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ORIGINAL PAPER

Fine mapping of *qGW1*, a major QTL for grain weight in sorghum

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Abstract

Key message **We detected seven QTLs for 100-grain weight in sorghum using an F_2 population, and delimited *qGW1* to a 101-kb region on the short arm of chromosome 1, which contained 13 putative genes.**

Abstract Sorghum is one of the most important cereal crops. Breeding high-yielding sorghum varieties will have a profound impact on global food security. Grain weight is an important component of grain yield. It is a quantitative trait controlled by multiple quantitative trait loci (QTLs); however, the genetic basis of grain weight in sorghum is not well understood. In the present study, using an F_2 population derived from a cross between the grain sorghum variety SA2313 (*Sorghum bicolor*) and the Sudan-grass variety Hiro-1 (*S. bicolor*), we detected seven QTLs for 100-grain weight. One of them, *qGW1*, was detected consistently over 2 years and contributed between 20 and 40 % of the phenotypic variation across multiple genetic backgrounds. Using extreme recombinants from a fine-mapping F_3 population, we delimited *qGW1* to a 101-kb region on the short arm of chromosome 1, containing 13 predicted gene models, one of which was found to be under purifying selection during domestication. However, none of the grain size candidate genes shared sequence similarity with previously cloned grain weight-related genes from rice. This study will facilitate isolation of the gene underlying *qGW1* and advance our understanding of the regulatory mechanisms of grain weight. SSR markers linked to the *qGW1* locus can be used for improving sorghum grain yield through marker-assisted selection.

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Introduction

Sorghum (*Sorghum bicolor* L. Moench) is an annual tropical C_4 grass of the Andropogoneae tribe in the Poaceae. It is one of the first domesticated and most important multipurpose cereal crops used for food, fodder, fiber, fuel, and building materials. It ranks fifth (after wheat, maize, rice, and barley) in terms of both production and area planted globally (http://www.fao.org). Sorghum withstands many abiotic stresses, such as drought and waterlogging, salt, lack of nutrients, heat, and cold. These characteristics make sorghum an important staple food for millions of rural families in arid and semi-arid regions of the world (Gilbert 2009; Paterson 2008). Given the increasing global

population, shrinking arable land area, and scarcity of water resources, people are expected to face serious global food shortage problems in the next 50 years (Diouf 2009; Khush 2005; Hibberd et al. 2008; Tilman et al. 2001). Therefore, breeding of high-yielding crop varieties, including sorghum, will have a profound impact on food security of the world.

Sorghum bicolor is a diploid species ($2n = 2x = 20$) with a haploid genome of ~730 Mbp (Paterson et al. 2009). Its genome is larger than that of rice (~389 Mbp; The International Rice Genome Sequencing Project 2005) but smaller than those of other major crops, such as wheat (~17,000 Mbp; Zhang et al. 2012a), maize (~2300 Mbp; Schnable et al. 2009), and sugarcane (2547–3605 Mbp; Bowers et al. 2003). Sorghum's genome shares considerable sequence similarity with those of sugarcane and maize (Paterson et al. 2009). These features make sorghum an attractive model plant for functional genomics and evolutionary studies of cereals in addition to rice.

Grain weight is one of the three major yield components (the other two being the number of grains per panicle and tiller number), and it also affects seedling germination and initial growth (Maranville and Clegg 1977). Therefore, an increase in grain weight is very important for sorghum yield. Grain weight is affected by grain length, width, and thickness. It is a complex quantitative trait controlled by multiple genes or quantitative trait loci (QTLs). To increase grain weight, we need to understand the genetic basis of its regulation.

To date, a number of rice QTL (genes) associated with grain weight have been isolated and characterized through map-based cloning, including *GS3* (Fan et al. 2006), *GW2* (Song et al. 2007), *qSW5* (Shomura et al. 2008)/*GW5* (Weng et al. 2008), *GIF1* (Wang et al. 2008), *GS5* (Li et al. 2011), *qGL3* (Zhang et al. 2012b), *GW8* (Wang et al. 2012), *TGW6* (Ishimaru et al. 2013), and *GS6* (Sun et al. 2013). These genes regulate cell proliferation and elongation through signaling pathways mediated by proteasomal degradation, phytohormones, and G proteins. Among these genes, *GS3*, *GS5*, *qSW5*/*GW5*, *GW8*, and *GIF1* were strongly selected for enhanced rice yield during rice domestication and breeding (Zuo and Li 2014). These studies may facilitate breeding of high-yielding rice varieties and provide useful information for studies of the genetic mechanisms of regulation of grain weight in sorghum. In sorghum, a number of grain size QTL mapping studies have been carried out (Paterson et al. 1995; Pereira et al. 1995; Rami et al. 1998; Brown et al. 2006; Feltus et al. 2006; Murray et al. 2008; Srinivas et al. 2009; Upadhyaya et al. 2012; Rajkumar et al. 2013) with QTLs for grain weight found on all chromosomes except 5. Mace and Jordan (2011) has conducted a meta-analysis based on the 48 sorghum QTL studies published from 1995 to 2010 and detected nine meta-QTL for grain weight on all sorghum chromosomes except 5 and 9. More recently, Zhang et al. (2015) detected four genomic regions significantly associated with grain size on chromosomes 4, 6, 7, and 10 using QTL mapping and GWAS study. However, none of these genes for grain weight have been finely mapped or cloned in sorghum.

To understand the genetic basis of sorghum grain weight regulation, in this study we aimed to (1) detect QTLs for 100-grain weight using an F_2 population derived from a cross between SA2313, a large-seed grain sorghum variety, and Hiro-1, a small-seed Sudan-grass variety; (2) determine whether a major QTL can be identified in three different crosses: SA2313 × Hiro-1, K-385 × SA2313, and ATx623 × SA2313; (3) fine-map *qGW1*, a major QTL on the short arm of chromosome 1.

Materials and methods

Plant materials and mapping populations

Four parental lines, SA2313 (PI276795), Hiro-1, ATx623, and K-385 (PI267128) were used in this study. SA2313 is a photoperiod-sensitive landrace with large grains. Hiro-1 is a variety of sudan-grass variety with small grains. ATx623 is a male-sterile line with smaller grains than SA2313. K-385 is a broom variety with small grains. SA2313 and K-385 were obtained from the National Plant Germplasm System (http://www.ars-grin.gov/npgs/index.html), and Hiro-1 and ATx623 were from Japan.

An F_2 population was developed using SA2313 as the female parent and Hiro-1 as the male parent. In the summer of 2012, the F_2 population (1300 individuals) was grown at the Shangzhuang experimental station of China Agricultural University at Beijing, China (39°N, 116°E). Because SA2313 is a photoperiod-sensitive variety, only 138 individuals headed and matured and were used for the initial QTL detection.

To confirm the initial mapping results, we grew the same F_2 population in the experimental fields at Sanya, Hainan Province, China (18°N, 109°E), in January 2013. Extreme individuals (47 plants with 100-grain weight of 1.21–2.11 g and 47 plants with 100-grain weight of 3.66–4.36 g) selected from the 1053 F_2 individuals were used for QTL analysis. Two other F_2 populations, ATx623 × SA2313 (72 individuals) and K-385 × SA2313 (109 individuals), were grown at Sanya in January 2014.

For fine mapping of the QTL *qGW1*, to exclude the genetic effect of the *qGW2* locus on chromosome 2, two F_2 plants from the SA2313 × Hiro-1 F_2 population, SH1246 and LJ455, with the heterozygous *qGW1* region and Hiro-1 type–homozygous *qGW2* region were selfed. The two F_3

mapping populations (218 and 189 individuals, respectively) were used for fine mapping of *qGW1*. The F_3 plants were grown at Sanya in January 2014.

The plants of all above populations with respective parents (20 individuals for each parent) were grown in rows at 40-cm intervals and 15 cm between individuals, 10 individuals were grown in each row; the fields and fertilization were managed following the standard local cultivation practice. To avoid cross-pollination, all plants were bagged before flowering. Main stem panicles were harvested 6 weeks after heading and sun-dried for 2 days. Seeds were threshed from the panicles and stored in dry containers at room temperature.

Trait evaluation

Freshly harvested seeds were dried in an oven at 42 °C for 3 days, the weight of 100 randomly selected fully filled grains was measured, and the average of three replications was recorded as 100-grain weight. Broad-sense heritability [$h^2 = V_G/V_P = (VF_2 - VF_1)/VF_2$] was calculated using Excel 2010.

Molecular marker analysis

DNA was extracted from fresh leaves using the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980) with minor modifications. A total of 639 pairs of sorghum simple sequence repeat (SSR) markers (Li et al. 2009; Yonemaru et al. 2009) distributed across the sorghum genome were used to screen for polymorphisms in the parental lines, and 127 polymorphic SSR primer pairs were used to genotype the F_2 populations. For the ATx623 × SA2313 population, we genotyped six polymorphic SSR markers on chromosome 1 (the *qGW1* region), nine markers on chromosome 2 (the *qGW2* region), and one marker on chromosome 4 (sorghum homolog of rice grain weight gene *GW2*). For the K-385 × SA2313 population, we genotyped nine polymorphic SSR markers in the *qGW1* region, nine markers in the *qGW2* region, and two markers in the region of the sorghum homolog of rice *GW2*.

To fine-map *qGW1*, additional SSR primers in the target region were identified (Li et al. 2009; Yonemaru et al. 2009), as well as designing four new SSR markers on the basis of the sorghum genome sequence (http://www.gramene.org/Sorghum_bicolor/Location). Primer information for the four newly developed SSR markers is listed in Table S1.

PCR reactions were carried out using an M13-tagged forward primer (Rampling et al. 2001) as previously described (Li et al. 2009). The reaction mixture (10 μl) contained 20 ng template DNA, 1 pmol forward primer, 5 pmol reverse primer, 5 pmol M13 (−29) primer, 1 μl of 10 × PCR buffer, 0.8 μl of 2.5 mM dNTPs, and 0.5 U Taq polymerase. PCR products were detected on a 6 % denaturing polyacrylamide gel with a LI-COR 4300-DNA sequencer (Li-COR, Lincoln, Nebraska, USA). The results were scored as either parental (A or B), heterozygous (H), or missing data (−).

Map construction and QTL mapping and fine mapping of *qGW1*

MapManager QTXb20 (http://mapmgr.roswellpark.org/mmQTL.html, Manly et al. 2001) was used to construct the linkage map and conduct the QTL analysis. Linkage map construction was performed with a LOD score threshold of 4.0 and a recombination frequency of 0.4. The Kosambi mapping function was used to calculate the map distances (Kosambi 1943). The linkage map was drawn using MapChart 2.0 (Voorrips 2002). Marker regression and simple interval mapping analysis ($P < 0.01$ significance threshold) were performed with the MapManager QTXb20 software, and a 1-LOD support interval was used as a confidence interval for the QTL location on the map. The QTL nomenclature was according to McCouch et al. (1997).

For fine mapping of *qGW1*, recombinant individuals in the F_3 population were identified, and extreme recombinants (the marker-recombinant individuals with grain weight smaller/larger than most smaller/larger marker-heterozygous individuals were extreme smaller/larger recombinants) were selected. Non-recombinants were used as a control and grouped as plants with the homozygous Hiro-1 genotype (CK1), heterozygous plants (CK2), and plants with the homozygous SA2313 genotype (CK3). The mean value and standard deviation of 100-grain weight were measured for 20 plants randomly chosen from each CK group. These values and those of the extreme recombinants were compared to determine the critical recombination points.

In silico search for sorghum homologs of rice grain size genes

The Phytozome *S. bicolor* genome sequence database V2.1 (http://phytozome.jgi.doe.gov/) was searched (BLASTN) with the sequences of the following rice genes: *GW2* (Song et al. 2007, *LOC_Os02g14720*), *qSW5/GW5* (Shomura et al. 2008; Weng et al. 2008, AB433345, *LOC_Os05g09520*), *TGW6* (Ishimaru et al. 2013, *LOC_Os06g41850*), *GS3* (Fan et al. 2006, *Os03g0407400*), *GS5* (Li et al. 2011, *LOC_Os05g06660*), *GW8* (Wang et al. 2012, *LOC_Os08g41940*), *GIF1* (Wang et al. 2008, *LOC_Os04g33740*) and *qGL3* (Zhang et al. 2012b,

Table 1 Grain traits of SA2313, Hiro-1, K-385, and ATx623

Trait	SA2313	Hiro-1	K-385	ATx623[b]
100-grain weight (g)	5.37 ± 0.03	1.41 ± 0.04[a]	1.50 ± 0.01[a]	2.03 ± 0.05[a]
Grain width (mm)	5.28 ± 0.15	2.80 ± 0.10[a]	3.13 ± 0.10[a]	3.59 ± 0.14[a]
Grain thickness (mm)	3.42 ± 0.13	2.21 ± 0.14[a]	2.30 ± 0.10[a]	2.22 ± 0.13[a]
Grain length (mm)	5.51 ± 0.20	5.33 ± 0.23	5.14 ± 0.13	4.16 ± 0.11[a]

For each trait, the mean values for the main panicles of 10 plants of Hiro-1, K-385, and ATx623 were compared with that for SA2313

[a] Significant difference from SA2313 at $P < 0.0001$

[b] The values for ATx623 were measured using seeds of the ATx623 × BTx623 cross

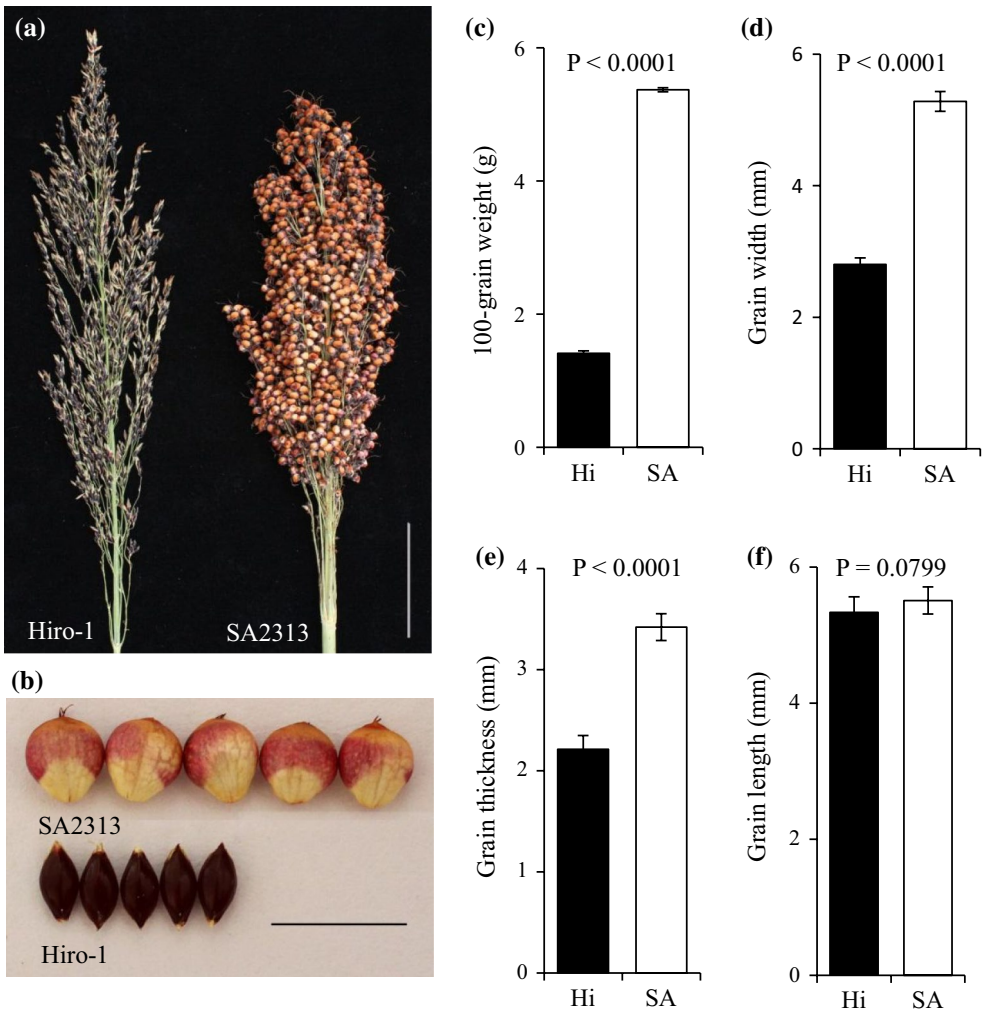


Fig. 1 Grain traits in Hiro-1 and SA2313. **a** Main panicle, *Scale bar* 5 cm, **b** Grains, *Scale bar* 1 cm, **c** 100-grain weight ($n = 10$ plants), **d** Grain width, **e** Grain thickness, **f** Grain length (**d–f**, $n = 10$ spikelets). All data in **c–f** are mean ± SD. *Hi* Hiro-1, *SA* SA2313

LOC_Os03g44500). Four rice genes for grain size mutations, *SRS1/DEP1* (Abe et al. 2010, *LOC_Os07g42410*), *SRS3* (Kitagawa et al. 2010, *LOC_Os05g06280*), *SRS5* (Segami et al. 2012, *LOC_Os11g14220*), *SG1* (Nakagawa et al. 2012, *LOC_Os09g28520*), and a maize grain size mutation gene *Mn1* (Cheng et al. 1996, GRMZM2G119689) were also included in this study.

Results

Phenotypic variation of 100-grain weight in parental lines and F_2 populations

We measured four grain traits and found that 100-grain weight, grain width, and grain thickness, but not grain

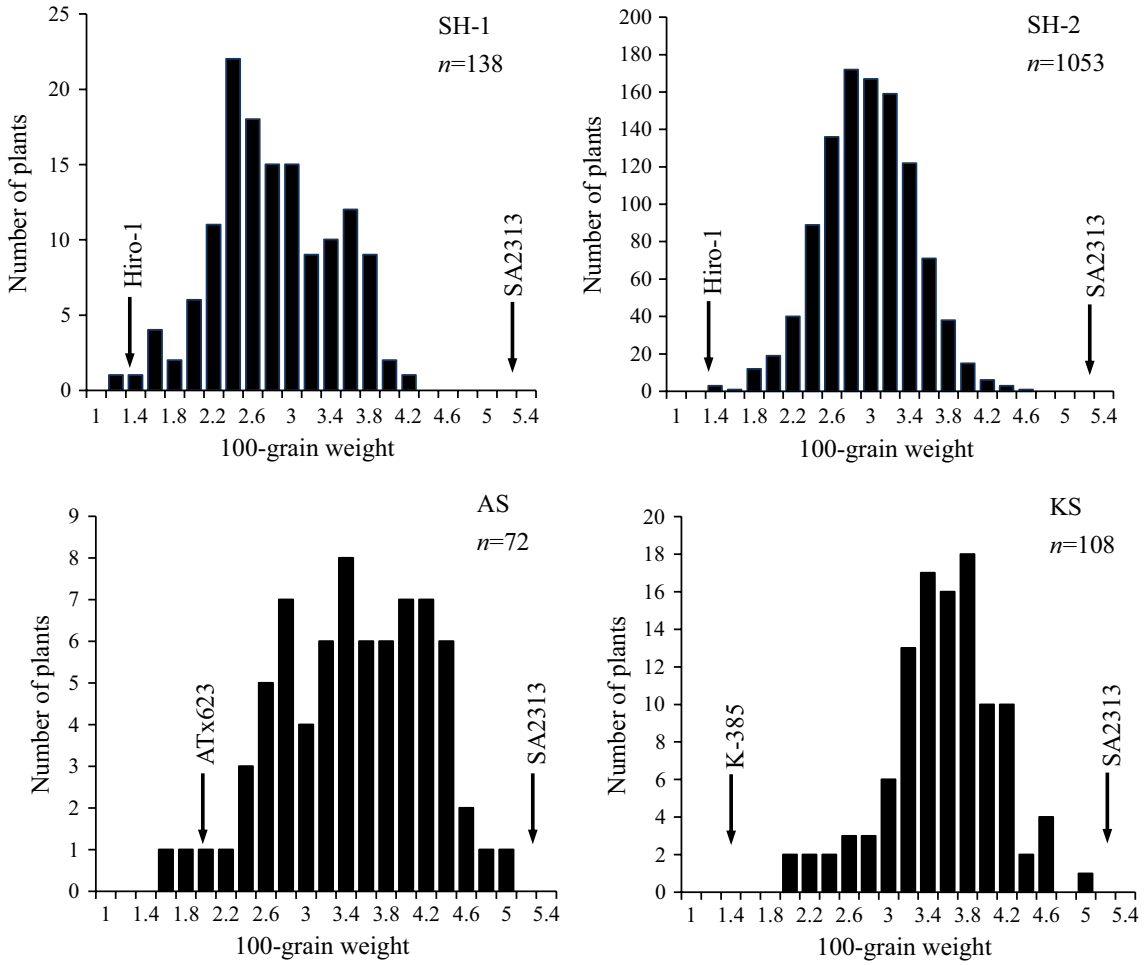


Fig. 2 Frequency distribution of 100-grain weight in three F_2 populations derived from different crosses. *SH* SA2313 × Hiro-1 (SH-1 was planted in Beijing and SH-2 in Sanya), *AS* ATx623 × SA2313, *KS* K-385 × SA2313, *n* population size. *Arrows* indicate the 100-grain weight values of each parent

length, were significantly different ($P < 0.0001$) between SA2313 and three other parental lines (Table 1; Figs. 1 and S1). We chose 100-grain weight as the target trait in subsequent analysis because, of all the traits measured, it had the largest difference between SA2313 and other lines and the smallest standard deviation (Table 1). The distribution of 100-grain weight was continuous and approximately normal in all four F_2 populations (Fig. 2), indicating quantitative inheritance of this trait.

Broad-sense heritability of 100-grain weight in the four F_2 populations

The broad-sense heritability was 0.946 in the SA2313 × Hiro-1 Sanya population, 0.917 in the ATx623 × SA2313 population, and 0.993 in the K-385 × SA2313 population. The broad-sense heritability could not be measured in the SA2313 × Hiro-1 Beijing population because the parental line SA2313 and F_1 plants did not head at the Beijing site.

QTL analysis for 100-grain weight in the four F_2 populations

A total of 127 polymorphic markers between SA2313 and Hiro-1 distributed across ten chromosomes were used to genotype 138 F_2 plants. The genotype data were used to construct a genetic linkage map (Fig. 3). The total length of the linkage map was 1873.5 cM with an average genetic distance of 14.8 cM between two adjacent markers. Three QTL were detected from the 2012 trial (Beijing) using the SA2313 × Hiro-1 population, *qGW1*, *qGW2*, and *qGW7*, at the 1 % significance level (Fig. 3; Table 2). *qGW1* (LOD = 7.04) was detected between markers SB00037 and SB00219 and explained the highest phenotypic variation (22 %); *qGW2* (LOD = 4.09) and *qGW7* (LOD = 2.20) explained 13 and 7 % of the phenotypic variation, respectively. For all three QTLs, the SA2313 allele increased 100-grain weight.

To confirm the results obtained in the 2012 trial, we analyzed 94 extreme individuals chosen from the 1053 F_2

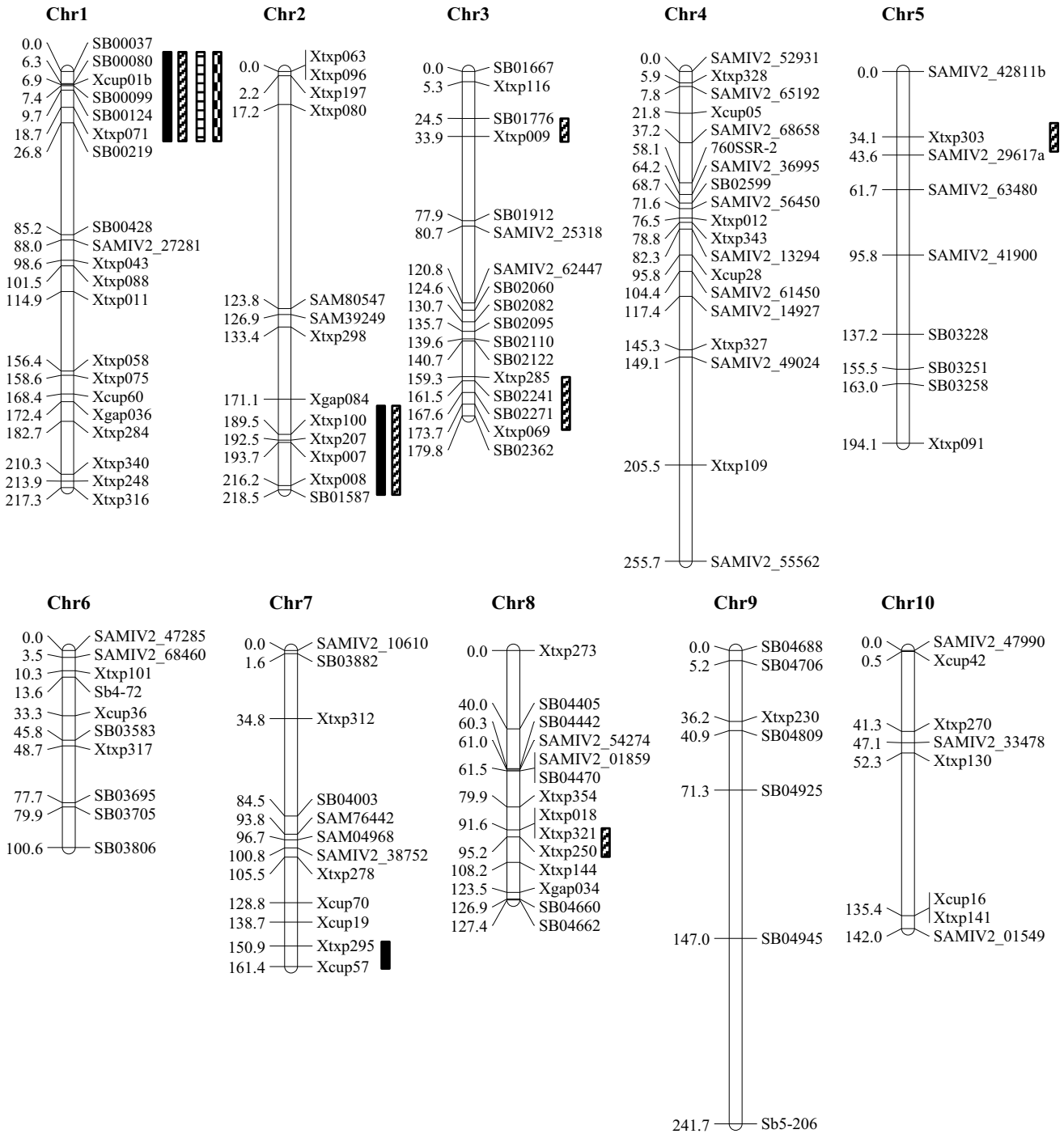


Fig. 3 A genetic linkage map of sorghum showing QTLs for 100-grain weight identified in the three F_2 populations. Chromosomes are represented as *vertical bars*, distances (cM) are shown on the *left* and markers on the *right*. QTLs significant at the 1 % level are shown on the right of the markers. *Shaded bars* represent QTLs detected in different populations. SH-1; SH-2; AS; KS

plants grown at the Sanya site (Fig. S2) and detected six QTLs at the 1 % significance level. *qGW1* (LOD = 10.23, explaining 40 % of phenotypic variation) and *qGW2* (LOD = 6.29, explaining 27 % of phenotypic variation) were detected at the same positions as in the Beijing population, whereas *qGW3.1* (LOD = 2.07, explaining 10 % of phenotypic variation), *qGW3.2* (LOD = 2.97, explaining 14 % of phenotypic variation), *qGW5* (LOD = 2.66,

Table 2 QTLs detected at $P < 0.01$ significant level for 100-grain weight in three F_2 populations

Cross	QTL name	Chr	Flanking markers	LOD	R^2 (%)	Increased effect	Additive effect	Dominant effect
SA2313 × Hiro-1 (2012)	*qGW1*	1	SB00037-SB00219	7.04	22	SA2313	−0.41	0.11
	qGW2	2	Xgap084-SB01587	4.09	13	SA2313	−0.35	−0.07
	qGW7	7	Xtxp295-Xcup57	2.2	7	SA2313	−0.21	−0.15
SA2313 × Hiro-1 (2013)	*qGW1*	1	SB00037-SB00219	10.23	40	SA2313	−0.77	−0.34
	qGW2	2	Xgap084-SB01587	6.29	27	SA2313	−0.73	0.25
	qGW3.1	3	SB01776-Xtxp009	2.07	10	SA2313	−0.29	0.52
	qGW3.2	3	Xtxp285-Xtxp069	2.97	14	SA2313	−0.55	0.1
	qGW5	5	Xtxp303-SAMIV2_29617a	2.66	13	SA2313	−0.5	0.09
	qGW8	8	Xtxp321-Xtxp250	2.55	12	SA2313	−0.32	0.55
ATx623 × SA2313	*qGW1*	1	SB00037-SB00219	3.3	20	SA2313	0.45	0.29
K-385 × SA2313	*qGW1*	1	SB00037-SB00219	6.4	24	SA2313	0.3	0.32

explaining 13 % of phenotypic variation), and *qGW8* (LOD = 2.55, explaining 12 % of phenotypic variation) were new (Fig. 3; Table 2). To assess *qGW1* in a different genetic background, we examined F_2 populations derived from the ATx623 × SA2313 and K-385 × SA2313 crosses, and found that *qGW1* was detected at the same genomic location as with the SA2313 × Hiro-1 population and explained 20 % (LOD = 3.30) and 24 % (LOD = 6.40) of phenotypic variation at the 1 % significance level, respectively. *qGW2* was detected in the K-385 × SA2313 population at only the 5 % significance level and explained 7 % of the phenotypic variation (data not shown), and it was not detected in the ATx623 × SA2313 population.

qGW1 had the highest R^2 value overall and was consistently detected in different F_2 populations and environments. This suggested that *qGW1* was a major QTL for 100-grain weight, and SA2313-derived alleles increased the grain weight of sorghum.

Search for sorghum homologs of rice grain size genes

We searched for sorghum genes homologous to 12 rice and one maize grain size genes (Table 3). Three homologs of rice *GW8* (Wang et al. 2012) were found in sorghum: Sobic.007G193500 in the *qGW7* region, Sobic.002G257900, in the *qGW2* region, and Sobic.003G406600 in the *qGW3.2* region. Two homologs of rice *qGL3* (Zhang et al. 2012b) were found in sorghum: Sobic.008G173900, within 3 Mb of *qGW8*, and Sobic.001G154900 on chromosome 1, within 3 Mb from *qGW1*. Two of five sorghum homologs of rice *GIF1* (Wang et al. 2008) were found to colocate with grain size confidence intervals (CI); Sobic.001G099700, closely linked to *qGW1*, and Sobic.003G440900 closely linked to *qGW3.2*. One homologous sorghum gene of rice *GS3* (Fan et al. 2006) was also found on chromosome 1, but greater than 30 Mb from *qGW1*, A sorghum homolog of *GW2* (Song et al. 2007) and *TGW6* (Ishimaru et al. 2013) was found on chromosome 4 and that of *GS5* (Li et al. 2011) and *qSW5/GW5* (Shomura et al. 2008; Weng et al. 2008) on chromosome 9, but we did not detect QTLs on these two chromosomes. The sorghum homologue of *SRS1/DEP2* (Abe et al. 2010, Sobic.002G374400) was located within the CI of *qGW2*; a sorghum homolog of rice *SRS3* (Kitagawa et al. 2010) was found on chromosome 9, but we did not detect QTL on this chromosome; one sorghum homolog for both rice *SRS5* (Segami et al. 2012, Sobic.001G107200) and maize *Mn1* (Cheng et al. 1996, Sobic.001G099700) were found on chromosome 1 and both were located within the primary interval of *qGW1*, but located outside of the fine mapped position of *qGW1*; and a sorghum homolog of rice *SG1* (Kitagawa et al. 2010, Sobic.002G226500) was found on chromosome 2 within 3 Mb of *qGW2*.

Fine mapping of *qGW1*

qGW1 was identified as the major QTL between the SB00037 and SB00219 markers in both F_3 populations (SH1246 and LJ455), and explained 30 % (LOD = 15.14) and 26 % (LOD = 10.19) of the phenotypic variation, respectively, which was consistent with the F_2 mapping results.

Using these flanking markers in both F_3 populations, we identified 109 recombinants around the *qGW1* locus. Using five markers between SB00037 and SB00219, we genotyped these recombinants and constructed a high-resolution map (Fig. 4a). The target region was narrowed down using 27 extreme recombinants divided into 13 groups according to their genotypes (G1–G13; Fig. 4b) and comparing them with non-recombinant controls (CK1, CK2, and CK3). G1 had smaller 100-grain weight than CK1 and delimited *qGW1* to a region downstream of SB00080. G9 had larger

Table 3 List of homologous genes of rice and maize grain size controlling genes in sorghum

Gene[a]	Homologous gene[b]	Functional annotation	Score	E-value	Sorghum Chromosome	Gene location	QTL detected in this study	Flanking markers location
GW8 (Wang et al. 2012)	Sobic.007G193500	Similar to Teosinte glume architecture 1 with SBP domain	297	3.70E−78	7	61,367,404	*qGW7*	61,119,144
						61,373,890		64,095,697
	Sobic.002G257900	Similar to Squamosa promoter-binding-like protein 18	252	1.40E−64	2	64,322,405	*qGW2*	64,095,678
						64,327,430		76,698,007
	Sobic.003G406600	Similar to Squamosa promoter-binding-like protein 2	167.2	4.50E−39	3	71,439,874	*qGW3.2*	67,805,692
						71,445,400		72,338,485
qGL3 (Zhang et al. 2012a, b)	Sobic.001G154900	Similar to Serine/thre-onine-protein phosp-hatase BSL2 homolog	380	8.80E−103	1	12,408,752	*qGW1*	1,000,898
						12,418,490		9,666,380
	Sobic.008G173900	Similar to Serine/thre-onine-protein phosp-hatase BSL2 homolog	318.7	2.50E−84	8	53,502,911	*qGW8*	50,413,982
						53,512,540		50,905,431
GS3 (Fan et al. 2006)	Sobic.001G341700	Similar to Grain length and weight protein	160	3.40E−37	1	55,720,607	*qGW1*	1,000,898
						55,726,010		9,666,380
GIF1 (Wang et al. 2008)	Sobic.004G166700	Similar to Cell wall invertase	767.7	0	4	50,935,604	–	–
						50,940,480		
	Sobic.001G099700	Similar to Beta-fructofuranosidase, insoluble isoenzyme 3 precursor	742.5	0	1	7,615,348	*qGW1*	1,000,898
						7,617,621		9,666,380
GW2 (Song et al. 2007)	Sobic.004G107300	Similar to RING-type E3 ubiquitin ligase	533.3	2.70E−149	4	10,287,591	–	–
						10,293,640		
TGW6 (Ishimaru et al. 2013)	Sobic.004G330200	Similar to Putative uncharacterized protein	1531	3.50E−107	4	65,698,515	–	–
						65,699,923		
GS5 (Li et al. 2011)	Sobic.009G053600	Similar to Putative serine carboxypep-tidase II	188.8	1.50E−45	9	5,449,707	–	–
						5,455,584		
qSW5/GW5 (Shomura et al. 2008; Weng et al. 2008)	Sobic.009G070000	IQ calmodulin-binding motif family protein, expressed	939	6.00E−63	9	8,208,073	–	–
						8,210,596		
SRS1/DEP2 (Abe et al. 2010)	Sobic.002G374400	Similar to COP1-interacting protein 7 (CIP7)-like	1222.2	0	2	73,190,883	*qGW2*	64,095,678
						73,199,290		76,698,007
SRS3 (Kitagawa et al. 2010)	Sobic.009G049400	Similar to Central motor kinesin 1	531.5	2.40E−148	9	4,952,041	–	–
						4,959,069		
SRS5 (Segami et al. 2012)	Sobic.001G107200	Similar to Tubulin alpha-2/alpha-4 chain	861.5	0	1	8,262,981	*qGW1*	1,000,898
						8,266,116		9,666,380
SG1 (Nakagawa et al. 2012)	Sobic.002G226500	Similar to Putative uncharacterized protein	462.9	1.40E−128	2	61,817,843	*qGW2*	64,095,678
						61,819,960		76,698,007
Mn1 (Cheng et al. 1996)	Sobic.001G099700	Similar to Beta-fructofuranosidase, insoluble isoenzyme 3 precursor	686.6	0	1	7,615,348	*qGW1*	1,000,898
						7,617,621		9,666,380

[a] The cloned rice and maize grain size controlling genes

[b] The founding of homologous genes in sorghum through blasting the CDS nucleotide sequence of the cloned genes with *Sorghum bicolor v2.1* genome database at http://phytozome.jgi.doe.gov

100-grain weight than CK1 and CK2 and delimited *qGW1* to a region upstream of Xtxp071. G12 had larger 100-grain weight than CK2 and CK3 and delimited *qGW1* to a region upstream of SB00193. Using this method, we concluded that G1 to G6 placed *qGW1* in a region downstream of SB00080, and G7 to G13 placed *qGW1* in a region upstream of SAMIV2_23738. As a result, *qGW1* was narrowed down to a 2693-kb region between SB00080 and SAMIV2_23738 (Fig. 4b).

Seven SSR markers (three publicly available and four newly developed; Table S1) were used to further screen 15 recombinants containing recombination points in this region. These recombinants fell into eight groups (Fig. 4c). G2 and G3 delimited *qGW1* to a region downstream of HLJ-10. The other six groups delimited *qGW1* to a region upstream of HLJ-18. Therefore, *qGW1* was delimited to a 101-kb region between HLJ-10 and HLJ-18 on the short arm of chromosome 1 (Fig. 4c).

Putative genes in the 101-kb region of interest

According to the sorghum genome database (http://phytozome.jgi.doe.gov), 13 predicted genes are located in this region in the BTx623 genome (Table 3). Among them, five genes encode proteins annotated as "putative uncharacterized protein" or "expressed protein of unknown function". Sobic.001G038500 encodes a transmembrane amino acid transporter. The protein encoded by Sobic.001G038700 is similar to LOC_Os03g60250 protein, which is a putative membrane-associated DUF588 domain-containing protein. Sobic.001G038800 encodes a protein weakly similar to SCAR-like protein 1. Both Sobic.001G038900 and Sobic.001G039000 encode Tubby C2 domain-containing proteins. Sobic.001G039100 encodes a putative 2OG-Fe oxygenase family protein. Sobic.001G039300 encodes a MSS1/TRME-ralated GTP-binding protein. Sobic.001G039400 encodes 40S ribosomal protein S26.

Based on the re-sequencing data of 44 wild, landraces and improved sorghum (Mace et al. 2013), only one gene, Sobic.001G038900, in our 101-kb region was found to be under purifying selection. This gene was sequenced across all four parental lines. Two synonymous SNPs in the 2nd exon and one 5 bp-deletion and one SNP in the 2nd intron were identified between the larger grain parent SA2313 in contrast to the small grain parents Hiro-1 and K-385. Hiro-1 and Atx623 also has four deletions, one insertion, and three SNPs in the 3′ UTR region (Fig. S3).

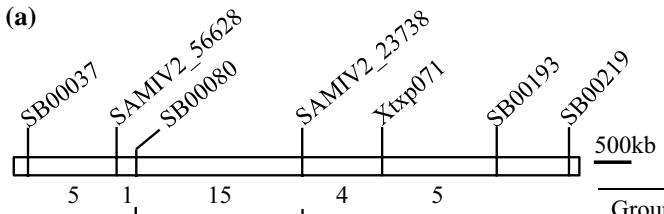
Discussion

In the present study, we detected seven QTLs for 100-grain weight in three F_2 populations. A major QTL, *qGW1*, was consistently detected in all F_2 populations; it had the largest LOD scores and highest explained phenotypic variation. According to the physical genome coordinates, *qGW1* colocates with a meta-QTL *QKWT_meta1.1* for kernel weight (between 2 and 7 Mb on SBI-01) detected by Mace and Jordan (2011) on the basis of the data from previous studies (Tuinstra et al. 1997; Rami et al. 1998; Murray et al. 2008). It is unlikely that *qGW1* is identical to the QTL for seed weight *QSwe-sbi01* near the SSR marker Xcup24 on chromosome 1, which explained 14.8 % of the phenotypic variation (Srinivas et al. 2009), because *QSwe-sbi01* is located approximately 4 Mbp from the *qGW1* flanking marker SB00219.

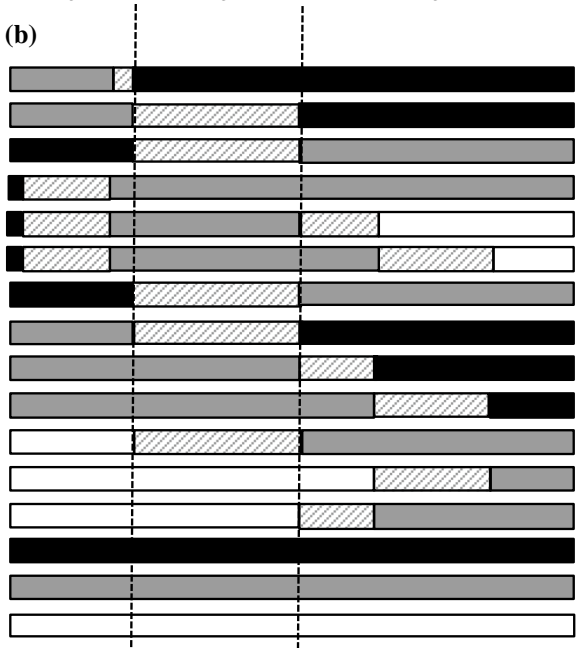
The chromosomal location of *qGW8*, which explained 12 % of phenotypic variation, co-located with a kernel weight QTL detected by Brown et al. (2006) (between 8 and 49 Mb on SBI-08). This QTL was also co-located with a kernel weight QTL detected by Sakhi et al. (2013) using an association mapping approach. Notably, Sobic.008G173900, a homolog of rice *qGL3* (Zhang et al. 2012b), was found within 3 Mb of *qGW8*.

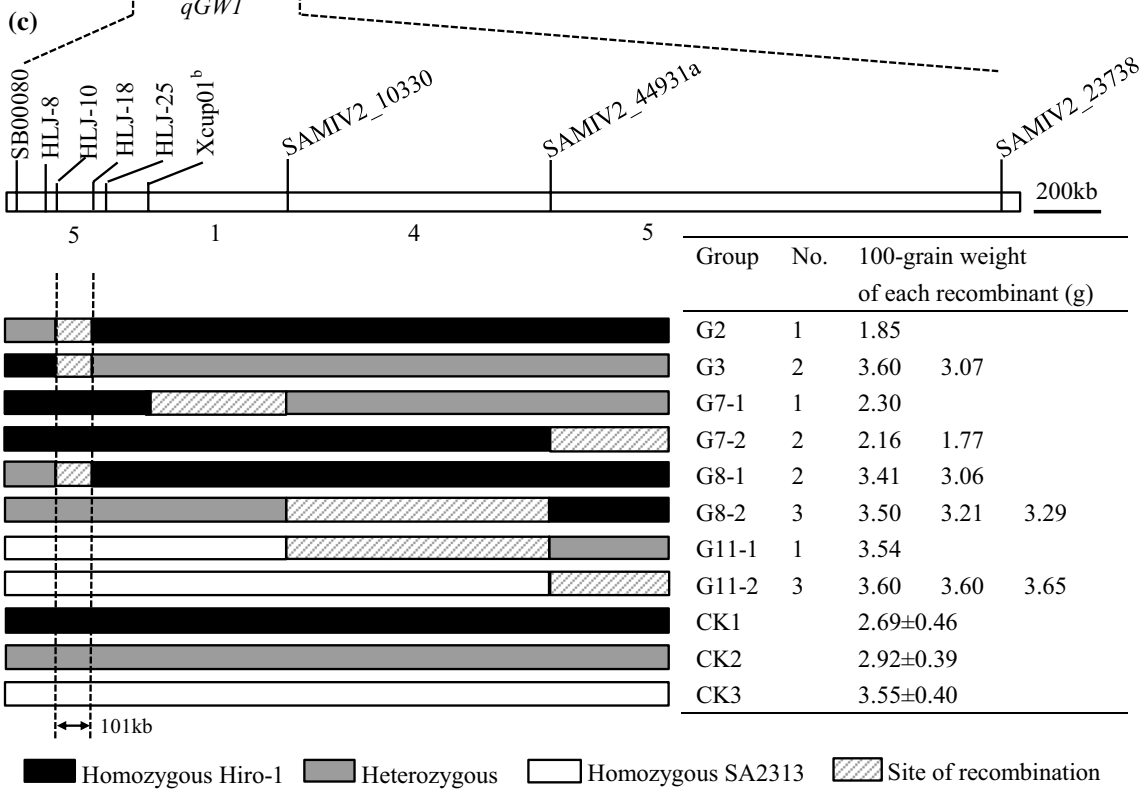
The "selective genotyping" approach reduces the time and cost of genotyping by selecting and genotyping only individuals from the high and low tails of the population distribution (Hillel et al. 1990; Dunnington et al. 1992; Plotsky et al. 1993; Tanksley 1993; Darvasi 1997). In this study, we used this approach with the 2013 trial data of the SA2313xHiro-1 population and detected two major QTLs (on chromosomes 1 and 2; the same QTLs as detected in the 2012 trial) and four new QTLs on chromosomes 3, 5, and 8 (detected only in the 2013 trial), all of which co-locate with QTL identified previously in the literature. The two QTL on chromosome 3 were found to co-locate with a QTL for grain weight per panicle detected by Shehzad and Okuno (2015) (*qGW3.1*) and a seed mass QTL detected by Paterson et al. (1995) (*qGW3.2*). *qGW5* was also found to co-locate with 100 grain weight QTL detected by Shehzad and Okuno (2015). As indicated by Charcosset and Gallais (1996), reducing the size of a QTL mapping population decreases the detection power and increases the QTL confidence interval and the risk of detecting false QTLs; therefore, the QTLs newly detected in the 2013 trial should be confirmed; however, their co-location with previously identified QTL provides further evidence for the association of these genomic regions with genes controlling grain size.

Because SA2313 is sensitive to photoperiod, only approximately 1/10 of its progeny in the trial planted in Beijing in 2012 headed and matured, in contrast to all plants maturing in the 2013 Sanya trial. No apparent differences in grain weight were observed between these two populations. No major genes for heading date have been found on chromosomes 3, 5, and 8 (Mullet et al. 2012) where the QTLs detected only with the 2013 trial data



Group	No.	100-grain weight of each recombinant (g)				
G1	1	1.89				
G2	1	1.85				
G3	2	3.60	3.07			
G4	2	3.23	3.34			
G5	2	3.37	3.36			
G6	1	3.48				
G7	3	2.30	2.16	1.77		
G8	5	3.41	3.50	3.21	3.29	3.06
G9	1	3.38				
G10	2	3.38	3.19			
G11	4	3.60	3.60	3.65	3.54	
G12	2	3.71	3.60			
G13	1	3.96				
CK1		2.69±0.46				
CK2		2.92±0.39				
CK3		3.55±0.40				





Group	No.	100-grain weight of each recombinant (g)			
G2	1	1.85			
G3	2	3.60	3.07		
G7-1	1	2.30			
G7-2	2	2.16	1.77		
G8-1	2	3.41	3.06		
G8-2	3	3.50	3.21	3.29	
G11-1	1	3.54			
G11-2	3	3.60	3.60	3.65	
CK1		2.69±0.46			
CK2		2.92±0.39			
CK3		3.55±0.40			

◀ **Fig. 4** Fine mapping of *qGW1* using a segregating F_3 population ($n = 407$ plants). **a** A high-resolution physical map of the *qGW1* region on chromosome 1. The numbers of recombinants between adjacent markers are indicated below the map. **b** The *qGW1* locus was narrowed down to SB00080–SAMIV2_23738 by analyzing the genotypes and phenotypes of the 27 recombinants. **c** The *qGW1* locus was delimited to a 101-kb interval between markers HLJ-10 and HLJ-18 using the developed polymorphic molecular markers and 15 recombinants. In the tables on the right, *Group* genotype category, *No.* the number of recombinants in each group, *CK1* homozygous for Hiro-1, *CK2* heterozygous, *CK3* homozygous for SA2313. Mean values ± SD ($n = 20$ randomly chosen plants) are shown for each CK

of the SA2313xHiro-1 population were located. However, although no major effect maturity genes have been reported to co-locate with these QTL, Mace and Jordan (2011) reported a meta-QTL for maturity *QDTFL_meta1.8* (between 7 amd 49 Mb on SBI-08), on the basis of the results of Srinivas et al. (2009) and Shiringani et al. (2010), in the *qGW8* region. The interaction between grain weight and heading date should, therefore, be studied in more detail.

Several studies have reported that the rice grain weight gene *GW2* (Song et al. 2007) may also have a considerable effect in other cereal crops. Li et al. (2010) isolated two maize homologs, *ZmGW2-CHR4* and *ZmGW2-CHR5*, and showed that they control some of the phenotypic variation for kernel size and weight; and in fact *ZmGW2-CHR4* is located within a consistent QTL for 100-kernel weight in maize (Li et al. 2010). Bednarek et al. (2012) have identified wheat *TaGW2* homologs in the A, B, and D genomes; and reported that *TaGW2-A* polymorphism affected grain width. In the present study, we found that Sobic.004G107300 is homologous to rice *GW2*; however, although no QTL was detected in the region close to Sobic.004G107300 on chromosome 4 in our three F_2 populations, this gene does co-locate with a QTL for 100-grain weight previously described by Shehzad and Okuno (2015).

In conclusion, we detected seven QTLs for 100-grain weight in three F_2 populations. *qGW1* was consistently detected in all F_2 populations and had higher LOD scores and explained phenotypic variation more than the other QTLs. We delimited *qGW1* to a 101-kb region harboring 13 putative genes on the short arm of chromosome 1. Our data provide useful information for understanding the molecular basis of grain weight in sorghum. The SSR markers linked to the *qGW1* locus can be used for improving sorghum grain yield through marker-assisted selection. Fine mapping of the *qGW1* locus creates a basis for cloning this grain weight–related gene and understanding its regulation.

Table 4 List of putative genes in the 101-kb target region from the sorghum genome database

Gene[a]/marker name	Location (bp)	Gene length (bp)	Description and functional annotation	v1.4 ID
HLJ-10	2,836,850–2,836,869			
Sobic.001G038200	2,833,794–2,838,571	4778	Putative uncharacterized protein	Sb01g003510
Sobic.001G038300	2,845,765–2,849,398	3634	Expressed protein	Sb01g003520
Sobic.001G038400	2,852,985–2,855,486	2502	Putative uncharacterized protein	Sb01g003530
Sobic.001G038500	2,877,213–2,879,897	2685	Transmembrane amino acid transporter protein	Sb01g003540
Sobic.001G038600	2,886,020–2,887,193	1174	Putative uncharacterized protein	Sb01g003550
Sobic.001G038700	2,893,379–2,895,150	1772	Similar to LOC_Os03g60250 protein	Sb01g003560
			Membrane associated DUF588 domain containing protein, putative, expressed	
Sobic.001G038800	2,894,377–2,904,726	10350	Weakly similar to SCAR-like protein1	Sb01g003570
Sobic.001G038900	2,908,192–2,909,750	1559	Expressed protein	Sb01g003580
			Tubby C 2	
Sobic.001G039000	2,910,974–2,912,168	1195	Similar to LOC_Os03g60210 protein	Sb01g003590
			Tubby C2	
Sobic.001G039100	2,911,960–2,914,798	2839	Oxidoreductase, 2OG-Fe oxygenase family protein, putative, expressed	Sb01g003600
Sobic.001G039200	2,918,041–2,918,466	426	Putative uncharacterized protein	Sb01g003610
Sobic.001G039300	2,927,176–2,931,986	4811	MSS1/TRME-related GTP-binding protein	Sb01g003620
Sobic.001G039400	2,932,773–2,935,443	2671	40S ribosomal protein S26	Sb01g003630
HLJ-18	2,937,611–2,937,630			

[a] The information and functional description of these genes were downloaded from http://phytozome.jgi.doe.gov

Author contribution statement CH designed the experiments; HL, CJ, LY, ESM, ZM, YN and DRJ performed the experiments; HL, CJ, MES and CH analyzed the data; and HL, CJ, ESM and CH wrote the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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