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Genetic Diversity and Association Analysis of Rice Genotypes for Grain Physical Quality Using iPBS, IRAP, and ISSR Markers

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ABSTRACT

The economic value of different rice varieties depends on their characteristics. Knowing the genetic control of the traits will help the breeder. Genetic diversity of 85 rice genotypes evaluated using six iPBS, one IRAP, and nine ISSR markers. The studied traits included the grain area, grain length, grain width, and diameter and grain perimeter, eccentricity of brown and white. The polymorphic alleles detected by each marker (varied from 3 to 8 alleles), and an average of 5.33 alleles per locus was observed. The iPBS1854 and iPBS2242 markers with 11 bands have the highest number of bands and the iPBS2240 and iSSR55 markers with 5 bands of the least band bands. The content of the polymorphic information varied from 0.018 (iPBS2241) to 0.241 (iPBS2240) and averaged 0.195. The iPBS2240 marker with high levels of polymorphic information identified as the best marker for genetic diversity evaluation. Regression analysis was performed between phenotypic traits and molecular data for association analysis. 54 alleles were identified for evaluated traits. Of these, in a normal condition of one allele linked to the area, length, width, the eccentricity of the brown rice grain. Also, two, three, four alleles associated with the area, length and width of the white grain. In Drought stress, one single allele correlated to the area, length and width of the brown rice grain. Eight and one alleles detected for exertion from canter and perimeter of the brown rice. Also, two, four, four and eight alleles associated with the area, length, width, from canter and perimeter of the heard rice grain, respectively. Among identified alleles, ISSR1-2, iPBS2241-2, ISSR16-4, ISSR55-1, ISSR57-1, iPBS2242-2 and iPBS2240-1 associated with several traits in both normal and stress conditions The presence of common alleles is probably due to the linkage of genetic locations which control these traits or pleiotropy. We suggest that linked markers with common traits be used for breeding programs.

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Introduction

Rice production cannot be easily developed and increased, as compared to other cereals, due to more specialized and capital intensive requirements. In addition, rice is not cultivated under any climatic conditions. Currently, in the world, its global cropland in the world and Iran is 159807722 hectares and 557687 hectares respectively. Among Asia's regions, Asia has about 90-91% of the world's total rice production, with only 8-9% of production being allocated to the rest of the world (FAO, 2018). Rice grain quality is a complex feature, which includes the quality, the quality of conversion and the quality of cooking and feed. These traits have always been cropped by plant breeders along with grain yield. Iranian people prefer aromatic rice with long grain length and low grain width. The quality and baking quality of rice grain are of great importance for Iranian breeders (Kordrostami *et al.,* 2015). Most of the agronomic and economic traits in plants are quantitative and are controlled by several genes or genomic regions with low effects. These traits show a general variation among individuals in a population, and in addition to having a large number of genes, they are heavily influenced by environmental factors (Collard *et al.,* 2008). In this regard, inform of genetic control,

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genomic position, and the amount of participation of each of the genes in the phenotypic traits explanation is one of the factors that improve the yield and quality of rice in the breeding programs (Rabie and Sabouri, 2008). It is important to note that morphological traits often have a limited number and may not show a true genetic relationship between genotypes. But, genetic variation is highly based on DNA polymorphism and is independent of environmental factors. In addition, when quantitative traits are investigated, a large number of samples are needed to evaluate genotypes. In contrast, when a polymorphic DNA analysis is performed, a small amount of sample can be informative. Therefore, molecular markers increase the efficiency of genotypes selection because their expression is independent of environmental effects, more convenient and reliable and need to less time (Kamushita *et al.,* 2008; Zheng *et al.,* 2008; Colagar *et al*., 2010; Rabie *et al.* 2013). Genetic analysis of traits related to apparent quality and baking in different cultivars of rice from six rice cultivars including Hashemi, Hasani, and Shahpsand cultivars, which are farmers in favor of curing quality, modified Kadus, Vandana with the origin of India and IR36. The studied traits included length, width, grain thickness, and grain form, as well as amylose content and grain gelatinization temperature. The genetic analysis of these traits indicated the existence of incomplete genetic dominance in controlling these traits, which indicates a high potential for selection for these traits. Talebi *et al.* (2016) used 9 varieties of native rice cultivars and 4 introduce cultivars and 20 microsatellite markers for evaluation of polymorphism and analysis of some of the traits related to pre-harvest germination of 21 native rice varieties. In their study, four markers (RM220, RM282, RM447, and RM320) associated with rice grain traits. Tabkhkar *et al.* (2011) used the rice genotypes (21 natives, 16 improved cultivars, 7 IRRI cultivars) to the evaluation of the allelic diversity of microsatellite markers that linked to rice quality. In their research, the content of the polymorphic information and the Shannon index were respectively 0.54 and 1.14, respectively. The aim of this study was to evaluate the molecular diversity between rice genotypes under normal conditions and drought stress in terms of physical quality of grain and identification of informative markers of each trait.

Materials and Methods

Plant materials

In order to study the allelic variation and analysis of the association of iPBS, IRAP and ISSR (Table1) markers with the rice grain quality, 85 genotypes (Table 2) cultivated in normal and drought conditions. Grain obtained were used for determination of physical grain quality traits. The studied traits included the area, length, width, diameter and perimeter, eccentricity of brown and white rice.

Table 1. Name, Annealing temperature and sequence of markers

Primer	Annealing temperature (°C)	Sequences
ISSR1	48-43	5'-AACAACAACAACAACAACG-3'
iPBS1854	60-55	5'-GCATCAGCCTGGACCAGTCCTCGTCC-3'
iPBS2238	60-55	5'-ACCTAGCTCATGATGCCA-3'
iPBS2241	55-50	5'-ACCTAGCTCATCATGCCA-3'
iPBS2240	60-55	5'-AACCTGGCTCAGATGCCA-3'
ISSR16	55-50	5'-CTCTCTCTCTCTCTCTG-3'
IRAP	57-52	5'-ACACACACACACACACACACACG-3'
ISSR809	64-59	5'-GAGGAGAGAGAGAGAGG-3'
ISSR45	55-50	5'-GTCGTCGTCGTCGTCGTCA-3'
ISSR48	55-50	5'-ACACACACACACACACACACATA-3'
iPBS2242	55-50	5'-GCCCCATGGTGGGCGCCA-3'
ISSR55	47-42	5'-TGATGATGATGATGATGAA-3'
ISSR57	67-62	5'-CTGGCATTTCCATTGTCGTCGATGC-3'
ISSR58	47-42	5'-TCTTCTTCTTCTTCTTCTG-3'
iPBS2237	60-55	5'-CCCCTACCTGGCGTGCCA-3'

Genotypic evaluation

All of the genotypes were planted in a greenhouse. Genomic DNA was extracted from the young leaves of each genotype. DNA extraction was performed by CTAB (Saghi Maroof *et al.,* 2005). Genomic DNA quality was evaluated using 0.8% agarose gel electrophoresis. Genomic DNA replication was performed with polymerase chain reaction and Thermo Cycler Device (BIO-RAD).

To prepare the PCR master in a volume of 10 µl for each sample, 1 µl PCR buffer (10x), 0.48 µl $MgCl_2$ (50 mM), 0.6 µl mix of 4 dNTP (2 mM), 1.5 µl of each primer (60 ng/µl), 5 units of *Taq* DNA Polymerase (0.20 µl), 2.5 µl of genomic DNA were mixed with 10 ng/ml concentration and 3.8 µl of sterilized distilled water. The PCR thermal program was performed according to Table 3. Electrophoresis of PCR products of ISSR, iPBS, and IRAP were performed on 1.5% agarose gel.

Statistical Analysis

The area, length, width, diameter and perimeter, the eccentricity of brown and white rice grains were evaluated using image processing methods by MATLAB software. Genetic diversity parameters were performed using POPGEN software. The correlation and regression analysis were done by SPSS software.

Table 2. Names of studied genotypes

No.	Genotype	No.	Genotype	No.	Genotype	No.	Genotype
1	IR14T132	23	IR58443-6B-10-3	45	CT 18614-4-1-2-3-2	67	IR 11A506
2	IR12T125	24	IR71896-3R-8-3-1	46	IR 04A216	68	IR 11A511
3	IR12T254	25	NSIC Rc 222	47	IR 05A272	69	IR 11A534
4	IR12T133	26	IR14L240	48	IR06A145	70	IR 11A546
5	IR12T260	27	IRRI 132	49	IR 09L204	71	IR 11A581
6	IR12T198	28	IR 43	50	IR08L216	72	IR 11N121
7	IR12T136	29	IRBL5-M[CO]	51	IR 09L324	73	IR 11N137
8	IR11T182	30	IRBLB-IT13[CO]	52	NSIC Rc 192	74	IR 11N169
9	IR11T185	31	IRBLI-F5	53	IR09N516	75	IR 11N239
10	IR11T200	32	IRBLKH-K3[CO]	54	IR 09N251	76	IR 11N313
11	IR11T210	33	IR 60080-46A	55	IR 10A227	77	IR12L201
12	IR11T219	34	IRBLK-KU[CO]	56	IR10A121	78	SAKHA 105
13	IR11T220	35	IRBLKM-TS[CO]	57	IR 10A199	79	B 40
14	IR12T148	36	IR 64683-87-2-2-3-3	58	IR10A231	80	UPL RI-7
15	IR12T246	37	IRBLKS-CO[CO]	59	IR 10A237	81	OM 6600
16	IR11T257	38	IRAT 112	60	IR 10A314	82	IR1552
17	IR11T258	39	IRBLSH-S[CO]	61	IR 10F221	83	PANT DHAN 19
18	IR12T122	40	IRBLTA2-IR64[CO]	62	IR 10L185	84	PR 113
19	CSR 90IR-2	41	IRBLTA-ME[CO]	63	IR10L139	85	IR 11C123
20	PSB RC 10	42	IRBLT-K59	64	IR 11A410	-	-
21	IR45427-2B-2-2B-1-1	43	IRBLZ5-CA[CO]	65	IR 11A479	-	-
22	IR55179-3B-11-3	44	IRBLZT-IR56[CO]	66	IR 11A501	-	-

Table3. Thermal program for amplification

Step	Time	Temperature (°C)	Cycles
Primary denaturation	5'	95	1
Denaturation	45"	95	
Annealing	45"	-	10
Polymerization	45"	72	
Denaturation	45"	95	
Annealing	45"	-	25
Polymerization	45"	72	
Final extension	5'	72	1

Results and Discussion

Genetic diversity and polymorphism information

The primers (six iPBS markers, one IRAP marker, and nine ISSR markers) used to analyze the genetic diversity of different rice genotypes could identify 135 bands. Of the 135 bands formed, 33.5% of the bands were polymorphic, and the average percentage of polymorphism was 47.5%, due to this polymorphism, it could be expected that these markers could be suitable in identifying rice genotypes. The iPBS1854 and iPBS2242 markers with 11 bands had the highest number of bands and the iPBS2240 and ISSR55 with five bands had the smallest band. The polymorphic alleles detected by each marker varied from 3 to 8 alleles, with an average of 5.33 alleles per locus, and the content of the polymorphic information varied from 0.078 (iPBS2241) to 0.241 (iPBS2240) and averaged 0.195. The number of effective alleles among the studied markers was different. The average number of effective alleles in total was 1.745 and varied from 1.151 to 1.895. The highest number of effective alleles for iPBS2240 primers was detected at 1.895, and the lowest number of effective alleles for the ISSR45 marker was detected at 1.151. One of the most important indicators for evaluating genetic diversity among genotypes is Shannon's genetic index. The estimation of the Shannon index showed that the variation varied from 0.208 to 0.661, and the iPBS2240 initiating compounds had the highest genetic variation of 0.656. The average genetic diversity index was 0.274. The iPBS2240 marker had high levels of polymorphic information content, genetic diversity index, Shannon index and the number of the effective alleles. So, it identified as the best marker for gene expression evaluation (Table 4). Tabkhkar *et al.* (2011) used Iranian and IRRI cultivars for evaluation of genetic diversity. In their research, the content of the polymorphic information and the Shannon index were 0.54 and 1.14, respectively. Cluster analysis based on UPGMA method and simple matching coefficient divided the cultivars into four groups. Kumar and Singh (2012) evaluated genetic variation among rice genotypes of using microsatellite markers. Among the 30 microsatellite markers used, they created a polymorphic microsatellite position and incorporated 231 total alleles into polymorphisms. The number of alleles per locus ranged from 5 to 17 variables and an average of 4.9 alleles per locus. The PIC value for 20 microsatellite markers varied from 0.074 to 0.092 stated that the molecular specification using the microsatellite marker can be used as an effective tool to reveal genotypic diversity with reasonable accuracy. Ashiq Rabbani *et al.* (2010) examined 14 rice cultivars by 30 selected markers from the whole rice genome. A total of 104 alleles were observed by 35 markers, which had 100% polymorphism. The number of alleles produced by each marker was between 2 to 6 and an average of 3.5 alleles. The multivariate information content ranged from 0.259 to 0.782 on an average of 0.571. The coefficient of similarity was between 0.99 and 0.1.

Association analysis in normal conditions

Based on the regression analysis, in normal and drought condition, 54 alleles were identified for the traits studied (Table 5, 6, 7 and 8). For the area of brown rice, one allele was detected, which had a negative and significant relationship with the area of the brown rice. Also, one allele identified for the length of the brown rice had a negative relationship and explained 6.6% of the phenotypic variation. The ISSR1-2 had a negative and significant correlation with the brown rice width that explained 8.2% of the phenotypic variation of the brown rice width. A similar allele was identified for the eccentricity of brown rice, which was significant at 5% probability. ISSR1-2 related to the rice diameter. A similar allele was identified for the perimeter of brown rice, which has a negative and significant relationship (Table 5).

For the white grain rice area, 2 informative alleles have been identified. The iPBS2241-2 and iPBS2242-1 allele had a negative and positive significant relationship with the white grain rice area. The iPBS2242-1 allele could explain 21.4% of the phenotypic variation of the white grain rice area. Three markers detected for white grain rice length, of which two ISSR16-4 and ISSR57-3 alleles were positive and significant and the iPBS2241-2 allele had a negative and significant relationship with white grain rice.

Table 4. Information of primer polymorphism, Shannon and genetic diversity index

Primers	Sequence (5' →3')	Bands	Polymorphic bands	Monomorphic bands	Polymorphism percentage	Polymorphic information content	Shannon index	Genetic diversity index	Effective alleles
ISSR1	5'-AACAACAACAACAACAACG	10	8	2	80	0.238	0.353	0.213	1.314
iPBS1854	5'-GCATCAGCCTGGACCAGTCCTCGTCC	11	8	3	72.72	0.196	0.378	0.337	1.388
iPBS2238	5'-ACCTAGCTCATGATGCCA	8	6	2	75	0.144	0.270	0.148	1.193
iPBS2241	5'-ACCTAGCTCATCATGCCA	8	4	4	50	0.078	0.256	0.147	1.191
iPBS2240	5'-AACCTGGCTCAGATGCCA	5	3	2	60	0.241	0.661	0.469	1.895
ISSR16	5'-CTCTCTCTCTCTCTCTG	9	6	3	66.66	0.201	0.482	0.328	1.574
IRAP	5'-ACACACACACACACACACACG	7	5	2	71.42	0.234	0.626	0.437	1.817
ISSR809	5'-GAGGAGAGAGAGAGAGG	7	5	2	71.42	0.201	0.208	0.125	1.208
ISSR45	5'-GTCGTCGTCGTCGTCGTCA	9	6	3	66.66	0.163	0.431	0.283	1.151
ISSR48	5'-ACACACACACACACACACACATA	6	4	2	66.66	0.241	0.649	0.458	1.861
iPBS2242	5'-GCCCCATGGTGGGCGCCA	11	7	4	63.63	0.208	0.475	0.307	1.493
ISSR55	5'-TGATGATGATGATGATGAA	5	3	2	60	0.142	0.369	0.246	1.408
ISSR57	5'-CTGGCATTTCCATTGTCGTCGATGC	8	6	2	75	0.176	0.365	0.214	1.284
ISSR58	5'-TCTTCTTCTTCTTCTTCTG	7	3	4	42.85	0.218	0.353	0.220	1.321
iPBS2237	5'-TCTTCTTCTTCTTCTTCTG	8	6	2	75	0.237	0.573	0.394	1.720
Total	-	135	80	39	997.02	2.930	4.449	4.22	22.43
Average	-	9	5.33	2.6	66.46	0.195	0.420	0.274	1.495

It should be noted that the ISSR57-3 allele could explain 22.9% of the variation in white grain rice. Four alleles were associated with white grain width, and all four identified alleles explained a high percentage of phenotypic variation. The iPBS2240-1 and iPBS2241-2 had a positive and significant relationship that explained 39.2% and 44.9% of phenotypic variation, respectively. ISSR57-6 and ISSR16-4 alleles had a negative relationship that explained 33.7 and 20.5 % of the phenotypic variation, respectively. ISSR57-6 had a positive and significant relationship at a probability level of 5% with Eccentricity of white rice and explained 27.4% of the phenotypic variation. Of the two alleles associated with the diameter of the white rice grains, the iPBS2241-2 had a negative and significant and iPBS2240-1, which had a positive and significant correlation with the thickness of white grain rice. Four markers were detected about the perimeter that linked to this quantitative trait. Two alleles had the positive relationship and two alleles were negatively related to this trait. The iPBS2241-2 and iPBS2240-1 alleles were related to the white grain diameter (Table 6).

Talebi *et al.* (2016) evaluated the allelic frequency and to analyze the relationship between the traits associated with pre-harvest germination with microsatellite markers, 4 markers (RM220, RM282, RM447, and RM320) were identified in relation to the grain trait. Rabiei and Ghareyazi (2005), identified two QTLs on chromosomes 5 and 7, respectively, between the RM437-RM289 and RM481-RM125 markers, which controlled the length, width, and shape of the grain. Kurd Rostami *et al.* (2015) have been trained to identify QTLs that are related to quality traits. For physical traits, 17 QTLs were detected, of which 2 were QTLs for grain width, 2 QTL for grain length, 4 QTL for grain shape and 3 QTLs for grain elongation.

Table 5. Multiple regression analysis between morphological traits (dependent variables) and alleles (independent variables) in brown grain rice under normal conditions.

Traits	Allele	Coefficient of regression	Standard error	F	R^2
Brown rice area	ISSR1-2	-10.817*	4.723	5264*	0.079
Brown rice length	ISSR1-2	-1.728*	0.784	4.31*	0.066
Brown rice width	ISSR1-2	-0.893*	0.383	5.431*	0.082
Eccentricity of brown rice	iPBS2242-7	-0.029*	0.012	6.159*	0.092
Brown rice diameter	ISSR1-2	-1.268*	0.557	5.190*	0.087
Brown rice perimeter	ISSR1-2	-0.216*	3.010	5.746*	0.086

Table 6. Multiple regression analysis between morphological traits (dependent variables) and alleles (independent variables) in white grains under normal conditions.

Traits	Allele	Coefficient of regression	Standard error	F	R^2
White grain area	iPBS2241-2	**-2.180	0.594	9.523**	0.135
	iPBS2240-1	0.971*	0.397	8.149**	0.214
White grain length	ISSR16-4	0.598**	0.195	5.456*	0.082
	iPBS2241-2	-0.816*	0.293	5.777**	0.161
	ISSR57-3	0.534*	0.235	5.838**	0.229
White grain width	ISSR16-4	-0.221**	0.046	15.746**	0.205
	ISSR57-6	-0.515**	0.128	12.236**	0.337
	iPBS2240-1	0.132**	0.046	12.705**	0.392
	iPBS2241-2	0.113**	0.046	11.839**	0.449
Eccentricity of white rice	ISSR16-4	0.021**	0.006	14.742**	0.195
	ISSR57-6	0.043*	0.017	11.300**	0.274
White grain diameter	iPBS2241-2	-0.248**	0.087	7.165**	0.105
	iPBS2240-1	0.149*	0.058	7.208**	0.194
White grain perimeter	iPBS2241-2	-0.716**	0.512	4.806*	0.073
	ISSR16-4	1.060**	0.357	5.396**	0.152
	ISSR57-3	1.072*	0.414	5.497**	0.218
	ISSR55-2	-0.955*	0.436	5.586**	0.278

Association analysis in drought conditions

In drought stress conditions, an allele associated with brown rice grain area. This allele had a negative and significant relationship with brown rice grain area. Only the identified allele had a negative relation with the length of the brown rice grain length and explained 7% of the phenotypic variation of the brown rice grain. ISSR1-2 allele had a negative and significant relationship with the brown rice grain width and justified 2.7% of the phenotypic variation. For the exertion of brown rice grain center, 8 alleles were identified, all of which were significant at 1% probability. Positive alleles of ISSR1-5, IPBS2237-6, ISSR57-6 and ISSR16-2, and alleles IPBS2238-1, ISSR55-1, IPBS2240-2, and ISSR57-1, had a negative relationship with the exertion of brown rice grain center. A similar allele was identified for QTLs of this trait, which has a negative and significant relationship with the rice brown grain perimeter (Table 7).

For white grain area, two informative alleles were identified. iPBS2241-2 allele has a negative and significant relationship and iPBS2242-1 allele has a positive and significant relationship with the white grain area. iPBS2242-1 allele alone could explain 19.8% of the phenotypic changes of the white grain area. Four alleles detected for the white grain length trait, of which three ISSR16-4, ISSR1-3, and ISSR57-3 alleles have a positive and significant relationship and the iPBS2241-2 allele had a negative and significant relationship with white grain length. It should be noted that the ISSR57-3 allele could explain 28.4% of the variation in white grain length. Four alleles were associated with white grain width, and all four identified alleles explained a high percentage of phenotypic variations. The iPBS2240-1 allele has a positive and significant correlation with heard rice grain width and explained 42.4% of the phenotypic changes, IPBS1854-6, ISSR57-6, and ISSR16-4 alleles, with an explanation of 34, 50 and 19.3% of phenotypic changes there was a negative relationship with white grain width. Of the 8 alleles associated with the exertion from the white grain center of the alleles, IPBS2241-3, ISSR16-4, and ISSR1-7 had a negative and significant relationship at a probability level of 1%, and other alleles (ISSR57-1, IRAP-4, IPBS2241-1, ISSR55-1, IPBS2242-2) had a positive and significant relationship at a probability level of 1%. About the white grain diameter, a linked allele was detected in QTLs of this trait, which had a positive and significant relationship with its (Table 8).

In general, under normal conditions, the ISSR1-2 allele has a negative and significant relationship with the area, length, width and diameter of the brown grain rice as well as the area, length, width, diameter and perimeter of the white grain rice. The ISSR16-4 allele was common in length, widths, eccentricity, and

perimeters of the white grain. Also, the iPBS2241-2 allele was common in grain size, grain length, grain width, and grain diameter and grain and iPBS2240-1 allele in area, width and diameter of the white grain. In conditions of drought stress, the ISSR1-2 allele was identified in common with area, width and perimeter of the heard rice grain, and the ISSR57-1 and ISSR55-1 alleles eccentricity of the brown and white grains. The ISSR16-4 allele was also associated with length, width, and eccentricity of the white grain. This suggests that the existence of common markers for traits may be due to the linkage of chromosomal loci controlling these traits or Pleiotropy.

Table 7. Multiple regression analysis between morphological traits (dependent variables) and alleles (independent variable) in rice genotypes in drought stress conditions for brown rice grains.

Traits	Allele	Regression coefficient	Standard error	F	R^2
Brown grain area	ISSR1-2	-10.044	4.735	4.49*	0.069
Brown grain length	iPBS2237-2	-1.13	0.531	4.581*	0.070
Brown grain width	ISSR1-2	-0.822	0.378	4.733*	0.072
Brown grain Eccentricity	iPBS2238-1	-0.091	0.031	23.48**	0.278
	ISSR55-1	-0.051	0.012	16.40**	0.354
	iPBS2240-2	-0.023	0.011	13.77**	0.412
	ISSR57-1	-0.066	0.015	12.07**	0.454
	ISSR1-5	0.043	0.021	11.78**	0.508
	iPBS2237-6	0.044	0.013	11.12**	0.544
	ISSR57-6	0.093	0.031	11.26**	0.588
	ISSR16-2	0.07	0.02	11.06**	0.621
White grain perimeter	ISSR1-2	-6.67	3.03	4.84**	0.074

Table 8. Multiple regression analysis between morphological traits (dependent variables) and alleles (independent variable) in white grains under drought stress conditions for white grains.

Traits	Allele	Coefficient of regression	Standard error	F	R^2
White grain area	iPBS2241-2	-2.080	0.603	8.46**	0.0122
	iPBS2242-1	0.999	0.417	7.42**	0.198
	ISSR16-4	0.621	0.191	4.96*	0.075
White grain length	iPBS2241-2	-1.012	0.293	6.26**	0.173
	ISSR1-3	0.527	0.231	5.87**	0.230
	ISSR57-3	0.487	0.231	5.76**	0.284
White grain width	ISSR16-4	0.052	-0.267	14.57**	0.193
	iPBS1854-6	0.052	-0.245	15.47**	0.340
	iPBS2240-1	0.053	0.190	14.47**	0.424
	ISSR57-6	0.151	-0.451	14.56**	0.501
White grain eccentricity	ISSR57-1	0.101	0.012	38.03**	0.384
	IPBS2241-3	-0.068	0.013	30.44**	0.504
	ISSR16-4	-0.020	0.010	27.01**	0.579
	IRAP-4	0.032	0.010	24.78**	0.632
	iPBS2241-1	0.109	0.031	24.28**	0.678
	ISSR55-1	0.023	0.010	22.69**	0.709
	iPBS2242-2	0.030	0.011	21.39**	0.731
	ISSR1-7	-0.037	0.015	21.10**	0.758
White grain diameter	iPBS2242-2	0.192	0.074	38.03**	0.099

Concluding remarks

Multiple regression analysis between rice grain traits and ISSR, iPBS and IRAP markers in normal conditions and drought stress showed a significant correlation between the data, during which a total of 54 markers were identified for rice grain traits in both conditions. Many of the alleles were able to explain more than 20% of the phenotypic variation of the traits, some of which were common among the traits. The presence of common alleles is probably due to the linkage of genetic locations which control these traits or pleiotropy. The primers used to study the genetic diversity of different rice genotypes could identify 135 loci, of which 80 were polymorphic. The iPBS1854 and iPBS2242 markers with 11 alleles had the highest number of bands and the iPBS2240 and ISSR55 markers with 5 bands had the smallest alleles. The iPBS2240 marker with high levels of polymorphic information content, Nie's diversity, Shannon diversity and effective allele number in this study were identified as the best markers for gene diversity evaluation.

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