doi:10.14720/aas.2022.118.4.2500 Original research article / izvirni znanstveni članek

Molecular diversity of rice (*Oryza sativa* L.) genotypes in Malaysia based on SSR markers

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Received January 14, 2022; accepted December 01, 2022.
Delo je prispelo 14. januarja 2022, sprejeto 1. decembra 2022

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Abstract: Rice crop improvement is determined by the degree of genetic variability and the heritability of favorable genes. A total of twenty-five SSR markers were used to measure the level of polymorphism and genetic variation among the 65 rice genotypes. Twenty-one of the twenty-five SSRs were discovered to be polymorphic, whereas the rest were determined to be monomorphic. A total of 91 alleles were found in 21 SSR markers, with an average of 4.00 alleles which ranged from 3 (RM335, RM551, RM538 RM190, RM242 and RM270) to 7 (RM263). The average PIC value was 0.62 ranging from 0.28 (RM 270) to 0.76 (RM 481). The rice genotypes were divided into nine primary clusters by a dendrogram based on NTSYS software's UPGMA analysis. The cluster analysis revealed that these genotypes were divided into nine clusters where cluster IB-1a has the most genotypes (31) followed by cluster IB-1b (24). The genotype BR24 and Utri as well as Pukhi and WANGI PUTEH had the highest dissimilarity coefficient values indicating genotype diversity. These accessions have a lot of genetic diversity among the constituents; thus, they could be used directly in a hybridization program to improve yield-related parameters.

Key words: molecular diversity; SSR markers; polymorphic information content; rice

Molekularna raznolikost genotipov riža (*Oryza sativa* L.) v Maleziji, določena na osnvi SSR označevalcev

Izvleček: Izboljšanje pridelka riža temelji na njegovi genetski variabilnosti in zmožnosti dedovanja primernih genov. Za meritev ravni polimorfizma in genetske variabilnosti med 65 genotipi riža je bilo uporabljenih 25 SSR označevalcev. Od 25 SSR je bilo 21 prepoznanih kot polimorfnih, med tem, ko so bili ostali določeni kot monomorfni. V 21 SSR označevalcih je bilo celokupno 91 alelov s poprečno vrednostjo 4,00, ki je variirala od 3 (RM335, RM551, RM538 RM190, RM242 in RM270) do 7 (RM263). Poprečna vrednost PIC je bila 0,62, z razponom od 0,28 (RM 270) do 0,76 (RM 481). Genotipi riža so se v dendrogramu razdelili v devet primarnih grozdov na osnovi programa NTSYS in UPGMA analize. Analiza grozdov je odkrila devet grozdov, kjer je grozd IB-1a vseboval največ genotipov (31), temu je sledil grozd IB-1b (24). Genotipi kot so BR24, Utri, Pukhi in WANGI PUTEH so imeli največji koeficient neenakosti, kar nakazuje veliko genetsko raznolikost. Te akcesije imajo v svojih lastnostih veliko genetsko raznolikost in bi lahko bile neposredno uporabljene v programih križanja za izboljšanje s pridelkom povezanih lastnosti.

Ključne besede: molekulaska raznolikost; SSR označevalci; informacija o polimorfnosti; riž

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1 INTRODUCTION

Rice is a key crop in the world, and its production is dependent on the development of superior varieties with greater productivity and adaptability, which is largely dependent on the presence of sufficient genetic variation in rice germplasm (Osekita et al., 2015). This is normally performed through hybridization following that in segregating populations in selecting plants with favorable qualities (Becerra et al., 2017)Chile, has a rice (Oryza sativa L.. Rice accessions include a large number of beneficial genes that rice breeders can employ to improve the crop and genetic heterogeneity exists among rice accessions allowing for a wide range of agricultural improvements. Any crop development effort needs genetic diversity because it aids genotype collection, monitoring, and identification. The generation of segregating progenies with significant genetic variety with potential development of recombinants for further selection and introgression of desirable genes from these diverse genotypes. A major goal in evolutionary biology has long been to characterize and quantify genetic variation. Analyzing morphological or molecular data can be used to estimate genetic diversity. One method to understanding their diversity is to use modern molecular technology (Linda Mondini et. al., 2009)

Any crop improvement initiative must start with a genetic diversity assessment since it aids in the development of improved recombinants. Genetic divergence across genotypes is significant in identifying parents with a wide range of characteristics. Genetic diversity can be measured using morphological traits, iso-enzymes, and DNA markers. The morphological changes in physical features that result from genetic differentiation may not be enough to identify between closely related species, races, or ecotypes. As a result, genetic characterization of natural resources is a vital step toward a better understanding of genetic resources and their use in future breeding programmes. Molecular markers have generally outperformed physical pedigree, heterosis and biochemical evidence in assessing genetic diversity (Nadeem et al., 2018). Factors affecting genetic gain include genetic variation available in breeding materials, heritability for traits of interest, selection intensity, and the time required to complete a breeding cycle. Genetic gain can be improved through enhancing the potential and closing the gaps, which has been evolving and complemented with modern breeding techniques and platforms, mainly driven by molecular and genomic tools, combined with improved agronomic practices (Xu et al., 2017). Genetic diversity is frequently quantified in terms of genetic distance or genetic similarity, both of which indicate the existence of genetic differences or similarities.

Microsatellite (SSR) markers are being one of the most reliable and effective DNA molecular markers. They are employed in a variety of applications including genetic diversity studies and rice breeding programmes (Bohra et al., 2017). Due to their multi-allelic and highly polymorphic nature even a small number of SSR markers can provide a better genetic diversity spectrum (Singh et al., 2016). The genetic diversity of crop kinds introduced throughout time fluctuates over time. SSR markers have been demonstrated to be a dependable technique for assessing genetic diversity in both wild and cultivated rice species as well as for detecting genetic polymorphism and genotype differentiation (Krupa, 2017). Microsatellite markers (SSR) are superior to other PCR-based markers used in genetic mapping research because they are highly informative, co-dominant in nature, highly reproducible, plentiful, easy to analyses and cost effective (Nadeem et al., 2018).

Genetic characterization clearly outperforms existing methods when it comes to detecting variety, both genotypes and genes (Koskey et al., 2018)there is paucity of data on rhizobia diversity and genetic variation associated with the newly released and improved mid-altitude climbing (MAC. Similarly, genetic characterizations using molecular technologies has a higher detection power than phenotypic approaches. This is due to the fact that molecular technologies reveal disparities in genotypes or the ultimate level of diversity embodied by an individual's DNA sequences that is unaffected by their environment. Standard characterization and evaluation of accessions can be carried out using a variety of ways, including classic procedures like the use of morphological character descriptor lists. They may also include agronomic performance evaluations under a variety of environmental situations. Genetic characterizations refer to the description of traits that follow a Mendelian inheritance pattern or that include specific DNA sequences. Molecular markers rather than morphological features can indicate significant differences between more direct, reliable, and effective technique for germplasm characterization, conservation, and management that is not impacted by environmental influences (Toppo et al., 2018). Molecular markers have been broadly utilised to investigate the genetic diversity and a wider selection of breeding materials that are less impacted by time, geographical, and environmental factors. Molecular markers can be used to identify genetic variation in rice cultivars (Yadav et al., 2017).

Therefore, the goal of the current study was to use SSR markers to examine the trend in genetic diversity in 65 rice genotypes. In view of the points, the current study was conducted to examine molecular diversity across 65

rice genotypes in order to discover varied genotypes that could benefit rice breeding programmes.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS AND DESIGN

The plant material for the present research work includes rice genotypes. Table 1 shows a complete list of genotypes used in the current study. The research evaluation took place at the Field 10, Universiti Putra Malaysia, Serdang, Selangor in 2019 The seedlings of 65 rice genotypes were raised on tray and appropriate agronomic practices were done. Twenty-one days old seedlings were transplanted in the plastic pot with three replications following randomized complete design. All the agronomic practices were carried out in the pot to grow a healthy crop**.**

2.2 MOLECULAR DIVERSITY ANALYSIS

A total 25 SSR markers were selected on the basis of polymorphism shown by markers in screening (Table 2).

2.3 ISOLATION OF GENOMIC DNA AND SCORING OF DNA BANDS

Young fresh leaves of individual genotypes were collected from 14 days old seedlings and the DNA was extracted using Doyle & Doyle (1987) CTAB extraction method with minor modifications. The DNA quality estimation was done using spectrophotometrically (Spectronic® Genesis™). The resulting ratio (OD260/OD280) was used to determine the nucleic acid purity in various DNA samples. A polymerase chain reaction (PCR) was used to amplify a specific region of total genomic DNA to a billion-fold in vitro. The Eppendorf Thermo-cycler (Mastercycler® X50) was used for all amplifications. On 2 % agarose gel, the amplified DNA products generated by SSR primers were resolved in TAE buffer [242 g Tris-base, 57.3 ml glacial acetic acid, and 100 ml 0.5 M EDTA (pH 8.0) diluted in distilled water and final volume made to 1000 ml]. Approximately 15 µl of PCR product was combined with 2 µl of 6X loading dye (bromophenol blue) and placed into an agarose gel slot for electrophoresis. The gels were loaded with 1µg of a 50 bp DNA marker to assess the molecular size of amplified products (Fermentas, USA). In a gel documentation system (Gel DocTM XR+, BIO-RAD, USA), the gels were visualized under

Table 1: List of genotypes used in the present study

Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes	Sl. No.	Genotypes
1	Pukhi	18	Dhala saitta	35	KUNYIT	52	BRRI dhan82
2	Panbira	19	Morich boti	36	GHAU	53	BRRI dhan72
3	Dharial	20	Saitta	37	LALAMG	54	BRRI dhan28
4	Utri	21	Lal Dular	38	MGAWA	55	BRRI dhan39
5	Luanga	22	Nayan moni	39	SUNGKAI	56	BRRI dhan42
6	Kaisa panja	23	Kalabokra	40	UGAN	57	BRRI dhan43
7	Vandana	24	HUA1003	41	TADOM	58	BRRI dhan46
8	Dular	25	Takanari	42	BANGKUL	59	BRRI dhan75
9	Sondhumoni	26	Kachalath	43	NMR151	60	BRRI dhan55
10	Hasikamli	27	Wkhi1	44	NMR152	61	BRRI dhan69
11	Dumai	28	Hukurikul193	45	MR297	62	B370
12	Parija	29	ML6	46	Putra 1	63	BINASAIL
13	Kataktara	30	ML9	47	Putra 2	64	BINA dhan7
14	Balirdia	31	Wanxiam-P10	48	MR 303	65	BINA dhan5
15	Binnatoa	32	RENGAN WANG	49	MR 309		
16	Parangi	33	PETEH PERAK	50	BR24		
17	Chengri	34	WANGI PUTEH	51	BRRI dhan48		

Table 2: List of primers used for varietal characterization of 65 rice genotypes

Sl no.	Markers	Forward primers (5'-3')	Reverse primers (3'-5')
1	RM1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
2	RM84	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC
3	RM424	TTTGTGGCTCACCAGTTGAG	TGGCGCATTCATGTCATC
4	RM174	AGCGACGCCAAGACAAGTCGGG	TCCACGTCGATCGACACGACGG
5	RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
6	RM 231	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
7	RM232	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
8	RM335	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG
9	RM551	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
10	RM168	TAGCAAGCTTGGAGAAGTGATGG	CAGAAGAAGTCAGCTCTATGCTTGG
11	RM87	CCTCTCCGATACACCGTATG	GCGAAGGTACGAAAGGAAAG
12	RM39	GCCTCTCTCGTCTCCTTCCT	AATTCAAACTGCGGTGGC
13	RM334	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
14	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
15	RM103	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATAGC
16	RM190	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG
17	RM481	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG
18	RM264	CATCTCCGCTCTCCATGC	GGAGTTGGGGTCTTGTTCG
19	RM434	GCCTCATCCCTCTAACCCTC	CAAGAAAGATCAGTGCGTGG
20	RM242	CACTCACACAAGCGACTGAC	CGCAGGTTCTTGTGAAATGT
21	RM216	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
22	RM202	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
23	RM270	GGCCGTTGGTTCTAAAATC	TGCGCAGTATCATCGGCGAG
24	RM277	CTGGTTCTGTCTGGGAGCAG	CTGGCCCTTCACGTTTCAGTG
25	RM453	GGCCAACGTGTGTATGTCTC	TTAATGCCAAGACGGATGGG

UV light after 1.5 hours of electrophoresis at a constant voltage of 80 volts. Finally, polymorphism was graded on the images of amplification products generated from all primers in order to assess genotype diversity.

2.4 STATISTICAL ANALYSIS

2.4.1 Molecular Diversity

Molecular Marker-based Genetic Diversity Analysis (MMGDA) has the ability to examine changes in genetic diversity through time and place. Each genotype and primer combination's band position in the comparative SSR profile was assessed using the gel images. This analysis includes SSR profiles from only genotype × primer combinations that gave steady amplification for all genotypes and no blank lane per unclear bands. The existence of a band was scored as a '1' while the lack of a band was scored as a '0,' resulting in the 0 and 1 matrix. The genetic similarity data among the 65 rice genotypes was generated using this binary data matrix.

The impact of different scales of measurement for different quantitative traits were decreased by standardising data for each trait separately prior to cluster analysis. Standardization was done by dividing the deviation of mean for a line from the mean for 65 genotype with the standard deviation for the given trait; the NTSYS (Rohlf Fj, 1987) software's STAND module was utilised to provide the information.

2.4.2 Genetic dissimilarity and cluster analysis based on UPGMA

NTSYS-pc version 2.2 W (Rohlf Fj, 1987) was used

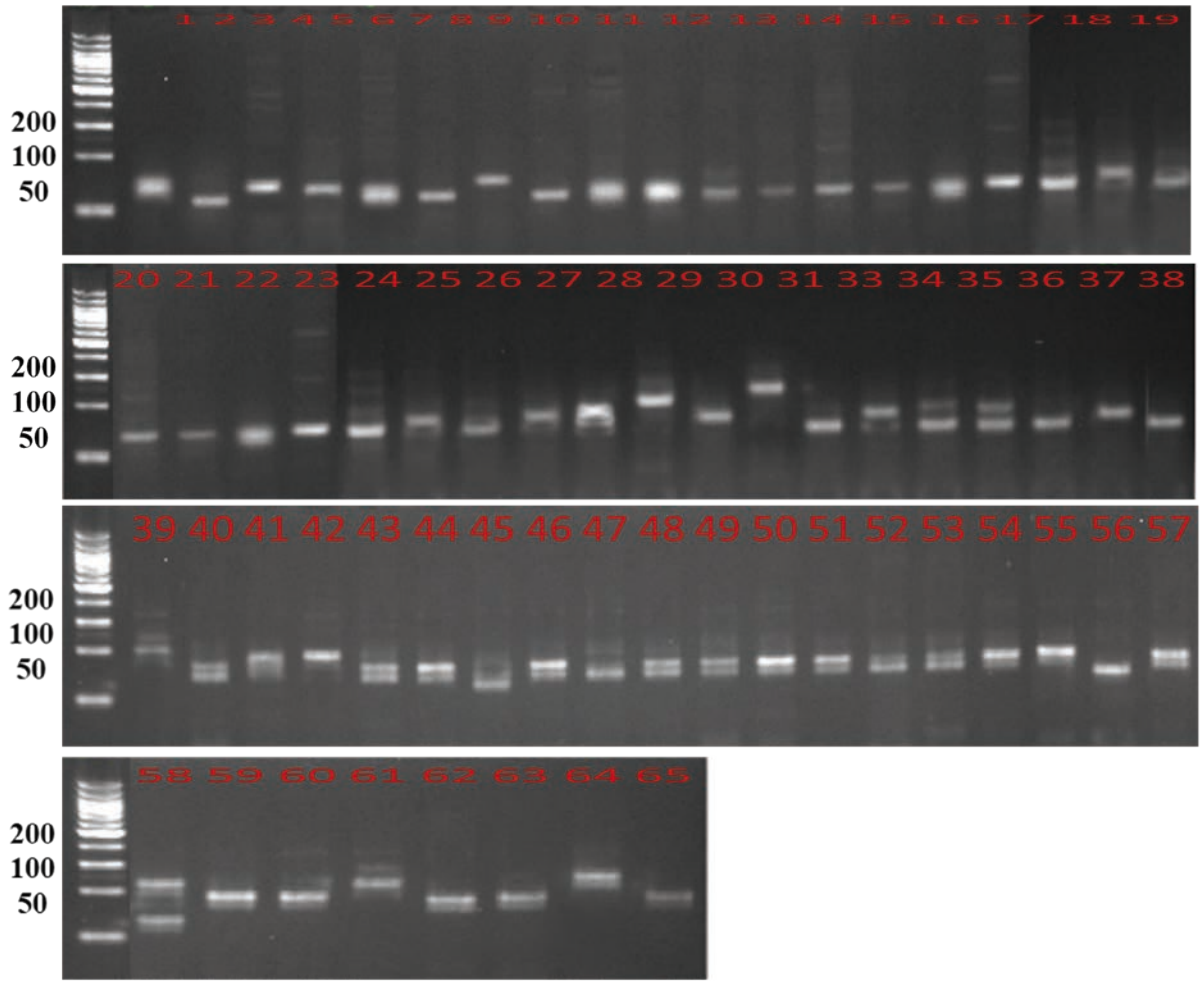

Figure 1: SSR banding profile obtained by marker RM481. Lane 1-65 represents rice genotype used in the present study; M = 50 bp DNA size marker

to analyse binary data matrix generated by polymorphism SSR markers. The Jackard's dissimilarity coefficient was calculated using the SIMQUAL programme. Cluster analysis was done using the dissimilarity matrix as an input. The Sequential Agglomerative Hierarchical Non-Overlapping (SAHN) module was used to do UPGMA-based clustering with Jackard's Coefficient of NTSYS-pc being used for dendrogram generation. The average distance between all individuals in the two groups was calculated using the unweighted pair-group technique with arithmetic averages (UPGMA) to connect clusters.

2.5 POLYMORPHIC INFORMATION CONTENT (PIC)

The ability of markers to detect polymorphisms is measured by their polymorphism information content (PIC). The number and frequency distribution of detectable alleles determine the PIC of a marker for identifying polymorphism within a population (Anderson et al., 1993)different populations are required to fulfill different objectives. Clones from the linkage map(s. PIC for the i_{th} marker is calculated as follows:

$$PIC = 1 - \Sigma P_{ij} \; (j = 1, 2, \ldots..n)$$

where P_{ij} is the frequency of the j_{th} pattern for the i_{th} marker and the summation extends over (n) patterns.

3 RESULTS

The molecular data on preferences of the genotypes were analyzed to find out the variation among genotypes for different characters. The following sub heads show

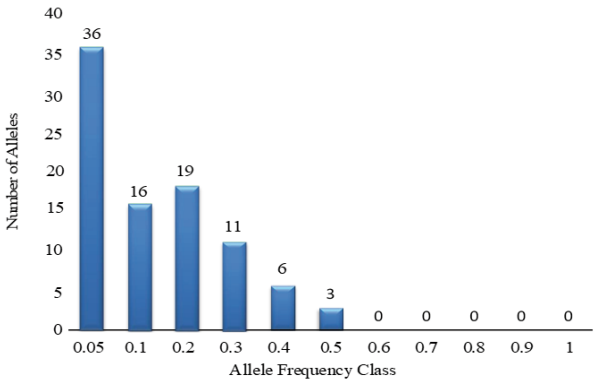

Figure 2: Histogram of allele frequency for all 91 alleles in the 65 rice genotypes

the experimental results from the current investigation for 65 traditional and improved genotypes.

3.1 ALLELIC POLYMORPHISM

The molecular diversity of 65 rice genotypes was assessed using an SSR marker. Out of 25 primers, the 21 primers created a polymorphic pattern that was repeatable, while the four monomorphic primers produced a monomorphic pattern. These SSR markers identified 91 alleles across all rice genotypes. The number of alleles per locus varied greatly amongst markers are ranging from 3 to 7 with an average of 4.00 alleles per locus (Table 3). The highest number of alleles (7) was detected in the marker RM263, and lowest number of alleles (3) were detected in the markers RM335, RM551, RM538 RM190, RM242 and RM270. Monomorphic markers identified on RMRM39, RM103, RM277 and RM453. The diverse genotypes and SSR markers used by different researchers could explain the discrepancies in average allele per locus. Rice genotypes shared a common major allele at 65 loci from 0.26 (RM202) to 0.74 (RM232). A moderate level of allele's frequency exists in these loci of rice genotypes with the average 0.42 (Table 3). Gel image showing SSR banding profile obtained by primer RM481 is presented in Figure 1. The rare alleles (frequency < 0.05) comprised 39.56 %, whereas intermediate (frequency 0.1-0.5) comprised 60.44 % and there were no abundant alleles (frequency > 0.5) (Figure 2). Out of 21 polymorphic, only one marker RM168 provided the highest number of 4 rare alleles, followed by RM174 (3), RM263 (3), RM232 (30, RM84 (20, RM424 (2), RM87 (2), RM481 (2), RM264 (2), RM216 (2), RM1 (1), RM231 (1), 335 (1),RM551 (1), RM334 (1), RM528 (1), RM190 (1), RM434 (1), RM242 (1), RM202 (1) and RM270 (1).

3.2 POLYMORPHIC INFORMATION CONTENT

The degree of polymorphism found by 21 loci markers could not be associated with the number of alleles or the Polymorphic Information Content (PIC) values in this investigation. The PIC values of several loci that produced equivalent numbers of alleles were not statistically different. For instance, 3 alleles were detected at each six loci on RM335, RM551, RM528, RM190, RM242 and RM270; 4 alleles were detected at each of the three loci on RM231, RM334 and RM434; 5 alleles were detected at each of the seven loci on RM1, RM424, RM174, RM87, RM481, RM264 and RM202, 6 alleles were detected at each of the four loci on RM84, RM232, RM168 and RM216, and 7 alleles were found of the loci on RM263. However, no significant difference was detected in their PIC values.

The average PIC value was 0.62 with values ranging from 0.28 (RM 270) to 0.76 (RM 481). (Table 3). The PIC values which reflect allele diversity and frequency among cultivars differed from site to locus. For all of the SSR loci, the PIC values derived from allelic diversity and genotype frequency were inconsistent. This meant that all of the genotypes included in the study were judged to be sufficiently diverse. The study found the highest PIC value 0.76 (RM481) followed by 0.75 (RM551), 0.72 (RM174, RM168 & RM434), 0.71 (RM263), 0.70 (RM216), 0.67 (RM424 & RM202), 0.65 (RM190), 0.64 (RM335), 0.62 (RM84 & RM232), 0.61 (RM87), 0.60 (RM528), 0.59 (RM264) and 0.58 (RM1). The PIC values of eighteen SSR markers found to be polymorphic and utilized in this study were more than 0.5 (Table 3). Low PIC values for the RM270 SSR marker may be the result of closely related genotypes while high PIC values for the RM481 SSR marker may be the result of diverse genotypes of cluster analysis implying that the shared allele distance and cluster analysis were appropriate methods for using SSR marker information. The study found that 18 SSR markers were considered to be the finest and highly informative. Therefore, it can be employed for molecular characterization and QTL analysis.

3.3 GENETIC RELATIONSHIPS

The rice genotype is rich reservoir of valuable genes that plant breeders exploit it for crop improvement. The primers have the ability to differentiate different rice genotypes based on the differences in their genomic region and their number of alleles. The average Genetic Diversity (GD) value was 0.69 with values ranging from 0.33 (RM270) to 0.81 (RM481) (Table 3). The significant rate

Table 3: Number of alleles, polymorphic information content and genetic diversity index for 25 simple sequence repeat (SSR) loci in the 65 rice genotypes

Marker	Chr. No	No. of observation	MAF	NA	RA	GD	PIC
RM1	1	53.00	0.32	5	1	0.66	0.58
RM84	1	53.00	0.39	6	2	0.70	0.62
RM424	2	65.00	0.46	5	2	0.74	0.67
RM174	2	65.00	0.58	5	3	0.80	0.72
RM263	2	65.00	0.69	7	3	0.76	0.71
RM 231	3	64.00	0.40	4	1	0.54	0.49
RM232	3	64.00	0.74	6	3	0.69	0.62
RM335	4	57.00	0.28	3	1	0.72	0.64
RM551	4	57.00	0.42	3	1	0.80	0.75
RM168	4	57.00	0.34	6	4	0.78	0.72
RM87	5	49.00	0.30	5	2	0.69	0.61
RM334	5	49.00	0.36	4	1	0.74	0.39
RM528	6	65.00	0.44	3	1	0.65	0.60
RM190	6	65.00	0.49	3	1	0.70	0.65
RM481	7	65.00	0.46	5	2	0.81	0.76
RM264	8	65.00	0.50	5	2	0.64	0.59
RM434	9	54.00	0.29	4	1	0.78	0.72
RM242	9	54.00	0.34	3	1	0.54	0.54
RM216	10	64.00	0.44	6	2	0.69	0.70
RM202	11	51.00	0.26	5	1	0.72	0.67
RM270	12	57.00	0.38	3	1	0.33	0.28
			0.42	91	36	0.69	0.62

MAF = Major Allele Frequency, NA = Number of alleles, Rare allele (RA) = Number of alleles that frequency < 0.05, GD = Gene Diversity, PIC = Polymorphic Information Content

of interchange of genetic materials across the rice genotypes tested particularly during their genetic improvement could explain the average genetic diversity value. Germplasm conservation, characterization, and breeding effects are all influenced by genetic diversity.

The genetic distance was calculated using the Unweighted Paired Group Method Using Arithmetic Averages (UPGMA) clustering method. UPGMA is a basic grouping algorithm for phylogenetic dendrograms based on genetic distance. Based on Jackard's dissimilarity coefficient the UPGMA was used to generate a dendrogram (Figure 3). Sixty-five rice genotypes were grouped into two main groupings (Table 4) based on dissimilarity coefficients: cluster I and cluster II (0.15). Cluster I was further sub-divided into two minor sub-groups IA and IB were further sub-divided into two sub-groups i.e. IA-1 and IA-2 (0.31) and IB-1 and IB-2 (0.32) respectively. Two minor sub-groups were created from the second main cluster i.e. IIA and IIB with dissimilarity coefficient 0.16. This indicated that the genotypes analyzed had a lot of variability. It is critical to have the most diverse genotypes when selecting desirable cultivars for use in breeding programmes.

The dissimilarity coefficient varies from 1 to 0, close to one shows high similarity while close to zero shows high dissimilarity. The average of dissimilarity coefficient varies from 0.77 to 0.51. The dissimilarity coefficient of all sixty-five genotypes is 0.61 on average. The highest dissimilarity coefficient varied between the genotype BR24 and Utri (0.9429) followed by the Pukhi and WANGI PUTEH (0.9286). The lowest value was found between Sondhumoni and Dumai (0.150). The most diverse genotype was Utri and Pukhi. These genotypes were grouped into nine clusters. Cluster indicated that 31 genotypes out of sixty-five belong to the cluster IB-1a followed cluster IB-1b which has 24 genotypes and cluster IB-1c with

Table 4: Grouping of sixty-five rice genotypes into different clusters based on Jackard's IJ coefficient

Cluster	Number of Genotypes	Name of the Genotypes
IA-1	1	Utri
IA-2	1	BRRI dhan42
IB-1a	31	Vandana, Parija, Luanga, Chengri, Nayan moni, Wkhi1, BRRI dhan55, Binatoa, BRRI dhan69, B370, ML6, BRRI dhan82, BINASAIL, BINA dhan7, Wanxiam-P10, RENGAN WANG, BRRI dhan72, BRRI dhan28, TADOM, BRRI dhan48,Dular,Kataktara, Sondhumoni, Dumai, Balirdia, Kaisa panja, Dharial, Parangi, Dhala saitta, Panbira, Hasikamli
IB-1b	24	Lal Dular, UGAN, HUA1003, ML9, PETEH PERAK, Kachalath, BR24, MR297, BRRI dhan39, BRRI dhan75, MR 309, BRRI dhan46, BINA dhan5, Hukurikul193, WANGI PUTEH, LALAMG, MGAWA, MR 303, GHAU, BANGKUL, NMR151,BRRI dhan43, KUNYIT, NMR152
IB-1c	3	Kalabokra, Takanari, Putra 1
IB-2	2	Morich boti, Saitta
IIA-1	1	SUNGKAI
IIA-2	1	Putra 2
IIB	1	Pukhi

3 genotypes. Cluster IA-1, IA-2, IIA-1, IIA-2 and IIB were monogenic in nature containing single genotypes each i.e. Utri, BRRI dhan42, SUNGKAI, Putra 2 and Pukhi, respectively.

3.4 ANALYZE THE POPULATION STRUCTURE

Using 21 SSR polymorphic markers across 65 rice genotypes the model-based clustering method was applied. The relatively high value of K for 65 genotypes was for K = 9 (Figure 4). At K = 9, the population structure analysis in the present study resulted in nine populations (Figure 5 & Table 5). The distribution of rice genotypes between genetic groups is quite different among genotypes. The mixture is most likely the product of a long history of breeding and domestication, both of which have had significant impacts on the diversity structure. Human-mediated gene flow may play an important role within a population due to breeding in rice for its self-fertilization nature. In other words, in the absence of human-mediated gene flow across populations by breeding one would expect a larger partitioning of diversity among rather than within populations in self-pollinated species.

In present study, the average distances between individuals in the same population (excluding for heterozygosity) were 0.3764 (population A), 0.4318 (population B), 0.6219 (population C), 0.3442 (population D), 0.4735 (population E), 0.2951 (population F), 0.5146 (population G), 0.3369 (population H), and 0.3114 (population I). The genetic diversity of different rice was assessed using average genetic distances (Table 5).

Data on genetic diversity and population structure can be utilised to create successful breeding processes for expanding the genetic base of commercial cultivars, identifying molecular markers, and preserving germplasm. In the generation of necessary derived varieties, distinguish, uniformity, and stability (DUS) testing of plant varietal characteristics as well as the discovery of molecular markers will be critical.

4 DISCUSSION

Molecular markers have been employed in genetic improvement programmes to examine genetic diversity to choose parents for cross-breeding, and learn about marker trait associations (Uba et al., 2021)West, Central, Southern and East Africa. The findings of DNA-based genetic diversity analyses could be used to develop effective breeding programmes aimed at extending the genetic base of commercially grown cultivars (Brumlop & Finckh, 2011)and poor neurodevelopment in children. We carried out a comprehensive literature review to examine the neurotoxicity of BaP. The data were used to identify potential point of departure (POD. The molecular diversity and characterization of rice genotypes were examined using twenty-five microsatellite simple sequence repeats (SSRs) in this study. SSR is more polymorphic than most other DNA markers as well as be-

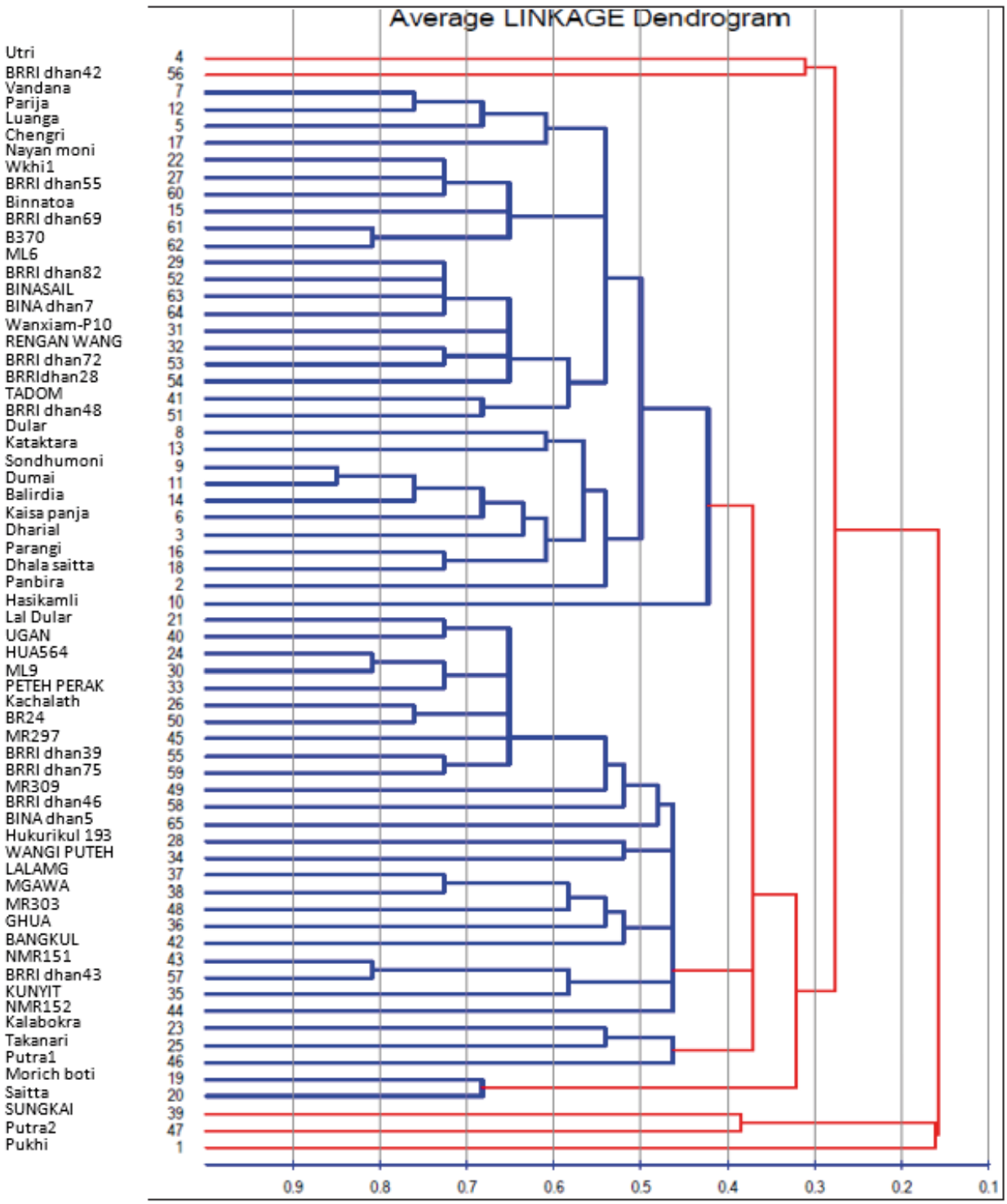

Figure 3: Cluster study utilizing the UPGMA method employing SSR fingerprint data and a Jaccard's IJ coefficient in 65 rice genotypes

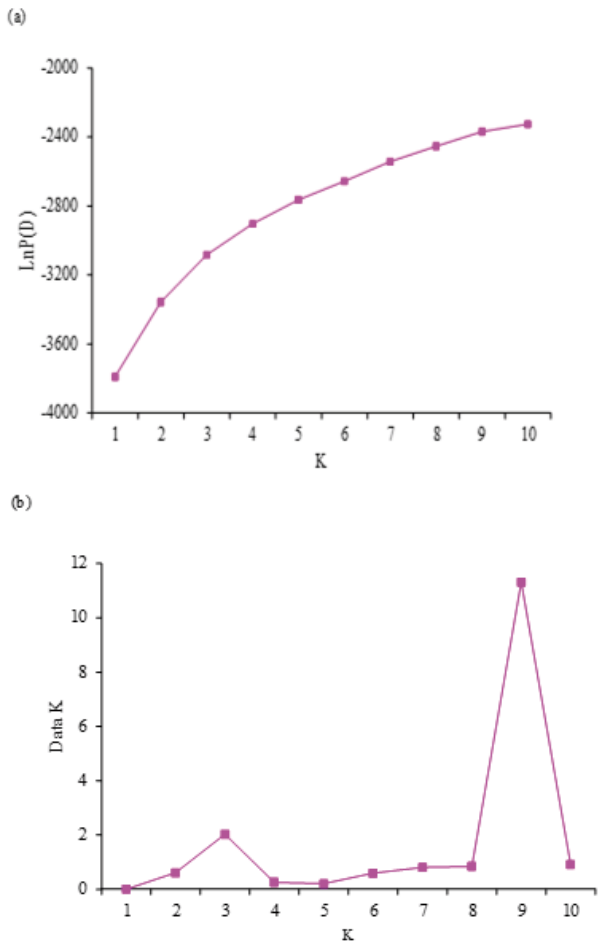

Figure 4: (a) The Bayesian log probability data [LnP (D)] by increasing K
(b) Magnitude of K as a function of K

ing co-dominant and having a bigger amount. As a result of SSR's high polymorphism information richness so that microsatellite markers have been used as molecular markers in fingerprinting (Sun et al., 2020)91 poplar cultivars belonging to four sections (Aigeiros, Tacamahaca, Populus and Turanga.

The SSR markers utilized in this study were both scorable and clear. Out of twenty-five markers used twenty-one were polymorphic allele and remaining four markers were monomorphic in nature. The number of alleles detected per marker were 3 to 7 with an average of 4.57. The alleles showed high degree of polymorphism, with major percent polymorphic bands in twenty-one SSR markers. It suggests that the genotypes used in present study shown as genetic divergence. Similar results were also reported (Ashraf et al., 2016) SSR primers were used to explore genetic variability of various rice landraces. However, when compared to values reported in other parts of the collections, this figure is extremely low. Thomson et al. (2010) reported that the average number of alleles per locus was 4.86, with the number of alleles per locus ranging from 3 to 8. The knowledge on these alleles in various genotypes will be tremendously useful in developing mapping populations for genome study and in applied breeding programmes. Molecular-based biological and geographical diversity differed in allelic richness, frequency of uncommon alleles, common and most frequent alleles, and group-specific unique alleles.

Genetic diversity is required for the selection of various plant breeding programmes and genomic diversity can be determined using a variety of methods. The environment has a significant impact on the expression of a variety of plant morphological features that are currently available and used to distinguish genotypes (Buzatti et al., 2019). Investigations at the molecular level reveal the true distinctions between genotypes. Molecular markers have been employed in genetic improvement programmes to research genetic diversity and to select parents for cross-breeding between parents with different backgrounds, as well as to determine marker trait associations (Gedil & Menkir, 2019).

This analysis showed the incidence of significant diversity in the traditional and improved rice genotypes studied. Based on the dendrogram the maximum degree of genetic resemblance between genotypes Sondhumoni and Dumai followed by BRRI dhan69 and B370, HUA564 and ML9 and NMR151 and BRRI dhan43. The most diverse genotype was Utri and Pukhi. Similar result was found also by (Nachimuthu et al., 2015) where the cultivars were grouped in two major groups and 14 sub-groups. As a result, the diversity of genotypes is critical for selecting suitable genotypes for use in breeding programmes.

The dissimilarity coefficient was calculated by Jackard IJ distance analysis, and result showed value ranged from 0 to 1. According to this dissimilarity coefficient we can understand the dendrogram and their relatedness. So, the highest diverse genotypes can be used as parents in breeding programme. The average of dissimilarity coefficient varies from 0.7689 to 0.5079. The total average of all sixty-five genotype's dissimilarity coefficient is 0.6119. The dissimilarity coefficient varied from the largest value 0.9429 between the genotype BR24 and Utri followed by the genotype value 0.9286 between the genotype Pukhi and WANGI PUTEH which shows high similarity between them and it may be expected that both of them may have arouse from the same parents. The lowest value 0.150 was found between Sondhumoni and Dumai. Similar result was reported by (Siva et al., 2013).

The power of distinction or polymorphism information content (PIC) is a useful statistic for comparing

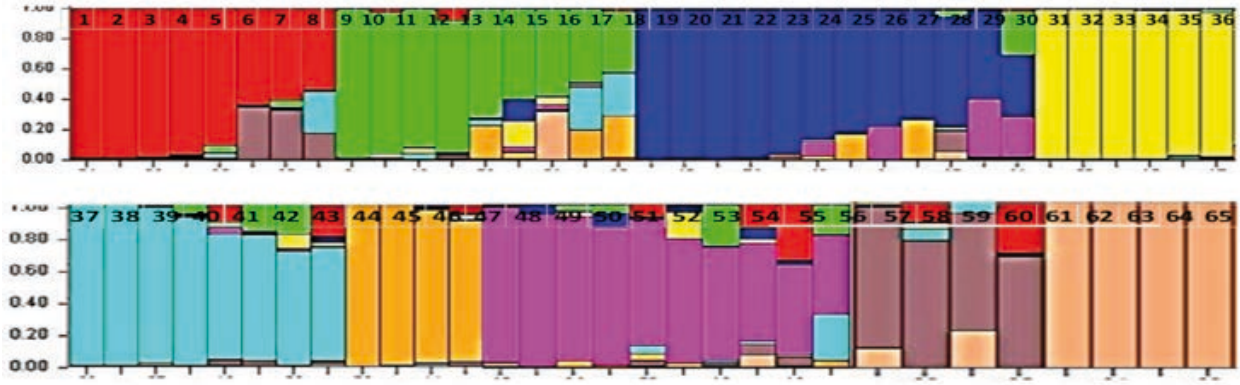

Figure 5: Model-based membership of 65 rice genotypes using STRUCTURE. Colors represent model based population for 9 inferred cluster

Table 5: The average distances between individuals of the same population (without heterozygosity)

P-A	P-B	P-C	P-D	P-E	P-F	P-G	P-H	P-I
0.3764	0.4318	0.6219	0.3442	0.4735	0.2951	0.5146	0.3369	0.3114

P-A = Population A, P-B = Population B, P-C = Population C, P-D = Population D, P-E = Population E, P-F = Population F, P-G = Population G, P-H = Population H, P-I = Population I

various markers (Serrote et al., 2020). This criterion's high levels indicate that the locus has a lot of polymorphisms and that uncommon alleles play a substantial influence in individual difference. Therefore, a marker with a high PIC will be particularly beneficial for differentiating genotypes with tight relationships. The allele diversity and frequency among genotypes are reflected in the PIC value (Ashraf et al., 2016). Each marker's PIC value can be calculated based on its alleles. All of the SSR loci investigated had different PIC values. Calculating PIC values for each of the SSR loci was used to assess the level of polymorphism among the 65 genotypes in the current investigation. In this study, the PIC values ranged from 0.28 (RM 270) to 0.76 (RM481) with an average of 0.52. PIC values of 0.5-mark markers are especially useful in genetic studies for determining the polymorphism rete of a marker at a particular locus. The presence of polymorphism between genotypes indicated that genetic variation exists at the molecular level. Microsatellite analysis was compared to three previous estimations in rice 0.26 to 0.65 with an average of 0.47 (Singh et al., 2014), 0.28-0.50 with a mean of 0.45 (Ashraf et al., 2016) and 0.239 to 0.765 with an average of 0.508 (Pathak et al., 2020). The number of alleles found was proportional to the PIC value of the locus. So PIC value is totally related polymorphism of markers. The highest PIC value of 0.76 at RM481 was shown to be the best marker for differentiating across rice cultivars. A number of other researchers have conducted similar investigations (Tarang et al., 2020).

5 CONCLUSIONS

Twenty-five SSR markers were used out of which twenty-one were found polymorphic. 21 polymorphic markers detected a total of 91 alleles among 65 rice genotypes, with an average of 4.00 alleles per polymorphism marker. The PIC values ranged from 0.28 to 0.76 and the highest PIC value of 0.76 was determined to be marker RM481 which was proven to be the best appropriate marker for discriminating among rice genotypes. The genetic divergence analysis divided 65 rice genotypes into nine groups with cluster IB-1a having the most cultivars (31) and clusters IB-1b and V having the least. Based on dendrogram the maximum degree of similarity was observed between genotype Sondhumoni and Dumai followed by BRRI dhan69 and B370, HUA564 and ML9 and NMR151 and BRRI dhan43. The most diverse genotype was Utri and Pukhi. The maximum value of the dissimilarity coefficient was discovered between the genotype BR24 and Utri (0.0429) and between Pukhi and WANGI PUTEH (0.9286) whereas lowest value was seen between Sondhumoni and Dumai (0.150) showing highly diverse genotypes. Breeders may attempt hybridization among the above genotypes that demonstrated the most diversity in the hopes of increasing genetic variability in rice and assisting in the development of promising rice genotypes. It is important to remember that both morphological and molecular techniques for studying genotypes have been demonstrated to be beneficial and one meth-

odology complements the other and offers a trustworthy result.

6 FUNDING

Under the research grant ID 6282519, the Bangladesh Agriculture Research Council, Ministry of Agriculture, Bangladesh [PIU-BARC, NATP-2] and University Putra Malaysia collaborated on this project.

7 ACKNOWLEDGEMENT

The authors acknowledge the Bangladesh Agricultural Research Council (BARC-Project of NATP Phase-II) the People's Republic of Bangladesh Ministry of Agriculture (MoA), and Bangladesh Rice Research Institute (BRRI). Also, to be thanked is Malaysia's Universiti Putra Malaysia (UPM).

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