Theor Appl Genet (2011) 123:859–867
DOI 10.1007/s00122-011-1632-6

ORIGINAL PAPER

Map-based cloning proves *qGC-6*, a major QTL for gel consistency of japonica/indica cross, responds by *Waxy* in rice (*Oryza sativa* L.)

Yan Su · Yuchun Rao · Shikai Hu · Yaolong Yang · Zhenyu Gao · Guanghen Zhang · Jian Liu · Jiang Hu · Meixian Yan · Guojun Dong · Li Zhu · Longbiao Guo · Qian Qian · Dali Zeng

Received: 8 November 2010 / Accepted: 31 May 2011 / Published online: 22 June 2011

Abstract In this study, one major QTL affecting gel consistency (GC) of japonica/indica cross was identified on chromosome 6 using a DH population. To understand the molecular mechanism that regulates GC in rice grains, the major QTL (*qGC-6*) was isolated through a map-based cloning approach utilizing chromosome segment substitution lines (CSSLs). Using 64 plants with extremely soft GC that were selected on recombinant break points between two SSR markers, RM540 and RM8200 in a BC4F2 population, *qGC-6* was mapped to a 60-kb DNA region between two STS markers, S26 and S27. These two markers were then used to further identify recombination break points. Finally, *qGC-6* was delimited in an interval of a 11-kb region. Gene prediction analysis of the 11-kb DNA sequence containing *qGC-6* identified only one putative ORF, which encodes granule-bound starch synthesis protein (Wx protein). Results of sequencing analysis and complementation experiment confirmed that this candidate ORF is responsible for rice GC. Genetic evidences revealed that *Wx* might contribute equally to the grain amylose content-controlling gene as well as gel consistency. This new information is important to breed rice varieties with improved grain quality.

Communicated by T. Sasaki.

Y. Su, Y. Rao and S. Hu contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1632-6) contains supplementary material, which is available to authorized users.

Y. Su · Y. Rao · S. Hu · Y. Yang · Z. Gao · G. Zhang · J. Liu · J. Hu · M. Yan · G. Dong · L. Zhu · L. Guo · Q. Qian (✉) · D. Zeng (✉)
State Key Laboratory of Rice Biology, China National Rice Research Institute, 31006 Hangzhou, China
e-mail: qianqian188@hotmail.com

D. Zeng
e-mail: dalizeng@126.com

Introduction

The yield of rice has been increased dramatically in the last century, but the poor eating and cooking quality (ECQ) of high yielding cultivars and hybrid rice represents a major problem for rice production (Liu et al. 2003). The improvement of rice ECQ has been increasingly demanded by consumers and has become a priority for rice breeders and geneticists (Itoh et al. 2003; Liu et al. 2003; Terada et al. 2000; Zeng et al. 2007). Rice ECQ is largely determined by three primary physical and chemical characteristics of the starch in the endosperm: amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) (Fan et al. 2005).

It has been known for some time that the granule-bound starch synthase (GBSS, EC 2.4.1.11), encoded by the *Waxy* gene (*Wx*), plays a very important role in determining AC in endosperm starch (Wang et al. 1995). In rice, two functional alleles of the *Wx* gene have been found to exist that correspond to the AC levels of *indica* and *japonica* rice varieties, respectively (Sano et al. 1986). Wx^a is widely distributed in *O. sativa* spp. *indica*, a subspecies with higher AC, whereas Wx^b is mainly observed in *japonica*, a subspecies with intermediate AC. A single nucleotide substitution of G-to-T at the splicing donor site of the first intron in Wx^b results in the inefficient splicing of the Wx^b pre-mRNA and the activation of two cryptic splice sites in exon 1. Thus, the abundance of mature *Wx* transcripts and

Wx protein in the Wx^b grains is about 10% of that in Wx^a (Cai et al. 1998; Frances et al. 1998; Isshiki et al. 1998; Zeng et al. 2007).

The inheritance of GT has been widely studied since the 1990s (He et al. 1999; Lin et al. 1994; Tian et al. 2009; Xu and Mo 1996). QTL mapping showed that GT may be controlled either by the alkali degeneration gene (*alk*) (Bao et al. 2002; Fan et al. 2005) or by the *Wx* gene (He et al. 1999). Umemoto et al. (2002) reported that the starch synthase IIa gene (*SSIIa*) was located at the *alk* locus on chromosome 6 in the rice genome (Umemoto et al. 2002). Gao et al. (2003) reported the map-based cloning of the *alk* locus that encoded SSIIa and verified that *ALK* gene mainly determined GT in rice. *ALK* also has minor effect on GC and some paste viscosity parameters. But GT has not yet been found to be correlated with AC, although *Waxy* (*Wx*) locus affected GT (Fan et al. 2005; Wang et al. 2007).

Tremendous efforts have also been made to understand the genetic basis of GC. Because of its significant negative correlation with AC, it has long been believed that GC is also controlled by *Wx* or another gene tightly linked to the *Wx* locus (He et al. 1999; Lanceras et al. 2000). Two QTLs on chromosome 6 and one on chromosome 7 were detected for GC by using RIL population derived from the cross KDML105×CT9993 (Lanceras et al. 2000). On the other hand, three QTLs, designated as *qGC-1*, *qGC-2* and *qGC-6*, were identified using a population consisting of 190 DH lines derived from an F_1 hybrid between WYJ2 and Zhenshan 97B. Two QTLs for amylose content and gel consistency were detected and mapped on chromosomes 6, respectively, using 285 BC_2F_2 plants developed from an interspecific cross between cv IR64 and *Oryza rufipogon* (Septiningsih et al. 2003). The QTL, *qGC-6* with the largest effect on GC was located in the interval between RM190 and RM510 on chromosome 6 (Tian et al. 2005; Zheng et al. 2008). He et al. (1999) identified two QTLs for GC on chromosomes 2 and 7 using a DH population consisting of 132 lines. Although these investigations largely contributed to the genetic basis of GC in rice, the basis for its molecular regulation remains unknown.

In this study, a QTL analysis for GC was carried out using a DH population derived from a typical japonica/indica cross, and a major QTL (*qGC-6*) for gel consistency was detected on chromosome 6. Development of chromosome segment substitution lines and map-based cloning approach were performed to clone this major QTL. Sequencing analysis and complementation experiment confirmed that the identified ORF for *qGC-6* encodes granule-bound starch synthesis protein (Wx protein), which proves that *Wx* locus is responsible for gel consistency of japonica/indica cross in rice.

Materials and methods

DH population

A typical *indica* cultivar 'TN1' and a typical *japonica* cultivar 'CJ06' of rice, *Oryza sativa* L., were used as parents to make hybrids. The anthers from F_1 plants were collected and cultured on the inducing medium SK3. After natural doubling or treatment with colchicine, doubled plants were obtained, which have been used for studies on whitebacked planthopper resistances (WBPH) and ligule length (Sogawa et al.2005; Sogawa et al. 2004; Zeng et al. 2009). The DH population and its parents were transplanted with a planting density of 20 cm × 20 cm in the experimental farm of China National Rice Research Institute, Hangzhou, China in the 2006 summer season. Each DH line was planted in six rows, with six plants in each row.

The development of CSSL

To obtain a pure genetic background and lessen the effect of other minor QTLs, the chromosome segment substitution lines were developed in this study. The plants carrying TN1 genotype at the flanking of *qGC-6* were selected to cross with CJ06 followed by four successive backcrossing. Simultaneously, the SSR marker, RM540 and RM8200, were initially used to identify plants having TN1 genotype in the backcrossed progenies. A set of 71 SSR markers (see Supplemental Table 1), which are distributed uniformly on previous linkage map (Sogawa et al. 2005; Sogawa et al. 2004; Zeng et al. 2009), were used to select individuals that contained as little TN1 DNA in the genetic background as possible in the BC_4F_1 families.

DNA extraction and PCR analysis

The preparation of genomic DNA for large screen to select recombinants was carried out as described by Sun et al. (2010). The refined DNA was extracted from fresh rice leaves using the CTAB method described by Murray and Thompson (1980). PCR was performed in 20 μl reactions containing 0.2 mM of each primer, 200 mM dNTP mix, 50 mM KCl, 10 mM TRIS–Cl, pH 8.3, 1.5 mM $MgCl_2$, 0.1% Triton X-100 and 1 unit of Taq polymerase. The PCR profile was: 94°C for 5 min for initial denaturation followed by 35 cycles of 94°C for 1 min, 55°C for 45 s, 72°C for 50 s, and 72°C for 10 min for final extension. The PCR reaction was performed in a PTC-225 tetrad (MJ Research, Watertown, MA). The PCR products were separated by electrophoresis on a 3.0–5.0% (w/v) agarose gel and stained with EB (ethidium bromide).

Marker development

Primers were designed around *qGC-6* on chromosome 6 to distinguish the CJ06 and TN1 alleles (see Supplemental Table 2). The STS and CAPS makers were developed based on the sequence differences between *indica* var. 93-11 and *japonica* var. Nipponbare (http://www.ncbi.nlm.nih.gov). The sequences were aligned using the SeqMan program of DNAstar (Gene-Codes) and insertions/deletions were identified. Primers flanking the indels were designed using the Primer Premier 5.0 program and tested on the parents.

Measuring the quality trait

Approximately 40 days after heading, rice grains were harvested, air dried and stored at room temperature for 3 months before milling. All the samples were dehusked in an electrical dehuller (Model B-76, China) and milled by sample miller (Model JB-20 Huangyan, China). The moisture content of all sample flours was balanced to about 12% with a range of 11.7–12.4%.

Twelve grains of milled rice were selected for measuring the alkali spreading values (ASV), and 10 g grains were ground to flour and used to measure AC and gel consistency (GC). Percent AC was measured as described previously with slight modification (Juliano 1971). Briefly, samples were boiled for 10 min in the volumetric flasks to completely disperse the grain powder and the optical density of the amylose-iodine blue was measured at 620 nm using a spectrophotometer. GC was measured using 100 mg of milled rice flour. The flour was first wetted with 0.2 ml of 95% ethanol containing 0.025% (w/v) thymol blue in 11 × 100 mm culture tubes, followed by adding 2 ml of 0.2 N KOH, and mixed vigorously. Tubes were covered with glass marbles, heated in a boiling water bath for 8 min, mixed again and kept in an ice water bath for 20 min. Finally, the tubes were laid horizontally against a ruled graphing paper and gel length was measured after 1 h. ASV was determined by incubating six milled grains in 10 ml of 1.7% KOH at 28°C for 23 h with two replicates. The degree of spreading was rated using the following 7-point semi-quantitative criteria: 1, grain not affected; 2, grain swollen; 3, grain swollen, collar incomplete and narrow; 4, grain swollen, collar complete and wide; 5, grain split, collar complete and wide; 6, grain dispersed, merging with collar; 7, grain completely dispersed and intermingled.

Data analysis and QTL mapping

Analysis of variance for all phenotypic characters was performed using the JMP statistical package, version 7.0 for Windows (SAS Institute Inc., Cary, NC). Interval QTL mapping was conducted by using the software of Mapmaker/QTL 1.1 for the gel consistency. The presence of a QTL was considered when an LOD score was larger than 2.4. The genetic variance explained by each QTL and QTL additive effect was calculated. The identified QTLs were named according to the standard nomenclature, as suggested (McCouch et al. 1997).

Complementation test

A genomic DNA fragment from TN1 of 8,381-bp, containing an entire *qGC-6* coding region, a 3,678-bp upstream region and a 1,224-bp downstream sequence, was inserted into the binary vector pCAMBIA1300 to generate the transformation plasmid p1300GC6 for complementation test. p1300GC6 and its control pCAMBIA1300 were introduced into *Agrobacterium tumefaciens* EHA105 by electroporation and then transferred into CJ06, performed as described previously (Zeng et al. 2007). To identify the *qGC-6* transgene in the transgenic lines, an Indel marker IndGC6 was developed based on the nucleotide difference between CJ06 and TN1 (see Supplemental Table 2).

Results

Phenotype performance of the cooking traits

Cooking traits including amylose content (AC), gel consistency (GC) and alkali spread value (ASV) of the parent lines (CJ06 and TN1) and the resulting DH population are summarized in Table 1. Significant differences were found between the two parents for AC and GC, while ASV of rice for CJ06 was similar to TN1. AC of rice for CJ06 was lower than that of TN1, whereas GC for CJ06 was higher than that of TN1. There were highly significant correlations among the three traits (Table 2). The GC of the *indica* parent TN1 was 29.5 mm, while the GC of the *japonica* parent CJ06 was 84.5 mm. A bimodal distribution of phenotypic values for GC was observed in the DH population (Fig. 1). It appeared, therefore, that GC was mainly controlled by a major locus plus some multi-minor loci.

Table 1 The cooking traits of rice grains including AC, GC and ASV of parents and DH populations

Trait	Parents		DH population			
	CJ06	TN1	Means	SD	Minimum	Maximum
AC	12.05	22.81	19.86	5.47	8.44	27.8
GC	84.5	29.5	51.9	23.63	25.0	100.0
ASV	7.0	6.9	6.73	0.42	5.5	7.0

Table 2 Correlation coefficients of the cooking traits in rice including AC, GC and ASV

Cooking traits	AC	GC
GC	−0.503**	
ASV	0.410**	−0.394**

** Significant at the level of 1%

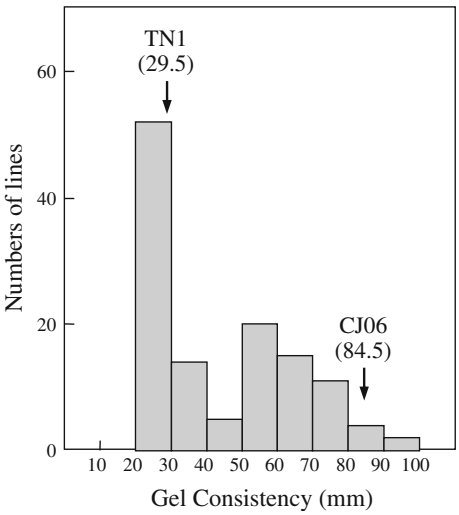

Fig. 1 The distribution of gel consistency in the DH population

QTL analysis for GC, AC and ASV

The linkage map used in this study was established at the China National Rice Research Institute. A total of 178 makers covered all 12 rice chromosomes with a total genetic distance of 1,674.8 cM, and an average distance of 9.41 cM between the adjacent markers. This linkage map has been widely used in the study of QTL mapping (Ma et al. 2009; Zeng et al. 2009; Zhang et al. 2008).

The whole genome was scanned for quantitative trait loci (QTLs) using Mapmaker/QTL1.1B with an LOD threshold of 2.4. Three QTLs for gel consistency were mapped on chromosome 2, 3 and 6, respectively, namely, *qGC-2*, *qGC-3* and *qGC-6* (Table 3, Fig. 2). The major QTL for GC, *qGC-6*, was detected at the interval between RM540 and RM587 on chromosome 6. Its proportion of phenotypic variation was over 52%, and the allele from 'TN1' decreased the phenotypic values for GC to about 32 mm. *qGC-2* and *qGC-3* had additive effects of −16.76 mm and −18.40 mm, respectively, indicating that 'TN1' alleles at these two loci also could decrease the phenotypic values for GC. Two QTLs for amylose content were detected on chromosome 3 and 6, and two QTLs were resolved for alkali spreading values on chromosome 2 and 6 (Table 3, Fig. 2).

Table 3 QTL identified for GC in the DH population

QTLs		Chr.	Interval	LOD	Var. %	Add.
GC	*qGC-2*	2	RM3732-RM492	2.96	17.8	−16.76
	qGC-3	3	RM514-RM85	3.05	14.6	−18.40
	qGC-6	6	RM540-RM587	12.17	52.4	−32.30
AC	*qAC-3*	3	RM514-RM85	2.56	16.5%	4.60
	qAC-6	6	RM540-RM587	17.63	81.4%	10.33
ASV	*qASV-2*	2	RM3732-RM492	2.62	13.7%	0.45
	qASV-6	6	RM540-RM587	4.86	22.4%	0.59

Development of CSSL

Based on QTLs analysis and previous genetic linkage map, one line from the DH population was selected to cross with CJ06, followed by four successive backcrossing. SSR markers, RM540 and RM8200, were used for the marker-assisted selection in the segregating progenies of each backcross generation. After four backcrosses with CJ06, the BC_4F_1 and BC_4F_2 generation were scanned with a set of 71 SSR markers, which distributed uniformly on previous linkage map. A plant, CSSL38-1-4, having the TN1 genetic background while carrying a homozygous introgression across the entire *qGC-6* region and devoid of any introgressions of *qGC-2* and *qGC-3* region (Fig. 3a, b), was selected. The GC for CSSL38-1-4 is dramatically decreased compared to its recurrent parent CJ06, but similar to TN1 (Fig. 3c).

Map-based cloning of *qGC-6*

Further QTL analysis indicated a peak for linkage with a maximum LOD score of 12.2 near RM587 on chromosome 6 (Fig. 4a). As a result, SSR marker RM540 and RM8200 across the *qGC-6* target region were used to determine recombination break points in the segregating progenies derived from the cross between CSSL38-1-4 and CJ06, and 396 selected plants from 4,032 BC_4F_2 progenies were transplanted in the field to generate enough seeds for evaluating their gel consistency and for further analysis. These plants show bimodal distribution with their phenotypic values for GC (Fig. 4b). To avoid using heterozygous plants or confounding plants, 64 plants with extremely soft gel consistency (GC $\geq$ 90 mm) were selected for fine mapping. Analysis of RM540 identified 30 recombination events between the marker and *qGC-6* on one side, while analysis of RM8200 detected 34 recombination events between the marker and *qGC-6* on the other side. The STS marker S13, S20 and S26 revealed seven, three and one recombinants, while marker S18 and S27 indicated six and two recombinants, respectively, on the other side (Fig. 4c).

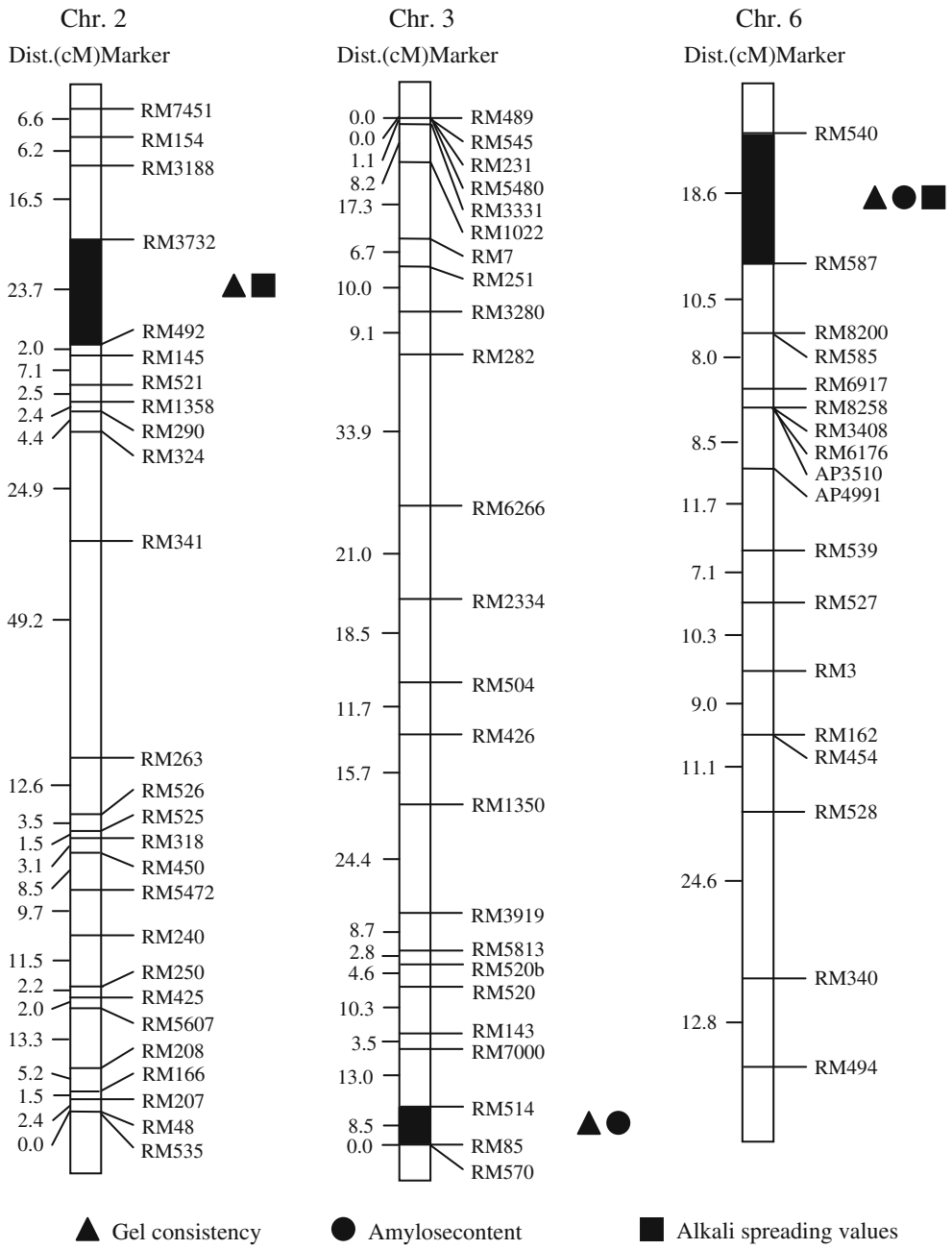

Fig. 2 QTLs of amylose content (AC), gel consistency (GC) and alkali spreading values (ASV) in the DH population. Map distances are expressed in cM on the left of the chromosome

Therefore, the *qGC-6* locus was finally delimited to a 60-kb DNA region between two STS markers, S26 and S27.

To narrow down the region of the *qGC-6* locus, the developed marker S26 and S27 were further used to identify recombination break points, and 27 selected plants from 7,427 BC_4F_2 progenies derived from the cross between CSSL38-1-4 and CJ06 were transplanted in the field to produce enough seeds for confirming their phenotypes. Seven plants with extremely soft gel consistency (GC $\geq$ 90 mm) and five plants with extremely hard gel consistency (GC $\leq$ 35 mm) were selected for fine mapping. Five developed markers were available to narrow down the region of *qGC-6* locus. IndGC6 co-segregated with *qGC-6*, with two recombinant events between P1 and *qGC-6*, and three between CAPS3 and *qGC-6*. Lines L3 and L5, which posses the TN1 alleles at S28 and CAPS6, had a hard gel consistency. Lines L6, L7 and L10, which carry CJ06 alleles at these loci, had a soft gel consistency (Fig. 4d). Thus, *qGC-6* was delimited to an interval of 11-kb region between markers S28 and CAPS6 on the BAC

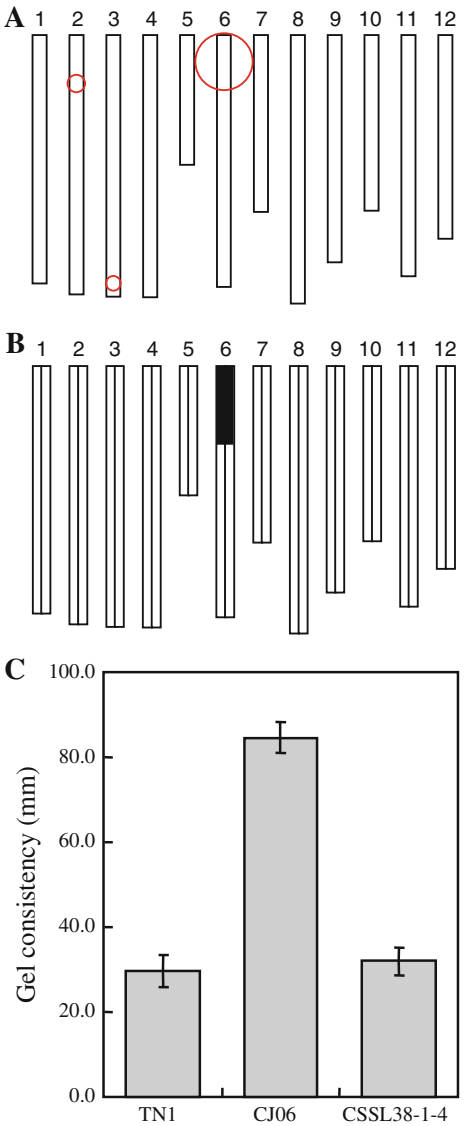

Fig. 3 The development of CSSL. **a** QTL analysis for GC of rice grain quality in the DH population. *Circle centers* indicate positions of QTLs on the rice chromosomes. *Circle* sizes indicate contributions to explained variation in GC of rice grain quality. *Black circles* indicate that the TN1 allele decreased GC; **b** graphical genotype of CSSL38-1-4 (a substitution line of chromosome 6). *Black bar* indicates the genome fragment from TN1; the other parts were from CJ06. **c** The GC for CJ06, TN1 and introgression CSSL38-1-4

clone P0679C08. Gene prediction analysis of the 11-kb DNA sequence containing *qGC-6* identified only one putative ORF, which encodes granule-bound starch synthesis protein (Wx protein).

Identification of *qGC-6*

Sequencing of this candidate ORF revealed the differences between TN1 and CJ06 (see Supplemental Fig. 1). A substitution of T by C in exon 9 leads to a non-synonymous change from serine to proline. A 12-bp deletion in 5′ UTR is also identified in TN1. The variation in length of a repeated sequence (i.e., CT microsatellite) within the rice *waxy* gene has been reported previously (Prathepha 2003). The $(CT)_{11}$ allele is found in TN1, whereas the $(CT)_{17}$ allele exists in CJ06. TN1 has the base G at the putative leader intron 5′ splice site, whereas CJ06 takes the base T at the site. The diversities between TN1 and CJ06 also include a 4-bp deletion, a 3-bp deletion and many substitutions in six introns.

To confirm that the identified ORF is indeed responsible for rice gel consistency, we carried out a genetic complementation experiment. The distribution of gel consistency in Fig. 4b shows a bi-modal distribution, and the ratio of hard gel consistency to soft was fitted nearly to 3:1. It indicates that the allele with hard gel consistency shows dominance to that of soft gel consistency in the rice grain. Thus, the plasmid p1300GC6 containing the entire ORF of TN1 allele and its regulatory sequences, which consist of a 3,479-bp coding sequence, a 3,678-bp upstream sequence including the regulatory region and untranslated region (UTR), and a 1,224-bp downstream region, was introduced into CJ06 plants through *Agrobacterium*-mediated transformation. The GC values were restored in transgenic plants carrying the p1300GC6 transgene (Fig. 5a). Those plants were identified with an Indel marker IndGC6, which could distinguish the transformants from CJ06 plants (Fig. 5b). All the six transgenic plants carrying the candidate gene have hard GC, whereas all the five plants transformed by pCAMBIA1300 (vector control) have soft GC (see Supplemental Table 3). Our data suggest that *Waxy* gene is also a major gene controlling GC in rice.

Discussion

Eating and cooking quality in rice is a complex trait determined by three primary physical and chemical characteristics of the starch in the endosperm: AC, GC and GT, all of which are typically quantitative traits. These are known in general to have low heritability, thus making these grain quality traits very difficult to investigate. Over the past few decades, although some studies have been conducted to characterize the genetic basis of GC and indicated that GC was negatively correlated to AC and determined by one major gene plus several minor loci (Bollich and Webb 1973; He et al. 1999; Kumar and Khush 1988; McKenzie and Rutger 1983; Tan et al. 1999; Tang and Khush 1991), no strong evidences have revealed the gene controlling GC directly. The major obstacle in GC analysis is the mapping population. Because GC is controlled by different loci, the mapping populations always showed continuous GC phenotype. Thus, proper population

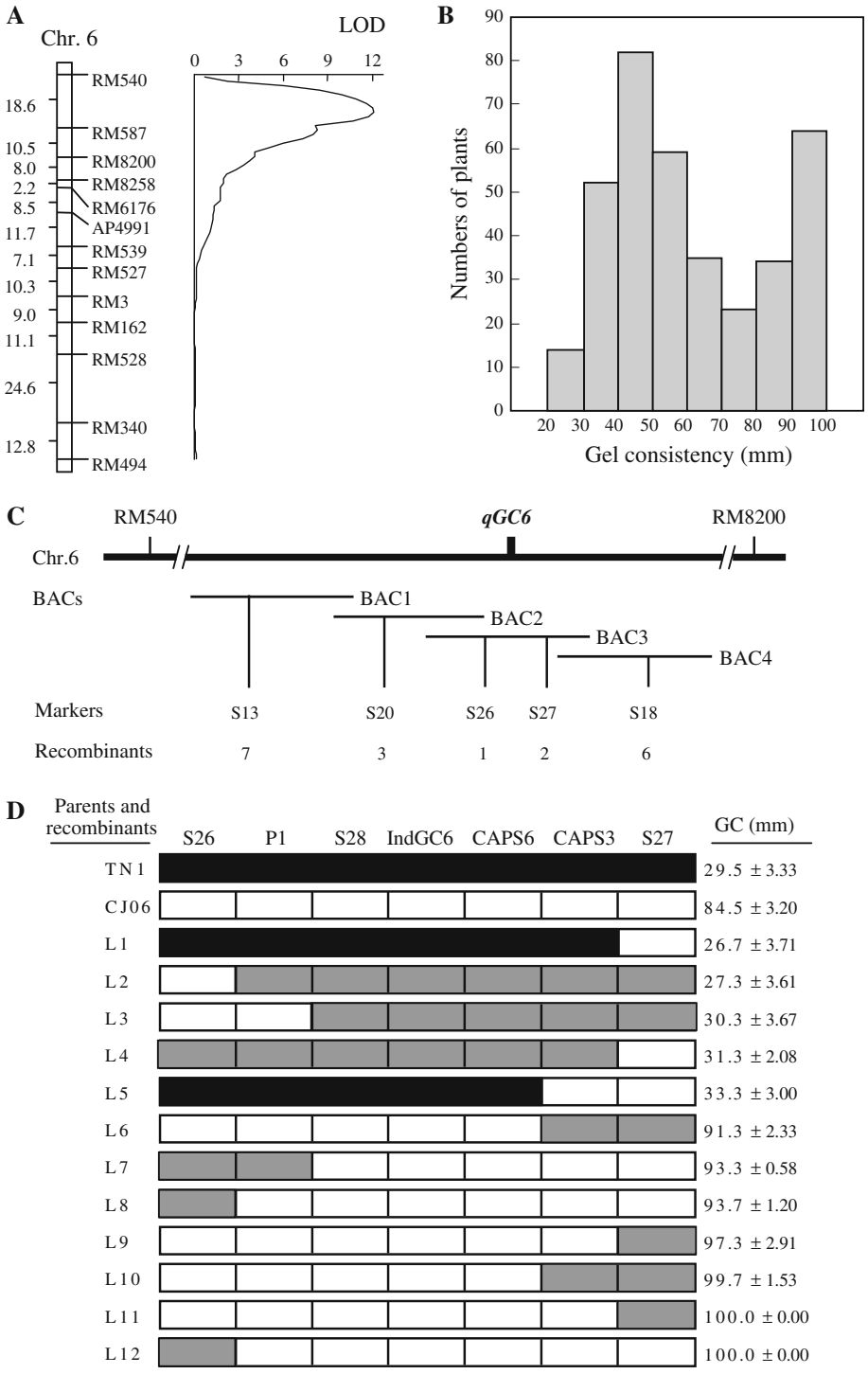

Fig. 4 Fine mapping of *qGC-6*. **a** Molecular linkage map of rice chromosome 6 showing the location of *qGC-6*. The genetic distance (Kosambi, centiMorgan) and marker name are shown on the left and right of the chromosome, respectively. **b** The phenotypic values for GC of each plant, which were selected from BC_4F_2 plants; 235 selected plants showed discontinuous and bimodal distributions for GC, one peak was at the low-GC (hard gel consistency) region and the other was at the high-GC (soft gel consistency) region. **c** The *qGC-6* locus was mapped on the short arm of chromosome 6 between markers RM540 and RM8200. A BAC contig spanned the *qGC-6* locus. The *numerals* indicate the number of recombinants identified from BC_4F_2 mutant plants. BAC1, P0538C01 BAC2, P0493C11; BAC3, P0679C08; BAC4, P0001H02. **d** Molecular marker genotypes and phenotypes of the recombinants for fine mapping. The *white*, *black* and *gray bars* denote the marker genotype of CJ06, TN1 and heterozygote, respectively. All the lines (L1–L12) displayed were the individuals from the BC_4F_2 population

development and powerful approach employment will be critical to isolate the gene controlling GC.

QTL analyses using genetic population derived from crosses of nearly isogenic line (NIL) or chromosome segment substitution line (CSSL) proved to be powerful tools for investigating the genetic and molecular bases of these quantitative traits (Xing et al. 2008; Xing and Zhang 2010; Zhou et al. 2009). In addition, several successful examples of QTL cloning resulted mainly from the development of corresponding NILs or CSSLs (Kojima et al. 2002; Song et al. 2007; Umemoto et al. 2002).

In this study, a DH population was first employed in the QTLs analysis to identify the genes affecting GC. Three QTLs for GC were identified on chromosome 2, 3 and 6, respectively, in which *qGC-6*, showed 52% variation explanation. Because the distribution of GC in the DH

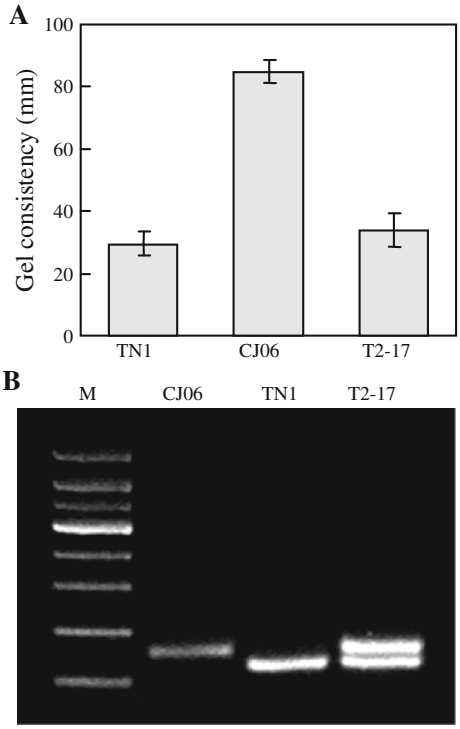

Fig. 5 Identification and confirmation of the *qGC-6* gene. **a** Phenotypic value of gel consistency in mature seeds of transgenic plant and the controls. **b** Identification of transgenic plants with the Indel marker IndGC6

population is continuous, which cannot be applied for further fine mapping, CSSLs were developed and used to isolate the *qGC-6* gene through map-based cloning approach. A combination of these approaches made us delimit *qGC-6* in a very small interval of a 11-kb region, which only harbors one putative ORF. From our results we find that combinations of different populations, DH and CSSLs, and different approaches, QTLs and Map-based cloning, proved that *Waxy* contributes equally to the grain gel consistency-controlling gene as well as amylose content, which might be utilized in other quantitative traits analysis.

Waxy gene (*Wx*) has been well known to play an important role in determining AC in endosperm starch (Wang et al. 1995). Two functional alleles of the *Wx* gene have been found corresponding to the AC levels of *indica* and *japonica* rice varieties (Sano et al. 1986), which were different at mRNA transcription (Cai et al. 1998; Frances et al. 1998). Because GC is significantly correlated to AC, *Wx* has been presumed to be the gene controlling GC as AC (He et al. 1999; Lanceras et al. 2000). But no direct evidence has been obtained. In our study, we mapped the *qGC-6* to a small region, which only contained the *Wx* gene model. In addition, the complementation experiment further confirmed that the *Wx* gene was responsible for rice GC. We think that the functional difference between the two *Wx* alleles might be caused by the diversities between these two varieties. For example, 29 SNPs and 4 indels were found between TN1 and CJ06, which were also reported previously (Bergman et al. 2001; Prathepha 2003). Cai et al. suggested a single nucleotide substitution of G-to-T at the splicing donor site of the first intron in Wx^b, resulting in the inefficient splicing of the Wx^b pre-mRNA and responsible for the functional divergence of these two alleles in controlling AC (Cai et al. 1998; Frances et al. 1998; Isshiki et al. 1998; Zeng et al. 2007).

In summary, the present study indicated that the *Waxy* allele was responsible for GC as well as AC. It also suggests that the *waxy* gene plays a crucial role in the improvement of eating and cooking quality of rice because it regulates both amylose content and gel consistency. These findings provide a well-defined target for quality improvement, especially in the improvement of amylose content and gel consistency. In particular, the polymorphic marker located at the gene locus will greatly facilitate the precise replacement of the *Wx* allele of the poor-quality parent using marker-assisted selection. This is particularly important for the improvement of hybrid parents to maintain high combining ability while improving grain quality.

Acknowledgments We thank Dr. Song Xianjun and Dr. Enrique R. Angeles for their critical comments on the manuscript. This work was supported by grants from the Ministry of Agriculture of China for transgenic research (No. 2011ZX08009, 2011ZX08001), the National Natural Science Foundation of China (No. 30771160, 30971760) and the Natural Science Foundation of the Zhejiang Province (No. R3090023) and Science and Technology Project, Zhejiang Province (2009C32047).

References

Bao JS, Sun M, Corke H (2002) Analysis of the genetic behavior of some starch properties in indica rice (*Oryza sativa* L.): thermal properties, gel texture, swelling volume. Theor Appl Genet 104:408–413

Bergman CJ, Delgado JT, McClung AM, Fjellstrom RG (2001) An improved method for using a microsatellite in the rice waxy gene to determine amylose class. Cereal Chem 78:257–260

Bollich CN, Webb BD (1973) Inheritance of amylose in two hybrid populations of rice populations of rice. Cereal Chem 50:631–636

Cai XL, Wang ZY, Xing YY, Zhang JL, Hong MM (1998) Aberrant splicing of intron 1 leads to the heterogeneous 5' UTR and decreased expression of waxy gene in rice cultivars of intermediate amylose content. Plant J 14:459–465

Fan CC, Yu XQ, Xing YZ, Xu CG, Luo LJ, Zhang Q (2005) The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled-haploid line population. Theor Appl Genet 110:1445–1452

Frances H, Bligh J, Larkin PD, Roach PS, Jones CA, Fu H, Park WD (1998) Use of alternate splice sites in granule-bound starch synthase mRNA from low-amylose rice varieties. Plant Mol Biol 38:407–415

Gao ZY, Zeng DL, Cui X, Zhou YH, Yan MX, Huang DN, Li JY, Qian Q (2003) Map-based cloning of the ALK gene, which controls the gelatinization temperature of rice. Sci China 46:661–668

He P, Li SG, Qian Q, Ma YQ, Li JZ, Wang WM, Chen Y, Zhu LH (1999) Genetic analysis of rice grain quality. Theor Appl Genet 98:502–508

Isshiki M, Morino K, Nakajima M, Okagaki RJ, Wessler SR, Izawa T, Shimamoto K (1998) A naturally occurring functional allele of the rice waxy locus has a GT to TT mutation at the 5' splice site of the first intron. Plant J 15:133–138

Itoh K, Ozaki H, Okada K, Hori H, Takeda Y, Mitsui T (2003) Introduction of Wx transgene into rice wx mutants leads to both high- and low-amylose rice. Plant Cell Physiol 44:473–480

Juliano BO (1971) A simplified assay for milled rice amylose. Cereal Sci Today 16:334–360

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. Plant Cell Physiol 43:1096–1105

Kumar I, Khush GS (1988) Inheritance of amylose content in rice (*Oryza sativa* L.). Euphytica 38:261–269

Lanceras JC, Huang ZL, Naivikul O, Vanavichit A, Ruanjaichon V, Tragoonrung S (2000) Mapping of genes for cooking and eating qualities in Thai jasmine rice (KDML105). DNA Res 7:93–101

Lin SY, Nagamura Y, Kurata N, Yano M, Minobe Y, Sasaki T (1994) DNA markers tightly linked to genes, Ph, alk and Rc. Rice Genet Newsl 11:108

Liu Q, Wang Z, Chen X, Cai X, Tang S, Yu H, Zhang J, Hong M, Gu M (2003) Stable inheritance of the antisense Waxy gene in transgenic rice with reduced amylose level and improved quality. Transgenic Res 12:71–82

Ma L, Bao J, Guo L, Zeng D, Li X, Ji Z, Xia Y, Yang C, Qian Q (2009) Quantitative trait Loci for panicle layer uniformity identified in doubled haploid lines of rice in two environments. J Integr Plant Biol 51:818–824

McCouch SR, Cho SR, Yano M, Paul E, Blinstrub M, Morishima HTK (1997) Report on QTL nomenclature. Rice Genet Newsl 14:11–13

McKenzie KS, Rutger JN (1983) Genetic analysis of amylose content, alkali spreading score, and grain dimensions in rice. Crop Sci 23:306–313

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucl Acids Res 8:4321–4326

Prathepha P (2003) Characterization of waxy microsatellite classes that are closely linked to the rice waxy gene and amylose content in Thai rice germplasm. Songklanakarin J Sci Technol 25:1–8

Sano Y, Katsumata M, Okuno K (1986) Genetic studies of speciation in cultivated rices. 5. Inter- and intra-specific differentiation in the waxy gene expression in rice. Euphytica 35:1–9

Septiningsih EM, Trijatmiko KR, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theor Appl Genet 107:1433–1441

Sogawa K, Qian Q, Zeng DL, Hu J, Zeng LJ (2005) Differential expression of whitebacked planthopper resistance in the japonica/indica doubled haploid rice population under field evaluation and seedbox screening test. Rice Sci 12:63–67

Sogawa K, Sun ZX, Qian Q, Zeng DL (2004) Phenotypic expression of whitebacked planthopper resistance in the newly established japonica/indica doubled haploid rice population. Rice Sci 11:155–160

Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623–630

Sun C, Chen G, Rao YC, Zhang GH, Gao ZY, Liu J, Ju PN, Hu J, Guo LB, Qian Q, Zeng DL (2010) A simple method for preparation of rice genomic DNA. Rice Sci 17(4):326–329

Tan YF, Li JX, Yu SB, Xing YZ, Xu CG, Zhang QF (1999) The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, *Shanyou 63*. Theor Appl Genet 99:642–648

Tang SX, Khush GS (1991) Genetic analysis of gel consistency in rice. Chin J Rice Sci 5:25–28

Terada R, Nakajima M, Isshiki M, Okagaki RJ, Wessler SR, Shimamoto K (2000) Antisense waxy genes with highly active promoters effectively suppress waxy gene expression in transgenic rice. Plant Cell Physiol 41:881–888

Tian R, Jiang GH, Shen LH, Wang LQ, He YQ (2005) Mapping quantitative trait loci underlying the cooking and eating quality of rice using a DH population. Mol Breed 15:117–124

Tian Z, Qian Q, Liu Q, Yan M, Liu X, Yan C, Liu G, Gao Z, Tang S, Zeng D, Wang Y, Yu J, Gu M, Li J (2009) Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. Proc Natl Acad Sci USA 106:21760–21765

Umemoto T, Yano M, Satoh H, Shomura A, Nakamura Y (2002) Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. Theor Appl Genet 104:1–8

Wang LQ, Liu WJ, Xu Y, He YQ, Luo LJ, Xing YZ, Xu CG, Zhang QF (2007) Genetic basis of 17 traits and viscosity parameters characterizing the eating and cooking quality of rice grain. Theor Appl Genet 115:463–476

Wang ZY, Zheng FQ, Shen GZ, Gao JP, Snustad DP, Li MG, Zhang JL, Hong MM (1995) The amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy gene. Plant J 7:613–622

Xing YZ, Tang WJ, Xue WY, Xu CG, Zhang QF (2008) Fine mapping of a major quantitative trait loci, qSSP7, controlling the number of spikelets per panicle as a single Mendelian factor in rice. Theor Appl Genet 116:789–796

Xing YZ, Zhang QF (2010) Genetic and molecular bases of rice yield. Annu Rev Plant Biol 61:421–442

Xu CW, Mo HD (1996) Qualitative quantitative analysis for inheritance of gelatinization temperature in indica rice. Acta Agron Sinica 22:385–391

Zeng D, Yan M, Wang Y, Liu X, Qian Q, Li J (2007) Du1, encoding a novel Prp1 protein regulates starch biosynthesis through affecting the splicing of Wxb pre-mRNAs in rice (*Oryza sativa* L.). Plant Mol Biol 65:501–509

Zeng DL, Hu J, Dong GJ, Liu J, Zeng LJ, Zhang GH, Guo LB, Zhou YH, Qian Q (2009) Quantitative trait loci mapping of flag-leaf ligule length in rice and alignment with ZmLG1 Gene. J Integr Plant Biol 51:360–366

Zhang J, Zhu YG, Zeng DL, Cheng WD, Qian Q, Duan GL (2008) Mapping quantitative trait loci associated with arsenic accumulation in rice (*Oryza sativa*). New Phytol 177:350–355

Zheng X, Wu JG, Lou XY, Xu HM, Shi CH (2008) The QTL analysis on maternal and endosperm genome and their environmental interactions for characters of cooking quality in rice (*Oryza sativa* L.). Theor Appl Genet 116:335–342

Zhou L, Chen L, Jiang L, Zhang W, Liu L, Liu X, Zhao Z, Liu S, Zhang L, Wang J, Wan J (2009) Fine mapping of the grain chalkiness QTL qPGWC-7 in rice (*Oryza sativa* L.). Theor Appl Genet 118:581–590