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Genetic diversity in traditional and modern allelopathic rice accessions revealed by minisatellite markers

M.S. Mazid*, A.S. Juraimi*, M.Y. Rafii[1], M.M. Hanafi[2], M.A. Hakim[1],
J. Ferdous[3] and S.H.Tan,
Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia,
UPM Serdang, Selangor DE 43400, Malaysia
E-mail: ashukur@upm.edu.my; msmazidupm@gmail.com

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ABSTRACT

This study aimed to assess the genetic diversity among the 46-allelopathic rice accessions from Bangladesh Rice Research Institute (BRRI) and Malaysian Agricultural Research and Development Institute (MARDI) and Africa using 20 minisatellite or inter simple sequence repeat (ISSR) markers. Out of 20 ISSR markers, 10-markers generated 188 polymorphic loci. The highest polymorphic loci (41) were found in accession BRRI dhan47, whereas, the lowest was in BR11 (22). Mean Shannon's information index and polymorphic information content were 0.37 and 0.19, respectively, in ten markers. The mean effective number of alleles in the 10-ISSR markers varied from 1.24 to 1.56. The number of polymorphic loci was highest (29) in WITA3 and lowest (24) in WITA12 accessions of African population. The cluster analysis revealed 10-main groups, including several subgroups at 0.31 similarity coefficient. The principal component analysis elucidated 36.94% complete variations in all accessions in this study. There was considerable genetic diversity in rice accessions in this study. The information about genetic diversity and relationship obtained from the study may be used to develop the allelopathic rice in future breeding programmes.

Key words: Allelopathy, analysis of molecular variance, dendrogram, genetic diversity, genetic similarities, inter-simple sequence repeats (ISSR), polymorphism, genetic resources, principal component analysis, rice accessions.

INTRODUCTION

Rice (*Oryza sativa* L.) is staple food of 3.0 billion people and its productivity is affected by numerous biotic and abiotic stresses (14). Weeds are one of the main biotic stresses. The rice growth and yield is reduced by weeds, a major constraint (30,31,43). Currently, weeds are controlled by herbicides (30) in spite of their several harmful effects (17,21,39). Hence, use of allelopathic rice variety, developed through genetic manipulation into rice cultivars seems to be the main alternative for weed management (3,5,40).

In rice variety development, the genetic diversity play important role in rice germplasm (16). Genetic variation is very important to make improvement in crop breeding (46). Moreover, the genetic diversity is very essential to effectively select the parents for hybridization and in breeding programme (45). Since, environmental factors

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*Correspondence author, [1]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, UPM Serdang, 43400 Serdang, Selangor, DE, Malaysia,[2]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, UPM Serdang, Selangor DE 43400, Malaysia,[3]Biotechnology divisions Bangladesh Rice Research Institute (BRRI), Gazipur-1701, Bangladesh.

influence the gene expression of different traits, therefore, evaluation of genetic variation merely based on morphological markers cannot assess the diversity (37,38).

Among various techniques, molecular markers are very useful to evaluate the genetic variability and study the evolutionary kinship (23). Molecular markers can detect the variations among the genotypes at molecular level compared to morphological analysis. Besides, they facilitates the assessment of genetic diversity, germplasm identification and varietal classification (41). Different molecular markers [random amplified polymorphic DNA (RAPD) (2), restriction fragment length polymorphism (RFLP) (36), amplified fragment length polymorphism (AFLP) (44) simple sequence repeat (SSR) (2,9), inter simple sequence repeat (ISSR) (1,2) and single nucleotide polymorphism (SNP) (19,45)] are available to assess the genetic variation in rice. Of these, ISSR markers are extensively used to determine the genetic diversity in crops (10,47). The ISSR marker influences the amplification of DNA segment, if it is in amplifiable distance between the two indistinguishable microsatellite repeat area oriented in reverse (48). As long primers (15-30 bp) are used for ISSR, thus high annealing temperature results in high reproducibility and rigidity (48). Such marker is considered impartial, nonaligned and does not need information of DNA sequences. This study aimed to (i) determine genetic diversity and variation in 46 allelopathic rice accessions and (ii) assess the genetic kinship among these rice accessions.

MATERIALS AND METHODS

Crop management : The research was conducted in net house (open field) in Field 2/ Universiti Putra Malaysia (UPM) [3º02′ North latitude and 101º42′ East longitudes and altitude was 31 m above sea level]. The rice accessions were grown in plastic buckets in the main season (August, 2015 to January, 2016). The allelopathic rice accessions were obtained from Malaysian Agricultural Research and Development Institute (MARDI), Bangladesh Rice Research Institute (BRRI) (24). These accessions were selected based on allelopathic research in past 20 years (4,18,21,26). Although, information regarding the allelopathic nature of these rice accessions is available, but information about their genetic diversity is scanty. Forty six rice accessions (including 27 local and 19 exotic) were taken and the treatments were replicated thrice in randomized complete block design (RCBD). Twenty five seeds of each accession were sown on August 13, 2015 in Petri dishes (9 cm dia.) lined with Whatman filter paper No. 1, at room temperature (30±2°C) with 12 h light and 10 mL distilled water was added per petri dish. Petri dishes were regularly monitored and if needed water were added. Six-days-old sprouted seeds were shifted from Petri dishes to plastic pots (14.25 cms dia and depth 25 cms) containing 12 kg sandy clay loam soil, (pH 5.8). In each pot 0.3, 0.4, 0.6 g urea was added as basal dose. Only, seven seedlings were grown in each pot for 30 days. Fertilizer management and other cultural practices were done as per guide lines (7).

Molecular markers: Ten ISSR markers with clear amplifications were identified from 20 ISSR markers for the genetic diversity study of 46 allelopathic rice accessions (Table 1).

Genotyping protocol: Total genomic DNA was extracted from 30 days old young rice leaves using slightly modified CTAB method (12). Total volume for polymerase chain reaction (PCR) was 15 µl. This PCR reaction volume consisted of : 70 ng DNA template (1 µl), PCR master mix (7.4 µl) (Dream Taq green PCR master mix-2X contained 4 mM $MgCl_2$, dNTPs, green buffer, Product code# K1082, Fermentas, Maryland, USA), primer (1 µl) and nuclease-free double-distilled water (5.6 µl). A PCR machine (My cycler thermo cycler, BIO RAD, USA) was used to get the PCR product. In order to run the PCR machine, we followed Touch Down protocol (22). There were 3-phases in this protocol. The temperature was fixed to 95ºC for 3 min before the phase one.

Phase I: The denaturation temperature was set to 95ºC for 30 s, annealing at T_m+10ºC for 45 s and the elongation temperature was adjusted to 72ºC for 60 s.

Phase II:, The temperature conditions were: for 30 s denaturation at 95ºC, for 45 s annealing at T_m-5ºC, and for 60 s the elongation at 72ºC.

Phase III:, The elongation temperature was fixed to 72ºC for 5 min. Phase one and two were repeated 10 and 20 times, respectively.

Finally, from each PCR product samples, 5 µl product was loaded on 1.5% agarose gel and gel was run inside electrophoresis machine containing 1% TBE buffer at 75V for 90 min. Using UVPRO Alpha Innotech gel documentation unit, the gel pictures were taken.

Table 1. List of ISSR markers used in this study.

S. No.	Oligo name (Primers)	Sequence	Tm (°C)
1	Primer_1	5′-AGA GAG AGA GAG AGA GT-3′	54.8
2	Primer_6	5′-ACA CAC ACA CAC ACA CT-3′	54.8
3	Primer_7	5′-GAG AGA GAG AGA GAG AT-3′	54.8
4	Primer_3	5′-AGA GAG AGA GAG AGA GYT-3′	56.5
5	Primer_11	5′-AGA GAG AGA GAG AGA GYA-3′	56.5
6	Primer_13	5′-ACA CAC ACA CAC ACA CYA-3′	56.5
7	Primer_15	5′-DBD ACA CAC ACA CAC AC-3′	55.6
8	Primer_5	5′-AGA GAG AGA GAG AGA GC-3′	57.2
9	Primer_8	5′-GAG AGA GAG AGA GAG AC-3′	57.2
10	Primer_14	5′-GAC AGA CAG ACA GAC A-3′	54.2

Statistical analysis

In this study, amplified fragments were scored as ‘1’ for the presence and ‘0’ for the absence of a band. A matrix was obtained from the analysis of molecular data and further statistical analyses were performed with the help of a programme POPGENE (version 1.31). Different genetic diversity parameters such as effective number of alleles (*Ne*), polymorphic information content (PIC), Shannon information index (*I*), Nei’s gene diversity (*H*), observed heterozygosity and percentage of polymorphic loci were calculated. Jaccard’s similarity coefficients were calculated using NTSYS-PC (version 2.1). The Jaccard’s similarity coefficients were used to construct a dendrogram applying the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). With the help of GENALEX 6.41 software (33) analysis of molecular variance (AMOVA) was performed which provided separation of difference among as well as within the population.

RESULTS AND DISCUSSION

Minisatellite markers analysis

Out of 20 minisatellites or inter simple sequence repeats (ISSR) markers, ten were polymorphic. The number of polymorphic loci was 188 revealed by10-polymorphic ISSR markers (Table 2, Fig. 1). From the analysis, 100 % polymorphic loci were found. The highest polymorphic loci (41) were observed in accession BRRI dhan47, while, it was lowest in BR11 (22) (Table 3). On an average, each accession produced 30.76 polymorphic loci. The number of largest and the smallest polymorphic loci were noticed in BRRI population by accessions BRRI dhan47 and BR11, respectively. On the other hand, the Acheh Puteh produced the lowest number of polymorphic loci, while, Anak Didek 3 and MR159 produced the largest number of polymorphic loci in MARDI population. Besides, the highest number of polymorphic loci (29) was found in WITA3 and; lowest (24) in WITA12 accessions of African population (Table 3). The genetic diversity in germplasms is the foundation of crop improvement. The magnitude of variation in the plant material, determines whether the germplasm could be used. The main goal of every genetic diversity study is to realize the prospect of ordering individual genotypes into different clades (23).

Table 2. Genetic divergence parameters in 10 ISSR markers.

List of primers	Average number of polymorphic Loci	Average PIC	Average effective number of allele	Average Nei's index	Average Shannon's index	Average observed heterozygosity
ISSR-1	19	0.14	1.24	0.17	0.29	0.00
ISSR-3	15	0.22	1.41	0.27	0.42	0.00
ISSR-4	20	0.17	1.31	0.21	0.34	0.00
ISSR-5	15	0.25	1.56	0.32	0.48	0.00
ISSR-6	21	0.21	1.42	0.26	0.40	0.00
ISSR-7	19	0.21	1.40	0.25	0.41	0.00
ISSR-10	20	0.18	1.38	0.23	0.35	0.00
ISSR-11	21	0.19	1.37	0.23	0.37	0.00
ISSR-12	17	0.17	1.31	0.21	0.35	0.00
ISSR-15	21	0.15	1.25	0.18	0.30	0.00

Data was calculated in di-allelic form.

Genetic diversity study based on phenotypic data do not provide the precise genetic variability due to the interactions between genotype and environment but also due to the unidentified genetic control of polygenic agronomic and morphological traits (42). Based on DNA markers, molecular characterization of germplasms is important tool to evaluate the genetic diversity (15). The ISSR markers successfully and effectively determined the genetic diversity in different rice genotypes (1,2,27). In this study, genetic diversity in 46 rice accessions was determined using ten ISSR markers. The results are comparable to Joshi *et al*. (20), where genetic diversity in 42 rice genotypes was determined using 11 ISSR markers. Genetic diversity of 41 bacterial blight resistant rice genotypes were assessed using 20 ISSR markers in glass house (27). Moreover, Lin *et al*. (25) used seven pairs of ISSR primers to assess the genetic diversity in 57 rice accessions. Likewise, the genetic diversity in 27 Hassawi rice genotypes was determined using 11 ISSR markers in green house (1). Under this study, in each primer the polymorphic loci ranged from 15 to

21 (mean 18.80) which is in agreement with Mazid *et al*. (27) but is higher than those in Samal *et al*. (35) and Raghavendra *et al*. (34). The polymorphic loci among the rice accessions varied from 22 to 41 (mean 30.76), which was lower than Mazid *et al*. (27).

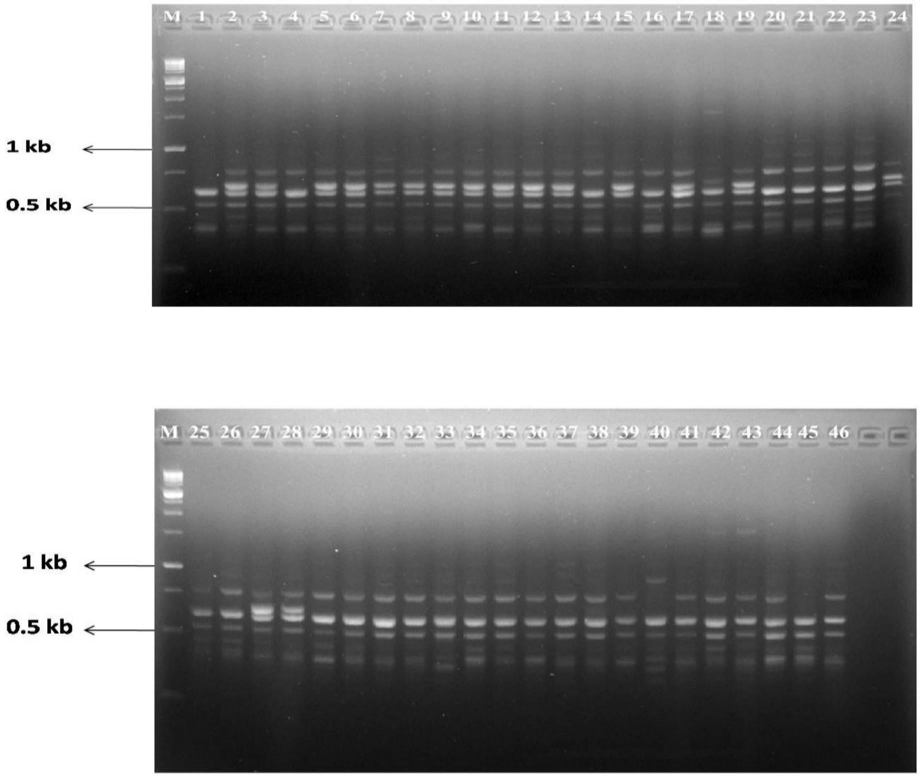

Figure 1. Polymorphism among rice accessions from VI to V46 using same ISSR marker, M=ladder.

Genetic variation

The mean effective number of alleles in 10-ISSR markers ranged from 1.24 to 1.56 (Table 2). The mean maximum and minimum Nei’s gene diversity was 0.32 and 0.17, respectively. The range of average Shannon’s information index was 0.29 and 0.48. Furthermore, the average polymorphic information content (PIC) varied from 0.14 for ISSR 1 to 0.25 for ISSR 5 (Table 2). In rice accessions, the lowest mean effective numbers of alleles was 1.30 in BRRI dhan57 and the highest 1.54 in MR159 (Table 3). The maximum value of both Shannon's information index and Nei's gene diversity were in MR159, while, the minimum were in BRRI dhan57. The mean PIC value was 0.24 and the highest (0.31) was in accessions Anak Didek 3 and the lowest (0.20) in BRRI dhan57 (Table 3). The range of mean effective number of alleles in ISSR markers in current study was lower than that of Mazid *et al.* (25). The range of Nei's gene diversity was 0.17 to 0.32 (mean 0.23), which was lower than in Mengistu (28) and Oladosu *et al*. (32). In this current study, mean Shannon information index ranged from 0.29 to 0.48 (mean 0.37) in which was higher than the value reported by Oladosu *et al*. (32) but lower than those in Mazid *et al*. (27). The PIC value varied from 0.14 to 0.25 (mean 0.19), which is lower than Mazid *et al*. (27) and Oladosu *et al*.(32). The range of mean effective numbers of alleles in rice accessions in the present study was lower than that of Mazid *et al.* (25). The Shannon's information index varied from 0.39 to 0.53 (mean 0.46) in, which was lower than Cao *et al*. (8) and Mazid *et al*. (27). Moreover, Nei's gene diversity ranged from 0.23 to 0.35 (mean 0.29), which was lower than de oliveira Borba *et al*. (2009) (11) and Mazid *et al*. (27). The PIC value ranged from 0.20 for BRRI dhan57 to 0.31 for Anak Didek3 with mean of 0.24, which was lower than in Lapitan *et al*. (24) and Mazid *et al*. (27).

In MARDI rice population, the maximum values for genetic diversity parameters (Nei's gene diversity, number of effective alleles and Shannon's information index) were found in rice accession MR159, whereas, these were minimum in accession Siam Er 54 (Table 3). The PIC value ranged from 0.22 to 0.31 in MARDI rice accessions. In BRRI rice population, the genetic diversity parameters were highest in accession BRRI dhan47 and lowest in BRRI dhan57. The mean PIC value in the BRRI rice accessions was 0.24 and it ranged from 0.28 for BRRI dhan47 to 0.20 for BRRI dhan57. The average effective numbers of alleles were 1.36 and it ranged between 1.35 and 1.37 in African population. Nei's gene diversity varied from 0.26 to 0.27. The maximum value (0.44) for Shannon's information index was found in accession WITA12, while it was lowest in WITA3 in African population. Accession WITA12 had the maximum PIC value (0.23) and the lowest (0.22) was in WITA3. Among the three populations (MARDI, BRRI, African), the highest PIC value (0.25) was found in MARDI population (Table 4). We found that genetic variability was present in accessions of three populations in this study. The MARDI population had highest PIC value (0.25) followed by BRRI population (0.24) and African population (0.23). The Shannon's index was highest in MARDI population (0.47), followed by BRRI population (0.45) and African population (0.44). The values in the current study were lower than the earlier reported values (27). The highest and the lowest Nei's gene diversity were found in MARDI (0.29) and African (0.27) population respectively. Mazid *et al*. (27) reported higher values for Nei's gene diversity than the values found in the current study populations. In this study, the mean genetic similarity coefficient in 46 rice accessions was 0.25, indicating the existence of high genetic variation. Our results are in conformity with Bhuyan *et al*. (6) who also reported mean genetic similarity of 0.25 in 24 rice genotypes using ISSR markers. In this study, all ISSR markers, population and rice accessions showed '0' value for the gene flow (Nm) and the heterozygosity.

Table 3. Genetic divergence parameters among 46 rice accessions by 10 ISSR markers.

Accessions	Number of polymorphic loci	PIC	Effective number of allele	Nei's index	Shannon index	Observed heterozygosity
Jambok	32	0.26	1.45	0.31	0.49	0.00
Manik	30	0.23	1.37	0.27	0.44	0.00
Siam Er 54	28	0.22	1.34	0.25	0.42	0.00
Wangi	30	0.23	1.37	0.27	0.44	0.00
Padang Gelap	31	0.25	1.43	0.30	0.48	0.00
Acheh Puteh	25	0.23	1.37	0.27	0.44	0.00
Pasir	32	0.25	1.43	0.30	0.48	0.00
Singgora	26	0.24	1.40	0.28	0.46	0.00
Merah Isi	29	0.24	1.39	0.28	0.45	0.00
Chatek Kuning	37	0.26	1.46	0.32	0.50	0.00
Anak Naga	36	0.26	1.45	0.31	0.49	0.00
Anak Didek 3	38	0.31	1.48	0.32	0.50	0.00
Seberang (MR77)	30	0.23	1.37	0.27	0.44	0.00
Sekencang (MR7)	36	0.26	1.45	0.31	0.49	0.00
Anak China	26	0.24	1.39	0.28	0.46	0.00
Anak Ikan China	29	0.24	1.40	0.29	0.46	0.00
Y1021	31	0.23	1.38	0.28	0.45	0.00
Amur	32	0.24	1.39	0.28	0.46	0.00
Jintan Puteh	29	0.22	1.35	0.26	0.43	0.00
MR84	35	0.27	1.48	0.32	0.50	0.00
MR127	35	0.25	1.43	0.30	0.48	0.00
MR85	37	0.26	1.46	0.32	0.50	0.00
MR159	38	0.28	1.54	0.35	0.53	0.00
MR14	28	0.23	1.38	0.28	0.45	0.00
MR73	27	0.23	1.37	0.27	0.44	0.00
MR59	32	0.26	1.45	0.31	0.49	0.00
MR15	32	0.24	1.39	0.28	0.46	0.00
BR-5331-93-2-8-4	30	0.23	1.37	0.27	0.44	0.00
BR-5615-9-1-2	33	0.24	1.41	0.29	0.46	0.00
BR17	31	0.25	1.43	0.30	0.48	0.00
BR11	22	0.21	1.33	0.25	0.41	0.00
BR21	27	0.23	1.37	0.27	0.44	0.00
BR25	31	0.23	1.38	0.28	0.45	0.00
BR26	30	0.23	1.37	0.27	0.44	0.00
BRRI Dhan29	29	0.24	1.40	0.29	0.46	0.00
BRRI Dhan57	25	0.20	1.30	0.23	0.39	0.00
BRRI Dhan40	24	0.23	1.36	0.27	0.44	0.00
Dular	33	0.24	1.41	0.29	0.46	0.00
BRRI Dhan42	27	0.21	1.33	0.25	0.41	0.00
BRRI Dhan46	34	0.26	1.47	0.32	0.50	0.00
BRRI Dhan47	41	0.28	1.52	0.34	0.52	0.00
BRRI Dhan62	31	0.23	1.38	0.28	0.45	0.00
BRRI Dhan64	30	0.26	1.47	0.32	0.50	0.00
BRRI Dhan39	33	0.24	1.41	0.29	0.46	0.00
WITA3	29	0.22	1.35	0.26	0.43	0.00
WITA12	24	0.23	1.37	0.27	0.44	0.00

Data was calculated in di-allelic form.

Table 4. Different genetic divergence parameters among three populations by 10 ISSR markers. Data was calculated in di-allelic form.

	Mean					
Population	Polymorphic loci number	PIC	Effective alleles	Nei's index	Shannon index	Observed heterozygosity
MARDI	31.51	0.25	1.41	0.29	0.47	0.00
BRRI	30.05	0.24	1.39	0.28	0.45	0.00
African	26.5	0.23	1.36	0.27	0.44	0.00

Cluster analysis

The Jaccard's genetic similarity coefficient revealed high genetic variability in test rice accessions. It ranged from 0.02 (between Acheh Puteh and BRRI dhan29) to 0.66 (between BRRI dhan40 and BRRI dhan46). The highest diversity was noticed between several Malaysian, Bangladeshi and African rice accessions. The similarity coefficient was 0.02 between the Malaysian rice Acheh Puteh and Bangladeshi modern rice BRRI dhan29 (The most diverse accessions), between MR14 and BR-5615-9-1-2, and between Malaysian rice MR14 and African rice WITA3, followed by similarity coefficient of 0.03 between the Malaysian traditional rice Jambok and Bangladeshi rice BR-5331-93-2-8-4. On the other hand, the highest similarity was found between the improved modern varieties. The maximum similarity (0.66) was noted between the Bangladeshi developed varieties BRRI dhan40 and BRRI dhan42, followed by those between Malaysian modern rice varieties MR84 and MR127 (0.57). A dendrogram (Fig. 2) was constructed with the UPGMA cluster analysis using the Jaccard's similarity coefficient that showed the genetic relationship in test rice accessions of this study. Rice accessions were clustered into10 main groups or clusters, with a similarity coefficient of 0.31. Cluster IX contained the maximum number of rice accessions followed by cluster II, cluster III and cluster VIII. The cluster I had three accessions, cluster V and cluster VI had two accessions and cluster IV, VII and X each had one accession (Fig. 2).

Name of rice accessions with respective groups or clusters including similarity are given (Table 5). The un-weighed pair group method with arithmetic mean (UPGMA) dendrogram largely grouped the 46 rice accessions into 10 major clusters, with similarity coefficient value of 0.31. Oladosu *et al.* (32) reported six main clusters, from 32 rice accessions applying 25 ISSR markers and Jaccard's genetic similarity coefficient. Moreover, using eight ISSR markers and Dice similarity co-efficient, Raghavendra *et al.* (34) found two main clusters from 66 rice varieties. In this study, grouping of rice accessions based on polymorphic ISSR markers showed that low to high genetic variability was present in studied rice accessions. The highest diversity was in Malaysian traditional rice accessions, this indicated the variations in their genetic design. Contrarily, modern rice accessions showed the highest similarity in this study due to common ancestry. These rice accessions were associated with ancestors i.e. had common parents, as revealed in their high genotypic relationships. For example, the maximum similarity was observed between rice accessions BRRI dhan40 and BRRI dhan42. The BRRI dhan40 was developed from a cross between BR10 and IR4595-4-1-15. While, BRRI dhan42 was developed from a cross between BR14 and IR 25588-7-3-1. Again BR10 was developed from a cross between IR5-114-3-1 and IR20. On the other hand BR14 was developed from a cross between IR5 and BR3. So, the cause of maximum similarity between BRRI dhan40 and BRRI dhan42 was the common ancestor IR5 (BRKB 2018). The 46 rice accessions were grouped into 10 major clusters through UPGMA-based clustering method with Jaccard's genetic similarity due to parental relationships. Another reason could be geographical distribution of the accessions. The results support the existence of region specific diversity, due to long term adaptability to climate and soil, over exploitation and several generations of selection (29). While, most of the Bangladeshi modern rice accessions were grouped into cluster II except WITA3 and WITA12 which were African. The reason could be similarity in genetic composition. However, not a single Malaysian rice accession was grouped with either Bangladeshi or African rice accessions.

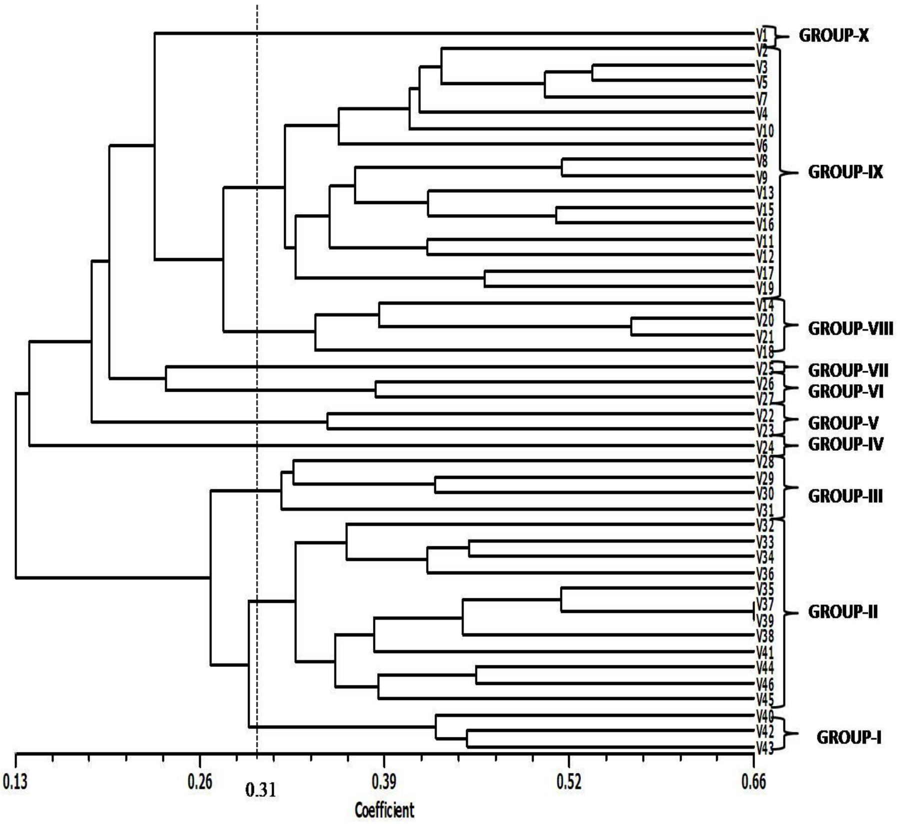

Figure 2. The figure illustrates the pattern of grouping that separated the 46 rice accessions into 10 groups at 0.39 similarity coefficient based on ISSR markers analysis. Note: V1= Jambok , V2= Manik , V3= Siam Er 54, V4=Wangi, V5= Padang Gelap, V6= Acheh Puteh, V7= Pasir, V8= Singgora, V9= Merah Isi, V10= Chatek Kuning, V11= Anak Naga, V12= Anak Didek 3, V13=Seberang (MR77), V14= Sekencang (MR7), V15= Anak China, V16= Anak Ikan China , V17= Y1021, V18= Amur, V19= Jintan Puteh, V20= MR 84, V21= MR127, V22= MR85, V23= MR159, V24= MR14, V25= MR73 (Makmur), V26= MR59, V27= MR15, V28= BR-5331-93-2-8-4, V29= BR-5615-9-1-2, V30= BR17, V31= BR11, V32= BR21, V33= BR25, V34= BR26, V35= BRRI Dhan29, V36= BRRI Dhan57, V37= BRRI Dhan40, V38= Dular, V39= BRRI Dhan42, V40= BRRI Dhan46, V41= BRRI Dhan47, V42= BRRI Dhan62, V43= BRRI Dhan64, V44= BRRI Dhan39, V45= WITA-3, V46= WITA-12.

Malaysian rice accessions either grouped with other Malaysian rice accessions or they formed their own individual groups indicating their different genetic composition. The reason of forming isolated group could be their parents were from different sources or they have different genetic structure than Bangladeshi or African rice accessions. Therefore, it is confirmed in this study that MARDI population was comparatively more divergent than BRRI and African rice populations. The present study findings are consistent with the results of Mazid *et al*. (27). The principal component analysis found 36.94% variability in all accessions in this study. Besides, the relationship between PCA and cluster analyses supported the results of genetic variations.

Table 5. Groups of 46-rice accessions according to cluster analysis with similarity range between accessions.

Group	Rice Accessions	Range of Similarity Coefficient (%)
I	BRRI dhan46, BRRI dhan62 and BRRI dhan64	42 to 45
II	BR21, BR25, BR26, BRRI dhan57, BRRI dhan29, BRRI dhan40, BRRI dhan42, Dular, BRRI dhan47, BRRI dhan39, WITA12 and WITA3	24 to 66
III	BR-5331-93-2-8-4, BR-5615-9-1-2, BR17 and BR11	26 to 43
IV	MR14	-
V	MR85 and MR159	35
VI	MR59 and MR15	39
VII	MR73	-
VIII	Sekencang, MR84, MR127 and Amur	33 to 57
IX	Manik, Siam Er 54, Padang Gelap, Pasir, Wangi, Chatek Kuning, Acheh Puteh, Singgora, Merah Isi, Seberang, Anak China, Anak Ikan China, Anak Naga, Anak Didek3, Y1021 and Jintan Puteh	20 to 54
X	Jambok	

Principal component analysis

The principal component analysis grouped 46 rice accessions into 10 clusters (Fig. 3). The group A of PCA plot consisted of BRRI dhan46, BRRI dhan64, BRRI dhan62, BR17 and MR59. This group was mostly similar to group I in cluster analysis, except BR17 and MR59. Group B of PCA plot consisted of BR21, BR25, BR26, BRRI dhan29, BRRI dhan57, BRRI dhan40, BRRI dhan42, BRRI dhan46, and BRRI dhan62, Dular, WITA3 and WITA12. This group B largely resembled to the group two (II) of cluster analysis except BRRI dhan47 and BRRI dhan39. Group C from the plot of PCA and group three (III) of cluster analysis had almost similar accessions except BR17 which assembled in group A in PCA. The common accessions between group C of PCA and group three (III) of dendrogram were BR-5331-93-2-8-4, BR-5615-9-1-2 and BR11. Accession MR14 belonged to group D of PCA and group four (IV) of cluster analysis. Group five (V) of cluster analysis had two members namely MR85 and MR159, which was similar to the group E of PCA plot except MR84. The accession MR15 was common in group six (VI) of cluster analysis and group F of PCA. However accession MR59 although formed group with MR15 in cluster analysis but it joined the group A in PCA. Moreover, group seven

(VII), ten (X) of cluster analysis and group J, I of PCA contained only one similar accession (group VII had MR73 and group X had Jambok). Nonetheless, there was a minor discrepancy between group eight (8) of cluster analysis and group G in PCA. In the group eight (VIII) of dendrogram had four accessions like Sekencang, Amur, MR84, and MR127. But rice accession MR84 was not in group G of PCA rather it joined the group E in PCA plot. A total of 16 accessions such as Manik, Siam Er 54, Wangi, Padang Gelap, Acheh Puteh, Pasir, Singgora, Merah Isi, Chatek Kuning, Anak Naga, Anak Didek 3, Seberang, Anak China, Anak Ikan China, Y1021 and Jintan Puteh formed group nine (IX) of dendrogram and group H in PCA plot.

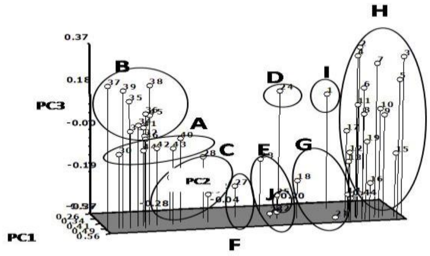

Figure 3. Three-dimensional plot of PCA depicting the pattern of kinships among 46 rice accessions and supporting the cluster analysis mostly based on ISSR markers analysis. Note: V1= Jambok , V2= Manik , V3= Siam Er 54, V4=Wangi, V5= Padang Gelap, V6= Acheh Puteh, V7= Pasir, V8= Singgora, V9= Merah Isi, V10= Chatek Kuning, V11= Anak Naga, V12= Anak Didek 3, V13=Seberang (MR77), V14= Sekencang (MR7), V15= Anak China, V16= Anak Ikan China , V17= Y1021, V18= Amur, V19= Jintan Puteh, V20= MR 84, V21= MR127, V22= MR85, V23= MR159, V24= MR14, V25= MR73 (Makmur), V26= MR59, V27= MR15, V28= BR-5331-93-2-8-4, V29= BR-5615-9-1-2, V30= BR17, V31= BR11, V32= BR21, V33= BR25, V34= BR26, V35= BRRI Dhan29, V36= BRRI Dhan57, V37= BRRI Dhan40, V38= Dular, V39= BRRI Dhan42, V40= BRRI Dhan46, V41= BRRI Dhan47, V42= BRRI Dhan62, V43= BRRI Dhan64, V44= BRRI Dhan39, V45= WITA3, V46= WITA12.

Analysis of molecular variance

The analysis of molecular variance indicated that larger variation existed among the rice accessions within the population (83%) followed by variation among the three populations (17%) (Table 6). This finding is in agreement with Mazid *et al.* (25)'s findings which showed higher variation among the genotypes within populations followed by variation among three populations. However, Girma (13) reported higher variation among the populations than that of within populations.

Table 6. Analysis of molecular variance (AMOVA) for 46 rice accessions by ISSR markers.

Source	df	SS	MS	Estimated variance	Variance (%)
Among Pops	2	181.404	90.702	5.395	17
Within Pops	43	1141.770	26.553	26.553	83
Total	45	1323.174		31.947	100

df-degrees of freedom; SS-sum square; MS-mean square.

CONCLUSIONS

This study aimed to determine the genetic diversity, variation and genetic kinship in 46 rice accessions. We found extensive genetic diversity in allelopathic rice accessions and also their kinship based on grouping. Genetically distant or diverse parent can create good heterosis, which is desirable in breeding programme. Agro-morphological evaluation of these accessions should be conducted. Results of this study should be combined with agro-morphological evaluation to develop superior allelopathic rice variety for weed control.

Conflict of interest

Authors declare that there is no conflict of interests concerning the publication of this work.

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