International Research Journal of BIOLOGICAL SCIENCES

Volume 01 | Issue 02 | 2019

SciRange PUBLICATIONS
www.scirange.com

SciRange

Open Access

International Research Journal of Biological Sciences

Research Article

pISSN: 2663-5968, eISSN: 2663-5976

Molecular Diversity Analysis of Some Selected BBRI Released Rice Varieties using SSR Markers

[1]M.A. Syed, [1]K.M. Iftehkaruddaula, [2]N. Akter and [1]P.S. Biswas

[1]Plant Breeding Division, Bangladesh Rice Research Institute (BRRI), Gazipur-1701, Bangladesh
[2]Plant Breeding Division, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701, Bangladesh

ARTICLE INFORMATION

Received: August 05, 2019

Accepted: September 30, 2019

Corresponding Author:
Dr. Md. Abu Syed,
Plant Breeding Division,
Bangladesh Rice Research Institute (BRRI), Gazipur-1701, Bangladesh
Tel: +88 01797129551

ABSTRACT

Rice is one of the most important staple food crops in the world. The experiment was conducted to estimate the genetic diversity and to reveal genetic relationships among rice cultivars. A total of 299 SSR markers were used covering the whole rice genome among arsenic sensitive and tolerant seven rice varieties. Summary statistics were performed using Power Marker and a dendogram was generated using the NTSYS-pc. The genetic distance was calculated using the Nei distance. A total of 405 alleles were detected where the number of alleles per marker ranged from 2 to 5, averaging of 2.45 alleles per locus. The band size for a given microsatellite locus varied between 73 bp (RM9) to 335 bp (RM539). PIC values ranged from 0.21 to 0.70, with an average of 0.36. Total 28 highly informative markers, 83 informative markers and 54 slightly informative markers were obtained which might be effectively used for genetic diversity and relationships study of rice. Seven rice varieties were constellated into three groups at the similarity coefficient of 0.49; where cluster-1 contained only the most arsenic tolerant variety BRRI dhan47, cluster-2 represents three varieties having moderate arsenic tolerance ability (BRRI dhan50, BRRI dhan54 and BRRI dhan55) and cluster-3 contained three arsenic sensitive varieties (BRRI dhan28, BRRI dhan29 and BRRI dhan45). The results of the genetic diversity would be useful to the selection of the parents for developing rice breeding program, especially in background selections during backcross breeding and for arsenic tolerant QTL identification.

Key words: Molecular diversity, cluster analysis, SSR markers, arsenic, rice

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops supporting more than half of the world population[1]. South Asia, one of the major centres for rice domestication, has been described as the "food basket" and "food bowl" of Asia[2]. Compared with other crop species, the genetic diversity in the world rice germplasm is quite large. Three subspecies, i.e., *Indica*, *Japonica* and *Javanica*, compose a large reservoir of rice germplasm including a variety of local landraces and cultivars[3,4]. The small portion of germplasm has been used in the breeding program though genetic resources of the world rice collection were in rich. As a result, close genetic makeup was observed in commercial rice variety[5].

Genetic similarity and diversity could be assessed by plant morphology, physiology, isozymes, storage protein profiles and DNA markers[6]. Among different DNA markers, SSR

markers have been extensively used as a powerful tool in variety protection[7], molecular diversity studies[8], QTL analysis, pedigree analysis and marker assisted breeding[9]. SSRs have been developed for many crop species, including wheat[10], maize[11], sorghum[12], tomato[13] etc. In rice, SSRs have been used to assess the genetic diversity of both wild and cultivated species[14-17]. SSR markers are particularly suitable for evaluating genetic diversity and relationships among closely related plant species, populations, or individuals[18]. SSR markers are widely used in rice research due to highly informative, mostly monolocus, co-dominant in nature and also cost effective [19]. Genetic diversity and polymorphisms of land races, local cultivars and diverse genetic stocks are frequently studied using SSRs. The objectives of the present study were to use SSR markers (1) to estimate the genetic variation and diversity, (2) to evaluate of the degree of polymorphism, (3) to suggest the most informative markers for marker assisted selection and (4) to reveal genetic relationships among seven modern rice cultivars released by Bangladesh Rice Research Institute (BRRI) between 1994 and 2011.

MATERIALS AND METHODS

The experiment was conducted at Marker Assisted Selection (MAS) laboratory of Plant Breeding Division of Bangladesh Rice Research Institute (BRRI).

Plant materials and markers: Seven rice cultivars were used in this investigation as shown in Table 1. These cultivars were used in this study to evaluate the use of SSR markers for identification of Arsenic (As) tolerant quantitative Trait Loci (QTL) and for the selection of As tolerant rice genotypes during breeding program. It was found that BRRI dhan47 showed high tolerance to arsenic and BRRI dhan50, BRRI dhan54 and BRRI dhan55 showed medium tolerance. On the contrary, BRRI dhan45 is the most susceptible cultivars followed by the other two varieties (BRRI dhan29 and BRRI dhan28) used in the present study[20]. A total of two hundred ninety-nine SSR markers were used in this study. Markers were selected based on their location to cover all the chromosomes uniformly maintaining more or less similar distance considering distances in base pair (bp).

Isolation of genomic DNA: Seeds were germinated in germination chamber at 30°C temperature. Three days after germination, germinated seeds were sown in earthen pots. The pots were then kept in a net house. Genomic DNA was isolated from young leaves of 30 days old seedlings following modified Miniscale method as described by Syed *et al.*[21]. DNA samples were evaluated both quantitatively and qualitatively using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and the concentration was determined using module Nucleic Acid, Software: 3.3.1.

Molecular analysis through SSR: Polymerase chain reaction (PCR) amplification was carried out in a 10 µL reaction mixtures, each reaction containing 25 ng of template DNA, 0.5 µM of each forward and reverse primer, 3 mM $MgCl_2$, 0.2 mM of dNTP mix, 1X PCR buffer and 1 unit of *Taq* DNA polymerase (Bio basic, Canada). Amplifications were performed by G-strom DNA Thermal Cycler (Gene Technology Ltd., England). The temperature cycles were programmed as initial denaturation at 94°C for 5 min, 35 cycles of 45 sec denaturation at 94°C, 45 sec annealing at 55°C, 1 min and 30 sec extention at 72°C and additional temperature of 72°C for 7 min for final extension and finally cooled at 4°C temperature. The 10X Loading dye was added to each well of the PCR plate and the amplified PCR products were subjected to electrophoresis in 8% polyacrylamide gel in 0.5X TBE buffer at 100 V for 1.30 to 2.15 h (depending on product size) along with a known DNA ladder. The gels were stained with ethidium bromide for 25-30 min and DNA fragments were visualized under UV light and gels were photographed using gel documentation system (BIO-RAD).

Data analysis and clustering: The molecular weights for each band were measured in base pairs using Alpha Ease FC 5.0 software (Alpha Innotech Corporation). Data of non-amplified and monomorphic SSR markers were not used for diversity study. One hundred and sixty-five polymorphic SSR markers distributed across 12 chromosomes were used for diversity analysis. Summary statistics, including the number of alleles per locus, major allele frequency and Polymorphic Information Content (PIC) values were determined using Power Marker

Table 1: List of Rice cultivars used in present investigation

Sl. No.	Name of cultivar	Pedigree	Progenitors	Year of release
1	BRRI dhan28	BR601-3-3-4-2-5	BR6(IR28)/Purbachi	1994
2	BRRI dhan29	BR802-118-4-2	BG90-2/BR51-46-5	1994
3	BRRI dhan45	BR5778-21-2-3	BR2/TETEP	2005
4	BRRI dhan47	IR63307-4B-4-3	IR51511-B-B-34-B/TCCP266-2-49-B-B-3	2007
5	BRRI dhan50	BR6902-16-5-1-1	BRRI dhan30/IR67684B	2008
6	BRRI dhan54	BR5999-82-3-2-HR1	BR1185-2B-16-1/BR548-128-1-3	2010
7	BRRI dhan55	IR73678-6-9-B	IR64/*Oryza rufipogon*	2011

version 3.25[22]. PIC values were calculated for each of the SSR loci with the following formula proposed by Anderson *et al.*[23].

$$PICi = 1 - \sum_{j=1}^{n} (Pij)^2 \quad (1)$$

where, n is the number of marker alleles for marker i and Pij is the frequency of the jth allele for marker i. The genetic distance was calculated using the Nei distance[24]. The allele frequency data from Power Marker version 3.25 was used to export the data in binary format (allele presence = 1; allele absence = 0) for analysis with Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc)[25]. The similarity matrix

was calculated with the Simqual subprogram using the Dice coefficient[26] and subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated using the program NTSYS-pc.

RESULTS AND DISCUSSION

Status of SSR markers: The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities. Keeping this in mind, a total of 299 SSR markers were used across the tested cultivars. Status of SSR markers was presented in Fig. 1. Among them, 27 markers did not amplified at all which might be due to the selection of these markers based on published *Japonica* rice base sequences[27] that might differ from *indica* type rice cultivars studied here. These markers might be useful for japonica type rice genotypes rather than *Indica* rice. In addition, 107 markers showed monomorphic in nature. Therefore, these 134 markers do not have importance on diversity and genetic relationship study of the cultivars. In case of closely related cultivars these phenomenon is common and were reported in many previous studies[5,15,17]. The rest 165 markers showed polymorphism among the tested modern rice cultivars that are commercially cultivated in Bangladesh.

Allelic diversity: A total of 405 alleles were detected. The average number of alleles per locus was 2.45, ranging from 2 to 5 across 165 SSR markers (Table 2). The highest number of allele (5) was observed in locus RM437 followed by 4 alleles for each 16 markers and 3 alleles for each 40 markers. The lowest number of alleles (2) was recorded for each 108 markers (Table 3). Similar values were reported by Joshi and Behera[28] (rang 2-6, mean 2.10), Prabakaran *et al.*[29] (range 2 to 3, mean 2.2), Pachuri *et al.*[30] (range 2-4, average 2.79 alleles), Zeng *et al.*[31] (range 2-7, average 3.10 alleles) and Singh *et al.*[32]

Table 2: Summary of allelic variation and polymorphic information content (PIC) values among studied rice genotypes for 165 SSR markers

	Total	Mean	Range
Alleles No.	405	2.45	2-5
Alleles size (bp)	-	-	73-335
Major allele frequency (%)	-	68.46	42.86-85.71
Allele size difference (bp)	-	-	3-109
PIC value	-	0.36	0.21-0.70

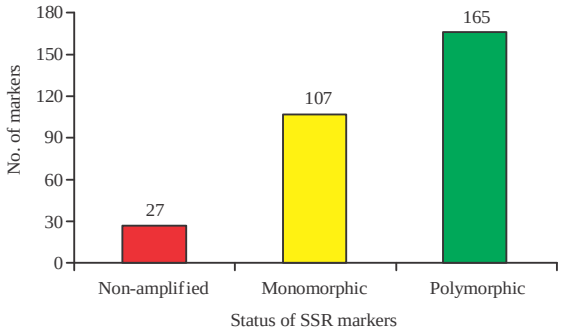

Fig. 1: Status of SSR markers used in this experiment among seven rice cultivars

(range 2-5, mean 3.11 alleles) but quite low compared with those reported Krupa *et al.*[33] (range 2 to 6, average 3.25 alleles), Matin *et al.*[34] (range 3-7, mean 4.4) and Siwach *et al.*[35] (range 1-8, mean 4.58). Comparatively low allele per locus might be due to the use of lower number of genotypes in the present study. Close genetic makeup would be another cause of low allele number those used in Pachuri *et al.*[30].

The overall size of PCR products amplified using 165 markers ranged from 73 bp (RM9) to 335 bp (RM539). The molecular allele size difference between the smallest and the largest allele for a given locus varied widely from 3 to 109 bp. There was a considerable range in allele frequency (42.86-85.71%); on an average 68.46% of the rice varieties shared a common allele (Table 2). Similar findings were observed by Singh *et al.*[32] (0.37-0.97, average 0.76) and Islam *et al.*[36] (0.37-0.97, average 0.77).

Polymorphism in SSR markers: PIC values, a reflection of allele diversity and frequency among the varieties, were calculated for all the markers. The PIC values for the microsatellite loci varied from 0.21 to 0.70 with a mean of 0.36 (Table 2). In this study, lower PIC value (0.36) indicated that the genotypes are not much diverged and are closely related. Total 28 highly informative marker (PIC >0.50) were found on different chromosomes (Table 4). The highest (5) highly informative markers was observed on chromosome 7 followed by 4 markers on each chromosome 1, chromosome 2, chromosome 5, 3 markers on chromosome 3 and 2 markers on

Table 3: List of number of allele per locus with their marker name identified in this study

No. of Allele/locus	No. of marker	Name of marker
5	1	RM437
4	16	RM493, RM9, RM472, RM154, RM232, RM85, RM548, RM334, RM481, RM180, RM336, RM248, RM464, RM222, RM209, RM229
3	40	RM490, RM212, RM486, RM529, RM211, RM279, RM423, RM263, RM526, RM250, RM208, RM570, RM442, RM119, RM252, RM470, RM317, RM567, RM159, RM598, RM538, RM31, RM589, RM204, RM549, RM541, RM125, RM234, RM18, RM337, RM152, RM210, RM316, RM23958, RM474, RM216, RM304, RM224, RM206, RM144
2	108	RM495, RM5, RM306, RM488, RM237, RM297, RM543, RM315, RM431, RM104, RM485, RM555, RM492, RM324, RM561, RM341, RM327, RM475, RM262, RM530, RM138, RM231, RM489, RM545, RM517, RM218, RM7, RM563, RM411, RM347, RM520, RM293, RM468, RM571, RM565, RM114, RM551, RM261, RM471, RM417, RM349, RM348, RM131, RM127, RM280, RM559, RM574, RM289, RM509, RM440, RM305, RM274, RM480, RM540, RM314, RM402, RM539, RM136, RM527, RM275, RM528, RM30, RM400, RM494, RM295, RM432, RM560, RM455, RM505, RM478, RM172, RM407, RM25, RM5556, RM126, RM6208, RM256, RM447, RM458, RM264, RM23679, RM23778, RM219, RM566, RM434, RM257, RM242, RM107, RM201, RM215, RM205, RM244, RM239, RM258, RM147, RM228, RM333, RM590, RM332, RM167, RM457, RM254, RM19, RM277, RM519, RM313, RM235, RM12
Total 165		

Table 4: Status of informative markers identified on the different chromosome in this study

Chromosome number	Highly informative marker (PIC>0.50)	Informative marker (0.50≥PIC≥0.25)	Slightly informative markers (PIC<0.25)
1	RM493, RM9, RM472, RM529; Total=4	RM495, RM490, RM5, RM297, RM212, RM486, RM104 Total =7	RM306, RM488, RM237, RM543, RM315, RM431 Total=6
2	RM154, RM423, RM263, RM526; Total=4	RM211, RM279, RM492, RM324, RM561, RM341, RM475, RM262, RM250, RM208, RM138 Total=11	RM485,RM555, RM327, RM530 Total=4
3	RM232, RM570, RM85; Total=3	RM489, RM545, RM517, RM7, RM347, RM520, RM293, RM571, RM565, RM442; Total =10	RM231, RM218, RM563, RM411, RM468, RM114; Total=6
4	RM252; Total=1	RM471, RM119, RM470, RM317, RM349, RM567; Total=6	RM551, RM261, RM417, RM348, RM131, RM127, RM280, RM559; Total=8
5	RM548, RM437, RM334, RM31; Total=4	RM159, RM574, RM598, RM440, RM305, RM538, RM480; Total =7	RM289, RM509, RM274 Total=3
6	RM589; Total=1	RM204, RM314, RM402, RM549, RM539, RM136, RM527, RM541, RM275, RM30; Total=10	RM540, RM528, RM400, RM494; Total=4
7	RM481, RM180,RM336, RM18, RM248; Total=5	RM295, RM125, RM432, RM455, RM234; Total=5	RM560, RM505, RM478, RM172; Total=4
8	RM337, RM152; Total=2	RM407, RM5556, RM210, RM256, RM458, RM264; Total =6	RM25, RM126, RM6208, RM447; Total=4
9	RM464; Total=1	RM316, RM23958, RM242; Total=3	RM23679, RM23778, RM219, RM566, RM434, RM257, RM107, RM201, RM215, RM205; Total=10
10	RM222; Total=1	RM474, RM216, RM239, RM258, RM304, RM228, RM333, RM590; Total =8	RM244, RM147; Total=2
11	RM209, RM229; Total=2	RM167, RM457, RM254, RM224, RM206, RM144 Total =6	RM332; Total=1
12	-	RM277, RM519, RM235, RM12; Total=4	RM19, RM313; Total=2
Total	28	83	54

chromosome 8 and chromosome 11. The lowest (1) highly informative markers were found on chromosome 4, chromosome 6, chromosome 9 and chromosome 10. Out of 83 informative markers (0.50≥PIC≥0.25), the highest (11) informative markers were found on chromosome 2. The second highest (10) informative markers were obtained at chromosome 3 and chromosome 6 followed by 8 markers on chromosome 10, 7 markers on chromosome 1& chromosome 5 and 6 markers on chromosome 4, chromosome 8 and chromosome 11. The lowest (3) informative markers were recorded on chromosome 9 followed by 4 markers on chromosome 12 and 5 markers on chromosome 7. The highest (10) slightly informative markers (PIC<0.25) were recorded on chromosome 9 while the lowest (1) informative markers were found on Chromosome 11. The PIC value observed in this study are comparable to many of the previous reports, for example, Singh *et al.*[32] (0.04 to 0.58, mean 0.29), Shah *et al.*[37] (0.05 to 0.60 with mean 0.37), Pachari *et al.*[29] (0 to 0.66 with an average 0.38), Salgotra *et al.*[38] (0.17 to 0.63 with a mean 0.40), Prabakaran *et al.*[28] (0.28 to 0.57 with a mean of 0.43), Becerra *et al.*[39] (0.12 to 0.83, mean 0.44), Sajib *et al.*[40] (14 to 0.71 with an average of 0.48), Krupa *et al.*[33] (0.22 to 0.79, mean 0.49), Rahman *et al.*[41] (0.16 to 0.84 with an average 0.49) and Jain *et al.*[42] (0.20 to 0.90 with an average of 0.56).

Table 5: Nei's genetic distance (below diagonal) values among studied rice genotypes

Cultivars	BRRI dhan28	BRRI dhan29	BRRI dhan45	BRRI dhan47	BRRI dhan50	BRRI dhan54	BRRI dhan55
BRRI dhan28	0.0000						
BRRI dhan29	0.4483	0.0000					
BRRI dhan45	0.3419	0.5043	0.0000				
BRRI dhan47	0.7059	0.7436	0.7458	0.0000			
BRRI dhan50	0.5565	0.4956	0.6261	0.7672	0.0000		
BRRI dhan54	0.4528	0.4857	0.5283	0.7009	0.4906	0.0000	
BRRI dhan55	0.5045	0.4818	0.5727	0.7321	0.4771	0.0571	0.0000

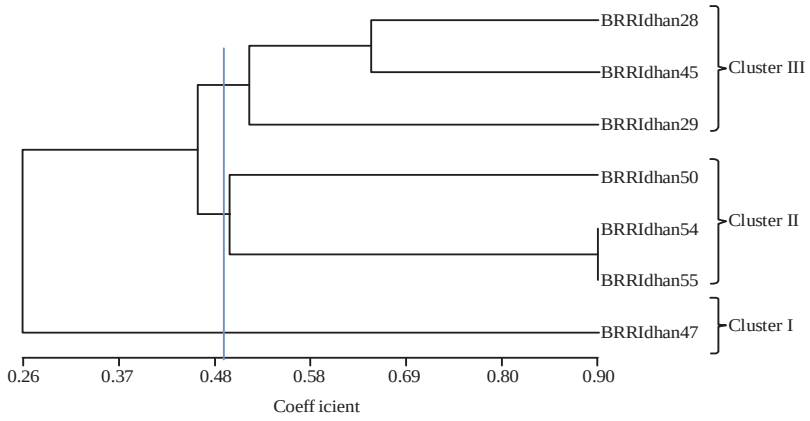

Fig. 2: Dendogram showing the genetic relationships among 7 rice cultivars using UPGMA cluster analysis of Dice genetic similarity coefficients generated from 165 SSR markers

Genetic distance-based analysis and cluster analysis: BRRI cultivars used in the present study have not been examined previously in terms of genetic relatedness using high number of SSR molecular markers covering all the 12 chromosomes of rice genome. The values of pair-wise comparisons of Nei's genetic distances (D) between genotypes were computed from combined data for the 165 primers, ranged from 0.0571 to 0.7672, whereas average genetic distance was 0.51 indicating a wide range of genetic variation among the genotypes (Table 5). Comparatively higher genetic distance was observed between BRRI dhan47 and BRRI dhan50 genotypes pair (0.7672) followed by BRRI dhan47 and BRRI dhan45 genotypes pair (0.7456) than the other combinations (Table 4). On the other hand, BRRI dhan54 and BRRI dhan55 cultivar pair showed the maximum similarity (0.0571).

The dendogram based on Dice similarity index and UPGMA method (Fig. 2) was obtained from the binary data that was deduced from the DNA profiles of the samples analyzed. Three distinct clusters were created from the analysis of the pooled SSR marker data at the similarity coefficient of 0.49. The cluster analysis showed high genetic variation among the rice cultivars studied, with similarity coefficient value ranging from 0.26 to 0.90. Cluster 1, Cluster 2 and Cluster 3 comprised one, three and three cultivars, respectively. Cluster 1 included only BRRI dhan47. While the second cluster was divided into two sub-clusters; the first one comprised two cultivars (BRRI dhan54 and BRRI dhan55) and the second sub-cluster included only BRRI dhan50. Cluster 3 was also divided into two sub-clusters; BRRI dhan29 alone grouped in a sub-cluster and remaining two cultivars (BRRI dhan28 and BRRI dhan45) had been grouped in another sub-cluster.

Shakil *et al.*[43] were assessed genetic diversity of 24 genotypes including BRRI varieties and coastal landraces of Bangladesh using 19 SSR markers where the cluster dendogram revealed 6 clusters at a cut off value of 32% similarities. Matin *et al.*[34] studied cluster analysis of the 12 deep water rice germplasms of Bangladesh and showed four major groups. Choudhury *et al.*[44] found two clusters within 24 indigenous and improved rice varieties in northeast India. Pachauri *et al.*[29] were made the UPGMA dendogram based on molecular marker analysis and clustered the 41 genotypes into four major clusters. Das *et al.*[45] found four groups among a set of 26 rice cultivars while Freeg *et al.*[46] reported that the 41 genotypes were divided into two groups at 0.44 similarity level.

Though the genetic divergence was not very high, but these studied cultivars showed considerable genetic diversity. Narrow genetic base of modern high yielding rice cultivars are

available from several countries, including Latin America[5,47], Japan[48], USA[49], Korea[50] and Taiwan[51]. This might be due to the selection pressure and adaptation capability of the specific genotype in the specific climatic conditions.

CONCLUSION

This study demonstrated that the tested samples possessed a considerable level of microsatellite variation. A total of 405 alleles were detected where an average number of alleles per locus was 2.45, ranging from 2 to 5 across 165 SSR markers. The PIC values for the microsatellite loci varied from 0.21 to 0.70 with a mean of 0.36. Comparatively higher genetic distance was observed between BRRI dhan47 and BRRI dhan50 genotypes pair followed by BRRI dhan47 and BRRI dhan45 genotypes pair than the other combinations. The identified informative and highly informative SSR markers could be effectively used in background selections during backcross breeding and also QTL identification for arsenic tolerance in rice by crossing between the most arsenic tolerant cultivar BRRI dhan47 and the most arsenic susceptible cultivar BRRI dhan45.

COMPETING INTEREST

The authors have declared that no competing interest exists.

REFERENCES

1. Ramkumar, G., A.K. Biswal, K.M. Mohan, K. Sakthivel and A.K.P. Sivaranjani *et al*., 2010. Identifying novel alleles of rice blast resistance genes Pik^h and Pita through allele mining. Int. Rice Res. Notes, 117: 4185.
2. Pachauri, V., N. Taneja, P. Vikram, N.K. Singh and S. Singh, 2013. Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.). Aust. J. Crop Sci., 7: 923-932.
3. Lu, H.M., A. Redus, J.R. Coburn, J.N. Rutger, S.R. Mccouch and T.H. Tai, 2005. Population structure and breeding patterns of 145 US rice cultivars base don SSR marker analysis. Crop Sci., 45: 66-76.
4. Garris, A.J., T.H. Tai, J.R. Coburn, S. Kresovich and S.R. McCouch, 2005. Genetic structure and diversity in *Oryza sativa* L. Genetics, 169: 1631-1638.
5. Herrera, T.G., D.P. Duque, I.P. Almeida, G.T. Núñez, A.J. Pieters, C.P. Martinez and J.M. Tohme, 2008. Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeats markers. Electron. J. Biotechnol., 11: 1-15.
6. Govindaraj, M., M. Vetriventhan and M. Srinivasan, 2015. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet. Res. Int., 2015: 14.
7. Alvarez, A., J.L. Fuentes, V. Puldón, P.J. Gómez, L. Mora, M.C. Duque, G. Gallego and J.M. Tohme, 2007.Genetic diversity analysis of Cuban traditional rice (*Oryza sativa* L.) varieties based on microsatellite markers. Genet. Mol. Biol., 30: 1109-1117.
8. Pervaiz, Z.H., M.A. Rabbani, I. Khaliq, R. Pearce and A. Malik, 2010. Genetic diversity associated with agronomic traits using microsatellite markers in Pakistani rice Landraces. Electron. J. Biotechnol., 13: 1-12.
9. Khatibani, L.B., B.A. Fakheri, M.H. Chaleshtori, A. Mahender, N. Mahdinejad and J. Ali, 2019. Genetic mapping and validation of Quantitative Trait Loci(QTL) for the grain appearance and quality traits in rice (*Oryza sativa* L.) by using Recombinant Inbred Line (RIL) Population. Int. J. Genomics, 2019: 1-13.
10. Jaiswal, S., S. Sheoran, V. Arora, U.B. Angadi and M.A. Iquebal *et al*., 2017. Putative Microsatellite DNA Marker-Based Wheat Genomic Resource for Varietal Improvement and Management. Front. Plant Sci., 8: 1-10.
11. Kumari, A., S. Sinha, K. Rashmi, S.S. Mandal and S. Sahay, 2018. Genetic diversity analysis in maize (*Zea mays* L.) using SSR markers. J. Pharmacogn. Phytochem., 2018: 1116-1120.
12. Billot, C., P. Ramu, S. Bouchet, J. Chantereau and M. Deu *et al*., 2013. Massive sorghum collection genotyped with SSR markers to enhance use of global genetic resources. PLoS ONE, 8: e59714.
13. Kaushal, A., A. Singh and S. Jeena, 2017. Genetic diversity in tomato (*Solanum lycopersicum* L.) genotypes revealed by simple sequence repeats (SSR) markers. J. Applied Nat. Sci., 9: 966-973.
14. Jiang, S.K., C. Huang, X. Zhang, J. Wang, W. Chen and Z. Xu, 2010. Development of a highly informative microsatellite (SSR) marker framework for rice (*Oryza sativa* L.) genotyping. Agric. Sci. China, 9: 1697-1704.
15. Masuduzzaman, A.S.M., M. Haque, M.M.E. Ahmed and C.K. Mohapatra, 2016. SSR marker-based genetic diversity analysis of tidal and flood prone areas in rice (*Oryza sativa* L.). J. Biotechnol. Biomater., 6: 241.
16. Islam, M.M., H.A. Begum, M.S. Ali, M. Kamruzzaman, S. Hoque and M.I. Hoque, 2017. DNA finger printing and genetic diversities in some Bangladeshi Aus rice (*Oryza sativa* L.) genotypes. SAARC J. Agric., 15: 123-137.
17. Aljumaili, S.J., M.Y. Rafii, M.A. Latif, S.Z. Sakimin, I.W. Arolu and G. Miah, 2018. Genetic diversity of aromatic rice germplasm revealed by SSR markers. BioMed. Rese. Int., 2018: 11.
18. Nadeem, M.A., M.A. Nawaz, M.Q. Shahid, Y. Doğan and G. Comertpay *et al*., 2018. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. Biotechnol. Biotechnol. Equip., 32: 261-285.

19. Vieira, M.L.C., L. Santini, A.L. Diniz and C.F. Munhoz, 2016. Microsatellite markers: What they mean and why they are so useful. Genet. Mol. Biol., 39: 312-328.
20. Iqbal, M., G.K.M.M. Rahman, G.M. Panaullah, M.M. Rahman and J.C. Biswa, 2016. Response of yield and yield attributes of different rice genotypes to soil arsenic. Res. Crop Ecophysiol., 11: 1-17.
21. Syed, M.A., K.M. Iftekharuddaula, M.A.K. Mian, M.G. Rasul, G.K.M.M. Rahmam, G.M. Panaullah, J.G. Lauren, J.M. Duxbury and P.S. Biswas, 2016. Main effect QTLs associated with arsenic phyto-toxicity tolerance at seedling stage in rice (*Oryza sativa* L.). Euphytica, 209: 805-814.
22. Liu, K. and S.V. Muse, 2005. Power marker: An integrated analysis environment for genetic marker analysis. Bioinformatics, 21: 2128-2129.
23. Anderson, J.A., G.A. Churchill, J.E. Autique, S.D. Tanksley and M.E. Sorreils, 1993. Optimizing parental selection for genetic-linkage maps. Genome, 36: 181-186.
24. Nei, M., 1973. The theory and estimation of genetic distance. In edited by Morton, N.E., Genetic structure of populations. Honolulu, HI: University of Hawaii Press, pp: 45-54.
25. Rohlf, F.J., 2002. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Setauket, NY: Exeter Software.
26. Dice, L.R., 1945. Measures of the amount of ecologic association between species. Ecology, 26: 297-302.
27. IRGSP (International Rice Genome Sequencing Project), 2005. The map-based sequence of the rice genome. Nature, 436: 793-800.
28. Joshi, R.K. and L. Behera, 2006. Identification and differentiation of indigenous non Basmati aromatic rice genotypes of India using microsatellite markers. Afr. J. Biotechnol., 6: 348-354.
29. Prabakaran, A., K. Paramasivam, T. Rajesh and D. Rajarajan, 2010. Molecular characterization of rice land races using SSR markers. Elect. J. Plant Breed., 1: 512-516.
30. Pachauri, V., N. Taneja, P. Vikram, N.K. Singh and S. Singh, 2013. Molecular and morphological characterization of Indian farmers rice varieties (*Oryza sativa* L.). Aust. J. Crop Sci., 7: 923-932.
31. Zeng, L., T.R. Kwon, X. Liu, C. Wilson, C.M. Grieve and G.B. Gregorio, 2004. Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. Plant Sci., 166: 1275-1285.
32. Singh, N., D.R. Choudhury, G. Tiwari, A.K. Singh and S. Kumar *et al*., 2016. Genetic diversity trend in Indian rice varieties: An analysis using SSR markers. BMC Genetics, 17: 127.
33. Krupa, K.N., H.E. Shashidhar, N. Dalawai, M. Reddy and H.V.K. Swamy, 2017. Molecular marker based genetic diversity analysis in rice genotypes (*Oryza sativa* L.) using SSR markers. Int. J. Pure Applied Biosci., 5: 668-674.
34. Matin, S., M. Ashrafuzzaman, M.M. Islam, S.U. Sikdar and N. Zobayer, 2012. Molecular marker based (SSR) genetic diversity analysis in deep water rice germplasms of Bangladesh. Int. J. Biosci., 2: 64-72.
35. Siwach, P., S. Jain, N. Saini, V.K. Chowdhury and R.K. Jain, 2004. Allelic diversity among Basmati and non-Basmati long-grain indica rice varieties using microsatellite markers. J. Plant Biochem. Biotechnol., 13: 25-32.
36. Islam, M.Z., M. Khalequzzaman, M.K. Bashar, N.A. Ivy, M.A.K. Mian, B.R. Pittendrigh, M.M. Haque and M.P. Ali, 2018. Variability Assessment of Aromatic Rice Germplasm by Pheno-Genomic traits and Population Structure Analysis. Scient. Rep., 8: 9911.
37. Shah, S.M., S.A. Naveed and M. Arif, 2013. Genetic diversity in Basmati and non-Basmati rice varieties based on microsatellite markers. Pak. J. Bot., 45: 423-431.
38. Salgotra, R.K., B.B. Gupta, J.A. Bhat and S. Sharma, 2015. Genetic diversity and population structure of Basmati rice (*Oryza sativa* L.) germplasm collected from North Western Himalayas using trait linked SSR markers. PLoS One, 10: e0131858.
39. Becerra, V., M. Paredes, E. Gutiérrez and C. Rojo, 2015. Genetic diversity, identification and certification of Chilean rice varieties using molecular markers. CHILEAN J. Agric. Res., 75: 267-274.
40. Sajib, A.M., M.M. Hossain, A.T.M.J. Mosnaz, H. Hossain, M.M. Islam, M.S. Ali and S.H. Prodhan, 2012. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.). J. Biol. Sci. Biotech., 1: 107-116.
41. Rahman, M.M., M.G. Rasaul, M.A. Hossain, K.M. Iftekharuddaula and H. Hasegawa, 2012. Molecular characterization and genetic diversity analysis of rice (*Oryza sativa* L.) using SSR markers. J. Crop Improv., 26: 244-257.
42. Jain, S., S.E. Mitchell, R.K. Jain, S. Kresovich and S.R. McCouch, 2003. DNA fingerprinting and phylogenetic analysis of Indian aromatic high quality rice germplasm using panels of fluorescent labeled microsatellite markers. In: Advance in Rice Genetics, Khush G.S., D.S. Brar and B. Hardy (Eds.). IRRI, Philippine, pp: 162-165.
43. Shakil, S.S., S. Sultana, M.M. Hasan, M.M. Hossain, M.S. Ali and S.D. Prodhan, 2015. SSR marker based genetic diversity analysis of modern rice varieties and coastal landraces in Bangladesh. Indian J. Biotechnol., 14: 33-41.
44. Choudhury, B., M.L. Khan and S. Dayanandan, 2013. Genetic structure and diversity of indigenous rice varieties (*Oryza sativ*a L.) in eastern Himalayan region of Northeast India. Springer Plus, 2: 228-237.
45. Das, B., S. Sengupta, S.K. Parida, B. Roy, M. Gosh, M. Prasad and T.K. Ghose, 2013. Genetic diversity and population structure of rice landraces from Eastern and North Eastern States of India. BMC Genet., 71: 1-14.

46. Freeg, H.A., G.B. Anis, A.A.A. Shousha, A.N.E. Banna and A.E. Sabagh, 2016. Genetic diversity among some rice genotypes with different drought tolerance based on SSR markers. Cercetari Agronomice in Moldova, 69: 39-50.
47. Rabelo, H.O., J.F.R. Guimarães, J.B. Pinheiro and E.F. Silva, 2015. Genetic base of Brazilian irrigated rice cultivars. Crop Breed. Applied Biotechnol., 15: 146-153.
48. Wanga, J., T. Jianga, D. Zoub, H. Zhao, Q. Li, H. Liub and C. Zhoud, 2014. Genetic diversity and genetic relationships of japonica rice varieties in Northeast Asia based on SSR markers. Biotechnol. Biotechnol. Equip., 28: 230-237.
49. Xu, Y., H. Beachell and S.R. McCouch, 2004. A marker-based approach to broadening the genetic base of rice in USA. Crop Sci., 44: 1947-1959.
50. Song, M.T., J.H. Lee, Y.S. Cho, Y.H. Jeon, S.B. Lee, J.H. Ku, S.H. Choi and H.G. Hwang, 2002. Narrow genetic background of Korean rice germplasm as revealed by DNA fingerprinting with SSR markers and their pedigree information. Korean J. Genet., 24: 397-403.
51. Lin, H.Y., Y.P. Wu, A.L. Hour, S.W. Ho, F.J. Wei, Y.I.C. Hsing and Y.R. Lin, 2012. Genetic diversity of rice germplasm used in Taiwan breeding programs. Bot. Stud., 53: 363-376.