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Analysing genetic control of cooked grain traits and gelatinization temperature in a double haploid population of rice by quantitative trait loci mapping

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Abstract Cooking quality in rice grains is a complex trait which requires improvement. Earlier reports show varying genetic influence on these traits, except for a common agreement on waxy (*Wx*) and alkali degeneration (*Alk*) loci on chromosome 6. The present study involved 86 doubled haploid lines derived from an *indica* × *japonica* cross involving IR64 and Azucena. Grain parameters viz., raw grain length (RGL), raw grain breadth (RGB), cooked grain length (CGL), cooked grain breadth (CGB), gelatinization temperature (GT), grain shape (RGS), length elongation ratio (LER) and breadth expansion ratio (BER) were subjected to mixed model mapping of quantitative trait loci (QTL). Segregation data of 175 markers covering a distance of 2395.5 cM spanning the entire genome were used. Fifteen main effect QTLs were detected spread over the genome, except on chromosomes 4, 8 and 11. Thirty epistatic interactions significantly influencing the traits were detected. Twelve of the main effect QTLs were involved in epistatic interactions. One main effect QTL associated with LER was detected near *Alk* locus. QTLs located for grain length on chromosomes 9 and 10 are reported for the first time. Detection of many epistatic loci and involvement of main effect QTLs in interactions demand for judicious selection of QTLs in marker-assisted selection programmes.

Keywords Cooked grain quality · Gelatinization temperature · Quantitative trait loci · Epistatic effects

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Introduction

Improvement of grain quality is one of the major objectives in rice (*Oryza sativa* L.) breeding. Grain quality as a whole is an integrated trait comprising of grain size, shape, aroma, texture and appearance, cooking, palatability, nutritional and processing qualities. A lot of genetic variability for these traits exists within the rice gene pool, with remarkable differences between two major subspecies viz., *indica* and *japonica*.

Yano and Sasaki (1997) suggested that rice grain quality is under the influence of quantitative alleles

showing continuous variation. Earlier genetic investigations reported monogenic (Chao 1928; Ramaiah et al. 1931), digenic (Bollich 1957) and trigenic (Ramiah and Parthasarathy 1933) inheritance for grain length besides polygenic inheritance for grain length and breadth (Jones et al. 1935; Chang 1974), but the results are inconclusive and limited (Govindaraj et al. 2005). Cooking quality of rice can be attributed to three major grain properties viz., grain elongation, volume expansion and water absorption (Ge et al. 2005). It is determined by the physicochemical properties of the endosperm viz., amylose content (AC) (Webb 1980; Juliano 1985), gel consistency (GC) (Cagampang et al. 1973) and gelatinization temperature (GT) (Little et al. 1958). Good cooking quality was associated with greater linear elongation, less volume expansion and less water absorption (Tang 1987). The raw grain dimensions like grain length and breadth, changes occurring to these traits on cooking and the temperature at which rice grain becomes gelatinous during cooking (GT) are measured for determining overall cooking quality (Govindaraj et al. 2005). GT is often indirectly estimated through alkali spreading value (ASV). ASV is inversely proportional to GT.

Since the rice grain quality traits have low heritability, it is particularly difficult for breeders to efficiently improve these traits using conventional selection methods. Modern molecular marker based approaches provide an opportunity of looking into the phenotypic variation in specialised segregating populations, in association with segregation of DNA markers distributed over the entire genome. In recent years, establishment of permanent segregating populations, such as doubled haploid (DH) lines or recombinant inbred lines (RIL), has provided researchers with genetically homozygous starting materials for genetic analyses such as QTL mapping. These populations have been widely employed to identify markers closely linked to quantitative trait loci (QTLs) for target traits. The identification of QTLs and the elucidation of their genetic control are essential for the development of efficient marker-assisted selection (MAS) aimed at improving breeding efficiency.

A brief scan of earlier studies on the genetic analyses of cooked grain traits reveals reports of a single major locus influencing AC, GC and GT (Bollich and Webb 1973; McKenzie and Rutger 1983; Kumar and Khush 1988; He and Lu 1993). Subsequently this locus was located on the short arm of chromosome 6 (Huang et al. 2000; Yan et al. 2001; Umemoto et al. 2002) and linked to waxy (*Wx*) gene (McKenzie and Rutger 1983; Sano 1984; Okagaki and Wessler 1988; Tan et al. 1999; Zhou et al. 2003). However, unlike AC and GC, the inheritance of GT was reported to be complex, showing quantitative variation (Beachell and Stansel 1963) and the expression of which is often modified by minor genes at other loci (Chang and Li 1991). Significant influence of another locus on chromosome 6, encompassing alkali degeneration (*Alk*) gene (Kudo 1968; He et al. 1999; Umemoto et al. 2002) encoding for soluble starch synthase II a (Gao et al. 2003) has also been reported.

Earlier, Ahn et al. (1993) mapped a QTL for cooked kernel elongation on chromosome 8 using RFLP markers. Bao et al. (2002) identified QTLs for AC, GC, GT and six paste viscosity parameters of grains in a DH population derived from IR64/Azucena. Aluko et al. (2004) reported pleiotropic influence of QTLs on chromosome 6 corresponding to the location of *Wx* gene on AC, ASV, grain length and length–breadth ratio. Ge et al. (2005) identified three QTLs for cooked grain elongation on chromosomes 2, 6 and 11, with substantial influence of a QTL near the *Wx* locus influencing linear elongation, volume expansion and water absorption. Govindaraj et al. (2005) described influence of QTLs on chromosome 6 for grain breadth and on chromosome 12 for cooked grain breadth by bulked segregant analysis. Li et al. (2003) described significant influence on GT by a QTL on chromosome 6, linked to *Alk* locus. Fan et al. (2005) and Tian et al. (2005) reconfirmed the presence of QTLs associated with GT, at this locus. Albeit the common understanding on the influences of *Wx* and *Alk* loci, previous works also reveal association of many major and minor loci with individual and/or epistatic effects on raw and cooked grain traits, which vary depending on the mapping populations used (Tan et al. 2000; Li et al. 2003; Rabiei et al. 2004; Wan et al. 2004; Fan et al. 2005; Ge et al. 2005; Jiang et al. 2005; Tian et al. 2005; Hagiwara et al. 2006).

The objectives of the present study was (a) to elucidate QTLs associated with the cooking quality in rice grains and with GT, and (b) to understand the genetic control of these QTLs in a DH line population derived from a cross between IR64 and Azucena, representing two subspecies, *indica* and *japonica*. This information can enrich the understanding on the

quantitative genetic control of these traits, for formulating efficient MAS strategies in future. To our knowledge this is the first study on this DH population for the grain traits investigated here. We report a few QTLs that have not been previously described for these traits.

Materials and methods

Plant materials

A set of 86 DH lines obtained from International Rice Research Institute, Metro Manila, Philippines was used in this study. The DH lines were generated by in vitro anther culture of F_1 between IR64, a semi-dwarf, heavy-tillering, high-yielding, widely grown variety adapted to irrigated rice-growing regions of Asia, and Azucena, a tall, sparse-tillering, low-yielding, long-grained aromatic variety adapted locally to upland conditions in the Philippines (Guiderdoni et al. 1992).

Phenotyping of grain traits

Freshly harvested matured seeds from the DH lines naturally cured to approximately 14% moisture content were used for the study. For each line, well dried uniform sized seeds were selected and husked. Length (RGL) and breadth (RGB) of ten unbroken raw kernels per replication were measured using a micrometre and expressed in millimetres. Three replications were maintained. Cooked grain traits were measured from the selected hulled kernels. Cooking was done by partial modification of the method by Azez and Shafi (1966). The selected kernels were placed separately in individual porous cloth bags, tied and presoaked in lukewarm water for 5 min. The soaked grains were cooked by immersing the cloth bags in boiling water for 17 min. The bags were taken out, water drained and the cooked grains were spread for drying at room temperature for 1 h. Length (CGL) and breadth (CGB) of ten unbroken cooked kernels per replication were measured in millimetres and averaged for three replications. GT was assessed indirectly as the ASV of hulled kernels as per modified procedure of Little et al. (1958). Thirty whole grains, were immersed in petri-plates containing 1.7% KOH in such a way that no two grains were in contact with each other. The plates were then incubated for 24 h at room temperature. The ASV were determined by visual scoring of the appearance of the grains and disintegration on a 1–7 linear scale, as 1 = grains not affected, 2 = grains swollen, 3 = grains swollen, collar incomplete and narrow, 4 = grain swollen, collar complete and wide, 5 = grains split or segmented, collar complete and wide, 6 = grain dispersed, merging with collar and 7 = grain completely dispersed and intermingled. Since ASV is inversely related to GT the higher value of ASV was taken for low GT and vice versa. A rating of 1.00–2.99 was taken as high GT (>74°C), 3.00–4.99 as intermediate (69–74°C) and 5.00–7.00 as low GT (55–68°C). Three derived traits, raw grain shape (RGS = RGL/RGB), linear elongation ratio (LER = CGL/RGL) and breadth expansion ratio (BER = CGB/RGB)) were also computed from original traits.

Genotyping

The molecular marker data developed for IR64/Azucena DH population (Huang et al. 1997) with 175 polymorphic markers comprising of 146 restriction fragment length polymorphisms (RFLP), three isozymes, 14 randomly amplified polymorphic DNA (RAPD) and 12 cloned genes was used. The map of 2395.5 cM length and covering the entire genome with an average marker distance of 13.7 cM, was analysed for identifying putative QTLs governing the traits involved in the study.

Data analysis

The phenotypic data were statistically analysed for variability and interrelations. The whole genome was scanned for putative main effect and epistatic QTLs using QTLMAPPER V.1.60 (Wang et al. 1999) using mixed model mapping procedures. In the analysis, likelihood ratio (LR) and *t*-test were combined to test the hypotheses on both the genetic effects and digenic epistatic effects. Estimates of main effect and epistatic QTLs were obtained by maximum likelihood estimation method. A Bayesian test was performed for the estimation of QTL effects and also for the estimating the probability of estimates. LR values corresponding to $P = 0.005$ were used for claiming the threshold for main effect and epistatic QTLs. Main effect QTLs were designated as per McCouch et al. (1997).

Results

Variability and association among traits

The statistical description of the grain parameters is presented in Table 1. Both the parents differed significantly for the traits studied. The female parent, Azucena had high CGB, GT, LER and BER, while IR64 had longer grains and high RGS. The DH lines showed normal distribution of traits except for RGB, RGS and BER, with substantial variation including transgressive variants.

Among the traits, RGL was positively correlated with RGB, RGS, CGL and CGB and negatively with LER. RGB was positively associated with CGB, but negatively with RGS and BER. CGL had high positive association with CGB, GT, LER and BER, while CGB showed significant positive association with BER. Besides the positive association with CGL, GT was also associated with LER. RGS was positively associated to BER but negatively to LER (Table 2).

Detection of putative main effect and epistatic QTLs

A total of 15 main effect QTLs distributed over all chromosomes except on 4, 8 and 11 were detected for the grain traits under study (Table 3). Further, 30 digenic QTL pairs were detected showing interaction effects on the traits, of which 12 pairs contained twelve main effect QTLs (Table 4). Eight pairs of digenic QTLs showed additive effects ranging from 1.5% (for intervals RZ448–RZ519/RG118–Adh1 for CGL) to 44.7% (for interval pair Sdh1–RG463/RG958–RG181 for CGL), while 24 QTL pairs showed epistatic interactions. The maximum contribution of epistatic effect was 27.5% exhibited by RZ678–RZ574/CDO127–RZ638 for RGB.

Table 1 Statistics of the grain quality parameters among IR64 × Azucena doubled haploid lines, along with parental means

Trait		Mean	Variance	Range	K–S value[a]	IR64	Azucena
Raw grain length (mm)	RGL	6.47	0.294	5.53–7.83	0.117**	7.10	6.73
Raw grain breadth (mm)	RGB	1.91	0.022	1.57–2.40	0.086*	1.87	2.00
Grain shape	RGS	3.41	0.114	2.48–4.25	0.050[ns]	3.81	3.37
Cooked grain length (mm)	CGL	9.19	0.829	7.60–11.57	0.151**	8.80	10.70
Cooked grain breadth (mm)	CGB	3.22	0.068	2.63–3.93	0.083[ns]	2.87	3.73
Length elongation ratio	LER	1.43	0.023	1.11–1.84	0.088*	1.24	1.59
Breadth expansion ratio	BER	1.69	0.026	1.22–2.15	0.078[ns]	1.54	1.87
Gelatinization temperature	GT	2.35	0.967	1.00–6.00	0.192**	1.67	5.00

[a] Kolmogorov–Smirnov test for normality

*, ** Significant at $P = 0.05$ and 0.01 respectively

[ns] Non-significant

Table 2 Pearson correlation coefficients between grain quality traits from 86 doubled haploid lines of IR64 × Azucena

	RGL	RGB	RGS	CGL	CGB	LER	BER	GT
RGL	1.000							
RGB	0.217*	1.000						
RGS	0.653*	−0.588*	1.000					
CGL	0.248*	0.123	0.089	1.000				
CGB	0.272*	0.262*	0.026	0.415*	1.000			
LER	−0.511*	−0.031	−0.413*	0.702*	0.198	1.000		
BER	0.062	−0.571*	0.504*	0.233*	0.631*	0.172	1.000	
GT	−0.167	−0.113	−0.061	0.472*	0.146	0.543*	0.195	1.000

* $P = 0.05$

RGL, raw grain length (mm); RGB, raw grain breadth (mm); RGS, grain shape; CGL, cooked grain length (mm); CGB, cooked grain breadth (mm); LER, length elongation ratio; BER, breadth expansion ratio; GT, gelatinization temperature

Table 3 Putative main effect QTLs detected by two-locus mixed model analysis for the grain parameters at the likelihood threshold of 7.88 at $P = 0.005$

Trait	Chrom	Interval	QTL	A_i	LOD	$h^2(A_i)$	Total $h^2(A)$
Raw grain length	2	RG437–RG544	*qRGL2*	0.174*	5.62	9.97	44.43
	9	Amy3ABC–RZ228	*qRGL9*	−0.219*	8.05	15.80	
	10	G1084–RG257	*qRGL10*	0.238*	9.60	18.66	
Raw grain shape	9	Amy3ABC–RZ228	*qRGS9*	−0.143*	11.39	16.82	25.55
	10	G1084–RG257	*qRGS10*	0.103*	5.39	8.73	
Cooked grain length	2	RG157–RZ318	*qCGL2*	−0.319*	9.36	12.12	57.33
	12	Sdh1–RG463	*qCGL12*	−0.616*	20.61	45.21	
Cooked grain breadth	1	RZ801–RG810	*qCGB1*	0.057*	4.25	4.30	48.86
	3	RZ337A–RZ448	*qCGB3*	−0.149*	19.70	29.40	
	7	CDO418–RZ978	*qCGB7*	−0.107*	12.30	15.16	
Length elongation ratio	6	RZ667–Pgi2	*qLER6*	0.046*	3.31	9.13	9.13
Breadth expansion ratio	3	RG104–RG348	*qBER3*	−0.062*	5.88	13.72	37.14
	3	RZ448–RZ519	*qBER3*	−0.081*	8.55	23.42	
Gelatinization temperature	2	RG256–RZ213	*qGT2*	−0.391*	5.61	14.41	29.80
	5	RZ70–RZ225	*qGT5*	0.404*	5.97	15.39	

* A_i values are the additive effects of the *i*th QTL, and are significant at $P = 0.005$ by two-tail probabilities of studentized *t* values. h^2 is the contribution of the QTL effect to the total phenotypic variation

Raw grain traits

Three major QTLs were detected on chromosomes 2, 9 and 10 for RGL, with a cumulative phenotypic contribution of 44.4%. Among these, the QTL on chromosome 10, *qRGL10*, had an LOD value of 9.6 with the highest individual contribution of 18.7%. Another QTL, *qRGL9* flanked by the markers Amy3ABC and RZ228 had an individual contribution of 15.8%. The QTLs, *qRGL2* and *qRGL10* were derived from IR64 and showed positive additive values, while *qRGL9* was derived from Azucena and showed negative additive values. No major QTLs associated with RGB could be detected. The RGS was found addressed by two QTLs residing separately on chromosomes 9 and 10. These QTLs (*qRGS9* and *qRGS10*) described a cumulative phenotypic variation of 25.6% for grain shape and shared the same marker intervals as that of *qRGL9* and *qRGL10*.

Among the six digenic interactions detected for RGL, two QTL pairs shared at least one locus on chromosome 2. Four digenic loci that exhibited significant additive actions included major QTLs *qRGL2*, *qRGL9* and *qRGL10*. One epistatic QTL loci on chromosome 2 (RG544–RG171) and on chromosome 5 (CDO105–RZ649) showed the highest epistatic influence (21.2%). Another digenic pair, consisting of QTLs on chromosomes 6 (RG433–Cat1) and 12 (AF6–RG457) exhibited an epistatic influence of 19.0% on the phenotypic variation of RGL. Five digenic interactions were detected for RGB. Among these, three loci showed high epistatic effects. Of these, one marker interval on chromosome 3 (RZ678–RZ574) in interaction with a locus on chromosome 11 (CDO127–RZ638) explained 27.5% of variation in the phenotype. This was followed by loci on chromosomes 1 (RG246–K5) and 7 (CDO38–RG351), and another pair on chromosome 7 viz., RG773–RG764 and CDO497–CDO418. Both these interacting pairs had individual contribution of 23.2% each. There were also five pairs of digenic loci influencing grain shape, of which, one pair involving *qRGS10* alone showed both additive and epistatic effects. Three pairs of loci, residing on chromosomes 1 (U10–RG 532) and 9 (G103–RZ 206), chromosomes 5 (RG 556–RZ 390) and 7 (RG 769–RZ 488) and on chromosomes 7 (CDO 497–CDO 418) and 12 (RG 958–RG 181) showed only epistatic effects, respectively explaining 13.0, 15.8 and 24.6% of the phenotype variation. One epistatic QTL pair involving two loci on chromosome 10 (G1084–RG257/RZ625–CDO93) shared same marker intervals as that of an epistatic QTLs of RGL.

Table 4 Epistatic QTLs detected by two-locus mixed model analysis for the grain parameters at the likelihood threshold of 7.88 at $P = 0.005$

Trait	Chrom	Interval (*i*)	Chrom	Interval (*j*)	LOD	A_i	A_j	AA_{ij}	$h^2(A_i)$	$h^2(A_j)$	$h^2(AA_{ij})$	Cumulative	
												Additive	Epistatic
RGL	2	**RG437–RG544**	2	RG157–RZ318	8.41	0.21	–	−0.14	14.1	–	6.2	51.5	67.9
	2	RG544–RG171	5	CDO105–RZ649	6.98	−0.18	–	−0.26	10.0	–	21.2		
	3	RG191–RZ678	3	Pgi1–CDO87	10.16	–	–	0.17	–	–	8.8		
	6	RG433–Cat1	12	AF6–RG457	10.14	–	–	0.25	–	–	19.0		
	9	**Amy3ABC–RZ228**	9	RG451–RZ792	10.73	−0.25	–	−0.17	20.2	–	8.6		
	10	**G1084–RG257**	10	RZ625–CDO93	6.66	0.23	–	0.13	17.3	–	4.9		
RGB	1	RG246–K5	7	CDO38–RG351	11.74	–	−0.05	−0.08	–	8.1	23.2	23.9	87.8
	3	RZ678–RZ574	11	CDO127–RZ638	11.21	0.04	0.03	0.09	5.5	3.7	27.5		
	5	RZ67–RZ70	8	AC5–RG418B	5.06	–	–	−0.05	–	–	8.8		
	7	RG773–RG769	7	CDO497–CDO418	9.39	–	−0.04	−0.04	–	6.7	5.2		
	9	CDO590–C711	12	RG341–AF6	16.99	–	–	−0.08	–	–	23.2		
RGS	1	U10–RG532	9	G103–RZ206	10.11	–	–	−0.13	–	–	13.0	36.7	60.7
	5	RG556–RZ390	7	RG769–RZ488	11.60	–	–	0.14	–	–	15.8		
	7	CDO497–CDO418	12	RG958–RG181	17.03	–	–	0.18	–	–	24.6		
	9	RZ228–RZ12	9	RZ792–RZ404	10.86	−0.13	–	–	11.9	–	–		
	10	**G1084–RG257**	10	RZ625–CDO93	5.46	0.16	−0.09	0.09	20.4	6.0	5.5		
CGL	2	**RG157–RZ318**	6	RZ398–RG213	9.85	−0.36	–	0.25	14.2	–	7.1	74.4	56.1
	2	RG157–RZ318	2	CDO686–Amy1AC	12.00	−0.35	–	–	13.9	–	–		
	3	RZ892–RG100	11	RG103–RG1109	16.16	–	–	−0.45	–	–	22.9		
	3	RZ448–RZ519	11	RG118–Adh1	15.70	0.12	–	−0.46	1.5	–	23.5		
	12	**Sdh1–RG463**	12	RG958–RG181	16.08	−0.63	–	0.16	44.7	–	2.7		
CGB	1	RG472–RG246	7	RZ488–RG511	16.26	–	–	0.14	–	–	22.6	67.8	26.6
	1	K5–U10	3	RZ448–RZ519	18.03	–	−0.17	–	–	34.3	–		
	1	RG146–RG345	9	Amy3ABC–RZ228	10.20	−0.05	0.11	0.04	2.7	14.1	1.9		
	3	**RZ337A–RZ448**	3	RG910–RG418A	6.13	−0.08	–	0.04	8.0	–	2.1		
	7	**CDO418–RZ978**	8	RZ143–RG20	14.55	−0.11	−0.04	–	14.4	2.3	–		
LER	6	**RZ667–Pgi2**	6	RG648–RG424	6.64	0.08	−0.05	0.04	27.2	11.3	6.0	38.4	6.0
BER	3	**RG104–RG348**	3	RG100–RG191	4.39	−0.05	–	–	10.7	–	–	10.7	0.0
GT	2	**RG256–RZ213**	3	RG104–RG348	5.13	−0.36	–	–	13.7			32.0	6.6
	5	**RZ70–RZ225**	6	Est–2Amp3	6.98	0.42	–	−0.25	18.3	–	6.6		

RGL, raw grain length (mm); RGB, raw grain breadth (mm); RGS, grain shape; CGL, cooked grain length (mm); CGB, cooked grain breadth (mm); LER, length elongation ratio; BER, breadth expansion ratio; GT, gelatinization temperature; LOD, likelihood of odds value

A_i and A_j are the additive effects of the marker intervals *i* and *j*, and AA_{ij} is the additive effect of digenic interaction between them. The additive effects shown are significant at probability of 0.005

$h^2(A_i)$ and $h^2(A_j)$ are the additive contributions of the marker intervals *i* and *j*, and $h^2(AA_{ij})$ is the epistatic contribution between them, towards the phenotype. Marker intervals in bold are the main effect QTLs showing interaction effects

Cooked grain traits

Two major QTLs derived from Azucena, having a cumulative contribution of 57.3%, were found to influence CGL significantly. One of these QTLs located on chromosome 12, *qCGL12*, with an LOD value of 20.6 described 45.1% of the phenotype variation with an additive value of −0.62. The other QTL on chromosome 2, *qCGL2*, bracketed by the markers RG157 and RZ318 had shown phenotypic contribution of 12.1%. Three major QTLs for CGB were found located on chromosomes 1, 3 and 7. Among

these, two were from Azucena and the third was from IR64. The Azucena derived QTLs, *qCGB3* and *qCGB7*, exhibited an individual contribution of 29.4 and 15.2%, respectively. The QTL from IR64, *qCGB1*, had relatively lower contribution (4.3%). A single QTL located on the short arm of chromosome 6, flanked between markers RZ667 and Pgi2, was found associated with LER and explained 9.1% of the total phenotypic variation for this trait. This QTL, *qLER6* with an LOD of 3.3 was IR64 derived. Two QTLs located on chromosome 3 addressed phenotypic variation for BER to an extent of 37.14%. These QTLs were found to be contributed by Azucena. Of these, *qBER3-2* flanked by markers RZ448 and RZ519, showed an individual contribution of 23.4% while the other, *qBER3-1* lying on the short arm of chromosome 3 was found explaining 13.7% of phenotype variation for BER.

Besides two main effect QTLs for CGL, five pairs of digenic epistatic loci were found to significantly influence the phenotype, of which two pairs included QTLs *qCGL2* and *qCGL12*. These pairs showed high additive and low epistatic effects. However, one epistatic QTL detected on chromosome 2 (RG157–RZ310) showed prominent additive contribution. There were five epistatic loci associated to CGB, of which two pairs included two main effect QTLs, *qCGB3* and *qCGB7*. The epistatic effect of *qCGB3* and its interacting locus RG910–RG418A on chromosome 3 was 2.1%. Three marker intervals on chromosome 1, RG 472–RG246, K5–U10 and RG146–RG345 and their corresponding interacting loci on chromosomes 7 (RZ488–RG511), 3 (RZ448–RZ519) and 9 (Amy3ABC–RZ228) showed epistatic (22.6%) and additive effects (34.3% and 14.1%), respectively. One epistatic QTL pair each, constituted by their main effect QTLs, *qLER6* and *qBER3* were found associated with LER and BER. *qLER6* along with a locus on chromosome 6 (RG648–RG424) showed an epistatic contribution of 6.0%, while that of *qBER3* and its interaction locus RG100–RG191 on chromosome 3 was 10.7%.

Gelatinization temperature

Two major QTLs were identified influencing GT with almost similar level of contributions, having been derived from either of the parents. The first, *qGT5* located at interval RZ70–RZ225 on chromosome 5 had explained 15.39% of the total phenotypic variation for GT. The other QTL, *qGT2* juxtaposed between markers RG256 and RZ213 on chromosome 2 described 14.41% of variation. *qGT2* was derived from Azucena and *qGT5* from IR64.

There were two digenically interacting pairs identified for this trait, both of which included the two main effect QTLs. Among these, *qGT5* only showed marginal epistatic effects in combination with an interval Est2–Amp3 on chromosome 6.

Discussion

Crop improvement for grain quality in rice is directed towards improving traits associated with consumer acceptance, nutritional quality and post harvest processing. The focal point of the present study was on the traits associated with physical changes occurring on cooking and GT. Fifteen main effect QTLs and thirty digenically interacting QTLs associated with raw and cooked grain quality and GT have been identified in this study. The results suggested that genetic action between different loci accounted for transgressive segregation of the traits, consistent with previous reports (Lark et al. 1995; Li et al. 1997; Yu et al. 1997). Transgressive segregation resulting in normal distribution for grain characteristics caused by linked QTLs had been reported by Hagiwara et al. (2006). It is relevant to mention that, many of these QTLs are at positions not previously reported. Twelve of the main effect QTLs showed interaction effects. In addition, most of the epistatic loci except main effect QTLs did not show perceptible additive effects. Few of the interacting loci were found to boost the influence of main effect QTLs. In our study the cumulative effect of epistatic QTLs was found more than that of the main effect QTLs in consistence with observations of Tan et al. (2001). However, these results were in contrast to Fan et al. (2005), who reported that important determinants of rice grain quality were main effect QTLs. They found that total effect of epistatic QTLs was smaller than the main effect QTLs, although epistatic QTLs did have sizeable influence on quality traits.

Main effect QTLs

The two major QTLs that determined the shape of grains, *qRGS9* and *qRGS10* were found co-localized

to *qRGL9* and *qRGL10* associated with RGL on their respective chromosomal locations. It is not surprising to note the co-localization of these QTLs, because shape of the grains determined by the ratio between the grain length and width and is predominantly influenced by the alleles determining grain length. Therefore, perhaps the genetic effect of these QTLs may be the same as reported by Xu et al. (2004). To our understanding, QTLs located on chromosomes 9 and 10 for RGL have not been previously reported. The location of *qRGL2*, the IR64 derived QTL coincided with a previously reported QTL for grain length (*gl2*) by Rabiei et al. (2004) and *qGL-2* by Wan et al. (2005).

The main effect QTL for LER, *qLER6*, localized on chromosome 6 was mapped near to the *Alk* locus, suggesting that *Alk* locus could be playing a role in linear elongation of rice grains. This QTL was found co-mapped with a QTL for cooked rice elongation previously reported by Wang et al. (2007). It is commonly acceded that *Wx* and *Alk* gene clusters play a major role in determining cooking, eating and textural quality in rice (He et al. 1999; Bao et al. 2001; Umemoto et al. 2002; Wan et al. 2004; Fan et al. 2005; Bao et al. 2006a, b; Wang et al. 2007). Both these genes are located on the short arm of chromosome 6 of about 40–50 cM apart (Fig. 1). However, the *qLER6* detected in this study seemed different from QTLs encompassing *Wx* locus influencing cooked grain elongation as reported by Tian et al. (2005) and Ge et al. (2005). *Wx* gene encodes for granule bound starch synthase 1, which produces amylose in non-glutinous rice kernels (Sano 1984), besides affecting the structure of amylopectin which primarilty determines rice eating quality (Villareal et al. 1997; Ball et al. 1998). There are two waxy alleles reported in rice, viz., Wx^a and Wx^b (Sano et al. 1986), Wx^a allele occurs in *indica*, while Wx^b in *japonica*. Wx^a increases amylose content dramatically in rice grain. There are two more alleles viz., Wx^{in} which shows intermediate behaviour of Wx^a and Wx^b, as well as Wx^{op}, which controls opaque or chalky endosperm. However, their distribution seems to be limited (Li et al. 2003). The *Alk* gene encodes for soluble starch synthase IIa (SSIIa) enzyme controlling alkali degeneration. It is implied that influence of *Alk* locus in confluence with Wx^a and Wx^b alleles controls on coked gran elongation in rice. No QTLs were however detected near *Wx* locus in the present study, probably because both the parents, IR64 and Azucena have little difference in amylose content (Bao et al. 2002). Perhaps this could have facilitated the detection of *qLER6* near *Alk* locus, in the absence of conspicuous variation at *Wx* region in this population. It is interesting to note that the main effect QTLs detected for length and breadth wise elongation of grains on cooking were not associated with the raw grain length and breadth per se. This may be suggestive of the fact that inherent factors determining the cooking properties are not influenced by the raw grain properties. These factors can become the ultimate determinants of grain quality (Ge et al. 2005).

In the present population, the variation in GT was found associated with two major QTLs and their interactions. One of these, *qGT2,* lies in close proximity to a QTL mapped by Lanceras et al. (2000) on chromosome 2. GT is a trait not only controlled by *Wx* but also by the *Alk* locus (Umemoto et al. 2002; Gao et al. 2003; Liu et al. 2006) and the gene encoding starch branching enzyme I (Q-enzyme I) (Nakamura et al. 1994) suggesting multigenic influence on this trait. GT is also related to paste viscosity of the rice grains which depends on the AC (Bao et al. 2002). In this context, it is relevant that *qGT2* mapped herein also correspond to a previously reported QTL for AC and *qGT5* with a QTL for paste consistency (Wang et al. 2007). Paste consistency is an important viscosity parameter determined as the difference between hot paste viscosity and cool paste viscosity.

Epistatic QTLs

A significant proportion (80.0%) of the identified main effect QTLs were involved in digenic interactions with background loci. Thus, the usual estimates of main effect of a QTL can be confounded by interactions, which may change according to genetic backgrounds, environments, and other factors, as reported in tomatoes and rice (Tankslesy and Hewitt 1988; Li et al. 1997). This means QTLs and the epistatic loci are interchangeable depending on the genetic backgrounds and probably environments where they are identified. Two main effect QTLs for RGL, *qRGL2* and *qRGL10* were found to have improved additive effects while interaction with other loci; while *qRGL9* had diminished effects. Similarly, *qCGL2*, *qGT5*, *qRGS10* and *qLER6* had increased additive effects, while *qCGL12*, *qCGB3*, *qCGB7*, *qGT2*, *qLER6* and

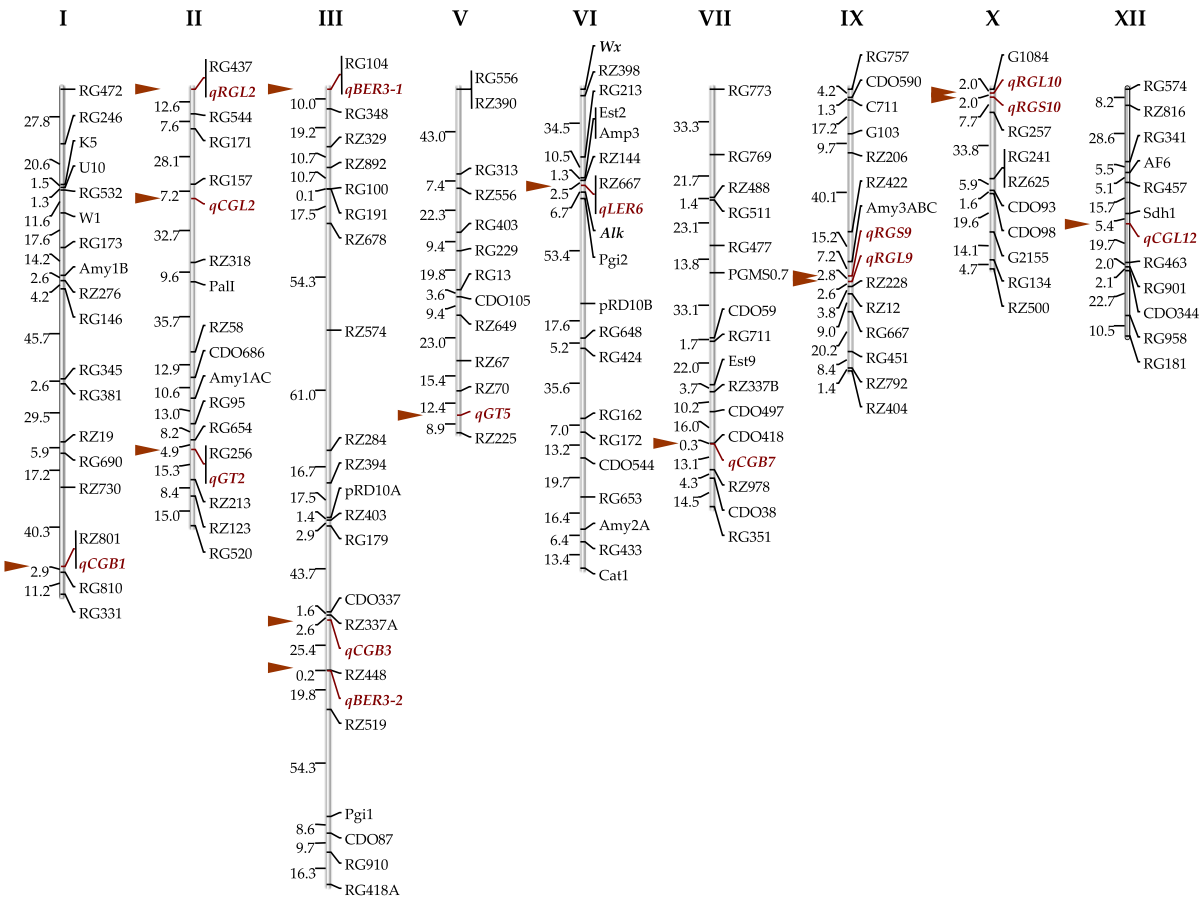

Fig. 1 Genomic positions of putative main effect QTLs associated with grain quality parameters in the doubled haploid line population of IR64 × Azucena. Relative positions of *Wx* and *Alk* loci are also shown

qBER3-1 had decreased additive effects. This suggested predominant inter-allelic modifications on the phenotype of grain quality traits (Fan et al. 2005).

One of the most important objectives of QTL mapping is genetic improvement of quantitative traits through MAS. Zhou et al. (2003) and Liu et al. (2006) have reported successful improvement of grain quality of Zhenshan 97, an elite *indica* cultivar, through MAS. However, limited efforts on MAS for rice quality traits have been reported, possibly because information on markers closely linked to target traits, gene action of QTLs and QTL × environment (QE) interaction are particularly lacking (Li 2001). Although the rice grain parameters are easily measurable, the continuous phenotypic segregation and low heritability seldom helps breeders to improve grain quality using conventional selection methods. This makes these traits good candidates for MAS (Wan et al. 2006). This study had shown that, besides main effect QTLs, epistatic QTLs play a crucial role in determining grain quality parameters. Even if the epistatic interactions of main effect QTLs limit their usefulness in MAS programmes (Tan et al. 2001), the pronounced individual additive effects of these QTLs are sufficient enough to recruit them for MAS. However, it is important to consider that background loci detected in segregating populations can play a large role in modifying the phenotypes under near-isogenic conditions (Zhou et al. 2003). Because of the interaction between different loci, the offspring phenotype will be largely influenced by the genetic background of the receptor line when marker-directed selection is carried out (Tan et al. 2001). The present study also confirmed that the QTL positions for grain quality parameters vary depending up on the population used as observed in many previous studies. Although this might complicate the utility of these QTLs, generalising marker combinations for specific QTLs need to be

done before formulating efficient MAS programmes. However, not many works has been done in resolving QTLs for grain quality traits across populations, which calls for further investigations. Information on more QTLs influencing grain traits may be highly relevant exclusively in culling out a few of them from the MAS programmes based on their ubiquity or quantum of influence on the target traits.

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