Plant Archives Volume 20 No. 2, 2020 pp. 6395-6400 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR DIVERSITY ANALYSIS OF DROUGHT TOLERANT RICE (*ORYZA SATIVA* L.) GENOTYPES USING SSR MARKERS

Riddha Dey, S.K. Singh, Prudhvi Raj Vennela, D.K. Singh, Shubhra Natasha Kujur, Sonali Habde, Yashraaj Sharma and Korada Mounika*

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, (U.P.) India.

Abstract

Genetic Diversity in rice provides a great opportunity for the plant breeders to develop novel and improved cultivars with desirable characteristics. In the present study, a total of 30 SSR markers were used across twelve drought tolerant rice genotypes for their characterization and divergence analysis. Among the thirty SSR markers used, only twenty three SSR markers showed polymorphism and the PIC value ranged from 0.551 (RM 11) to 0.305 (RM 307) with a mean value of 0.38. Variation in the number of alleles ranged from 2 to 3 with an average of 2.217. A total of 51 microsatellite alleles were amplified from twelve genotypes and this demonstrates considerable variability among genotype. RM 11 was found the best marker for the identification of twelve genotypes as demonstrated by the PIC values. A dendogram was constructed using UPGMA which represents four clusters. Through dendogram analysis, highest similarity was observed between genotypes IR 95786-9-2-1-2 and IR 93827-29-1-1-2. The most diverse cultivars were MTU1010 and CN 2044. Further studies for comparative mapping and marker assisted selection (MAS) for drought tolerant rice genotypes can be done easily through the highly informative markers identified in this study.

Key Words: Dendogram, Molecular diversity, SSR markers and UPGMA.

Introduction

Rice (*Oryza sativa* L.), a member of Poaceae family and sub family Oryzoidea holds a prime position in Indian Agriculture and plays a significant role in Indian economy being the staple food for two thirds of the world population. Rice is the first food crop whose entire genome sequence is accessible and it has comparatively small genome size of 430 Mb (Causse *et al.,* 1994) because of the availability of a high-precision genomic sequence and highly saturated molecular markers. It is an ideal model plant for the study of grass genetics and genome organization.

Study of genetic diversity helps in the development of superior recombinants which is a major part of crop improvement program. Crosses between parents with maximum genetic divergence are generally the most responsive for genetic improvement (Arunachalam, 1981). The selection of agronomically suitable diverse parents for hybridization is important for getting desired recombinants in the segregating generations (Pandey, 2017).

***Author for correspondence :** E-mail : monikakorada@gmail.com

The significant advancements in molecular biology have shifted the focus of assessment of genetic diversity from relying on morphological markers to using molecular markers. A molecular marker is a DNA sequence that is readily detected and whose inheritance can be easily monitored. Recently, DNA profiles based on various molecular markers have been widely applied across different fields (Primrose *et al*., 2010). Conventionally, genetic diversity is estimated by the D^2 analysis, metroglyph and principal component analysis using morphological traits. D^2 technique is based on multivariate analysis developed by Mahalanobis 1936, measures the amount of genetic diversity in a given population in respect of several characters and assesses relative contribution of different components to the total divergence (Zahan *et al.,* 2008).

Materials and Methods

The present investigation was performed during *Kharif* 2016 (at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P., India). The molecular analysis was done

Table 1: Description of Genotypes of rice used in the study.

Sl. No.	Genotypes	Source	Sl. No.	Genotypes	Source
1.	IR95785-15-2-1-2	IRRI, Philippines	7.	PR37160-8-3-1-1-1-1	IRRI, Philippines
2.	IR95786-9-2-1-2	IRRI, Philippines	8.	MTU-1010 (Check)	IRRI, Philippines
3.	IR93827-29-1-1-2	IRRI, Philippines	9.	NDR-97 (Check)	NDUAT,Faizabad
4.	IR95836-14-3-1-2	IRRI, Philippines	10.	IR93827-29-1-1-4	IRRI, Philippines
5.	SAHBHAGIDHAN	IRRI, Philippines	11.	CN-2044	IRRI, Philippines
6.	PR37956-3B-44-1	IRRI, Philippines	12.	IR93809-101-2-2-2	IRRI, Philippines

Isolation of genomic DNA

at the Molecular Biology Laboratory (Niche Area Lab) of the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.), India. The experimental material for this investigation comprised of twelve genotypes. The details of genotypes used in the present study are listed in table 1.

Young leaves of 12 days old rice seedlings were collected and immediately stored at -20^{0}C till further processing. The DNA was extracted following CTAB extraction method according to Doyle and Doyle (1987)

Polymerase chain reaction (PCR)

Polymerase chain reaction was performed to selectively amplify *in vitro*, a specific segment of the total genomic DNA to a billion fold (Mullis *et al*., 1986). The most essential requirement of PCR is an availability

Table 2: Description of SSR markers used in the study.

Sl. No.	Micro-satellite locus	Chrom-osome No.	Forward /Reverse	Sequence 5' ———> 3'	Anneal ing Tem-perature
1	RM307	4	ForwardReverse	GTACTACCGACCTACCGTTCACCTGCTATGCATGAACTGCTC	5752
2	RM124	4	ForwardReverse	ATCGTCTGCGTTGCGGCTGCTGCATGGATCACCGAGCTCCCCCC	6062
3	RM507	5	ForwardReverse	CTTAAGCTCCAGCCGAAATGCTCACCCTCATCATCGCC	5253
4	RM413	5	ForwardReverse	GGCGATTCTTGGATGAAGAGTCCCCACCAATCTTGTCTTC	5252
5	RM161	5	ForwardReverse	TGCAGATGAGAAGCGGCGCCTCTGTGTCATCAGACGGCGCTCCG	6060
6	RM178	5	ForwardReverse	TCGCGTGAAAGATAAGCGGCGCGATCACCGTTCCCTCCGCCTGC	5962
7	RM334	5	ForwardReverse	GTTCAGTGTTCAGTGCCACCGACTTTGATCTTTGGTGGACG	5452
8	RM133	6	ForwardReverse	TTGGATTGTTTTGCTGGCTCGCGGAACACGGGGTCGGAAGCGAC	5562
9	RM510	6	ForwardReverse	AACCGGATTAGTTTCTCGCCTGAGGACGACGAGCAGATTC	5254
10	RM454	6	ForwardReverse	CTCAAGCTTAGCTGCTGCTGGTGATCAGTGCACCATAGCG	5454
11	RM162	6	ForwardReverse	GCCAGCAAAACCAGGGATCCGGCAAGGTCTTGTGCGGCTTGCGG	6060
12	RM125	7	ForwardReverse	ATCAGCAGCCATGGCAGCGACCAGGGGATCATGTGCCGAAGGCC	6060
13	RM11	7	ForwardReverse	TCTCCTCTTCCCCCGATCATAGCGGGCGAGGCTTAG	5353
14	RM455	7	ForwardReverse	AACAACCCACCACCTGTCTCAGAAGGAAAAGGGCTCGATC	5452
15	RM118	7	ForwardReverse	CCAATCGGAGCCACCGGAGAGCCACATCCTCCAGCGACGCCGAG	6262
16	RM408	8	ForwardReverse	CAACGAGCTAACTTCCGTCCACTGCTACTTGGGTAGCTGACC	5457
17	RM152	8	ForwardReverse	GAAACCACCACACCTCACCGCCGTAGACCTTCTTGAAGTAG	5652
18	RM25	8	ForwardReverse	GGAAAGAATGATCTTTTCATGGCTACCATCAAAACCAATGTTC	4949
19	RM44	8	ForwardReverse	ACGGCAATCCGAACAACCTCGGGAAAACCTACCCTACC	5354
20	RM284	8	ForwardReverse	ATCTCTGATACTCCATCCATCCCCTGTACGTTGATCCGAAGC	5354
21	RM433	8	ForwardReverse	TGCGCTGAACTAAACACAGCAGACAAACCTGGCCATTCAC	5252
22	RM447	8	ForwardReverse	CCCTTGTGCTGTCTCCTCTCACGGGCTTCTTCTCCTTCTC	5654
23	RM316	9	ForwardReverse	CTAGTTGGGCATACGATGGCACGCTTATATGTTACGTCAAC	5449
24	RM105	9	ForwardReverse	GTCGTCGACCCATCGGAGCCACTGGTCGAGGTGGGGATCGGGTC	6262
25	RM215	9	ForwardReverse	CAAAATGGAGCAGCAAGAGCTGAGCACCTCCTTCTCTGTAG	5254
26	RM474	10	ForwardReverse	AAGATGTACGGGTGGCATTCTATGAGCTGGTGAGCAATGG	5252
27	RM271	10	ForwardReverse	TCAGATCTACAATTCCATCCTCGGTGAGACCTAGAGAGCC	4856
28	RM171	10	ForwardReverse	AACGCGAGGACACGTACTTACACGAGATACGTACGCCTTTG	5452
29	RM484	10	ForwardReverse	TCTCCCTCCTCACCATTGTCTGCTCCCCTCTCTCTCTCTC	5456
30	RM552	11	ForwardReverse	CGCAGTTGTGGATTTCAGTGTGCTCAACGTTTGACTGTCC	5252

of a pair of short (typically 20-25 nucleotides) primers having sequence complementary to either end of the target DNA segment (called template DNA) supposed be synthesized in large amount. A total of thirty simple sequence repeat (SSR) markers were used for studying molecular diversity and denoted in table 2. After the completion of the PCR, the products were stored at -20ºC until the gel electrophoresis was done.

Table 3: Alleles and polymorphism information content (PIC) of SSR primers used in the present study.

Primer	Allele	PIC
RM 307	2	0.305
RM 124	2	0.356
RM 507	2	0.346
RM413	2	0.305
RM 161	2	0.373
RM 178	2	0.318
RM 334	2	0.346
RM 133	2	0.373
RM 510	3	0.505
RM 454	3	0.526
RM 162	2	0.368
RM 125	3	0.449
RM 11	3	0.551
RM 455	2	0.375
RM 118	2	0.346
RM 408	2	0.368
RM 152	3	0.438
RM 25	2	0.356
RM 284	2	0.346
RM 447	2	0.359
RM 316	2	0.368
RM 105	2	0.373
RM 215	2	0.375

Visualization of amplification products

The amplified DNA products generated through SSR primers were resolved through electrophoresis. For electrophoresis, 15µl of the PCR product was mixed with 2µl of 6X loading dye (bromophenol blue) and loaded in the slot of agarose gel. Gel electrophoresis was carried out at a constant voltage of 65 V for about 3.5 hours and the gels were visualized under a UV light source in a gel documentation system (Gel Doc™ XR+, BIO-RAD, USA).

Scoring the PCR amplified fragments

Band position in comparative SSR profile for each genotype and primer combination was scored from the respective gel images. The amplified fragments were scored as '1' for the presence and '0' for the absence of a band generating the 0 and 1 matrix.

Data Analysis

Standardization was done by dividing the deviation of mean for a line from the mean for 12 genotypes with the standard deviation for the given trait; the STAND module of NTSYS software was used to furnish the same.

Table 4: Grouping of twelve genotypes of rice into four clusters on the basis of dendogram analysis.

Sl. No.	Cluster	No. of Genotypes	Genotypes
1	Cluster-I	2	1.IR 95785-15-2-1-211.CN 2044
2	Cluster-II A	4	2.IR 95786-9-2-1-23.IR 93827-29-1-1-24.IR 95836-14-3-1-25.Sahbhagidhan
3	Cluster-II B-1	3	6.PR37956-3B-44-17.PR37160-8-3-1-1-1-18.MTU1010-Check
4	Cluster-II B-2	3	9.NDR-9710.IR 93827-29-1-1-412.IR 93809-101-2-2-2

Table 5: Jackard dissimilarity coefficient.

	1	2	3	4	5	6	7	8	9	10	11	12	Average D^2
1		0.6875	0.8056	0.8649	0.8235	0.7714	0.7419	0.8529	0.8611	0.8286	0.6774	0.8056	0.7928
2			0.5484	0.7429	0.6452	0.750	0.7941	0.8286	0.6970	0.6970	0.7714	0.7143	0.7160
3				0.5484	0.6563	0.7568	0.6875	0.6875	0.7059	0.6667	0.8108	0.7895	0.6967
4					0.5517	0.750	0.6774	0.5862	0.7353	0.7353	0.8056	0.750	0.7043
5						0.5667	0.6071	0.70	0.7188	0.7576	0.8919	0.8378	0.7051
6							0.5517	0.5517	0.5806	0.8108	0.8718	0.7222	0.6985
7								0.6429	0.5714	0.750	0.8571	0.80	0.6983
8									0.6207	0.6207	0.9189	0.6452	0.6959
9										0.5517	0.80	0.5806	0.6748
10											0.60	0.5806	0.6908
11												0.6250	0.7845
12													0.7137

1= IR 95785-15-2-1-2, 2= IR 95786-9-2-1-2, 3= IR 93827-29-1-1-2, 4= IR 95836-14-3-1-2, 5= Sahbhagidhan, 6= PR 37956-3B-44-1, 7= PR 37160-8-3-1-1-1-1, 8= MTU1010-Check, 9= NDR-97, 10= IR 93827-29-1-1-4, 11= CN 2044, 12= IR 93809-101-2-2-2

The binary data matrices generated by polymorphic SSR markers were subjected to further analysis using NTSYS-pc version 2.11W. The SIMQUAL program was used to calculate the Jackard's coefficient. Unweighted pair-group average (UPGMA) based clustering was done using SAHN module of NTSYSpc for dendrogram construction.

Polymorphic information content (PIC)

PIC for SSR markers was calculated as per the formula: PIC= 1-Pij2, where PICi is the polymorphic information contents of a marker i and the summation extends over n patterns.

Results and Discussion

In the present diversity among twelve drought tolerant rice cultivars was done using thirty polymorphic SSR markers. Among them twenty three markers showed polymorphism and cluster analysis was done to construct dendogram using Jackard's dissimilarity coefficient.

PIC value

The PIC value is an evidence of diversity and frequency among the varieties. The alleles and PIC details of each markers are listed in table 3. The largest PIC value was observed for locus RM 11 (0.551) followed by RM 454 (0.526) and lowest for RM 413 (0.305). PIC values ranged from 0.551 to 0.305 with a mean value of 0.38. Variation in the number of alleles ranged from 2 to 3 with an average of 2.217. A total of 51 microsatellite alleles were amplified from 12 genotypes and this demonstrates considerable variability among genotype. Similar results were reported by Wong *et al.*, (2009) and Singh *et al*., (2016).

Dendogram analysis

A dendogram Fig. 2 based on Jackard's similarity coefficient was constructed using UPGMA. The genetic divergence was assessed based on D^2 statistics. Twelve rice genotypes were grouped into two main clusters *i.e.* cluster I (2 genotypes) and cluster II (10 genotypes) with similarity coefficient 0.46. Cluster II was further sub-

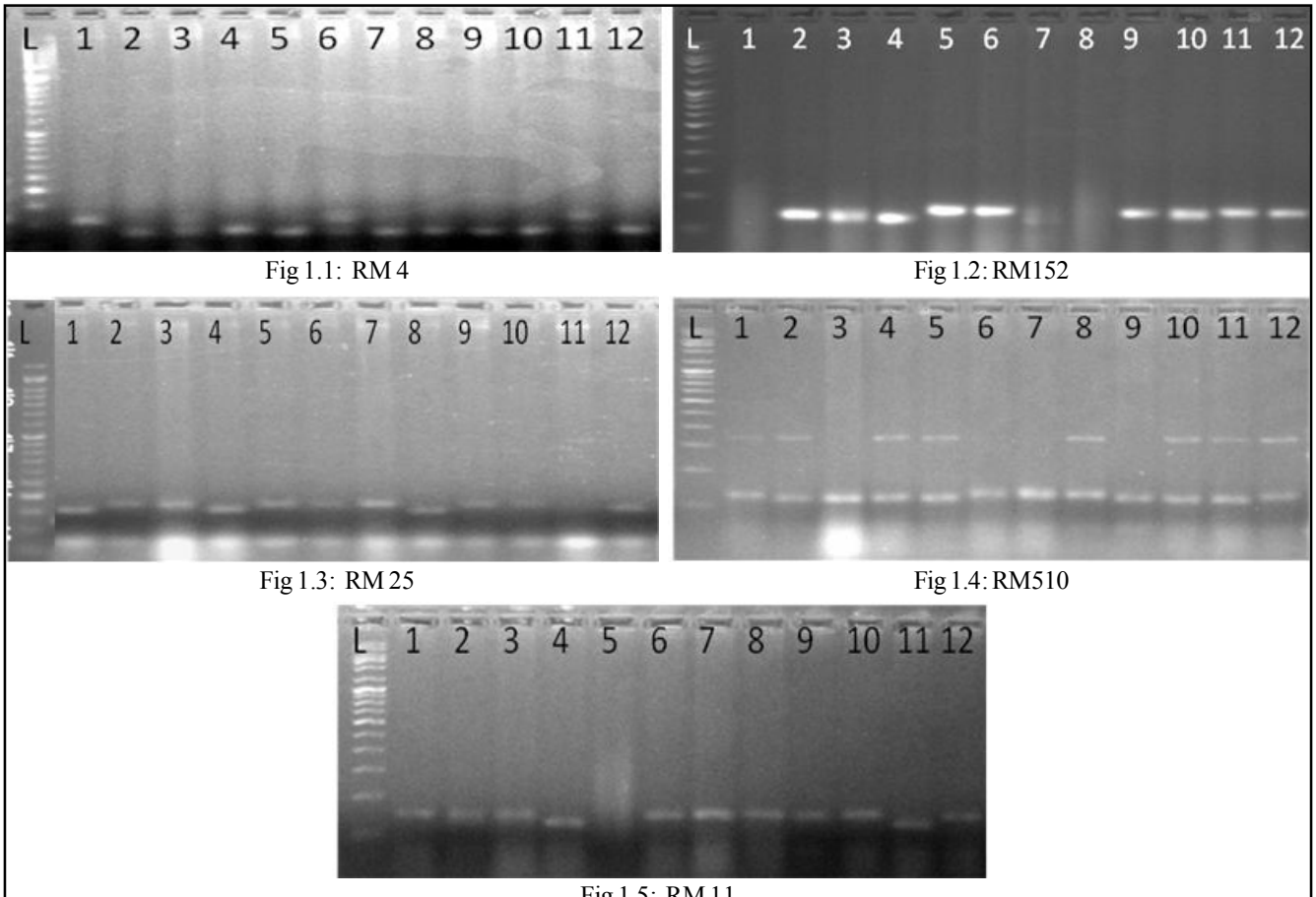

Fig 1.1: RM 4 Fig 1.2: RM152

Fig 1.3: RM 25 Fig 1.4: RM510

Fig 1.5: RM 11

1= IR 95785-15-2-1-2, **2**= IR 95786-9-2-1-2, **3**= IR 93827-29-1-1-2, **4**= IR95836-14-3-1-2, **5**= Sahbhagidhan, **6**= PR37956-3B-44-1, **7**= PR37160-8-3-1-1-1-1, **8**= MTU1010-Check, **9**= NDR-97, **10**= IR93827-29-1-1-4, **11**= CN 2044, **12**= IR 93809-101-2-2-2.

Fig. 1: SSR Markers obtained in 12 rice genotypes. Lane 1-12 represents rice genotypes (Fig: 1.1-1.5)

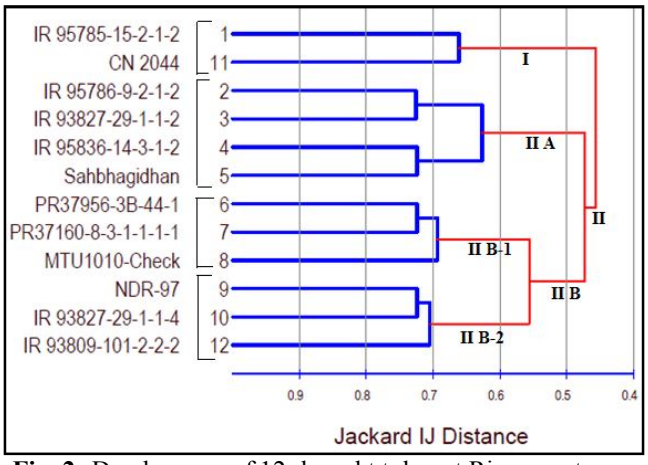

Fig. 2: Dendrogram of 12 drought tolerant Rice genotypes.

divided into two minor sub-groups IIA (4 genotypes) and IIB with similarity coefficient 0.48. Cluster IIB were further sub-divided into two subgroups *i.e.* IIB-1 (3 genotypes) and IIB-2 (3 genotypes) with similarity coefficient 0.56. The details of the clusters are tabulated in the table 4. This indicates presence of considerable diversity in the genotypes studied. Similar results were reported by Shahriar *et al.,* (2014), when assessed the genetic diversity of thirty four rice genotypes through 3 polymorphic SSR markers.

Jackard's dissimilarity coefficient

A dissimilarity matrix was used to determine the level of diversity among the cultivars studied. The average of dissimilarity coefficient varies from 0.7928 to 0.6748. The total average of dissimilarity coefficient of all 12 genotypes is 0.7143. The values are represented in Table 5. These results are similar to the findings of Sajib *et al.,* (2012) when assessed 12 elite aromatic rice genotypes through 24 SSR markers. A dissimilarity matrix was used to determine the level of dissimilarity among the cultivars studied. The dissimilarity coefficient varies from one to zero, closer to one shows higher disssimilarity while closer to zero shows higher similarity.

The most genetically dissimilar genotypes were observed to be MTU1010 (Check) and CN 2044 (0.9189) followed by CN 2044 and Sahbhagidhan (0.8919), PR37956-3B-44-1 and CN 2044 (0.8718), IR 95785-15-2-1-2 and IR 95836-14-3-1-2 (0.8649), and lastly IR 95785-15-2-1-2 and NDR-97 (0.8611). This shows high dissimilarity between the genotypes. The least genetically dissimilar genotypes were observed to be IR 95786-9-2-1-2 and IR 93827-29-1-1-2 (0.5484), IR 93827-29-1-1-2 and IR 95836-14-3-1-2 (0.5484) followed byIR 95836-14-3-1-2 and Sahbhagidhan(0.5517),PR37956-3B-44-1 and PR37160-8-3-1-1-1-1 (0.5517) and lastly NDR-97 and IR 93827-29-1-1-4 (0.5517) showing that they are highly similar with each other and it may be expected that both of them may have arouse from the same parents. Similar results were reported by Babu *et al.,* (2014), Sonkar *et al.,* (2016). Thus, SSR markers proves to be a potential tool in the identification and characterization of genetically distant cultivars from various sources.

Conclusion

The study of 12 genotyps with 30 SSR markers showed the largest PIC value for locus RM 11 (0.551) and lowest for RM 413 (0.305). Twelve rice genotypes were grouped into two main clusters, I and II with similarity coefficient 0.46. Cluster II is sub divided into two minor sub-groups IIA and IIB, and with similarity coefficient 0.48. Cluster IIB were further sub-divided into two subgroups *i.e.* IIB-1 and IIB-2 with similarity coefficient 0.56. Cluster I consists of 2 genotypes, cluster IIA consists of 4 genotypes. Cluster IIB-1 and IIB-2 consists of 3 genotypes each. This indicates a presence of considerable diversity in the genotypes studied. On the basis of dendogram the highest similarity observed between genotypes IR 95786-9-2-1-2 and IR 93827-29-1-1-2 followed byIR 93827-29-1-1-2 and IR 95836-14-3-1-2.The most diverse cultivar was MTU1010-Check and CN 2044.

Acknowledgement

The authors are thankful to Central laboratory (Niche area lab), Institute of Agricultural Science, Banaras Hindu University, Varanasi for the lab facilities.

References

Arunachalam, V. (1981). Genetic divergence in plant breeding. *Indian J. Genet*., **14:** 226-236.

Babu, B.K., V. Meena, V. Agarwal and P.K. Agrawal (2014). Population structure and genetic diversity analysis of Indian and exotic rice (*Oryza sativa* L.) accessions using SSR markers. *Mol. Biol. Rep.,* **41(7):** 4328-39.

Causse, M., T.M. Fulton, Y.G. Cho, S.N. Ahn and J. Chunwongse (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics,* **138:** 1251-1274.

Doyle, J.J. and J.L. Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bulletin,* **19:** 11-15.

Mullis, K., S. Faloona, R. Scharf and Saiki, Horn, Erlich (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symp. Quant. Biol.,* **51:** 263-273.

Pandey, S., D. Behura, R. Villano and D. Naik (2000). Economic Cost of Drought and Farmers' Coping Mechanisms: A

Study of Rainfed Rice in Eastern India. *IRRI Discussion Paper Series,* pp. 1–35.

Primrose, S., M. Woolfe and S. Rollinson (2010). Food forensics: methods for determining the authenticity of foodstuffs. *Trends Food Sci.Tech.,* **21:** 582-590.

Sajib, M.A., M. Hossain, A.T.M.J. Mosnaz , H. Hossain, M.M. Islam, M.S. Ali, S.H. Prodhan (2012). SSR marker-based molecular characterization and genetic diversity analysis of aromatic landreces of rice (*Oryza sativa* L.) *J. Bio. Sci. Biotech.,* **1(2):** 107-116.

Shahriar, M.H., A.H.K. Robin, S.N. Begum and A. Hoque (2014). Diversity analysis of some selected rice genotypes through SSR- based molecular markers. *J. Bangladesh Agril. Univ.,* **12(2):** 307-311.

Singh, N., D.R. Choudhury, G. Tiwari, A.K. Singh, S. Kumar, K. Srinivasan, R.K. Tyagi, A.D. Sharma, N.K. Singh and R. Singh (2016). Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. *BMC Genet.,* **17:** 127. doi: 10.1186/s12863-016-0437-7.

Sonkar, S., S.K. Singh, P.R. Vennela and D.K. Singh (2016). Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using SSR markers, *IJAEB,* **9(1):** 45-51, DOI Number: 10.5958/2230-732X.2016.00007.3.

Wong, S.C., P.H. Yiu, S.T.W. Bong, H.H. Lee, P.N.P. Neoh, A. Rajan (2009). Analysis of Sarawak Bario rice diversity using microsatellite markers. *Am. J. Agri. Biol. Sci.,* **4(4):** 298-304.

Zahan, M.I., M.S.R. Bhuiyan and M.S. Hossain (2008). Genetic divergence in Oleiferous Brassica species. *J. Sher-e-Bangla Agric. Univ.,* **2(1):** 1-6.