International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(4): 1046-1050

Received: 01-05-2020
Accepted: 05-06-2020

Abhinav Sao
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Praveen Panigrahi
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Prafull Kumar
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Hemant Sahu
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Abhinav Sao
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Molecular Distinguishness among rice (*Oryza sativa* L.) landrace of Central India using microsatellite markers

Abhinav Sao, Praveen Panigrahi, Prafull Kumar and Hemant Sahu

DOI: https://doi.org/10.22271/chemi.2020.v8.i4h.9741

Abstract
Molecular characterization of the genotypes gives precise information about the extent of genetic diversity which helps in the development of an appropriate breeding program. A total of 16 microsatellite (SSR) markers distributed across the rice genome were used for molecular characterization and discrimination of 100 local landraces of rice. The molecular data revealed a total of 24 alleles in 100 rice accessions, The number of alleles per locus generated by each marker ranged from 1 to 5 alleles with the mean of 1.5 alleles per locus and an average polymorphism information content (PIC) of 0.387. This suggests that these markers could be potentially used for molecular characterization of rice accession from various sources. Microsatellite markers (SSR) are also used to detect the genetic similarity of accessions of rice under study. The genetic similarity coefficient ranged from 0.76-1.00 as revealed by UPGMA cluster analysis using the 16 SSR markers. A total of four distinct groups resulted at a cut-off similarity coefficient of 0.83 among the 100 rice accessions. Allelic variability among the SSR markers was high enough to categorize landraces and to catalogue the genetic variability observed for future use. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programs, including gene mapping, and ultimately for emphasizing the importance of marker-assisted selection (MAS) in rice improvement.

Keywords: Genetic diversity, microsatellite markers, PIC values, rice landraces

Introduction
Rice is the world's largest food crop, providing the daily caloric needs of more than half of the global population. South Asia, one of the major centers for rice domestication, has been described as the "food basket" and "food bowl" of Asia. Cultivated rice is one of the most polymorphic crop species, and is composed of several ecological groups, frequently referred to as subspecies (Oka, 1988) [7]. One of the important approaches to rice breeding is hybridization and subsequent selection. Parents' choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary. Asian rice (*Oryza sativa* L.) has been cultivated for an estimated 10,000 years (Liu *et al*., 2007) [3] and currently feeds more than one third of the world's population. Growth and development of agricultural resources is mostly depending on genetic diversity among different crop plants and it is estimated that not even 15% of the potential diversity has utilized. This implies that thousands of valuable allelic variations of traits of economic significance remain unutilized (Hossain *et al*., 2007) [2]. Therefore, landraces of distinct genetic structure are a good promise for the future rice crop improvement. Thus, identification of genotypes and their inter-relationships is vital.
Landraces harbor a great genetic potential for rice improvement. Unlike high-yielding varieties (whose variability is limited due to homozygosity), the landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period of time. This aids in the adaptation of landraces to wide agro ecological niches and they also have unmatched qualitative traits and medicinal properties. This rich variability of complex quantitative traits still remains unexploited. Landraces are also important genetic resources for resistance to pests and fungal diseases Collection and evaluation of landraces are an integral part of the pre-breeding process carried out by rice breeders (Vaughan 1991; Siddiq *et al*. 2005) [15, 13].

Simple sequence repeat is an important tool for genetic variation identification of germplasm (Powell *et al*., 1996; Ma *et al*., 2011) [9, 4]. The morphological marker characters may be qualitative or quantitative in nature as they may be governed by one or more genes. The quantitative characters are influenced by environment, which indicates that such characters are not stable hence cannot be used as marker trait whereas; qualitative traits may be used as morphological markers with low reliability in characterization of germplasm because they are less influenced by environment. The molecular markers are DNA based marker and it represents the genetic constitution of any individual. DNA of any individual does not influenced by environment; hence the DNA based markers are supposed to be stable marker to diagnose any trait. Molecular markers are powerful tools in the assessment of genetic variation, in the elucidation of genetic relationships within and among species, and have demonstrated the potential to detect genetic diversity and to aid in the management of plant genetic resources. In the present study, 100 accessions of rice are used for molecular characterization and genetic diversity study. The present study addresses the utility of SSR markers in revealing genetic relationships at the molecular level among local landraces of rice collected from Bastar plateau zone of Chhattisgarh, India which is a hot spot of biodiversity.

In Bastar region of Chhattisgarh rice is grown predominantly during *kharif* season as rainfed crop having 2.39 million hectare area but the productivity of this crop is very low, 08.53 qt/ha. Rice based cropping systems are in existence and farmers raise traditional rice varieties and still adopt organic farming. Safari, Gurmatia, Sathka, Bhata Mokdo, Chudi Dhan etc. are among these traditional varieties. According to the traditional healers of Bastar region many of these medicinal rice varieties are used in traditional medicine system for treatment of rheumatism, skin infections, paralysis, diabetes etc. (Oudhia, 2006) [8]. The Bastar region is also known for its rich floristic diversity and tribal culture. The rice cultivated in the region is globally known for variability in grain size, aroma, and medicinal value. The interaction between tribal farmers and the terrestrial heterogeneity of the landscape, presenting diverse ecologies, has resulted in the evolution of a very large number of landraces and farmer's varieties with variability for most traits and suitability for diverse agro-ecologies in staple food crops such as rice, converting the region into a very important center of genetic diversity for rice (Singh, 2013) [14].

Materials and Methods

The present investigation was carried out during the *Kharif*, 2015 at Research cum Instructional Farm, S.G. College of Agriculture and Research Station, Kumhrawand, Jagdalpur, Bastar, Chhattisgarh, India. The latitude and longitude of Jagdalpur can be projected as 19° 4'0" N and 82° 2'0" E, respectively. The city is nestled on the Bastar Plateau and is positioned at a height of around 552 meters from the sea level. The experimental materials comprised of hundred local landraces of rice collected from Bastar Plateau zone (Table 1). A detailed study of morphological differences among this landraces is done and also subjected to molecular characterization to judge molecular diversity.

Table 1: List of hundred local landraces of rice used in the study

S. No.	Accession Name	S. No.	Accession Name	S. No.	Accession Name	S. No.	Accession Name
1	Olesar	29	Ram-Laxman	57	Dhadhar Dhan	85	Phara dhaan
2	Lochai	30	Aasanchudi	58	Baadichudi	86	Baans Kontiya
3	Pakhiya Dhaan	31	Sonpuri	59	Pakhiyadhaan	87	Kurso Bhog
4	Khuji Dhaan	32	Idiraghotiya	60	Begnidhaan	88	Muthiya
5	Baadshah Bhog	33	Safurlochai	61	Motilur	89	Umarichudi
6	Kukdimundi	34	Mayurdhaan	62	Rakhidhaan	90	Khudbudi
7	Gongel	35	Pandri Lochai	63	Machripoti	91	Kaatamehar
8	Sofa Kaanan	36	Haldigaathi	64	Laalbargi	92	Aanjan
9	Madras Chudi	37	Vishnubhog	65	Dogarkaabri	93	Laal Banso
10	Kumda Phool	38	Pilkosnai	66	Shivdharohar	94	Rani Kaajar
11	Gada Khuta	39	Gangabaaru	67	Guthiya	95	Kusum Jhopa
12	Kaakad Kado	40	Jhumra	68	Bhokva dhaan	96	Photki Dhaan
13	Naani Chudi	41	Masuridesi	69	Jondranakti	97	Jatiya
14	Baasta Bhog	42	Mokdo	70	Badekhuji	98	Kaalamaali
15	Milkoor Mail	43	Dubraj	71	Kabrodhan	99	Gechi Dhaan
16	Baudi	44	Hansa Dubraj	72	Bhatamokdo	100	Goydi
17	Bagdi Chudi	45	Kurludhaan	73	Sendursenga		
18	Turejagadakhuta	46	Madiadhaan	74	Sela		
19	Bhayar	47	Teenkormail	75	Kolyara		
20	Sonasaari	48	Baadigoydi	76	Rangchudi		
21	Baadilochai	49	Haldijeera	77	Meharlaldhan		
22	Jeeradhaan	50	Pandrisatka	78	Dengichudi		
23	Gurmatiya	51	Kukdi	79	Mundrichudi		
24	Haldigodi	52	Bahiyakhuta	80	Basomati		
25	Adgadhaan	53	Kaalaumari	81	Manki dhaan		
26	Ghotiya Dhaan	54	Chiradhaan	82	Sargiphool		
27	Keraphool	55	Dhaagan	83	Kantabargi		
28	Sorchubaadi	56	Karigrass	84	Rang gadakhuta		

Genomic DNA isolation

Total genomic DNA was extracted and purified from 15 day old seedling leaves collected from at least 2-3 seedlings from each lines, using modified CTAB method described by Zheng *et al*. 1995. The quality of genomic DNA sample was assessed by 1% agarose (Sigma A9539) gel electrophoresis at 5V/cm. Sixteen SSR markers (Table 2) were selected from the

list of panel of 30 SSR markers displayed at the Rice Genes web site; http:// www.gramene.org/microsat/ssr.html.
The Polymerase Chain Reaction (PCR) was conducted in a reaction solution of total 10 µl prepared by mixing 1 µl of 50 ng per µl concentration of template DNA, with 9 µl of cocktail (Table 3).

Table 2: Sixteen microsatellite markers used for molecular characterization across 100 local landraces of rice.

S. No.	SSR Primers	Chromosome number	PRIMER SEQUENCES	
			FORWARD 5' → 3'	REVERSE 5' → 3'
1	RM495	1	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC
2	RM283	1	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG
3	RM514	3	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
4	RM124	4	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC
5	RM413	5	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
6	RM161	5	TGCAGATGAGAAGCGGCGCCTC	TGTGTCATCAGACGGCGCTCCG
7	RM133	6	TTGGATTGTTTTGCTGGCTCGC	GGAACACGGGGTCGGAAGCGAC
8	RM162	6	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG
9	RM125	7	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
10	RM455	7	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
11	RM408	8	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
12	RM152	8	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG
13	RM44	8	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
14	RM447	8	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
15	RM484	10	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
16	RM277	12	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG

Table 3: PCR mix for one reaction

Reagent	Stock concentration	Volume (µl)
PCR buffer with 15 mM $MgCl_2$	10X	1.0
dNTPs (Mix)	1mM	0.8
Nuclease Free Water	-	5.95
Taq polymerase	3 U/µl	0.25
DNA template	50 ηg/µl	1.0
Total 10		

Electrophoretic separation and visualization of amplified products
5% polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified microsatellite products After electrophoresis gel stained with Ethidium Bromide (10 µl in 200 ml distilled water) were visualized under UV.

SSR data statistical analysis
Only clear and unambiguous SSR markers were scored. The banding pattern of population developed by each set of primer was scored separately. The size of amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 100 base pairs (bp) DNA ladder. Particular base pair position was scored as "1" and absence of band for that particular base pair position was scored as "0". Polymorphism information content (PIC) was calculated, according to the method of Anderson *et al.* (1993):

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where, P_{ij} is the frequency of the j^{th} allele for the i^{th} marker, and is summed over n alleles. Genetic similarities were estimated from the matrix of binary data using Jaccard coefficient. The similarity coefficients were used for cluster analysis of the rice cultivars utilizing the Unweighted Pair Group Method with Arithmetic Averages (UPGMA). The analysis and dendrogram showing the distance-based interrelationship among the genotypes construction were performed using the NTSYS-pc version 2.02 (Rohlf, 1999).

Results and Discussions
SSR Polymorphisms
A total of 16 SSR markers (primers) were used for molecular characterization and discrimination of 100 accessions of rice. After analysing the data generated from 16 microsatellite markers (SSR), a total of 24 alleles were detected in 100 rice accessions. The number of alleles per locus generated by each marker ranged from 1 to 5 alleles with an average of 1.5 alleles per locus. The highest number of alleles (5) was detected in the locus RM152 and the lowest number of alleles (1) was detected on each of locus RM16, RM455, RM495, RM447, RM484, RM413, RM283, RM514, RM125, RM277, RM133, RM124 and RM162. only three SSR marker (RM44, RM408 & RM152) showed polymorphic reaction with polymorphism information content (PIC) values of 0.04, 0.53 and 0.59, respectively. There is a co-relation also found in our study between higher number of alleles and PIC values. Similar results of PIC values was also found by Nadia *et al.* 2014. This suggests that these markers could be potentially used for molecular characterization of rice accession from various sources. The gel images of amplified fragments produced by primer RM152, RM44 and RM408 are presented in Fig. 1.

Table 4: List of 16 microsatellite markers with their chromosomal locations, number of alleles and allele size found among 100 rice accessions

S. No.	SSR Primers	Chromosome number	Annealing temperature	Number of alleles	Allele size (bp)
1	RM495	1	55	1	170
2	RM283	1	61	1	120
3	RM514	3	55	1	110
4	RM124	4	67	1	110
5	RM413	5	53	1	120
6	RM161	5	61	1	160
7	RM133	6	63	1	160
8	RM162	6	61	1	140
9	RM125	7	63	1	130
10	RM455	7	57	1	160
11	RM408	8	55	4	100, 120, 130, 140.
12	RM152	8	53	5	100, 110, 115, 120, 130.
13	RM44	8	53	2	100, 120
14	RM447	8	55	1	135
15	RM484	10	55	1	140
16	RM277	12	55	1	110

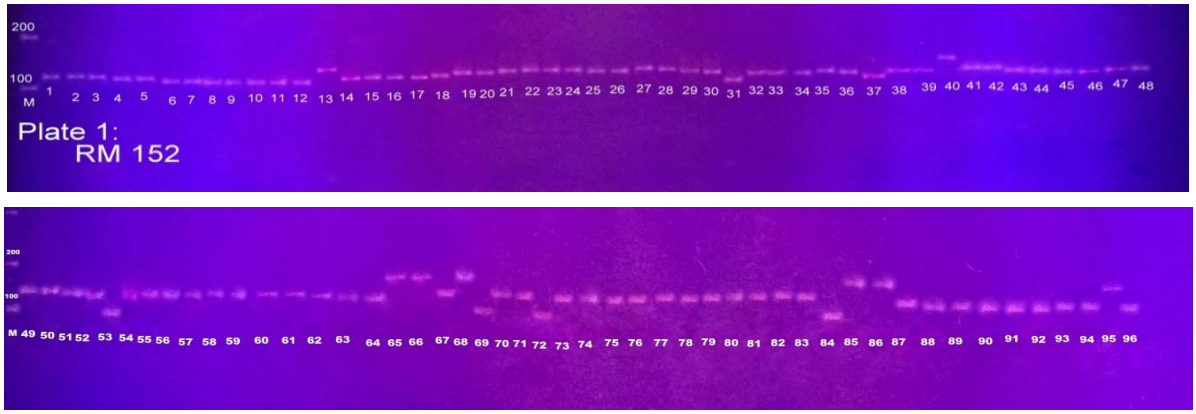

Fig 1: Images of gel obtained from UV transilluminator

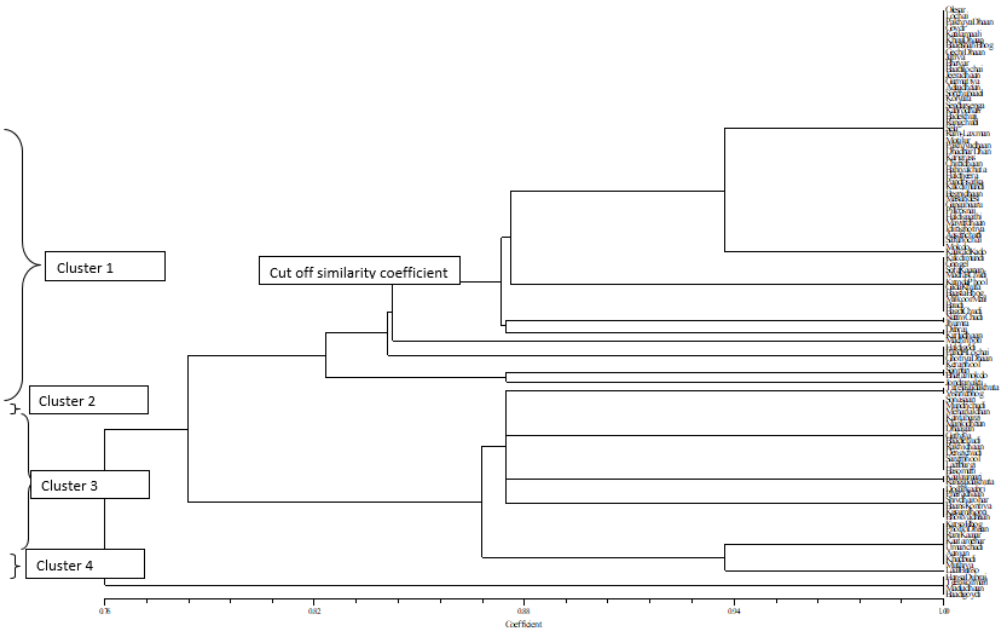

Fig 2: Molecular dendrogram depicting the distribution of genotypes

Clustering of landraces

Microsatellite markers (SSR) are also used to detect the genetic similarity of accessions of rice under study. The genetic similarity coefficient ranged from 0.76-1.00 as revealed by UPGMA cluster analysis using the 16 SSR markers. A total of four distinct groups resulted at a cut-off similarity coefficient of 0.83 among the 100 rice accessions. The dendogram shows a clear separation of the rice accessions into four groups (Fig. 2). The accessions that are derivatives of genetically similar dropped in one group. Group I had maximum accessions (61). Sonpuri, Bhatamokdo and Jondranakti formed Group II. On the other hand, Group III has 32 accessions.

In Group I Haldigodi, Pandrilochai, Ghotiyadhaan and Keraphool were found in duplicate (i.e. 100% similarity) while they exhibited 84% similarity with rest of the

accessions of group I. Machripoti is nearly 84.3% similar to rest of the accessions of group I (i.e. from Olesar to Kurludhan). This group is further divided into two subgroups. With 87.5% genetic similarity group IA included accessions from Olesar to Bagdichudi whereas group IB included accessions from Naanichudi to Kurludhaan. In Group II, Jondranakti is nearly 87.7% similar with Sonpuri and Bhatamokdo whereas Sonpuri and Bhatamokdo are duplicate (i.e. 100% similarity. Group III is further divided into two sub groups. With 86.8% genetic similarity Group III A included accessions from Turejagadakhuta to Bhokvadhan, Whereas Group III B included Kursobhog to Lalbanso. In Group IV, Hansadubraj, Teenkormail, Madiadhaan and Baadigoydi were found in duplicate (i.e. 100% similarity). Thus, SSR markers provide adequate power of resolution to discriminate between rice accessions and it could serve as a potential tool in the identification and characterization of genetically distant cultivars from various sources.

The present investigation addresses the utilization of 16 microsatellite markers to reveal genetic polymorphism and ensures unambiguous identification of 100 accessions of rice. The mean allele (1.5 alleles) across 16 loci obtained in our study was comparable with the result reported by Meti *et al.* (2013) [5]. In contrast, the mean value from our study is somewhat lower than the results observed in previous diversity studies, having 3 to 9 alleles, with an average of 4.53 alleles per locus for 30 microsatellite markers (Hossain *et al*., 2007) [2]. Similar result was observed in earlier report by Rahman *et al.* (2012) [10] who found an average of 4.18 alleles per locus. In this study, the larger range of similarity values for cultivars revealed by microsatellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs. With the aid of microsatellite makers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes from different clusters to get hybrid varieties with the highest heterosis (Sajib *et al.,* 2012) [12].

Conclusion

The present study clearly indicated that microsatellite markers are useful in assessing genetic diversity in rice genotypes. Genetically distinct genotypes were found in different clusters though they might have some morphological similarities. The cluster analysis had clearly showed some landraces having different names showed much similarity among themselves in UPGMA cluster analysis which might be due to different names assigned by farmers to landraces based on phenotypic differences though they are genetically related to each other. Genetically distinct landraces present in different clusters can be successfully used as parents in hybridization programmes.

Acknowledgement:

The authors thankfully acknowledge the Dharohar samiti, a farmer's organization of Golawand village of Kondagaon district, Chhattisgarh, India for providing the valuable seeds of local landraces of rice and also to S.G. College of Agriculture and Research Station, (Indira Gandhi Krishi Vishvavidyalaya) Jagdalpur (India) for providing all essential resources for carrying out research work.

Funding: This study was a student's (Mr. Praveen Panigarhi) post graduate research programme under the supervision of Dr. Abhinav Sao funded by Indira Gandhi Krishi Vishvavidyalaya, Raipur (India).

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. Optimizing parental selection for genetic linkage maps. Genome.1993; 36:181–186.
2. Hossain MZ, Rasul MG, Ali MS, Iftekharuddaula KM, Mian MAK. Molecualar characterization and genetic diversity in fine grain and aromatic landraces of rice using microsatellite markers. Bangladesh J Genet. Pl. Breed. 2007; 20(2):1-10.
3. Liu L, Lee GA, Jiang L, Zhang J. The earliest rice domestication in China. Antiquity 2007; 81:313.
4. Ma H, Yin Y, Guo ZF, Cheng LJ, Zhang L, Zhong M, Shao GJ. Establishment of DNA fingerprinting of Liaojing series of japonica rice. Middle-East Journal of Scientific research. 2011; 8(2):384-392.
5. Meti N, Samal KC, Bastia DN, Rout GR. Genetic diversity analysis in aromatic rice genotypes using microsatellite based simple sequence repeats (SSR) marker. African Journal of Biotechnology. 2013; 12(27):4238-4250.
6. Nadia I, Mohiuddin AKM, Sultana S, Ferdous J. Diversity analysis of indica rice accessions (*Oryza sativa* L.) using morphological and SSR markers. Annals of Biological Research.2014; 5(11):20-31.
7. Oka HI. Origin of cultivated rice. Japan Scientific Societies Press. Elsevier, Tokyo, 1988.
8. Oudhia P. Ignored Medicinal Rice in Chhattisgarh needing immediate attention. http://www.Ecoport.org. 2006.
9. Powel W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A. Comparison of RFLP, RAPD, AFLP and SSR markers for germplasm analysis. Molecular Breeding. 1996; 2(3): 225-238.
10. Rahman MM, Rasaul MG, Hossain MA, Iftekharuddaula KM, Hasegawa H. Molecular Characterization and Genetic Diversity Analysis of Rice (*Oryza sativa* L.) Using SSR Markers. Journal of Crop Improvement. 2012; 26(2):244-257.
11. Rohlf FJ. NTSY Spc: Numerical Taxonomy System, ver. 2.1. Exeter Publishing, Ltd. Setauket, NY. 1999.
12. Sajib MA, Hossain MM, Mosnaz ATMJ, Hossain H, Islam MM, Ali MS *et al*. SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.). J Bio Sci. Biotech. 2012; 1(2):107-116.
13. Siddiq EA, Saxena S, Malik SS. Plant genetic resources: food grain crops. In: Dhillon BS, Saxena S, Agrawal A, Tyagi RK. eds. Indian Society of Plant Genetic Resources, New Delhi, Narosa Publishing House, New Delhi, India. 2005, 27–57.
14. Singh A K. Probable Agricultural Biodiversity Heritage Sites in India: XV. The Bastar Region. Asian Agri-History. 2013; 17(1):3–24.
15. Vaughan DA. Choosing rice germplasm for evaluation. Euphytica 1991; 54: 147–154.
16. Zheng K, Huang N, Bennet J, Khush GS. PCR-based marker assisted selection in rice breeding. IRRI Discussion Paper Series No. 12, International Rice Research Institute, P.O. Box 933, Manila, Philippines, 1995, 16-18.