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Identification and validation of a new grain weight QTL in rice

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ABSTRACT. The genetic control of grain weight (GW) remains poorly understood. Quantitative trait loci (QTLs) determining the GW of rice were identified using a natural GW mutant, sgw. Using a segregating population derived from sgw (low GW) and cultivar 9311 ('9311'; indica, high GW), the chromosome segment associated with GW was detected on the short arm of chromosome 7. To validate and further refine the locus, QTL analysis based on F_2 and F_3 populations was conducted, and a single major QTL (designated as *qsgw7*) affecting the 1000-grain weight of paddy rice was identified on the short arm region of rice chromosome 7 between simple sequence repeat (SSR) markers RM21997 and RM22015, where 4 bacterial artificial chromosome clones, OJ1339_F05, P0506F02, P0011H09, and P0519E12, were present. Analysis of the near isogenic line for *qsgw7* (NIL*qsgw7*) showed that the grain length, width, and volume of paddy rice in NIL*qsgw7* were significantly lower than those in '9311' and that the 1000-grain weight, grain length, width, volume, and chalkiness of brown rice in NIL*qsgw7* were significantly lower than those in '9311'. These results suggested that the *qsgw7* gene,

which was identified in this study, may be a new GW-related QTL that could affect GW and grain shape, especially grain plumpness.

Key words: Rice; 1000-grain weight; QTL; *qsgw7*; NIL

INTRODUCTION

Grain weight (GW) is an important factor that is related to grain yield potential and cooking qualities of rice (*Oryza sativa* L.). The loci that control GW have been identified on each of the 12 rice chromosomes (http://www.gramene.org). With the progress of molecular genetic maps, some major quantitative trait loci (QTLs) for GW or size have been identified, such as *GS3* on chromosome 3 (Fan et al., 2006), *GW2* on chromosome 2 (Song et al., 2007), *qSW5/GW5* on chromosome 5 (Shomura et al., 2008; Weng et al., 2008), and *GS5* on chromosome 5 (Li et al., 2011). However, it is difficult for breeders to improve grain size efficiently using phenotypes because the traits are quantitatively inherited (McKenzie and Rutger, 1983). Therefore, the identification of new genes (QTLs) conferring grain size variation would provide more valuable targets in breeding applications (Wang et al., 2011).

Near isogenic lines (NILs) and introgression lines (ILs) are developed by advanced backcrossing in combination with marker-assisted selection (MAS). The use of NILs or ILs is an effective method of characterizing QTLs in detail (Zhou et al., 2010). Some QTLs in rice were cloned by map-based cloning using NIL materials, such as *Gn1a* for grain number, *qGY2-1* for grain yield, and *qSH1* for seed shattering (Ashikari et al., 2005; He et al., 2006; Konishi et al., 2006). In addition to the classical NILs that contained a small introgressed fragment in an isogenic background, a residual heterozygous line (RHL) or a heterogeneous inbred family (HIF) that differed at the target QTL region in an inbred background of the parent (Tuinstra et al., 1997; Yamanaka et al., 2005) was widely used to perform QTL fine mapping and cloning. However, the effects of some QTLs that were detected in a primary population may disappear in one or more homogenous backgrounds (Kandemir et al., 2000; Lecomte et al., 2004). It was suggested that an independent evaluation of the QTL effects, using either independent samples or QTL-NILs, should be undertaken before performing further studies (Dai et al., 2005; Salvi and Tuberosa, 2005).

In this study, a low GW natural mutant, designated sgw, was identified in the indica rice (*Oryza sativa* L. ssp. indica) '9311', a high-yielding cultivar in China with a large GW (Zou et al., 2008), in the field in Jiangxi Agricultural University, Nanchang, China. Here, we produced F_2 and F_3 populations derived from sgw x 9311. The objectives of this study were 1) to isolate the QTLs responsible for GW; 2) to validate the QTLs associated with the phenotype; and 3) to evaluate the effect of the locus on GW-related traits including grain length, width, thickness, and volume under the near isogenic genetic background, which may provide a starting point for the functional characterization of the GW gene.

MATERIAL AND METHODS

Plant materials

A natural mutant, designated sgw, was identified in the indica rice cultivar 9311

in the field in Jiangxi Agricultural University, Nanchang, China (28.68°N, 115.89°E) in 2010 (2010NC), which had a lower GW than 9311. To analyze the genetic basis for the phenotype change, a survey was conducted between sgw and 9311 using 376 simple sequence repeat (SSR) markers that were evenly distributed on the 12 rice chromosomes.

To isolate the QTLs responsible for GW and to develop NILs for the locus, a cross was made between sgw and 9311 in 2010NC. The hybrid of sgw x 9311 was planted in Tengqiao, Hainan Province, China (18.2°N, 108.9°E) in 2010 (2010HN). Subsequently, a small self-pollinated population consisting of 50 plants (numbered S01-S50) was obtained in 2011NC and used to analyze the chromosome(s) associated with GW. In this analysis, a GW locus (tentatively designated *qsgw7*) was identified on chromosome 7 with a series of single plants (S10, S15, S23, and S34). Meanwhile, S15 (designated NILqsgw7), which carried the critical region of the chromosomal segment from sgw, was used to verify the effects of the target interval on GW and related traits in 2011HN and 2012NC.

To validate *qsgw7*, an F_2 population consisting of 67 individuals derived from S18 was grown in an experimental paddy field in 2011HN for genetic analysis. S18 contained a heterozygous segment near the microsatellite locus RM21997 on chromosome 7 that covered the target locus, but the rest of the genome was homozygous. Thirty F_3 plants for each of the 67 individuals of the F_2 population were planted in the 2012NC. Field management followed essentially the normal agricultural practice.

Phenotypic assessment

The 1000-GW of paddy rice was evaluated for the self-pollinated population, the F_2 population, and the F_3 population. Each plot of 30-day-old seedlings was laid out with 20 cm between plants and 30 cm between rows. Grains harvested from individual plants were dried naturally after harvesting, and then water was used to remove those that had not been fully filled. The fully filled grains were re-dried in an oven at 30°C for 24 h. After drying, the GW was measured on 3 replicates of 100 randomly selected grains. The estimated GW was converted to the 1000-GW of paddy rice by multiplying the average weight of 100 randomly selected grains by 10.

Molecular marker development and QTL mapping

Genomic DNA was extracted following Dellapporta et al. (1983) from fresh leaves of the 2 parents (sgw and 9311) and the 67 F_2 progeny of S18. Polymerase chain reactions (PCRs) and subsequent amplicon separation by electrophoresis were performed according to Chen et al. (1997). SSR and insertion and deletion (in/del) markers were developed from a nucleotide basic local alignment search tool (BLASTN) alignment between the genome sequences of the japonica cultivar Nipponbare and the indica cultivar 9311. PCR primers were designed using the Primer Premier 5.0 software. The genomic sequence was obtained from the Rice Genome Research Program (RGP) (http://rgp.dna.affrc.go.jp/), the International Rice Genome Sequencing Project (IRGSP) (http://www.rgp.dna.affrc.go.jp/IRGSP/index.html), and the indica sequence (http://rice.genomics.org.cn/rice/index2.jsp).

QTL analysis for the 1000-GW of paddy rice for the F_2 population was carried out using

the QTL IciMapping v2.2 Mapping software (Li et al., 2008), applying a threshold logarithm of odds (LOD) of 3.0. QTL nomenclature followed the recommendations of McCouch and the Committee on Gene Symbolization, Nomenclature and Linkage (CGSNL) (Rice Genetics Cooperative, 2008).

NIL analysis

NIL*qsgw7* and 9311 (control) were sown on December 4, 2011, at the Hainan Province site. Their seedlings were transplanted on January 4 and were grown under natural conditions in a completely randomized block design with two replications in Tengqiao, Hainan Province. Each plot consisted of two rows that were separated by 30 cm, with each row consisting of 10 plants that were separated by 20 cm. Crop management followed the normal procedures for rice. At maturity, the grains of eight plants from the middle of each plot were bulked harvested and air-dried, and the grains that were not fully filled were removed by selection with water. A sample of fully filled grains was re-dried in an oven at 30°C for 24 h. The weight of three samples of 100 paddy rice and brown rice was used to estimate the 1000-GW. Twenty randomly chosen paddy rice and brown rice from each sample were aligned lengthwise along a vernier caliper to measure grain length and arranged by breadth to measure grain width. The grain thickness was determined for each paddy rice and brown rice individually using a vernier caliper (Guanglu Measuring Instrument Co. Ltd, China), and the values were averaged and used as the measurements for NILqsgw7 and 9311.

The same material was also sown on May 20, 2012, at the Nanchang site. Their seedlings were transplanted on June 19 and grown under the natural conditions in a completely randomized block design with 2 replicates, using the same field plot design as above. The crop management and evaluation of the 1000-GW, grain length, grain width, and grain thickness were the same as above.

RESULTS

Phenotypic variation and genetic analysis of sgw and 9311

The inbred progeny of sgw showed the same phenotype as the low GW mutant, indicating that the mutant trait was not caused by the environment but the hereditary gene mutation. The 1000-GW of sgw (28.10 g) differed obviously from that of the normal GW variety, 9311 (32.35 g) in 2010NC (Figure 1).

To conduct the genetic analysis of sgw, a total of 376 SSR markers (Temnykh et al., 2001; McCouch et al., 2002) distributed throughout the genome were used to survey the polymorphisms between sgw and 9311, and 16 markers showed clear polymorphisms between sgw and 9311. These polymorphic makers represented 13 chromosome fragments on chromosomes 2, 3, 4, 5, 6, 7 (2 segments), 8, 9, 11, and 12 (3 segments) and were used to analyze the genotype of small self-pollinated populations of sgw x 9311.

Chromosomal location of GW

To determine the chromosomal location of the QTLs for GW, the 50 self-pollinated

population plants of sgw x 9311 were evaluated for their 1000-GW of paddy rice in 2011NC. The polymorphic markers were also genotyped in these plants. A single plant, S15, which contained the least homozygosity from sgw in the 9311 genetic background (three segments on chromosomes 7, 9, and 12), was identified (Figure 2). The 1000-GW of paddy rice in S15 was significantly lower than that in 9311 ($P < 0.01$) (Figure 3). To dissect the genetic factors underlying the low GW in S15, another three plants (S10, S23, and S34), which included some of the three different chromosome segments, were selected, and all showed the same 1000-GW of paddy rice as 9311 (Figure 3). The graphical genotype of the four plants indicated that the chromosome 7 segment (marker RM21997) could be linked to the 1000-GW of paddy rice (Figure 2). This GW gene in rice was tentatively designated *qsgw7*.

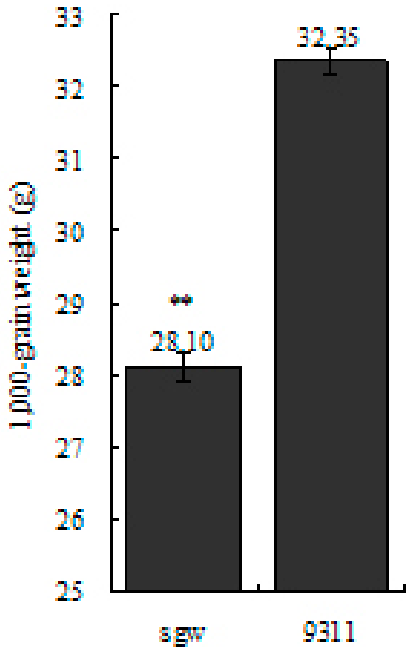

Figure 1. 1000-grain weight data of paddy rice for sgw and 9311 in 2010NC. **Significant difference at the 0.01 level according to the *t*-test.

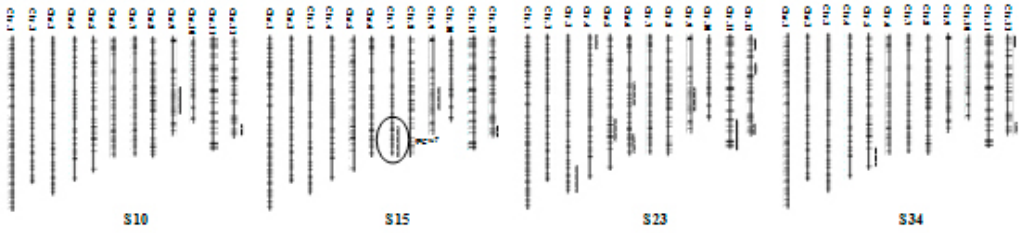

Figure 2. Graphical genotype of four NILs (S10, S15, S23 and S34). Black bar indicates the genome fragment from sgw chromosome segments; the other parts were from 9311. Black circle indicates that the 9311 allele increases GW.

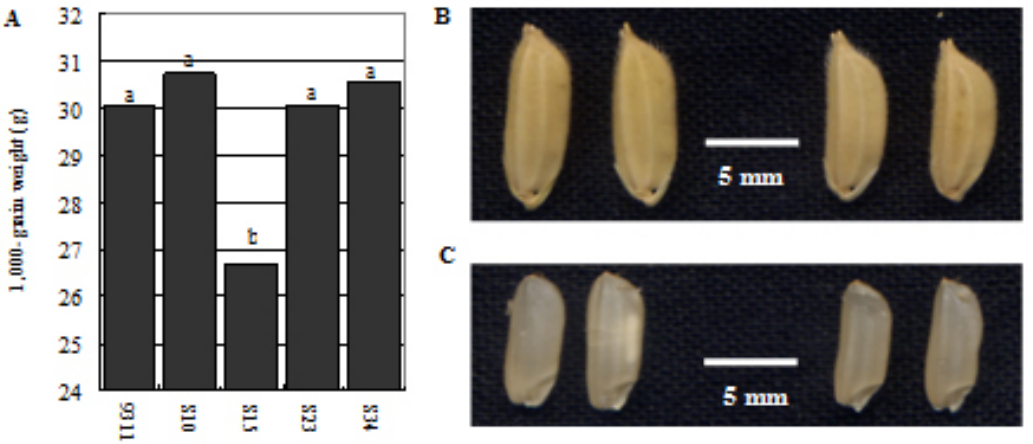

Figure 3. 1000-grain weight data of paddy rice for four NILs (S10, S15, S23, and S34) and 9311 (**A**) and grain shape of 9311 (left) and S15 (right) (**B**, **C**). **A.** Different letters on the bars signify a significant difference at the 0.01 level. **B.** Grains prior to dehulling. **C.** After dehulling.

QTL validation and a detailed map of *qsgw7*

To validate the position of *qsgw7*, an F_2 population was derived from S18, which was heterozygous for the critical region but homozygous throughout most of the rest of the genome except for a region on chromosome 9 (Figure 4). The 1000-GW of paddy rice segregated continuously among the 67 segregants and varied between 26.86 and 32.54 g (Figure 5).

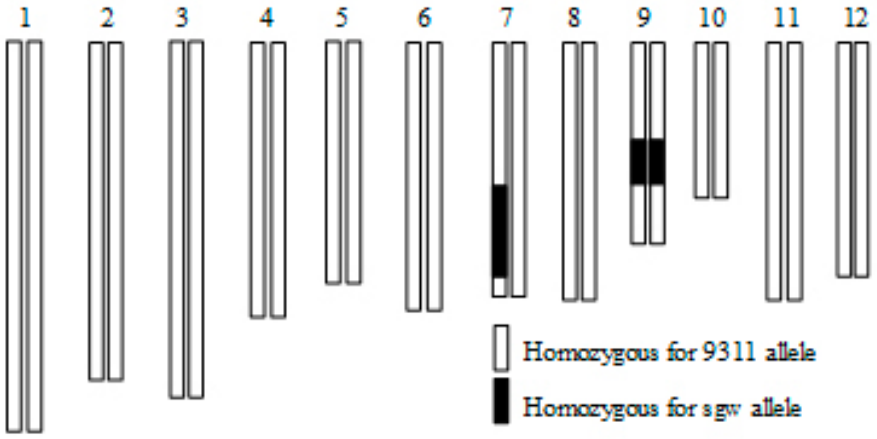

Figure 4. Graphical genotypes of S18.

To further refine the locus, 5 new polymorphic markers (RM455, RM351, RM21899, RM22015, and RM22048) around RM21997 were developed, and a single QTL affecting the 1000-GW of paddy rice was identified on the short arm of rice chromosome 7 between SSR

markers RM21997 and RM22015 (Figure 6). The peak LOD value for *qsgw7* (LOD = 10.37) was found adjacent to SSR marker RM22015, and the phenotypic variance was 62.34%, with the positive allele contributed by 9311 (Table 1).

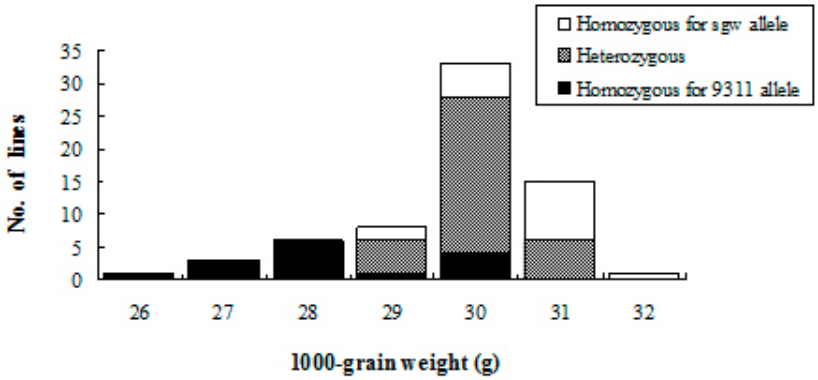

Figure 5. Frequency distribution of 1000-grain weigh of paddy rice in the F_2 population derived from S18. Three genotype classes of qsgw7 assessed by progeny tests are as indicated.

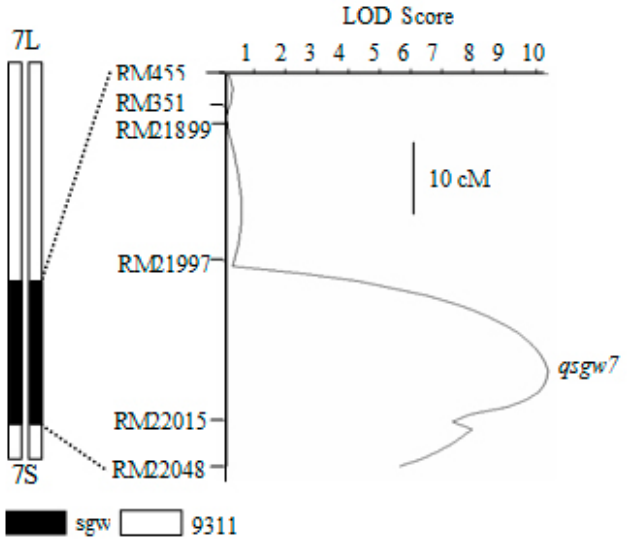

Figure 6. LOD score of the QTL controlling 1000-grain weight of paddy rice on chromosome 7 in segregating population. The genetic map was reconstructed with six DNA markers, and the vertical axis indicates the genetic loci examined. The horizontal axis indicates the LOD score.

Table 1. QTL analysis for 1000-grain weight of paddy rice in self-pollinated population of S18.

Trait	Chr.[a]	Left marker	Right marker	Add[b]	LOD	PVE (%)[c]
1000-grain weight of paddy rice	7	RM21997	RM22015	-1.46	10.37	62.34

[a]Chromosome; [b]additive effect; [c]phenotypic variation explained by the QTL.

In the F_3 families from 67 F_2 plants, the 1000-GW of paddy rice displayed 3 distinct phenotypic groups: 17 families with uniformly large GW, 15 families with uniformly small GW, and 35 families with both large and small GW. The ratio of the three groups fit well with the expected ratio (1:2:1) of single locus Mendelian segregation ($\chi^2 = 5.38$, $P > 0.05$). Clearly, the three distinct phenotypic classes corresponded to the three genotypes of the F_2 individuals at the *qsgw7* locus: homozygote for the 9311 allele (large GW), homozygote for the sgw alleles (small GW), and heterozygote (Figure 5). Using the three phenotypic classes as a marker, *qsgw7* was directly delimited by the SSR markers RM21997 and RM22015 (Figure 3). This interval comprised four bacterial artificial chromosome clones, OJ1339_F05, P0506F02, P0011H09, and P0519E12, according to the physical map of the region (http://ricegaas.dna.affrc.go.jp).

Grain shape of NIL*qsgw7*

In 2011HN and 2012NC, S15 was selected as an NIL for *qsgw7* (NIL*qsgw7*), which carried the chromosomal segment from sgw that included the critical region, using MAS. As shown in Table 2, the 1000-GW of paddy rice of NIL*qsgw7* was 13.7 and was 16.5% lower than that of 9311 after maturing in 2 different environments. The grain shape analysis showed that the grain length and width of paddy rice in NIL*qsgw7* were also significantly lower than in the rice cultivar 9311; the grain volume of paddy rice in NIL*qsgw7* was 11.6 and was 10.4% lower than in rice 9311 in 2 environments.

The 1000-GW of brown rice in NILqsgw7 was 22.0 and was 22.0% lower than in 9311 in 2 environments. The length and width of brown rice were significantly lower than those in 9311 in 2 environments; the grain volume of brown rice in NIL*qsgw7* was 24.7 and was 19.4% lower than in 9311 in 2 environments (Table 2).

Table 2. Comparisons of 1000-grain weight and related traits between 9311 and NILqsgw7 in 2011HN and 2012NC.

Traits	2011HN			2012NC		
	9311	NILqsgw7	Δ[a]	9311	NILqsgw7	Δ
1000-grain weight of paddy rice (g)	33.53	28.93**	-0.137	33.66	28.09**	-0.165
Grain length of paddy rice (mm)	9.76	9.48**	-0.029	9.88	9.46**	-0.043
Grain width of paddy rice (mm)	3.02	2.86**	-0.053	3.09	2.84**	-0.081
Grain thickness of paddy rice (mm)	2.19	2.10	-0.041	2.10	2.14	0.019
Grain volume of paddy rice (mm^3)	64.48	56.99**	-0.116	64.11	57.43**	-0.104
1000-grain weight of brown rice (g)	26.33	20.53**	-0.220	27.51	21.46	-0.220
Grain length of brown rice (mm)	7.17	6.94**	-0.032	7.19	6.87**	-0.045
Grain width of brown rice (mm)	2.70	2.34**	-0.133	2.65	2.37*	-0.106
Grain thickness of brown rice (mm)	1.93	1.73*	-0.104	1.95	1.85	-0.051
Grain volume of brown rice (mm^3)[b]	37.36	28.12**	-0.247	37.25	30.03*	-0.194
Rate of chalky rice (%)	16	10**	-0.375	31	8**	-0.742

[a]Δ = (NILqsgw7-9311)/ 9311; [b]grain volume was given by grain length x grain width x grain thickness. *Significance level of 5%; **significance level of 1%.

DISCUSSION

Increasing rice productivity to ensure sufficient food has been an intense topic of research for a long time. Studies in recent years have shown that MAS is an effective approach for reducing the cost and improving the efficacy and accuracy of selection for plant breeding

(Ribaut and Hoisington, 1998; Young et al., 1999). However, there are only a small number of reports on quantitative trait improvement, especially for yield-related traits, even though numerous QTLs for yield and associated traits have been identified in rice. The instability of QTL expression and the lack of reliable markers are two critical barriers to large-scale utilization of MAS in high-yield rice breeding (Zhu et al., 2011).

GW directly and obviously affects yield. Breeders tend to select plants with large seed size for high yield and appropriate grain size for milling yield and market preferences. Viewed from the perspective of mechanics, the larger the GW, the more favorable it is for farmers. GW is generally extremely significantly correlated with cooking qualities and the physical appearance (Stangoulis et al., 2007). Therefore, it might be possible to obtain higher yields by designing an ideal GW. Using molecular marker maps and QTL mapping technology, numerous QTLs linked to rice GW were reported (Lin et al., 1996; Cui et al., 2003; Ishimaru, 2003). However, until now, only a few QTLs for GW had been isolated. One of the reasons is that rice GW is controlled by a polygenic system. Another reason is the lack of genetic populations suitable for GW QTL isolation. To elucidate the genetic basis of important agronomic traits, NILs or ILs have been developed (Eshed et al., 1995; Ishimaru et al., 2005; Dai et al., 2008). These NILs and ILs were used to confirm QTLs that were putatively detected in primary populations, such as F_2s, recombinant inbred lines (RILs), or backcross inbred lines (BILs). Additionally, NILs and ILs could also contribute to the analysis of the physiological functions of QTLs and to the identification of the underlying genes by map-based strategies (Fan et al., 2006).

In this study, a natural low GW mutant, sgw, was used to analyze the genetic basis of GW. We chose to detail its map location on the short arm of chromosome 7, and we showed that it was located between the SSR markers RM21997 and RM22015. An NIL that carried an sgw chromosomal segment corresponding to *qsgw7* in the 9311 genetic background was analyzed to clarify the grain-related traits of this locus. Compared to the 1000-GW of 9311, the 1000-GW of paddy rice in NIL*qsgw7* was 13.7 and 16.5% lower in 2 environments (Table 2). The GW of paddy rice is mainly determined by the length, width, thickness, and volume. In NIL*qsgw7*, the grain length, width, and thickness of paddy rice were significantly lower than those in 9311 in 2 environments. Brown rice analysis showed that the 1000-GW, grain length, width, and volume of brown rice in NIL*qsgw7* were significantly lower than those in rice 9311 in 2 environments. These results suggested that the lower GW in NIL*qsgw7* might be determined by the inferior kernel shape (grain length, width, and thickness), especially the grain volume. In other words, grain plumpness might play an important role in GW formation. In addition, another NIL, S10, showed no difference in GW and related traits compared with those of 9311 in 2 environments (data not shown), which indicated that *qsgw7* may not only affect the GW but also the grain shape.

We also identified a GW QTL, *qTGW7*, on the short arm of chromosome 7 in our previous study, but this QTL only responsible for GW and did not affect grain length and width (Bian et al., 2010); Shao et al. (2012) described the fine mapping of *GS7* on the short arm of chromosome 7, but the QTL was only responsible for grain shape and not for GW in rice. Moreover, these two QTLs were not located in the same region as *qsgw7*. These results indicated that the gene identified in this study may be a new GW-related QTL.

The most significant finding in our study was delimiting the *qsgw7* gene to the short arm of chromosome 7. QTL analysis using F_2 and F_3 progenies derived from S18 showed that *qsgw7* was directly delimited by SSR markers RM21997 and RM22015 (adjacent to SSR

marker RM22015), where 4 bacterial artificial chromosome clones, OJ1339_F05, P0506F02, P0011H09, and P0519E12 were present. Moreover, the NIL*qsgw7* carrying this QTL not only provided an opportunity for map-based cloning of this important grain yield QTL but also supplied a useful inbred line to improve GW. These achievements benefit the understanding of the genetic control of rice GW and the goal of using *qsgw7* for high yield breeding with MAS. Further analyses to validate the effect of *qsgw7* on other grain-related traits or yield and to identify the *qsgw7* gene are ongoing.

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