Agronomy Research **20**(S1), 1104–1116, 202
https://doi.org/10.15159/AR.22.050

Genetic diversity analysis of Indonesian rice germplasm (*Oryza sativa* L.) with simple sequence repeat markers

A.N. Puspito[1], S. Nabilah[1], D.M.A. Imam Buqori[2], S. Hartatik[2], Kyung-Min Kim[3] and M. Ubaidillah[4,*]

[1]University of Jember, Graduate School of Biotechnology, Jember, ID6812 East Java, Indonesia
[2]University of Jember, Faculty of Agriculture, Study Program of Agronomy, Jember, ID6812 East Java, Indonesia
[3]Kyungpook National University, College of Agriculture and Life Science, School of Applied BioSciences, Division of Plant Biosciences, Daegu, South Korea
[4]University of Jember, Faculty of Agriculture, Study Program of Agrotechnology, Jember, ID6812 East Java, Indonesia
[*]Correspondence: moh.ubaidillah.pasca@unej.ac.id

Received: April 5th, 2022; Accepted: July 20th, 2022; Published: August 18th, 2022

Abstract. The characterization of germplasm provides information on the regional rice genetic diversity and variety kinship classification. This study aimed to provide information on the agro-morphological traits and genetic diversity of fifty local rice varieties from Java and Borneo Island in Indonesia. The variability of thirteen agronomic traits showed the differentiation among the accessions, while the phenotypic traits were grouped into six clusters. The genotyping characterization was conducted using SSR (Simple Sequence Repeats) markers (22 microsatellites), and continued with genetic diversity and Polymorphism Information Content (PIC) analysis. The agro-morphological clustering based on Ward's Hierarchical constructed six sub-clusters. The PC1 and PC2 had 86.3% of the total percentage. The UPGMA method was used to construct six different groupings, as the correlation between each group and its collecting source was significant. Furthermore, the UPGMA dendrogram clustered the 50 accessions into six main clusters, while the PIC showed a polymorphism value range of 0.41–0.74. RM162 located on chromosome 5, which was considered as the best marker for fifty-one genotypes. At the same time, the lowest PIC value of 0.41 was observed in RM431 located in chromosome 1. This classification can be helpful as a detailed information for plant breeders to characterize and select the germplasm, while conducting backcrosses between rice accessions.

Key words: agro-morphological, diversity, germplasm, rice, SSR.

INTRODUCTION

Over half of the world's population consumes rice as a primary food (Garris et al., 2005; Ghneima et al., 2008; Hokazono et al., 2009; Sabu et al., 2009; Singh et al., 2016). Rice is classified into other two large groups, namely *indica* and *japonica* subspecies

(Zaidi et al., 2006; Agrama et al., 2010). Rice plant is a mostly-cultivated cereal crop in Asia, especially in Indonesia, because of its wide range of ecological, geographical and climate aspects (Ni et al., 2002). The country has several diversities, including landraces, cultivar, and wild accession. This condition occurs as Indonesia is a tropical country that has a strategic geographical position located on the equator. Indonesia is known as the largest archipelagic country in the world with more than seventeen islands, as each of which has its own uniqueness and characteristics according to the landscape. Furthermore, there are over seventeen thousand rice genetic resources served as a potential natural source.

The climatic condition of Indonesia helps the production of several local rice diversities, including the ones resistant to biotic stress (brown planthopper, blast, and blight disease) (Park et al., 2022). The utilization of local germplasm is relatively low, as more than thirty-five thousand accessions have been characterized through continuous natural selection, resulting in gradual changes among the crosses of genetically related accessions during the evolutionary time (Caicedo et al., 2007; Pachauri et al., 2013). As a consequence, the adaptation process produced new rice varieties with diverse morphology, physiology, genetic, and habitat (Vaughan et al., 2008; Jasim Aljumaili et al., 2018).

Before the green revolution policy, farmers in each region have been planting and cultivating the germplasm rice for generations and centuries. This policy has a substantial negative effect due to rapid replacement of local varieties with the modern ones. However, some of them are still planted by farmers because of their adaptation and competition potential in sub-optimal natural conditions (Pandey et al., 2015).

Genetic diversity provides an opportunity to obtain high benefits through rice breeding program opportunity to improve the yield and characterization quality and quantity (Chakravarthi & Naravaneni, 2006; Varshney et al., 2008; Huang et al., 2010; Yadav et al., 2013; Roy et al., 2016; Skipars et al., 2021). The characterization of local germplasm is extremely important to determine the superior genes potential from local germplasm, while genetic diversity helps ensure the efficiency level (Prysiazhniuk et al., 2020; Karimah et al., 2021; Shanina & Likhodeyevsky, 2021). Several studies have been conducted on these concepts in many countries of the world. However, there is a lack of information sources to describe the genetic diversity of rice germplasm on a local scale (Thomson et al., 2009). In addition, this information is also required to provide a detailed picture of the rice genetic diversity in a country, besides its complex interactions with the germplasm potential (Barry et al., 2007).

Therefore, this study used 22 SSR markers distributed from chromosome number 1 until 12 to examine the genetic diversity of 50 genotypes, and their efficacy was compared to the utilized rice germplasm.

MATERIALS AND METHODS

Plant material

In this study, we collected fifty sources of rice germplasm from Java and the Borneo Islands of Indonesia. In Java Island, the samples were obtained from East Java, Central Java, Banten, and West Java Provinces. Meanwhile on Borneo Island, the samples were obtained from Central Borneo and East Borneo Provinces, as shown in Table 1. The control varieties included the *Japonica* (*Nagdong*, *Black Madras*, and *Bekinju*) and *Indica* (*Tetep* and *TN1*) subspecies. The experiment was designed to focus on the insect

standard handling, plant geometry, and fertilizer application. All of these activities were conducted under rainy lowland conditions.

Table 1. List of varieties

No	Accession name	Origin	No	Accession name	Origin
1	*Tetep*	Vietnam	26	*Hoing*	Banten
2	*Madras*	Korea	27	*Ketan Hitam 1*	West Java
3	*Bekinju*	Korea	28	*Ketan Keuyeup*	West Java
4	*Nampyeong*	Korea	29	*Genjah Batu*	West Java
5	*TN1*	China	30	*Si Rendet*	West Java
6	*Kali Culuk*	East Java	31	*Botel*	West Java
7	*Merah SP*	East Java	32	*Pulut Rigoti*	West Java
8	*Bondowoso 1*	East Java	33	*Ketan Putri*	West Java
9	*Inpago 8IPB*	East Java	34	*Aek Sibundong*	West Java
10	*Gogo Niti 2*	East Java	35	*Batang Paiman*	West Java
11	*Mansur*	East Java	36	*Bondoyudo*	West Java
12	*Merah Wangi*	East Java	37	*Cigeulis*	West Java
13	*MS Pendek*	East Java	38	*Cilamaya Muncul*	West Java
14	*Genjah Nganjuk*	East Java	39	*Ciliwung*	West Java
15	*Super Manggis*	East Java	40	*Cisantana*	West Java
16	*Kropak*	East Java	41	*Cisokan*	West Java
17	*Inpago*	East Java	42	*Cempo Selamet*	Middle Java
18	*Sereh*	Banten	43	*Cempo Telouluk*	Middle Java
19	*Waren*	Banten	44	*Mamas*	Middle Java
20	*Pare Jaketra*	Banten	45	*Cempo Wulut*	Middle Java
21	*Pare Caok*	Banten	46	*Hawara Batu*	Middle Java
22	*Bulu Putih*	Banten	47	*Kero*	Middle Java
23	*Pandan Ungu*	Banten	48	*Ketan Nangka*	Middle Java
24	*Roti*	Banten	49	*Ketan Benjar*	Middle Borneo
25	*Amas*	Banten	50	*Pulut Hitam*	East Bormeo

Morphology clustering and characterization

After 20 days of rice seedling (DAS), we transplanted the rice germplasm to the experimenting field at Jember University, located on 100–200 masl with rainfall level of 2.396 mm year^{-1} and humidity of 84%–95%. Fifty accessions were cultivated during the rainy season from October, 2017 to April, 2018 in 1×1 m plots (5 lines/accession) with a 0.25 m spacing and 0. m spacing between the rows using a block design. We used the Standard Evaluation System to describe the detailed morphology of rice after the standard agronomy (Allhgholipour et al., 2014). The phenotypic data included traits, such as the plant height (Ht), culm length (CL), culm's internode length (CmIL), panicle length (PL), number of panicles per plant (PnP), leaf length (LL), leaf width (LW), flag leaf length (FLL), flag leaf width (FLW), and ligule length (LgL). Other traits include grain length (GrL), grain width (GW), and grain length to width (GrLW). The total phenotypic data from these samples were analyzed further using a Principal Component Analysis (PCA) to reveal the critical parameter correlation matrix data (Roy et al., 2016). Then, the Ward's Hierarchical Clustering was used to group the agro-morphological characteristics. We also used PAST 3 software to examine the parameter clustering and characterization.

Genotyping characterization

The fifty leaf samples were collected for DNA isolation from young leaf tissue using the SDS method (Thomson et al., 2007). Using a UV Reader for 30 minutes, we examined the DNA quality at 100 V in a 1.7% agarose gel containing 1 x TAE buffer (Tris-acetate-EDTA). The DNA concentration was determined using a NanoDrop Spectrophotometer (Nanovue Plus-UK) and the purified DNA was diluted to 100 ng using TE buffer to preserve its quality, when stored at –20 °C. The total PCR reaction obtained a volume of 10 μl, which included 5 μl PCR Nexpro master mix reaction, 3 μl DDH2O water, 1 μl for forward and reverse primer, and 1 μl DNA template. The 22 SSR markers mapped by McCouch et al. (2002) were used to distribute the 12 chromosomes (Table 2) and cover the genome for each sample.

Table 2. List of 22 SSR markers

Primer No	Locus	Chr.	Repeat motif	PCR primer (5' to 3')	
1	RM431	1	(AG)16	F	TCCTGCGAACTGAAGAGTTG
				R	AGAGCAAAACCCTGGTTCAC
2	RM259	1	(CT)17	F	TGGAGTTTGAGAGGAGGG
				R	CTTGTTGCATGGTGCCATGT
3	RM154	2	(GA)21	F	ACCCTCTCCGCCTCGCCTCCTC
				R	CTCCTCCTCCTGCGACCGCTCC
4	RM452	2	(GTC)9	F	CTGATCGAGAGCGTTAAGGG
				R	GGGATCAAACCACGTTTCTG
5	RM489	3	(ATA)8	F	ACTTGAGACGATCGGACACC
				R	TCACCCATGGATGTTGTCAG
6	RM55	3	(GA)17	F	CCGTCGCCGTAGTAGAGAAG
				R	TCCCGGTTATTTTAAGGCG
7	RM307	4	(AT)14(GT)21	F	GTACTACCGACCTACCGTTCAC
				R	CTGCTATGCATGAACTGCTC
8	RM124	4	(TC)10	F	ATCGTCTGCGTTGCGGCTGCTG
				R	CATGGATCACCGAGCTCCCCCC
9	RM334	5	(CTT)20	F	GTTCAGTGTTCAGTGCCACC
				R	GACTTTGATCTTTGGTGGACG
10	RM161	5	(AG)20	F	TGCAGATGAGAAGCGGCGCCTC
				R	TGTGTCATCAGACGGCGCTCCG
11	RM162	6	(AC)20	F	GCCAGCAAAACCAGGGATCCGG
				R	CAAGGTCTTGTGCGGCTTGCGG
12	RM11	7	(GA)17	F	TCTCCTCTTCCCCCGATC
				R	ATAGCGGGCGAGGCTTAG
13	RM118	7	(GA)8	F	CCAATCGGAGCCACCGGAGAGC
				R	CACATCCTCCAGCGACGCCGAG
14	RM408	8	(CT)13	F	CAACGAGCTAACTTCCGTCC
				R	ACTGCTACTTGGGTAGCTGACC
15	RM284	8	(GA)8	F	ATCTCTGATACTCCATCCATCC
				R	CCTGTACGTTGATCCGAAGC
16	RM404	8	(GA)33	F	CCAATCATTAACCCCTGAGC
				R	GCCTTCATGCTTCAGAAGAC
17	RM215	9	(CT)16	F	CAAAATGGAGCAGCAAGAGC
				R	TGAGCACCTCCTTCTCTGTAG

Table 2 (continued)

18	RM171	10	(GATG)5	F	AACGCGAGGACACGTACTTAC
				R	ACGAGATACGTACGCCTTTG
19	RM552	11	(TAT)13	F	CGCAGTTGTGGATTTCAGTG
				R	TGCTCAACGTTTGACTGTCC
20	RM536	11	(CT)16	F	TCTCTCCTCTTGTTTGGCTC
				R	ACACACCAACACGACCACAC
21	RM19	12	(ATC)10	F	CAAAAACAGAGCAGATGAC
				R	CTCAAGATGGACGCCAAGA
22	RM277	12	(GA)11	F	CGGTCAAATCATCACCTGAC
				R	CAAGGCTTGCAAGGGAAG

Moreover, the DNA samples were amplified using the T100 Thermal Cycler (Bio-Rad-US). The PCR program was performed in 5 minutes of predenaturation at 95 °C, 30 seconds of denaturation at 95 °C, 30 seconds of annealing at 55–60 °C, a minute of extension at 72 °C, and 5 minutes of final extension at 72 C. The samples were conducted on a 1.7% agarose gel at 100V for 40 minutes using electrophoresis.

Statistical and genetic diversity analysis

The visible DNA bands were appropriated to the molecular weight and scored using the Allele's format, which represented the band presence as number one and band absence as zero number, until the DNA tape had the lowest size (Sajib et al., 2012). The genetic diversity, allele frequency, and PIC value (Polymorphic Information Content) were analyzed using the PowerMarker V3.25 (Liu & Muse, 2005). The PIC value was used to represent the polymorphism value in a variety of ways. The allele score matrixes were used to develop a phylogeny tree by applying the UPGMA method. However, the tree was constructed using the NTSYPC program and Jaccard's coefficient to determine the relationship among the varieties (Pandey et al., 2015).

RESULTS AND DISCUSSION

Morphological characterization

This study was conducted to characterize the agronomic trait variability of the samples collected from different provinces in Indonesia (Banten, Borneo, East Java, Central Java, and West Java). The variety evaluation in 13 morphological characters revealed the highest value of plant height trait (187.40 cm) in *Cempo Selamet* variety and the lowest value in Ciliwung variety (53.60 cm). This condition can be attributed to the number of panicles per plant, ranging from 5 to 48 per plant. Based on the grain characteristics, GrL was found among 5.09–7.53 cm and GrW was among 1.56–3.41 cm. As shown in Table 3, the average of grain size was discovered at 3.06, which indicates that the grain is on the medium side.

The Principal Component Analysis (PCA) of 13 traits is presented from PC1 to PC5 (Eigenvalue > 1) The analysis was conducted to identify the most important variables contributing to the total agro-morphological variation of the samples. At the same time, the multivariate method visualizes the accessions clustering that depends on the component loading with the multivariate method (Singh et al., 2016). The detailed

information about these values is enlisted in Table 4. The PC1 and PC2 represent the distribution of the 50 accessions, besides the number and names of the accessions listed in Table 1. However, the results showed that the PC1 and PC2 only obtained 86.3% of the total percentage. The first principal value was 71.5% of the percentage variance. This condition was characterized by high positive loading, containing Ht, CL, FLL and LL. The second principle was 14.8%, with high positive loadings of FLL and LL. Another trait, namely PnP, obtained a high positive loading of 0.86, as listed in PC3 (Table 4).

Table 3. Agro-morphological characters of 50 accessions

Agronomic traits	Mean ± SD	Min	Max	% CV
Ht	114.47 ± 13.17	53.60	187.40	12.32
CL	86.83 ± 12.71	28.00	163.40	15.87
CmIL	9.52 ± 0.51	1.60	51.00	5.03
LL	39.56 ± 5.51	23.90	87.90	14.17
LW	1.40 ± 0.06	0.70	2.56	4.93
FLL	30.61 ± 3.60	12.10	87.60	13.29
FLW	1.26 ± 0.08	0.90	2.00	6.32
PL	25.24 ± 2.55	13.10	39.80	9.78
PnP	20.65 ± 2.03	5.00	48.00	11.82
GrL	6.42 ± 0.11	5.09	7.53	1.79
GrW	2.15 ± 0.06	1.56	3.41	2.84
GrLW	3.06 ± 0.11	1.65	4.40	3.64
LgL	1.44 ± 0.07	0.40	2.60	5.87

Note: Ht = plant height; CL = culm length; cmIL = culm internode length; LL = leaf length; LW = leaf width; FLL = flag leaf length; FLW = flag leaf width; PL = panicle length; PnP = panicle per plant; GrL = grain length; GrW = grain width; GrLW = grain length to width; LgL = ligule length.

Table 4. Principal component analysis

Characteristics	PC1	PC2	PC3	PC4	PC5
Ht	0.755	-0.071	-0.321	0.518	0.108
CL	0.605	-0.279	0.251	-0.653	-0.172
CmIL	0.079	0.101	0.163	0.172	0.554
LL	0.145	0.529	0.233	-0.293	0.578
LW	0.003	-0.005	-0.007	-0.014	0.002
FLL	0.184	0.774	0.063	0.076	-0.547
FLW	0.002	0.002	-0.005	-0.004	0.009
PL	0.032	0.054	-0.093	0.027	0.036
PnP	0.041	-0.158	0.860	0.428	-0.126
GrL	-0.002	0.002	-0.021	-0.009	0.024
GrW	0.001	0.002	-0.012	-0.014	0.008
GrLW	-0.003	-0.003	0.011	0.017	0.000
LgL	0.001	0.003	-0.008	0.000	-0.011

Note: Ht = plant height; CL = culm length; cmIL = culm internode length; LL = leaf length; LW = leaf width; FLL = flag leaf length; FLW = flag leaf width; PL = panicle length; PnP = panicle per plant; GrL = grain length; GrW = grain width; GrLW = grain length to width; LgL = ligule length.

The PCA visualization could assist in suggesting the most appropriate genotype selection to improve the yield based on the component loading value. The distribution of the separated accessions is presented in scare plots of PC1 and PC2 (Fig. 1).

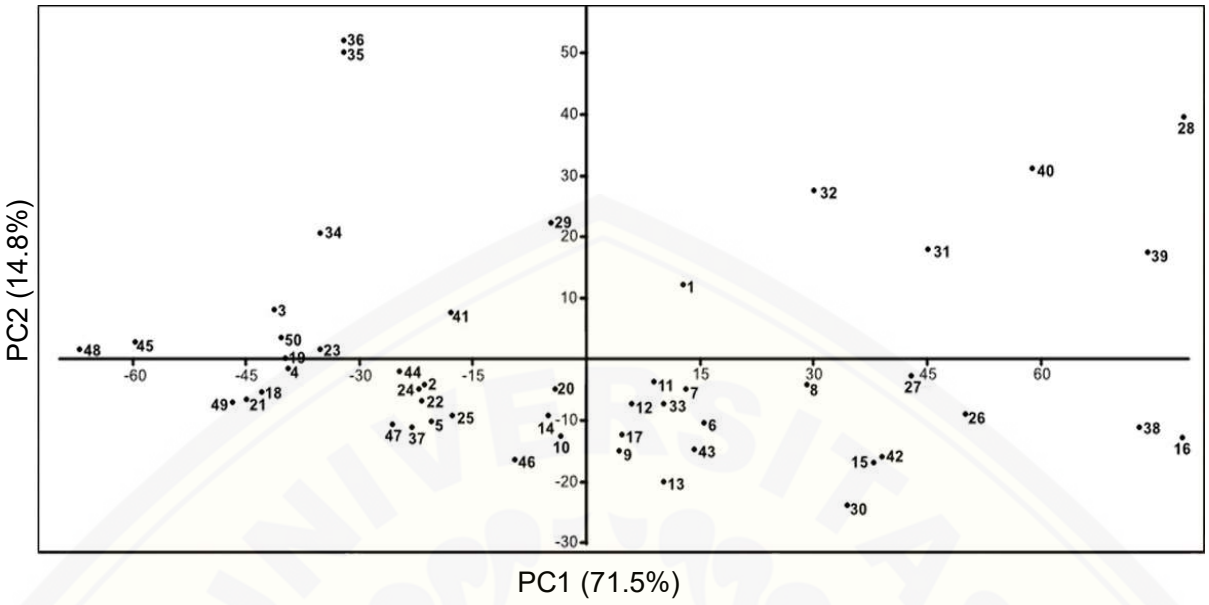

Figure 1. Principal Component Analysis with PC1 and PC2 value: (1) *Tetep*; (2) *Madras*; (3) *Bekinju*; (4) *Nampyeong*; (5) *TN1*; (6) *Kali Culuk*; (7) *Merah SP*; (8) *Bondowoso* 1; (9) *Inpago 8IPB*; (10) *GogoNiti 2*; (11) *Mansur*; (12) *Merah Wangi*; (13) *MS Pendek*; (14) *Genjah Nganjuk*; (15) *Super Manggis*; (16) *Kropak*; (17) *Sereh*; (18) *Waren*; (19) *Pare Jaketra*; (20) *Pare Caok*; (21) *Bulu Putih*; (22) *Pandan Ungu*; (23) *Roti*; (24) *Amas*; (25) *Inpago*; (26) *Ketan Hitam 1*; (27) *Ketan Keuyeup*; (28) *Genjah Batu*; (29) *Si Rendet*; (30) *Ketan Benjar*; (31) *Cempo Salamet*; (32) *Cempo Telouluk*; (33) *Mamas*; (34) *Cempo Wulut*; (35) *Hawara Batu*; (36) *Kero*; (37) *Ketan Nangka*; (38) *Pulut Hitam*; (39) *Hoing*; (40) *Botel*; (41) *Pulut Rigoti*; (42) *Ketan Putri*; (43) *Aek Sibundong*; (44) *Batang Paiman*; (45) *Bondoyudo*; (46) *Cigeulis*; (47) *Cilamaya Muncul*; (48) *Ciliwung*; (49) *Cisantana*; (50) *Cisokan*.

The plots with high-value loading were presented on the right, namely *Mamas*, *Mansur*, *Merah Wangi*, *Merah SP*, *Sereh, Inpago 8IPB*, *Aek Sibundong*, and *Kali Culuk*. Meanwhile, plots with low positive loading were presented on the left, namely *Cilamaya Muncul*, *Ketan Nangkam Inpago*, *Amas*, *Pandan Ungu*, and *Hawara Batu*. The agro-morphological clustering based on Ward's Hierarchical Cluster is shown in Fig. 2.

Two larger groups were constructed, containing two sub-clusters tagged as IA, IB, IIA, and IIB. Two sub-clusters of IB were created, using *Black Madras* and *Bekinju* varieties, while *Nampyeong* and *TN1* varieties were used as controls. However, *Pandan Ungu* and *Amas* were very closed to *Black Madras*. At the same time, *Cisokan* was very closed to *Bekinju* as *Japonica* subspecies control. The other sub-cluster, i.e. IIA, was also divided into two smaller sub-clusters. *Cempo Telouluk* variety was very closed to *Tetep* as a variety control, and other varieties, such as *Bondowoso 1, Ketan Keuyeup, Ketan Putri, Super Manggis*, and *Ketan Benjar*. Furthermore, the IA and IIB sub-clusters only contain one smaller sub-cluster.

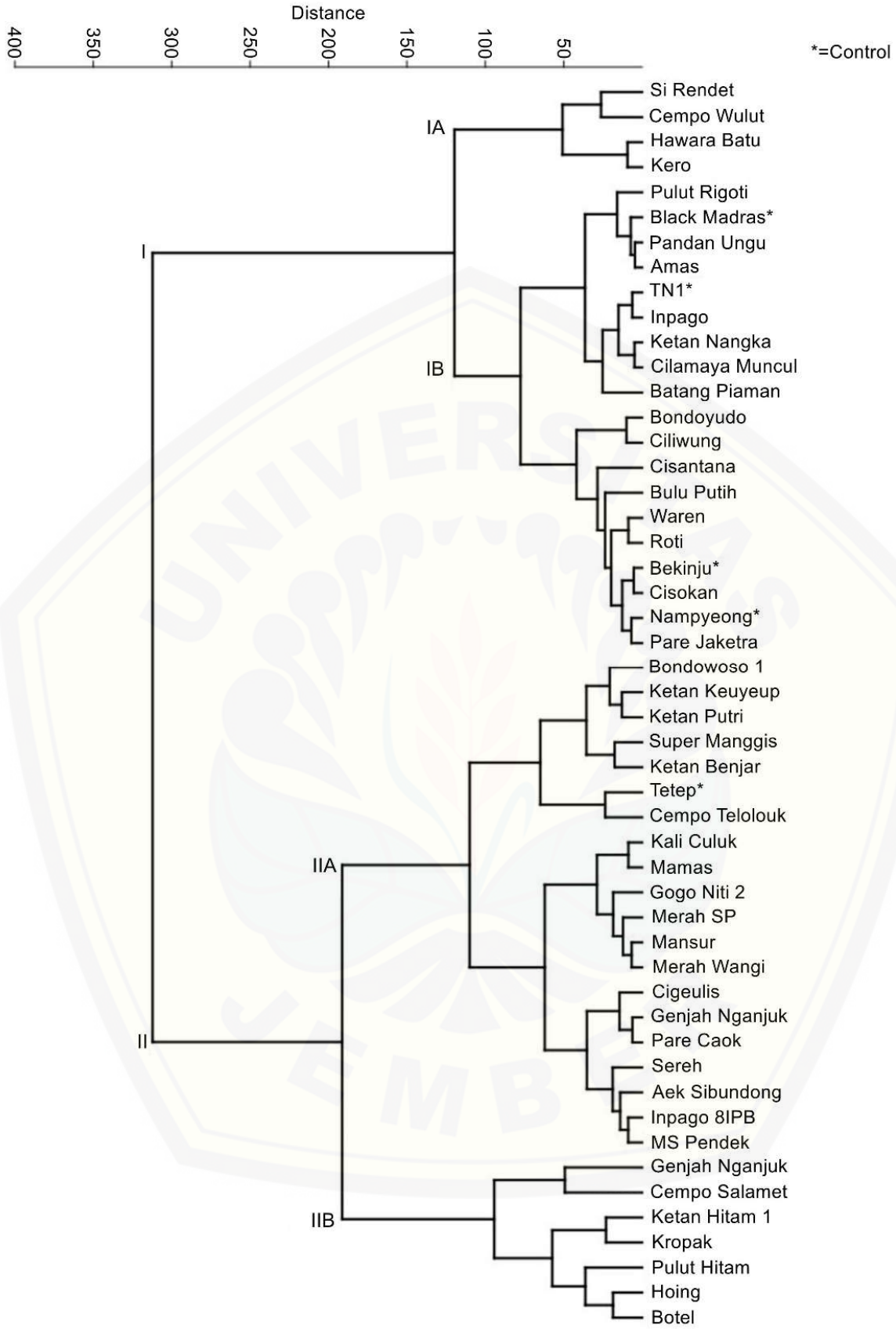

Figure 2. The 50 accessions grouping based on the agro-morphological characters with Ward's hierarchical clustering.

The SSR markers and genetic diversity analysis

The 22 markers used in the 50 accessions mostly showed polymorphic characteristics. The total amplified fragments (alleles) was 1074 of 22 primers with a mean of 48.8 alleles for each locus. The detailed information is presented in Table 5.

The Pi value showed the average frequency of alleles in each locus at 0.23–0.49. Therefore, PIC can indicate the polymorphism value of a molecular marker. The highest value of 0.74 was found in RM162, followed by RM408 with 0.72 and RM171 with 0.69. Therefore, RM162, located on the chromosome 5, is considered as the best marker due to high level of polymorphism compared to others. However, the lowest PIC value of 0.41 was observed in RM431 located in the chromosome 1. This level was determined using a mean value of 0.56, whereas a value of > 0.5 showed high polymorphism and a value of < 0.5 showed low polymorphism. Other studies also obtained similar results at 0.23–0.84, with an average of 0.61 (Brondani et al., 2006), or varied among 0.45–0.65, with an average of 0.57 (Roy et al., 2016), and among 0.28–0.98, with an average of 0.63 (Jasim Aljumaili et al., 2018). These values are markedly higher than the values obtained in this study.

Table 5. Summary of SSR analysis

No	Marker	Amplified Fragment (alleles)	Pi (frequency alleles)	PIC (polymorphic information content)
1	RM431	52	0.34	0.41
2	RM259	48	0.48	0.52
3	RM154	67	0.44	0.42
4	RM452	43	0.42	0.64
5	RM489	51	0.25	0.65
6	RM55	47	0.46	0.52
7	RM307	55	0.27	0.62
8	RM124	39	0.38	0.66
9	RM334	62	0.30	0.50
10	RM161	51	0.25	0.59
11	RM162	44	0.29	0.74
12	RM11	48	0.47	0.55
13	RM118	39	0.39	0.64
14	RM408	45	0.30	0.72
15	RM284	49	0.49	0.52
16	RM404	54	0.35	0.62
17	RM215	43	0.42	0.63
18	RM171	40	0.39	0.69
19	RM552	70	0.23	0.44
20	RM536	46	0.45	0.59
21	RM19	38	0.38	0.71
22	RM277	43	0.43	0.62

Genetic diversity depends on the gene recombination, mutation, and selection due to capable of creating new varieties. Furthermore, genetic diversity is explored to identify the potential genes that can be used to repair germplasm (Allhgholipour et al., 2014). Every crop improvement program is the primary source of variability, which acts as a reservoir for identifying superior alleles controlling the agronomic and qualitative key parameters by mapping the allele mining associations (Nachimuthu et al., 2015). The cluster analysis results of fifty genotypes based on Jaccard's Coefficient successfully classified the genotypes into two main clusters. In contrast, the genetic similarity among the genotypes was 0.2–1.0. Group I consisted of 5 genotypes, while Group II consisted of with two sub-clusters (IIA and IIB). Group IIA consisted of A (5 genotypes) and B (16 genotypes). In addition, Group IIB consisted of C (5 genotypes), D (1 genotypes), and E (8 genotypes). The detailed UPGMA dendrogram with the genetic relationship among accessions is presented in Fig. 3.

Five control varieties, namely *Tetep, Bekinju, Nampyeong, Black Madras*, and *TN1*, were distributed in group II. The *TN1* and *Bekinju* varieties were closed to *Bondowoso 1,*

Merah SP, Merah Wangi, and *MS Pendek*. Other varieties, including *Black Madras, Tetep,* and *Nampyeong*, were closed to *Bulu Putih* and *Pare Caok*. The method divided them into six genetic clusters confirmed to correspond with the *Japonica* and *Indica* subspecies.

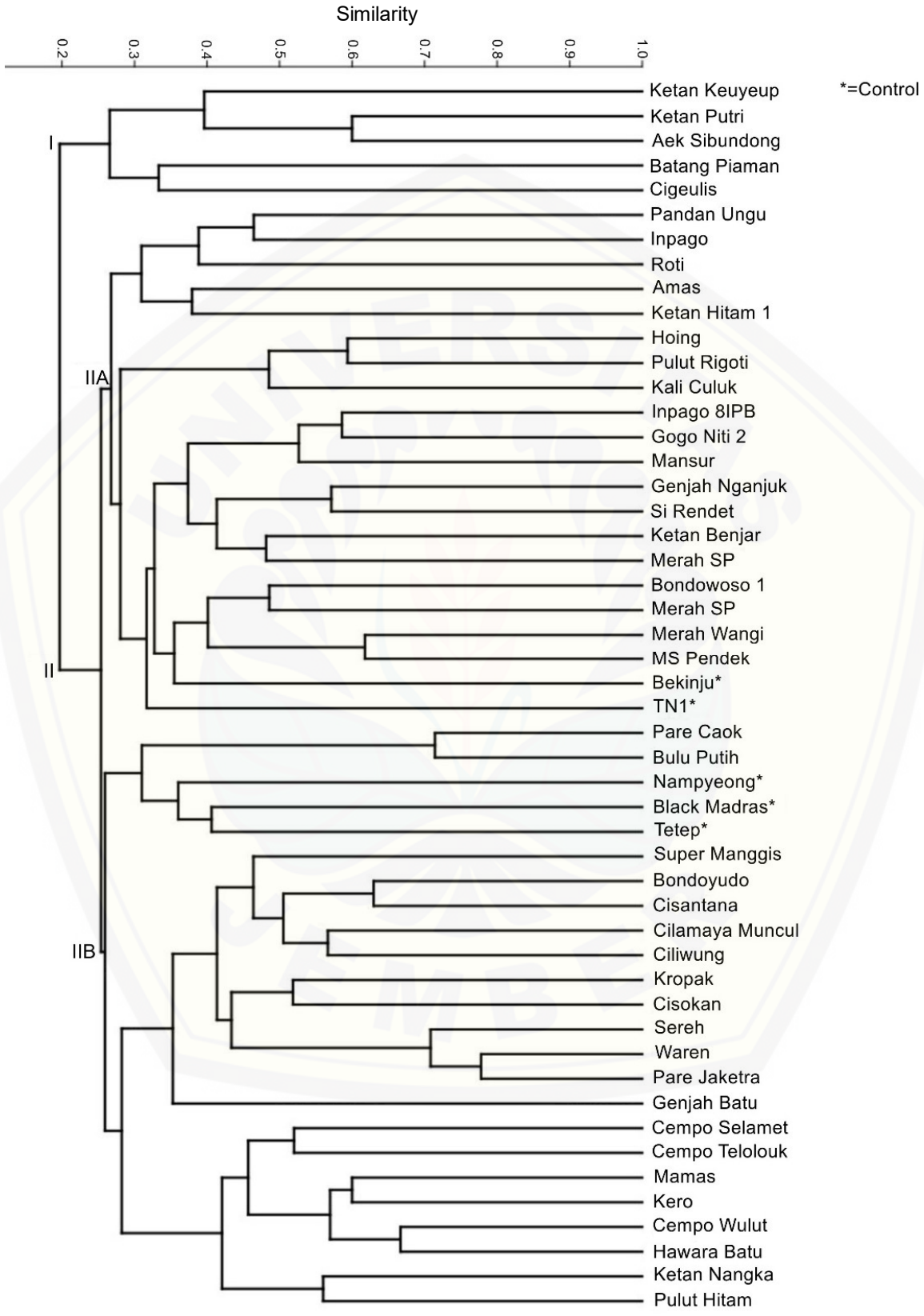

Figure 3. The 50 genotypes grouping based on the 22 SSR markers with UPGMA method.

Therefore, it can be deduced that the Principal Coordinate Analysis (Fig. 4) indicates the separated fifty accessions based on their genetic relationship with the majority group closed to the *Indica* subspecies, rather than the *Japonica* subspecies.

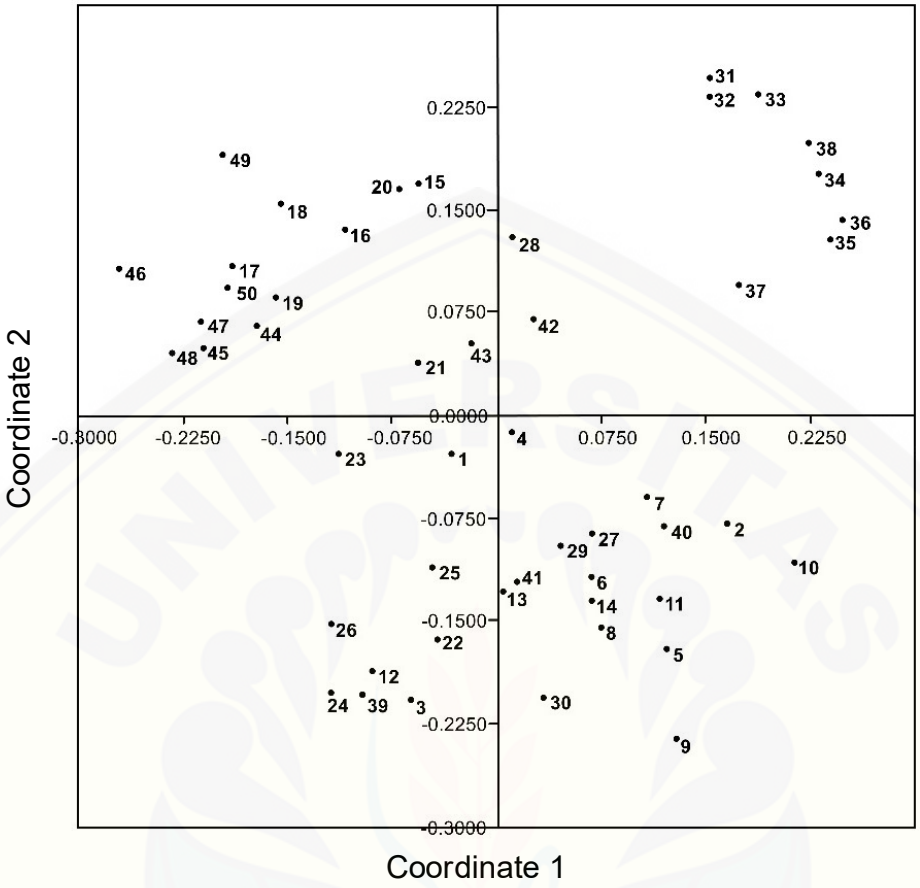

Figure 4. Principal Coordinate Analysis: (1) *Tetep*; (2) *Madras*; (3) *Bekinju*; (4) *Nampyeong*; (5) *TN1*; (6) *Kali Culuk*; (7) *Merah SP*; (8) *Bondowoso 1*; (9) *Inpago 8IPB*; (10) *GogoNiti 2*; (11) *Mansur*; (12) *Merah Wangi*; (13) *MS Pendek*; (14) *Genjah Nganjuk*; (15) *Super Manggis*; (16) *Kropak*; (17) *Sereh*; (18) *Waren*; (19) *Pare Jaketra*; (20) *Pare Caok*; (21) *Bulu Putih*; (22) *Pandan Ungu*; (23) *Roti*; (24) *Amas*; (25) *Inpago*; (26) *Ketan Hitam 1*; (27) *Ketan Keuyeup*; (28) *Genjah Batu*; (29) *Si Rendet*; (30) *Ketan Benjar*; (31) *Cempo Salamet*; (32) *Cempo Telouluk*; (33) *Mamas*; (34) *Cempo Wulut*; (35) *Hawara Batu*; (36) *Kero*; (37) *Ketan Nangka*; (38) *Pulut Hitam*; (39) *Hoing*; (40) *Botel*; (41) *Pulut Rigoti*; (42) *Ketan Putri*; (43) *Aek Sibundong*; (44) *Batang Paiman*; (45) *Bondoyudo*; (46) *Cigeulis*; (47) *Cilamaya Muncul*; (48) *Ciliwung*; (49) *Cisantana*; (50) *Cisokan*.

CONCLUSIONS

The thirteen agronomic trait variabilities showed the differentiation among the accessions, while phenotypic traits found were grouped into six clusters. The genotyping characterization was conducted using SSR (Simple Sequence Repeats) markers (22 microsatellites), continued with the genetic diversity and Polymorphism Information Content (PIC) analyses. The agro-morphological clustering based on Ward's Hierarchical constructed six subclusters with the PC1 and PC2 obtained 86.3% of the total percentage.

ACKNOWLEDGMENTS. Authors would like to thank the Islamic Development Bank (IDB) Project and Center for Development of Advanced Sciences (CDAST) University of Jember, Indonesia for funding this project.

REFERENCES

Agrama, H.A., Yan, W., Jia, M., Fjellstrom, R. & McClung, A.M. 2010. Genetic structure associated with diversity and geographic distribution in the USDA rice world collection. *Natural Science* **2**, 247.

Allhgholipour, M., Farshdfar, E. & Rabiei, B. 2014. Molecular characterization and genetic diversity analysis of different rice cultivars by microsatellite markers. *Genetika* **46**, 187–198.

Barry, M.B., Pham, J.L., Noyer, J.L., Courtois, B., Billot, C. & Ahmadi, N. 2007. Implications for in situ genetic resource conservation from the ecogeographical distribution of rice genetic diversity in Maritime Guinea. *Plant Genetic Resources* **5**, 45–54.

Brondani, C., Caldeira, K.D.S. & Borba, T.C.O. 2006. Genetic variability analysis of elite upland rice genotypes with SSR markers. *Crop Breeding and Applied Biotechnology* **6**, 9–17.

Caicedo, A.L., Williamson, S.H., Hernandez, R.D., Boyko, A., Fledel-Alon, A., York, T.L., Polato, N.R., Olsen, K.M., Nielsen, R., McCouch, S.R., Bustamante, C.D. & Purugganan, M.D. 2007. Genome-wide patterns of nucleotide polymorphism in domesticated rice. *PLoS Genet.* **3**(9), 1745–56. doi: 10.1371/journal.pgen.0030163

Chakravarthi, B.K. & Naravaneni, R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (Oryza sativa L.). *African Journal of Biotechnology* **5**, 684–688.

Garris, A.J., Tai, T.H., Coburn, J., Kresovich, S. & McCouch, S. 2005. Genetic structure and diversity in Oryza sativa L. *Genetics* **169**, 1631–1638.

Ghneim, H.T., Posso, D.D., Pérez, A.I., Torrealba, N.G., Pieters, A.J., Martinez, C.P. & Tohme, J.M. 2008. Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. *Electronic Journal of Biotechnology* **11**, 3–4.

Huang, M., Xie, F.M., Chen, L.Y., Zhao, X.Q., Jojee, L. & Madonna, D. 2010. Comparative analysis of genetic diversity and structure in rice using ILP and SSR markers. *Rice Science* **17**, 257–268.

Hokazono, S., Hayashi, K. & Sato, M. 2009. Potentialities of organic and sustainable rice production in Japan from a life cycle perspective. *Agronomy Research* **7**(I), 257–262.

Jasim, A.S., Rafii, M.Y., Latif, M.A., Sakimin, S.Z., Arolu, I.W. & Miah, G. 2018. Genetic diversity of aromatic rice germplasm revealed by SSR markers. *BioMed research international*, **2018.**

Karimah, A.Z., Siswoyo, T,S., Kim, K.M. & Ubaidillah, M. 2021. "Genetic Diversity of Rice Germplasm (Oryza Sativa L.) of Java Island, Indonesia." *Journal of Crop Science and Biotechnology* **24**(1), 93–101. https://doi.org/10.1007/s12892-020-00063-

Liu, K. & Muse, S.V. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**, 2128–2129.

McCouch, S.R., Teytelman, L. & Xu, Y. 2002. Development and mapping of 2240 new SSR markers for rice (Oryza sativa L.). *DNA research* **9**, 199–207.

Nachimuthu, V.V., Muthurajan, R. & Duraialaguraja, S. 2015. Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in Oryza sativa L. *Rice* **8**, 1–25.

Ni, J., Colowit, P.M. & Mackill, D.J., 2002. Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science* **42**, 601–607.

Pachauri, V., Taneja, N., Vikram, P., Singh, N.K. & Singh, S. 2013. Molecular and morphological characterization of Indian farmers rice varieties (Oryza sativa L.). *Australian Journal of Crop Science* **7**, 923–932.

Pandey, S.K., Das, A., Rai, P. & Dasgupta, T. 2015. Morphological and genetic diversity assessment of sesame (Sesamum indicum L.) accessions differing in origin. *Physiology and molecular biology of plants* **21**, 519–529.

Park, J.R., Kim, E.G., Jang, Y.H., Jan, R., Farooq, M., Ubaidillah, M. & Kim, K.M. 2022. Applications of CRISPR/Cas9 as New Strategies for Short Breeding to Drought Gene in Rice. *Front Plant Sci*. Feb **24**(13), 85044 doi: 10.3389/fpls.2022.850441

Prysiazhniuk, L., Honcharov, Y., Chernii, S., Hryniv, S. & Melnyk, S. 2020. The use of DNA markers for the evaluation of maize lines and hybrids based on cytoplasmic male sterility. *Agronomy Research* **18**(Special Issue 2), 1424–1432

Roy, S., Marndi, B.C., Mawkhlieng, B., Banerjee, A., Yadav, R.M., Misra, A.K. & Bansal, K.C. 2016. Genetic diversity and structure in hill rice (Oryza sativa L.) landraces from the North–Eastern Himalayas of India. *BMC genetics* **17**, 1–15.

Sabu, K.K, Abdullah, M.Z., Lim, L.S. & Wickneswari, R. 2009. Analysis of Heritability and Genetic Variability of Agronomically Important Traits in Oryza Sativa x O. Rufipogon Cross. *Agronomy Research* **7**(1), 97–102.

Sajib, A.M., Hossain, M., Mosnaz, A.T.M.J., Hossain, H., Islam, M., Ali, M. & Prodhan, S.H. 2012. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landreces of rice (Oryza sativa L.). *Journal of BioScience & Biotechnology* **1**, 107–106.

Shanina, E.P. & Likhodeyevsky, G.A. 2021. Evaluation of interspecific potato breeding material with a complex of genes of immunity to potato virus y using molecular markers. *Agronomy Research* **19**(1), 224–231.

Singh, N., Debjani, R.C., Tiwari, G., Singh, A.K., Sundeep, K., Srinivasan, K., Tyagi, R.K., Sharma, A.D., Singh, N.K. & Singh, R. 2016. Genetic diversity trend in Indian rice varieties: An analysis using SSR markers. *BMC Genetics* **17**, 1–13. 10.1186/s12863-016-0437-7

Šķipars, V., Ruņģis, D., Ločmele, I., Seile, S., Mežaka, I. & Legzdiņa, L. 2021. Evaluation of Morphological Traits, Genetic Diversity and Major Resistance Genes in Barley Subpopulations Cultivated under Organic and Conventional Farming Systems. *Agronomy Research* **19**(4), 1981–98.

Thomson, M.J., Polato, N.R., Prasetiyono, J., Trijatmiko, K.R., Silitonga, T.S. & McCouch, S.R. 2009. Genetic Diversity of Isolated Populations of Indonesian Landraces of Rice (Oryza Sativa L.) Collected in East Kalimantan on The Island of Borneo. *Rice*. **2**, 80–92.

Thomson, M.J., Septiningsih, E.M., Suwardjo, F., Santoso, T.J., Silitonga, T.S. & McCouch, S.R. 2007. Genetic diversity analysis of traditional and improved Indonesian rice (Oryza sativa L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics* **114**, 559–568.

Varshney, R.K., Thiel, T., Sretenovic-Rajicic, T., Baum, M., Valkoun, J., Guo, P., Grando, S., Ceccarelli, S. & Graner, A. 2008. Identification and validation of a core set of informative genic SSR and SNP markers for assaying functional diversity in barley. *Molecular Breeding* **22**, 1–13.

Vaughan, D.A., Lu, B.R. & Tomooka, N. 2008. The evolving story of rice evolution. *Plant science* **174**, 394–408.

Yadav, S., Singh, A., Singh, M.R., Goel, N., Vinod, K.K., Mohapatra, T. & Singh, A.K. 2013. Assessment of genetic diversity in Indian rice germplasm (Oryza sativa L.): use of random versus trait-linked microsatellite markers. *Journal of genetics* **92**, 545–557.

Zaidi, M.A., Narayanan, M., Sardana, R., Taga, I., Postel, S., Johns, R., McNulty, M., Mottiar, Y., Mao, J., Loit, E. & Altosaar, I. 2006. Optimizing tissue culture media for efficient transformation of different indica rice genotypes. *Agronomy Research* **4**, 563–575.