by agro-morphological and genetic characterization. This agro-morphological cluster in this study that the closeness of each accession was not linked to the region. These data similar to the results found by Roy et al. (2016) that the PCA and Ward's cluster grouped hill rice accession did not follow a genotype geographical origin.

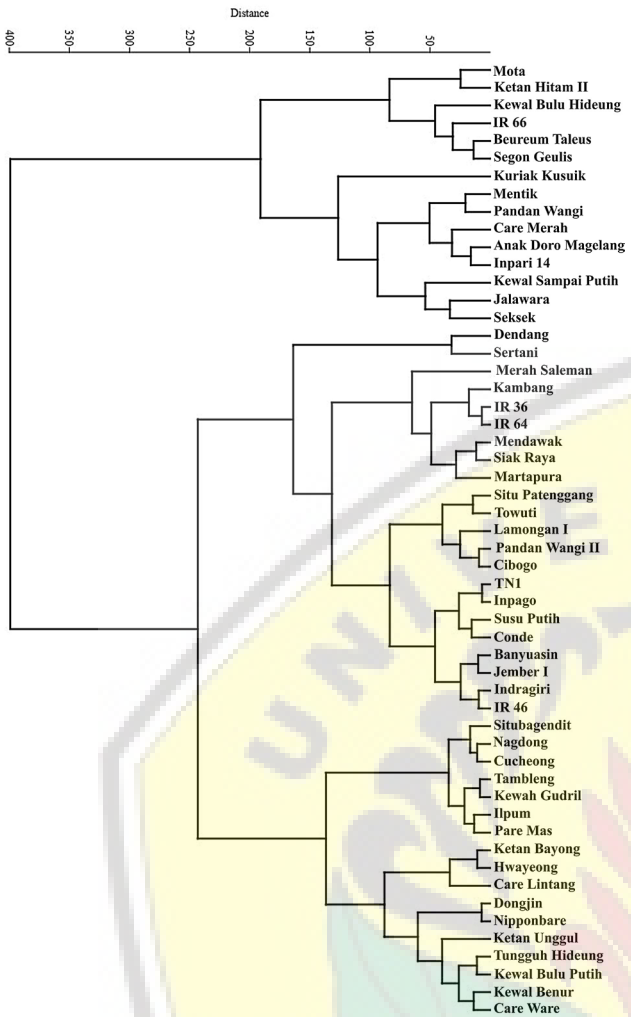

Fig. 3 Clustering agro-morphological 15 characters based on the ward's hierarchical methods

From the result of genetic data, the average of PIC values in this study is 0.83. The average of these values differs from those found in previous studies. Krupa et al. (2017) conducted three microsatellite analyses on rice and found PIC values 0.34–0.88 with an average of 0.56. It was reported that the value ranged from a low of 0.10 to 0.90 with an average of 0.71 (Ming et al. 2010), 0.00 to a high of 0.90, and averaged 0.76 (Kanawapee et al. 2011), 0.47–0.88 with an average 0.71.

Allele number reflects genetic diversity, and the richness of genetic diversity can give diversity information about the accessions in the region. This study, had a range of 2–15 alleles, with an average of 9.2 per locus. The average of these values differs from those found in previous studies. Becerra et al. (2017) reported the total allele number as 183 alleles from 249 accessions analyzed by 30 SSR in China. Aljumaili et al. (2018) found from 53 Malaysian aromatic rice accessions the alleles per locus were calculated to be between 2.40 and 3.35 on average.

Glazmann et al. (1987) reported that, genetic classification of rice-based on two broad groups which are *Indica* and *Japonica* group. The UPGMA-based dendrogram using a complete linkage method and Jaccard's similarity coefficient was obtained from the binary where the genotypes that are derivatives of genetically similar types clustered together by deducing from the DNA profiles of the samples (Sajib et al. 2012). The clustering showed that closeness of each accession was not linked to the region and no geographic differentiation was presented in one group (Thomson et al. 2009), although the geographical distance is one of the factors affecting the genetic relationship (Wright 1943).

Genetic grouping reveals a clear divide of the accessions into two groups *Indica* and *Japonica* group rather than by agro-morphological clustering. *Indica* group controls distributed into Cluster IA were IR 66, IR 64, IR 46, IR 36, and TN1. One *Japonica* control group distributed into Cluster

Table 3 Statistical analysis based on the Genotype Data

Marker	Chr	Repeat motif	alleles	Ho	He	PIC	Marker	Chr	Repeat motif	alleles	Ho	He	PIC
RM431	1	(AG) 16	11	0.00	0.97	0.95	RM11	7	(GA) 17	2	0.00	0.69	0.67
RM259	1	(CT) 17	9	0.00	0.91	0.89	RM118	7	(GA) 8	14	0.00	0.90	0.88
RM154	2	(GA) 21	13	0.07	0.70	0.68	RM408	8	(CT) 13	12	0.00	0.95	0.92
RM452	2	(GTC) 9	15	0.00	0.96	0.94	RM284	8	(GA) 8	4	0.00	0.79	0.78
RM489	3	(ATA) 8	8	0.00	0.88	0.82	RM404	8	(GA) 33	7	0.02	0.85	0.83
RM55	3	(GA) 17	9	0.00	0.89	0.85	RM215	9	(CT) 16	13	0.00	0.93	0.91
RM307	4	(AT) 14 (GT) 21	10	0.04	0.79	0.73	RM171	10	(GATG) 5	12	0.00	0.92	0.90
RM124	4	(TC) 10	7	0.00	0.86	0.81	RM552	11	(TAT) 13	11	0.00	0.80	0.79
RM334	5	(CTT) 20	5	0.00	0.78	0.77	RM536	11	(CT) 16	3	0.00	0.74	0.71
RM161	5	(AG) 20	9	0.00	0.89	0.86	RM19	12	(ATC) 10	13	0.00	0.94	0.93
RM162	6	(AC) 20	9	0.02	0.88	0.87	RM277	12	(GA) 11	7	0.00	0.88	0.85

Heterozigosity (Ho) Expected Heterozigosity or Genetic diversity (He) Polymorphic Information Content (PIC)

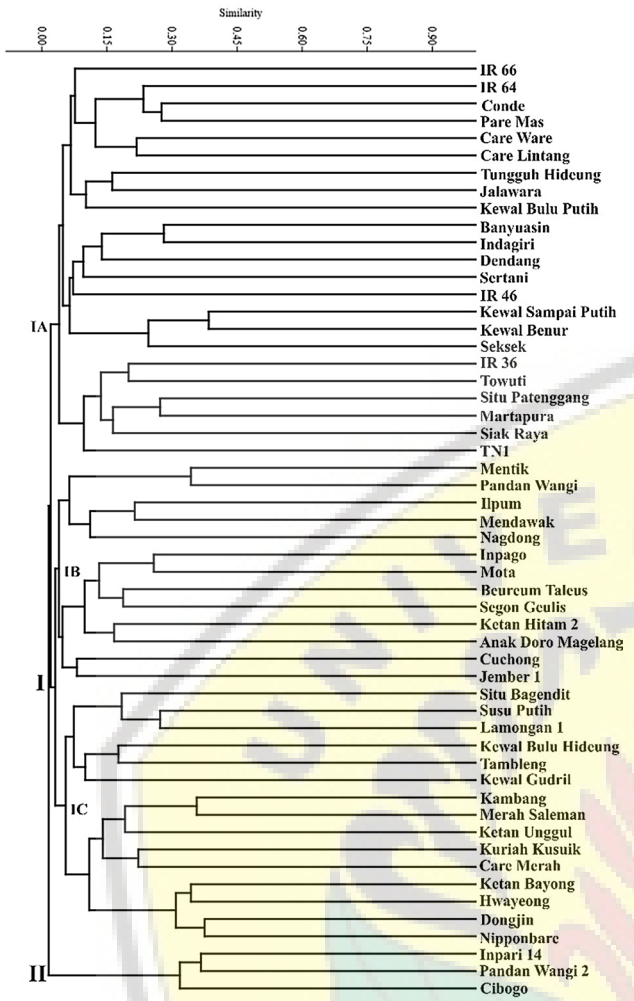

Fig. 4 Clustering 54 genotypes based on the UPGMA methods with Jaccard's Coefficient

IB were Ilpum, Nagdong, and Cuchong, while another *Japonica* control group consisted of Hwayeong, Dongjin and Nipponbare were formed in IC. Group IB and IC had assumed closeness to the *Japonica* group. The *Japonica* group showed a higher percentage (55%) compared to the *Indica* group (45%). These results are almost similar to the comparison in the study of 130 traditional Indonesian varieties analyzed using the *Japonica* isoenzyme group of 65% while the large *Indica* group 34%, but this is contrary to 246 Indonesian landraces that the *Indica* ratio (68%) is higher than the *Japonica* group (32%) (Glaszmann et al. 1987) and the study of 3670 Indonesian varieties analyzed using 11 isoenzymes which are the Indica group which is 68% while *Japonica* has a percentage of 28% (Khush et al. 2003). The utilization of genetic information for the assembly of new improved varieties in Indonesia is focused mainly on crossing the germplasm of *Indica* rice derived from IR rather than *Japonica* (Thomson et al. 2007).

Conclusion

Java is the potential island to explored of germplasm that is great significance for future breeding not only for a breeder for the local farmers. In this study, an effort was made to collect 54 rice to assess their morphology trait and genetic diversity. The phenotypic expression of each region makes different each accession to the others. Clustering based on the morphology trait indicated is not the linkage effect of regions. The result of this study proved the benefit of 22 SSR markers that polymorphism to assess genetic diversity and closeness of each accession. The abundant genetic diversity of Java local rice are considerable based on future rice breeding program and improve yield production.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest regarding the publication of the paper.

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