

## Additive Effects of QTLs Related to Grains in Rice

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**Abstract:** Advanced backcross QTL analysis was used to identify quantitative trait loci for grain-related traits in a population derived from a cross between IR64 and Tarom Molaei. Parental survey was done by 235 SSR markers, among them 114 markers with clear polymorphic bands were employed for genotyping of 59 advanced backcross lines. Using multiple interval mapping, a total of 11 putative QTLs were detected, of which 5 were for grain length (GL), 3 for grain width (GW), 3 for grain shape (GS). Additive by additive digenic epistasis effects observed for GL and GW. The present study indicated that favorable QTL alleles from the traditional varieties of rice can be transferred into an improved rice variety by advanced backcross QTL strategy and molecular breeding.

**Key words:** Advanced backcross, grain-related traits, QTL mapping, Rice (*Oryza sativa* L.).

### 1. Introduction

Rice is one of the most important world crops, and has now become a model system in genome studies among monocot species, because of its small genome size and the availability of high-density molecular marker linkage maps (Causse *et al.* 1994). The improvement of rice grain quantity and quality are the main objectives for many programs and these two traits show continuous phenotypic variation in rice progenies and are controlled by quantitative trait loci (QTLs) (Yano and Sasaki, 1997).

The development of molecular genetic maps in rice [McCouch *et al.* 2002; Causse *et al.* 1994] and statistical methods [Kao and Zeng 1997; Visschere *et al.* 1996] have facilitated the identification of quantitative trait loci (QTLs) controlling important quantitative characters. Many studies in rice have identified QTLs for grain-related traits using different DNA markers, statistical methods and

populations [Arif 2002; Lin *et al.* 1998; Li *et al.* 1997; Rabiei *et al.* 2004; Redona and Mackill 1998; Tan *et al.* 2000]. Tsunematsu *et al.* (1996) mapped two QTLs for grain length on chromosomes 3 and 7, and two other QTLs for grain width on chromosomes 5 and 9. Redona and Mackill (1998) found seven, four and three QTLs for grain length, grain breadth and grain shape, respectively, and reported that grain shape was mostly controlled by loci on chromosomes 3 and 7. Zheng *et al.* (2007), detected one QTL for grain length, two QTLs for grain width and one QTL for grain length to width ratio (grain shape), among them one QTL from each trait mapped on chromosome 7.

Advanced backcross analysis has been proposed as an approach for combining marker-based QTL discovery with elite variety improvement (Tanksley and Nelson 1996). This strategy uses molecular markers to identify beneficial alleles from unadapted germplasm and has

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been successfully applied in detecting and transferring valuable QTLs from unadapted germplasm into elite breeding lines for several crop plants like tomato (Tanksley et al. 1996), and rice (Xiao et al. 1998).

The objectives of the present study were to detect and map QTLs conferring grain-related traits using the simple sequence repeat (SSR) markers using advanced backcross lines (designated BC<sub>2</sub>F<sub>5</sub>) derived from an indica × indica cross between IR64 and Tarom molaei (hereafter referred to as TM).

## 2. Materials and Methods

**Mapping population:** QTL mapping was done with 59 BC<sub>2</sub>F<sub>5</sub> lines, derived from a cross between IR64 and TM. This population was grown in 2006 at Chaparsar Rice Research Station, a station linked to Rice Research Institute of Iran (RRII) in Mazenderan province, Iran

**Phenotypic measurements:** Field management was conducted similar to that of normal rice cultivation. Only eight plants in the middle of each plot were selected for scoring the traits. Grain shape (GS) was estimated on the basis of Grain length (GL) to grain width (GW) ratio.

**DNA extraction, PCR analysis and SSR assay:** DNA suitable for PCR analysis was extracted using a simplified and modified miniscale procedure [Fotokian 2004]. DNA from the two parents of the cross was surveyed for polymorphisms using 235 SSR markers. PCR amplification for polymorphism assay was performed with some modification in method used by Arif (2002).

**SSR Linkage map construction and QTL mapping:** A molecular-marker linkage map was constructed using Mapmaker/EXP 3.0 (Lincoln et al. 1992) based on data consisting of 114 SSRs marker loci. The kosambi mapping function was used to transform the recombination frequency to genetic distances (centi-Morgan, cM) (kosambi 1944). Chromosomal locations of putative QTLs were determined by multiple interval mapping (MIM) using version 2.5 of QTL Cartographer (wang *et al.* 2006). QTLs were detected with a logarithm of odds (LOD) threshold of 3.0. QTL cartographer analysis was also performed to detect digenic epistasis interaction between detected QTLs. The percentage of explained phenotypic variation (PV%) associated with each significant QTL was calculated from the regression of each marker/phenotype combination by a coefficient of determination (R<sup>2</sup>). The additive effect of a putative QTL was equal to the half of difference between two homozygous classes.

## 3. Results and Discussion

**Traits performance and distribution:** Large segregation and continuous distribution were observed for four traits (Table 1), suggesting quantitative inheritance of the studied characters and the feasibility of collected data for QTL mapping.

Significant differences ( $P < 0.05$  based on *t* test) were detected between two parents for all the quantitative traits, with exception of GW, listed in Table 1.

Table 1. Descriptive statistics of grain length (GL), grain width (GW), and Grain shape (GS), in parents and BC<sub>2</sub>F<sub>5</sub> lines.

Traits	BC <sub>2</sub> F <sub>5</sub> lines	Parents (Mean ± SD)	
	(Mean ± SD)	IR64	TM
GL	9.56 ± 0.72	9.4 ± 0.63a <sup>1</sup>	11 ± 0.56b
GW	2.35 ± 0.34	2.5 ± 0.28a	2.4 ± 0.25a
GS	4.15 ± 0.68	3.76 ± 0.52a	4.42 ± 0.46b

<sup>1</sup> a and b indicate a significant difference (t-test,  $p < 0.05$ ).

Marker segregation and genetic linkage map: Out of 235 microsatellite markers which covered the whole genome of rice, 114 (48.5%) showed polymorphism between recurrent parent "IR64" and donor parent "TM" and were used for genotyping of BC<sub>2</sub>F<sub>5</sub> lines. The highest polymorphism with 15 markers was found in chromosome 2, followed by the chromosomes 1 and 3 (11 markers). On an average, there were 9.5 markers on each chromosome, varying from six markers to chromosomes 5 and 9 to 15 markers on chromosome 2. One hundred and twelve loci were skewed towards IR64, while two loci were skewed towards TM. Among the scored SSR bands, 90% were derived from IR64 while only 10% came from TM. The percentage of the IR64 genome in each line ranged from 57% to 93% with a geometrical average of 81%. The correlation between the length of the linkage map for each chromosome and the number of markers on the map was significant ( $r=0.65$ ,  $p<0.05$ ). The linkage map had a total map length of 1692.6 cM with an average interval of 14.8 cM. This value is lower than the minimum required level of 20 cM for QTL mapping (Lander and Botstein, 1989).

QTL analysis: A total number of 11 QTLs were detected for three traits, of which five were for GL, three for GW, and three for GS (Table 3). One of these QTLs showed  $R^2$  higher than 20%. This QTL (*QGw2a*) could be considered as major genes.

Grain length (GL): Five QTLs (*QGI2*, *QGI7*, *QGI11*, *QGI12a*, and *QGI12b*) were detected for GL. These QTLs jointly explained 68.3% of the total variation for GL. Eighteen percent of phenotypic variation for GL could be explained by digenic epistasis interaction. The remaining unexplained phenotypic variation might be due to environmental effects or to undetected QTLs with relatively smaller phenotypic effects. The QTL *QGI12* alone explained 41% of

variation. Amongst the identified QTLs related to GL, QTLs on chromosomes 2 and 7 had favorable alleles from the donor parent TM, and the QTL on chromosomes 2, 11 and 12 had the favorable alleles from the cultivated parent IR64 (Table 2).

Grain width (GW): Three QTLs were mapped for GW. These three QTLs with the large effects were *QGw2a*, *QGw2b*, and *QGw1* that individually explained 13%, 8% and 12% of the total phenotypic variation, and had an additive effect of 0.2, -0.05 and 0.05, respectively (Table 3).

Grain shape (GS): Three QTLs were detected for GS, which jointly explained 16.5% of the total phenotypic variation. The QTLs *QGs2a* and *QGs2b* on chromosome 2 were located in the same intervals as the loci for GL.

Detection of digenic epistasis: Pairwise epistatic interactions were examined between all detected QTLs (a total of 120 interactions). Among the examined epistatic interactions, one digenic (two-locus) epistatic interactions were identified (Table 3), which could be grouped into two distinct between-region interactions (each region can contain one or more marker locus). The epistatic interactions which was identified between QTL-linked regions, it was detected between two regions on chromosome 2 and 7 for GL (*QGI2a* \* *QGI7*) (Table 2).

QTL analysis of agronomic traits in rice is well documented, which facilitates comprehension and permits comparisons of the results from different research groups. It is difficult to make comparisons among results obtained by studies performed with different types of markers, populations and environments. Identification of QTLs that have already been detected in other populations or mapped in the classical linkage map lends additional confidence to the importance of individual loci, as showed for *QGs2a* in the interval of RM475-RM263 on chromosome 2 for GS by Rabiei *et al.* (2004), and *QGI2*, which is detected in the

interval of RM250-RM208 on chromosome 2 for GL by Arif (2002).

The marker locus RM4A on chromosome 12 accounted for 41% of the GL phenotypic variation, which means this chromosomal region has a major effect on GL. Marker-assisted selection for this trait could help the identification and selection of best performing lines.

Although it is difficult to detect epistatic interactions among markers in small population [Yano and Sasaki 1997], in this study, One additive by additive interaction was detected for GL. Small number of identified interactions between QTLs demonstrated that most of the identified QTLs exerted their effects independent of the other QTLs. Further investigations, including the use of larger populations or the construction of isogenic lines, are necessary to determine the exact nature of these interactions. For breeding purposes, QTLs which do not have epistatic interactions are desirable.

It is likely that there were more QTLs with minor effects which were not detected in this study, and could possibly be detected if a larger population was used. Furthermore, some of the remaining variation could be due to epistatic interactions among QTL-linked and unlinked markers. The results support the hypothesis that quantitative traits are often controlled by the effects of a few major QTLs which act in concert with a number of smaller-effect QTLs [Paterson et al. 1991]. In this study, the finding that only a few major QTLs affected traits related to rice grain indicates that a rapid response to directional selection for these traits are expected.

Although the effect of some detected QTLs were quite small (Table 2), their discovery in the present study demonstrates the ability of the marker analysis to uncover cryptic genetic variation that otherwise would have been masked by the large genetic difference between the parents [Foolad and Chen 1999].

Statistical threshold is very important in QTL analysis because the numbers of detected QTLs would be different when using different thresholds in QTL analysis. Most of the thresholds employed in published QTL analyses of rice have been between LOD 2.0 and 3.0 in MAPMAKER/QTL [Yano and sasaki 1997]. Although it is difficult to detect QTLs using a high threshold in smaller populations [Lin *et al.* 1998], the LOD we used here is a relatively high (LOD = 3.0), in order to avoid false positive, to identify all putative QTLs. The confirmation of these putative QTLs using advanced backcross progeny will still be necessary.

In this study, the genetic effect of QTLs detected was additive, because of the pattern of used population which was an advanced backcross. The dominance effect of QTLs related to grain-related characters has been reported. Redona and Mackill (1998) reported the additive or partial dominance effects for QTLs controlling grain dimensions. Further, Singh and Singh (1985) had also indicated that both additive and non-additive gene actions were important for grain shape.

In addition to MIM, simple interval mapping (SIM) and composite interval mapping (CIM) were also performed to scan the chromosome regions. The QTLs were detected by SIM and CIM confirmed the presence of the QTLs detected by MIM.

The present study suggests that favorable QTL alleles could be transferred from traditional relatives of rice into an elite rice variety for improvement of grain-related traits by advanced backcross QTL strategy and SSR markers.

Table 2. The chromosome location, markers interval, LOD score, additive effect and the percentage of explained variance ( $R^2$ ) by QTLs of grain-related traits in BC<sub>2</sub>F<sub>5</sub> lines derived from 'IR64' × 'TM'.

Trait	Chr. <sup>a</sup>	Markers Interval <sup>b</sup>	QTL <sup>c</sup>	Add. Effect <sup>d</sup>	R <sup>2</sup> (%) <sup>e</sup>	LOD <sup>f</sup>
Grain length (GL)	2	<u>RM475</u> -RM263	<i>QGI2a</i>	0.49	14	4.3
	2	<u>RM250</u> -RM208	<i>QGI2b</i>	-0.19	7	4.4
	7	RM18- <u>RM172</u>	<i>QGI7</i>	-0.17	1	3.38
	11	<u>RM120</u> - <u>RM202</u>	<i>QGI11</i>	0.31	5.3	2.9
	12	<u>RM4A</u> -RM19	<i>QGI12</i>	0.09	41	3.3
		Digenic epistasis	<i>QGI2 * QGI7</i>	-1.7	18	2.3
Grain Width (GW)	1	<u>RM431</u> -RM14	<i>QGw1</i>	0.05	12	5.8
	2	<u>RM485</u> - <u>RM236</u>	<i>QGw2a</i>	0.2	13	24
	2	<u>RM263</u> - <u>RM525</u>	<i>QGw2b</i>	-0.05	8	8.4
Grain Shape (GS)	1	<u>RM200</u> -RM220	<i>QGs1</i>	-0.28	5	3.11
	2	<u>RM475</u> -RM263	<i>QGs2a</i>	-0.38	6.1	3.37
	2	<u>RM250</u> -RM208	<i>QGs2b</i>	0.14	5.4	3.26

<sup>a</sup> Chromosome.

<sup>b</sup> underlines markers are those found significant for the putative locus.

<sup>c</sup> QTLs nomenclatures on the basis of McCouch et al. (1997).

<sup>d</sup> -ve or +ve additive effect contributed by TM or IR64.

<sup>e</sup> phenotypic variance explained by each QTL, or digenic epistasis.

<sup>f</sup> The logarithm of odds (LOD).

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