

## Allelic diversity and association analysis for grain quality traits in exotic rice genotypes

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**ABSTRACT:** The present research aims to study the association and allelic diversity of linked microsatellite markers to grain quality QTLs of 84 exotic rice genotypes. To this end, 9 microsatellite markers (RM540, RM539, RM587, RM527, RM216, RM467, RM3188, RM246, RM5461) were used in which a total of 61 alleles were identified with a mean of 6 alleles per locus. The polymorphism information content (PIC) varied from 0.542 (RM540) to 0.812 (RM3188) for SSR markers. Cluster analysis was performed using UPGMA method and genotypes were divided into five groups. Furthermore, based on regression analysis, for rice grain quality properties in flooding conditions as long as drought stresses, 10 alleles were identified. Of these, four alleles with gelatinization temperature, an allele with protein content under flooding conditions, and three alleles with protein content and three alleles with gelatinization temperature were related under drought stress. It should be noted that the RM216-C and RM5461-D alleles were commonly identified in several traits. The presence of common markers for traits is probably due to the consistency of chromosomal locus controlling these traits or pleiotropy. The results of this study may imply that the important identified alleles for example RM216-A for gelatinization temperature ( $R^2=30.1\%$ ) can be used in rice quality improvement programs.

**KEYWORDS:** Association analysis, Genetic variation, Grain physicochemical quality, Polymorphic Information Content (PIC).

### INTRODUCTION

Rice (*Oryza sativa* L.) as one of the most important crops is grown in more than 110 countries. About 90 percent of the world's rice is produced on the Asia. Rice ranked second after wheat in cultivating and supplying calories [19]. Rice in Iran is grown over 600000 hectares, with an average yield of up to 4.5 tons per hectare [8]. Considering the importance of rice in the diet as a result of population growth, researchers have been working on plans to increase yield. One of the ways to increase the rice yield per unit area is to produce high yielding varieties with low to medium plant heights. Hence, the quality of cooking and marketability is relatively

favorable. Because landrace varieties of rice have a low yield and mostly are susceptible to pests and diseases, but farmers still prefer to cultivate them for their excellent cooking quality and aroma. They are derived from high nutritional value in the market, while the improved cultivars that have been introduced so far have been delayed due to the poor quality of physical and chemical degradation gradually. Therefore, it is necessary at the beginning of the rice breeding programs to study the genetic structure of the quality characteristics of rice, because the success of the breeding programs depends greatly on the selection of best parent [12]. Genetic

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diversity is the basis of plant breeding, so breeders would have a chance of success in their breeding program if the variety and chance of choosing the appropriate materials are available to them [1]. Today, in order to determine the genetic diversity among populations, in addition to traditional methods (based on morphological and phylogenetic markers and characteristics), modern molecular instruments and markers, including DNA markers, are used. Therefore, the polymorphism obtained from these genetic markers is one of the parameters that can be evaluated for studying populations and understanding the genetic differences between them [2]. SSRs or microsatellites markers are one of the most suitable markers for genetic variation of plants. [13]. Musyoki et al [14] studied the genetic diversity of selected genotypes of rice (*Oryza sativa* L.) from Kenya and Tanzania based on amylose content and gelatinization temperature using microsatellite markers. The number of alleles varied from 2 to 4, and the mean of the alleles in each locus was 2.75. The polymorphism information content was from 0.2920 (RM202) to 0.6841 (RM141) with an average of 0.4697. Cluster analysis classified the genotypes into two groups. The researchers suggested that the microsatellite markers associated with QTLs controlling the gelatinization temperature and amylose content could be used more effectively in studies on the diversity of rice genotypes [9].

Palanga et al [17] evaluated genetic variation of 30 rice genotypes and 8 microsatellite markers related to rice chemical quality. A total of 45 alleles were detected with an average of 5.63 alleles per locus. The number of alleles for markers varied from 3 alleles for RM204 to 8 alleles for RM342A and RM190. The cluster analysis by UPGMA method divided the genotypes into five main groups. The results of this study showed that the use of SSR markers is suitable for determination of quality of cooking and the aroma of rice cultivars. He et al [7] stated that the inheritance of the characteristics affecting the cooking quality is much more complicated than other agronomic characteristics. They have a major genes and a small effect gene for the amylose content on chromosomes 5 and 6 for the gel consistency of two QTLs on chromosomes 2 and 7, and for the gelatinization temperature of a single genes and a small genes of the effect on the chromosome 6 locus. Zhang et al [21] determined that the diversity of the waxy gene was highly correlated with the variation in baking quality and oral rice quality. In this study, a series of isogenic lines carrying four different alleles of the *wx* gene were

studied. These isogenic lines showed very high variation in amylose content, gelatinization temperature, starch viscosity and thermodynamic properties. The aim of this research was to evaluate the molecular diversity among rice genotypes under flooding and drought stress conditions in terms of traits related to chemical quality of rice and identification of informative markers for traits related to chemical quality of rice, including gelatinization temperature, amylose and protein content in exotic rice germplasm.

## MATERIALS AND METHODS

### Plant materials

In order to study the allelic variation and analysis of the relationship between the SSR markers related to the chemical quality of rice under flooding and drought conditions, an experiment was conducted at Gonbad Kavous University. The rice seeds harvested from the national project in Gonbad Kavous University in (year 2016) were used (Table 1). The seeds were cultivated in two separate environments, flooding and drought condition. Nitrogen fertilizer at 150 kg/ha and Phosphorous at 100 kg/ha were applied. Seeds were used for measuring of traits one month after harvest. Under stress conditions, field irrigation was discontinued 40 days after planting. The soil moisture was measured four times in 10 day intervals. Soil moisture values were 8, 18, 24, 32 and 4%, respectively.

In this research, gelatinization temperature [11], the amylose content [8] and protein content [3] were investigated on the grains of rice cultivars harvested under flooding and drought conditions.

### Extract Genomic DNA and PCR

Genomic DNA was extracted from the young leaf of 85 genotypes which were planted in the greenhouse by CTAB method [18]. Genomic DNA quality was evaluated using 0.8% agarose gel electrophoresis. Genomic DNA amplification was performed with Polymerase Chain Reaction and Thermo Cycler Device (BIO-RAD). Selection of SSR primers were done based on gramene site ([www.gramene.org](http://www.gramene.org)) information and previous studies which reported QTLs related to rice grain quality traits (Table 2).

To prepare the reaction mixture in a volume of 10  $\mu$ L for each sample, 1  $\mu$ L PCR buffer (10 times), 0.48  $\mu$ L MgCl<sub>2</sub> (50 mM), 0.6  $\mu$ L mix of 4 dNTPs (2 mM), 0.75  $\mu$ L of each

**Table 1.** Names of studied genotypes

No	Genotype	Origin	No	Genotype	Origin	No	Genotype	Origin	No	Genotype	Origin
1	IR14T132	IRRI	23	IR58443-6B-10-3	Philippines	45	CT 18614-4-1-2-3-2	IRRI	67	IR 11A506	IRRI
2	IR12T125	IRRI	24	IR71896-3R-8-3-1	Philippines	46	IR 04A216	IRRI	68	IR 11A511	IRRI
3	IR12T254	IRRI	25	NSIC Rc 222	IRRI	47	IR 05A272	IRRI	69	IR 11A534	IRRI
4	IR12T133	IRRI	26	IR14L240	IRRI	48	IR06A145	IRRI	70	IR 11A546	IRRI
5	IR12T260	IRRI	27	IRRI 132	IRRI	49	IR 09L204	IRRI	71	IR 11A581	IRRI
6	IR12T198	IRRI	28	IR 43	IRRI	50	IR08L216	IRRI	72	IR 11N121	IRRI
7	IR12T136	IRRI	29	IRBL5-M[CO]	IRRI	51	IR 09L324	IRRI	73	IR 11N137	IRRI
8	IR11T182	IRRI	30	IRBLB-IT13[CO]	IRRI	52	NSIC Rc 192	IRRI	74	IR 11N169	IRRI
9	IR11T185	IRRI	31	IRBLI-F5	IRRI	53	IR09N516	IRRI	75	IR 11N239	IRRI
10	IR11T200	IRRI	32	IRBLKH-K3[CO]	IRRI	54	IR 09N251	IRRI	76	IR 11N313	IRRI
11	IR11T210	IRRI	33	IR 60080-46A	IRRI	55	IR 10A227	IRRI	77	IR12L201	IRRI
12	IR11T219	IRRI	34	IRBLK-KU[CO]	IRRI	56	IR10A121	IRRI	78	SAKHA 105	Egypt
13	IR11T220	IRRI	35	IRBLKM-TS[CO]	IRRI	56	IR 10A199	IRRI	79	B 40	Indonesia
14	IR12T148	IRRI	36	IR 64683-87-2-2-3-3	Philippines	58	IR10A231	IRRI	80	UPL RI-7	IRRI
15	IR12T246	IRRI	37	IRBLKS-CO[CO]	IRRI	59	IR 10A237	IRRI	81	OM 6600	IRRI
16	IR11T257	IRRI	38	IRAT 112	IRRI	60	IR 10A314	IRRI	82	IR1552	IRRI
17	IR11T258	IRRI	39	IRBLSH-S[CO]	IRRI	61	IR 10F221	IRRI	83	PANT DHAN 19	India
18	IR12T122	IRRI	40	IRBLTA2-IR64[CO]	IRRI	62	IR 10L185	IRRI	84	PR 113	IRRI
19	CSR 90IR-2	IRRI	41	IRBLTA-ME[CO]	IRRI	63	IR10L139	IRRI	85	IR 11C123	IRRI
20	PSB RC 10	IRRI	42	IRBLT-K59	IRRI	64	IR 11A410	IRRI			
21	IR45427-2B-2-2B-1-1	IRRI	43	IRBLZ5-CA[CO]	IRRI	65	IR 11A479	IRRI			
22	IR55179-3B-11-3	IRRI	44	IRBLZT-IR56[CO]	IRRI	66	IR 11A501	IRRI			

**Table 2.** list of SSR markers

Motif	T <sub>m</sub>	Primer sequences	SSR Marker
(AG) <sub>16</sub>	55	gccttctgctcatttatgc ctaggcctgccagattgaac	RM540
(CTT) <sub>18</sub>	55	acgcgaacaattaacagcc ctttgctaccagtagatccage	RM587
(TAT) <sub>21</sub>	55	gagcgtcctgtttaaaccg agtagggtatcacgcatccg	RM539
(GA) <sub>17</sub>	55	ggctcgatctagaaaatccg ttgcacaggttgcgatagag	RM527
(CT) <sub>18</sub>	55	gcatggccgatggtaaag tgtataaaaccacagcca	RM216
(TC) <sub>21</sub>	55	ggtctctctctctctctctc ctcctgacaattcaactcgcg	RM467
(CT) <sub>12</sub>	50	tcacgagtcgttcgttcttg cttgcgtctcaagtgggtgag	RM3188
(CT) <sub>20</sub>	55	gagctccatcagccattcag ctgagtgctgctgcgact	RM246
(TC) <sub>19</sub>	55	gcctccatataaaccggc agcgaagggaacacgacag	RM5461

**Table 3.** Information content of polymorphism and cultivar variation for the primers studied

Marker name	No. of bands	PIC	Nie Genetic diversity	Major Allele Frequency
RM540	4	0.542	0.6306	0.4268
RM587	7	0.5953	0.633	0.5556
RM539	5	0.6104	0.6581	0.5062
RM527	7	0.7372	0.7679	0.3810
RM216	8	0.8045	0.8276	0.2469
RM467	6	0.7612	0.7909	0.3250
RM3188	7	0.8120	0.835	0.2432
RM246	8	0.7931	0.8172	0.2821
RM5461	9	0.8015	0.8248	0.2500
Total	61	6.4582	6.7851	3.2168
Mean	6	0.718	0.7538	0.357

forward and reverse primer (60 ng/μl), 5 units of *taq* DNA Polymerase (0.20 μl), 2.5 μl of genomic DNA at a concentration of 10 ng/μl and 3.8 μl of sterilized distilled water which mixed up together. The PCR cycling conditions were as initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 45 s, annealing temperature of each primers pair for 40 s, 72 °C for 45 s and final extension at 72 °C for 5 min. The PCR products were loaded into a vertical electrophoresis and 6% polyacrylamide gel, and then the gels were stained using silver nitrate method [4].

### Statistical Analysis

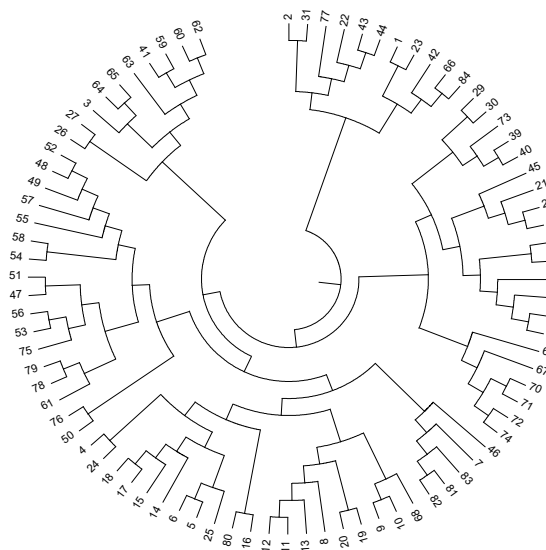
Correlation and relationship between traits and markers recorded using regression relationships by SPSS software. Quality characteristics as dependent variable and scoring of amplified DNA as independent variable were considered. Genetic diversity statistics were calculated using Power Marker software [10].

## RESULTS and DISCUSSION

### Genetic diversity

In this research, genetic variation of rice was investigated using nine SSR-linked to QTLs controlling rice grain quality properties.

The number of observed alleles in each locus is a good indicator of genetic variation [15]. Generally, the used primers to analyze the genetic diversity were able to detect 61 alleles with an average of 6 alleles per locus (Table 3). Considering this polymorphism, it can be expected that these markers can be used as a powerful tool for identification and distinction of different rice genotypes. RM5461 and RM540 amplified the highest (9) and the least (4) alleles, respectively. The polymorphic information content varied from 0.55 to 0.8120 and was an average of 0.71 per locus. The Nie Genetic diversity varies from 0.063 to 0.833, and the primer of RM3188 had a value of 83.3 and the highest genetic diversity. Victoria et al [13] used 24 rice cultivars and 164 microsatellite markers to evaluate the genetic diversity of the traits related to grain quality in rice. In their study, the polymorphism information content varied from 0.18 for RM420 to 0.91 for the RM473B and with an average of 0.86 for each locus. In a report by Musyoki et al [12] to study of genetic diversity of traits related to the chemical quality of rice grain, the polymorphism information content varied from 0.2920 (RM202) to 1.6841 (RM141)



**Figure 1.** Grouping the genotypes studied based on the SSR markers

with an average of 46.077. Also, in the study of Palanga et al [15] in evaluating the genetic diversity of 30 rice genotypes and 8 microsatellite markers related to rice chemical quality, the polymorphism information content varied from 0.39 to 0.83, and cluster analysis using UPGMA genotype. The studied groups were divided into five main groups.

### Cluster analysis

Generally, cluster analysis is used to find actual categories as well as reduce the number of data. In other words, the goal is to identify fewer groups. Therefore, individuals that are more similar to each other are grouped together. Furthermore, it can be said that cluster analysis is the important method for evaluation of similarity between genotypes in a germplasm.

In cluster analysis based on the molecular data and the UPGMA method, finally five groups were determined based on the dendrogram (Fig. 1). In the first group, 11 genotypes (2, 31, 77, 22, 43, 44, 1, 23, 42, 66 and 84), in the second group, 21 genotypes (29, 90, 73, 39, 40, 45, 21, 28) 34, 32, 33, 38, 37, 35, 36, 69, 67, 70, 71, 72 and 74) were considered. In the third group, there are 25 genotypes (46, 7, 83, 81, 82, 68, 10, 9), in the fourth group, 16 genotypes (50, 76, 61, 78, 79, 75, 53, 56, 47, 51, 54, 58, 55, 57, 49, 48 and 52) and in the fifth group 10 genotypes (26, 27, 3, 64, 65, 63, 41, 68, 60 and 62). Chamani Mohasses et al [17] studied the genetic variation of 9 lines of rice using SSR and ISSR markers. Grouping

of lines was performed by UPGMA method in 3 groups, which consisted of 1, 3 and 5 genotypes, respectively. Victoria et al [18] also investigated the genetic diversity of rice quality traits using microsatellite markers. Cluster analysis assigned rice cultivars in three groups.

### Association Analysis

Regarding the regression analysis of rice quality, two conditions of flooding and drought stress were identified for the studied traits (Table 4). Under flooding conditions, an allele associated with rice protein content was identified which had a positive and significant relationship with gelatinization of rice. For rice gelatinization temperature, 4 alleles associated with QTLs of this trait were located, of which RM327-C, RM467-C and RM216-A alleles had a positive and significant relationship and the RM467-E allele had a negative and significant correlation to gelatinization temperature.

Under drought stress conditions, for the grain protein, two alleles associated with this trait were detected; RM216-C allele had a positive and significant correlation with the protein content of rice grain, while the RM5461-D allele had a negative and significant relationship with the trait. Three alleles were identified for the rice gelatinization temperature. The negative and positive relationship between allele RM5461-D, the RM5461-C and RM216-C was found with this trait respectively. Verma et al [19] studied the relationship analysis of rice cooking quality characteristics using SSR markers. The polymorphic information content was 316 (RM21945) to 0.73 (RM252) with an average of 0.505. In this study, two related markers for amylose content (RM190 and RM11), two related markers for gel consistency (RM21945 and RM169), and four gel consistency markers (RM169, RM11, RM12 and RM21) were identified as informative markers for these traits.

Nikzadeh Talebi et al [16] used 34 varieties of landrace and improved rice cultivars and 20 microsatellite markers to evaluate the allelic frequency and association analysis of some of the traits related to pre-harvest germination. In their study, four markers (RM220, RM282, RM447 and RM320) were identified that linked to grain traits and explained 47 percent of grain traits variation.

Also, association mapping of 48 rice genotypes for drought tolerance traits showed that 82 SSR markers have a high correlation to root traits such as root length, root diameter and root dry weight. The results of this study showed that under stress conditions, RM29, RM3843,

**Table 4.** Multiple Regression Analysis Between Chemical Rice Characteristics (dependent variable) and SSR Markers (Independent Variables) under Flooding and Drought Conditions

Conditions	traits	Allele	The regression coefficient	standard error	F	R <sup>2</sup>
flooding	Protein	RM216-C	0.023	0.008	8.68**	0.096
		RM327-C	0.525	0.194	15.57**	0.16
	Gelatinization temperature	RM467-E	-0.246	0.083	12.021**	0.229
		RM467-C	0.182	0.083	9.79**	0.269
		RM216-A	0.066	0.033	8.63**	0.304
Drought	Protein	RM216-C	0.027	0.005	21.59**	0.208
		RM5461-D	-0.012	0.004	15.61**	0.278
	Gelatinization temperature	RM216-C	0.132	0.034	15.56**	0.149
		RM5461-D	-0.247	0.084	11.73**	0.205
		RM5461-C	0.185	0.085	9.75**	0.240

RM318, RM5720, RM170, RM585, RM540 and RM36 are linked to root characteristics [6].

## CONCLUSION

RM216-C allele was detected in terms of the grain protein content of rice grain under flooding conditions and drought stress and gelatinization temperature under drought stress conditions. Also, the RM5461-D allele was associated with rice grain protein content and rice gelatinization temperature in terms of drought stress, which could indicate that the presence of common markers for traits may be due to the association of chromosomal locus controlling these traits or Pleiotropy. The primers used to analyze the genetic diversity of different rice genotypes were 61 polymorphic loci. The RM5461 (9) and RM540 (4) have the highest and lowest alleles, respectively. The polymorphism information content varied from 0.542 (RM540) to 0.812 (RM3188) for SSR markers. According to these results, polymorphic loci could be expected to serve as a powerful tool in identifying and distinct of different rice genotypes.

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## تنوع آلی و تجزیه ارتباط برای صفات کیفیت دانه در ژنوتیپ های خارجی برنج

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### چکیده

در این بررسی تنوع ژنتیکی و تجزیه ارتباط ارقام برنج برای نشانگرهای پیوسته به کیفیت شیمیایی بذر ۸۴ ژنوتیپ خارجی برنج مورد ارزیابی قرار گرفت. برای این منظور از ۹ نشانگر ریزماهواره (RM540، RM539، RM587، RM527، RM216، RM467، RM3188، RM246، RM5461) استفاده شد. از این تعداد نشانگر در کل ۶۱ مکان چند شکل با میانگین ۶ آلل در هر مکان شناسایی شد. میزان شاخص محتوای اطلاعات چندشکلی برای این از ۰/۵۴۲ (RM540) تا ۰/۸۱۲۰ (RM3188) برای نشانگرهای SSR متغیر بود. جهت تعیین روابط ژنتیکی بین ژنوتیپها، تجزیه خوشه‌ای به روش UPGMA انجام و ژنوتیپها به ۵ گروه تقسیم شدند. بر اساس تجزیه رگرسیون داده‌های مرتبط با کیفیت برنج در مجموع شرایط نرمال و تنش خشکی ۱۰ آلل برای صفات مورد بررسی شناسایی شد که از این تعداد چهار آلل با درجه حرارت ژلاتینه شدن، یک آلل با میزان پروتئین در شرایط نرمال و سه آلل با میزان پروتئین دانه برنج و سه آلل با درجه حرارت ژلاتینه شدن در شرایط تنش خشکی مرتبط بودند. قابل ذکر است آلل‌های RM216-C و RM5461-D به صورت مشترک در چندین صفت شناسایی شدند. وجود نشانگرهای مشترک برای صفات احتمالاً به دلیل پیوستگی مکان‌های کروموزومی کنترل کننده این صفات و یا پلیوتروپی می‌باشد. پس از تایید نتایج این بررسی می‌توان در برنامه‌های اصلاحی برای کیفیت برنج استفاده نمود.

**کلمات کلیدی:** تجزیه ارتباط، تنوع ژنتیکی، صفات فیزیکی‌شیمیایی دانه، محتوای اطلاعات چند شکلی