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ORIGINAL PAPER

Mapping of quantitative trait loci and development of allele-specific markers for seed weight in *Brassica napus*

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Abstract Seed weight is an important component of grain yield in oilseed rape (*Brassica napus* L.), but the genetic basis for the important quantitative trait is still not clear. In order to identify the genes for seed weight in oilseed rape, QTL mapping for thousand seed weight (TSW) was conducted with a doubled haploid (DH) population and an F_2 population. A complete linkage map of the DH population was constructed using 297 simple sequence repeat (SSR) markers. Among nine TSW QTLs detected, two major QTLs, *TSWA7a* and *TSWA7b*, were stably identified across years and collectively explained 27.6–37.9% of the trait variation in the DH population. No significant epistatic interactions for TSW detected in the DH population indicate that the seed weight variation may be primarily attributed to additive effects. The stability and significance of *TSWA7a* and *TSWA7b* were further validated in the F_2 population with different genetic backgrounds. By cloning *BnMINI3a* and *BnTTG2a*, two *B. napus* homologous genes to *Arabidopsis thaliana*, allele-specific markers were developed for *TSWA5b* and *TSWA5c,* two TSW QTLs on A5, respectively. The importance of the major and minor QTLs identified was further demonstrated by analysis of the allelic effects on TSW in the DH population.

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Introduction

Oilseed rape (*Brassica napus*) is one of the most important oil crops worldwide. The seed size and/or weight of oilseed rape has been considered as one of the most important trait, because the seed is not only the productive organ for life cycle but also the storage of oil and proteins, the predominant products of the crop. First, seed weight is one of the three direct components (silique per plant, seeds per silique and seed weight) of plant grain yield. It is positively correlated with plant productivity (Clarke and Simpson 1978; Butruille et al. 1999; Shi et al. 2009). Second, seed size may also be correlated with oil content and protein content (Morgan et al. 1998; Lionneton et al. 2004; Adamskia et al. 2009). Lastly, large seeds normally have better adaptability during germination, and seedlings from large seeds may be superior over ones from small seeds in competitive survival rates (Geritz et al. 1999; Adamskia et al. 2009). Therefore, understanding of the genetic bases of seed size and/or weigh formation is of great interest in the improvement of grain yield and quality in oilseed rape.

In spite of the importance of seed size and/or weigh of oilseed rape, there were few genetic studies in *B. napus*. Quantitative genetic analysis showed that seed weight has relatively high heritability compared with other seed yield-component traits (Liu and Liu 1987; Qi et al. 2004; Shi et al. 2009). With the development of molecular marker techniques, few studies on QTL mapping for seed weight have been carried out in *B. napus*. Quijada et al. (2006) detected three QTLs (located on N7, N17 and N19) for

seed weight at four populations evaluated at two locations for 2 years. No common QTLs could be detected across populations. Udall et al. (2006) identified 6, 4 and 5 QTLs of seed weight at Hua Doubled Haploid (DH) population, SYN DH and testcross population, respectively, and found only one QTL located on N14 with stable effect across populations and environments. Recently, Shi et al. (2009) identified 159 QTLs for seed weight in *B. napus* based on the analysis of two (TNDH and RC-F_2) populations in ten natural environments. However, only four major QTLs were detected and only one, qSW.A7-2, detected in ten environments.

In model plant *Arabidopsis*, progress has been made to identify the molecular regulators of seed size with mutations and misexpression experiments in the past decade. Alonso-Blanco et al. (1999) mapped 11 QTLs relevant to seed size, first showing the genetic complexity of the trait in the species. More recently, the molecular mechanism of seed size determination has been studied through mutant analyses. For example, mutations in *TTG2* (*Transparent Testa Glabrous* 2) gene, which affect flavonoid pigmentation in the seed coat, usually reduce seed weight (Johnson et al. 2002; Garcia et al. 2005). Larger seeds were observed in the mutants of floral homeotic gene *AP2* (*APETELA2*) or *ARF2* (*Auxin Response Factor* 2) (Jofuku et al. 2005; Ohto et al. 2005; Schruff et al. 2005). Luo et al. (2005) identified two small seed mutants, viz. *IKU2* (*HAIKU2*) and *MINI3* (*MINISEED3*), and proposed, for the first time, a framework to assemble a genetic pathway for seed size control. Considering the close relationships between *B. napus* and *Arabidopsis*, large numbers of homologues of the seed size regulators would be expected available in oilseed rape.

Over the past several years, genetic maps in *B. napus* have been constructed with various types of molecular markers (e.g. Piquemal et al. 2005; Qiu et al. 2006; Sun et al. 2007; Westermeier et al. 2009). However, integration of the maps remains a challenge because of the insufficient common marker information. Great efforts have been made to develop simple sequence repeat (SSR) markers in *Brassica* research community due to its transferable nature and the fact that it is easy to handle (Plieske and Struss 2001; Suwabe et al. 2002; Lowe et al. 2004; Iniguez-Luy et al. 2008; Cheng et al. 2009). Although publicly available SSR markers have been increased steadily, there is still few QTL mapping studies containing enough SSR markers to allow a transverse comparison between populations so far.

With the long-term goal of understanding the genetic basis of seed weight control, the present study was focused on identification of major QTLs for seed weight in oilseed rape. A SSR-based linkage map was constructed to facilitate the comparison among this type of study. A candidate gene cloning approach was applied to develop allele-specific markers in order to take advantage of the wealthy sequence information in *Arabidopsis* and *Brassica* databases. The comprehensive results presented here provide valuable information for future marker-assisted selection (MAS) of seed weight breeding and map-based cloning of the candidate genes in *B. napus*.

Materials and methods

Plant materials

Two segregation populations were used for mapping and trait analyses in this study. The first one is a DH population of 238 individual DH lines, which were produced from microspore culture of F_1 buds of the cross between SW Hickory, a spring-type *B. napus* variety and a kind gift from SvalÖf Weibull AB, Sweden, and JA177, a winter-type *B. napus* pure line. A random subset of 190 DH lines was sampled for whole genome linkage map construction and for mapping QTL of seed weight. The second one is an F_2 population including 190 individuals derived from the cross between winter-type *B. napus* pure lines J7046 and J7005 and it was used for mapping of the major QTLs detected in the first population. Henceforward the first population will be referred as SJ DH population and the second F_2 population.

Field trails and trait evaluation

The DH lines together with their parental lines and F_1 hybrid were grown in two consecutive years in 2007–2008 and 2008–2009. The field experiment followed a randomized complete block design with three replications. Each line was planted in two rows and 11–12 plants in each row, with a distance of 21 cm between plants within each row and 30 cm between rows.

The F_2 population, together with its two parental lines and F_1 hybrid, was grown in the 2006–2007 season. The field planting was arranged as a complete random design with total 20 plots including two plots for each parent and the F_1 hybrids, respectively, and 16 plots for F_2 plants. Each plot consisted of three rows and 11–12 plants in each row were finally grown at a distance of 21 cm between plants within each row and 30 cm between rows. In total, there were about 530 F_2 plants and 70 for each parental line and F_1 hybrids, respectively. One hundred and ninety F_2 plants were randomly sampled from the F_2 population for trait evaluation and genotyping.

All materials were grown in winter-type oilseed rape growing season on the experimental farm of Huazhong Agriculture University, Wuhan, China. The field management followed essentially regular breeding practice.

Matured seeds were threshed by hand from open-pollinated plants. The cleaned seeds were air-dried for at least

4 weeks. Seed weight of each plant was measured based on 500 fully developed seeds with three replications. The average seed weight was then converted to 1,000-seed weight (TSW) for easy comparison with other studies. The means of TSW of 10–15 plants from each plot were used for trait evaluation of parents, F_1 and SJ DH lines.

Molecular marker and linkage map

Primer sequences for SSR markers were obtained from various resources including http://www.ukcrop.net/perl/ace/search/BrassicaDB (Lowe et al. 2004), http://www.brassica.info/ssr/SSRinfo.htm (prefixed by Ra, Ol, Na, BN, BRMS and MR), and http://www.rapedata.cn/marker/ (prefixed by BrGMS). In addition, primer sequences prefixed by BRAS and CB were obtained from electronic supplementary material of Piquemal et al. (2005), prefixed by "s" from Qiu et al. (2006), prefixed by FITO from electronic supplementary material of Iniguez-Luy et al. (2008), and prefixed by BnGMS from the electronic supplementary material of Cheng et al. (2009), respectively. Primer pairs prefixed by BoGMS and BnEMS were developed from *B. oleracea* genome sequences and *B. napus* EST sequences, respectively (see supplementary material for the primer sequences).

All primers were synthesized by GeneRay Biotech (Shanghai, China) and subjected to polymorphism screening between SW Hickory and JA177. The polymorphic primers were used for genotyping of the SJ DH lines. The protocol for the analysis of SSR markers was described by Cheng et al. (2009). When a primer pair generated more than one polymorphic locus, an alphabetic letter was given behind the primer code to distinguish different loci. For example, BnEMS178 has two genetic loci in the SJ DH population that were named BnEMS178A and BnEMS178B, respectively. The χ^2 test was used to assess goodness-of-fit to the expected segregation ratio for each marker.

Linkage analysis with all markers was performed using MAPMAKER 3.0 (Lincoln et al. 1992). A minimum log likelihood of the odds (LOD) score of 9.0 and a maximum distance of 30 cM were used to group loci into linkage groups (LG). The order within each LG was determined by the commands of *order*, *try*, and *ripple*. LG assignment was based on common marker loci from *B. napus* mapping populations as described by Parkin et al. (1995), Lowe et al. (2004), Piquemal et al. (2005), Qiu et al. (2006) and Cheng et al. (2009). Genetic distances between loci were calculated using the Kosambi mapping function (Kosambi 1944).

QTL mapping and statistical analysis

QTLs were detected using the composite interval mapping (CIM) with the Windows version of QTL Cartographer V2.0 (Wang et al. 2004). A forward–backward stepwise regression was performed to choose co-factors before QTL detection. A window size of 10 cM around the test interval, where the co-factors were not considered, was chosen with $P_{in} = 0.05$ and $P_{out} = 0.05$ (model 6 of QTL Cartographer). Default LOD threshold values of 2.0 was used to declare the presence of a QTL. QTL confidence intervals were determined by 1-LOD intervals surrounding the QTL peak. The QTLs within overlapped confidence intervals between environments and populations were assumed to be the same ones.

Epistatic interactions among loci in SJ DH population were estimated using QTLNetwork 2 (Yang et al. 2007). The 2D genome scans were conducted with $P < 0.05$ significance threshold based on 1,000 permutations.

The heritability (h^2) of TSW in SJ DH population was calculated as: $h^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{gl}/n + \sigma^2_e/nr)$, where σ^2_g is genotypic variance, σ^2_{gl} variance due to genotype by environment interaction, σ^2_e error variance; n number of environments, and r number of replications. The estimates of σ^2_g, σ^2_{gl} and σ^2_e were obtained from an analysis of variance (ANOVA) with environment considered as a random effect. The heritability (h^2) of TSW in F_2 population was calculated as: $h^2 = (V_{F2} - 1/2(V_{P1} + V_{P2}))/V_{F2}$, where V_{F2}, V_{P1} and V_{P2} were phenotypic variance of F_2, P_1 and P_2, respectively.

Development of allele-specific markers for seed weight

A candidate gene cloning strategy was applied to isolate the homologous genes of *Arabidopsis thaliana* from parental lines of the SJ DH population. The genomic fragments corresponding to the coding sequence (CDS) of *TTG2* and *MINI3*, respectively, were amplified by the following primer pairs:

TTG2F	5′-ATGGATGTGAAAGAGAGTGAAAGAA-3′
TTG2R	5′-TTAAATGGCTTGATTAGAATGTTGTG-3′
MINI3F	5′-ATGAATGCTTTTGATGGAACCTAC-3′
MINI3R	5′-CTAAAGGTTGAGACCAAAGTTGAGA-3′

The PCR products were cloned into pMD18-T vector (Takara Corporation, Japan) according to the manufacturer's instructions. The M13F and M13R universal primers and the BigDye Terminator Cycle Sequencing v3.1 (Applied Biosystems, Foster City, CA, USA) were used for sequencing. Sequences were aligned using the computer program SEQUENCHER 4.1.2 (Gene Codes Corporation, Ann Arbor, MI, USA). Allele-specific markers were designed according to the nucleotide variations between parental lines and used for the genotyping of the SJ DH lines and linkage analysis.

Prediction and alignment of putative proteins

Total RNA was extracted from leaves of *B. napus* using TRIZOL (Invitrogen, Paisley, UK) and was converted into first-strand cDNA following the manufacturer's instruction (TIANScript RT Kit, Beijing, China). The *TTG2* and *MINI3* cDNAs were amplified from the first-strand cDNA and then cloned into pMD18-T vector for sequencing. The sequences of cDNA and genomic DNA of *Brassica* in public database were aligned to predict putative proteins of TTG2 and MINI3, using the web-based software, InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/) and Clustalw2 (http://www.ebi.ac.uk/Tools/clustalw2/).

Results

Construction of the linkage map

A total of 297 molecular markers, corresponding to 327 SSR loci, were mapped onto 19 LGs in the SJ DH mapping population, covering 2,011.1 cM according to the Kosambi function (Fig. 1). The 19 LGs were aligned to the public linkage maps by shared SSR markers, where LGs A1–A10 represent the ten chromosomes in A genome (*B. rapa*) and C1–C9 the nine in C genome (*B. oleracea*) of *B. napus*, respectively (http://www.brassica.info/resource/maps/lg-assignments.php). One hundred and one SSR loci (30.9%) showed distorted segregation ration ($P < 0.01$) in the DH population. Among them, 36 loci with distorted segregation skewed towards the male parent SW Hickory and the rest towards the female parent JA177. This was consistent with previous reports in other DH populations of *B. napus* (Ferreira et al. 1994; Uzunova et al. 1995; Cheung et al. 1997; Chen et al. 2007). Loci with distorted segregation tended to cluster on LGs A4, C2, C3, C5 and C9.

In this study, 135 polymorphic SSR markers including 91 primer pairs prefixed by BoGMS and 44 by BnEMS were developed (see supplementary material) and mapped onto the linkage maps developed from the SJ DH population. The new SSR markers were assigned to 143 polymorphic loci and distributed on all LGs (Fig. 1). The distribution of these SSR loci on the linkage map showed some relationship with their origin. BoGMS type loci developed from *B. oleracea* tended to be more commonly distributed in the C genome (i.e. LGs C1 to C9; 57 out of 96, 59.4%), while BnEMS type loci developed from *B. napus* tended to be evenly distributed in the A and C genome components (i.e. 24 loci on LGs A1 to A10 and 23 loci on C1 to C9).

Among 162 publicly available SSR markers used in this study, 86 have been previously mapped on published linkage maps (Suwabe et al. 2002; Lowe et al. 2004; Piquemal et al. 2005; Qiu et al. 2006; Cheng et al. 2009). Current mapping results were compared with published linkage maps to identify common markers located on a same LG among different mapping experiments. A marker appeared in a same LG in more than two experiments (including the present one) was regarded as a common marker. Most of the mapped markers (70 out of 86, 81.4%) could be located on the same LG in this study, showing good transferability of SSR markers. The other SSR markers used in this study (including newly developed ones) were mapped on a linkage map for the first time and distributed on all LGs.

Fig. 1 The genetic linkage (LG) map and QTLs for seed weight ▶ identified in SJ DH population in 2007 and 2008. The *bar* to the *left* of the LG denotes the confidence interval for the QTL and the *triangle* the QTL peak position

Phenotypic analysis of TSW

Significant differences between the parents were detected based on *t* test for seed weight in both populations (Table 1), in which SW Hickory (or J7046) exhibited heavier seed weight than JA177 (or J7005). Continuous distributions and transgressive segregations in SJ DH and F_2 populations suggested a quantitative inherence pattern for TSW (Table 1; Fig. 2). The high heritability of TSW was observed in both populations, which was consistent with previous studies (Udall et al. 2006; Shi et al. 2009).

QTL mapping and epistasis effects analysis of TSW in the SJ DH population

CIM was used to detect QTL for TSW in SJ DH population. A total of nine QTLs were identified for TSW on six LGs (A1, A2, A5, A7, A10 and C4) in 2007 and 2008, which explained 3.7–20.8% of the phenotypic variation individually (Table 2; Fig. 1). Notably, two of these, *TSWA7a* and *TSWA7b* on LG A7, were identified in both years and showed the largest effects and collectively explained 27.6–37.9% of the total seed weight variation. The QTL *TSWA7a* located on the marker interval BoGMS715-BnEMS858 accounted for 17.1% of the trait variation in 2007 and around 18% in 2008. The peak of the QTL was shifted slightly in 2 years but had overlapping confidence intervals. In addition, the SW Hickory allele at this locus increased TSW by 0.14–0.17 g in both years (Table 2). The QTL *TSWA7b* also had a sizable effect and could explain from 20.8% of the variation in 2007 and 9.9% of the variation in 2008, with the allele from SW Hickory increasing TSW by 0.12–0.15 g. The peak of *TSWA7b* was stably located at 101.1 cM in 2 years (Table 2). The remaining seven QTLs were detected in only 1 year. The effects of

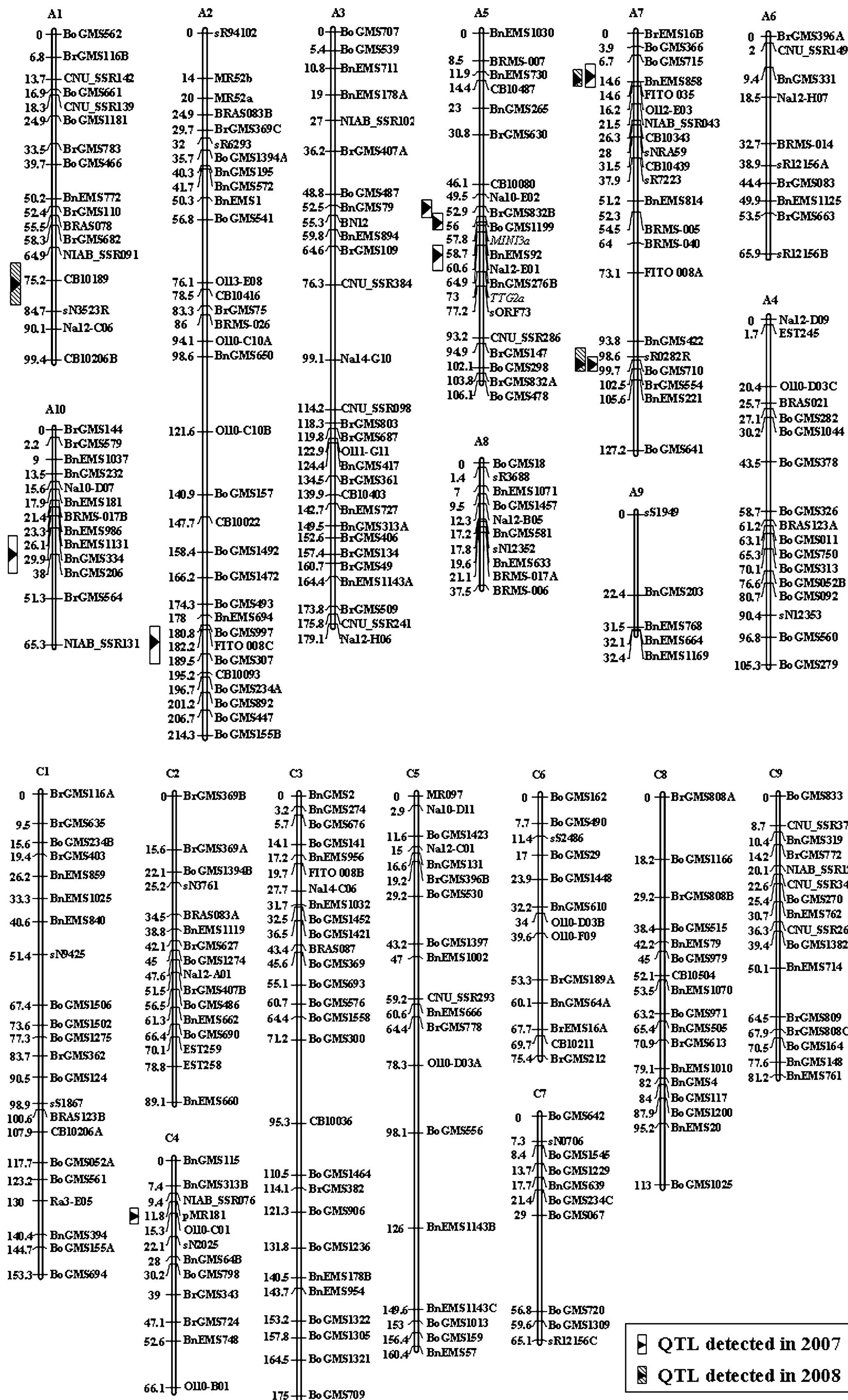
A1
A2
A3
A5
A7
A6
A4
A10
A8
A9
C1
C2
C3
C5
C6
C8
C9
C4
C7
QTL detected in 2007
QTL detected in 2008

Table 1 Descriptive statistics of 1,000-seed weight (g) in parents, F_1 and segregating populations

Population	Parents		F_1	Segregating populations		h^2_B (%)
	P_1	P_2		Mean ± SD	Range	
DH (2007)	3.04 ± 0.24 A	2.11 ± 0.14 B	2.54 ± 0.17	2.55 ± 0.33	1.90–3.80	82.9
DH (2008)	2.69 ± 0.18 a	2.38 ± 0.12 b	2.58 ± 0.09	2.74 ± 0.39	1.85–4.12	
F_2 (2006)	4.26 ± 0.25 A	2.68 ± 0.06 B	3.44 ± 0.09	3.03 ± 0.37	2.18–4.35	76.5

Within the same year, different uppercase and lowercase letters after numbers indicate a significant difference at the 0.01 and 0.05 probability level among the two parents based on *t* test, respectively

P_1 female parents (SW Hickory in DH and J7046 in F2 population, respectively), P_2 male parents (JA177 in DH and J7005 in F2 population, respectively)

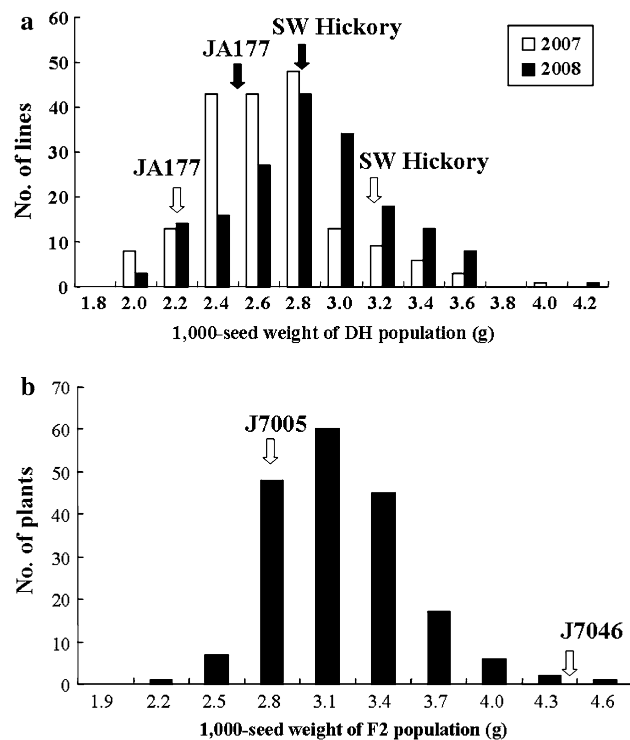

Fig. 2 Distribution of the 1,000-seed weight in SJ DH population derived from the cross of SW Hickory × JA177 (**a**) and F_2 population derived from the cross of J7046 × J7005 (**b**). *Arrows* the means of 1,000-seed weights of the parents corresponding to the progeny populations

these QTLs were relatively small, with their contributions ranging from 3.7 to 8.9% of the phenotypic variation. The alleles of SW Hickory acted positively at *TSWA5a*, *TSWA5b*, *TSWA5c*, *TSWA10* and *TSWC4*, whereas negatively at *TSWA1* and *TSWA2* (Table 2).

Analysis of the epistasis effects on TSW was conducted using the software program QTLNetwork 2. No significant epistatic interactions were detected in the SJ DH population (data not shown), indicating that the seed weight variation in the SJ DH population may primarily be controlled by additive effects.

Validation of the major TSW QTLs on A7 in different populations

In order to confirm the stability of major QTLs for TSW on A7 across different genetic backgrounds, a local genetic map of the F_2 population was constructed and seed weight data collected from the field experiment of 2006–2007 growing season for QTL mapping. Two QTLs corresponding to the locations of *TSWA7a* and *TSWA7b* were, respectively, detected and collectively explained 18.0% of the total seed weight variation. The allele from J7046 was in the direction of increasing seed weight at *TSWA7b* while decreasing at *TSWA7a* (Table 2; Fig. 3).

In a recent study, Shi et al. (2009) mapped several QTLs for seed weight on A7 and one of these shared a same maker sR0282R, corresponding to *TSWA7b* detected in this study (Fig. 3). The fact that the major QTLs for TSW on A7 were detected in different populations with diverse genetic backgrounds points to the conservative nature of the A7 loci for seed weight, thus providing clear targets for future studies.

To develop closer markers for the two major TSW QTLs on A7, sequence databases were searched for homologous region in *B. rapa*. Two *B. rapa* BACs on A7, KBrB084P16 and KBrH001J06, were identified to be located at the vicinity of QTL *TSWA7a* and *TSWA7b*, respectively. BAC-specific SSR markers were then developed for mapping in SJ DH population (Table 3; Fig. 3). Two SSR markers, *I0509* from KBrB084P16 and *J0609* from KBrH001J06, were mapped close to *TSWA7a* and *TSWA7b*, respectively (Fig. 3). *I0509* and *J0609* were located exactly on the *TSWA7a* and *TSWA7b* QTL peaks at 2007, respectively, and they were shifted slightly in relation to the QTL peaks in 2008 (Fig. 3). Rescanning QTLs for TSW by including these two markers (with the same method and parameter settings) resulted in higher LOD scores of *TSWA7a* (from 10.36 to 11.43) and *TSWA7b* (from 10.37 to 11.13) in 2007. Furthermore, the peaks of *TSWA7a* and *TSWA7b* also shifted slightly to the direction of *I0509* and *J0609* loci (Fig. 3).

Table 2 The QTLs for seed weight detected in the SJ DH and F_2 populations

Population	Year	QTL	Interval	Peak	Marker	LOD	A	R^2 (%)[a]
DH	2007	*TSWA2*	FITO008C-BoGMS307	185.8	FITO 008C	2.89	−0.08	6.0
		TSWA5a	BrGMS832B-BoGMS1199	53.1	BrGMS832B	2.74	0.08	5.0
		TSWA5b	BoGMS1199-*MINI3a*	57.8	*MINI3a*	3.88	0.09	7.0
		TSWA5c	BnGMS276-*TTG2a*	68.1	BnGMS276B	3.91	0.09	7.1
		TSWA7a	BoGMS715-BnEMS858	13.0	BnEMS858	10.36	0.14	17.1
		TSWA7b	BoGMS710-BrGMS554	101.1	BrGMS554	10.37	0.15	20.8
		TSWA10	BnGMS334-BnGMS206	37.9	BnGMS206	2.43	0.08	4.6
		TSWC4	Ol10C01- sN2025	15.8	Ol10C01	2.20	0.07	3.7
	2008	*TSWA1*	CB10189-sN3523R	77.2	CB10189	4.73	−0.12	8.9
		TSWA7a	BoGMS715-BnEMS858	14.2	BnEMS858	9.99	0.17	17. 8
		TSWA7b	BoGMS710-BrGMS554	101.1	BrGMS554	5.57	0.12	9.9
F_2	2006	*TSWA7a*	BRMS 036A-FITO 035A	14.2	FITO 035A	4.58	−0.26	12.7
		TSWA7b	Ra2G08-sR0282R	109.9	sR0282R	2.55	0.13	5.4

QTL nomenclature uses the trait name initials followed by the LG number; an alphabetical letter a or b or c is added if more than one QTL are identified in one LG

Interval the smallest marker interval flanking peak position, *Peak* map position (cM) of peak LOD scores, *Marker* the closest marker to the peak, *A* additive effect; positive effects indicate that the allele from female increases the value of the seed weight (g/1,000-seed weight)

[a] Proportion for the phenotypic variation explained by the QTL

Development of allele-specific markers for TSW

In *Arabidopsis*, *TTG2* and *MINI3* were shown to play an important role in the control of seed size and weight (Garcia et al. 2005; Luo et al. 2005). To explore the possibility of utilizing *TTG2* and *MINI3* as allelic markers for TSW, experiments were set up to isolate the homologous sequences of the two genes in *B. napus*. By searching NCBI nucleotide database, two *B. rapa* BAC clones, AC232555 and AC189531, were identified containing sequences highly similar to *Arabidopsis TTG2* and *MINI3*, respectively. Interestingly, the two BAC clones both belong to the A5 chromosome (http://www.brassica.info/resource/sequencing/status.php). Based on the sequence information, genomic fragments corresponding to *TTG2* and *MINI3* were amplified from the parents of the SJ DH population. Sequences of 10–20 clones of each gene from each parent were aligned and then compared with the homologous sequences of *B. rapa* and *Arabidopsis*. It was found that the amplified fragments for those two putative genes from *B. napus* shared high similarities to that of *B. rapa* BAC clones (98–99% similarity) as well as to that of *Arabidopsis* (80% for *TTG2* and 74% for *MINI3*).

To ensure that the cloned genomic DNA fragments indeed represented the candidate gene of TTG2 in *B. napus*, cDNA sequences of TTG2 were cloned from several *B. napus* lines including JA177. Different transcript variants for TTG2 were identified (data not shown). The predicted amino acids from the most abundant sequence among the transcript variants and the genomic DNAs from two parents as well as the *B. rapa* BAC AC232555 shared high similarity with *Arabidopsis* TTG2 (Fig. 4). The predicted TTG2 in *B. napus* and *B. rapa*, named BnTTG2a and BrTTG2, respectively, contain two WRKY domains and nearby conserved residues (Fig. 4), which is the signature structure for TTG2 in *Arabidopsis* (Johnson et al. 2002).

Similar procedure was followed to determine the homologous gene of MINI3 in *B. napus*. Putative MINI3 in *B. rapa* and *B. napus* were predicted by comparing the DNA sequences of *B. rapa* BAC AC189531 and cloned DNA fragments in this study with AtMINI3. The predicted amino acids of *Brassica rapa* and *B. napus* shared very high similarity (99%) and moderate similarity with AtMINI3 (Fig. 5). As in *Arabidopsis*, the putative MINI3 genes in *B. rapa* (named BrMINI3) and *B. napus* (named BnMINI3a) contain a single WRKY domain and nearby conserved residues (Fig. 5).

Taken together, *BnTTG2a* and *BnMINI3a* identified in this study are very likely members of the homologues genes of *Arabidopsis TTG2* and *MINI3* located at the A genome.

To further demonstrate the locations of *BnTTG2a* and *BnMINI3a* in *B. napus* genome, mapping the two genes was attempted with the SJ DH population. By careful examination of the DNA sequences, a SNP marker for *BnTTG2a* and a CAPs marker for *BnMINI3a* were developed (named *TTG2a* and *MINI3a*, respectively) based on nucleotide differences between the parents (Table 3; Fig. 6). The two markers were subsequently mapped on LG A5 in the SJ DH population (Fig. 1). *MINI3a* was

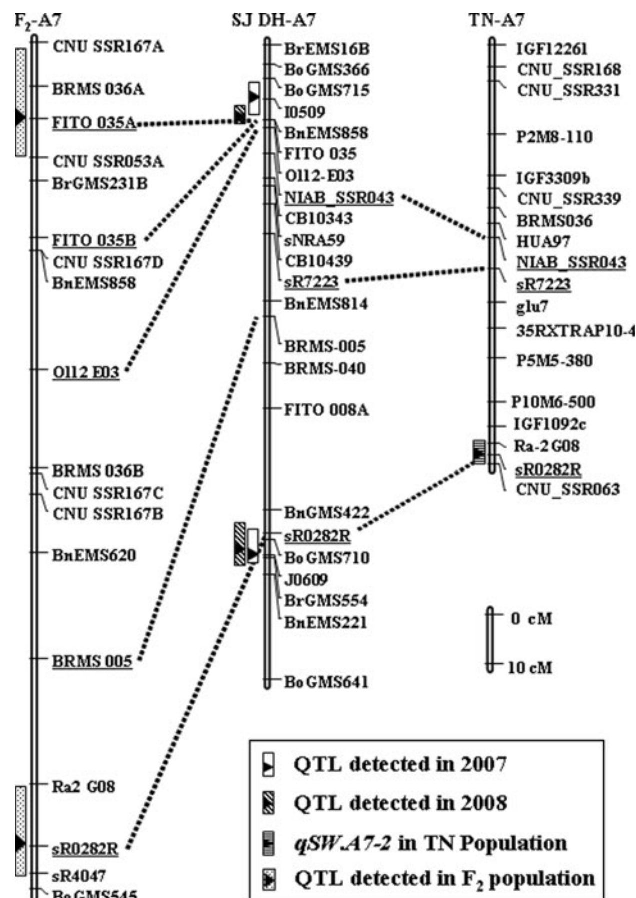

Fig. 3 Comparison of QTLs on LG A7 among different populations. The QTLs in F_2 and SJ DH populations were identified in the study; the QTL *qSW.A7-2* in TN Population was reported by Shi et al. (2009). Alignment of LG A7 from different populations is indicated with common markers (*underline*)

colocalized with QTL *TSWA5b* contributing about 7% of TSW variation (Table 2). The position of *TTG2a* was shifted about 5 cM from the QTL peak of *TSWA5c*, which was adjacent with *TSWA5b* and exhibited a similar additive effect to *TSWA5b* (Table 2). The identification of *TTG2a* and *MINI3a* as allelic-specific markers for TSW will facilitate the further exploration of genetic components for seed weigh control in *Brassica* species.

Combined effects of QTLs on TSW in the SJ DH population

The major effects of *I0509* and *J0609* loci on phenotypic variation were examined first. The lines in the SJ DH population were grouped based on the genotypes at these two particular loci and the mean of TSW was calculated. For *I0509* locus, TSW of the group containing the allele with positive additive effects from SW Hickory (i.e. genotype AA) were significantly higher than that of JA177 (genotype BB) in both years (Table 4). Similar trend was observed for locus *J0609* (Table 4). A clear and stable detection of genetic effects from these loci are consistent with the results that the seed weight is predominantly controlled by additive effect in the SJ DH population (Table 2). The two loci on A7 are likely important determinants for seed weight in *B. napus*.

The combined effects of all the QTLs for seed weight were further examined. The DH lines were grouped according to their genotypes at A7 and A5 QTLs and their seed weights were compared (Table 4). Because the three QTLs on A5 were tightly linked together and few recombination among them were observed in the DH lines (data not shown), the three loci on A5 were regarded as one single unit to simplify the genotypic categorization. Thus, the three loci could result in eight genotypic groups in the SJ DH population (Table 4).

Two conclusions could be drawn from the data in the lower part of Table 4. First, when all three positive additive alleles were present, the seed weights were significantly higher than the groups with only one A7 major locus plus A5 loci regardless the allelic status, clearly showing the importance of two A7 loci. Second, although the QTLs on A5 were only detected in 2007, its effects on seed weight could not be neglected. By looking at the data in 2008, TSW in group I (all three containing positive alleles) was significantly higher than all groups with only one or null A7 locus, while group II (two A7 loci plus null A5 loci) showed similar TSW phenotype with groups III and IV (only one A7 major locus plus A5 loci). It was obvious that the average seed weight was determined by the number of favorable alleles as well as the relative contribution of

Table 3 Primer sequences of the molecular markers developed in the study

Marker name	Marker type	Forward primer sequence	Reverse primer sequence
I0509	SSR	5′-ATCATGATGACTTTTGCAATG-3′	5′-GCTCTTGGTAACATAAAATCG-3′
J0609	SSR	5′-GTTGGTTAAAATCGTGTATGC-3′	5′-CCTACAAAAAGCAATAACGTG-3′
MINI3a	CAPS (*Pst*I)	5′-AGACCATAACAATCACCGAACC-3′	5′-ACACGATCAATCTCTGGTTCATT-3′
TTG2a	SNP	5′-CCGCGGGTGATTCATCTAAG-3′	5′-GGAAGCTAAAAAATAAAGAGTTAAA-3′

CAPS cleaved amplified polymorphic sequence, *SNP* single nucleotide polymorphism

```
BnTTG2a_P2  MDVKESERNVVAKPVASRPSCSS-VRTFTDLLADSVTVSPQSNCHETVDASIIPKTERFKQPASASVSSPR--VEGSGDVKSCDD-SESK 86
BrTTG2      MDVKESERKVVAKPVASRPSCSS-VRTFTDLLADSVTVSPQSNCHETVDASIIPKTERFKQPASASVSSPR--VEGSGDVKSCDD-SESK 86
BnTTG2a_P1  MDVKESERKVVAKPVASRPSCSS-VRTFTDLLADSVTVSPQSNCHETVDASIIPKTERFKQPASASVSSPR--VEGSGDVKSCDD-SESK 86
AtTTG2      MEVNDGERVVIAKPVASRPSSSSGFRTFTELLTDSVTVSPQTTCHEIVDAAIRPKTLRFNQPVAASVSCPRAEVKGIGNGMSCDDDSDSR 90
            *:*::.** *:*********.** .****:**:********:.*** ***:* *** **:**.:****.**  *:* *:  **** *:*:

BnTTG2a_P2  SYVIYKPKAKLVSQATVSALANMA-------WIKREAVAYGKRVSQGTHLAVPNLVPRVPTFKESETSIGDRSYVDGYNWRKYGQKQVKG 169
BrTTG2      SYVIYKPKAKLVSQATVSALANMA-------WIKREAVAYGKRVNQGTHRAVPNLVPRVPTFKESETSTGDRSYVDGYNWRKYGQKQVKG 169
BnTTG2a_P1  SYVIYKPKAKLVSQATVSALANMA-------WIKREAVAYGKRVNQGTHRAVPNLVLRVPTFKESETSTGDRSYVDGYNWRKYGQKQVKG 169
AtTTG2      NYVVYKPKAKLVSKATVSALANMLQGNRQQTWRQSEAVSYGKSVSQGTHRAGPNLVQKVPSFTESETSTGDRSSVDGYNWRKYGQKQVKG 180
            .**:*********:*********        * : ***:*** *.**** * ****  :**:*.***** **** ****************

BnTTG2a_P2  SDSPRGYYKCTHPKCPVKKRVERSSMGGHVSEIVYQGEHNHSKPSCPLPRRASSSSSSGFQTP-----SEESMGQEPN-----PLWSDQ- 248
BrTTG2      SDSPRGYYKCTHPKCPVKKSVERSSMGGHVSEIVYQGEHNHSKPSCPLLRRASSSSSSGFQTP-----SEGSMGEEPN-----PLWSNQ- 248
BnTTG2a_P1  SDSPRGYYKCTHPKCPVKKRVERSSMGGHVSEIVYQGEHNHSKPSCPLPRRASSSSSSGFQTP-----SEGSMGEEPN-----PLGSNQ- 248
AtTTG2      SECPRSYYKCTHPKCPVKKKVER-SVEGQVSEIVYQGEHNHSKPSCPLPRRASSSISSGFQKPPKSIASEGSMGQDPNNNLYSPLWNNQS 269
            *:.**.************* *** *: *:****************** ****** *****.*      ** ***::**     ** .:*

BnTTG2a_P2  --------EKMNEGCVITPFEFAVPRTANSTGGTSDSGCRSSQCDERELDDPSRSKTSMKNETQSSEAGVSQSSGESDSLEDGFKWRKYG 330
BrTTG2      --------EKMNEGCVITPFEFAVPRTANSTGGTSDSGCRSSQCDERELDDPSRSKTSMKNETQSSEAGVSQSSGESDSLEDGFKWRKYG 330
BnTTG2a_P1  --------ERINEGCVIIPFEFAVLRTANSTGGTSDSGCRSGQCDERELDDPSRSKTSMKNEKQSSDGGVSQSSGESDSLEDGFKWRKYG 330
AtTTG2      NDSTQNRTEKMSEGCVITPFEFAVPRSTNSNPGTSDSGCKSSQCDEGELDDPSRSKR-RKNEKQSSEAGVSQGSVESDSLEDGFRWRKYG 358
                    *::.***** ****** *::**. *******:*.**** *********   ***.***:.****.* *********:*****

BnTTG2a_P2  QKAVGGNAYPRSYYRCTSVNCRARKRVERASDDSRAFITTYEGKHNHHHLQLRPPTSSTLSFSSPQHSNQAI 402
BrTTG2      QKAVGGNAYPRSYYRCTSVNCRARKRVERANDDPKAFITTYEGKHNHHHLQLRPPTSSTLPFSSPQHSNQAI 402
BnTTG2a_P1  QKAVGGNAYPRSYYRCTSVNCRARKRVERASDDSRAFITTYVGKHNHHHLLLRPPSSSTLPTTF-------- 394
AtTTG2      QKVVGGNAYPRSYYRCTSANCRARKHVERASDDPRAFITTYEGKHNHH-LLLSPPSSSTLPFNSPQLSKQTI 429
            **.**************.******:****.**.:****** ****** * * **:****. .
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Fig. 4 Alignment of putative protein sequences of TTG2 from *Brassica* and *Arabidopsis*. BrTTG2 is putative protein of *B. rapa*. WRKY domain defined by the conserved amino acid sequence WRKYGQK, together with a C_2H_2 motif, is highlighted in *black boxes*

```
BrMINI3     MNAFDGTYRGVRTCWAAPSSPSPRSLLAMLNQGDNNDVVDQINEIFPQA------NHQPEQRSSLRERVAARVEFNLPPLETQNNR-PFA 83
BnMINI3a_P2 MNAFDGTYRGVRTCWAAPSSPSPRSLLAMLNQGDNNDVVDQINEIFPQA------NHQPEQRSSLRERVAARVEFNLPPLETQNNR-PFA 83
BnMINI3a_P1 MNAFDGTYRGVRTCWAAPSSPSPRSLLAMLNQGDNNDVVDQINEIFPQT------NHQPEQRSSLRERVAARVEFNLPPLETQNNR-PFA 83
AtMINI3     MSDFDENFIEMTSYWAPPSSPSPRTILAMLEQTDNG--LNPISEIFPQESLPRDHTDQSGQRSGLRERLAARVGFNLPTLNTEENMSPLD 88
            *. ** .:   : : **.*******::****:* **.   :: *.*****        ..*. ***.****:**** ****.*:*::*  *:

BrMINI3     AFFRNPSTTVP-SPLVLISPGFSPSAMLQFPNTFIDPSHMILPSPVANGGPPEAVESSGADHATMMISNND----PMHVALP-------- 160
BnMINI3a_P2 AFFRNTSTTVP-SPLVLISPGFSPSAMLQFPNTFIDPSHMILPSPVANGGPPEAVESSGADHATMMISNND----PMHVALP-------- 160
BnMINI3a_P1 AFFRNPSTTVP-SPLVLISPGFSPSAMLQFPNTFIDPSHMILPSPVANGGPPEAVESSGADHATMMISNND----PMHVALP-------- 160
AtMINI3     AFFR--SSNVPNSPVVAISPGFSPSALLHTPNMVSDSSQIIPPSSATNYGPLEMVETSGEDNAAMMMFNNDLPYQPYNVDLPSLEVFDDI 176
            ****  *:.** **:* *********:*: ** . *.*::* **..:* ** * **:** *:*:**: ***    * :* **

BrMINI3     -------------------PQQVAAFESGPALN-ETDLINMEIDR---------------KNEDEEEYKEDEDEEHNIVDELDAE----- 210
BnMINI3a_P2 -------------------PQQVAAFESGPALN-ETDLINMEIDR---------------KNEDEEEYKEDEDEEHNIVDELDAE----- 210
BnMINI3a_P1 -------------------PQQVAAFESGPALN-ETDLINMEIDR---------------KNEDEEEYKEDEDEEHNIVDELDAE----- 210
AtMINI3     ATEESFYIPSYEPHVDPIGTPLVTSFESELVDDAHTDIISIEDSESEDGNKDDDDEDFQYEDEDEDQYDQDQDVDEDEEEEKDEDNVALD 266
                                 .  *::***  . : .**:*.:* ..               ::***::*.:*:* :.:  :* * :

BrMINI3     ---PSSPKRRKFGESTMIGATRSCKSQRVILQMETEENNPDDGFFWRKYGQKVVKGNPNPRSYYKCTYTACDVKKHVERGAEDVKFLLVT 297
BnMINI3a_P2 ---PSSPKRRKFGESTMIGATRSCKSQRVILQMETEENNPDDGFFWRKYGQKVVKGNPNPRSYYKCTYTACDVKKHVERGAEDVKFLLVT 297
BnMINI3a_P1 ---PSSPKRRKFGESTMIGATRSCKSQRVILQMETEENNPDDGFFWRKYGQKVVKGNPNPRSYYKCTYTACDVKKHVERGAEDVKFLLVT 297
AtMINI3     DPQPPPPKRRRYEVSNMIGATRTSKTQRIILQMESDEDNPNDGYFWRKYGQKVVKGNPNPRSYFKCTNIECRVKKHVERGADNIKLVVTT 356
               *..****::  *.******:.*:**:*****::*:**:**:*******************:***   * *********:::*:::.*

BrMINI3     YDGIHEHDPPAARGSSSSGLKGQYSSSVSQDHNNHRT------------VPPSSSSASEALRFFPSSLDPPVDMTQFYMTGLAKLPSLPV 375
BnMINI3a_P2 YDGIHEHDPPAARGSSSSGLKGQYSSSVSQDHNNHRT------------VPPSSSSASEALRFFPSSLDPPVDMTQFYMTGLAKLQSLPV 375
BnMINI3a_P1 YDGIHEHDPPAARGSSSSGLKGQYSSSVSQDHNNHRT------------VPPSSSSASEALRFFPSSLDPPVDMTQFYMTGLAKLPSLPV 375
AtMINI3     YDGIHNHPSPPARRSNSSSRNRSAGATIPQNQNDRTSRLGRAPPTPTPPTPPPSSYTPEEMRPF-SSLATEIDLTEVYMTGISMLPNIPV 445
            *****:* .*.** *.**.  : .  .:::.*::*:: :            .**.** :.* :* * *** .  :*:*:.****:: * .:**

BrMINI3     YQNHGLMNWNNEPEIDRVIPDGTEVFKGIRDRLNLNFGLNL 416
BnMINI3a_P2 YQNHGLMNWNNEPEIDRVIPDGTEVFKGIRDRLNLNFGLNL 416
BnMINI3a_P1 YQNHGLMNWNNEPEIDRVIPDGTEVFKGIRDRLNLNFGLNL 416
AtMINI3     YENSGFMYQNDEPTMN-AMPDGSDVYDGIMERLYFKFGVDM 485
            *:* *:*  *:** :: .:***::*:.** :** ::**:::
```

Fig. 5 Alignment of putative protein sequences of MINI3 from *Brassica* and *Arabidopsis*. BrMINI3 is putative protein of *B. rapa*. WRKY domain defined by the conserved amino acid sequence WRKYGQK, together with a C_2H_2 motif, is highlighted in *black boxes*

individual locus, which was consistent with simple additive effects of QTLs in seed weight control.

Likewise, other minor QTLs other than the ones on A5 and A7 also played a role in seed weight control. By examining all the TSW QTLs genotypes in the SJ DH population, one line (#75) possessing all superior alleles at nine QTLs loci was identified with the highest TSW across 2 years (3.8 g in the year of 2007 and 4.1 in 2008). On the contrary, line #87 possessing all unfavorable alleles at all QTLs loci was identified with the lowest seed weight in 2 years (1.9 g in the year 2007 and 2.0 in 2008). The result suggested that it is possible to apply the marker information to assisted selection for seed weight improvement.

Discussion

In recent years, SSRs have been recognized as a preferable type of molecular markers for tagging genes and genetic analysis due to its advantages over many other types of markers, such as transferability among populations or related species, codominant nature and low costs (Plieske

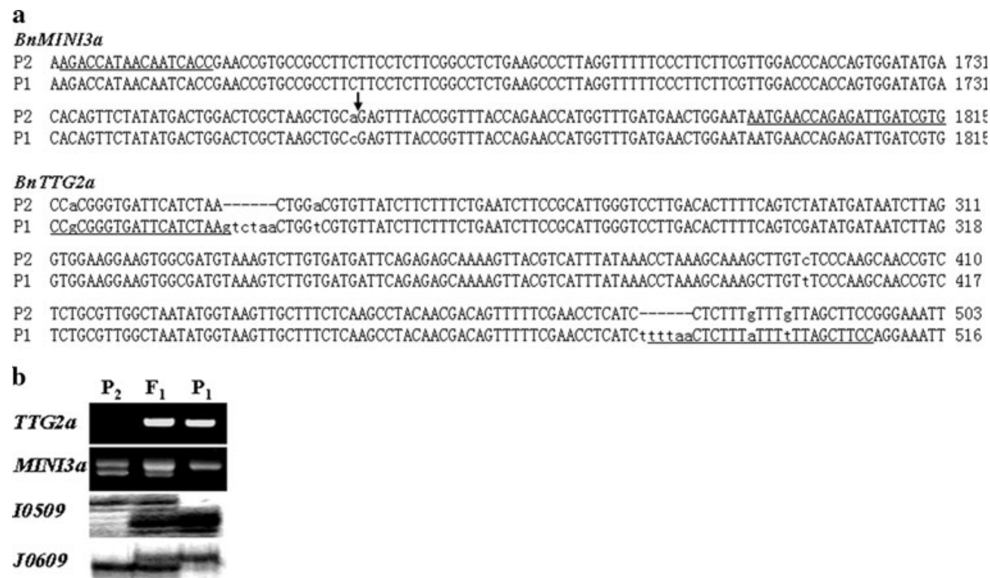

Fig. 6 Development of allele-specific markers for seed weight. **a** Comparison of the partial genomic nucleotide sequences of *BnMINI3a* and *BnTTG2a* genes cloned from SW Hickory (*P1*) and JA177 (*P2*). *Underlined* is the sequence of allele-specific primer; *lowercase letter* indicates the nucleotide variances between SW Hickory and JA177; *arrowhead* indicates the restriction site variation of *Pst*I. **b** PCR products amplified from SW Hickory, JA177 and their hybrid (F_1) using the allele-specific markers. The PCR products are separated by electrophoresis in 2.0% agarose gels and stained with ethidium bromide for *TTG2a* and *MINI3a*, whereas in 6% denaturing polyacrylamide gels and stained with silver for *I0509* and *J0609*

and Struss 2001; Suwabe et al. 2002, 2008; Lowe et al. 2004; Cheng et al. 2009). In the present study, a SSR map of the SJ DH population showed a good compatibility and high repeatability with published linkage maps in *B. napus*. Most of SSRs in this study detected only one locus, and could therefore be useful as anchor markers to compare and align maps derived from different populations.

Despite the importance of seed weigh in the determination of total plant grain yield, little is known about the genetic mechanism that determines the final size and weight of seeds in oil *Brassica* crops. Genetic studies in major crop species including rice, tomato, soybean, maize, barley and wheat using QTL mapping indicated that relatively few loci showed significant effects on seed weight compared to other quantitative traits (Paterson et al. 1995; Doganlar et al. 2000; Coventry et al. 2003; Groos et al. 2003; Doebley et al. 1994; Hyten et al. 2004). So far only few of the QTLs for seed weight in crops have been cloned (Fan et al. 2006; Weng et al. 2008).

In the present study, two of nine QTLs detected in the SJ DH population, *TSWA7a* and *TSWA7b*, were mapped at the top and bottom of LG A7 across environments and collectively explained 27.6–37.9% of the seed weight variation. The stability and significance of the two QTLs were later validated in the F_2 population with different genetic background. By further adding two SSR markers derived from two *B. rapa* BACs to the vicinity of *TSWA7a* and *TSWA7b*, our results thus provided tightly linked markers to those major QTLs. Quijada et al. (2006) detected a major QTL for seed weight, *SW7.1*, on the top of A7, which was also detected at the same marker interval of pW194aE-pX104aH by Udall et al. (2006), The two QTLs may present a same candidate region for seed weight, as they were located at a same marker interval in two different mapping populations that shared a common parent, P1084. However, the direct relationship between the QTLs identified previously and in this study cannot be discerned due to the lack of shared markers between the maps. Recently, Shi et al. (2009) reported a consensus QTL *qSW.A7-2* on LG A7, explaining 9.0–20.5% of the seed weight variation across ten natural environments and two related populations of oilseed rape. The peak of *qSW.A7-2* was flanked by two SSR markers Ra2-G08 and sR0282R, where the QTL *TSWA7b* detected in this study was located (Fig. 3). Most recently, Basunanda et al. (2010) mapped a QTL for seed weight around the common marker Ra2-G08 on A7 in two different DH populations and two corresponding populations of backcrossed test hybrids. These results suggest that the QTL *TSWA7b* is stably expressed in different genetic backgrounds and environments, making it a valuable target for molecular cloning and in breeding for seed weight improvement.

With the information on seed size regulators in *Arabidopsis*, two homologous genes, *BnMINI3a* and *BnTTG2a*, were identified and mapped on the LG A5 in this study. *BnMINI3a* co-segregated with QTL *TSWA5b* and *BnTTG2a* was located at the closest marker interval of *TSWA5c* (Fig. 1; Table 2), thus providing putative candidate genes

of the QTLs in *B. napus*. *TSWA5b* and *TSWA5c* were detected only in 2007 field environment but their effects on TSW could still be detected in a combined analysis of QTL effects (Table 4), indicating that such minor QTLs cannot be neglected.

In *Arabidopsis*, the mutation at either *TTG2* or *MINI3* was found to have significant effects on seed size and weight (Garcia et al. 2005; Luo et al. 2005), while the two candidate gene loci in *B. napus* only contributed a sizable effect to seed weight (Table 2). This is understandable considering the genomic complexity of amphidiploid *B. napus*, in which there could be as many as six copies corresponding to an individual homologous region in *Arabidopsis* available (Lysak et al. 2005). Further studies are needed to link the identified polymorphisms between parents in these two genes with their phenotypic contributions to seed weight in *B. napus*.

Table 4 Effects of individual or combined locus on seed weight in the SJ DH population as revealed by allelic genotype grouping

Group	Genotype			*N*	1,000-seed weight (g)	
	I0509	J0609	A5		Mean ± SD (2007)	Mean ± SD (2008)
Single locus effect[a]						
I0509	AA			84	2.72 ± 0.34 A	2.94 ± 0.36 A
	BB			101	2.41 ± 0.27 B	2.61 ± 0.36 B
J0609		AA		92	2.71 ± 0.34 A	2.91 ± 0.39 A
		BB		98	2.40 ± 0.26 B	2.61 ± 0.34 B
MINI3a			AA	90	2.61 ± 0.36 a	2.80 ± 0.42 a
			BB	90	2.50 ± 0.31 b	2.73 ± 0.36 a
TTG2a			AA	91	2.62 ± 0.36 A	2.79 ± 0.43 a
			BB	99	2.49 ± 0.31 B	2.72 ± 0.35 a
Multiple locus effect[b]						
I	AA	AA	AA	19	2.97 ± 0.37 a	3.18 ± 0.43 a
II	AA	AA	BB	19	2.76 ± 0.29 b	3.01 ± 0.32 ab
III	BB	AA	AA	12	2.60 ± 0.26 bc	2.80 ± 0.39 bc
IV	AA	BB	AA	13	2.58 ± 0.22 c	2.85 ± 0.26 bc
V	BB	AA	BB	23	2.54 ± 0.24 cd	2.76 ± 0.31 c
VI	AA	BB	BB	14	2.49 ± 0.23 cd	2.79 ± 0.28 bc
VII	BB	BB	AA	23	2.38 ± 0.22 de	2.52 ± 0.34 d
VIII	BB	BB	BB	23	2.24 ± 0.25 e	2.45 ± 0.33 d

AA and *BB* designate the allelic genotype same as parent SW Hickory and parent JA177 at a particular locus, respectively, *N* sample size for each genotypic group

[a] Within a group and same year, different uppercase or lowercase letters indicate a significant difference at the 0.01 and 0.05 probability level based on *t* test, respectively

[b] The three QTLs tightly linked on A5 were treated as one locus for genotypic grouping. For group I–VIII, means followed by a same letter indicate no significant difference at the 0.05 probability level based on Duncan-test

The three QTLs, *BnMINI3a, BnTTG2a*, and another adjacent QTL *TSWA5a* were identified individually within a relatively small region (about 15 cM) on A5 (Fig. 1; Table 2). The existence of three loci for seed weight on A5 was supported by several lines of evidence. First, the results of mapping *BnMINI3a* and *BnTTG2a* were consistent from both SSR marker analysis and candidate gene cloning and mapping (Figs. 1, 6; Table 2). Second, comparison mapping with *Arabidopsis* homologous genome showed that the three QTLs on A5 correspond to fragments from *Arabidopsis* chromosome 5 (for *TSWA5a*), chromosome 1 (for *BnMINI3a*) and chromosome 2 (*BnTTG2a*), respectively (data not shown). However, due to the primary mapping nature of the present study, the conclusion that the three QTLs are completely independent still awaits studies of fine mapping and comparison of near isogenetic lines with each separated locus. Such a close link of the three loci and limited sampling size in the present study could also result in overestimated additive effect, which has been reflected in combined effect analysis (Table 4) of three individual loci.

Genetic analysis in several crops have clearly shown that, in addition to single locus QTLs, epistatic QTLs also play an important role in the genetic basis of yield-related traits (Lark et al. 1995; Maughan et al. 1996; Yu et al. 1997). However, analysis about epistatic interactions for seed weight in *B. napus* has not been reported yet. In the present study, analysis of epistatic interactions indicated that seed weight variation in the SJ DH population was primarily controlled by simple additive effects. This was further supported by comparison of the seed weights in groups of DH lines with different QTL genotypes (Table 4). Furthermore, two lines with favorable or unfavorable alleles at detected all QTLs in the SJ DH population were identified to show extreme seed weights stably. Thus, it could be possible for breeders to reliably predict performance of seed weight from QTL allele's information only. Such a genetic pattern provides breeders an opportunity to improve seed weight of oilseed rape through a pyramiding approach.

In conclusion, the QTLs identified in this study are well suitable to MAS due to no significant epistatic interactions that could interfere with each other in selection process. The molecular markers tightly linked to major QTLs on A7 and the allele-specific markers for *BnMINI3a* and *BnTTG2a* on A5 will prove useful for introgression and positional cloning of seed weight genes.

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