Indian J. Genet., 74(2): 238-242 (2014)
DOI: 10.5958/0975-6906.2014.00161.8

Short Communication

Genetic diversity in upland rice of Assam assessed through SSR markers

Sunayana Rathi*, R. N. S. Yadav, K. Pathak[1] and R. N. Sarma[1]

Centre for Studies in Biotechnology, Dibrugarh University, Dibrugarh; [1]Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat 785 013

(Received: July 2013; Revised: September 2013; Accepted: February 2014)

Abstract

The genetic diversity in 100 upland rice germplasm of Assam, was analyzed using 120 SSR markers, of which 98 (81.66%) showed useful polymorphism with a total of 183 alleles. The number of alleles ranged from one to three with an average value of 1.867 per locus. The markers showed an average effective number of alleles to be 1.73, average Nei's gene diversity was 0.393, and average Shannon's Information Index was 0.556. The effective number of alleles and Shannon's information index showed positive correlation with the observed number of alleles. The resolving power of the 98 SSR primers ranged from 0.72 for primer RM38 to 2.82 for primer RM592. SSR generated an average similarity of 0.460 with similarity ranging from 0.07 to 1.00. Sufficient diversity was detected among the accessions. A basic molecular database was created for upland rice genotypes, which will be useful to identify and map useful genes that are harbored by these germplasms.

Key words: Upland rice, SSR markers, polymorphism information content, gene diversity

Northeast India is considered as one of the primary centers of origin of rice plant representing a rich source of genetic diversity and reservoir of valuable gene system. Upland rice in Assam is synonymous to *ahu* rice, which is sown in the month of February and March/April, in upland situation of plains as well as in hills. Despite the availability of several high yielding varieties, the landraces of *ahu* rice are very popular among the farmers. These landraces of upland rice represent a unique and critical source of genetically variable traits for rice improvement. In a race against genetic erosion, local upland rice germplasms along with other breeding materials are collected and usually, characterized at morphological level. However, such characterization is neither exhaustive nor complete to facilitate their uses in rice breeding programme. In recent years, a vast array of molecular markers are increasingly used for efficient characterization and classification of germplasm. Among the various classes of molecular markers available, microsatellite markers (SSR markers) are the most popular one because of their abundance, high discriminatory power, co-dominance, ease and economy of use in both manual and automated systems [1]. The present investigation was undertaken to study the nature and extent of diversity in a set of 100 upland rice accession using SSR markers to assist breeders for their effective utilization in breeding programme.

One hundred germplasms, comprising local upland rice of Assam, advanced breeding lines and high yielding varieties were used from the collection of rice germplasm maintained at the Regional Agricultural Research Station, Titabar, AAU, Assam. DNA from leaf was extracted using a modified protocol [2]. Initially, 120 microsatellite primer pairs were used for analyzing the diversity, based on the published map information (http://www.gramene.org/microsat/ssr.html). Finally, 98 SSRs, showing useful polymorphism were selected for analyzing the variability in 100 genotypes. Amplification of DNA was performed in 20 µl reactions consisting of 20-50 ng

*Corresponding author's e-mail: rathisunayana@yahoo.co.in
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

Table 1. Genetic characteristics of 100 upland rice germplasm lines based on SSR data

S.No.	Locus name	na	ne	I	O_{Ho}	O_{He}	h	Rp	S.No.	Locus name	na	ne	I	O_{Ho}	O_{He}	h	Rp
1	RM323	1	1	0	1	0	0	1.06	51	RM340	2	1.676	0.593	0.98	0.02	0.495	2.04
2	RM151	1	1	0	1	0	0	1.1	52	RM439	2	1.962	0.6833	1	0	0.495	2
3	RM158	1	1	0	1	0	0.4898	1.96	53	RM103	2	1.814	0.641	1	0	0.48	2
4	RM600	2	1.993	0.691	1	0	0.4898	2	54	RM125	2	1.393	0.4559	1	0	0.48	2
5	RM129	2	1.95	0.68	1	0	0.2449	2	55	RM214	2	1.676	0.593	0.98	0.02	0.493	2.04
6	RM9	2	1.676	0.593	0.98	0.02	0.2449	2.04	56	RM11	2	1.814	0.641	1	0	0.493	2
7	RM128	1	1	0	1	0	0.4488	1.92	57	RM180	2	1.748	0.6191	1	0	0.476	2
8	RM315	2	1.51	0.521	0.99	0.01	0.4488	2	58	RM182	2	1.676	0.593	0.98	0.02	0.476	2.04
9	RM104	2	1.98	0.688	1	0	0.4278	2	59	RM320	2	1.962	0.6833	1	0	0.5	2
10	RM14	2	1.651	0.583	1	0	0.4278	2	60	RM234	1	1	0	1	0	0.5	1.98
11	RM154	2	1.956	0.682	0.99	0.01	0.4032	2.02	61	RM429	2	1.962	0.6833	1	0	0.257	2
12	RM423	2	1.873	0.659	1	0	0.4032	2	62	RM172	2	1.962	0.6833	1	0	0.257	2
13	RM174	2	1.95	0.68	1	0	0.2637	2	63	RM152	2	1.962	0.6833	1	0	0.243	2
14	RM27	2	1.814	0.641	1	0	0.2637	2	64	RM38	1	1	0	1	0	0.243	0.72
15	RM341	2	1.855	0.653	1	0	0.4968	2	65	RM25	1	1	0	1	0	0	1.94
16	RM475	2	1.98	0.688	1	0	0.4968	2	66	RM72	2	1.937	0.6769	1	0	0	2
17	RM106	2	1.956	0.682	0.99	0.01	0.4758	2.02	67	RM339	2	1.962	0.6833	1	0	0.435	2
18	RM8	2	1.7	0.602	1	0	0.4758	2	68	RM531	2	1.651	0.5833	0.98	0.02	0.435	2.04
19	RM6	2	1.748	0.619	1	0	0.4032	2	69	RM149	2	1.676	0.593	0.98	0.02	0.403	2.04
20	RM48	2	1.748	0.619	1	0	0.4032	2	70	RM264	2	1.962	0.6833	1	0	0.403	2
21	RM231	2	1.98	0.688	1	0	0.375	2	71	RM285	2	1.956	0.6819	0.99	0.01	0.495	2.02
22	RM218	2	1.736	0.615	0.97	0.03	0.375	2.06	72	RM321	1	1	0	1	0	0.497	1.96
23	RM251	2	1.814	0.641	1	0	0.4352	2	73	RM566	2	1.814	0.641	1	0	0.476	2
24	RM282	2	1.937	0.677	1	0	0.4352	2	74	RM434	2	1.956	0.6819	0.99	0.01	0.476	2.02
25	RM347	2	1.7	0.602	1	0	0.4992	2	75	RM257	2	1.962	0.6833	1	0	0.403	2
26	RM156	2	1.98	0.688	1	0	0.4992	2	76	RM278	2	1.956	0.6819	0.99	0.01	0.403	2.02
27	RM426	2	1.972	0.686	1	0	0.3432	2	77	RM205	2	1.987	0.6899	1	0	0.484	2
28	RM55	2	1.95	0.68	1	0	0.3432	2	78	RM474	2	1.956	0.6819	0.99	0.01	0.484	2.02
29	RM293	2	1.748	0.619	1	0	0.2268	2	79	RM222	2	1.962	0.6833	1	0	0.471	2
30	RM422	2	1.748	0.619	1	0	0.2268	2	80	RM311	2	1.814	0.641	1	0	0.259	2
31	RM307	1	1	0	1	0	0.2975	1.96	81	RM467	1	1	0	1	0	0.495	1.94
32	RM518	2	1.962	0.683	1	0	0.2975	2	82	RM184	1	1	0	1	0	0.497	1.32
33	RM142	1	1	0	1	0	0.4968	1.94	83	RM258	2	1.956	0.6819	0.99	0.01	0.42	2.02

34	RM252	2	1.368	0.44	1	0	0.4968	2	84	RM171	2	1.962	0.6833	1	0	0.428	2
35	RM241	2	1.962	0.683	1	0	0.4998	2	85	RM496	2	1.835	0.6474	0.98	0.02	0.442	2.04
36	RM303	2	1.962	0.683	1	0	0.4998	2	86	RM286	2	1.956	0.6819	0.99	0.01	0.435	2.02
37	RM124	2	1.962	0.683	1	0	0.5	2	87	RM167	2	1.962	0.6833	1	0	0.495	2
38	RM349	2	1.962	0.683	1	0	0.5	2	88	RM536	2	1.93	0.675	0.99	0.01	0.497	2.02
39	RM153	2	1.962	0.683	1	0	0.3942	2	89	RM209	2	1.956	0.6819	0.99	0.01	0.498	2.02
40	RM592	3	2.759	1.057	0.561	0.439	0.3942	2.82	90	RM21	2	1.962	0.6833	1	0	0.499	2
41	RM249	2	1.835	0.647	0.98	0.02	0.42	2.04	91	RM206	2	1.736	0.615	0.99	0.01	0.32	2.02
42	RM169	2	1.962	0.683	1	0	0.42	2	92	RM144	2	1.748	0.6191	1	0	0.32	2
43	RM430	2	1.845	0.651	0.99	0.01	0.4758	2.04	93	RM20A	2	1.962	0.6833	1	0	0.412	2
44	RM305	2	1.835	0.647	0.98	0.02	0.48	2.04	94	RM19	2	1.814	0.641	1	0	0.412	2
45	RM480	2	1.814	0.641	0.98	0.02	0.2571	2.04	95	RM247	2	1.393	0.4559	1	0	0.32	2
46	RM225	2	1.962	0.683	1	0	0.2571	2	96	RM277	2	1.962	0.6833	1	0	0.32	2
47	RM276	2	1.814	0.641	1	0	0.3942	2	97	RM235	2	1.962	0.6833	1	0	0.115	2
48	RM3	2	1.393	0.456	1	0	0.3942	2	98	RM12	2	1.393	0.4559	1	0	0.115	2
49	RM162	1	1	0	1	0	0.48	1.92	Mean		1.87	1.734	0.5565	0.992	0.008	0.393	1.97
50	RM345	1	1	0	1	0	0.48	1.96	St. Dev		0.37	0.348	0.2386	0.045	0.045	0.124	

na=Number of alleles; ne=Effective number of alleles; I=Shannon's information index; O_{Ho}=Observed Homozygosity; O_{He}=Observed Heterozygosity; h=Nei's gene diversity; Rp=Resolving Power

DNA, 0.25 mM dNTP, 0.2 µM each primers, and 0.5 unit of *Taq* DNA polymerase. Thermocycling was carried out in a GeneAmp PCR System 2400 and the 'touchdown' amplification conditions were: 94°C for 5 min; 2 × (94°C for 1 min, 65°C for 1 min and 72°C for 2 min); 2 × (94°C for 1 min, 62°C for 1 min and 72°C for 2 min); 4 × (94°C for 1 min, 59°C for 1 min and 72°C for 2 min); 25 × (94°C for 1 min, 55°C for 1 min and 72°C for 2 min); and a final extension of 72°C for 5 min. Amplified products were mixed with loading dye and were separated in 3% agarose gels by electrophoresis. The average number of alleles (na), effective number of alleles (ne), total number of alleles, the percentage of polymorphic loci, observed homozygosity, observed heterozygosity, Shannon's information index (I), Nei's gene diversity (h), were quantified between accessions for genetic diversity assessment using POPGENE v 1.32 [3]. The UPGMA dendrogram was constructed using the NTsyspc program ver. 20.2a [4].

Table 1 summarizes the results obtained based on the analysis of the 100 upland rice genotypes using 98 polymorphic SSR loci with a total of 183 alleles. The number of alleles ranged from one to three with an average value of 1.867 per locus. Kibria *et al.* [5] reported in aromatic rice that the number of alleles ranged from one to two per microsatellite locus. The effective number of alleles (ne) ranged from 1.0 (11 markers) to 2.759 in RM592, with an average of 1.734 for the 98 SSR loci (Table 1). This effective number of alleles is positively correlated with the observed number of alleles ($r = 0.904$, $P < 0.001$). This means that the proportion of homozygotes would decrease, or heterozygosity/polymorphism would increase, with increase in the actual number of alleles at a locus in the analyzed rice germplasm. The average value of I (Shannon diversity index) was 0.556 and ranged from 0.0 to 1.056. For those loci with $I > 1$, the number of alleles was found to be ≥ 3, and for those loci with $I < 1$, the number of alleles was found to be ≤ 3 (Table 1). Thus, I estimate showed positive correlation with the allele number ($r = 0.968$, $P < 0.001$), and therefore, I values can be used to quantify the diversity or polymorphism of SSR markers. Zhang *et al.* [6] reported average Shannon's index of 0.462 in weedy rice with microsatellite markers which is lower and is consistent with the present findings. The observed homozygosity estimated showed that 73 loci exhibited 100 % homozygosity and the average observed homozygosity was 99% (Table 1). Similarly, average heterozygosity recorded for the 98 loci and was found

to be as low as 8%. This high rate of observed homozygosity and low heterozygosity is expected because of self-fertilized nature of rice. All of the populations are genetically diverse, with Nei's gene diversity (h) ranging from 0.0 to 0.5 with an average of 0.393 (Table 1). Tabkhkar *et al.* [7] reported Nei's gene diversity for microsatellite loci ranging from 0.63 in RM314 to 0.81 in RM276 with an average of 0.72 which was higher, while Kibria *et al.* [5] obtained an average of 0.119 in aromatic rice genotypes which was smaller than the present findings. The resolving power of the 98 SSR primers ranged from 0.72 for RM38 to 2.82 for RM592 with an average of 1.972 (Table 1). The marker RM592 with highest resolving power was able to distinguish more number of genotypes, while RM38 with lowest resolving power could distinguish less number of genotypes. Sujatha *et al*. [8] reported the resolving power of five SSR primers of rice ranging

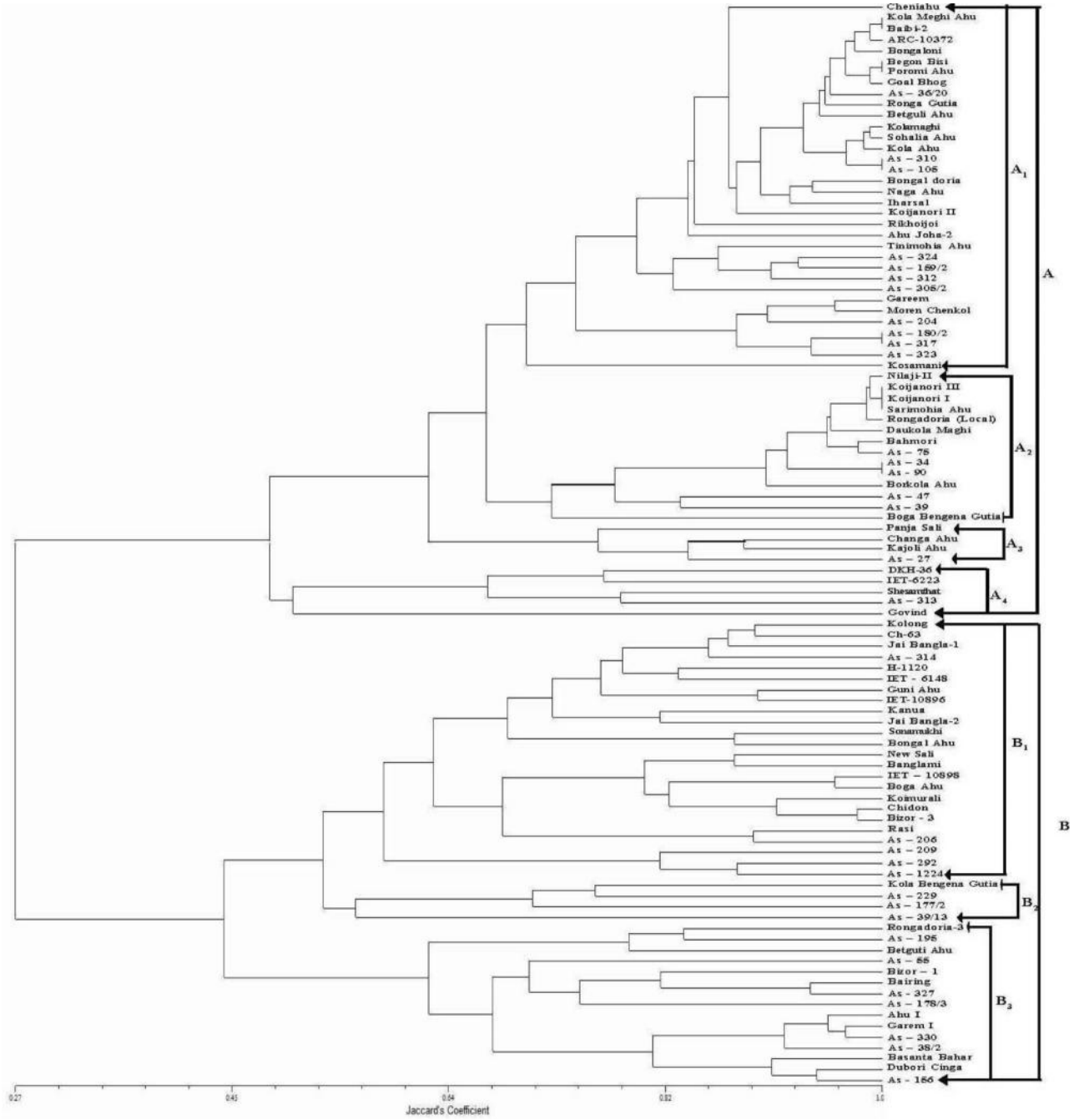

Fig. 1. UPGMA based dendrogram of 100 upland rice genotypes using Jaccard's coefficient of similarity based on 98 SSR markers

from 0.77 to 1.8 in 32 wild rice accessions. The number of alleles, effective number of alleles and Shannon's information index showed positive correlation with the resolving power. From this study, we can conclude that based on number of alleles, effective number of alleles, Shannon's diversity index and resolving power RM592 is the best SSR primer. Based on SSR marker the indices of genetic relationship on pair-wise Jaccard's coefficient of similarity among all the genotypes, using SSR data, were calculated (data not presented). SSRs generated an average similarity of 0.460 ranging from 0.071 to 1.0.

One hundred genotypes were used to make dendrogram using Unweighted Pair Group Method of Arithmetic Means (UPGMA) (Fig. 1). The dendrogram revealed two major clusters (A and B) at a similarity coefficient of 27%. The cluster A was the largest one with 57 genotypes, could be divided into four sub-clusters (A_1, A_2, A_3 and A_4) at a similarity value of 49%. The sub-cluster A_1 comprised of 34 genotypes at a similarity value of 76.6% out of which 'Cheniahu' was isolated from the rest. Cheniahu' is a dormant genotype with highest duration of dormancy [9]. The genotypes 'Kola Meghi Ahu' and 'Baibi-2', 'Begon Bisi' and 'Poromi Ahu', 'As-310' and 'As-105', 'As-180/2' and 'As-317' were 100% similar. The sub-cluster A_2 comprised of 14 genotypes at a similarity value of 68%. Among these genotypes, Rongadoria is a most popular landrace of Assam and recommended for cultivation in direct seeded upland situation. The sub-cluster A_3 comprised of 4 genotypes at a similarity value of 62%. The sub-cluster A_4 comprised of 5 genotypes at a similarity value of 50%. This cluster comprised of improved, advance breeding line, HYV and local germplasms.

Forty three genotypes were grouped together in cluster B, at a similarity value of 44% and was subdivided into three sub-clusters (B_1, B_2 and B_3). The sub cluster B_1 consists of 24 genotypes at a similarity value of 52%. This cluster also comprised of improved, advance breeding line, HYV and local germplasms. Among these genotypes, Banglami is a most popular landrace of lower Assam due to its capacity to withstand moisture stress. The sub-cluster B_2 comprised of 4 genotypes at a similarity value of 56%. The sub cluster B_3 consists of 15 genotypes at a similarity value of 62%. Cophenetic correlation (0.92) studies revealed SSR data had a very good fit. This confirms that SSR dendrogram was representative of original similarity matrix [10]. Such a correlation provides clear picture of diversity pattern. However, SSR markers were unable to produce any group specific band/allele to distinguish the upland genotypes of Assam, necessitating the use of more SSR primers for such study.

Sufficient genetic diversity was revealed between the genotypes indicating potentiality of SSR markers to distinguish closely related genotypes. Proper phenotyping is necessary to identify various useful traits so that these marker data could be utilized in a breeding programme for effective utilization of diverse germplasm of this region.

References

1. **McCouch S. R., Temnykh S., Lukashova A., Coburn J., DeClerck G., Cartinhour S., Harrington S., Thomson M., Septiningsih E., Semon M., Moncada P. and Li J.** 2001. Microsatellite markers in rice: abundance, diversity and applications. *In*: Khush, G. S., Brar, D. S. and Hardy, B. (eds), Rice Genetics IV. International Rice Research Institute, Manila, pp. 117-136.
2. **Plaschke J., Ganal M. W. and Roder M. S.** 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet., **91**: 1001-1007.
3. **Yeh F. C., Yang R. C., Boyle T. B. J.** 1999. POPGENE, version 1.32 Microsoft window-based freeware for population genetic analysis (Univ of Alberta, Canada).
4. **Rohlf F. J.** 2000. NTSYSpc: Numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Publications, New York, USA.
5. **Kibria K., Nur F., Begum S. N., Islam M. M., Paul S. K., Rahman K. S. and Azam S. M. M.** 2009. Molecular marker based genetic diversity analysis in aromatic rice genotypes using SSR and RAPD markers. Int. J. Sustain. Crop Prod., **4**: 23-34.
6. **Zhang L., Dai W., Wu C., Song X. and Qiang S.** 2012. Genetic diversity and origin of Japonica- and Indica-like rice biotypes of weedy rice in the Guangdong and Liaoning provinces of China. Genet. Resour. Crop Evol., **59**: 399-410.
7. **Tabkhkar N., Rabiei B. and Sabouri A.** 2012. Genetic diversity of rice cultivars by microsatellite markers tightly linked to cooking and eating quality. ACJS, **6**: 980-985.
8. **Sujatha K., Rai V. P., Kaladhar K., Ram T. and Sarla N.** 2004. Selected ISSR and SSR markers reveal phylogenetic relationship among wild *Oryza* species. *In:* Rice Genet. Newslett., **21**: 1-4.
9. **Rathi S., Baruah A. R., Chowdhury R. K., Sarma R. N.** 2011. QTL Analysis of Seed Dormancy in Indigenous Rice of Assam, India. Cereal Res. Commun., **39**: 137-146.
10. **Sneath P. H. A. and Sokal R. R.** 1973. Numerical Taxonomy. W.H. Freeman and Co., San Fransisco, USA.