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RESEARCH ARTICLE

Variation of grain quality characters and marker-trait association in rice (*Oryza sativa* L.)

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Abstract. A set of 24 genotypes were studied for 17 grain quality characters and validated with the reported associated rice microsatellite markers with grain quality characters. Using 23 polymorphic markers distributed across 11 chromosomes marker-trait associations were studied. The percentage of polymorphism information content (PIC) of the markers ranged between 54.0 and 86.7. Eight markers with >80% and seven markers with >70% of PIC were found to be efficient in differentiating the studied grain quality characters. A total of 37 significant marker-trait associations ($P \leq 0.09$) were found with R^2 ranging from 4.70% to 43.80%. Eight markers a (RM246, RM11, RM241, RM16427, RM421, RM3, RM234 and RM257) showed association with more than one character suggesting their utility for the selection for grain quality characters which can be deployed in the rice crop improvement programmes.

Keywords. grain quality; microsatellite markers; association; validation.

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop and primary energy source of more than half the population of the world. Rice grain dimensions, cooking characters, hulling per cent (HULL) and milling per cent (MILL) are important criteria for developing new varieties. Cooking characteristics are largely determined by the properties of the starch that makes up 90% of milled rice (RKMP 2014). Starch is a major component of endosperm of rice consisting of amylose and amylopectin. Amylose is made up of linear molecule composed of α (1,4) linked glycosidic chains and has three physicochemical characteristics namely amylose content (AC), gel consistency (GC) and gelatinization temperature (GT), which are important criteria influencing processing quality and cooking of milled rice (Little *et al.* 1958; Cagampang *et al.* 1973; Juliano 1985). While evaluating the quality of rice grain, AC is considered as one of the important indicators and is determined as ratio of AC present in endosperm to the total starch. The AC in rice is commonly categorized as waxy/glutinous (0–5% of amylose), low AC (<20% of amylose), intermediate AC (between 21% and 25% of amylose) and high AC (>25% of amylose) (Kongseree and Juliano 1972). The waxy gene plays a key role in amylose synthesis by encoding enzyme granule bound starch synthesis, which is located on chromosome 6 (He *et al.* 1999; Tan *et al.* 1999; Fan *et al.* 2005). Percentage of AC in the paddy flour is determined by the modified method (Juliano 1971). GC per cent is the measure of tendency of the cooked rice to harden on cooling and is determined by heating a small portion of rice in a diluted alkali. Based on the GC values, rice can be categorized as soft (61–100 mm), intermediate (41–60 mm) and hard (26–40 mm) (Kongseree and Juliano 1972). The grain chalkiness is chalky proportion of the grain and is measured based on the standard evaluation system (SES 2013). Elongation ratio (ER) and water uptake (WU) are cooking parameters of grain and are determined as per the proposed protocol (Juliano and Bechtel 1985). The ER was determined by dividing the length of cooked kernel to length of uncooked kernel. The polished rice is considered as a poor source in terms of micronutrients, although wide genotypic variability exists for zinc (Zn) and iron (Fe) content in brown and polished grains (Bouis and Welch 2010; Gregorio 2002).

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Simple sequence repeats (SSR) or rice microsatellite (RM) markers are abundant, codominant and multi-allelic in nature and are valuable genetic markers in rice (Temnykh *et al.* 2001). RM markers play an important role in marker-assisted selection, marker-assisted back crossing, marker-assisted introgression, diversity analysis and fingerprinting for rice improvement programmes. The present study was carried out to validate the reported RM markers linked to the quality traits in a set of rice genotypes for facilitating the rice molecular breeding programmes. However, before going for the direct application of these reported markers, validation is a prerequisite for their use in marker-assisted selection for efficient and precision breeding. Hence, efforts were made in this study to validate the reported RM markers for grain quality characters.

Materials and methods

Location of the study

This experiment was designed and executed in the experimental farm of Indian Institute of Rice Research, Hyderabad, located at 17.53°N latitude and 78.27°E longitude, 545 m altitude, with a mean temperature of 31.2°C and mean annual precipitation of 988.3 mm. The soil pH was recorded as 8.53 and 8.58, before and after planting, respectively. The experiment was performed in a randomized complete block design (RCBD) in the field.

Plant material

The 24 rice genotypes used in this study were collected from ICAR-IIRR, among which Chittimutyalu is categorized as an aromatic landrace while others as non aromatic (table 1).

Twenty-six days old seedlings were transplanted in the experimental field in an area of 22 m^2. A set of 60 plants were planted arranged in four rows each consisting of 15 plants with a spacing of 20 × 15 cm. To maintain the varietal purity, two line gap was given between the genotypes as well in the replications. In this study, the recommended cultural methods were used for rice cultivation (IRRI 2015). The fertilizers, nitrogen (N), phosphorus (P) and potassium (K) were used in the experimental plot as per the recommended doses per hectare (N:P:K in ratio 5:3:2) (Boualaphanh *et al.* 2011).

Grain quality characters

After harvesting, 250 g of fresh paddy seeds were collected from each genotype to carry out the grain quality analysis and discoloured, damaged, unfilled, different sized and shaped grains were discarded. The grain quality of the rice was studied as 17 characters namely, HULL, MILL, grain dimensions and linear elongation on cooking, assessment of Fe and Zn content in both brown and polished rice and other biochemical traits in rice genotypes.

The rice samples were dehusked using laboratory model dehusker and milled with rice miller (McGill type) and the HULL and MILL of rice were recorded. The head rice recovery per cent (HRR) was determined based on the methodology proposed (Khush *et al.* 1979). Kernel length (KL) and breadth (KB) of the paddy were measured by using vernier calipers based on which length/breadth ratio (L/B ratio) was determined (Yadav and Jindal 2007). The grain dimensions and quality characters, namely WU, kernel length after cooking (KLAC), ER, volume expansion ratio (VER), alkali spreading value (ASV), AC per cent and GC were measured by standard methods (Madhubabu *et al.* 2017). For estimation of Fe and Zn in grains, each line was separated and 20 g of seed was subjected to energy dispersive X-ray fluorescent spectrophotometer (ED-XRF) (Rao *et al.* 2014).

Genotyping with SSR markers

Tissue samples from 15-day-old seedlings were used for DNA isolation following the protocol (Zheng *et al.* 1995).

Table 1. Genotypes used in this study.

	Genotype	Cross combination		Genotype	Cross combination
1	Akshyadhan	BR827-35/SC5109-2-2	13	Rasi	TNI/CO29
2	Chittimutyalu	Land race	14	Salivahana	RP5-32/Pankaj
3	MTU1010	Krishnaveni/IR64	15	BPT5204	GEB24/TN1//Mahsuri
4	Dhanarasi	B32Sel.4/*O.rufipogon*/B127	16	Sampada	Vijaya/C14-8
5	DRR Dhan 38	BPT5204/KMR-3	17	Sasyasree	IR8/TKM6
6	DRR Dhan 39	CSR3/Kasturi	18	Savitri	Pankaj/Jagannatha
7	IR64	IR 5657-33-2-1/IR 2061-465-1-5-3	19	Swarna	Vasistha/Mahsuri
8	Jaya	TN1/T141	20	Tella Hamsa	HR12/TN1
9	Lalat	OBS677/IR2071//Vikram/W1263	21	Triguna	Swarnadhan/RP1579-38
10	Mandya Vijaya	Sona/Mahsuri	22	Varadhan	Swarna/IET9314//BR327-36
11	NDR97	N22/Ratna	23	Varalu	WGL20471/CR544-1-2
12	PR113	IR8/RP2151-173-1-8//IR8	24	IET26264	SambaMahsuri/Chittimutyalu

Quantification of DNA was done with NanoDrop (Thermo Fisher Scientific, Wilmington, USA). In the present study, we have chosen microsatellite markers which are efficient in estimating the diversity analysis among the parental lines, cultivars and inbred lines in rice and to identify the rice genotypes with desired qualities (Enoki *et al.* 2002). A set of 50 RM markers spanning on 12 chromosomes were used to identify polymorphism among the selected genotypes. Of the 50 SSR markers, 23 markers were observed to show distinct polymorphism. A 15 μL polymerase chain reaction (PCR) mixture consists of 30 ng/μL of template DNA, 1× PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01 mg mL^{-1} gelatin), 2.5 mM of $MgCl_2$, 5 pm of forward and reverse primers, and 0.05 mM of dNTPs and 1 U *Taq* polymerase (GeNei). PCR chain consisted of five major steps, i.e. initial denaturation of template DNA at 96°C for 5 min followed by 35 cycles of PCR (Applied Biosystems, Foster City, USA) amplification, including a 30 s denaturation at 96°C, 30 s of annealing at 58°C; 1 min elongation at 72°C, followed by final extension at 72°C for 10 min. Three per cent agarose gel was used to separate amplicons consisting of ethidium bromide in an submarine electrophoresis unit at 90 V for 1 to 2 h followed by gel documentation where the gel was exposed to UV transillumination for visualization and estimation of the respective amplicon bands with the help of standard DNA marker/ladder (GeneRuler 100-bp ladder) (Balaji *et al.* 2012).

The RM markers with clear-cut polymorphism among the amplicons of the desired parents/checks were used for further analysis of genotypes and for association of the quality traits in rice. The PIC value representing the relative informativeness of each marker was calculated for all the markers (Ni *et al.* 2002). The parameters such as genetic variability, genotypic coefficient of variation (GCV) phenotypic coefficient of variation (PCV) and broad-sense heritability (h^2) were estimated (Johanson *et al.* 1955).

Statistical analysis

The mean data recorded from genotypes of RCBD were subjected to analysis of variance (ANOVA), heritability, PCV and GCV using SPAR 2.0 software (Sangeetha *et al.* 2008). Association between variations in quality characters and markers were calculated using mixed linear model (MLM) and generalized linear model (GLM) implemented in TASSEL v3.0 (Yu *et al.* 2006; Bradbury *et al.* 2007). Pearson correlation coefficient and heat map were analysed using *R* statistical software (3.2.0).

Results and discussion

Grain quality characters

Grain chalkiness was observed as one of the important quality characters; in the present study, 13 genotypes showed very occasional chalkiness (VOC) while in the remaining genotypes it was completely absent (table 2). The mean values of grain quality and genetic parameters of 24 rice genotypes are given in table 3. The mean values of HULL of the genotypes ranged from 72.3 (NDR97) to 81.8 (Varalu) with an average of 78.3. MILL ranged from 62.5 (Jaya) to 75.5 (Varalu), with an average of 70.2. HRR per cent of the genotypes ranged from 41.0 (DRR Dhan 38) to 70.9 (Rasi) with an average of 60.5. KL of the genotypes ranged from 3.8 (Chittimutyalu) to 7.4 (DRR Dhan 39) cm with an average of 5.8 cm and KB of the genotypes ranged from 1.8 (Lalat) to 2.5 (IR64) cm with an average of 2.1 cm. Ratio of L/B of the genotypes ranged from 2.0 (Chittimutyalu) to 3.6 (DRR Dhan39) with an average of 2.8. KL and ratio of L/B were high in DRR Dhan 39. Chittimutyalu showed the lowest values of KL and ratio of L/B. VER of the genotypes ranged from 4.4 (Akshydhan) to 5.8 (Savitri) mm with an average of 5.2 mm. WU of the genotypes ranged from 98.3 (Varalu) to 290.0 (PR113) mL with an average of 187.5 mL.

Table 2. Genotypes and grain characters.

	Genotype	GT	Grain chalkiness	Aroma		Genotype	GT	Grain chalkiness	Aroma
1	Akshyadhan	LB	A	NS	13	Rasi	MS	VOC	NS
2	Chittimutyalu	SB	VOC	SS	14	Salivahana	SB	A	NS
3	MTU1010	LS	A	NS	15	BPT5204	MS	A	NS
4	Dhanarasi	SB	VOC	NS	16	Sampada	MS	A	NS
5	DRR Dhan 38	MS	A	NS	17	Sasyasree	LS	A	NS
6	DRR Dhan 39	LS	A	NS	18	Savitri	SB	VOC	NS
7	IR64	LS	VOC	NS	19	Swarna	SB	VOC	NS
8	Jaya	SB	VOC	NS	20	Tella Hamsa	LS	A	NS
9	Lalat	LB	A	NS	21	Triguna	LS	VOC	NS
10	Mandya Vijaya	MS	A	NS	22	Varadhan	MS	VOC	NS
11	NDR97	LS	VOC	NS	23	Varalu	LS	VOC	NS
12	PR113	LS	VOC	NS	24	IET26264	MS	VOC	NS

GT, grain type; LB, long bold; LS, long slender; MS, medium slender; SB, small bold; A, absent; VOC, very occasionally chalkiness; NS, nonscented; SS, super scented.

Table 3. Genetic parameters of genotypes of rice (*Oryza sativa* L.).

	Grain quality component	Range	Mean ± SD	CD	SE	CV	Coefficient of variation		Heritability (%)
							PCV	GCV	
1	HULL	72.30–81.76	78.32 ± 2.02	3.46	1.21	2.67	3.38	2.07	37.49
2	Milling per cent	62.53–75.50	70.21 ± 3.53	5.83	2.04	5.03	6.50	4.11	40.00
3	HRR	40.96–70.93	60.51 ± 9.34	6.11	2.14	6.11	16.23	15.03	85.80
4	Kernel length (mm)	3.75–7.40	5.77 ± 0.74	0.67	0.23	7.04	14.16	12.28	75.29
5	Kernel breadth (mm)	1.75–2.46	2.10 ± 0.16	0.44	0.15	12.56	12.97	3.22	6.16
6	L/B ratio	2.01–3.59	2.83 ± 0.46	0.57	0.20	12.11	19.02	14.66	59.43
7	Volume expansion ratio (mm)	4.43–5.80	5.18 ± 0.39	0.54	0.19	6.35	9.15	6.59	51.86
8	Water uptake (mL)	98.33–290.00	187.50 ± 55.79	9.88	3.46	3.19	29.87	29.70	98.86
9	Kernel length after cooking (mm)	8.10–12.96	10.60 ± 1.43	2.92	1.02	16.68	19.17	9.44	24.27
10	Elongation ratio	1.36–2.83	1.87 ± 0.32	0.57	0.20	18.42	22.85	13.52	35.01
11	Alkali spreading value	4.00–7.50	5.68 ± 1.10	0.97	0.34	10.34	21.19	18.50	76.19
12	Amylose content per cent	22.43–31.8	26.12 ± 2.26	4.76	1.67	11.04	12.50	5.85	21.93
13	Gel consistency (mm)	21.33–75.00	43.48 ± 16.27	5.21	1.82	7.26	37.89	37.18	96.33
14	Fe content in brown rice (ppm)	7.05–12.56	9.16 ± 1.42	0.50	0.17	3.24	20.62	20.37	97.54
15	Zn content in brown rice (ppm)	10.97–23.89	17.19 ± 3.89	0.27	0.09	0.95	24.08	24.06	99.84
16	Fe content in polished rice (ppm)	1.47–5.04	2.18 ± 0.78	0.22	0.08	5.99	36.15	35.64	97.25
17	Zn content in polished rice (ppm)	6.25–21.18	11.58 ± 3.14	0.25	0.09	1.33	27.13	27.10	99.76

KLAC of the genotypes ranged from 8.1 (DRR Dhan 38) to 13.0 (Jaya) with an average of 10.6 mm. ER of the genotypes ranged from 1.4 (Jaya) to 2.8 (DRR Dhan 39) with an average of 1.9. ASV of the genotypes ranged from 4.0 (DRR Dhan 39 and Akshydhan) to 7.5 (Varalu) with an average of 5.7. AC per cent of the genotypes ranged from 22.4 (Chittimutyalu) to 31.8 (NDR97) with an average of 26.1. GC of the genotypes ranged from 21.3 (Sampada) to 75.0 with an average of 43.5 (Jaya) mm. The grain Fe content in brown rice ranged from 7.05 ppm (Jaya) to 12.53 ppm (Chittimutyalu) with an average of 9.18 ppm; and the Zn content in brown rice ranged from 10.97 ppm (MandyaVijaya) to 23.89 ppm (Chittimutyalu) with an average of 17.01 ppm. Zn and Fe content was lost from the grains during polishing.

Table 4. List of polymorphic microsatellite (RM) markers and PIC (%) values for association of amylase content in rice.

	Marker	Chromosome	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Approximate size of the allele in bp	PIC (%)
1	RM246	1	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT	95–110	74.3
2	RM1	1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	90–110	60.9
3	RM573	2	CCAGCCTTTGCTCCAAGTAC	TCTTCTTCCCTGGACCACAC	200–220	72.2
4	RM262	2	CATTCCGTCTCGGCTCAACT	CAGAGCAAGGTGGCTTGC	140–160	80.8
5	RM85	3	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC	90–105	86.7
6	RM232	3	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG	160–180	73.4
7	RM241	4	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG	120–130	71.9
8	RM16427	4	CTCCTCATGTCGCTGATTCTTGG	CCGAGATCTACCTCTTGCTGTCC	290–300	54.0
9	RM164	5	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC	230–280	86.7
10	RM421	5	AGCTCAGGTGAAACATCCAC	ATCCAGAATCCATTGACCCC	220–230	54.0
11	RM3	6	ACACTGTAGCGGCCACTG	CCTCCACTGCTCCACATCTT	105–160	83.3
12	RM276	6	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA	90–160	86.7
13	RM190	6	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	110–120	63.9
14	RM402	6	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG	110–120	70.7
15	RM11	7	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	110–180	86.7
16	RM234	7	TTCAGCCAAGAACAGAACAGTGG	CTTCTCTTCATCCTCCTCCTTGG	110–120	74.3
17	RM339	8	GTAATCGATGCTGTGGGAAG	GAGTCATGTGATAGCCGATATG	150–180	57.6
18	RM284	8	ATCTCTGATACTCCATCCATCC	CCTGTACGTTGATCCGAAGC	110–120	76.3
19	RM6543	9	AGCGGGCTCCTGAACAGTCTACC	CCATGCAAGAACGCGATCACC	80–90	57.6
20	RM257	9	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	110–160	60.9
21	RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	430–470	81.6
22	RM235	12	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC	95–105	86.0
23	RM247	12	TAGTGCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG	130–190	57.6

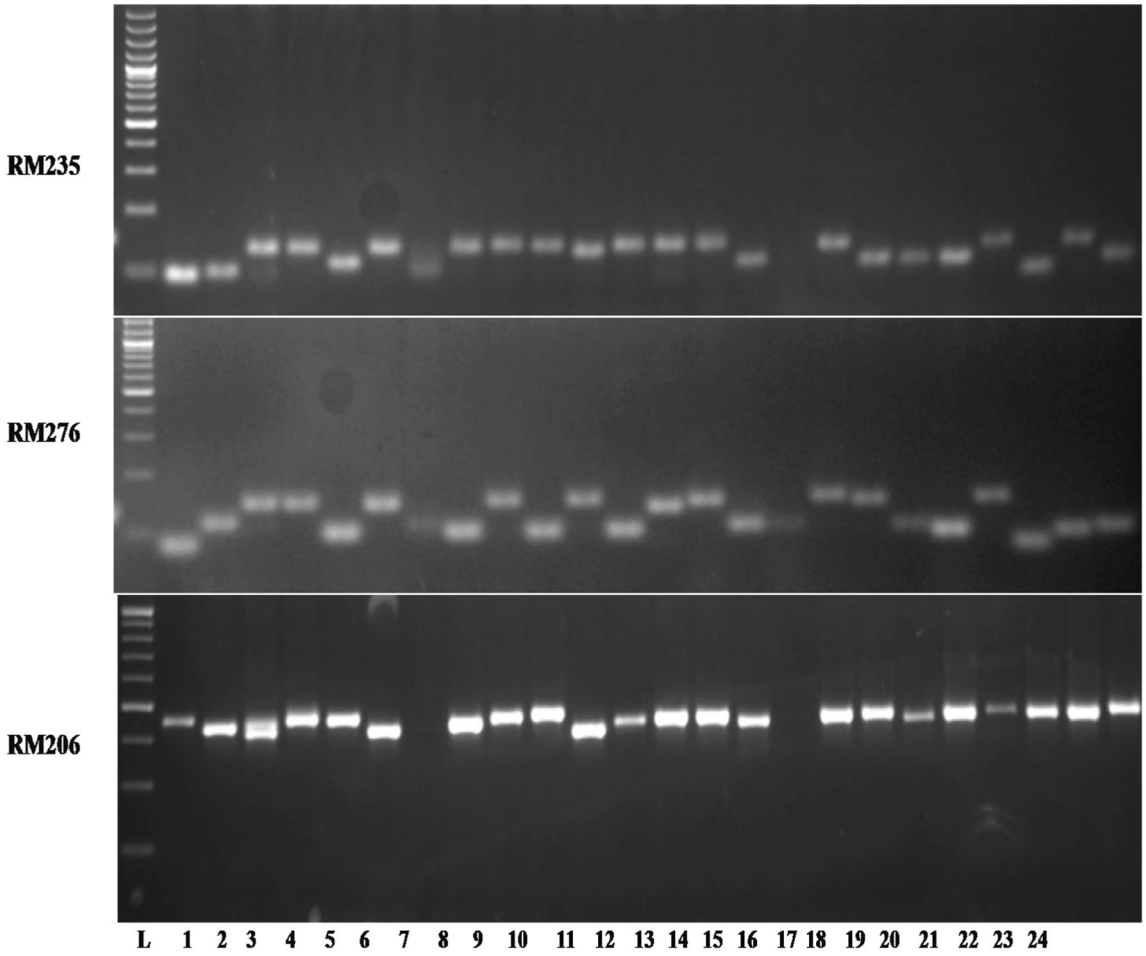

Figure 1. Polymorphism among the genotypes using SSR markers RM235, RM276 and RM206.

After polishing, the Fe content in rice grain ranged from 1.47 ppm (PR113) to 5.04 ppm (Chittimutyalu) with an average of 2.18 ppm; and the Zn content ranged from 6.25 ppm (MandyaVijaya) to 21.18 ppm (Chittimutyalu) with an average of 11.58 ppm.

Among the 24 genotypes, Akshyadhan showed the lowest VER and ASV. Chittimutyalu has shown the lowest KL, L/B ratio and lower per cent of AC. However, Chittimutyalu was identified to possess high Fe and Zn contents in both brown and polished rice. DRR Dhan 38 was observed with less per cent of HRR, KLAC, and ASV and DRR Dhan 39 was observed to possess high KL, L/B ratio and ER. In other genotypes, i.e. Jaya was identified with higher values in terms of KLAC and GC but with lesser values with respect to MILL and ER. Whereas NDR97 was observed with lower values in terms of HULL and higher value with respect to AC per cent. Varalu was observed with higher values in terms of HULL, MILL and ASV and lower values in terms of WU.

Genetic variability in rice accessions

The genetic variation among the rice accessions traits is represented in table 3. It was observed that the PCV was higher than the GCV for all the studied traits. Highest GCV was recorded for GC (37.18), however high GCV and PCV were observed for Fe and Zn content in polished rice, WU and Zn content in brown rice. The highest GCV and PCV values indicated that the selection can be performed for further improvement of these characters (Talukdar *et al.* 2017). The lowest GCV and PCV was observed with respect to HULL, KL, MILL, AC per cent, VER and KLAC, indicating limited scope for improvement within the experimental materials of the study. The selection was dependent not only on variability but also on heritability of the concerned trait (Talukdar *et al.* 2017). No distinctive differences were observed between PCV and GCV for the traits, namely GC, WU, Zn content in polished, Fe and Zn content in brown rice. The heritability of the traits ranged from 6.16 to 99.84 with a

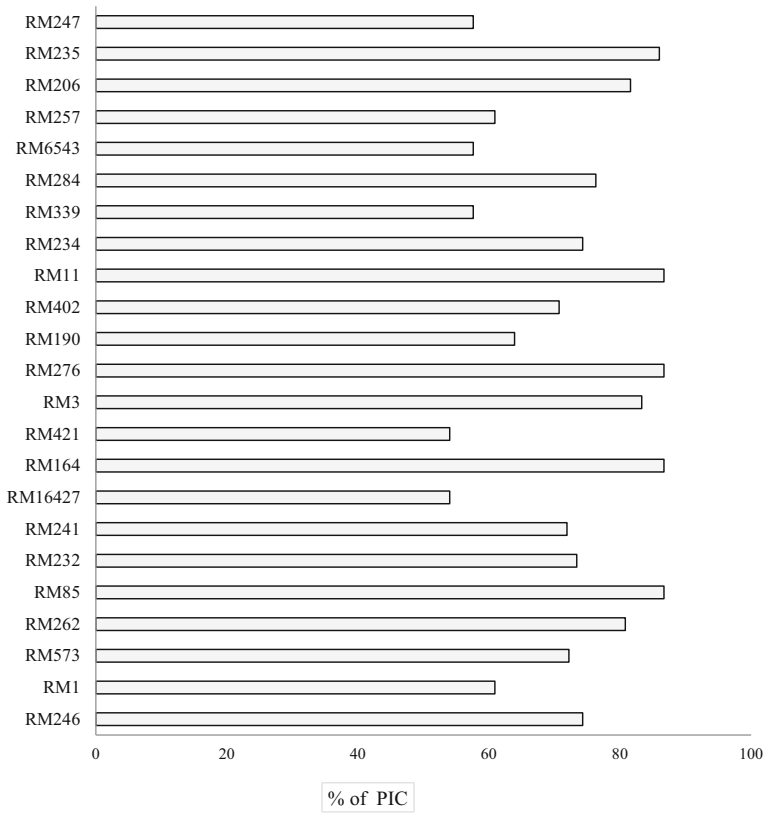

Figure 2. Microsatellite markers and PIC (%) values for association in rice.

mean value of 64.88. Highest heritability was observed for Zn and Fe content in both polished and brown rice, WU, GC and HRR per cent. The low heritability observed for the KB indicates the influence of the environment and ineffectivenss of this trait for selection (Akinwale *et al.* 2011; Pandey *et al.* 2012). High genetic advance combined with high heritability and low genetic advance combined with low heritability was recorded for all the traits corroborating the reported observations (Parikh *et al.* 2012). Phenotypic correlation coefficient in general was higher in comparison with genotypic correlation coefficient (table 3) indicating strong inherent association among the traits. The results indicated low environmental influence and predominance of genetic factors controlling variability for these traits as reported earlier (Madhubabu *et al.* 2017; Talukdar *et al.* 2017).

Analysis of PIC with SSR markers

Of the 50 SSR markers, 23 markers distributed over all the chromosomes have shown distinct polymorphism in 24 genotypes (table 4). The polymorphism among the genotypes are shown in figure 1. The percentage of PIC of these markers ranged between 54.0 and 86.7. Of the 23 markers, eight (RM262, RM85, RM164, RM3, RM276, RM11, RM206 and RM235) have shown >80.0% of PIC while seven markers (RM246, RM573, RM232, RM241, RM402, RM234 and RM284) showed >70.0% of PIC (figure 2).

Correlation studies

The HULL had negative correlation with Fe content in brown rice and AC; however, the HULL has shown significant positive correlation with MLL. HRR per cent had positive correlation with ASV. The milling per cent had significant positive correlation with HRR per cent. During the milling, the breakage of the kernels is caused due to the stress cracks and it influences the HULL, MILL and HRR per cent. The major factors responsible for breaking are variety of rice, management of post-harvest operations, drying conditions and other operational conditions (Ban 1971; Kunze 1979; Bautista *et al.* 2000; Cnossen and Siebenmorgen 2000). KL was observed to show significant positive correlation with L/B ratio, KLAC and ER supported the previous reports (Rehal *et al.* 2017). However, the KL was found to be negatively correlated with Fe content in polished rice (Sarwar *et al.* 1998). Negative correlation was also observed between KB and ratio of length to breadth. Significant positive correlation was observed between Fe content of brown rice and polished rice, whereas positive correlation was observed between GC and KLAC. Endosperm contains Fe and is also present in both brown and

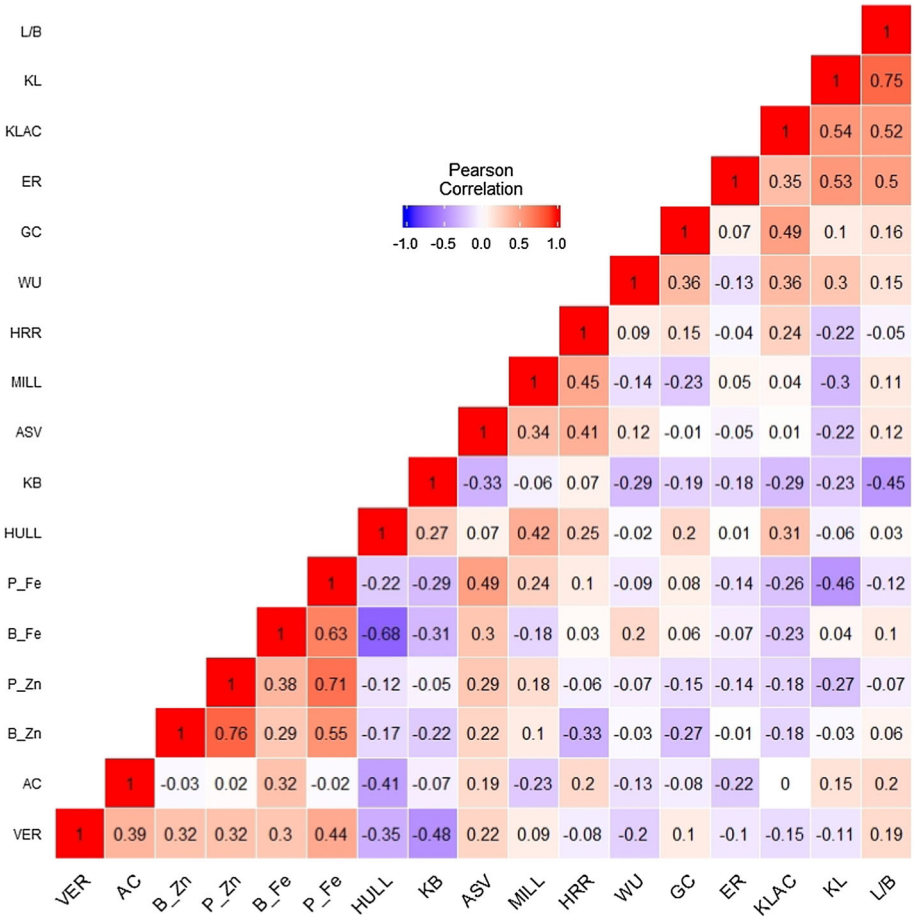

Figure 3. Pearson correlation coefficient of quality characters for genotypes of rice. HULL, hulling per cent; MILL, milling per cent, HRR, head rice recovery per cent; KL, kernel length (mm); KB, kernel breadth (mm); L/B, length/breadth ratio; VER, volume expansion ratio (mm); WU, water uptake (mL); KLAC, kernel length after cooking (mm); ER, elongation ratio (mm); ASV, alkali spreading value; AC, amylose content per cent; GC, gel consistency (mm); B_Fe, iron content in brown rice, B_Zn, zinc content in brown rice, P_Fe, iron content in polished rice, P_Zn: zinc content in polished rice.

polished rice. Hence, positive correlation was observed between Fe content of both brown and polished rice. The ER is positively correlated with L/B ratio. The Zn content of the brown rice was positively correlated with Fe content of polished rice and Zn content of polished rice. The Fe content of polished rice had significant positive correlation with ASV (figure 3).

Marker-trait association analysis

Marker-trait association analysis identified a total of 37 significant marker-trait associations ($P \leq 0.09$) for 17 characters with R^2 (percentage of the total variation explained) ranging from 4.70 to 43.80% (table 5). In this study, GLM and MLM tests were observed with different outcome since MLM uses both population structure and kinship for analyses, whereas GLM considers only population structure. The GLM-QQ and MLM-QQ plots demonstrated a close to perfect distribution of scores from the reference line (figure 4).

After the analysis of GLM, 33 microsatellite marker trait associations were observed to be associated with the grain quality characters (table 5). Among these 33 markers trait associations, a single marker (RM11) was associated with HULL on chromosome 7 with a variation of 21.82%. The grain quality is a complex quantitative trait controlled by numerous genes with low heritability, significantly influenced by the environment (Pandey *et al.* 2012). Three markers showed significant association with HRR per cent on chromosomes 1, 2 and 3, among which RM262 on chromosome 2 was observed with highest variation (30.36%). In this study, five markers showed significant association with KL on chromosomes 4, 5, 6 and 7, among which RM421 on chromosome 5 indicated highest variation (32.59%). Two markers, RM190 on chromosome 6 and RM234 on chromosome 7 were associated with KB with variation of 14.63% and 20.32%, respectively. Similar reports were observed where two major QTL were identified for grain length and grain width on chromosomes 3 and 8 (Rabiei *et al.* 2004). For ratio of L/B, we found five significant marker-trait associations on chromosomes 1, 4, 5, 6

Table 5. Significant marker-trait association identified in rice genotypes.

Trait	Chromosome	*F* value	*P* value	R^2 (%)	MS effect	MS error	*df* error
HULL							
Significant marker							
RM11	7	2.93	0.08	21.82	10.34	3.53	21
Nonsignificant marker							
RM421	5	1.5E-04	0.99	0.00	6.5E-04	4.31	22
HRR							
Significant marker							
RM246	1	3.17	0.09	12.60	252.56	79.67	22
RM262	2	4.58	0.02	30.36	304.38	66.50	21
RM16427	4	5.43	0.03	19.80	397.12	73.10	22
Nonsignificant marker							
RM284	8	0.00	1.00	0.04	0.43	95.45	21
RM232	3	0.02	0.90	0.08	1.521	91.08	22
Kernel length							
Significant marker							
RM241	4	2.97	0.07	22.03	1.39	0.47	21
RM421	5	10.64	0.00	32.59	4.10	0.39	22
RM3	6	3.89	0.04	27.05	1.70	0.44	21
RM402	6	4.59	0.04	17.26	2.17	0.47	22
RM11	7	2.82	0.08	21.15	1.33	0.47	21
Nonsignificant marker							
RM246	1	0.01	0.94	0.02	0.00	0.57	22
RM85	3	0.06	0.94	0.59	0.04	0.60	21
Kernel breadth							
Significant marker							
RM190	6	3.77	0.07	14.63	0.10	0.03	22
RM234	7	5.61	0.03	20.32	0.13	0.02	22
Nonsignificant marker							
RM284	8	0.01	0.99	0.05	1.7E-04	0.03	21
RM206	11	0.06	0.94	0.60	0.00	0.03	21
L/B ratio							
Significant marker							
RM246	1	4.68	0.04	17.55	0.89	0.19	22
RM241	4	2.96	0.07	22.00	0.56	0.19	21
RM421	5	3.72	0.07	14.48	0.73	0.20	22
RM3	6	3.52	0.05	25.09	0.63	0.18	21
RM11	7	5.35	0.01	33.75	0.85	0.16	21
Nonsignificant marker							
RM6543	9	0.01	0.91	0.06	0.00	0.23	22
RM232	3	0.00	0.96	0.01	6.9E-04	0.23	22
Volume expansion ratio							
Significant marker							
RM276	6	2.77	0.09	20.87	0.37	0.14	21
Nonsignificant marker				0.00			
RM246	1	0.00	0.95	0.02	7.6E-04	0.16	22
Kernel length after cooking							
Significant marker							
RM246	1	7.29	0.01	24.89	11.81	1.62	22
RM16427	4	3.51	0.07	13.75	6.52	1.86	22
RM3	6	4.36	0.03	29.33	6.96	1.60	21
RM11	7	8.18	0.00	43.80	10.39	1.27	21
RM235	12	4.41	0.03	29.59	7.02	1.59	21
Nonsignificant marker							
RM276	6	0.23	0.80	2.14	0.51	2.21	21
RM262	2	0.28	0.76	2.59	0.61	2.20	21
Elongation ratio							
Significant marker							
RM85	3	3.43	0.05	24.60	0.28	0.08	21
RM241	4	7.26	0.00	40.88	0.47	0.07	21
Nonsignificant marker							
RM190	6	0.00	0.96	0.01	3.3E-04	0.11	22
RM1	7	0.01	0.93	0.04	9.5E-04	0.11	22

Table 5 (*contd*)

Trait	Chromosome	*F* value	*P* value	R^2 (%)	MS effect	MS error	*df* error
Alkali spreading value							
Significant marker							
RM246	1	10.03	0.00	31.32	8.76	0.87	22
RM234	7	3.53	0.07	13.82	3.87	1.10	22
Nonsignificant marker							
RM3	6	0.03	0.97	0.28	0.04	1.33	21
RM85	3	0.13	0.88	1.25	0.18	1.32	21
Amylose content per cent							
Significant marker							
RM421	5	3.07	0.09	12.24	14.45	4.71	22
RM284	8	2.95	0.07	21.94	12.94	4.39	21
RM6543	9	6.08	0.02	21.64	25.53	4.20	22
Nonsignificant marker							
RM402	6	0.05	0.83	0.22	0.26	5.35	22
RM11	7	0.19	0.83	1.76	1.04	5.52	21
Gel consistency							
Significant marker							
RM246	1	3.17	0.09	12.58	766.67	242.17	22
RM232	3	3.50	0.07	13.73	836.92	238.97	22
RM164	5	3.23	0.06	23.53	716.91	221.93	21
RM11	7	5.30	0.01	33.53	1021.61	192.91	21
Nonsignificant marker							
RM276	6	0.08	0.93	0.72	22.02	288.11	21
RM234	7	0.03	0.86	0.14	8.70	276.62	22
Fe content in brown rice							
Significant marker							
RM257	9	5.01	0.04	6.15	5.08	1.02	11
RM206	11	3.47	0.08	4.70	3.89	1.12	12
Nonsignificant marker							
RM235	12	0.00	0.98	0.00	0.00	1.47	12
Zn content in brown rice							
Significant marker							
RM164	5	8.03	0.01	14.67	51.27	6.38	12
Nonsignificant marker							
RM276	6	0.00	0.98	0.00	0.00	8.15	12
Fe content in polished rice							
Significant marker							
RM257	9	14.3	0.00	20.04	2.81	0.19	12
Nonsignificant marker							
RM262	2	0.00	0.98	0.00	0.00	0.27	12

R^2 (%), percentage of the total variation explained.

and 7, among which RM11 on chromosome 7 showed highest variations (33.75%) as in previous reports (Bai *et al.* 2010), indicating QTL for ratio of kernel L/B on chromosomes 2, 3, 5 and 7. Similar reports (Talukdar *et al.* 2017) detected RM346 marker on chromosome 7 for ratio of kernel L/B; other statement (Lin *et al.* 1995) has identified 12 QTL for rice grain dimensions, including five for KL, two for KB, and five of kernel L/B on chromosomes 5, 6 and 7 indicating the possibility of identification of theses markers for the targeted traits in comparisons with the previous reports.

Single marker, RM276 on chromosome 6 was associated with VER with variation of 20.87%. For KLAC, five significant marker-trait associations were found on chromosomes 1, 4, 6, 7 and 12, among which RM11 on chromosome 7 showed highest variation (43.80%). Two markers, RM85 and RM241 showed significant association for ER on chromosomes 3 and 4, with a variation of 24.60% and 40.88%, respectively. ASV has significant marker-trait association with RM246 on chromosome 1 and RM234 on chromosome 7 with a variation of 31.32% and 13.82%. Three markers (RM421 on chromosome 5 with a variation of 12.24%, RM284 on chromosome 8 with a variation of 21.94% and RM6543 on chromosome 9 with a variation of 21.64%) showed significant association with respect to AC per cent. Not much variation and significant differences were observed for the markers, RM284 and RM6543 on chromosomes 8 and 9, respectively. Four markers showed significant association for GC on chromosomes 1, 3, 5 and 7, among which RM11 on chromosome 7 was observed with highest variation (33.53%). Two markers, RM257 and RM206 showed significant association with Fe content in brown rice on chromosomes 9 and 11, with a variation of 6.15 and 4.70%. A single marker, RM164 on chromosome 5 was associated with Zn content in brown rice with a

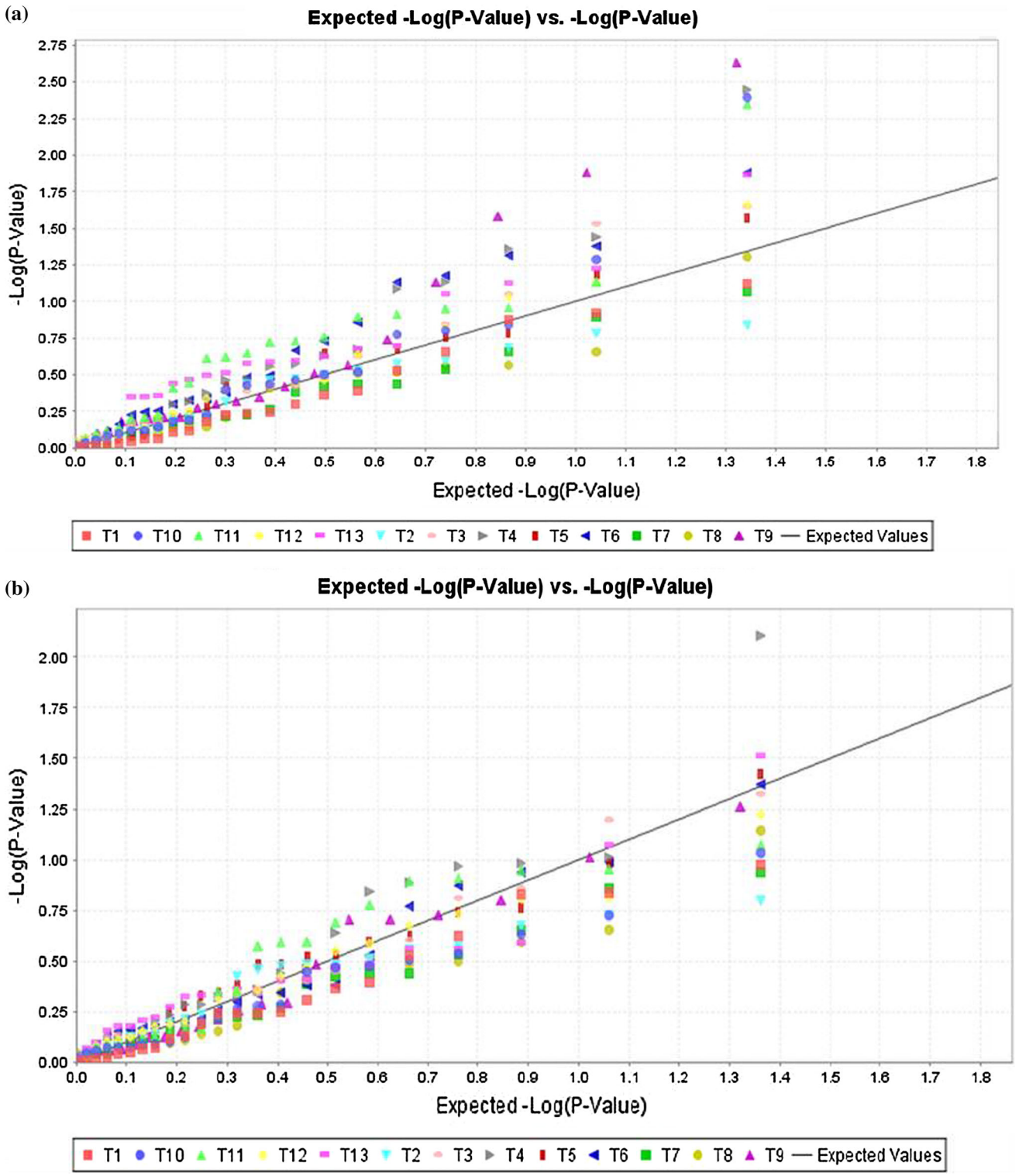

Figure 4. Quantile-quantile (QQ) plots of quality characters using GLM and MLM tests. (a) GLM-QQ plot, (b) MLM-QQ plot.

variation of 14.67%. Fe content in brown rice has significant marker-trait association with RM257 on chromosome 9 with a variation of 20.04%. From earlier reports (Kiranmayi *et al.* 2014), two markers (RM3322 on chromosomes 5 and RM7488 on chromosomes 6) were associated with both Fe and Zn content in brown rice grains. The present study has resulted in the identification of markers (RM246 on chromosome 1; RM241 and RM16427 on chromosome 4; RM421 on chromosome 5; RM3 on chromosome 6; RM11 and RM234 on chromosome 7; RM 257 on chromosome 9)

Table 6. Single and pleiotropic effects of the SSR markers with quality characters of rice.

Marker	Pleiotropic effects
RM246	HRR; length/breadth; kernel length after cooking; alkali spreading value; gel consistency
RM241	Kernel length; length/breadth; elongation ratio
RM16427	HRR; kernel length after cooking
RM421	Kernel length; length/breadth; amylose content per cent
RM3	Kernel length; length/breadth; kernel length after cooking
RM11	HULL; kernel length, length/breadth; kernel length after cooking; gel consistency
RM234	Kernel breadth; alkali spreading value
RM257	Iron content in brown rice, Iron content in polished rice.
Marker	**Single effect**
RM262	HRR
RM85	Elongation ratio
RM232	Gel consistency
RM164	Gel consistency, zinc content in brown rice
RM276	Volume expansion ratio
RM190	Kernel breadth
RM402	Kernel length
RM284	Amylose content per cent
RM6543	Amylose content per cent
RM235	Kernel length after cooking
RM206	Iron content in brown rice

associated with pleiotropic effects (table 6). These molecular markers would enable to assess the grain quality characters of rice (Blair and McCouch 1997; Joshi *et al.* 2000).

In conclusion, the present study indicated that 23 RM markers associated with 17 rice grain quality characters and found association for 14 traits with all the markers. Of the 23 markers, eight have shown >80.0% of PIC and seven markers with >70.0% of PIC, confirming the robustness of these markers for varietal identification and differentiation. A total of 37 significant marker-trait associations for 14 characters with R^2 ranging from 4.70 to 43.80% were detected. Seven markers showed association with several characters suggesting pleiotropic effect of the loci. The markers RM246 (HRR per cent, ratio of L/B, KLAC, ASV and GC), RM11 (HULL, KL, ratio of L/B, KLAC, GC), RM241 (KL, ratio of L/B and ER), RM16427 (HRR per cent, KLAC), RM 421 (KL, ratio of L/B, AC per cent), RM3 (KL, ratio of L/B, KLAC), RM234 (KB, ASV) and RM257 (Fe content in brown rice and Fe content in polished rice) showed pleiotropic effects. The results suggest that these markers could be efficiently used for the identification of rice genotypes with desired quality as well nutritional quality characters indicating their utility in the rice crop improvement programmes.

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