Journal of Agricultural Science; Vol. 8, No. 9; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Mapping of QTLs Controlling Grain Shape and Populations Construction Derived from Related Residual Heterozygous Lines in Rice

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Received: July 3, 2016 Accepted: July 28, 2016 Online Published: August 15, 2016

doi:10.5539/jas.v8n9p104 URL: http://dx.doi.org/10.5539/jas.v8n9p104

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Abstract

Grain shape is usually characterized by grain length (GL), grain width (GW), grain thickness (GT) and length to width ratio (LWR), and controlled by quantitative trait locus (QTL). In this paper, QTL analysis was performed using an F_2 population and an F_8 recombinant inbred line (RIL) population from a cross Xiang743/Katy. A total of 38 QTLs for grain shape were detected and eight of them were repeatedly identified in both populations. Seven for GL, five for GW, five for GT, and eight for LWR were detected in F_2 population, explaining totally phenotypic variance of 94.51%, 61.52%, 54.33% and 91.84%, respectively. Five for GL, three for GW, and five for LWR were detected in RIL population, explaining totally phenotypic variance of 39.83%, 37.52% and 36.71%, respectively. Many QTLs were located in similar intervals, contributing to complicated trait correlation. A few QTLs were mapped in intervals coincided with previously cloned genes associated with grain size. Two residual heterozygous lines (RHLs) were selected out on the basis of newly identified loci, populations derived from RHLs were constructed for fine mapping QTLs associated with grain shape.

Keywords: *Oryza sativa* L., grain shape, recombinant inbred line (RIL), quantitative trait loci (QTLs), residual heterozygous line (RHL)

1. Introduction

Grain shape, closely correlated with grain weight, a major component of grain yield in rice (Lin and Wu. 2002), is usually characterized by GL (the maximum distance of a grain from top to bottom), GW (the most widest distance between two sides within a grain), GT (the most thickest distance within a grain) and LWR (GL was divided by GW with a grain). Grain shape affects appearance quality and cooking quality of rice, which are very important for consumers due to people's preference. Grain shape is a complex trait, numerous studies showed that grain shape and weight were controlled by quantitative trait loci (QTLs) (Huang et al., 2013; Tan et al., 2000). So far, hundreds of QTLs underlying grain shape and weight have been identified and were scattered throughout twelve rice chromosomes (www.gramene.org).

Due to close correlation among grain shape, a lot of genes/QTLs for grain size were mapped in similar intervals (Huang et al., 2013). That indicated that some genes/QTLs regulated seed development with pleiotropism and/or QTLs were distributed on chromosomes with clusters. In addition, some major QTLs were frequently detected in different populations across various environments. For example, two major QTLs for GL and grain weight were repeatedly identified on chromosome three and seven respectively in many independent studies (Bai et al., 2010; Li et al., 2004; Qiu et al., 2012; Shao et al., 2010; Shao et al., 2012; Thomson et al., 2003; Xiao et al., 1998). These QTLs could be utilized for improving grain shape in rice breeding program, but with consideration of pleiotropism.

Clone and characterization of more QTLs/genes underlying grain shape enhanced our understanding of seed development. To date, nine QTLs have been cloned (Huang et al., 2013). Of them, four for GL, including *GS3* (Fan et al., 2006; Mao et al., 2010), *GL3/GL3.1* (Peng et al., 2012; Qiu et al., 2012), and *GL7/GW7* (Wang et al.,

2015a; Wang et al., 2015b); Four for GW, including *GW2* (Song et al., 2007), *GW5/qSW5* (Shomura et al., 2008; Weng et al., 2008), *GS5* (Li et al., 2011), and *GW8* (Wang et al., 2012); Two for grain weight, including *GS2* (Che et al., 2015; Duan et al., 2015; Hu et al., 2015) and *GW6* (Jun et al., 2015).

In previous studies, NILs or CSSLs were usually adopted to fine map and clone complex traits related QTLs. Besides, Residual heterozygous line (RHL) provided an alternative way to fine map QTL, RHL was referred to as heterozygous in target region but homozygous elsewhere in the genome, which was equivalent to an F_1 individual derived from cross of a pair of NILs. Some yield related QTLs were fine mapped using the method (Yu et al., 2008; Su et al., 2010). In this paper, an F_2 population and an RIL population from a cross Xiang743/Katy were used to map QTLs for grain shape and secondary segregating populations were constructed, which lay a foundation for fine mapping and cloning grain shape related gene/QTLs.

2. Materials and Methods

2.1 Rice Materials

The genetic materials included Katy and Xiang743, a variety of Hunan landrace rice, and two populations from a cross Xiang743/Katy. One population consisted of 186 F_2 plants, and the other contained 176 F_8 recombinant inbred lines (RILs) obtained by single-seed descendent method. In 2014, the RIL population was grown in Changsha (Hunan Province, China). Each line was represented by three rows of three plants each, with a randomized block design. The middle individual plant was marked for extracting genomic DNA and seeds were harvested for evaluating grain traits.

2.2 Measurements of Grain Traits

Twenty fully filled grains were lined up along a vernier caliper to measure grain length, after which grains were arranged in the breadth to measure width. GT were measured individually. The values were averaged and used as measurements for length, width and thickness of individual grain. LWR of the grain was calculated as GL divided by GW.

2.3 DNA Extraction and Molecular Marker Analysis

DNA was extracted at seedling stage, following protocol described by Lu and Zheng (1992). SSR primers were amplified in a total of 10 μL reaction systems containing 1 μL of 10×SSR buffer, 0.5 μmol/L of each primer, 0.25 mmol/L dNTPs, 0.5 unit of *Taq* DNA polymerase and 10-30 ng genomic of DNA. Samples were pre-denatured at 94 ^{o}C for 2 mins, followed by 30 cycles of 45 s at 94 ^{o}C, 45 s at 55 ^{o}C and 45 s at 72 ^{o}C, and a final extension at 72 ^{o}C for 8 mins. Polymerase Chain Reaction (PCR) products were detected using silver staining on 6% nondenaturing polyacrylamide gel.

2.4 Construction of Linkage Maps

Based on information from Gramene database (www.gramene.org), SSR primers evenly distributed on twelve rice chromosomes were selected for survey of polymorphism between Xiang743 and Katy. The primers with clear and polymorphic bands were employed for genotyping Xiang743/Katy population. Map construction was carried out as follows: Firstly, the markers were assigned to rice chromosome according to the maps of from www.gramene.org, thus all primers were categorized into twelve groups. Secondly, MAPMAKER ver. 3.0b (Lincoln, 1992) was employed to identify the most probable marker order within each group. The threshold of LOD = 3 was used to determine linkage group. The genetic map distance in centiMorgans (cM) was calculated using Kosambi function.

2.5 Data Analysis and QTL Mapping

Correlation coefficients were calculated to determine correlation between traits using DPS 14.10. Windows QTL Cartographer 2.5 was used to detect QTLs underlying GL, GW, GT and LWR. Composite interval mapping (CIM) was performed to claim putative QTLs. QTL analysis was performed with 1000 permutations at the 0.05 probability level. A threshold of LOD > 3.0 was used to declare presence of a putative QTL. Multiple interval mapping (MIM) was conducted to analyze QTL interaction between putative QTLs for single trait (Basten et al., 2002). QTLs were named following method of McCouch et al. (1997).

3. Results

3.1 Distributions of Trait Measurements in F_2 and RIL Populations

The trait distributions of parents and two populations are shown in Figures 1 and 2. The parents showed large differences in GL and GW and slight differences in GT and LWR. All of trait distributions in F_2 population were similar with those in RIL population, but larger variation occurred in RIL population than in F_2 population.

Continuous distribution and transgressive segregation were simultaneously observed in all of the traits in two populations, suggesting that these traits were controlled by multiple loci.

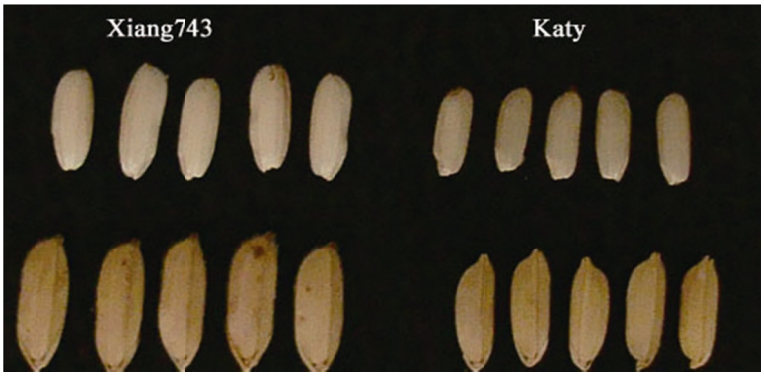

Figure 1. Rice grains and brown rice of parents

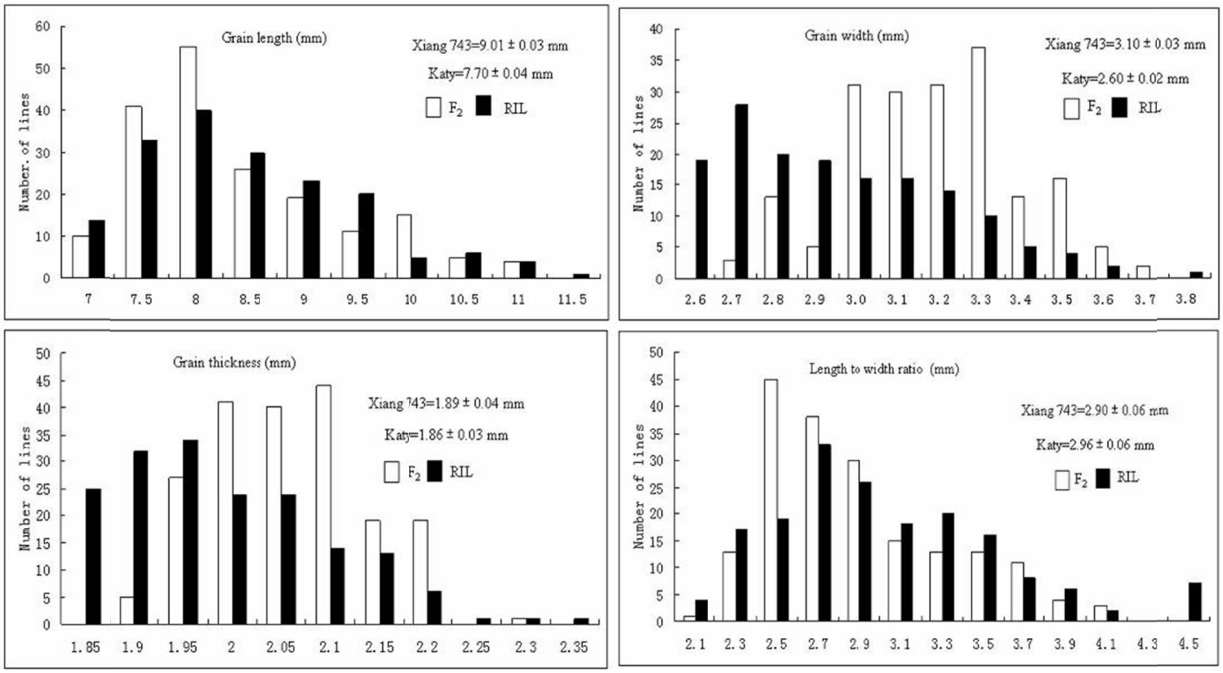

Figure 2. Trait distributions of grain length, grain width, grain thickness and length to width ratio in F_2 and RIL populations

3.2 Correlation Analysis between Grain Traits

Correlation analysis was presented in Table 1. The results showed that these distributions in F_2 population were highly similar with those in RIL population. GL was significantly positive correlation with GW and LWR, and was not correlated with GT. GW was significantly positive correlation with GT and LWR. GT was significantly positive correlation with LWR.

Table 1. Correlation analysis of grain length, grain width, grain width and length to width ratio in F_2 and RIL populations

Trait	GW	GT	LWR
GL	-0.52**/-0.37**	0.06/0.04	0.93**/0.86**
GW		0.49**/0.46**	-0.80**/-0.78**
GT			-0.17*/-0.21*

Note. **: Significant difference at p = 0.01 level; *: Significant difference at p = 0.05 level.

3.3 Construction of Genetic Linkage Map

For F_2 population, a genetic linkage map with 129 polymorphic SSR markers evenly distributed on twelve chromosomes was constructed, spanning 2051.1 cM, with adjacent markers ranging from 4.3 to 36.5 cM and an average interval of 15.1 cM (Figure 3). The map was used to identify QTLs for cold tolerance in previous study (Liu et al., 2015).

For RIL population, the same sets of markers were used to construct genetic linkage map. In brief, 121 out of 129 informative markers genotyping F_2 population were employed to genotype RIL population, resulting in a linkage map of spanning 1797.8 cM, ranging from 0.6 to 39.9 cM and an average of 14.8 cM between adjacent markers (Figure 3). The order of SSR markers in linkage map of RIL population was almost consistent with that in F_2 population.

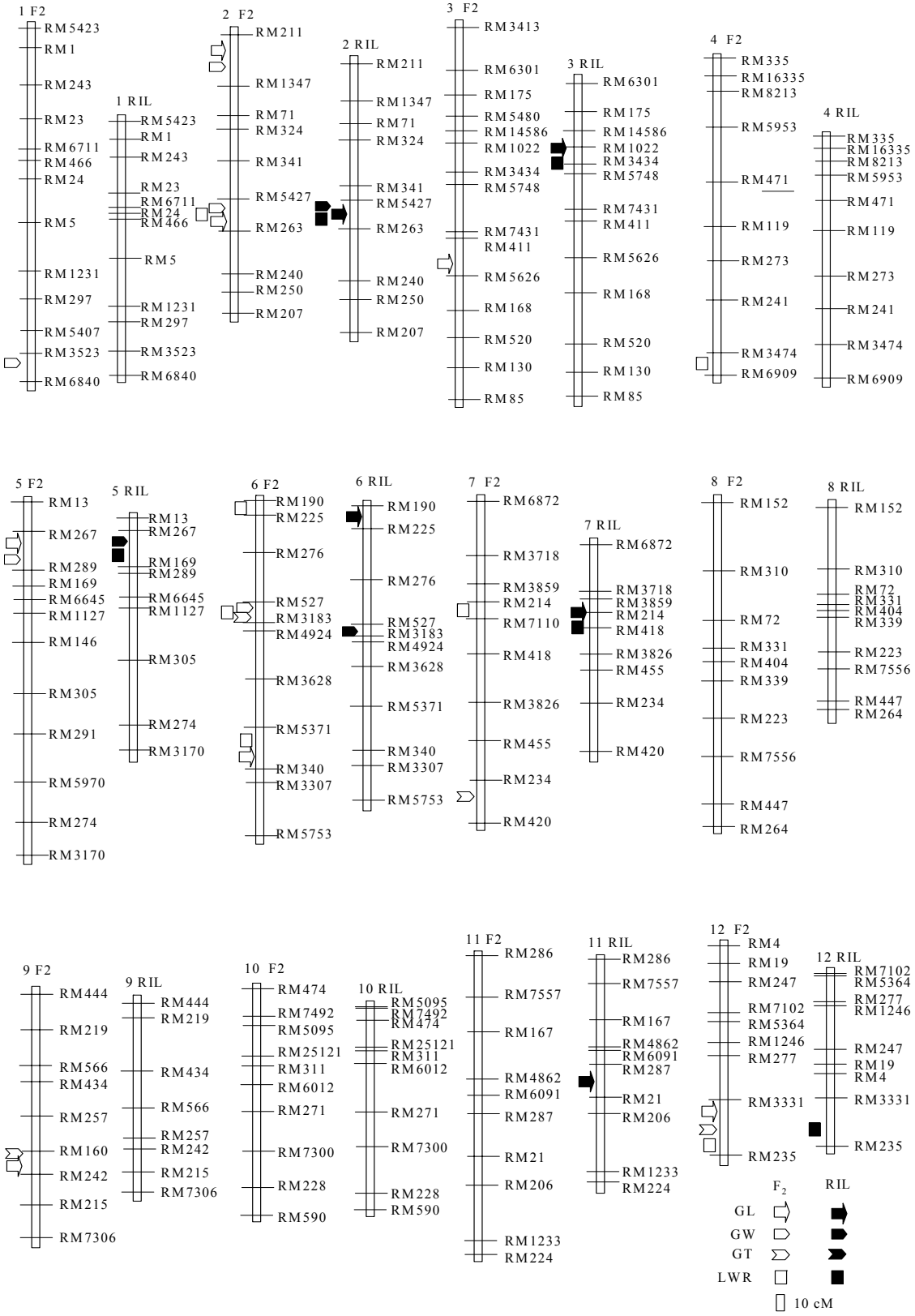

Figure 3. Locations of QTLs for grain length, grain width, grain thickness and length to width ratio in linkage map of F_2 and RIL populations. Only regional linkage map where QTLs were detected was shown in F_2 population

3.4 QTL Analysis in F_2 and RIL Populations

A total of 38 QTLs for grain shape were identified and distributed throughout all chromosomes except chromosome eight and ten in F_2 and RIL populations (Table 2), eight of which were repeatedly identified in both populations. A total of 25 QTLs were identified in F_2 population, among them, seven for GL, five for GW, five for GT and eight for LWR, explaining phenotypic variance of 94.51%, 61.52%, 54.33% and 91.84%, respectively. A total of thirteen QTLs were identified in RIL population, among them, five for GL, three for GW and five for LWR, explaining phenotypic variance of 39.83%, 37.52% and 36.71%, respectively.

3.4.1 Grain Length

Seven QTLs for grain length were detected in F_2 population (Table 2). Of them, two QTLs (*qGL-3-1* and *qGL-12*) were located in interval of RM411-RM5626 and RM3331-RM235 on chromosome three and twelve, explaining phenotypic variance of 5.26% and 28.03%, respectively. The both alleles from Xiang 743 acted to increase GL. Five of the rest from Katy alleles contributed to increase GL. The largest effect of *qGL-6-1* explained phenotypic variance of 35.6% with LOD score of 7.35, followed by *qGL-5* and *qGL-9*, with phenotypic variance of 22.34% and 12.78% and LOD score of 9.27 and 3.72, respectively. Two minor QTLs (*qGL-2-1* and *qGL-2-2*) were located on chromosome two, explaining phenotypic variance of 5.58% and 7.43%, respectively.

Five QTLs for grain length, located in five regions on five chromosomes, were detected in RIL population (Table 2). Two QTLs (*qGL-6-2* and *qGL-11*) from Xiang743 alleles increased GL, explaining phenotypic variance of 7.86% and 10.58%, respectively. Three of the rest, including *qGL-2-2*, *qGL-3-2* and *qGL-7*, increased GL from Katy alleles.

Thus, a total of eleven QTLs were detected, one QTL, *qGL-2-2*, was detected in both populations, the remaining ten were observed in only one of the populations.

3.4.2 Grain Width

Five QTLs for grain width were detected (Table 2), of them, three QTLs (*qGW-2-2*, *qGW-5* and *qGW-6*), located on chromosome two, five and six, respectively, were detected in both populations, alleles from Xiang743 acted to increase GW. The *qGW-5* showed the largest effect in both populations, with LOD score of 15.49 and 6.55 and phenotypic variance of 23.64% and 20.23%, respectively. The remaining two QTLs, *qGW-1* and *qGW-2-1*, were detected only in RIL population, alleles from Katy increased GW, with LOD score of 3.28 and 4.04 and phenotypic variance of 8.19% and 5.42%, respectively.

3.4.3 Grain Thickness

Five QTLs for grain thickness were detected in F_2 population (Table 2). The *qGT-12* was located on chromosome twelve, allele from Katy increased GT, with phenotypic variance of 8.35% and LOD score of 3.24. Four of the rest from Xiang743 alleles played positive roles in increasing GT. The *qGT-5*, located on chromosome five, explained phenotypic variance of 18.17%. The *qGT-6*, located on chromosome six, explained phenotypic variance of 9.8%. Two QTLs, *qGT-7* and *qGT-9*, located on chromosome seven and nine, explained phenotypic variance of 12.12% and 8.72%, respectively.

3.4.4 Grain Length to Width Ratio

Nine QTLs for grain length to width ratio were detected (Table 2). Of them, four QTLs, namely *qLWR-2*, *qLWR-5*, *qLWR-7* and *qLWR-12*, were detected in F_2 and RIL populations. In both cases, three QTLs (*qLWR-2*, *qLWR-5* and *qLWR-7*) from Katy alleles increased grain length to width ratio, the *qLWR-7* from Xiang 743 allele increased grain length to width ratio. It was worthy noting that these four QTLs were located in intervals as the same as QTLs for grain length and grain width as described above. This is quite understandable because length to width ratio was a secondary trait derived by dividing grain length by grain width. The remaining five, including four QTLs (*qLWR-4*, *qLWR-6-1*, *qLWR-6-2* and *qLWR-6-3*), detected in F_2 population, and one QTL (*qLWR-3*), detected in RIL population.

3.5 Epistatic Interaction in F_2 and RIL Populations

To clarify epistatic interaction between putative QTLs, MIM analysis was performed for each of grain shape in F_2 and RIL populations. The results showed that no significant QTL interactions were observed among putative QTLs.

3.6 Populations Construction Derived from RHLs

On the basis of primary map, one line carrying heterozygous segment in interval of RM527-RM3183 on chromosome six, named RHL1, which was newly identified and related to grain width, grain thickness and grain

length to width ratio, were selected out from RIL population. One lines carrying heterozygous segment in interval of RM3331-RM235 on chromosome tweleve, named RHL2, which was newly identified and related to grain length, grain thickness and grain length to width ration, were selected out from RIL population.

3.6.1 Populations Derived from RHL1

RHL1 was genotyped by 121 SSR markers evenly distributed on tweleve chromosomes. Results showed that all of the chromosomes contained both Xiang743' and Katy' genotype. Among them, genotypes of 47 SSR markers were from Xiang743, genotypes of 66 SSR markers were from Katy, 8 SSR markers were heterozygous and divided into two regions, including target regions of RM527-RM3628 on chromosome six and RM335-RM8213 on chromosome three. RHL1 self-crossed and developed an F_2 population consisted of 231 individual plants, which would be used for genetic dissection in grain width, grain thickness and grain length to width ratio.

3.6.2 Populations Derived from RHL2

Similarly, RHL2 was genotyped by 121 SSR markers evenly distributed on tweleve chromosomes. Except chromosome two carried only Katy genotype, the rest chromosomes contained both Xiang743' and Katy' genotype. Genotypes of 52 SSR markers were from Xiang743, genotypes of 60 SSR markers were from Katy, 9 SSR markers were heterozygous, including target region of RM1246-RM235, which comprised five markers, the remaining four markers were scattered on chromosome two, three, seven and ten, respectively. RHL2 self-crossed and developed an F_2 population consisted of 262 individual plants, which would be used for genetic dissection in grain length, grain thickness and grain length to width ratio.

Table 2. Putative QTLs for grain length, grain width, grain thickness and length to width ratio in F_2 and RIL populations

Trait	Chromosome	QTLs	interval	F_2 population				RIL population			Candidate gene[d]
				A[a]	D[b]	LOD	R^2 (%)[c]	A[a]	LOD	R^2 (%)[c]	
GL	2	qGL-2-1	RM1347-RM71	-0.33	0.013	3.27	5.58				
	2	qGL-2-2	RM5427-RM263	-0.32	-0.21	3.29	7.43	-0.03	3.44	8.76	GW2
	3	qGL-3-1	RM411-RM5626	0.27	0.15	3.38	5.26				GL3/GL3.1
	3	qGL-3-2	RM1022-RM3434					-0.03	5.21	10.25	
	5	qGL-5	RM267-RM169	-0.6	-0.08	9.24	22.34				GW5/qSW5 or GS5
	6	qGL-6-1	RM5371-RM340	-0.68	-0.77	7.35	35.6				GW6
	6	qGL-6-2	RM190-RM225					0.03	4.08	7.86	
	7	qGL-7	RM214-RM418					-0.03	3.47	7.47	
	9	qGL-9	RM257-RM242	-0.11	0.68	3.72	12.78				GW9.1
	11	qGL-11	RM287-RM206					0.03	4.77	10.58	
	12	qGL-12	RM3331-RM235	0.93	-0.68	6.09	28.03				
GW	1	qGW-1	RM3523-RM6840	0.09	-0.06	3.28	8.19				qGRL1
	2	qGW-2-1	RM1347-RM71	0.07	-0.05	4.04	5.42				
	2	qGW-2-2	RM5427-RM263	0.13	-0.05	9.85	14.52	0.01	4.34	9.51	GW2
	5	qGW-5	RM267-RM169	0.16	-0.02	15.49	23.64	0.01	6.55	20.23	GW5/qSW5 or GS5
	6	qGW-6	RM527-RM3183	0.12	-0.03	7.81	11.63	0.01	3.45	11.37	
GT	5	qGT-5	RM267-RM169	0.05	-0.02	7.21	18.17				GW5/qSW5 or GS5
	6	qGT-6	RM527-RM3183	0.03	-0.01	4.52	9.8				
	7	qGT-7	RM234-RM420	0.03	0.04	3.85	12.12				GL7/GW7
	9	qGT-9	RM257-RM242	0.03	0.02	3.3	8.72				GW9.1
	12	qGT-12	RM3331-RM235	-0.02	-0.03	3.24	8.35				
LWR	2	qLWR-2	RM5427-RM263	-0.22	-0.1	7.25	13.55	-0.2	5.67	11.38	GW2
	3	qLWR-3	RM1022-RM3434					-0.2	6.96	11.38	
	4	qLWR-4	RM3474-RM6909	0.15	-0.07	3.07	4.01				
	5	qLWR-5	RM267-RM169	-0.33	-0.03	12.52	27.12	-0.16	4.16	7.1	GW5/qSW5 or GS5
	6	qLWR-6-1	RM190-RM225	0.11	0.1	3.08	4.78				
	6	qLWR-6-2	RM527-RM3183	-0.22	-0.11	5.68	14.93				
	6	qLWR-6-3	RM5371-RM340	-0.3	-0.33	4.21	29.21				GW6
	7	qLWR-7	RM214-RM418	-0.14	-0.01	3.31	4.43	-0.13	3.61	5.1	
	12	qLWR-12	RM3331-RM235	0.38	-0.28	5.59	19.41	0.14	3.03	5.35	

Note. [a] Additive effect of the allele from Xiang743 compared with Katy; [b] Dominance effect; [c] Percentage of phenotypic variation explained by the QTL; [d] Chromosomal location of each QTL was compared to genes for grain size in previous studies.

4. Discussion

In the study, two populations from a cross Xiang743/Katy were used for mapping QTLs underlying GL, GW, GT and LWR. The trait distributions in F_2 and RIL populations were highly similar, suggesting that major QTLs could stably express in different populations.

Compared to QTLs detected in F_2 population, fewer QTLs were detected in RIL population, but eight QTLs were repeatedly identified in both populations, including one for GL, three for GW and four for LWR. No QTLs for GT were detected in RIL population. It is likely that there is little difference in GT between Xiang743 and Katy. Furthermore, the effects of QTLs detected in RIL population were generally smaller than those detected in

F_2 population. The LOD > 3 was used to declare a putative QTL. Thus, a few loci with smaller effect were not grouped into it.

Many QTLs had pleiotropic genetic effects on grain shape (Qiu et al., 2012), which has been confirmed by cloning and characterizing these loci (Huang et al., 2013). In this paper, correlation analysis was conducted in F_2 and RIL population, results showed high consistency in two populations. Three pairs of QTLs for GL and GW were detected in interval of RM1347-RM71 and RM5427-RM263 on chromosome two, RM267-RM169 on chromosome five, respectively, in F_2 population. One pair of QTLs for GL and GW was detected in interval of RM5427-RM263 on chromosome two in RIL population. Their effects acted as the opposite direction, which might contribute to significantly negative correlation between GL and GW. Four pairs of QTLs for GL and LWR were detected in interval of RM5427-RM263 on chromosome two, RM267-RM169 on chromosome five, RM5371-RM340 on chromosome six, and RM3331-RM235 on chromosome twelve, respectively, in F_2 population. Three pairs of QTLs were detected in interval of RM5427-RM263 on chromosome two, RM1022-RM3434 on chromosome three, and RM214-RM418 on chromosome seven, respectively, in RIL population. Their effects acted as the same direction, which was consistent with significantly positive correlation between GL and LWR. Two pairs of QTLs for GW and GT were located in interval of RM267-RM169 on chromosome five and RM527-RM3183 on chromosome six in F_2 population, they had the same direction, which was in accordance with significantly positive correlation between GW and GT. Three pairs of QTLs for GW and LWR were detected in interval of RM5427-RM263 on chromosome two, RM267-RM169 on chromosome five and RM527-RM3183 on chromosome six, respectively, in F_2 population. Two pairs of QTLs for GW and LWR were located in interval of RM5427-RM263 on chromosome two and RM267-RM169 on chromosome five in RIL population. Their effects acted as the opposite direction, which explained significantly negative correlation between GW and LWR. Three pairs of QTLs for GT and LWR were identified in interval of RM267-RM169 on chromosome five, RM527-RM3183 on chromosome six and RM3331-RM235 on chromosome twelve, respectively, in F_2 population. Their effects acted as the opposite direction, which was consistent with significantly negative correlation between GT and LWR. Thees results demonstrated that many QTLs showed pleiotropic effects and contributed complex trait correlations, which should be taken into account in rice breeding programs.

Compared to QTLs identified in previous studies, a few QTLs in this paper were located in similar regions with previously fine mapping and/or cloned ones. For example, the *qGL2-2* for GL, *qGW2-2* for GW and *qLWR-2* for LWR, located in interval of RM5427-RM263, resided on the location of *GW2*, which might be the right candidate of *GW2* (Song et al., 2007). The *GL-3-1* for GL overlapped with position of *GL3/GL3.1* (Peng et al., 2012; Qiu et al., 2012). Four QTLs (*qGL-5* for GL, *qGW-5* for GW, *qGT-5* for GT, *qLWR-5* for LWR), located in interval of RM267-RM169, coincided with *GW5/qSW5* or *GS5*, which might be the candidate of *GW5/qSW5* or *GS5* (Li et al., 2011; Shomura et al., 2008; Weng et al., 2008). The *qGL-6-1* for GL and *qLWR-6-3* for LWR, located in interval of RM5371-RM340, was consistent with the location of *GW6* (Jun et al., 2015). The *qGT-7* for GT overlapped with region of *GL7/GW7* (Wang et al., 2015; Wang et al., 2015). The *qGL-9* for GL and The *qGT-9* for GT, located in interval of RM257-RM242, overlapped with region of *GW9.1* for grain weight (Xie et al., 2008). The *qGW-1* for GW coincided with *qGRL1* for GL (Singh et al., 2012). Some loci were newly identified, including the interval of RM190-RM225 for GL and LWR, RM527-RM3183 for GW, GT and LWR, RM214-RM418 for GL and LWR, RM3331-RM235 for GL, GT and LWR, RM1022-RM3434 for GL and LWR, and so on.

In this paper, the parents were not extremely contrasting in grain length, grain width, grain thickness and grain length to width ratio. But results showed that previously cloned QTLs related to grain shape were almost detected in two populations, which suggested that identified QTLs in this paper were trustable. On the basis of these results, two RHLs, heterozygous in interval of RM527-RM3183 on chromosome six and RM3331-RM235 on chromosome tweleve respectively, were selected out from RIL population, a series of RHLs with overlapping heterozygous segment in the target regions had been selected from segregating populations derived from self-crossing of the two RHLs, which paved the way for fine mapping of QTLs associated with grain size.

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