



Full Length Article

Scanning QTLs for Grain Shape using Two Sets of Introgression Lines in Rice

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Abstract

Grain shape determines both appearance quality and grain yield. In this study, two sets of introgression line populations derived from three parents were used to dissect the genetic basis of grain shape and illustrate the genetic background effects on QTL expression. Among the three parents, IR75862 was the common donor parent, and Ce258 and Zhongguangxiang1 were two recipient parents. The two introgression line populations were evaluated for grain shape traits in the summer of 2012 at Jingzhou, China. A total of 23 QTLs were detected in all 12 chromosomes except chromosome 1, 3 and 9 for grain length, grain width, grain thickness and length to width ratio, 11 and 12 detected in Ce258 and Zhongguangxiang1 background, respectively. They explained 6.95–38.27% of phenotypic variation. Among these QTLs, only three (26%) named qGL8, qLWR5 and qLWR10 stably expressed in both genetic backgrounds, clearly indicating that genetic background had large effects on QTL expression for grain shape. IR75862 alleles at these QTLs decreased GL and LWR in both backgrounds. Our findings will facilitate the gene cloning and breeding application for improvement of grain yield and quality in future. © 2017 Friends Science Publishers

Keywords: Rice; Introgression lines; Grain shape; Quantitative trait locus; Genetic background

Introduction

Rice (*Oryza sativa* L.) is a very important crop in the world, providing staple food for 60% of the world's people (Zhang, 2007; Kumar *et al.*, 2013). More than 40% more rice will be required in rice consumption area in next half century (Khush, 2005). Besides, with the improvement of living standard, grain quality has been paid more attention by consumers than ever before. Grain shape is a very important appearance quality trait, and has large effect on milling quality and grain yield. Consumers at different areas prefer rice of different grain (de-hulled seeds) shapes (Luo *et al.*, 2004). Thus, rice grain shape became an important breeding trait (Li *et al.*, 2004a). Furthermore, rice is considered a model plant for genetics and genomics of crops (Delseny *et al.*, 2001; Paterson *et al.*, 2005). Using comparative genomics method, some grain shape genes in maize and wheat have been cloned (Li *et al.*, 2010a, b; Su *et al.*, 2011; Bednarek *et al.*, 2012).

Grain shape is determined by grain length (GL), grain

width (GW), length to width ratio (LWR) and grain thickness (GT). The former three traits have high heritability, while the heritability of GT is low (Hu *et al.*, 2013). All of them are typical quantitative traits controlled by multiple genes distributed on all 12 chromosomes (<http://archive.gramene.org/ql/>) and affected by environmental factors. With the development of rice functional genomics and molecular marker technology, researchers have mapped many QTLs for grain shape using different types of populations (Tan *et al.*, 2000; Li *et al.*, 2004b; Raibiei *et al.*, 2004; Bai *et al.*, 2010; Li *et al.*, 2011a; Yan *et al.*, 2014; Chen *et al.*, 2016; Liu *et al.*, 2016). Some QTLs with large effect were successfully fine mapped and cloned in different rice germplasms by map-based cloning approach. *GS3* and *GL3.1* both affect GL. A C-A mutation in the second exon and a rare allele in these two gene result in long grain (Fan *et al.*, 2006, 2008; Takano-Kai *et al.*, 2009; Mao *et al.*, 2010; Qi *et al.*, 2012; Zhang *et al.*, 2012; Hu *et al.*, 2012; Lu *et al.*, 2013). Both *GW2* and *qSW5/GW5* control GW (Song *et al.*, 2007; Weng *et al.*, 2008; Shomura

et al., 2008). 1-bp and 1212-bp deletions in these two genes cause loss of function, and finally increase GW. All of these four genes were negative regulators of grain shape or size, while the other four genes are positive regulators. *GS2/GL2* encodes a transcriptional regulator Growth-Regulating Factor 4 (Zhang *et al.*, 2013; Hu *et al.*, 2015b; Che *et al.*, 2015). A TC-AA mutation in the miR396c target site increases the expression of *GS2*, and leads to large grain size. *GS5* and *GW8* encode a putative serine carboxypeptidase and the SBP-family transcription factor *OsSPL16*, respectively (Li *et al.*, 2011b; Wang *et al.*, 2012b). The expression levels are positively associated with GW. *GL7/GW7* encodes a TONNEAU1-recruiting motif protein (Shao *et al.*, 2012; Qiu *et al.*, 2012; Wang *et al.*, 2015a, b). Copy number Variations are associated with changes in expression of *GL7/GW7*, resulting in slender grains. Although more and more genes for grain shape have been characterized, only a few genes have been successfully used in molecular breeding for improving grain shape (Wang *et al.*, 2012a, b). Therefore, identification of more favorable genes/QTLs for grain shape from rice germplasms is very important and essential to develop high yield and quality varieties in molecular breeding.

Although many genes were detected to have large effects on grain shape, the applications of them on molecular breeding aren't always successful, because of insistence of genetic background between mapping population and breeding population. Many researches indicated that expression of QTLs for many traits is largely affected by genetic background, such as panicle size, drought tolerance and salt tolerance (Liao *et al.*, 2001; Mei *et al.*, 2006; Xie *et al.*, 2008; Cheng *et al.*, 2012a, b; Wang *et al.*, 2013 and 2014; Qiu *et al.*, 2015). Although a lot of QTLs for rice grain shape have been reported, our understanding of the effect of genetic background on detection of QTL for grain shape is very limited. Some special mapping populations derived from common parents have advantage to dissect the effect of genetic background on QTL expression, such as reciprocal recombinant inbred line (Zhao *et al.*, 2016), reciprocal introgression line (IL) (Wang *et al.*, 2014), two or more introgression lines with common parents (Peng *et al.*, 2014; Qiu *et al.*, 2015), for QTL identified in different backgrounds could be compared and common QTLs detected in several backgrounds could be mined.

In our previous study, two sets of IL populations were developed derived from a common donor parent, IR75862-206-2-8-3-B-B-B (IR75862), and two recipient parents, Ce258 and Zhongguangxiang1 (ZGX1) (Qiu *et al.*, 2015). We used these two sets of ILs to illustrate the genetic basis of grain shape. The objectives of this study were to (1) detect QTLs for grain shape traits, (2) evaluate genetic background effect on QTL detection and mine QTLs independent from genetic background. The results will give us useful information to improve rice grain shape in molecular breeding.

Materials and Methods

Plant Materials and Field Experiment

Two sets of IL populations were used in this study. They were derived from IR75862 as common donor parent, and Ce258 and ZGX1 as recipient parents, respectively. IR75862 is a tropical *japonica* glutinous variety with high zinc and iron concentration and short grains introduced from IRR1 (Impa *et al.*, 2013). Ce258 and ZGX1 are two elite *indica* variety with long grains. IR75862 was crossed with Ce258 and ZGX1, respectively, and then the F₁ individuals were backcrossed with the recurrent parents for one time. The BC₁F₁ progenies were self-crossed without selections for nine generations to BC₁F₁₀. Ultimately, two sets of introgression lines (ILs) were developed for QTL mapping, each comprising 200 lines in Ce258 background (Ce258-ILs) and ZGX1 background (ZGX1-ILs), respectively.

A total of 403 lines, including 400 ILs and three parents, were planted in the summer season of 2012 on the farm of College of Agriculture, Yangtze University, Jingzhou, China (30.2°N, 112.7°E). Jingzhou is located in the middle of Yangtze River, and is a main production area of rice in China. Seeds were sown on May 10 and transplanted on June 5 with a randomized complete block design. Each IL was planted in three rows and ten individuals in each row at spacing of 20 cm × 20 cm with two replications. All field management followed local farmers' practices.

Trait Measurements

Five individuals in the second row of each IL were harvested, bulked and stored for at least three months. One hundred full-filled seeds were randomly chosen to evaluate grain shape traits. GL was estimated for three times by lined up 10 grains end-to-end. GW was measured for three times by arranging 10 grains in the breadth. 20 grains were individually measured for GT using an electronic digital caliper (Yaxing Precision Co. Ltd., HongKong, China). LWR was calculated as GL by GW. The average of grain shape traits were used for phenotype analysis.

DNA Extraction and Marker Analysis

Young leaves of about 30 individuals per IL were bulked and used to extract DNA using CTAB method with minor modifications (Murray and Thompson, 1980). Total of 128 and 133 SSR markers evenly distributed on rice genome (<http://archive.gramene.org/markers/>) were selected for genotyping ILs for Ce258-ILs and ZGX1-ILs, respectively. Among them, 81 markers were common to the two populations (Fig. 2). Marker locations on linkage maps were according to published rice linkage map (Temnykh *et al.*, 2001; McCouch *et al.*, 2002).

Data Analysis

Statistical description and correlations among different traits were analyzed by Statistica 5.5 (Morales, 2001). Inclusive composite interval mapping method (ICIM) in QTL IciMapping 3.2 were used for QTL mapping (Li *et al.*, 2007). The permutation method based on 1000 runs of randomly shuffling the trait values was used to obtain empirical thresholds (Churchill and Doerge, 1994).

Results

Grain Shape Performance of Parents and Two Introgression Line Populations

Significantly differences were observed among three parents for grain shape traits (Table 1). The donor parent IR75862 had significantly shorter grains and smaller LWR than those of the two recurrent parents Ce258 and ZGX1. For GW,

Table 1: Statistical descriptions of four grain shape traits in two sets of IL populations derived from a common donor, IR75862, and two recipient parents, Ce258 and ZGX1

Traits	Ce258	ZGX1	IR75862	P1-P3	P2-P3	Ce258-ILs			ZGX1-ILs		
	(P1)	(P2)	(P3)			Mean±SD	Range	CV(%)	Mean±SD	Range	CV(%)
GL	9.07	9.77	8.49	0.58**	1.28**	9.91±0.47	8.65-11.78	4.74	9.19±0.41	7.26-10.81	4.39
GW	2.18	2.39	2.41	-0.23*	-0.02	2.31±0.21	1.79-3.31	9.05	2.07±0.18	1.50-3.15	8.49
GT	2.01	1.79	2.08	-0.07	-0.29*	1.96±0.16	1.24-2.42	8.17	1.77±0.13	1.23-2.22	7.56
LWR	4.16	4.08	3.52	0.64**	0.56**	4.33±0.40	2.36-5.52	9.19	4.48±0.32	2.68-5.46	7.21

*and **, represent significant levels at $P \leq 0.05$ and 0.01 , respectively; GL: grain length, GW: grain width, GT: grain thickness, LWR: length to width ratio

Table 2: Correlation coefficients of four grain shape traits in two sets of ILs populations derived from a common donor, IR75862, and two recipient parents, Ce258 and ZGX1

	GL	GW	GT	LWR
GL				
GW	-0.10			
GT	0.12	0.43**		
LWR	0.55**	-0.85**	-0.33**	

Data under and above the diagonal are correlation coefficients in Ce258-ILs and ZGX1-ILs, respectively; *and **, represent significant levels at $P \leq 0.05$ and 0.01 , respectively; GL: grain length, GW: grain width, GT: grain thickness, LWR: length to width ratio

Table 3: QTLs affecting four grain shape traits in two sets of ILs populations derived from a common donor, IR75862, and two recipient parents, Ce258 and ZGX1

Background	Trait	QTL	Chr.	Marker interval	LOD	A ^a	R ² (%) ^b	grain shape genes ^c
Ce258	GL	<i>qGL8</i>	8	RM284-RM80	3.63	-0.60	29.30	<i>GW8</i>
		<i>qGL12</i>	12	RM3409-RM235	3.24	-0.19	7.96	
	GW	<i>qGW5</i>	5	RM413-RM289	6.02	0.38	34.41	<i>SRS3, GS5, qSW5/GW5</i>
		<i>qGW6</i>	6	RM3-RM528	8.11	-0.52	38.27	
		<i>qGW8</i>	8	RM284-RM80	3.66	0.27	24.50	<i>GW8</i>
	GT	<i>qGT7</i>	7	RM11-RM336	5.56	-0.24	12.61	
		<i>qGT10</i>	10	RM467-RM3773	3.49	0.34	18.50	
	LWR	<i>qLWR2</i>	2	RM154-RM211	3.04	-0.16	7.01	<i>GW2</i>
		<i>qLWR5</i>	5	RM413-RM289	2.89	-0.35	22.17	<i>SRS3, GS5, qSW5/GW5</i>
		<i>qLWR7</i>	7	RM427-RM11	3.96	-0.42	20.58	
<i>qLWR10</i>		10	RM222-RM311	2.85	-0.33	20.61		
ZGX1	GL	<i>qGL5</i>	5	RM153-RM413	2.67	-0.45	13.57	<i>SRS3, GS5, qSW5/GW5</i>
		<i>qGL7</i>	7	RM455-RM234	9.05	-0.41	28.47	<i>GL7/GW7</i>
		<i>qGL8</i>	8	RM284-RM210	6.81	-0.34	15.95	<i>GW8</i>
		<i>qGL10</i>	10	RM147-RM590	8.11	-0.50	20.07	
	GT	<i>qGL11</i>	11	RM332-RM167	2.71	0.21	9.46	
		<i>qGT5.1</i>	5	RM509-RM430	2.63	-0.19	18.38	
		<i>qGT5.2</i>	5	RM161-RM26	2.60	0.19	16.52	
		<i>qGT8</i>	8	RM284-RM210	2.86	0.07	16.52	<i>GW8</i>
	LWR	<i>qGT11</i>	11	RM260-RM287	2.70	0.07	6.95	
		<i>qLWR4</i>	4	RM348-RM349	2.75	-0.53	19.68	
<i>qLWR5</i>		5	RM413-RM405	6.05	-0.72	23.94	<i>SRS3, GS5, qSW5/GW5</i>	
		<i>qLWR10</i>	10	RM222-RM311	4.56	-0.73	23.90	

GL: grain length, GW: grain width, GT: grain thickness, LWR: length to width ratio; ^a The additive effect is the effect associated with substitution of Ce258 and ZGX1 alleles by IR75862 alleles; ^b Phenotypic variation explained by the QTL; *GW2* (Song *et al.*, 2007), *SRS3* (Kitagawa *et al.*, 2010), *GS5* (Li *et al.*, 2011b), *qSW5* (Shomura *et al.*, 2008), *GW5* (Weng *et al.*, 2008), *GL7* (Wang *et al.*, 2015b), *GW7* (Wang *et al.*, 2015a), *GW8* (Wang *et al.*, 2012b)

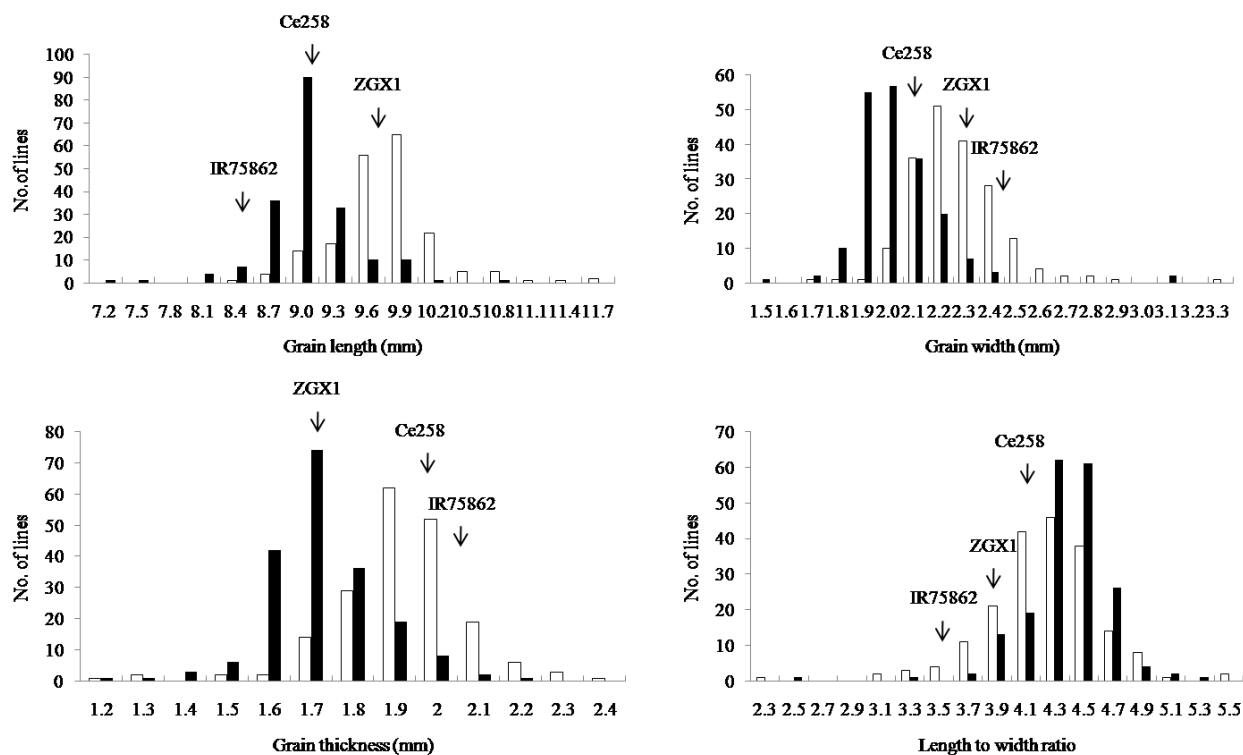


Fig.1 Frequency distributions of four grain shape traits in the two sets of IL populations derived from a common donor, IR75862, and two recipient parents, Ce258 and ZGX1. White and black bars represent IL populations with Ce258 and ZGX1 backgrounds, respectively

IR75862 was almost same with ZGX1 but significantly wider than that of Ce258. For GT, IR75862 was similar to Ce258 and they were thicker than that of ZGX1.

All grain shape traits exhibited continuous distributions with transgressive segregations in both two IL populations (Table 1; Fig. 1), indicating that all measured traits are controlled by multiple genes.

Correlations of Traits in the Two Introgression Line Populations

Correlation coefficients between two traits were demonstrated in Table 2. In both backgrounds, LWR was significantly negatively correlated with GW and GT, indicated that slender and thin grains had bigger LWR. GT had positive correlation with GW, suggesting that wider grain filled better.

There were some inconsistent correlations among traits in the two populations. For example, positive correlation was observed between GL and LWR in only Ce258-ILs, while GL was negatively correlated with GW and GT only in ZGX1-ILs. These differences may come from genetic background variations.

QTL Scanning for Grain Shape Traits

QTLs for GL: Two and five QTLs for GL were detected on Ce258-ILs and ZGX1-ILs, respectively (Table 3; Fig. 2),

located on chromosome 5, 7, 8, 10, 11 and 12. The phenotypic variations caused by each QTL were around 7.96–29.30%, respectively. Among these QTLs, *qGL8* detected in Ce258-ILs had the biggest phenotypic variation rate. IR75862 alleles at all loci decreased GL except for *qGL11*.

QTLs for GW: Three QTLs controlling GW were identified in Ce258-ILs, located on chromosome 5, 6 and 8 (Table 3; Fig. 2). Each QTL explained 24.50–38.27% of phenotypic variance, respectively. Among them, *qGW6* contributed most. IR75862 alleles at all loci increased GW except for *qGW6*. There was no QTL detected in ZGX1-ILs, probably because of no difference between that of ZGX1 and IR75862.

QTLs for GT: Six QTLs affecting GT were found in the two populations (Table 3; Fig. 2). They were located on chromosome 5, 7, 8, 10 and 11. Two QTLs were identified in Ce258-ILs and four in ZGX1-ILs, respectively. QTL individually explained 6.95–18.50% of phenotypic variance, respectively. Among these QTLs, *qGT10* detected in Ce258-ILs had the largest phenotypic variation rate. All positive alleles of QTL came from IR75862 except for *qGT7* and *qGT5.1*.

QTLs for LWR: Seven QTLs influencing LWR were scanned in the two populations, four in Ce258-ILs and three in ZGX1-ILs, respectively (Table 3; Fig. 2). They were located on chromosome 2, 4, 5, 7 and 10. The QTLs explained up to 23.94% of phenotypic variance,

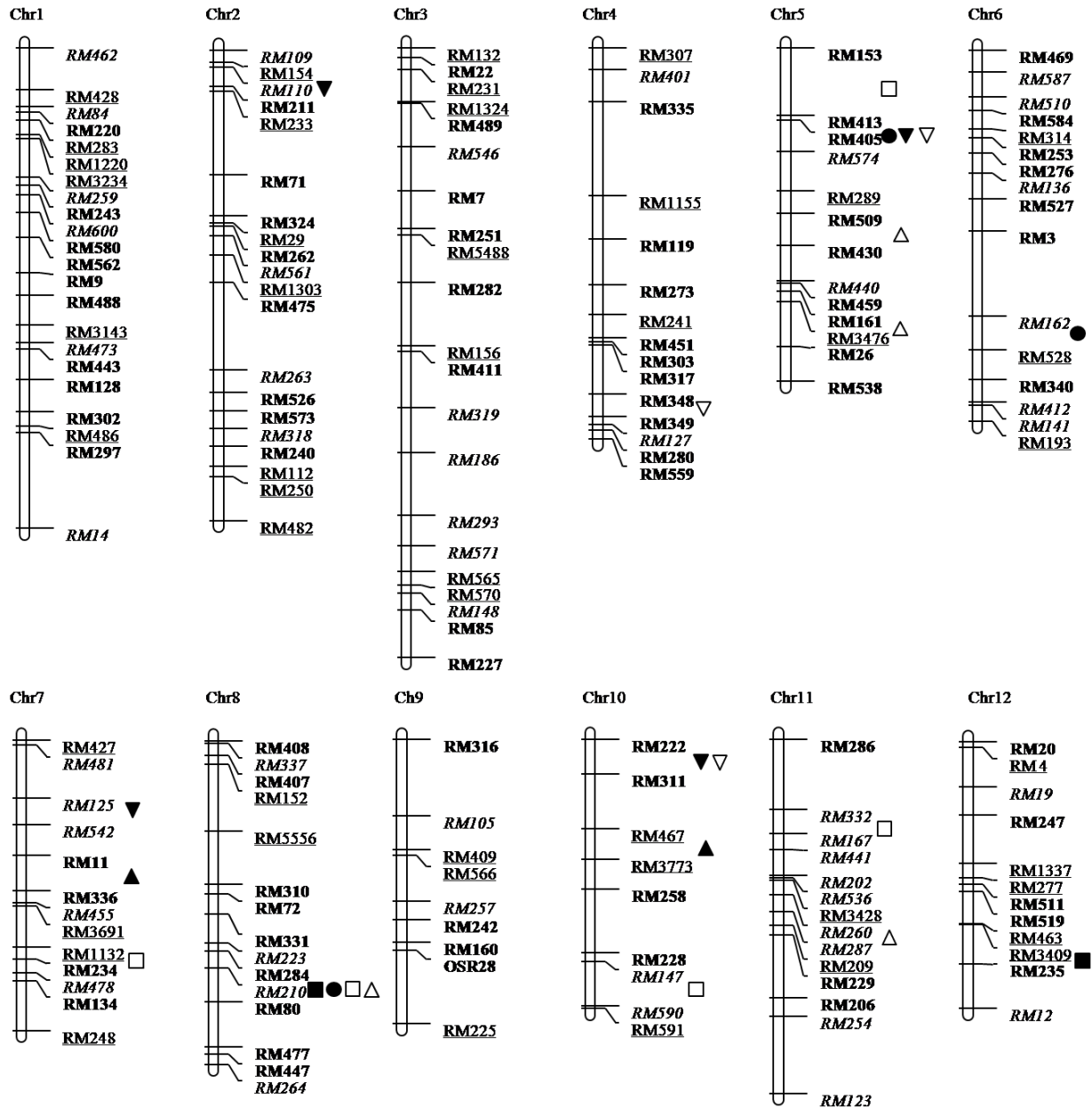


Fig. 2. Linkage maps and locations of QTLs affecting four grain shape traits identified in two sets of IL populations derived from a common donor parent, IR75862, and two recipient parents, Ce258 and ZGX1.

■ ● ▲ ▼ represent QTLs for GL, GW, GT and LWR in Ce258-ILs, while □ ○ △ ▽ represent QTLs for GL, GW, GT and LWR in ZGX1-ILs. Underline stands for markers in Ce258-ILs only, italic for markers in ZGX1-ILs only, and bold for markers common in the two introgression lines

respectively. Among these QTLs, *qLWR5* detected in ZGX1-ILs contributed most. All negative alleles of QTL came from IR75862.

QTLs Common Detected in Both Backgrounds

Twenty-three QTLs affecting four grain shape traits were identified in two sets of ILs. Among them, three QTLs

(26%) were mapped in both genetic backgrounds (Table 3; Fig. 2). *qGL8* was identified in the region flanked by marker RM284 and RM80, where IR75862 allele decreased GL in both backgrounds. *qLWR5* and *qLWR10* were detected in the region of RM413-RM289 and RM222-RM311, respectively. IR75862 at both loci decreased LWR at both genetic backgrounds.

Discussion

A lot of evidences indicated that effect of genetic background on QTL mapping is very large. The QTL effects in different backgrounds were diverse when different recipient parents were used (Yousef and Juvik, 2002). It was reported that below twenty percent of the QTLs for yield and related traits, resistance to biotic and abiotic stresses were detected in reciprocal ILs (Mei *et al.*, 2006; Xie *et al.*, 2008; Cheng *et al.*, 2012; Wang *et al.*, 2014). Till now, very little researches reported genetic background effect on QTL expression for grain shape, and very few QTL for grain shape independent from genetic background was identified. It's uncertain whether it will be successful when a QTL is applied in molecular breeding for improving quality, because no one knows if the QTL still works in a different background. In this study, only 3 out of 23 QTLs for grain shape (26%) were identified in both backgrounds, clearly indicating overwhelming genetic background effects on QTL detection for grain shape. Therefore, if QTL mapping information is wanted to apply to rice molecular breeding for improving grain shape, much attention should be paid. It is essential that QTL mapping and molecular breeding are carried out in the same genetic background. Fortunately, these three QTLs independent from background have largely potential value applied in molecular breeding for improving rice quality, for it will still work in other breeding populations and could be higher possibly successful in molecular breeding than other QTLs.

In this study, 23 QTLs were identified for grain shape traits using two sets of IL populations with the same donor parent. They were compared with those previous studies (<http://archive.gramene.org/qlt/>). Some QTLs were located in the same region with cloned genes. For example, *qLWR2* in the region of RM154-RM211 for LWR was in the same region of *GW2* (Song *et al.*, 2007). *qGL5* in the region of RM153-RM413 for GL and *qGW5* and *qLWR5* for GW and LWR in the region of RM413-RM289 were mapped together with *SRS3*, *GS5*, *qSW5/GW5* (Weng *et al.*, 2008; Shomura *et al.*, 2008; Kitagawa *et al.*, 2010; Li *et al.*, 2011b). *qGL7* flanked between RM455 and RM234 for GL was mapped together with *GL7/GW7* (Wang *et al.*, 2015a, b). *qGL8*, *qGW8* and *qGT8* flanked between RM284 and RM210 for GL and width and thickness were mapped in the same region of *GW8* (Wang *et al.*, 2012b). Besides, some QTLs were mapped near the regions in previous studies. *qGT5.1* in the region of RM509-RM430 and *qGT5.2* in the region of RM161-RM26 for GT were located near *qGT-5-1* (Li *et al.*, 2011a). *qGT7* in the region of RM111-RM336 for GL and *qLWR7* flanked between RM427 and RM11 for LWR were located in the adjacent region of *qGL7-2* and *qLWR7* (Li *et al.*, 2011a). *qGL10* in the region of RM147-RM590 for GL was identified near *qGL-10* (Li *et al.*, 2011a). *qLWR10* flanked between RM222 and RM311 was located in the same region of *qGW-10* (Li *et al.*, 2011a). *qGT10* in the region of RM467-RM3773 for GT was

mapped in the adjacent region of *qGT10* (Yan *et al.*, 2014). *qGT11* flanked between RM260 and RM287 for GT was located in the same region of *qGT11* (Yan *et al.*, 2014). Whether the above QTLs identified in this study and the reported grain shape QTLs/genes are the same genes will need to be further clarified after fine mapping and cloning of the QTLs.

Improving rice shape is one of the most important goals of most rice breeding programs worldwide. However, grain shape traits are all complex traits controlled by a large number of QTLs/ genes, and it's difficult for us to select high-yield rice variety with good grain shape. Fortunately, rice has a large number of germplasms, which are gene carriers with all kinds of alleles (Tansley and McCouch, 1997). There are plenty of novel alleles distributed in unique subspecies. For instance, favorable alleles of *SH1*, *NRT1.1B* exist only in *indica* (Konishi *et al.*, 2006; Hu *et al.*, 2015a), while favorable alleles of *GL7/GW7* and *GW8* distribute mainly in *tropical japonica* (Wang *et al.*, 2012b; Wang *et al.*, 2015a, b). Identification and use of these alleles in rice molecular breeding can greatly speed up breeding proceeding. In the present study, the genome of a *tropical japonica* variety (IR75862) was introgressed into two *indica* varieties (Ce258 and ZGX1) genome. 6 QTLs (26%) of IR75862 (*qGL11*, *qGW6*, *qGT5.2*, *qGT8*, *qGT10* and *qGT11*) can increase GL and GT and decrease GW. It's possible to develop new high-yield rice varieties with good grain shape through introgressing and pyramiding the IR75862 alleles of these loci into Ce258 and ZGX1 by marker-assisted selection. Furthermore, there were three QTLs (*qGL8*, *qLWR5* and *qLWR10*) commonly detected in the two populations, indicating that they are independent from genetic background.

Conclusion

Although IR75862 alleles at all loci decrease grain shape, researchers can use mine novel alleles from rice germplasms, and apply them into molecular breeding for improving rice quality. They are highly valuable genetic recourses for quality improvement in rice.

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