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Short Communication

Association analysis for grain quality traits in rice

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Abstract

Association analysis was conducted on a panel of accession of indigenous rice using SSR markers for nine traits related to grain and cooking quality parameters of rice. The value of PIC ranged from 0.316 (RM21945) to 0.738 (RM252) with an average of 0.505 for all the genotypes under study. A previously identified marker linked to amylose content, RM 190 on chromosome 6, showed a strong association for amylose content in this class of rice. Another marker on chromosome 7, RM11, also showed an association with amylose content. Two markers (RM169 and RM21945 with two alleles) showed associations with gel consistency. Four significant associations for gelatinization temperature were detected by four markers (RM11, RM12, RM21 and RM169). A total of six associations were detected for grain length, by five markers. For decorticated grain length 12 number of association was detected by seven markers. For grain length after cooking 11 associations were detected by six markers. Two associations were detected for grain width. Four associations were detected for decorticated grain width by three markers. Two associations were detected for grain width after cooking on chromosomes 4 and 5. The present study also validated the two linked markers, RM21936 for grain length and RMw513 for grain length after cooking, for use in MAS. The study indicates the presence of novel QTLs for a few traits under consideration. The study reveals the feasibility of undertaking association analysis in conjunction with germplasm characterization.

Key words: Association mapping, SSR marker, grain quality, cooking quality and indigenous rice

Rice is the important staple food crop for more than half of world population. In recent years due to rise in living standard, people started demanding quality rice with good eating and cooking quality asttributes with varied preferences across different regions. Breeding efforts are more focused on improving the quality of rice to meet out the specific type of quality rice demand of consumers as well as potential market demand for various commercial uses. Grain quality in rice is determined by many factors, such as, grain appearance, nutritional value, cooking and eating quality. Grain quality can be considered as physical, chemical, cooking and nutritional quality groups. Grain quality traits are controlled by major and minor quantitative trait loci (QTLs), implying that the genetic mechanisms underlying quality traits are complex. QTL analyses have identified several markers linked to grain quality (Shao et al. 2010; Ram et al. 2011; Fan et al. 2005; Lestari et al. 2009 and Tabkhar et al. 2012). But validation of those markers is essential to add value to those markers in a diverse set of germplasm before using them in marker aided breeding programme. Such information is very limited in rice particularly for quality traits. Therefore, the present investigation was carried out to find association of markers for quality traits.

The experimental material comprised of 65 genotypes of rice (Table 1). Among them, 24 genotypes were landraces and 41 were HYVs and advanced breeding lines. Pure seeds of 65 genotypes, collected from Instrumental Cum Research (ICR) farm and Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat were divided into three lots and from each lot data was recorded for each grain quality parameter under study at three different time interval. Within each lot each observation was

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Table 1. List of genotypes studied

Genotype	Pedigree	Genotype	Pedigree	Genotype	Pedigree	Genotype	Pedigree
IRRI		BokulJoha	Savitri/Badsabhog	KMJ Bora-9	Landrace	Panindra	Pankaj/Jagannath//Negheri Bao
IR 58025A	Male sterile line	Bor Malbhog	Landrace	KMJ-13A-1-12-3	Mahsuri/Luit	Piyoli	Pankaj/Mahsuri
IR 68897A	Male sterile line	Chilarai	IR 24/CR 44-118-1	Koimurali (Kmj M 142)	Landrace	Prafulla	Akisali/Kushal
IR 79156A	Male sterile line	Dikhow	Chilarai/Kalinga III	Kola Joha	Landrace	Ranga Bas	Landrace
Swarna Sub-1	Swarna with QTL Sub 1	DubariBao	Landrace	Kolong	Chilarai/Kalinga III	Ranga Bordhan	Landrace
IRRI, IARI		Gandhari	Advanced breeding line	Konjoha	Landrace	SadiyaLahi	Landrace
IR 64	IR5657-33-2-1/ IR2061-65-1-5-5	Gopinath	Pusa 2-21/IR 36	Konjoha boingaigaon	Landrace	Satyaranjan	IET 9711/IET 11161
IR 36	IR1561-228-1-2/ IR1737//CR	Jalashree	Pankaj/FR 13A	Konjoha Moran-2	Landrace	Saudang Bora-2	Landrace
Assam		Jalkunwari	Pankaj/FR 13A	Kapilee	Heera/Annada	SialiJoha	Landrace
Aghoni Bora	Gandhibora/KMJ 1-52-2	Joria	Landrace	Luit	Heera/Annada	Terabali	Landrace
Basant Bahar		Joymoti	Jaya/Mahsuri	Lakhman Dhan	Landrace	**Manipur**	
Basundhara	IET 9711/IET 11161	Jyotiprasad	K 343-29-1-1/Suweon 334	ManoharSali	Lati Sail/Guachari	Black Rice	Indigenous to Manipur
Betguti (Big)	Landrace	Kanaklata	Jaya/Mahsuri	Mohan	Advanced breeding line	**Malaysia**	
Betguti Small	Landrace	KetekiJoha	Savitri/Badsabhog	Moinagiri	Landrace	Mahsuri	Taichung65/ MayangEbos 6080/2
Bharoti	IR64/Mahsuri	KharikaJoha	Landrace	Moniram	Pankaj/Mahsuri	**Thailand**	
Bhogali	Ghewbora/KMJ 1-52-2	kmj 1-19-1	IR 8/Manohar Sali	MSE-9	Pureline of M. Sali	Padumoni (KDML 105)	Introduction from Thailand
Bishnuprasad	K 343-29-1-1/Suweon 334	KMJ Bora-52	Landrace	Mulagabhru	Jaya/Mahsuri	**ANGRAU**	
Boga Bordhan	Landrace	KMJ Bora-74	Landrace	NBR-2	Tainung Sen Glutinous 2/Joymoti	Sambha Mahsuri	GEB24/T(N)1//Mahsuri

recorded thrice and mean was considered as data for each replication. Amylose content, gelatinization temperature (GT) and gel consistency were determined (as per the procedure given by Cagampang et al. 1973; Sawbhagya and Bhattacharya 1979). Grain length, decorticated grain length, grain width and decorticated grain width were measured as per standard procedure. The total genomic DNA from each of the genotypes was extracted following the protocol of Plaschke et al. (1995) with slight modification. Twenty SSR markers selected from 36 primers were used to analyze the genetic variability for grain quality in all the genotypes (Table 1). The amplification conditions were based on the procedure of Rathi et al. (2014). SSR data scored as '1' for the presence of product and '0' for absence. SSR data were analyzed by using the software package NTSYS-pc Version 2.1 (Rohlf, 2000). Data on individual phenotypic trait were regressed on whole 1-0 binary marker data and association declared significant if $p<0.05$.

The analysis of variance revealed significant distinct variation among the genotypes for all amylose content, gel consistency, grain length, and decorticated grain length, grain length after cooking, grain width, decorticated grain width and grain width after cooking. Out of 20 primers 14 were polymorphic and six were monomorphic. The polymorphic primers yielded 32 alleles. The primers RM9, RM12, RMw513 and RM349 produced three alleles each and the rest produced two alleles. The number of alleles produced was less than that reported earlier (Wan et al. 2006). This might be attributed to genotypic difference and gel matrix used. The value of PIC of the marker ranged from 0.316 (RM21945) to 0.738 (RM252) with an average of 0.505 for all the genotypes under study, which reflect the better discriminatory power of these primers. Average PIC value, reflection of locus diversity, was higher in landraces (0.540) than HYVs (0.505) which indicated that markers under study were more efficient and useful to distinguish landraces and HYVs and other genotypes. The average similarity index between the genotypes indicated that landraces were also diverse.

Dendrogram revealed the existence of two major clusters and some class specific grouping pattern in genotypes under study. No strong correlation of clustering pattern with grain quality for studied parameters was observed. However, a grouping of six aromatic landraces was observed in cluster B. The clustering pattern also supported the relatedness of Manohar Sali with its two selections from the Manohar Sali in the same cluster. The another sub group composed of Mahsuri, Mahsuri derivatives (Sambha Mahsuri, Swarna Sub-1, Bharoti, Mulagabhru, Piyoli) along with IR 64. Mahsuri and Sambha Mahsuri showed more than 85% similarity. The clear cut divergence of NBR-2 from other genotype from other genotype might be attributed to the fact that NBR-2 is a hybrid genotype developed from the cross, Tainung Sen Glutinous 2/ Joymoti, which share no commonality with the materials under study except Joymati. Most of the members of cluster B2 were found to be similar in grain aroma and decorticated grain length. Information generated through cluster analysis based on genotypic data could be efficiently used in breeding rice varieties for improving grain quality traits.

Association analysis

Marker trait association analysis for various characters identified 47 significant associations ($P < 0.05$), with the R^2 values ranging from 4.5 to 32.7% (Table 2). Three associations were detected for amylose content, the two being on chromosome 6 by RM190, explaining 33.4 % of variation altogether. Another marker on chromosome 7, RM 11, also showed an association with amylose content. These two markers RM190 and RM11 together explained for 38% variation for amylose content in indigenous rice genotypes. The amylose content is under the control of the waxy (*Wx*) gene on chromosome 6 (Aluko et al. 2004; Wang et al. 2007) and Keipro et al. (2008) identified RM190 linked to the waxy locus. By detecting a strong association for amylose content, the present investigation has also validated the linkage of RM190 with amylose content indicating it's potentially for immediate use in MAS. Three QTLs for grain length were detected on chromosomes 3, 4 and 7 and *qGL4b*, had been fine mapped between RM5586 and RM3524 on chromosome 4 (Kato et al. 2011). Another association for grain length detected on chromosome 4 by RM252

Table 2. Association identified between SSR markers and grain quality parameters

S.No.	Trait	Marker	Chromo-some	P	R^2	S.No.	Trait	Marker	Chromo-some	P	R^2
1	AC	RM11_150	7	0.042	0.049	25	DGL	RM496_280	10	0.003	0.121
2	AC	OSR19_125	6	0	0.212	26	DGL	RM496_267	10	0	0.257
3	AC	OSR19_103	6	0.003	0.122	27	DGL	RM9_136	1	0	0.203
4	GL	RM21936_91	7	0.033	0.056	28	DGL	RM9_125	1	0	0.17
5	GC	RM169_180	5	0.011	0.083	29	GLAC	RM11_150	7	0.001	0.144
6	GC	RM21945_292	7	0.011	0.083	30	GLAC	RM11_135	7	0.001	0.14
7	GC	RM21945_287	7	0.035	0.054	31	GLAC	RM341_180	2	0	0.237
8	GT	RM11_150	7	0.02	0.069	32	GLAC	RM341_160	2	0	0.224
9	GT	RM12_170	12	0.05	0.045	33	GLAC	RM242_225	9	0	0.182
10	GT	RM21_160	11	0.036	0.053	34	GLAC	RM242_200	9	0	0.234
11	GT	RM169_165	5	0.009	0.091	35	GLAC	RM496_280	10	0.001	0.138
12	GL	RM252_216	4	0.006	0.099	36	GLAC	RM496_267	10	0	0.26
13	GL	RM341_160	2	0.021	0.067	37	GLAC	RM9_136	1	0	0.191
14	GL	RM242_225	9	0.043	0.049	38	GLAC	RM9_125	1	0	0.157
15	GL	RM242_200	9	0.007	0.097	39	GLAC	RMw513_260	5	0.025	0.063
16	GL	RM496_267	10	0.032	0.056	40	GW	RM252_206	4	0.01	0.088
17	DGL	RM11_150	7	0.002	0.127	41	GW	OSR19_103	6	0.043	0.049
18	DGL	RM11_135	7	0.003	0.116	42	DGW	RM252_206	4	0.016	0.074
19	DGL	RM12_190	12	0.05	0.045	43	DGW	RM169_165	5	0.024	0.064
20	DGL	RM341_180	2	0	0.195	44	DGW	OSR19_125	6	0.01	0.087
21	DGL	RM341_160	2	0	0.247	45	DGW	OSR19_103	6	0.005	0.103
22	DGL	RM337_170	8	0.012	0.082	46	GWAC	RM252_216	4	0.039	0.051
23	DGL	RM242_225	9	0	0.239	47	GWAC	RM169_165	5	0.002	0.126
24	DGL	RM242_200	9	0	0.327						

P = Probability value; R^2 = Coefficient of variation. *AC = Amylose content; GC = Gel consistency; GT= Gelatinization temperature; DGL = Decorticated grain length; GLAC = Grain length after cooking; GW = Grain width; DGW = Decorticated grain width; GWAC = Grain width after cooking

which is near to QTL for grain length, *qGL4* [9], thereby confirming the location of a gene for grain length on chromosome 4. The marker RM21936 detected one association on chromosome 7 for grain length which explained 5.6 % of variation. The marker RM21936 was identified to be linked with QTL for grain length, explaining 20% variation (Shao et al. 2010), thereby suggesting a strong potentiality of RM21936 marker to use in MAS. Maximum variation (32.7 %) was explained by the marker RM242 on chromosome 9 for decorticated grain length. For grain length after cooking, 11 associations were detected, of which association of markers on chromosomes 2 and 9 with grain length after cooking as reported by otherss (Ge et al. 2005; Yang et al. 2013). Present investigation also confirms the presence of a QTL for decorticated grain width on chromosome 5 by the marker RM169 with that of Zhang et al. (Zhang et al. 2013). Two associations were detected for grain width after cooking on chromosome and also confirmed the presence of a QTL for cooked grain width on chromosome 5 (Ge et al. 2005). The present study highlighted the utility identification of marker-trait associations along with diversity analysis for use in MAS. But the associations of other markers without any published report of such association needs to validated through bi-parental approach. Moreover, use of association mapping approach with more marker coverage across the genome is required for more precise location of gene and marker validation.

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