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Genetic dissection of large grain shape in rice cultivar 'Nanyangzhan' and validation of a grain thickness QTL (*qGT3.1*) and a grain length QTL (*qGL3.4*)

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Abstract Grain shape is an important agronomic trait in rice, which influences the yield and quality. In order to dissect the genetic basis of the large grain shape in 'Nanyangzhan', a recombinant inbred line (RIL) population derived from Nanyangzhan (NYZ) and Zhenshan 97B (ZS97) was used to map quantitative trait loci (QTLs) for grain length (GL), width (GW), thickness (GT), length-to-width ratio (LWR) and kilo-grain weight (KGW). A total of 53 QTLs were detected and distributed on 11 chromosomes in 2 years. Among those, QTLs for GW and GL showed a concentrated distribution on chromosome 2 and chromosome 3, respectively. NYZ, the parent with large grain shape, carried 44 alleles showing positive effects on the studied traits. In addition, the near-isogenic lines (NILs) of two novel QTLs, *qGT3.1* and *qGL3.4*, were constructed with the background of ZS97. Results showed that NIL-$qGT3.1^{NYZ}$, the NIL carrying homozygous *qGT3.1* regions from NYZ, showed an increased value of 0.12 mm in grain thickness on average as compared to NIL-$qGT3.1^{ZS}$. Similarly, NIL-$qGL3.4^{NYZ}$ increased the length of each grain by 0.47 mm on average as compared to NIL-$qGL3.4^{ZS}$. Taken together, these results would be of great use in breeding rice cultivars with desirable grain shape.

Keywords QTL · Grain shape · Grain thickness · Grain length · *Oryza sativa* L

Introduction

Rice (*Oryza sativa* L.) is a prominent cereal crop and major staple food all around the world. Among various agronomic traits of rice, grain shape is a key component, which not only determines grain weight and yield, but also affects the end-use quality of rice (Huang et al. 2013). In breeding programs, grain shape characters including grain length (GL), width (GW), thickness (GT) and length-to-width ratio (LWR) are importantly considered for the evaluation of grain weight and final yield. Therefore, elucidating the genetic basis of grain shape would be of great interest for breeding cultivars with high yield and quality.

During the past two decades, great efforts have been made to understand the genetic basis of grain shape. It is well-evident that the four components of grain shape are typical quantitative traits controlled by polygenes (Xing and Zhang 2010; Zuo and Li 2014). To date, a huge number of quantitative trait loci (QTLs) regulating GL, GW, GT, and LWR have been reported, which were distributed over all the 12 chromosomes. Among those, a few QTLs such as *GS2*/*GL2* (Hu et al. 2015; Che et al. 2015; Duan et al. 2015), *GS3* (Fan et al. 2006), *GL3*/*GL3.1* (Qi et al. 2012; Zhang et al. 2012), *GLW7* (Si et al. 2016), *GW2* (Song et al. 2007), *qSW5*/*GW5*

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(Shomura et al. 2008; Weng et al. 2008), *GS5* (Li et al. 2011), *GW6a* (Song et al., 2014), *TGW6* (Ishimaru et al. 2013), *GW8* (Wang et al. 2012a), and *GS7*/*GW7* (Wang et al. 2015a, b) have been cloned, which have greatly increased our knowledge regarding the genetic basis of grain shape. However, more attention should be paid to mining novel QTL/genes to further expand the understanding of the regulatory mechanisms of grain shape.

Rice cultivars with large grain shape are valuable germplasm resources, which contain novel alleles having great contribution to grain shape. For instance, *GW2*, a major QTL conferring grain width and weight, was isolated from WY3, *a japonica* variety with a KGW value of 41.9 ± 1.3 g (Song et al. 2007). *GL3*/*GL3.1*, a major QTL for grain length and weight, was identified in WY3, and N411, a *japonica* variety with a KGW value of 72.13 ± 2.32 g (Qi et al. 2012; Zhang et al. 2012). Likewise, *GS2*/*GL2*, a major QTL for GL, GW, GT and KGW, was isolated from three *indica* cultivars (BDL, RW11, Judali) with extra large grain shape (Hu et al. 2015; Che et al. 2015; Duan et al. 2015). Therefore, identifying and isolating novel alleles for grain shape from cultivars with large grain shape would not only help in elucidating the genetic basis and molecular mechanisms of grain shape, but also provide valuable gene resource for rice breeding programs.

In this study, a recombinant inbred line (RIL) population derived from two *indica* cultivars, Zhenshan 97B (ZS97) and Nanyangzhan (NYZ) was developed, and used to dissect the genetic basis of the extremely large grain shape in NYZ. QTLs for GL, GW, GT, LWR and KGW were mapped, and two QTLs conferring GT (*qGT3.1*) and GL (*qGL3.4*) were validated in the near-isogenic lines (NILs) background, respectively. These results would facilitate the future breeding of high-yielding and good-quality rice varieties.

Materials and methods

Population development and field experiments

A population of 190 RILs was developed from a cross between two *indica* varieties viz., Zhenshan 97B (ZS97) and Nanyangzhan (NYZ), followed by a series of self-crosses using the single-seed decent method. NYZ has extra large grains, while ZS97 possesses small grains. The RILs F_7 and F_8 were planted at the experimental farm of Huazhong Agricultural University in Lingshui (Hainan) during 2001 growing seasons, and Wuhan (Hubei) during 2002 growing seasons.

The NILs for *qGT3.1* and *qGL3.4* were constructed by backcrossing the selected F_9 lines with the recurrent parent, ZS97, for four times, followed by a self-cross. Two pairs of NILs, NIL-$qGT3.1^{NYZ}$ and NIL-$qGT3.1^{ZS}$, and NIL-$qGL3.4^{NYZ}$ and NIL-$qGL3.4^{ZS}$, were produced. NIL-$qGT3.1^{NYZ}$ and NIL-$qGT3.1^{ZS}$ were the NILs carrying homozygous *qGT3.1* regions from NYZ and ZS, respectively, while NIL-$qGL3.4^{NYZ}$ and NIL-$qGL3.4^{ZS}$ were the NILs carrying homozygous *qGL3.4* regions from NYZ and ZS, respectively. The two pairs of NILs were planted at the experimental farm of Huazhong Agricultural University in Wuhan (Hubei) during 2011 growing seasons.

Trait evaluation

After harvesting, paddy rice was air-dried and stored at room temperature for at least 3 months before testing. Fully filled grains were used for measuring the GL, GW, GT and KGW. GL and GW were measured as described in Tan et al. (2000). LWR was calculated as the ratio of GL to GW. GT was determined for each grain individually using an electronic digital caliper (Guanglu Measuring Instrument Co. Ltd., China) with a precision of 0.1 mm, and thirty grain values were averaged and used for data analysis. KGW was calculated based on 400 grains and replicated for three times.

Molecular marker development and analysis

For genotyping of the RIL population, simple sequence repeat (SSR) markers were identified from the Gramene database (www.gramene.org).

DNA was extracted according to the CTAB method with minor modification (Murray and Thompson 1980). Marker analysis was performed according to Sun et al. (2013).

Construction of genetic linkage map

A total of 574 SSR markers were used to screen for the polymorphic markers between two parents (ZS97 and NYZ) and 185 markers were identified. Among those, 106 markers distributed evenly over all 12 rice chromosomes were employed to screen the RIL F_7 lines. A linkage map was constructed using the program

Mapmaker/Exp 3.0 (Lincoln et al., 1992) with the Kosambi mapping function.

Data analysis

Correlation analysis was performed using the data analysis module in Microsoft Office Excel 2007. QTL analysis was performed by composite interval mapping (CIM) using the software package QTLCartographer V2.5 (Wang et al. 2012b) with a logarithm of odds (LOD) threshold of 2.5.

Results

Phenotypic variation and correlation in the RIL population

A significant difference was observed between the two parents, ZS97 and NYZ, regarding all the five studied traits (Fig. 1; Table 1). Averaged across 2 years, the large-grain parent (NYZ) had a GL value of 12.1 mm, GW of 3.6 mm, LWR of 3.3 and KGW of 40.1 g. In contrast, the small-grain parent (ZS97) had an average value of 7.9 mm for GL, 3.3 mm for GW, 2.3 for LWR and 25.6 g for KGW. The GT values of NYZ and ZS97 were 2.54 mm and 2.09 mm, respectively, in 2002. All the five traits showed continuous variation, followed the normal distribution and displayed transgressive segregation in the RIL population during 2 years.

All the four grain shape factors, GL, GW, LWR and GT, were significantly and positively correlated with KGW in both years (Table 2). The highest correlation coefficient was observed between GL and KGW, with a value of 0.641 in 2001 and 0.695 in 2002. GT was also positively correlated with GL ($r = 0.410$) and GW ($r = 0.328$) in year 2002, while no correlation was observed between GL and GW.

QTLs identified in the RIL population

GL

A total of 17 QTLs for GL were detected in 2 years (Table 3; Fig. 2). The phenotypic variance explained by each QTL ranged from 2.53 to 33.04%. Two QTLs (*qGL3.2* and *qGL3.3*) located between MRG2204 and RM411 on chromosome 3 accounted for 33.04% and 31.81% of GL variation in 2001 and in 2002, respectively. Three QTLs viz., *qGL3.1*, *qGL3.4* and *qGL10*, were repeatedly detected, and explained 8.55%, 11.32% and 3.35% variation in 2001, and 11.24%, 11.50% and 8.58% variation in 2002, respectively. Two QTLs (*qGL11.1* and *qGL11.2*) located at adjacent regions on chromosome 11 accounted for 4.00% of the GL variation in both years. The remaining 10 QTLs were identified only in a single year, and explained less than 10% variation.

GW

In total, 12 QTLs for GW were detected in 2 years (Table 3; Fig. 2), and the variation explained by each QTL ranged from 4.36% to 22.05%. The QTL *qGW2.4*, located between RM183 and RM526 on chromosome 2, was repeatedly detected in both years and explained 22.05% and 11.6% of GW variation in 2001 and 2002, respectively. *qGW2.2*, the other repeatedly detected QTL on chromosome 2, explained 4.71% variation in 2001 and 6.79% in 2002. Two other QTLs (*qGW2.3* and *qGW2.6*) located on chromosome 2 explained 11.7% and 10.6% variation in 2001 and 2002, respectively. *qGW4*, the QTL flanked by RM317 and RM255 on chromosome 4, and *qGW6.1*, the QTL flanked by RM585 and RM557 on chromosome 6, accounted for 14.17% and 13.57% variation in 2001, respectively. The remaining 6 QTLs were detected only in a single year, and accounted for less than 10% variation.

LWR

In total, eight QTLs were detected for LWR in 2 years (Table 3; Fig.2). Among those, *qLWR3.2*, located between MRG2204 and RM156 on chromosome 3, explained 27.85% of phenotypic variation in 2001 and 33.19% in 2002. The other three repeatedly detected QTLs, *qLWR2.1*, *qLWR3.3* and *qLWR5*, explained 10.35%, 4.40% and 8.44% variation in 2001, and 7.68%, 7.29% and 8.11% variation in 2002, respectively. The QTL flanked by RM517 and RM473D on chromosome 3, *qLWR3.1*, accounted for 10.33% variation in 2001. The remaining 3 minor QTLs explained less than 10% variation, and were only detected in 2001.

KGW

Totally, 11 QTLs for KGW were detected in 2 years (Table 3; Fig. 2). Among those, *qKGW3.1*, located

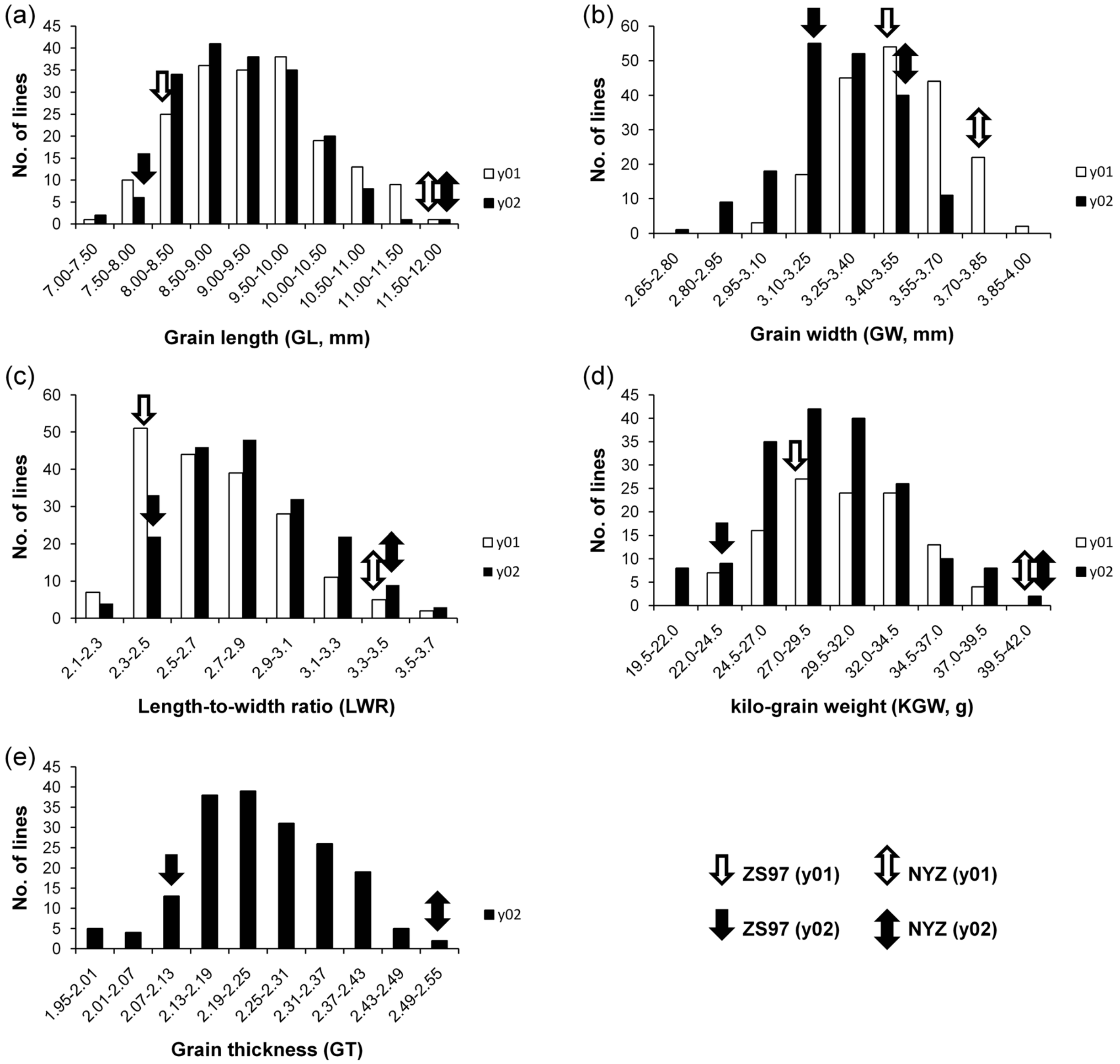

Fig. 1 Frequency distribution of RIL population for GL, GW, LWR, KGW and GT during 2001 and 2002 growing seasons. y01, year 2001; y02, year 2002

between MRG2204 and RM156 on chromosome 3, explained 21.43% of KGW variation in 2001 and 17.80% in 2002. The remaining QTLs were only identified in a single year, and the variance explained by each QTL ranged from 3.58% to 13.66%.

GT

Five QTLs for GT were detected in year 2002 (Table 3; Fig. 2), which were distributed on chromosomes 3, 4 and 9. The QTL *qGT3.1*, located between RM545 and RM517 on chromosome 3, explained 16.94% of phenotypic variation. The variation explained by each of the other four QTLs was less than 10%.

Validation of *qGT3.1* and *qGL3.4* in the NIL background

Among the five studied traits, GT received less attention in previous studies. Therefore, in order to validate the

Table 1 Descriptive statistics of GL, GW, LWR, KGW and GT in two parents (ZS97 and NYZ) and RIL population during 2001 and 2002 growing seasons

Traits	Year	Parents		RIL Population	
		ZS97	NYZ	Mean	Range
GL (mm)	2001	8.1 ± 0.1	12.1 ± 0.1	9.3	7.3–12.1
	2002	7.7 ± 0.1	12.1 ± 0.1	9.2	7.0–12.1
GW (mm)	2001	3.5 ± 0.0	3.7 ± 0.1	3.5	3.0–4.0
	2002	3.2 ± 0.1	3.5 ± 0.1	3.3	2.7–3.7
LWR	2001	2.3 ± 0.0	3.3 ± 0.0	2.7	2.1–3.7
	2002	2.4 ± 0.1	3.4 ± 0.1	2.8	2.2–3.7
KGW (g)	2001	27.5 ± 0.3	39.7 ± 0.5	30.3	22.0–39.4
	2002	23.7 ± 0.1	40.6 ± 0.9	29.4	19.6–40.3
GT (mm)	2002	2.09 ± 0.08	2.54 ± 0.07	2.24	1.97–2.56

genetic effect of QTLs conferring GT, the NIL of *qGT3.1* was constructed, which showed the largest effect in the RIL population. An F$_9$ line carrying homozygous genotypes from NYZ at markers RM545 and RM517 was backcrossed with the recurrent parent ZS97 for four times, and a BC$_4$F$_1$ line was produced. The genetic background of the BC$_4$F$_1$ line was evaluated with 92 markers evenly covering the whole genome. The result revealed that the line carried two other heterozygous regions not conferring GT on the short arms of chromosomes 1 and 10, except for the target region (data not shown). Thus, the line was self-crossed to produce the two homozygous NILs, NIL-*qGT3.1*^{NYZ} and NIL-*qGT3.1*^{ZS}, to evaluate the genetic effect of *qGT3.1*. As shown in Fig. 3(a), significant difference in grain thickness was observed between the two NILs. Compared to NIL-*qGT3.1*^{ZS}, NIL-*qGT3.1*^{NYZ} displayed an increased value by 0.12 mm in grain thickness.

Of the four QTLs conferring grain length on chromosome 3, the QTL located on the terminal region of the long arm, *qGL3.4*, was selected to validate its effect in the NIL background. The development process of the two homozygous NILs, NIL-*qGL3.4*^{NYZ} and NIL-*qGL3.4*^{ZS}, was similar to that of NIL-*qGT3.1*^{NYZ} and NIL-*qGT3.1*^{ZS}, except that the genotype of *qGL3.4* was determined by RM570 and RM442. As shown in Fig. 3(b), significant difference in grain length existed between the two NILs. Compared to NIL-*qGL3.4*^{ZS}, NIL-*qGL3.4*^{NYZ} displayed an increased value by 0.47 mm in grain length.

Table 2 Correlations of GL, GW, LWR, KGW and GT in 2001 and 2002 growing seasons

	KGW01	KGW02	GL01	GL02	GW01	GW02	LWR01	LWR02	GT02
KGW01									
KGW02	0.718**								
GL01	0.641**	0.616**							
GL02	0.622**	0.695**	0.836**						
GW01	0.315**	0.427**	0.010	0.023					
GW02	0.210*	0.402**	−0.065	−0.047	0.737**				
LWR01	0.430**	0.349**	0.885**	0.727**	−0.453**	−0.395**			
LWR02	0.396**	0.340**	0.710**	0.832**	−0.387**	−0.588**	0.804**		
GT02	0.516**	0.655**	0.328**	0.410**	0.345**	0.328**	0.132	0.156	

GL01, GL in 2001; GL02, GL in 2002; GW01, GW in 2001; GW02, GW in 2002; LWR01, LWR in 2001; LWR02, LWR in 2002; KGW01, KGW in 2001; KGW02, KGW in 2002; GT02, GT in 2002

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

Table 3 QTLs identified for GL, GW, LWR, KGW and GT in the RIL population during 2001 and 2002 growing seasons

Traits	Chr[a]	QTL	Interval	y01[b]			y02		
				LOD	Additive[c]	R^2(%)[d]	LOD	Additive	R^2(%)
GL	1	*qGL1.1*	RM297-RM543				5.93	−0.2105	6.61
	1	*qGL1.2*	RM302-RM102				5.60	−0.2260	7.51
	2	*qGL2.1*	RM322-RM521	4.01	0.2071	4.85			
	2	*qGL2.2*	RM526-RM221	4.29	−0.1832	3.75			
	2	*qGL2.3*	RM497-RM530	3.73	−0.1776	3.56			
	3	*qGL3.1*	RM517-RM473D	4.89	−0.3481	8.55	3.21	−0.3110	11.24
	3	*qGL3.2*	MRG2204-RM156				21.34	−0.4577	31.81
	3	*qGL3.3*	RM156-RM411	27.93	−0.5406	33.04			
	3	*qGL3.4*	RM570-RM442	11.37	−0.3195	11.32	9.59	−0.2783	11.50
	5	*qGL5.1*	RM574-RM169	6.60	−0.2319	6.11			
	5	*qGL5.2*	RM509-RM430	8.14	−0.2792	8.60			
	6	*qGL6.1*	RM136-RM527	5.71	−0.2236	4.91			
	6	*qGL6.2*	RM541-MRG2498	5.98	−0.2126	4.62			
	10	*qGL10*	RM304-RM484	3.54	0.1849	3.35	6.09	0.2528	8.58
	11	*qGL11.1*	RM202-RM287	4.80	−0.1919	4.00			
	11	*qGL11.2*	RM287-RM229				3.30	−0.1657	4.00
	11	*qGL11.3*	RM206-RM187	2.70	−0.1524	2.53			
GW	2	*qGW2.1*	RM322-RM521				3.33	−0.0504	7.38
	2	*qGW2.2*	RM301-RM475	2.50	−0.0418	4.71	3.71	−0.0507	6.79
	2	*qGW2.3*	RM262-RM183	4.51	−0.0660	11.70			
	2	*qGW2.4*	RM183-RM526	9.66	−0.0843	22.05	5.90	−0.0650	11.60
	2	*qGW2.5*	RM530-RM166				5.46	−0.0614	10.60
	2	*qGW2.6*	RM482-RM498				3.36	−0.0440	5.59
	4	*qGW4*	RM317-RM255	8.05	0.0710	14.17			
	6	*qGW6.1*	RM585-RM557	4.70	−0.0716	13.57			
	6	*qGW6.2*	RM136-RM527	5.41	−0.0563	9.01			
	6	*qGW6.3*	RM527-RM541				2.61	−0.0393	4.36
	7	*qGW7*	RM125-RM214				3.21	−0.0454	5.90
	8	*qGW8*	RM502-RM447	2.94	0.0413	4.62			
LWR	2	*qLWR2.1*	RM322-RM521	5.72	0.0965	10.35	4.87	0.0833	7.68
	2	*qLWR2.2*	RM301-RM475	6.59	0.0913	8.70			
	3	*qLWR3.1*	RM517-RM473D	5.87	−0.1243	10.33			
	3	*qLWR3.2*	MRG2204-RM156	19.81	−0.1595	27.85	18.72	−0.1744	33.19
	3	*qLWR3.3*	RM570-RM442	4.25	−0.0639	4.40	5.65	−0.0823	7.29
	4	*qLWR4*	RM252-RM317	4.58	−0.0717	5.51			
	5	*qLWR5*	RM574-RM169	5.41	−0.0868	8.44	3.40	−0.0854	8.11
	11	*qLWR11*	RM202-RM287	4.97	−0.0703	5.23			
KGW	1	*qKGW1.1*	RM246-RM473A	3.88	−1.0584	7.54			
	1	*qKGW1.2*	RM543-RM302				2.50	−0.7923	3.58
	1	*qKGW1.3*	RM102-RM315				3.41	−0.9952	5.58
	2	*qKGW2.1*	RM183-RM526				6.58	−1.5017	12.93
	2	*qKGW2.2*	RM525-RM497				3.15	−0.9075	4.61
	3	*qKGW3.1*	MRG2204-RM156	8.28	−1.8082	21.43	10.08	−1.7420	17.80

Table 3 (continued)

Traits	Chr[a)]	QTL	Interval	y01[b)]			y02		
				LOD	Additive[c)]	R^2(%)[d)]	LOD	Additive	R^2(%)
	3	*qKGW3.2*	RM156-RM411	6.36	−1.4775	13.66			
	6	*qKGW6.1*	RM136-RM527	5.94	−1.4327	12.21			
	6	*qKGW6.2*	MRG2498-RM454				4.26	−1.0957	6.24
	6	*qKGW6.3*	RM454-MRG5298				3.59	−0.9643	5.04
	10	*qKGW10*	RM484-RM228				2.51	0.8408	3.56
GT	3	*qGT3.1*	RM545-RM517				8.39	−0.0582	16.94
	3	*qGT3.2*	RM156-RM411				4.44	−0.0312	7.68
	4	*qGT4.1*	RM317-RM255				5.17	0.0346	9.47
	4	*qGT4.2*	RM255-RM348				3.91	0.0315	8.06
	9	*qGT9*	RM296-RM321				3.62	−0.0290	6.43

a) Chr, chromosome

b) y01, 2001; y02, 2002

c) Additive, additive effect of QTL: positive values indicate that alleles from ZS97 increase the trait scores, and negative values indicate that alleles from NYZ increase the trait scores

d) R^2 , variation explained by QTL

Discussion

QTLs for grain shape and KGW identified in the RIL population

In this study, a total of 53 QTLs for grain shape and KGW were identified from the RIL population derived from the two parents (NYZ and ZS97) differing substantially in their grain shape. It was clearly observed that QTLs for GW were mainly located on chromosome 2, while QTLs for GL were mainly detected on chromosome 3 (Table 3, Fig. 2). Among those, the region flanked by MRG2204 and RM411 on chromosome 3 was responsible for GL and KGW in both years and for GW and GT in 2002 only (Fig. 2). *GS3*, a major QTL for GL and KGW and a minor QTL for GW and GT, showed the same location (Fan et al. 2006). Therefore, *GS3* was likely to be the candidate gene underlying the region. The region flanked by RM322 and RM521 on chromosome 2 was responsible for GL in 2001, GW in 2002 and LWR in both years (Fig. 2). Previously, a major QTL for GW, *GW2*, was observed in this region by Song et al. (2007). Thus, *GW2* was likely to be the candidate gene for GW. In addition, the region flanked by RM183 and RM526 on chromosome 2, and the region flanked by RM574 and RM169 on chromosome 5, showed the same locations with the cloned *GS2*/*GL2* and *SW5*/*GW5*, respectively (Shomura et al. 2008; Weng et al. 2008; Hu et al. 2015; Che et al. 2015; Duan et al. 2015). The remaining QTLs were detected in a single year and no cloned genes for grain shape were co-located.

Accumulation of QTLs showing positive effects contributing to the large grain shape

Rice cultivars with extra large grain shape are valuable resources, which are believed to carry a few novel and major genes for grain shape. Several studies have been conducted to dissect the genetic basis of large grain shape, in which two genes conferring GL, *GS3* and *GL3*/*GL3.1* (Kato et al. 2011; Zhang et al. 2011; Qi et al. 2012; Zhang et al. 2012; Zhang et al. 2013), two genes conferring GW, *GW2* and *qSW5*/*GW5* (Song et al. 2007; Zhang et al. 2011; Ying et al. 2012; Zhang et al. 2013; Gao et al. 2015b), and a pleiotropic gene conferring GL, GW and GT, *GS2*/*GL2* (Hu et al. 2015; Che et al. 2015; Duan et al. 2015), were frequently detected. In this study, NYZ contributed to 45 of the total 54 QTLs showing positive effects identified in the RIL population (Table 3). Among those, *qGL3.2*, *qGL3.3*, *qGW2.1* and *qGW2.4* showed the same locations with *GS3*, *GW2* and *GS2*/*GL2*, and all the four alleles from NYZ increased the value of

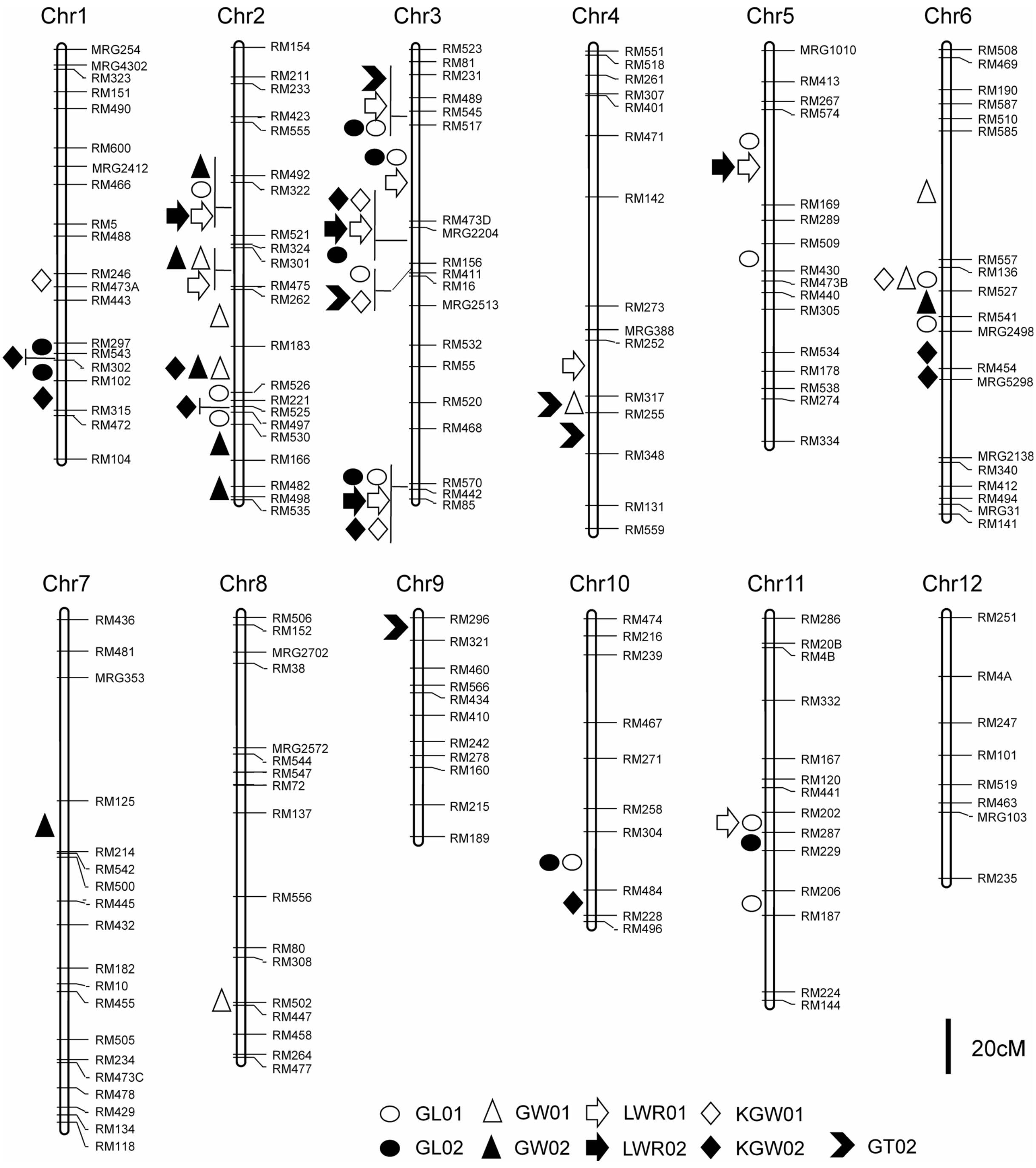

Fig. 2 Distribution of putative QTLs for GL, GW, LWR, KGW and GT identified in the RIL population during 2001 and 2002 on the linkage map. GL01, QTLs for GL in 2001; GL02, QTLs for GL in 2002; GW01, QTLs for GW in 2001; GW02, QTLs for GW in 2002; LWR01, QTLs for LWR in 2001; LWR02, QTLs for LWR in 2002; KGW01, QTLs for KGW in 2001; KGW02, QTLs for KGW in 2002; GT02, QTLs for GT in 2002

GL and GW. In addition, a total of 17 QTLs were detected for GL, and 14 alleles from NYZ showed positive additive effects. Among those, the alleles from NYZ of all the four QTLs for GL on chromosome 3 showed positive effects, and accounted for more that 50% of the variation in 2 years (Table 3).

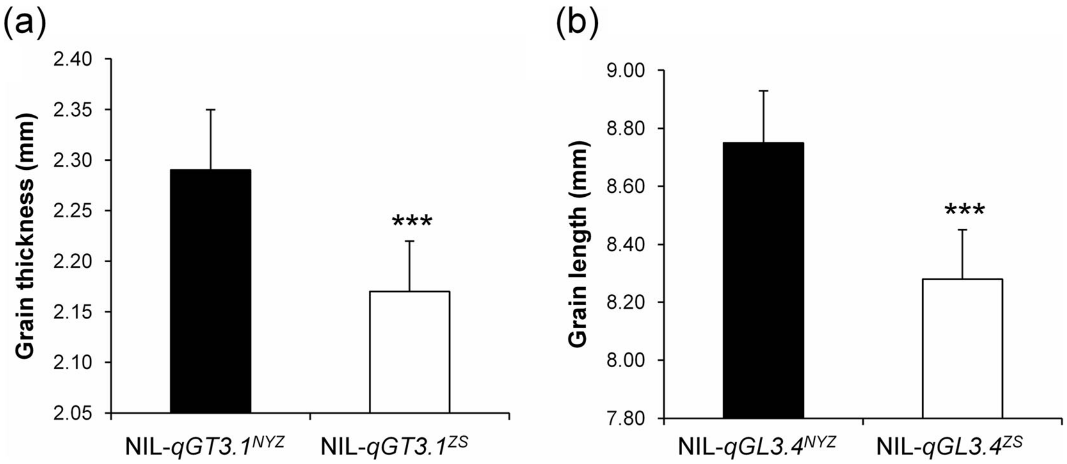

Fig. 3 Grain thickness of NIL-*qGT3.1*^{NYZ} and NIL-*qGT3.1*^{ZS} (**a**) and grain length of NIL-*qGL3.4*^{NYZ} and NIL-*qGL3.4*^{ZS} (**b**). *** significant at $P < 0.001$

Therefore, the large grain shape of NYZ might be the combined influence of numbers of QTLs showing positive effects.

Validation of *qGT3.1*and *qGL3.4* in the background of ZS97

GT, an important determinant of grain shape, has received less attention in the past compared with the rest of the three determinants. A few studies have been performed to dissect the genetic basis of GT (Zhang et al. 2011, Ying et al. 2012, Liu et al. 2015). However, only one QTL (*qGT8*) was finely mapped to a region of 11.2 kb, which contained *GW8*, a gene conferring grain width and yield (Gao et al., 2015a). In addition, *GS3* and *GS2*/*GL2*, two major genes conferring GL, also affect the GW and GT (Fan et al. 2006, Hu et al. 2015). In the present study, five QTLs were detected for GT (Table 3). Among those, *qGT3.1*, located between RM545 and RM517 on chromosome 3, accounted for the highest (16.94%) phenotypic variation (Table 3). In order to validate the effect of *qGT3.1*, two homozygous NILs, NIL-*qGT3.1*^{NYZ} and NIL-*qGT3.1*^{ZS} were developed with the background of ZS97. Results showed that NIL-*qGT3.1*^{NYZ} displayed an increased value by 0.12 mm in grain thickness than NIL-*qGT3.1*^{ZS} , demonstrating that the *qGT3.1* region from NYZ could improve grain thickness (Fig. 3).

Until now, several genes conferring GL have been cloned, of which two major genes, *GS3* and *GL3*/*GL3.1* are on chromosome 3 (Fan et al. 2006, Qi et al. 2012, Zhang et al. 2012). Among the 17 QTLs for GL, all the 4 accounting for over 10% of the variation were distributed on chromosome 3 (Table 3, Fig. 2). *qGL3.2* and *qGL3.3* were located to the surrounding regions of *GS3*, while *qGL3.1* was mapped to the adjacent region of *qGL3.2*. Only *qGL3.4* was away from the two cloned genes and on the terminal region of chromosome 3; thus, it was selected to validate its effect under the background of ZS97. Results showed that the GL value of NIL-*qGL3.4*^{NYZ} was 0.47 mm longer than that of NIL-*qGT3.1*^{ZS} , demonstrating that the *qGT3.1* region from NYZ did, indeed, have a positive effect on grain length (Fig. 3).

Breeding rice cultivars with desirable grain shape, good quality and high yield

The Asian cultivated rice consists of two major subspecies, *indica* and *japonica*. Typical *indica* cultivars have long and slender grains, while typical *japonica* cultivars have short and round grains, which are desired by different people in different regions or countries (Unnevehr 1992, Juliano and Villareal 1993). Many rice cultivars cannot meet a balance among grain shape, quality and yield. For example, NYZ, the parent with large grain in this study, showed high chalkiness and low yield (data not shown). Nevertheless, approved rice cultivars in China show moderate grain, better quality and higher yield. Therefore, breeding rice cultivars with a combination of desirable grain shape, good quality and high yield is of great challenge and significance Although the genetic basis and molecular mechanism underlying grain shape is not quite clear, a few genes have been cloned and many QTLs have been identified and even finely mapped. In this study, 5 QTLs for GL (*qGL3.1*, *qGL3.2*, *qGL3.3*, *qGL3.4* and *qGL10*), 2 QTLs for GW (*qGW2.2* and *qGW2.4*), 4 QTLs for LWR (*qLWR2.1*, *qLWR3.2*, *qLWR3.3* and *qLWR5*) and 1 QTL for KGW (*qKGW3.1*) were stably detected in 2 years, or accounted for over 20% of the variation (Table 3). In addition, *qGT3.1* and *qGL3.4* were further validated in the NIL background. With so many cloned genes and identified QTLs for grain shape, selective combinations of some would produce cultivars with good combinations of desirable grain shape, good quality and high yield.

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