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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY ANALYSIS OF DIFFERENT RICE CULTIVARS BY MICROSATELLITE MARKERS

Mehrzad ALLHGHOLIPOUR[1*], Ezatollah FARSHDFAR[2], Babak RABIEI[3]

1* Department of Plant Breeding, Rice Research Institute of Iran (RRII), PRasht/Iran
2 Department of Agronomy & Plant Breeding, Faculty of Agricultural Sciences, Razi University, Kermanshah, Iran
3 Department of Agronomy & Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

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A total of 52 rice SSR markers well distributed on 12 chromosomes were used to characterize and assess the genetic diversity among ninety four rice genotypes. The total number of polymorphic alleles was 361 alleles with the average of 5.86 alleles per SSR locus. The study revealed that some markers such as RM276 and RM5642 on chromosome 6 and RM14 and RM1 on chromosome 1 have more than 9 observed alleles compared to other primers like RM16, RM207, RM208 and RM317 with 3-4 alleles. The highest and lowest PIC values were observed for primers RM276 (0.892 and RM208 (0.423) respectively. Using Shannon´s diversity index, a mean genetic diversity of 1.641 was obtained from the analysis, indicating a high level of genetic variation among these cultivars. Cluster analysis using the complete linkage method based on jaccard similarity coefficient revealed that all genotypes were classified to nine clusters at genetic similarity level of 0.01-0.75, which contained 12, 16, 2, 18, 3, 6, 16, 10 and 11 varieties, respectively. Results of discriminant analysis showed that the nine cluster groups were confirmed at high levels of correct percent (96.8) and revealed true differences among these clusters. As a final result from this study, we selected eight cultivars from different cluster including Daylamani, Tarom mohali (landrace rice cultivars), RI1843046, Back cross line, RI184472, RI184421 (promising cultivars), Line 23 and IR50 (IRRI lines) as parents. All of the selected cultivars will be arranged in complete diallel design to obtain combining

Corresponding author: Mehrzad Allhgholipour,' Department of Plant Breeding, Rice Research Institute of Iran (RRII), P.O Box: 1658, Postal Code: 41996-13475/ Rasht/Iran

abilities, gene effects and heterosis for each important morphology and physico-chemical characters.

Key words: Cluster analysis, genetic diversity, rice cultivars, SSR marker

INTRODUCTION

Rice (*Oryza sativa* L.) genetic resources are widely available in the worldwide (CHARKRAVARTHI and NARAVANENI, 2006). Rice landraces are precious genetic resources, because they contain huge genetic variability which can be used to complement and broaden the gene pool of advanced genotypes (KOBAYASHI *et al*., 2006). The extent of genetic diversity in a crop population depends on recombination, mutation, selection and random genetic drift. Mutation and recombination bring new variations to a population, whereas selection and genetic drift remove some alleles, often from agronomically important lines. Exploring diversity in a landrace collection is very important for identifying new genes and further improvement of the germplasm (BRONDANI *et al*., 2006; JAYAMANI *et al*., 2007; THOMSON *et al*., 2007). The selection of parental lines plays a vital role in developing ideal combinations. Therefore, it is essential to study the relationship and genetic diversity among parental lines in rice. In fact, plant breeders often select parental lines in combinations with morphological trait and pedigree information. However, this breeding method is less effective and accurate due to environmental effect. Molecular markers have been widely used to study the genetic variation and diversity of breeding materials, which were less influenced by temporal, spatial and environmental conditions (HAMZA *et al*., 2004; SUN *et al*., 2001). Several types of molecular markers are available for the extent of genetic variation in rice. Among the so many types of these molecular markers, microsatellite markers detect a significantly higher degree of polymorphism in rice and are especially suitable to evaluate the genetic diversity among closely related rice cultivars (MIAH *et al.,* 2013). From the view point of rice breeders, it is preferable to identify and use donors of important traits from within the same of subspecies or cultivar group. For the application of marker assisted selection (MAS) within a subspecies, it is important to obtain information on the genetic diversity within a rice subspecies over different genome regions. The excellent attributes of SSR markers and the availability of over 18000 markers in rice (MCCOUCH, 2005) make it possible to obtain this information. The main objectives of this research were to assess the genetic diversity among 94 rice genotypes that were widely used in rice breeding programs and to identify the heterotic pattern and selecting the best parental lines for developing the new rice varieties with high performance.

MATERIALS AND METHODS

A total of ninety four rice genotypes comprising landraces, pure lines, breeding and foreign cultivars notably adapted to environment conditions of Giulan provinces were chosen in the present study. These cultivars were obtained from the Rice Research Institute of Iran (RRII)[1] and also from the International Rice Research Institute (IRRI)[2]. A detailed description of the materials used in this study is shown in Table 1. Fifty two SSR markers (Table 2) were selected as a subset of SSR markers previously used to assay genetic diversity of wild rice and weedy rice by specific polymerase chain reaction (PCR) conditions (SONG *et al*., 2003; SONG *et al*., 2006; CAO *et al*., 2006). Detailed information of primer sequences is available at http://www.gramene.org/microsat/ssr.txt.

Table 1 a Name, origin of country and pedigree of rice genotypes used in this study

S.N	Genotypes	Pedigree	Origin
1	Sangejo	Local cultivar	Guilan,Iran
2	RI18430-30-1-2-1-1	Saleh / Hashemi	RRII[a], Iran
3	Mohammadi	Local cultivar	Mazandran, Iran
4	Salari	Local cultivar	Guilan,Iran
5	Alikazemi	Local cultivar	Guilan,Iran
6	Hassansaraiee	Local cultivar	Guilan,Iran
7	Alamitaroom	Local cultivar	Guilan,Iran
8	Anbarbo	Local cultivar	Guilan,Iran
9	Daylamani	Local cultivar	Mazandran, Iran
10	Shahpasand	Local cultivar	Mazandran, Iran
11	Garib	Local cultivar	Guilan,Iran
12	Domsefid	Local cultivar	Guilan,Iran
13	Domzard	Local cultivar	Guilan,Iran
14	Gharib sia Rayhani	Local cultivar	Guilan,Iran
15	Tarom mohali	Local cultivar	Mazandran, Iran
16	Tarom Amiri	Local cultivar	Mazandran, Iran
17	Binam	Local cultivar	Guilan,Iran
18	Hassani	Local cultivar	Guilan,Iran
19	Hashemi	Local cultivar	Guilan,Iran
20	Domsia	Local cultivar	Guilan,Iran
21	Abjiboji	Local cultivar	Mazandran, Iran
22	RI18430-40-1-1-1-1	Saleh / Hashemi	RRII, Iran
23	Ghashenge	Local cultivar	Guilan,Iran
24	Champa Bodar	Local cultivar	Guilan,Iran
25	RI18430-46-1-2-1-1	Saleh / Hashemi	RRII, Iran
26	RI18430-52-1-2-1-2	Saleh / Hashemi	RRII, Iran
27	RI18430-20-1-2-2-1	Saleh / Hashemi	RRII, Iran
28	RI18430-56-1-2-3-1	Saleh / Hashemi	RRII, Iran
29	Ramezanali Tarom	Local cultivar	Mazandran, Iran
30	RI18431-4-2-3-1-1	Saleh / Abjiboji	RRII, Iran
31	RI18431-12-2-3-2-2	Saleh / Abjiboji	RRII, Iran
32	RI18431-21-1-3-2-2	Saleh / Abjiboji	RRII, Iran
33	Gohar	Imroved cultivar	Basmati, India
34	RI18431-29-1-2-2-1	Saleh / Abjiboji	RRII, Iran
35	Line 23	Introduction	IRRI[b], Philippines
36	Line 830	Introduction	IRRI, Philippines
37	Line 831	Introduction	IRRI, Philippines
38	Line 840	Introduction	IRRI, Philippines
39	Line 841	Introduction	IRRI, Philippines
40	Neda	Imroved cultivar	Mazandran, Iran
41	Nemat	Imroved cultivar	Mazandran, Iran
42	Dorfak	Imroved cultivar	Guilan,Iran
43	Kadous	Imroved cultivar	IRRI, Philippines
44	Saleh	Imroved cultivar	Guilan,Iran
45	Sepidrood	Imroved cultivar	Guilan,Iran
46	Khazar	Imroved cultivar	Guilan,Iran
47	Shiroodi	Imroved cultivar	Mazandran, Iran
48	RI18432-9-1-3-2-1	Saleh / Mohammadi	RRII, Iran
49	RI18432-23-1-2-2-1	Saleh / Mohammadi	RRII, Iran
50	RI18432-28-1-3-2-1	Saleh / Mohammadi	RRII, Iran

[a] Rice Research Institute of Iran
[b] International Rice Research Institute

Table 1 b Name, origin of country and pedigree of rice genotypes used in this study

S.N	Genotypes	Pedigree	Origin
51	RI18432-67-2-1-3-2	Saleh / Mohammadi	RRII[a], Iran
52	RI18432-30-2-2-3-1	Saleh / Mohammadi	RRII, Iran
53	Taychoung	Introduction	China
54	Fojiminori	Introduction	China
55	Usen	Introduction	Egypt
56	IR36	Introduction	IRRI[b], Philippines
57	IR58	Introduction	IRRI, Philippines
58	IR28	Introduction	IRRI, Philippines
59	IR30	Introduction	IRRI, Philippines
60	IR50	Introduction	IRRI, Philippines
61	IR60	Introduction	IRRI, Philippines
62	Zinet	Introduction	Egypt
63	RI18434-7-1-2-3-1	Saleh / Hassani	RRII, Iran
64	RI18434-10-1-2-2-2	Saleh / Hassani	RRII, Iran
65	RI18435-7-1-3-2-1	Saleh / Alamitaroom	RRII, Iran
66	RI18435-10-2-2-3-1	Saleh / Alamitaroom	RRII, Iran
67	Line 44	PR27137-CR153	IRRI, Philippines
68	Line 45	PSBRC44(IR59468-B-B-3-2)	IRRI, Philippines
69	18431 / Abjiboji	Saleh / Abjiboji // Abjiboji	RRII, Iran
70	RI18436-8-1-2-2-1	Saleh / Hassansaraiee	RRII, Iran
71	RI18436-15-1-2-3-2	Saleh / Hassansaraiee	RRII, Iran
72	RI18436-11-1-2-2-2	Saleh / Hassansaraiee	RRII, Iran
73	RI18437-12-2-1-2-1	Saleh / Salari	RRII, Iran
74	RI18437-42-1-3-3-2	Saleh / Salari	RRII, Iran
75	RI18437-6-1-2-2-1	Saleh / Salari	RRII, Iran
76	RI18437-10-1-3-2-1	Saleh / Salari	RRII, Iran
77	RI18439-1-2-3-2-1	Saleh / Gharib	RRII, Iran
78	RI18439-9-1-2-2-1	Saleh / Gharib	RRII, Iran
79	RI18439-20-1-2-3-2	Saleh / Gharib	RRII, Iran
80	RI18439-16-1-2-1-1	Saleh / Gharib	RRII, Iran
81	RI18440-1-1-2-1-2	Sepidrood / Abjiboji	RRII, Iran
82	IRFAON2010-216	Introduction	IRRI, Philippines
83	RI18440-2-1-2-3-1	Sepidrood / Abjiboji	RRII, Iran
84	RI18441-3-1-2-3-2	Sepidrood / Hashemi	RRII, Iran
85	IIRON2010-112	Introduction	IRRI, Philippines
86	RI18442-1-1-1-2-1	Sepidrood / Hassansaraiee	RRII, Iran
87	RI18442-10-2-1-2-3	Sepidrood / Hassansaraiee	RRII, Iran
88	RI18442-22-2-1-3-1	Sepidrood / Hassansaraiee	RRII, Iran
89	IIRON2010-410	Introduction	IRRI, Philippines
90	RI18443-3-1-2-3-1	Sepidrood / Mohammadi	RRII, Iran
91	RI18443-10-2-2-3-1	Sepidrood / Mohammadi	RRII, Iran
92	RI18444-6-2-1-3-2	Sepidrood / Alamitaroom	RRII, Iran
93	RI18445-24-1-1-2-1	Sepidrood / Hassani	RRII, Iran
94	RI18447-2-1-2-1-1	Sepidrood / Gharib	RRII, Iran

[a] Rice Research Institute of Iran

[b] International Rice Research Institute

Table 2. SSR marker used in this study, observed and effective number of alleles and polymorphic information content for all primers

S.N	SSR Marker	Chromosome Number	Na[a]	Ne[b]	I[c]	PIC[d]
1	RM 1	1	11	7.124	2.105	0.830
2	RM 3	6	8	6.346	1.941	0.842
3	RM 7	3	6	4.128	1.527	0.758
4	RM 11	7	8	4.588	1.708	0.782
5	RM 14	1	11	6.598	2.087	0.857
6	RM 16	3	4	3.830	1.364	0.739
7	RM 17	12	8	5.691	1.888	0.824
8	RM 55	3	4	3.278	1.274	0.695
9	RM 103	6	7	4.697	1.644	0.787
10	RM 104	1	6	4.708	1.664	0.780
11	RM 119	4	6	4.443	1.618	0.775
12	RM 125	7	5	2.779	1.212	0.640
13	RM 128	1	6	4.945	1.665	0.798
14	RM 152	8	6	3.495	1.408	0.714
15	RM 164	5	6	5.191	1.718	0.807
16	RM 168	3	5	2.604	1.132	0.616
17	RM 171	10	6	5.032	1.662	0.801
18	RM 190	6	9	5.070	1.765	0.802
19	RM 202	11	7	5.364	1.772	0.819
20	RM 204	6	6	4.186	1.556	0.761
21	RM 205	9	6	3.356	1.385	0.702
22	RM 207	2	4	2.494	1.011	0.599
23	RM 208	2	3	1.689	0.708	0.423
24	RM 209	11	7	3.221	1.440	0.690
25	RM 215	9	7	5.436	1.803	0.816
26	RM 219	9	6	4.455	1.611	0.776
27	RM 223	8	7	4.663	1.674	0.786
28	RM 239	10	4	2.689	1.100	0.638
29	RM 240	2	9	7.324	2.077	0.864
30	RM 250	2	6	4.246	1.598	0.765
31	RM 252	4	7	3.421	1.508	0.708
32	RM 253	6	8	4.347	1.667	0.770
33	RM 255	4	5	3.144	1.293	0.682
34	RM 257	9	6	3.725	1.453	0.732
35	RM 258	10	8	6.281	1.951	0.841
36	RM 262	2	7	4.107	1.619	0.765
37	RM 276	6	13	9.154	2.353	0.892
38	RM 283	1	7	5.409	1.788	0.815
39	RM 309	12	9	6.914	2.034	0.855
40	RM 316	9	8	5.335	1.821	0.813
41	RM 317	4	4	3.412	1.308	0.716
42	RM 340	6	5	3.060	1.292	0.673
43	RM 445	7	9	4.998	1.798	0.800
44	RM 475	2	7	3.712	1.593	0.731
45	RM 484	10	8	5.022	1.786	0.801
46	RM 491	12	6	4.275	1.595	0.748
47	RM 549	6	6	4.370	1.563	0.771
48	RM 551	4	8	6.281	1.920	0.846
49	RM 592	5	10	7.149	2.116	0.860
50	RM 5371	6	7	5.828	1.832	0.828
51	RM 5642	5	12	8.604	2.260	0.885
52	W 2 R	6	7	4.795	1.668	0.792
Mean	**-**	**-**	**7**	**4.750**	**1.641**	**0.766**

[a] Observed number of alleles, [b] effective number of alleles, [c] shannon diversity index,
[d] polymorphism information content

Rice genomic DNA was extracted from 21-day-old seedling leaves collected from at least 2-3 seedlings in each cultivar, according to the modified CTAB method (MURRAY and THOMPSON, 1980). The polymerase chain reaction (PCR) was performed in a total volume of 10µl per reaction containing 2 µl of template DNA(5ng / µl), 0.5µl of forward and reverse primers (5 µM stock concentration), 1.2 µl dNTPs (1mM), 0.14µl Taq polymerase (5 U/µl), 0.48µl ofMgCl2 (50 mM) and 1µl 10×PCR buffer. The PCR amplification was carried out on a thermal cycler at an initial temperature of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s (primer annealing occurred with most of the primers while some were adjusted), 72°C for 2 min and at least 72°C for 5 min and then stored at 4°C. The PCR products were separated by electrophoresis in 10% polyacrylamide gel (PAGE) with 0.8% cross-linker (ratio of bis-acrylamide to acrylamide) in 0.5 × tris-borate EDTA (TBE) buffer. The resolved PCR bands were detected by staining with ethidium bromide (EtBr). The genotypes were manually scored using the binary coding system, '1' for presence of band and '0' for absence of band. Genetic similarities were estimated from the matrix of binary data using jaccard similarity coefficient. To infer genetic relationships and phylogeny, the similarity coefficients were used for cluster analysis of the rice cultivars utilizing the complete linkage method. The analysis and dendrogram construction were performed using the NTSYS-pc ver 2.02 (ROHLF, 1999). Fisher´s linear discriminant analysis (FISHER, 1936) using PROC STEPDISC in SAS program (SAS INSTITUTE, 2008) was employed to determine the predicted group membership and percentage of original cases correctly classified for cluster analysis outputs. Polymorphism information content (PIC) was calculated for each marker, according to the method of ANDERSON *et al*. (1993): $PIC_i = 1 - \sum P_{ij}^2$ Where, P_{ij} is the frequency of the *j*th allele for *i*th marker, and is summed over *n* alleles. For further confirmation, Shannon diversity index (HUTCHENSON, 1970), effective number of alleles (Ne) and observed number of alleles (Na) (KIMURA and CROW, 1964) were also calculated using the POPGENE software ver. 1.32 (YEH *et al*., 1997).

RESULTS

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. In the present investigation, 52 rice microsatellites RM or SSR markers well distributed on 12 chromosomes were used to characterize and assess the genetic diversity among 94 pure rice cultivars from different regions and origins. All 52 rice microsatellites showed polymorphism between 94 rice cultivars. All studied varieties were pure and showed one band for all studied markers. A total of 361 bands were scored and of which no bands were found to be monomorphic. The Shannon diversity index (I), effective number of alleles (Ne) and observed number of alleles (Na) for each SSR locus and PIC values are shown in Table 2. The study revealed that some markers such as RM276 and RM5642 on chromosome 6 and RM14 and RM1 on chromosome 1 have more than 9 observed alleles compared to other primers like RM16, RM207, RM208 and RM317 belonging to different chromosomes with 3-4 alleles. Many studies have reported significantly greater allelic diversity of microsatellite markers than other molecular markers (MCCOUCH *et al*., 2001). The PIC values, a reflection of allele diversity and frequency among the cultivars, also varied from one locus to another. The PIC values derived from allelic diversity and frequency among the genotypes were not uniform for all the SSR loci tested. Lower PIC value may be the result of closely related genotypes and higher PIC values might be the result of diverse genotypes. The PIC value for the SSR loci ranged from 0.423 to 0.892 with

an average of 0.766 (Table 2). The genetic diversity of each SSR locus appeared to be associated with the number of alleles detected per locus. The highest and lowest PIC values were observed for primers RM276 (0.892) on chromosome 6 and RM208 (0.423) on chromosome 2 respectively. Some SSR markers such as RM5642 (0.885), RM240 (0.864) and RM592 (0.860) had high PIC values and were located in subsequent ranking. Using Shannon´s diversity index, an overall genetic diversity of 1.641 was obtained from the analysis, indicating a high level of genetic variation among these cultivars. The lowest diversity was for RM208 and highest value was for RM276 as same as PIC values. The observed number of alleles (Na) in the 94 rice cultivar varied from 3 to 13, with an average of seven. The effective number of alleles (Ne) ranged from 1.689 to 9.154, with an average of 4.750 (Table 2). The high PIC values suggested that SSR markers were polymorphic markers and suitable to detect the genetic diversity of these rice cultivars at the DNA level. The Shannon index was relatively high for each SSR markers, indicating that a relatively great genetic diversity exists in these rice cultivars. Cluster analysis was performed using the complete linkage method to group the studied varieties based on jaccard similarity coefficient. Nine clusters were formed at genetic similarity level of 0.01-0.75 (Figure 1), which contained 12, 16, 2, 18, 3, 6, 16, 10 and 11 cultivars, respectively. Each cluster distinguishes the genotypes clearly from the others. Cophenetic correlation coefficient between similarity matrix from the jaccard coefficient and output matrix from the dendrogram of cluster analysis was 0.94 (the highest value rather than other similarity coefficients) indicating that the used similarity coefficient and cluster analysis method were suitable to use the information derived from SSR markers. All of the Iranian local rice varieties are located in first two clusters excluding *Mohammadi* and *Champabodar* that they are grouped in cluster six with some pure lines. This grouping result is in agreement with pedigree information. The Iranian local rice cultivars have same genetic background and for this reason, all of them are engaged near each other in the same cluster. Seven pure lines which they have already been procured from crossing between *saleh* and two local cultivars (*Hashemi* and *Abjiboji*) were located in first two clusters. In fact, these new pure lines have some morphological and phisyco-chemical characteristics as same as local rice cultivars. This is a noticeable point in rice breeding programs, because they are like traditional cultivars and for this reason they were accepted by rice farmers for cultivation. A number of pure lines including RI184347 and RI1843410 (*Saleh* × *Hassani*), RI184357 and RI1843510 (*Saleh* × *Ahlamitaroom*), plus *Taychoung*, *Fujiminori* and line No.44 were located in cluster four. A backcross line which it is developed in hybridization program in RRII was also placed in this cluster. This new line was obtained from *Saleh* × *Abjiboji* combination that *Abjiboji,* as a local cultivar was recurrent parent in this cross. In contrast, some pure lines and improved rice cultivars are placed in clusters five (3 cultivars) and six (6 cultivars), respectively. All of the pure lines in cluster five and eight were obtained from *Sepidrood* and *Saleh* as improved varieties in RRII, Iran. Interestingly, all of the improved and pre-released rice cultivars in RRII are located in cluster seven excluding *khazar* and RI1843223 (*Saleh* × *Mohammadi*) that these two cultivars are grouped in cluster three. Cluster eight had all of the breeding lines which were developed in IRRI, Philippines. Finally, three introduction rice varieties and eight pure lines which were obtained from *sepidrood* and local rice cultivars are placed in cluster nine. Different clustering pattern have also been reported by different methods of diversity analysis in some previous studies (SEETHARAM *et al*., 2009; ZHANG, 2010).

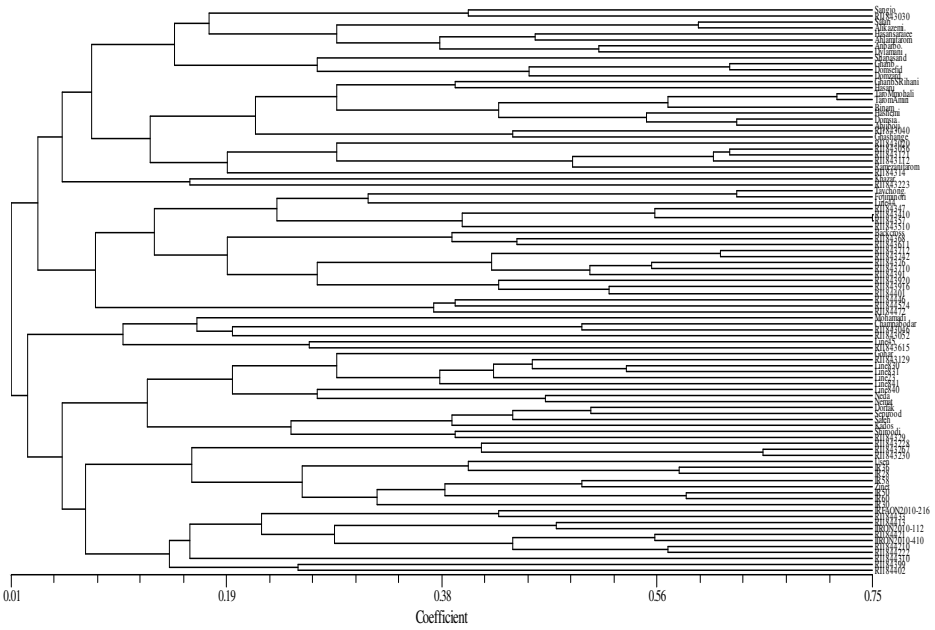

Figure 1 Dendrogram of cluster analysis from the jaccard similarity coefficient and complete linkage (Furthest neighbor) method to group 94 rice genotypes based on 52 SSR markers data

To determine of the real and correcting groups that were obtained from cluster analysis using complete linkage method and jaccard similarity coefficient, the step-wise discriminant analysis was employed. This multivariate technique developed by Fisher (FISHER, 1936) provides an effectiveness and powerful method for this purpose. Discriminant analysis result is presented table 3. Results showed that the nine cluster groups were confirmed at high levels of correct percent (96.8) and revealed true differences among these clusters. Only the probability of incorrect grouping percent was 3.2 percent. In six groups (including 1, 3, 4, 5, 8 and 9) all genotypes were correctly located inside each cluster, while in three clusters (2, 6 and 7), the probability of correct classification was 93.7, 83.3 and 93.8 percent respectively. In fact, three cultivars including *RI184314*, *line 45* and *RI184329* are incorrectly located in cluster 2, 6 and 7 respectively, while these three genotypes are correctly related to clusters 3, 9 and 8 respectively.

Table 3 Discriminant analysis of 9 clusters of 94 rice genotypes based on SSR markers

		Predicted group membership									
	Cluster	1	2	3	4	5	6	7	8	9	Total
Count	1	12	0	0	0	0	0	0	0	0	12
	2	0	15	1	0	0	0	0	0	0	16
	3	0	0	2	0	0	0	0	0	0	2
	4	0	0	0	18	0	0	0	0	0	18
	5	0	0	0	0	3	0	0	0	0	3
	6	0	0	0	0	5	0	0	0	1	6
	7	0	0	0	0	0	0	15	1	0	16
	8	0	0	0	0	0	0	0	10	0	10
	9	0	0	0	0	0	0	0	0	11	11
%	1	100	0	0	0	0	0	0	0	0	100
	2	0	93.7	6.3	0	0	0	0	0	0	100
	3	0	0	100	0	0	0	0	0	0	100
	4	0	0	0	100	0	0	0	0	0	100
	5	0	0	0	0	100	0	0	0	0	100
	6	0	0	0	0	0	83.3	0	0	16.7	100
	7	0	0	0	0	0	0	93.8	6.2	0	100
	8	0	0	0	0	0	0	0	100	0	100
	9	0	0	0	0	0	0	0	0	100	100

96.8% of original grouped cases correctly classified

DISCUSSION

The current results indicated that SSR markers are of an indispensable complementation to pedigree analysis in identification of parental groups. In general, the pedigree analysis is considered to have no effect on selection and mutation. Therefore, pedigree analysis can't reveal the relationship between progeny and their parents exactly. On the contrary, SSR markers can detect genetic variation at DNA level. Furthermore, the lines without any clear pedigree record can also be classified into their corresponding parental groups by SSR markers. In other words, combination of pedigree analysis and SSR markers will be helpful in more reliable grouping. A number of studies reported that DNA markers are the most promising technique used to diversity analysis and to differentiate among genotypes at species and subspecies level (O'NEILL *et al*., 2003). Since molecular studies represent the actual genotypic constituents and are independent of environment, so we can consider it as the most powerful method of diversity analysis. Considering this view, we can suggest morphological genetic diversity as second choice of diversity analysis. So the method which provide accurate assessment of genetic diversity and efficiently group the genotypes will be utilized to select the best parents in future breeding programs.

BEYENE *et al.* (2005) also suggested morphological traits as relatively less reliable and efficient for precise discrimination and analysis of their genetic relationships then molecular diversity. Despite this, morphological traits are important for its fast, simple and as a general approach for assessing genetic diversity. It was found that ranking using physiological genetic

distances showed insignificant rank correlation with both the ranking of SSR marker based distances and the ranking of morphological genetic distances. In breeding program, generally parents are selected based on the genetic divergence for obtaining transgressive segregants and superior genotypes. Selection of parental lines for hybridization can be done by inclusion of distant parents (ZHI-ZHOU *et al*., 2012). Breeding program perform better if parents are selected based on specific objectives considering positive common criterion as additional benefit. Moreover, selection of parents from each cluster and crossing them in a series of diallel cross were proved to be highly fruitful (RAHMAN *et al*., 2011).

As a final result from this study, we selected eight cultivars including *Daylamani, Tarom mohali* (landrace rice cultivars*), RI1843046, Back cross* line*, RI184472, RI184421* (promising cultivars)*, Line 23 and IR50* (IRRI lines) as parents among 94 rice genotypes. Each cultivar as a parent picks up from different cluster excluding cluster three with two cultivars. All of the selected cultivars should be arranged in complete diallel design to obtain combining abilities, genetic effects and heterosis for each important morphology and physico-chemical characters.

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REFERENCES

ANDERSON, J.A., G.A. CHURCHILL, J.E. AUTRIQUE, S.D. TANNKSLEY, M.E. SORRELLS (1993): Optimizing parental selection for genetic linkage map. Genome. *36*: 181-186.

BEYENE, Y., A.M. BOTHA, A.A. MYBURG (2005): A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland maize. African J. Bio. *4*: 586-595.

BRONDANI, C., T.C.O. BORBA, P.H.N. RANGEL, R.P.V. BRONDANI (2006): Determination of genetic variability of traditional varieties of Brazilian rice using microsatellite markers. Genet. Mole. Biol. *29*: 676-684.

CAO, Q., B.R. LU, H. XIA, J. RONG, F. SALA, A. SPADA, F. GRASSI (2006): Genetic diversity and origin of weedy rice (*Oryza sativa* f. spontanea) populations found in north-eastern china revealed by simple sequence repeat (SSR) markers. Ann. Botany. *98*: 1241- 1252.

CHAKRAVARTHI, B.K. and R. NARAVANENI (2006): SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). African J. Biotech*. 5*: 684-688.

FISHER, R.A. (1936): The use of multiple measurements in taxonomic problems. Ann. Eugenics. *76*: 619-922.

HAMZA, S., W.B. HAMZA, A. REBAI, M. HARRABI (2004): SSR-based genetic diversity assessment among tunisian winter barley and relationship with morphological traits. Euphytica**.** *135*: 107-118.

HUTCHENSON, K. (1970): A test for comparing diversities based on the shannon formula. J. Theo. Biolog. *29*: 151-154.

JAYAMANI, P., S. NEGRAO, M. MARTINS, B. MACAS, M.M. OLIVERIA (2007): Genetic relatedness of portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci. *47*: 879-886.

KIMURA, M. and J.F. CROW (1964): The number of alleles that can be maintained in a finite population. Genetics. *49*: 725-738.

KOBAYASHI, A., E. KAWORU, F. SHUICHII, T. NAGAMINE (2006): Microsatellite markers revealed the genetic diversity of an old japanese rice landrace 'Echizen'. Genet. Res. and Crop Evolution. *53*: 499-506.

MCCOUCH, S. R. (2005): Diversifying selection in plant breeding. PLOS Biology. *2*: 1507-1512.

MCCOUCH, S. R., S. TEMNYKH, A. LUKASHOVA, J. COBURN, G. DECLERCK, S. CAETINHOUR, S. HARRINGTON, M. THOMSON, E. SEPTININGSI, M. SEMON, P. MONCADA, L. JIMING (2001): Microsatellite markers in rice: Abundance, diversity and applications. In: Rice Genetics IV. IRRI. Manila, Philippines. 117-135.

MIAH, G., M.Y. RAFII, M.R. ISMAIL, A.B. PUTEH, H.A. RAHIM, K.N. ISMAIL, M.A. LATIF (2013): A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. Int. J. Mol. Sci. *14*: 22499-22528.

MURRAY, M. G. and W.F. THOMPSON (1980): Rapid iIsolation of high molecular weight plant. Nucleic Acids Research. *8*: 4321-4325.

O'NEILL, R.R., J. SNOWDON, W. KOHLER (2003): Population genetics aspects of biodiversity. Progress in Botany. *64*:115-137.

RAHMAN, M.M., A. HUSSAIN, M.A. SYED, A. ANSARI, M.A.A. MAHMUD (2011): Comparison among clustering in multivariate analysis of rice using morphological traits, physiological traits and simple sequence repeat markers. American-Eurasian J. Agric. and Environ. Sci. *11* (6): 876-882.

ROHLF, F.J. (1999): NTSYSpc: Numerical taxonomy system, ver. 2.1. Exeter Publishing, Ltd. Setauket, NY.

SAS INSTITUTE, Inc. (2008): SAS 9.2 Copyright 2002–2008. SAS Institute Inc., Cary, North Carolina, USA.

SEETHARAM, K., S. THITUMENI, K. PARAMASIVAM (2009): Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters. African J. Biotech. *18*: 2050-2059.

SONG, Z. P., W. ZHU, J. RONG, X. XU, J.K. CHEN, B.R. LU (2006): Evidences of introgression from cultivated rice to *Oryza rufipogon* (Poaceae) populations based on SSR fingerprinting: Implications for wild rice differentiation and conservation. Evolutionary Ecology. *20*: 501-522.

SONG, Z. P., X. XU, B. WANG, J.K. CHEN, B.R. LU (2003): Genetic diversity in the northern most rice (*Oryza rufipogon* Griff.) populations estimated by SSR markers. Theo. Appl. Genet. *107*: 1492-1499.

SUN, C. Q., X.K. WANG, Z.C. LI, A. YOSHIMURA, N. IWATA (2001): Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theo. Appl. Genet. *102*: 157-162.

THOMSON, M. J., E.M. SEPTININGSHIN, F. SUWARDJO, T.J. SANTOSO, T.S. SILITONGA, S.R. MCCOUCH (2007): Genetic diversity analysis of traditional and improved indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. Theo. Appl. Genet. *114*: 559-568.

YEH, F.C., R.C. YANG, T. BOYLE, Z.H. YE, J.X. MAO (1997): POPGENE: the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada. [Available at http://www.ualberta.ca/~fyeh/]

ZHANG, C. (2010): Cluster analysis on japonica rice (*Oryza sativa* L.) with good eating quality based on SSR markers and phenotypic traits. Rice Sci. *17*: 111-121.

ZHI-ZHOU, H., X. FANG-MING, C. LI-YUN, M.A. DELA-PAZ (2012): Genetic diversity of tropical hybrid rice germplasm measured by molecular markers. Rice Sci. *19* (3): 193−201.

MOLEKULARNA KARAKTERIZACIJA I ANALIZA GENETIČKE DIVERGENTNOSTI RAZLIČITIH KULTIVARA PIRINČA KORIŠĆENJEM MIKROSATELITSKIH MARKERA

Mehrzad ALLHGHOLIPOUR[1*], Ezatollah FARSHDFAR[2], Babak RABIEI[3]

[1]* Odelenje za oplemenjivanje biljaka, Institut za pirinač Irana (RRII) Rasht/Iran
[2] Odelenje za poljoprivredu i oplemenjivanje biljaka, Fakultet za poljoprivredne nauke, Razi Univerzitet, Kermanshah, Iran
[3] Odelenje za poljoprivredu i oplemenjivanje biljaka, Facultet poljoprivrednih nauka ,Univerzitet Guilan, Rasht, Iran.

Izvod

Korišćena su ukupno 52 SSR markera pirinča dobro rasporešena na 12 hromozoma u karakterizaciji genetičke divergentnosti 94 genotipa pirinča. Utvršena su 361 polimorfna alela sa prosečno 5.86 alela/ SSR. Markeri RM276 i RM5642 na hromozomu 6 i markeri RM14 i RM1 na hrmozomu 1 imaju više od 9 alela u poređenju sa ostalim prajmerima kao što su RM16, RM207, RM208 i RM317 sa 3 – 4 alela. Najviše i najniže PIC su utvrđene vrednosti su dobijene za prajmere RM276 (0.892 i RM208 (0.423). Analiza grupisanja (*cluster* analiza) korišćenjem potpunog metoda ukopčanosti zasnovanoj *na jaccard* koeficijentu sličnosti je potvrdila das u svi genotipovi klasifikovani u 9 klastera pri nivou genetičke sličnosti od 0.o1 – 0.75, sa 12, 16, 2, 18, 3, 6, 16, 10 i 11 genotipova. Rezultati diskriminantne analize pokazuju das u devet klaster grupa potvrđene na visokom nivou korigovanog koeficijenta (96.8) što pokazuje stvarnu razliku između tih klastera (grupa). Kao finalni rezltat odabrano je osam kultivara iz različitih klastera (grupa). Svi odabrani kultivari će biti uključeni u potpun dialelni set u cilju utvrđivanja kombinacione sposobnosti, efekta gena i heterozisa za svaku od značajnih morfoloških i fizičko – hemijskih osobina.

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