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Genetic diversity of rice cultivars by microsatellite markers tightly linked to cooking and eating quality

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Abstract

Improving cooking and eating quality of rice is one of the important objectives of many breeding programs. The study of genetic diversity in specific regions of rice genome using molecular markers is an important index that can be used for the application of marker assisted selection (MAS) in rice breeding programs. In this study, 48 rice genotypes were grouped using 7 microsatellite (SSR) markers tightly linked to major QTLs controlling three major components of rice cooking and eating quality (i.e. amylose content, gelatinization temperature and gel consistency). The number of polymorphic alleles produced by each microsatellite marker ranged from 3 alleles at RM314 locus to 10 alleles at RM276 locus. The total number of polymorphic alleles was 41 alleles with the average of 5.86 alleles per SSR locus. Effective number of alleles varied from 2.68 to 5.25 alleles at RM314 and RM276, respectively, with an average of 3.74 alleles per locus. The average heterozygosity based on Nei's gene diversity was 0.72 indicating high genetic variation among the studied varieties. Cluster analysis with UPGMA method based on simple matching (SM) similarity coefficient divided the genotypes into four groups and separated the landrace cultivars with good cooking and eating quality (based on Iranian taste) from others. Cophenetic correlation coefficient between similarity matrix and cophentic matrix was 0.93 indicating that the used similarity coefficient and cluster analysis method were suitable to use the information derived from markers to group rice genotypes. Results of this research indicated that microsatellite markers linked to genes or QTLs controlling grain cooking and eating properties are suitable tools for marker assisted selection (MAS) to identify rice grain quality.

Keywords: Cluster analysis; grain quality; genetic diversity; marker assisted selection; rice.
Abbreviations: AC- amylose content; GC- gel consistency; GT- gelatinization temperature; GBSS- granule-bound starch synthase; MAS- marker assisted selection; QTL- quantitative trait loci; SSR- simple sequence repeats; SSIIa- soluble starch synthase IIa; UPGMA- unweighted pair group method with arithmetic averages.

Introduction

Rice (*Oryza sativa* L.) is a staple food crop in the world and accounts for 21, 14 and 21% of global energy, protein and fat supply, respectively (Kennedy and Burlingam, 2003). Therefore, improving the grain quality is very important for rice breeders. Starch is the major component of polished rice (~95% of dry weight). Three physicochemical characteristics of starch i.e. amylose content (AC) (Webb, 1980; Juliano, 1985), gel consistency (GC) (Cagampang et al*.,* 1973) and gelatinization temperature (GT) (Little et al*.,* 1985) determine eating, cooking and processing quality of rice. Rice starch consists of two forms of glucose polymers namely amylose and amylopectin. Amylose is considered the most important predictor of sensory quality in rice (Fitzgerald et al., 2008). Cooked rice with low AC is sticky and soft, as AC increases, rice becomes firmer (Kennedy and Burlingam, 2003). It has been well documented through molecular mapping studies that there are many QTLs with major effects on chromosome 6 which is responsible for cooking and eating quality variations (Table 1). The *Waxy* gene (*wx*) on the short arm of chromosome 6, encodes a granule-bound starch synthase (GBSS) that plays a key role in the amylose synthesis of rice (Tan et al*.,* 1999; Fan et al*.,* 2005). The GT of rice flour is controlled by the alkali degeneration locus (*alk*) encoding a soluble starch synthase IIa (SSIIa) isoform (Fan et al., 2005; Bao et al., 2006). Shu et al. (2006) demonstrated that there are two separate loci that control GT characteristics, the first major QTL identified earlier by Tan et al. (1999) is coincident with the gene *alk2(t)* with genetic distance of 3.93 cM from the *wx* gene and the second QTL identified at *alk* locus region linked with SSR marker RM276. QTLs for GC are associated with *wx* locus (Tan et al., 1999; Fan et al., 2005; Fitzgerald et al., 2008). Improving cooking and eating quality traits is complex because of their polygenic inheritance and environmental interactions (Lapitan et al., 2007; Ordonez et al*.,* 2010). In addition to genetic complexity, rice cooking and eating quality is also largely affected by environmental factors, cultural practices, and postharvest practices such as air temperature during ripening, the amount of fertilizer, irrigation management, grain-drying after harvest, and cooking methods (Lestari et al., 2009). The genetic complexity of cooking and eating quality, as well as the difficulty in accurate evaluation of cooking and eating quality at early breeding generations, has constrained the development of rice varieties with high cooking and eating quality (Lestari et al., 2009). PCR-based markers especially SSR markers are efficient, time consuming and cost-effective approaches for quality evaluation of rice. Microsatellites have been used for comparative analysis of specific regions of rice

Table 1. Some QTLs detected in rice chromosome 6 which are responsible for cooking and eating quality variations (Gramene database[a]).

No.	SSR markers	Trait name	QTL	Parents	Previous studies
1	RM170	GC	*gc6a*	Zhenshan 97/H94	Fan et al. (2005)
		AC	-	IR64/IRGC105491	Septiningsih et al. (2003)
2	RM190	AC	*ac6a*	Zhenshan 97/H94	Fan et al. (2005)
		GC	*gc6b*	Zhenshan 97/H94	Fan et al. (2005)
		GT	*asv6a*	Zhenshan 97/H94	Fan et al. (2005)
		AC	*amy6*	Caiapo/IRGC103544	Aluko et al. (2004)
		GT	*alk6-1*	Caiapo/IRGC103544	Aluko et al. (2004)
3	RM204	GT	-	-	He et al. (1999)
4	RM253	AC	*ac6b*	Zhenshan 97/H94	Fan et al. (2005)
		GC	*gc6c*	Zhenshan 97/H94	Fan et al. (2005)
		GT	*asv6b*	Zhenshan 97/H94	Fan et al. (2005)
		AC	-	IR64/IRGC105491	Septiningsih et al. (2003)
		GC	-	IR64/IRGC105491	Septiningsih et al. (2003)
		GT	-	Huangyu B/II32 B	Shu et al. (2006)
		GT	*alk6-2*	Caiapo/IRGC103544	Aluko et al. (2004)
5	RM276	GT	*asv6c*	Zhenshan 97/H94	Fan et al. (2005)
		GT	-	Huangyu B/II32 B	Shu et al. (2006)
6	RM314	GC	*gc6c*	Zhenshan 97/H94	Fan et al. (2005)
		GT	*alk2(t)*	Huangyu B/R3027	Shu et al. (2006)
		AC	-	IR64/IRGC105491	Septiningsih et al. (2003)
		GC	-	IR64/IRGC105491	Septiningsih et al. (2003)
7	RM584	AC	*gAC-6a*	Chuan7/Nanyangzhan	Lou et al. (2009)
		AC	*ac6b*	Zhenshan 97/H94	Fan et al. (2005)

[a] available on http://www.gramene.org/

genome (Kumar et al., 2011) and quality evaluation of rice varieties (Bergman et al., 2001; Lapitan et al., 2007; Kibria et al., 2009; Lestari et al., 2009). Lapitan et al. (2007) suggested that information on genetic diversity of specific regions of rice genome can be very useful for gene mapping and for the application of marker assisted selection (MAS) in breeding programs. Lestari et al. (2009) observed that as nucleotide differences among genotypes are a major source of heritable variation, molecular markers derived from them should provide an effective measure of genotypic variation and hence phenotypic differences among varieties. In the present study, we used seven SSR markers which were tightly linked to major QTLs of AC, GC and GT (localized on the short arm of chromosome 6) to evaluate the genetic variation of 48 rice varieties with various starch physicochemical properties. The objective of this research was to determine the potential of these markers for distinguishing genotypes with various amylose classes and to suggest the most informative markers for marker assisted selection.

Results and discussion

Polymorphism of microsatellite markers

All SSR motifs used in the present study were polymorphic and produced varying number of alleles with different size ranges (Table 2). A total of 41 alleles were detected among the 48 rice genotypes with an average of 5.86 alleles per locus. The number of alleles per locus ranged from 3 in RM314 to 10 in RM276 (Table 2). This value was similar to the average of 5.89 per microsatellite locus reported by Lapitan et al. (2007), while it was higher than the average of 4.23 alleles per locus reported by Ghneim et al. (2008) for Venezuelan rice cultivars and the average of 3 alleles per locus reported by Kibria et al. (2009) using microsatellite markers linked to genes controlling rice grains aroma. Furthermore, the average number of alleles per locus obtained in the present study was smaller than that reported in previous studies. For example Kuroda et al. (2007) reported an average of 9.28 alleles per locus over 7 SSR loci and/or Shefatur Rahman et al. (2009) who recorded 6.33 alleles per locus using a small set of three SSR markers on 34 varieties. The overall size of amplified products ranged from 93bp in locus RM276 to 169bp in locus RM584. The effective number of alleles varied from 2.68 in locus RM314 to 5.25 in locus RM276 with an average of 3.74 alleles which was much higher than the average of 2.1903 alleles reported by Kibria et al. (2009). Nei's gene diversity for microsatellite loci ranged from 0.63 in RM314 to 0.81 in RM276 with an average of 0.72 that was higher than the average of 0.524 reported by Ghneim et al. (2008) using 48 SSR markers in 11 Venezuelan rice varieties and the average of 0.50 recorded by Bounphanousay et al. (2008) using 24 SSR markers. Also, Kibria et al. (2009) obtained an average of 0.119 in aromatic rice genotypes using three SSR markers that was smaller than that recorded in the current research.

Cluster analysis and genetic relationships

Cluster analysis was performed using the UPGMA method to group the studied varieties based on simple matching similarity coefficient (supplementary data). Four clusters were formed at genetic similarity level of 0.76-0.84 (Fig 1), which contained 20, 2, 4 and 22 varieties, respectively. Cophenetic correlation coefficient between similarity matrix from the simple matching coefficient and output matrix from the dendrogram of cluster analysis was 0.93 (the highest value rather than other similarity coefficients) indicating that the used similarity coefficient and cluster analysis method were suitable for using the information derived from SSR markers to group the rice varieties. Group I was comprised of 18 Iranian landrace varieties and two improved lines, IR64 and Gill1, at about 80% similarity coefficient. All genotypes in this group were classified as aromatic rice with good cooking and eating properties with intermediate AC, except Gill1 with high AC. IR64 a cultivar with different origin

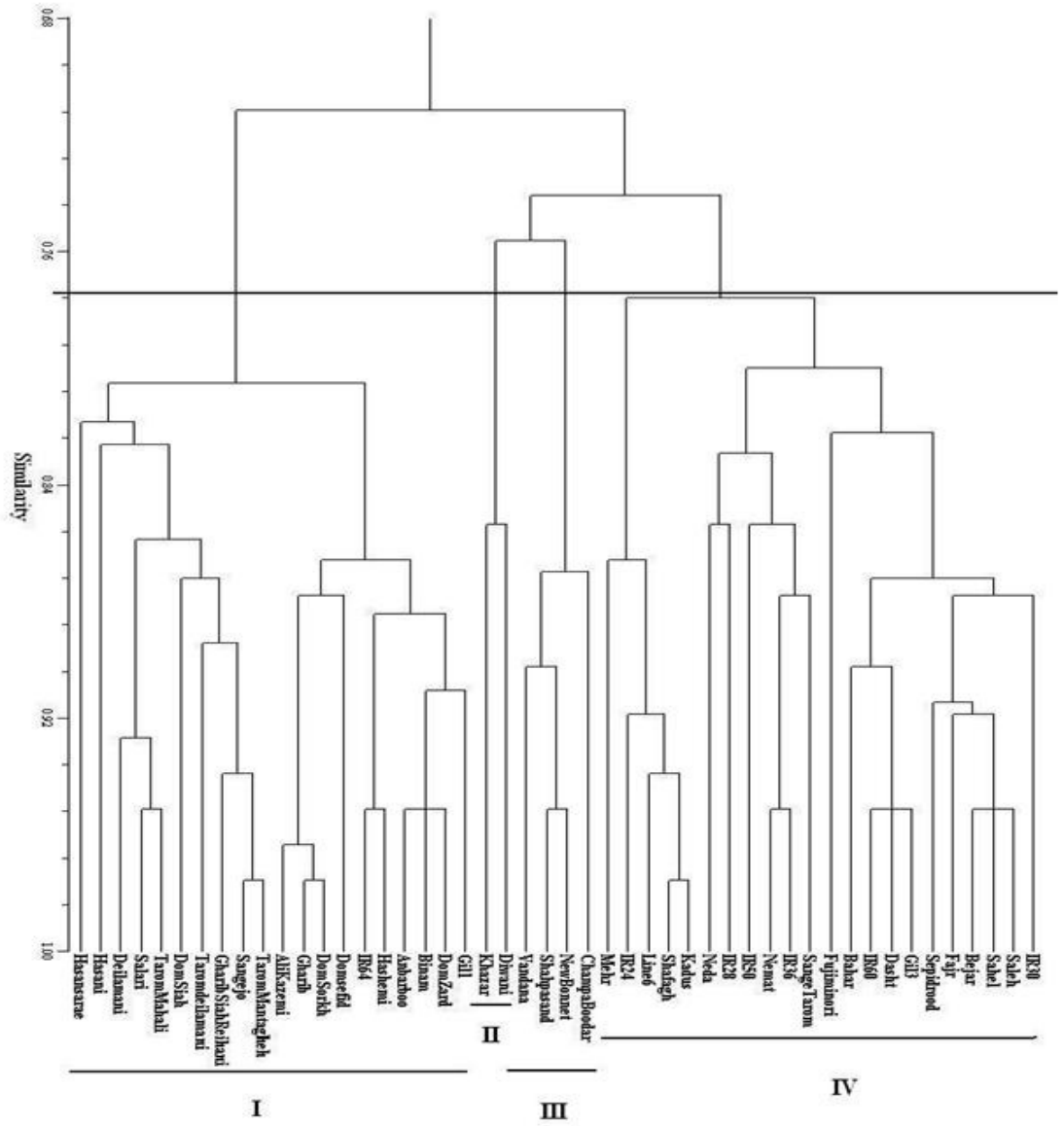

Fig 1. Dendrogram of cluster analysis by UPGMA method based on simple matching coefficient of 7 microsatellite markers linked to grain quality characteristics in 48 studied rice genotypes.

showed intermediate AC, GC and GT and good cooking and eating quality (Fitzgerald et al., 2008). Group II contained two genotypes, Khazar and Diwani with different origins at similarity coefficients of 85%. In group III, four varieties with high AC were classified at similarity coefficients of about 86.4%. Improved rice lines and IRRI lines were grouped in cluster IV at similarity coefficients of 79.5%. These varieties had intermediate to high AC except IR24 and Fujiminori which had low AC. The results of this research showed that genotypes with the same origins were clustered into the same classes. Also genotypes with the same amylose classes were grouped into the same clusters. Thus, the SSR markers used were applicable for evaluation of genetic variation and cooking and eating quality traits of rice varieties. Moumeni et al. (2003) determined the genetic relatedness of popular local cultivars and blast-resistance donor germplasm using fingerprints derived from simple sequence repeat (SSR) and plant defense gene markers. They reported that within-group similarities for the traditional and improved cultivars were greater than 80% and 75%, respectively. Lapitan et al. (2007) reported that SSR markers can distinguish quality rice subspecies and classified cultivars with the same cooking and eating quality. Also Kibria et al. (2009) grouped 14 aromatic rice genotypes with SSR markers in two main clusters and the dendrogram revealed that the genotypes that are derivatives of genetically similar type clustered together.

Table 2. Characteristics of microsatellite markers linked to three major components of rice cooking and eating quality (amylose content, gelatinization temperature and gel consistency) in all studied rice genotypes.

Locus	Motif	Expected size	Allele Size (bp)	Sample size	Number of total alleles	Number of effective alleles	Heterozygosity*
RM170	(CCT)7	121	110-134	46	5	3.09	0.68
RM190	(CT)11	124	109-140	42	7	3.97	0.75
RM204	(CT)44	169	149-162	38	5	3.27	0.69
RM253	(GA)25	-	111-141	41	7	4.79	0.79
RM276	(AG)8A3(GA)33	149	93-145	47	10	5.25	0.81
RM314	(GT)8(CG)3(GT)5	118	111-128	47	3	2.68	0.63
RM584	(CT)14	169	135-169	48	4	3.1	0.68
Mean					5.86	3.74	0.72
SD					2.34	0.97	0.07

*Computed based on Nei's gene diversity index

Table 3. Characteristics of studied rice varieties.

No.	Variety	Country of origin	AC (%)	GT	GC (mm)	Quality	No.	Variety	Country of origin	AC (%)	GT	GC (mm)	Quality
1	Domsephid [a]	Iran	19.9	3.5	60	High	25	Nemat [a]	Iran	26.82	7	30	Mediocre
2	Domzard [a]	Iran	20.1	3.4	68	High	26	Kadous [a]	Iran	23.25	3.3	52	Mediocre
3	Domsorkh [a]	Iran	19.9	5.9	60	High	27	Neda [a]	Iran	25.07	6.2	30	Poor
4	Domsiah[c]	Iran	23	4	53	High	28	Sepidrood [a]	Iran	26.4	7	30	Poor
5	Sange-Tarom [c]	Iran	22	5	42	High	29	Line 6 [a]	Iran	22.7	3	44	-
6	Tarommahali [a]	Iran	19.9	4.2	50	High	30	Shafagh [b]	Iran	21.69	3.17	92	Mediocre
7	Taromdeilamani [c]	Iran	23.04	3.2	41.75	High	31	Fajr [b]	Iran	22.9	6	68	Good
8	Tarom-Mantagheh [c]	Iran	20.2	3.6	48	High	32	Sahel [b]	Iran	23.2	6.5	45	Mediocre
9	Hasani [a]	Iran	20.4	6.1	50	Good	33	Mehr [c]	Iran	25	7	-	Mediocre
10	Champaboodar [a]	Iran	27.7	3.2	40	Mediocre	34	Bahar [c]	Iran	21.8	5	60	Mediocre
11	Binam [a]	Iran	20.7	3.2	57	Good	35	Bejar [c]	Iran	23.2	-	-	-
12	Shahpasand [a]	Iran	26.1	3	68	Good	36	Gill 1 [c]	Iran	28.3	5.5	-	Mediocre
13	Sangjo [a]	Iran	21.3	3	60	High	37	Gill 3 [c]	Iran	25.4	6	-	Poor
14	Salari [a]	Iran	21.7	3.3	60	Good	38	IR24 [d]	Philippines	18	<3	82	-
15	Gharib-Siahreihani [a]	Iran	17.8	4	50	Mediocre	39	IR28 [d]	Philippines	27	<3	32	-
16	Hashemi [a]	Iran	20.1	3.2	52	High	40	IR30 [d]	Philippines	26	3-5	32	-
17	Anbarboo [a]	Iran	20.9	3	60-65	Good	41	IR36 [d]	Philippines	23	3-5	30	-
18	Hasansaraei [a]	Iran	20.2	3.1	55	High	42	IR50 [d]	Philippines	23	3-5	45	-
19	Alikazemi [a]	Iran	16.7	4.9	70	Good	43	IR60 [d]	Philippines	26	<3	42	-
20	Gharib [a]	Iran	20.1	3.6	70	Mediocre	44	IR64 [d]	Philippines	21	3-5	65	Good
21	Deilamani [a]	Iran	20.4	3.2	60	Good	45	Diwani	Syria	-	-	-	-
22	Saleh [a]	Iran	26.7	7	30	Mediocre	46	Vandana [d]	India	25	3	45	-
23	Khazar [a]	Iran	22.4	3	55	Mediocre	47	New Bonnet	USA	-	-	-	-
24	Dasht [a]	Iran	26.85	7	30	Mediocre	48	Fujiminori	Japan	-	6	-	-

References of quality traits data: [a]Allahgholipoor and Mohammad Salehi (2001), [b]Nasiri et al. (2004), [c]Rice Research Institute of Iran, [d]IRRI database: www.iris.irri.org

Materials and methods

Plant materials

Plant materials of this research were 48 different rice varieties, including 21 Iranian landrace and 16 improved lines, 3 upland rice varieties with different origin, 1 *japonica* variety and 7 IRRI improved rice lines, which were received from Rice Research Institute of Iran (RRII) and International Rice Research Institute (IRRI). The studied varieties together with their cooking and eating quality characteristics are presented in Table 3. For preparation of leaf samples and DNA extraction, 10 seeds from each variety were planted in pots and leaf samples of young seedlings were prepared after 25 days and kept at -80°C.

Genomic DNA extraction

DNA was extracted from the rice seedlings according to the modified CTAB method (Saghai-Maroof et al*.,* 1984). DNA quality was checked by electrophoresis in an agaroz gel and quantification was accomplished using a spectrophotometer.

Microsatellite markers and PCR amplification

Seven SSR markers RM170, RM190, RM204, RM253, RM276, RM314 and RM584 located in vicinity of two genes, *wx* and *alk,* were used in this study. All markers had been previously mapped to the short arm of chromosome 6 on the Cornell Rice SSR 2001 map (Temnykh et al., 2001). These markers were closely linked to QTLs controlling AC, GC and GT (Table 1). DNA samples were amplified in 10 µl reaction volumes containing of 2 µl template DNA (5 ng), 5 µl ddH_2O, 1 µl PCR buffer (10X), 0.48 µl $MgCl_2$ (50 mM), 0.6 µl dNTPs (2 mM), 0.4 µl of each primer (60 ng) and 0.12 µl of Taq DNA Polymerase (5 U/µl). PCR was carried out in a thermal cycler (Perkin–Elmer-Gene Amp PCR System 9700, USA) to the cycle profile: Initial denaturation at 94°C for 4 min, 40 cycles of 1 min denaturation at 94°C, 30 sec annealing at 55°C or 61°C (depending on the marker used)

and 1 min extension at 72°C, and then 4 min at 72°C for the final extension.

Electrophoresis

PCR products were subjected to vertical electrophoresis (BioRad Sequi-Gen©) and 6% polyacrylamide gel containing urea and separated from each other. The gels were stained with silver nitrate method (Bassam et al., 1991) and scanned. All experiments were performed in the Biotechnology Laboratory of the Agricultural Biotechnology Research Institute of North of Iran (ABRINI).

Data analysis

All studied varieties were pure and showed one band for all studied markers. The genotypes were manually scored using the binary coding system, '1' for presence of band and '0' for absence of band. Simple matching similarity coefficient (Sokal and Michener, 1958) was calculated using the SIMQUAL subprogram in NTSYS-pc software ver. 2.02e (Rohlf, 1998) to construct a dendrogram using the UPGMA method. Genetic polymorphism values and effective number of alleles (Kimura and Crow, 1964) were calculated using the POPGENE software ver. 1.32 (Yeh and Boyle, 1997). Gene diversity index was evaluated by Nei (1973) method according to equation 1:

$$H_i = 1 - \sum_{j=1}^{n} P_{ij}^2 \quad (1)$$

Where P_{ij} is the frequency of *j*th allele for *i*th marker and n is the number of observed alleles in the studied population.

Conclusion

Results of this research indicated that the use of microsatellite markers linked to grain quality traits especially the markers tightly linked to *wx* and *alk* genes can distinguish rice varieties from each other for quality characteristics. Measuring quality traits of rice varieties (i.e. amylose content, gel consistency and gelatinization temperature) are time consuming and expensive. Furthermore, some quality traits like gelatinization temperature can be measured only in limited time (maximum 3 month after harvest). In rice breeding programs especially in initial generations, there are so many lines but only a few seeds are obtained from each line, so direct measurement of cooking and eating quality of lines is difficult. Therefore, it is necessary to suggest alternative methods. The results also showed that microsatellite markers linked to genes or QTLs controlling grain quality properties are suitable tools for marker assisted selection (MAS) to select rice lines with high quality.

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