RESEARCH NOTE

QTL detection of rice grain quality traits by microsatellite markers using an *indica* rice (*Oryza sativa* L.) combination

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Introduction

The gelatinization temperature (GT), gel consistency (GC) and amylose content (AC) are the three major rice traits that are directly related to cooking and eating quality (Little *et al*. 1958). GT is a physical trait responsible for cooking time and the capacity to absorb water during the processes cooking, and the temperature at which starch irreversibly loses its crystalline order during cooking. The GC is responsible for softness, and the AC is responsible for texture and appearance in rice. Hence, regulating AC in rice has been a major concern of rice breeders. To facilitate the development of new varieties with high cooking and eating qualities, it is necessary to understand the genetic bases of such traits.

Lanceras *et al*. (2000) found four QTLs for AC on chromosomes three, four, six and seven. These QTLs accounted for 80% of phenotypic variation observeded in AC. Two QTLs on chromosome six, and one on chromosome seven were detected for GC, which accounted for 57% of phenotypic variation. Umemoto *et al*. (2002) confirmed this and demonstrated that the *alk* locus encodes the enzyme soluble starch synthase IIa. Bao *et al*. (2002) and Lanceras *et al*. (2000) found the effect of the *wx* region on GC. However, He *et al*. (1999) and Bao *et al*. (2002) showed that GC is controlled by two QTLs with minor effects.

Zhou *et al*. (2003) improved the eating and cooking quality of Zhenshan 97 by introgressing the waxy gene region from Minghui 63 (*wx*-MH), a restorer line that had medium AC, soft GC and high GT. Li *et al*. (2004) identified four QTLs for AC, three for GT and five for GC using backcross-inbred lines. The four QTLs for AC were located on chromosomes three, four, five and six. The QTL on chromosome six covered the *wx* gene region and mainly contributed to the variance between *japonica* and *indica* varieties (Li *et al*. 2004). The other QTLs were probably minor factors controlling AC. Fan *et al*. (2005) detected a total of 12 main-effect QTLs for the three traits, with a QTL corresponding to the *wx* locus showing a major effect on AC and GC, and a QTL corresponding to the *alk* locus having a major effect on GT. Yong *et al*. (2006) found seven different QTLs including two for AC, three for GT, two for GC at six chromosomal regions controlling complex traits related to rice grain quality. Wada *et al*. (2006) mapped four QTLs for AC on chromosomes three, seven, nine and 12. One QTL for texture was identified on chromosome three. Yong *et al*. (2006) also reported that a major QTL for AC, qAC-6 was located on chromosome six. Using 110 polymorphic SSR markers and 209 recombinant inbred lines Amarawathi *et al*. (2008) mapped QTLs related to AC, GC and GT on seven different chromosomes.

Grain quality is an economically important trait in rice, and any information about the genetic mechanisms governing grain quality traits will be useful for the rice breeders. Unfortunately, such information on Iranian rice germplasm is limited. In this study, we developed a linkage map with 74 simple sequence repeat (SSR) markers. Genetic linkage map covered 1231.5 cM of rice genome. The results showed that AC, GC and GT are predominantly influenced by two intervals on chromosome eight. The RM4955-RM152 interval was found to contain the QTLs that explained the most variation in AC, GC and GT. qGT-8a, qGC-8a and qAC-8a are identified as major genes and explained 20.21%, 24.22% and 22.10% of the total phenotypic variance, respectively. Markers of this interval might be useful in marker-assisted breeding to improve rice genotypes.

Materials and methods

Two parent rice varieties Tarommahalli (TAM) and Khazar (KHZ) were used in this experiment. The former is a traditional variety with high grain quality with low AC, a soft GC

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and a high GT, and the latter is of poor quality because of high AC, a hard GC and a low GT.

A total of 365 SSR primer pairs were surveyed based on their polymorphism between two parents. Simple and composite interval QTL mapping related to quality traits was conducted using the QTL Cartographer version 2.5 (Basten *et al*. 2001) software. A minimum LR score of 9.2 was used for the identification of putative QTLs, and the percentage of total phenotypic variation and additive effects explained by each QTL for grain quality traits were calculated.

The GT was determined using the method of Little *et al*. (1958) with minor modifications. Each sample was tested three times, and each time, six intact milled grains were put in a weighing boat, to which 10 ml of 1.7% KOH was added. The degree of disintegration and transparency of paste dissolved out of the kernels were evaluated using a 7-point scale as described by Little *et al*. (1958) via visual observation in seven categories from one (unaffected) to seven (completely dissolved).

The GC was measured in duplicate according to the method of Cagampang *et al*. (1973). 100 mg of rice flour was weighed in a 10 mm × 110 mm culture tube, to which 0.2 ml of 0.95% ethanol containing 0.025% thymol blue was added to prevent clumping of the powder during gelatinization (Cagampang *et al*. 1973). Two ml of 0.2 M KOH solution was added to each tube and vortexed thoroughly, then the tubes were placed in boiling water bath for 8 min. The gel occupied about 2/3 of the tubes. After standing at room temperature for 5 min; tubes were placed on ice for 20 min, and then laid down horizontally on a table surface. After 1 h, the total distance that the gel had extended down the side of the tubes was measured and the length (mm) from the bottom of the tube to the top of the gel was reported as the measure of GC. The longer the distance corresponded softer is the gel.

AC was estimated using the procedure of Juliano (1971) with minor modification (Williams *et al*. 1958; Perez and Juliano 1978). A set of 20 polished grains were ground to a fine powder with mortar and pestle, and sieved through a 0.40 mm screen. Rice flour weighing 100 mg was extracted overnight in a solution of 1 ml absolute ethanol and 9 ml 1 N NaOH. This suspension was heated in a boiling water bath for 10 min, followed by cooling to room temperature. Samples were diluted to 100 ml with distilled water. A 5 ml sample suspension was added to 50 ml distilled water in a 100 ml flask and 1 ml of 1 M acetic acid was added to acidify the sample along with 1.5 ml idionic solution (0.2% I_2 in 2% KI). Distilled water was added to a volume of 100 ml and the suspension was mixed well and then kept for 20 min. As a control, 5 ml of 0.09 M NaOH solution was used to replace the sample suspension. This control solution was used to calibrate to 0 at 620 nm with a spectrophotometer. Samples with known values of high, medium, and low AC were used to draw the standard amylose curve. The AC of sample relative to the sample dry weight was determined by comparison to this standard curve.

Results and discussion

Frequency distribution in the $F_{2:3}$ for segregating phenotypic classes for the three grain quality traits studied are shown in figure 1. AC and GC were measured on a quantitative scale and showed continuous variation with normal distribution. The $F_{2:3}$ families showed transgressive segregation for all three traits. Two QTL of relatively small effects were identified, which were qGT-1 and qGT-8 explaining 5.52% and 9.82% of the variance, respectively. At qGT-8 locus, the allele from TAM had a positive effect, while at qGT-1 locus the pattern of effects was reversed (table 1).

Only one QTL for GC was detected (qGC-4), which accounted for 7.91% of the phenotypic variance. At qGC-4 locus, the allele from TAM had a negative effect on GC (table 1). A total of four QTLs were resolved, designated as qAC-5, qAC-8a, qAC-8b and qAC-12 which explained 11.33%, 20.21%, 14.45% and 13.33% of the variance, respectively. The QTL with largest effect (qAC-8a) was located at the interval RM4955-RM152 on chromosome eight (table 1; figure 2). For all the QTLs, the alleles from TAM had negative effects, except for qAC-12 which had positive effect.

Four QTLs were found responsible for GT, which were qGT-4, qGT-8a, qGT-8b and qGT-11, explaining 20.11%, 24.22%, 19.34% and 25.10% of the variance, respectively. The QTL with largest effect (qGT-11) was located at interval between RM144 and RM1341 on chromosome 11 (table 1; figure 2). For all the QTLs, the alleles from TAM had positive effect. A total of eight QTLs were identified for GC using QTL Cartographer version 2.5, one each on chromosomes five and six, and two each on chromosomes four, eight and 12. The most effective QTL, qGC-8a with a LOD score of 9.30 was located on the chromosome eight between SSR markers RM4955 and RM152 (figure 2), and explained 22.10% of the phenotypic variation. There were two important QTLs for GC located on chromosomes 8 and 12, designated as qGC-8b and qGC-12b, respectively. The qGC-8b locus mapped on chromosome eight in the interval RM152-RM8264 (figure 2) with a LOD score of 10.52 and explained 16.40% of the phenotypic variation, whereas qGC-12b was located in the marker interval RM7627-RM1337 (figure 2) on chromosome 12 with a LR score of 14.48 and explained 16.56% of the phenotypic variation. The positive alleles for three loci (qGC-6, qGC-12a and qGC-12b) were contributed by the KHZ (table 1; figure 2).

Our results showed that AC, GC and GT are predominantly influenced by two QTLs on chromosome eight (qGT-8a, qGT-8b, qGC-8a, qGC-8b, qAC-8a and qAC-8b). The qGT-8a, qGC-8a and qAC-8a were identified as major genes and explained 20.21%, 24.22% and 22.10%

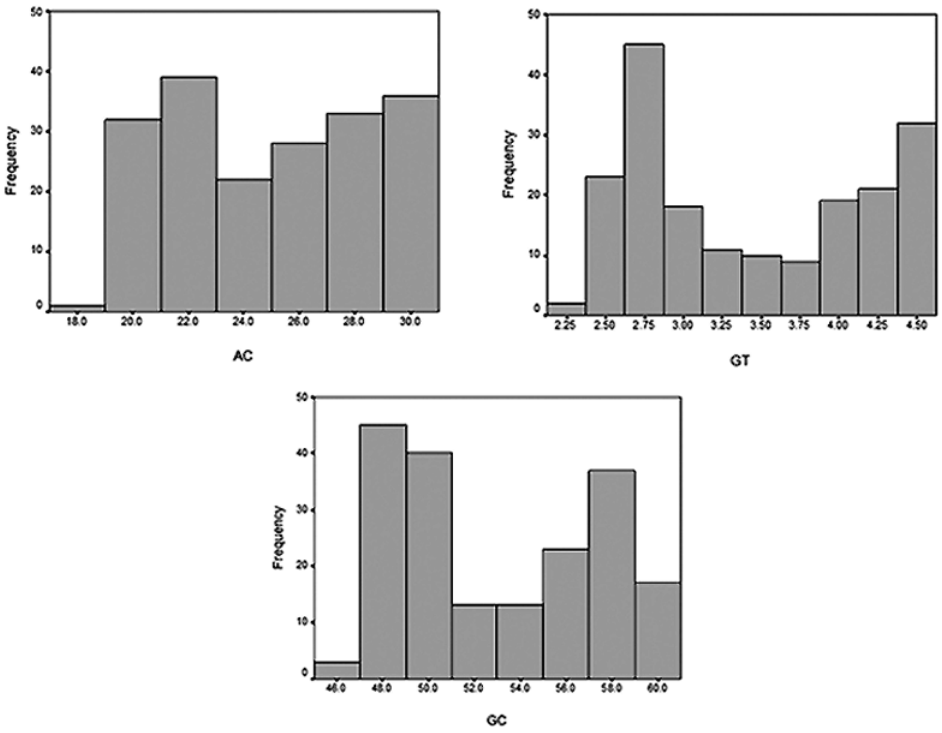

Figure 1. Frequency distribution of rice grain quality traits in Iranian rice population.

Table 1. Putative QTLs for salt tolerance in seedling stage of the F_2 population derived from TAM and KHZ.

Traits	QTL[a]	Chromosome	Flanking markers	LR	a[b]	d[c]	d/a	PEV[d]	Dpe[e]
			Interval mapping						
GT	qGT-1	1	RM543-RM8231	9.879	−0.27	0.27	−1.00	5.52	KHZ
	qGT-8	8	RM4955-RM6208	13.945	0.18	0.19	1.06	9.82	RAM
GC	qGC-4	4	RM6589-RM5473	12.412	−1.61	1.70	−1.06	7.91	TAM
			Composite interval mapping						
AC	qAC-5	5	RM480-RM3345	11.53	−0.53	−1.01	1.91	11.33	TAM
	qAC-8a	8	RM4955-RM152	12.53	−0.78	−0.94	1.21	20.21	TAM
	qAC-8b	8	RM152-RM8264	14.26	−0.91	−0.74	0.81	14.45	TAM
	qAC-12	12	RM276-RM7626	10.90	1.82	−1.54	−0.85	13.33	KHZ
GT	qGT-4	4	RM6589-RM5473	11.75	0.16	−0.38	−2.38	20.11	TAM
	qGT-8a	8	RM4955-RM152	15.82	0.28	−0.01	−0.04	24.22	TAM
	qGT-8b	8	RM152-RM8264	16.80	0.27	0.03	0.11	19.34	TAM
	qGT-11	11	RM144-RM1341	14.65	0.50	−0.41	−0.82	25.10	TAM
GC	qGC-6	6	RM5371-RM340	12.80	0.60	2.27	3.78	18.11	KHZ
	qGC-4a	4	RM5642-RM5709	14.07	−1.70	2.39	−1.41	14.15	TAM
	qGC-4b	4	RM5709-RM551	13.21	−2.24	1.86	−0.83	15.42	TAM
	qGC-5	5	RM480-RM3345	9.37	−0.20	−1.36	6.80	14.81	TAM
	qGC-8a	8	RM4955-RM152	9.30	−0.81	−0.88	1.09	22.10	TAM
	qGC-8b	8	RM152-RM8264	10.52	−0.53	−1.13	2.13	16.43	TAM
	qGC-12a	12	RM276-RM7626	14.32	2.56	−0.95	−0.37	15.21	KHZ
	qGC-12b	12	RM7627-RM1337	14.48	2.49	−1.39	−0.56	16.56	KHZ

[a]QTLs are named by abbreviations plus chromosomal number.
[b]Additive effect.
[c]Dominant effect.
[d]Percentage of total phenotypic variance explained by the QTL.
[e]Direction of phenotypic effect, TAM and KHZ indicate Tarommahalli and Khazar, respectively.

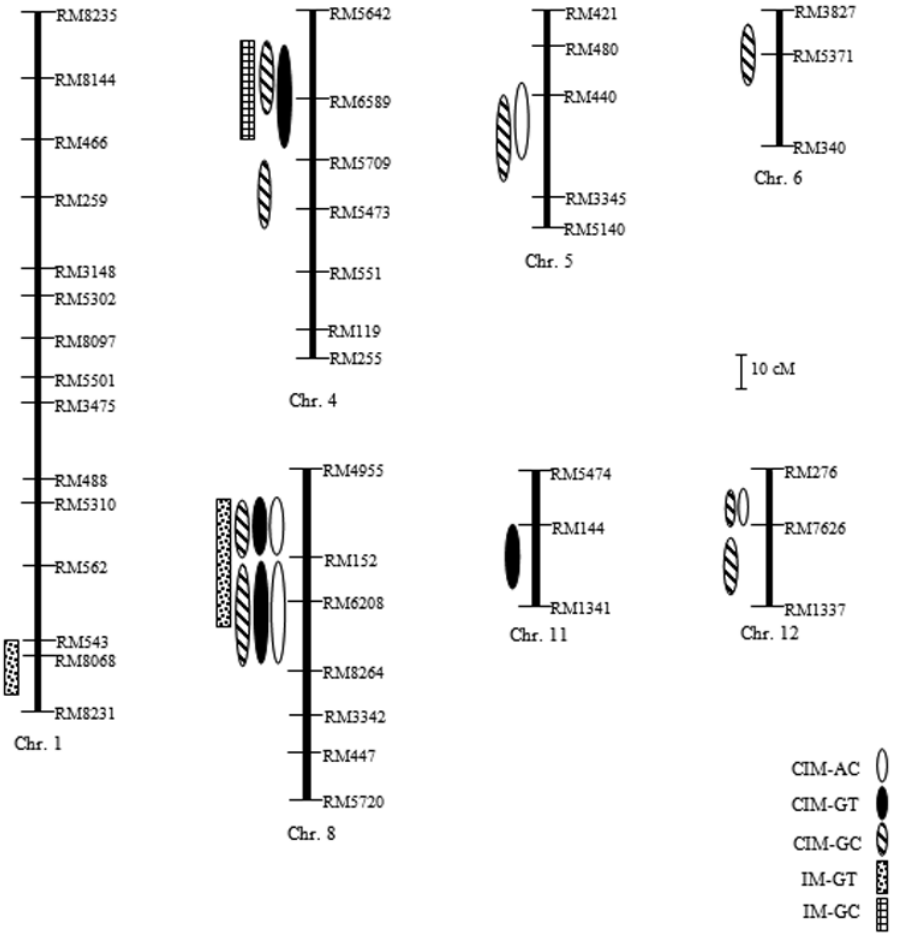

Figure 2. The position of QTL for traits representing grain quality of Iranian rice population.

of the total phenotypic variance, respectively. The RM4955-RM152 interval (figure 2) is found to be most informative with the QTLs related to AC, GC and GT. These results were inconsistent with Tan *et al*. (1999), He *et al*. (1999), Lanceras *et al*. (2000), Umemoto *et al*. (2002) and Tian *et al*. (2005) who reported that GC was controlled by the *alk* gene on chromosome six. It was possibly due to the specifically Iranian germplasm used in our study. We mapped QTLs related to quality traits on Iranian aromatic rice (for example TAM) for the first time. In this study, some of QTLs related to quality traits were not detected. Precise detection of QTLs for quality traits in Iranian rice population remained a problem due to less SSR markers and low density linkage map and thus it is suggested that further study should be performed with more SSR markers and perpetual mapping population. Henceforth, it is necessary to analyse genes for rice grain quality traits in other varieties using new molecular methods and to map different genes for quality traits of rice. Further, although some of the quality related QTLs identified through the study of progeny from crosses between TAM and KHZ have been analysed at the molecular level, these QTLs could not be characterized in detail because of the lack of appropriate advanced backcross progenies or NILs. Further genetic and molecular analysis of these QTLs may provide a clue to understand the divergent features in the genetic control mechanisms of the quality traits of Iranian rice population.

However, our result showed that GC influenced by a gene on chromosome six (qGC-6). We detected three separate QTLs on chromosomes four, five and 12 for GC. Thus, this study clearly demonstrates that a region of chromosome eight plays a major role in determining these commonly measured cooking qualities. Several groups reported that a major QTL for AC was mapped on *wx* locus region of chromosome six (He *et al*. 1999; Tan *et al*. 1999; Lanceras *et al*. 2000; Li *et al*. 2004; Fan *et al*. 2005; Tian *et al*. 2005). Lang and Buu (2004) analysed parental genotypes with 20 SSR primers in chromosome six. One primer pair for the locus *wx*F-R showed association with AC. In this study four QTLs were mapped for AC, of which one each were on chromosomes five and 12, and two were on chromosome eight. Any QTL related to AC was not detect on chromosome six but one QTL identified for AC (qAC-5) on chromosome five that might be the same loci detected by Tanaka *et al*. (1998) and Li *et al*. (2004). Tanaka *et al*. (1998) reported three other QTLs on chromo-

somes two, three and six using DH lines and Li *et al*. (2004) also mapped three other QTLs on chromosomes three, four and six using the BIL lines.

In conclusion, based on the comparison of chromosomal location, the result showed that some QTLs are likely in the same locus as in previous study. However, it is difficult to determine whether QTLs are in the same locus or are tightly linked. Therefore, further analysis, including fine mapping of some QTLs (qAC-5 and QTLs of RM4955-RM152 interval) using common markers, cloning and sequence comparison of these QTLs, will be required to answer these questions. This experiment achieved a considerable enhancement of an available detection of AC in $F_{2:3}$ populations. Plant breedering for effective selection can utilize the identification of a major locus for AC located nearby a microsatellite marker. Other microsatellite marker could be used to trace the flow of genes or quantitative traits loci of interest in rice and to make predictions about the outcome of crossing and selection programme that will increase the future efficiency of cultivar development. Zhou *et al*. (2003) using marker-assisted selection in three generations of backcrossing followed by one generation of selfing, introduced the *wx*-MH fragment from Minghui 63 into Zhenshan 97B. The introduction of this fragment has greatly improved the cooking and eating quality of the rice grains. The results of these experiment increased our understanding of the genetic components of grain quality, which in turn will help rice breeders formulate strategies for improving the cooking and eating qualities of rice.

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