ORIGINAL PAPER

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

Chuchuan Fan · Guangqin Cai · Jie Qin · Qingyuan Li · Minggui Yang · Jianzhong Wu · Tingdong Fu · Kede Liu · Yongming Zhou

Received: 18 January 2010/Accepted: 4 June 2010/Published online: 24 June 2010 © Springer-Verlag 2010

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 F₂ 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 F₂ population with different genetic backgrounds. By cloning BnMINI3a and BnTTG2a, two B. napus homologous genes to Arabidopsis thaliana, allelespecific markers were developed for TSWA5b and TSWA5c, two TSW QTLs on A5, respectively. The importance of the

Communicated by H. Becker.

C. Fan, G. Cai and J. Qin contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1388-4) contains supplementary material, which is available to authorized users.

C. Fan \cdot G. Cai \cdot J. Qin \cdot Q. Li \cdot M. Yang \cdot J. Wu \cdot T. Fu \cdot K. Liu (\boxtimes) \cdot Y. Zhou (\boxtimes)

National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China e-mail: kdliu@mail.hzau.edu.cn

Y. Zhou e-mail: ymzhou@mail.hzau.edu.cn major and minor QTLs identified was further demonstrated by analysis of the allelic effects on TSW in the DH population.

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-F₂) 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-Luv 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 $P_{\rm in} = 0.05$ and $P_{\rm out} = 0.05$ (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: $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{g1}^2/n + \sigma_e^2/nr)$, where σ_g^2 is genotypic variance, σ_{g1}^2 variance due to genotype by environment interaction, σ_e^2 error variance; *n* number of environments, and *r* number of replications. The estimates of σ_g^2 , σ_{g1}^2 and σ_e^2 were obtained from an analysis of variance (ANOVA) with environment considered as a random effect. The heritability (h^2) of TSW in F₂ 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 F₂, P₁ and P₂, 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'-ATGGATGTGAAAGAGAGTGAAAGA
	A-3′
TTG2R	5'-TTAAATGGCTTGATTAGAATGTTGT
	G-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, Inter-ProScan (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 ratio (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;

Fig. 1 The genetic linkage (LG) map and QTLs for seed weight \blacktriangleright 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

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.

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

1	41	A2		A3		A5			A7	A6
0 -	Bo GMS562	0 sR94102	2	0 ft Bo GM	5707	0 Bn	EMS 1030	0 -	f-BrEMS16B	0 HBrGM\$3%A
6.8-	BrGMS116B			5.4 Bo GM	\$539	8.5 HBF	MS-007	- 6.7	Bo GMS715	2 CNU_SSR149
13.7-	CNU_SSR142	14 🕂 MR52b		IU.8 BREMIS	67H	119-18n	EMS730	146 -	BnEMS858	9.4 BnGMS331
16.9	Bo GMS661	20 - MR52a		19 BnEMS	5178A	22 12	510487 CD1620CE	146	FITO 035	18.5 Nal2-H07
249	Bo CMS1181	24.9 - BRAS0	BB	27 HNIAB_	55R102	23 T Ba	GN15205	16.2 21.5	NIAB SSR043	
22 2/	B-CM6793	29.7 + BrGMS 32 ☆sR6293	309C			30.8 HBr	GMS630	26.3	CB10343	32.7-BRMS-014
39.7-	Bo GMS466	35.7 1 Bo GMS	1394A	JO.2 BIGME	MU/A			28 1 31.5	CB10439	38.9 sR12156A
		40.3 1 BnGMS	572			46.1 - CE	310080	37.9	sR7223	44.4 BrGM5083
50.2-	BnEMS772	50.3 H BnEMS	1	48.8 HB0 GMD 52.5 HBnGM	5487 579	A 49.5 - Na	10-E02	512 -	BnEMS814	49.9 BnEMS 1125
55.5	BRAS078	56.8 - Bo GMS	541	55.3∰BN12		56 ABo	GMS852B GMS1199	523 545	BRMS-005	53.5 BrCMS663
58.3	BrGM5682			59.87 BnEM2 646 TBrGM2	5894 5109	57.8 - Mi	INI3a FINE02	64 -	BRMS-040	FE O BITIER
64 9 [€]	NIAD_SSILGI					60.6 Na	12-E01	731 -	FITO M8A	022 0 SIGSION
75.2-	- CB10189	76.1 H OII3-E0	8	76.3 CNU_S	SR384	64.9 Bn	GMS276E	3		
84.7-	sN3523R	83.3 BrGMS	75			77.2] [1]	G2a RF73			A4
90.1-	Nal2-C06	86 BRMS-0	026			937 L C		۶		0 开Nal2-D09 1.7 开EST245
00.4	CB10206B	94.1 ++ OHU-CI 98.6	IUA 650	00 1 No14 C	10	949 TBr	GMS147	• 93.8 - ⊠98.6 -	BnGM5422	
33.44	0-CD10200D			33.1-4-11a14-G	-10	102.1 Bo	GMS298	BD99.7 /	Bo GMS710	
						106.1 Bo	GMS852A GMS478	102.54	BrGM\$554 BrEM\$221	20.4 Oll0-D03C
A	10			1142 CNU_S	SR098			10000		25.7 H BKASU21
0-	BrGMS144	121.6 H OLLO-CI	1 0B	118.3 HBrGMS	687 687	18				30.2 Bo GMS1044
227	BnEMS 1037			122.9∭OII1-G	11	 	Chicio	127.2-	₩B₀ GM\$641	43 5 B. CMC279
13.5-	BnGMS232			124.4' BnGM 134.5/11:BrGM	5417 1361	1.4 开sF	3688			472 10 (1123) (0
15.6	Na10-D07 BnEMS181	140.9 🕂 Bo GMS	157	139.9 HCB104	ຮັ	7. 魚野	EMS 107 I	ļ	49	
214	BRMS-017B	147.7 CB1002	2	142.7 BnEMS	5727	12.3 A N	al2-B05	" O-	f ^{₅\$1949}	58.7 Bo GMS326
$\begin{bmatrix} 23.3 \\ 26.1 \end{bmatrix}$	BnEMS986			149.5 BrGMS	406	17.2 B	nGMS581			63.1 Bo GMS011
29.9	BnGMS334	158.4 HB. GMS	1492	157.4 BrGM9	5134 :40	17.81 sr 19.6 B	12362 nEMS633			65.3 Bo GMS750
⊔ 38 [/]	BnGMS206	166.2 - Bo GMS	1472	164.4 BnEMS	61143A	211 B	RMS-017A	L		70. P. Bo GMS313 76.6 Bo GMS052B
513-	BrGM\$564	174.3 - Bo GMS	493		200	37.5 V BI	KM15-006	22.4-	BnGMS203	80.7/ Bo GMS092
		178 BnEMS	694	175.8 CNU_S	SR241			31.5-	BnEMS768	90.4 sN12353
65.3-	NIAB_SSRI31	180.8 180.8 FITO 0	997 . D8C :	179.1 Nal2-H	106			32.1	BnEMS664	96.8 Bo GM\$560
		189.5 Bo GMS	307					32.4	BnEMS1169	105.3 Bo GMS279
		195.2 CB1009	3 2344							
		1.001 /1								
		201.2 /∐\BoGMS	892							
		201.2 Bo GMS 206.7 Bo GMS	892 447							
		201.2 1 Bo GMS 206.7 Bo GMS 214.3 Bo GMS	892 447 155B							
CI		201.2 1 Bo GMS 206.7 Bo GMS 214.3 Bo GMS	892 447 3155B	3	C	5	66		(*	C 9
CI	BrGM\$116A	201.2 Bo GMS 206.7 Bo GMS 214.3 Bo GMS C2 0.0 By CMS360B	892 447 6155B C.	3 2. BaciMis?	Ci A	; 1. NTR097	C6	- CRICICO	C8	C9
cı ⁰ ∰	BrGMS116A	201.2 206.7 Bo GMS 214.3 Bo GMS C2 0 BrGMS369B	892 447 155B 0 - f 3.2 2	3 - BnGM52 - BnGM5274	0 2.9	5 - MR097 Na10-D11	C6 0 ∰ I	30 GMS162	C8 0 ∰BxGM5808.4	С9 А 0-Д-Во СМ5833
CI 0 1 95	BrGMS116A BrGMS635	201.2 1 Bo GMS 206.7 Bo GMS 214.3 Bo GMS C2 0 BrGMS369B	892 447 155B 0 - 3.2 / 5.7 /	3 BnGMS2 BnGMS274 BoGMS676	0 2.9 116	- MR097 Nal0-D11 - Bo GMS1423	C6 0 - F F 7.7 - F F	36 GMS162 36 GMS490	C8 0	C9 A 0 + Bo GM\$833 8.7 + CNU 558372 CNU 558372
CI 0 + 1 95 + 1 15.6 + 1	BrGMS116A BrGMS635 Bo GMS234B BrGM5403	201.2 1 Bo GMS 206.7 Bo GMS 214.3 Bo GMS C2 0 BrGMS369B 15.6 BrGMS369A	892 4447 0155B 0 - 3.2 / 5.7 / 14.1 -	3 BnGM52 BnGM5274 Bo GM5676 Bo GM5141 BnFM5956	0 - 2.9 - 11.6 - 15 - 2	5 - MR097 - Nal0-D11 - Bo GMS1423 - Nal2-C01	C6 0 - 1 7.7 - 1 114 - s 17 - 1	30 CMS162 30 CMS490 S2486 30 CMS29	C8 0	C9 A 0 Bo GMS833 8.7 CNU_55R372 10.4 BnGM5319 1.42 BnGM5772
Cl 0 +	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrGMS403 BrLMS889	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CMS C2 0 -/ BrGMS369B 15.6 -/ BrGMS369A 22.1 -/ Bo GMS1394B	892 447 1558 0 3.2 7 5.7 / 14.1 - 17.2 7 19.7 /	3 BnGMS2 BaGMS74 BaGMS74 BaGMS76 BaEMS956 FITO 008B	0 2.9 11.6 15 16.6	MR097 Nal0-D11 - Bo GMS1423 Nal2-C01 Br.GMS131 Br.GMS136B	C6 0 + H 7.7 + H 114 + s 17 + H 73 9 - H	30 GMS162 30 GMS490 32 486 30 GMS29 30 GMS29 30 GMS29	C8 0 + BrGM58084 18.2 - Bo GMS1166	C9 A 0 Bo GMS833 8.7 CNU SSR372 10.4 BnGMS319 14.2 BnGMS719 14.2 MIAB_SSR129
Cl 0 + 1 95 + 1 15.6 + 1 19.4 + 1 26.2 + 1 33.3 + 1	BrGMS116A BrGMS635 BrGMS2348 BrCMS403 BrEMS859 BrEMS859 BrEMS1025	201.2 // Bo CMS 206.7 // Bo CMS 214.3 Bo CMS C2 0 BrGMS369B 15.6 BrGMS369A 22.1 Bo GMS1394B 25.2 - sN3761	892 447 1555 3.27 5.7 14.1 17.27 19.7 27.7	3 BnGMS2 BnGMS274 BoGNS576 BoGNS576 BnEMS956 FITO 008B Na14:C06	0 - 2.9 - 11.6 - 15 - 16.6 - 19.2 -	MR097 Na10-D11 - Bo GMS1423 Na12-C01 BaCMS131 BaCMS396B - Bo GMS530	C6 0 + H 7.7 + H 11.4 + s 17 + H 23.9 + H	30 GMS162 30 GMS490 52486 30 GMS29 30 GMS1448	C8 0 + BrGM5808/ 18.2 + Bo GM51166 29.2 + BrGM5808E	C9 A 0 Bo GMS833 8.7 - CNU 55R372 10.4 BrGMS319 14.2 BrGMS377 20.1 - NIAB 55R129 22.6 - CNU 55R343 3 - 5, J BrGMS770
Cl 0 + : 95 - : 15.6 - : 19.4 - : 26.2 - : 33.3 - : 40.6 - :	BrGMS116A BrGMS635 BrGMS2348 BrGMS403 BrEMS859 BrEMS1025 BrEMS840	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CMS C2 0 -/ BrCMS369B 15.6 -/ BrCMS369A 22.1 -/ Bo CMS1394B 25.2 *N3761 34.5 -/ BRA5083A	892 447 1555 3.2 5.7 14.1 17.2 27.7 31.7 32.5	3 BnCMS2 BnCMS274 BnCMS576 BnEMS956 FITO 008B Nal4-C06 BnEMS 1032 (Bo CMS1452	0 - 2.9 - 11.6 - 15 - 16.6 - 19.2 - 29.2 -	5 MR097 Na10-D11 - Bo GMS1423 Na12-C01 BaCMS131 BrCMS396B - Bo GMS530	C6 0 + H 7.7 + H 11.4 + s 17 + H 23.9 + H 32.2 + H	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS1610 D10 D029	C8 0 + BrGM5808/ 18.2 - Bo GM51166 29.2 - BrGM5808E	C9 A 0 Bo GMS833 8.7 - CNU_SSR372 10.4 BoGMS319 5.42 BoGMS3772 20.1 - NIAB_SSR129 22.5 4 BoGMS770 30.7 BoLMS762
Cl 95	BrGMS116A BrGMS635 BrGMS234B BrGMS403 BrrEMS859 BrrEMS8025 BrrEMS840	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CMS C2 0 -/ BrCMS369B 15.6 -/ BrCMS369A 22.1 -/ Bo CMS1394B 25.2 */0761 34.5 -/ BRA5083A 38.8 -/ BrEMS1119	892 447 155B 0 - 327 5.7 141 - 17.2 19.7 19.7 27.7 - 31.7 32.5 36.5	3 BnGMS2 BaGMS274 BaGMS76 BaGMS141 BhEMS966 FITO 008B Nal4 C06 BhEMS 1032 BaGMS1421	0 2.9 11L6 15 16.6 19.2 29.2	- MR097 Nal0-D11 - Bo CMS1423 Nal2-C01 BnCMS131 BrCMS396B - Bo CMS307	C6 0 + H 7.7 + H 11.4 + s 17 + H 23.9 + H 32.2 + H 33.4 (10) 34.6 (10)	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS1448 30 GMS1448 2010-003B 2010-003B 2010-003B	C8 0 - BrGMS808A 18.2 - Be GMS1166 29.2 - BrGMS808F 38.4 - Be GMS515	C9 A 0 Bo GMS833 8.7 CNU_5SR372 10.4 BnGMS319 14.2 BrGMS772 20.1 VNLB 5SR263 25.4 Bo GMS270 30.7 BhEMS762 36.3 CNU_5SR263 0.4 UNUSSR263
Cl 95	BrGMS116A BrGMS635 Bo CMS234B BrGMS2403 BrrEMS859 BrrEMS859 BrrEMS840 sr0425	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CM	892 447 155B 0 3.27 5.7 14.1 - 17.2 7 19.7 27.7 - 31.7 32.5 36.5 43.4 43.4 43.4	3 BnGMS2 BnGMS274 BoGMS274 BoGMS76 BoGMS141 BnEMS956 FITO 008B Na14-06 BnCMS1421 BoGMS1421 BRGMS260	0 - 29 - 29 - 11.6 - 15 - 7 16.6 / 19.2 - 29.2 - 43.2 - 47 - 47 - 47 - 47 - 47 - 47 - 47 - 4	- MR097 Na10-D11 - Bo GM51423 Na12-C01 Br.GM5133 Br.GM5396B - Bo GM5530 - Bo GM51397 NBr.EM51002	C6 0 + H 7.7 + H 11.4 * s 17 + H 23.9 + H 32.2 + H 32.2 + H 33.4 (0)	3o GMS162 3o GMS490 52486 3o GMS29 3o GMS1448 3nGMS610 D10-D03B D110-F09	C8 0	C9 A 0 Bo GM5833 8.7 CNU_SSR372 10.4 Bo.GM5319 1.42 B.GM5712 20.1 VNLAB SSR129 22.6 CNU_SSR343 25.4 Bo GM5270 30.7 B.EM5762 36.3 CNU_SSR263 39.4 Bo GM5132
Cl 95	BrGMS116A BrGMS635 Bo CMS234B BrGMS2403 BrEMS859 BrEMS859 BrEMS840 st0425	201.2 // Bo CMS 206.7 // Bo CMS 214.3 // Bo CM	892 447 155B 0 3.27 5.7 141 19.7 27.7 - 31.7 31.7 31.5 36.5 43.4 45.6 55.1	BnGMS2 BnGMS74 BoGMS74 BoGMS76 BoGMS141 BnEMS956 FITO 008B Na14-C06 BnGMS1421 BoGMS1421 BoGMS1421 BoGMS1421 BoGMS1421 BoGMS1421	$\begin{array}{c} 0 \\ 0 \\ -2.9 \\ 11.6 \\ -15 \\ 19.2 \\ 29.2 \\ -29.2 \\ -43.2 \\ -47 \\ -7 \end{array}$	MR097 Na10-D11 Bo GMS1423 Na12-O11 BnGMS131 BnGMS130 BnGMS130 Bo GMS1397 BnEMS1002	C6 0 H 7.7 H 11.4 s 17 H 23.9 H 32.2 H 34 C 39.6 C	3o GMS162 3o GMS490 52486 3o GMS29 3o GMS1448 3nGMS610 D10-D03B D110-F09 3rGMS189A	C8 0 BrGM58084 18.2 Bo GM51166 29.2 BrGM5808F 38.4 Bo GM5515 42.2 BrEM579 45 Bo GM5979 52.1 CB10504	C9 A 0 Bo GM5833 8.7 CNU_SSR372 10.4 Bo.GM5319 5. 142 Bo.GM5712 20.1 CNLB_SSR373 25.4 Bo.GM5710 30.7 Bo.EM5762 36.3 CNU_SSR363 39.4 Bo.GM51382 50.1 Bo.EM5714
Cl 95	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrEMS859 BrEMS80 SN8425 Bo GMS1506	201.2 // Bo CMS 206.7 // Bo CMS 214.3 // Bo CMS 200 // BrCMS369A 22.1 // Bo CMS1394B 25.2 // No12 34.5 // BrCMS637 38.8 // BrCMS67 45 // Bo CMS1274 45 // Bo CMS1274 47 // Bo CMS127	892 447 0 555B 3.2 / 5.7 / 14.1 - 17.2 / 14.1 - 17.2 / 3.17 - 3.17 - 3.15 / 3.15 / 3.15 / 3.15 / 3.16 / 3.17 / 3.17 / 3.17 / 3.16 / 3.17 / 3.1	3 BnGMS2 BnGMS274 BoGMS274 BoGMS76 BoGMS141 BnFMS956 FITO 008B Na14-C06 BnFMS1032 BoGMS1421 BrCMS1421 BrCMS1421 BoGMS1421 BoGMS1421 BoGMS1421 BoGMS1421 BoGMS1421	$\begin{array}{c} 0 \\ 0 \\ -2.9 \\ 11.6 \\ 15 \\ 16.6 \\ 19.2 \\ 29.2 \\ -43.2 \\ 43.2 \\ 47 \\ 59.2 \\ -59.2 \\ \end{array}$	- MR097 Na10-D11 Bo GMS1423 Na12-O11 BnGMS131 BnGMS396B Bo GMS530 BnEMS1002 CNU_SSR293	C6 0 + I 11.4 + s 17 + I 32.2 + I 34 + C 39.6 + C 53.3 + I 60.1 + F	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS1448 30 GMS610 D110-F09 31 GMS64A	C8 0	C9 A 0 Bo GM5833 8.7 CNU 55R372 10.4 BnGM5319 5. 142 BrGM5712 20.1 WILAB 55R129 21.6 CNU 55R343 32.54 Bo GM5136 30.7 BrEM5762 36.3 CNU 55R263 39.4 Bo GM51382 50.1 BnEM5714
Cl 95	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrEMS859 BrEMS80 sN8425 Bo GMS1506 Bo GMS1506 Bo GMS1502	201.2 // Bo CMS 206.7 // Bo CMS 214.3 // Bo CMS 201.1 // Br CMS369A 22.1 // Bo CMS1394B 25.2 // No12 34.5 // Br CMS1394B 25.2 // No12 34.5 // Br CMS1274 47.6 // Na12-A01 51.5 // Bo CMS1274 47.6 // Na12-A01 51.5 // Bo CMS4076 51.5 // Bo CMS4076 51.5 // Bo CMS4076	892 447 0	3 BnCMS2 BnCMS274 BoCMS274 BoCMS276 BoCMS141 BnEMS956 FITO 008B Na14-C06 BnEMS 1032 BoCMS1421 BRA5087 BoCMS1421 BRA5087 BoCMS4693 BoCMS576 BoCMS576 BoCMS1558	$\begin{array}{c} 0 \\ - \\ 2.9 \\ 11.6 \\ - \\ 15 \\ 29.2 \\ - \\ 43.2 \\ - \\ 47 \\ - \\ 59.2 \\ - \\ 60.6 \\ - \\ 61.6 \\ - \\ \end{array}$	- MR097 Na10-D11 - Bo GMS1423 Na12-O11 BaGMS131 BaGMS396B - Bo GMS530 - Bo GMS530 - Bo GMS530 - BaEMS1002 - CNU_SSR293 BaEMS566 BaCMS778	C6 0 + I 11.4 * s 17 + I 23.9 + I 32.2 + I 33.4 < C 39.6 < C 53.3 + I 60.1 + I 67.7 + Z	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS1448 30 GMS610 D110-F09 31 GMS64 30 GMS64A 30 GMS64A	C8 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS8087 38.4 Bo GMS815 42.2 BrEMS79 45 Bo GMS979 52.1 CB10504 53.57 BrEMS1070 63.2 Bo GMS971 53.4 Bo GMS971 53.57 BrEMS1070	C9 A 0 Bo GM5833 8.7 CNU_5SR372 10.4 BnGMS319 5.142 BrGMS772 20.1 WILAB 5SR129 21.6 CNU_5SR343 32.54 Bo GM5270 30.7 BnEM5762 36.3 CNU_5SR263 39.4 Bo GM51382 50.1 BnEM5714 645 BrGM5809
Cl 95 15.6 26.2 33.3 40.6 51.4 51.4 77.3-()	BrGMS116A BrGMS635 Bo GMS2348 BrGMS403 BrEMS859 BrEMS80 sN9425 Bo GMS1506 Bo GMS1502 Bo GMS1502 Bo GMS1275	201.2 // Bo CMS 206.7 // Bo CMS 214.3 // Bo CM	892 447 1155B 0	3 - BnGMS2 - BcMS274 - BoCMS274 - BoCMS276 - BoCMS141 - BnEMS986 - FITO 008B - Nal4-C06 - BnEMS 1032 - BoCMS1421 - BRAS087 - BoCMS1421 - BRAS087 - BoCMS545 - BoCMS569 - BoCMS576 - BoCMS569 - BoCMS576 - BoCMS569 - BoCMS569 - BoCMS569 - BoCMS569 - BoCMS569 - BoCMS576 - BoCMS569 - BoCMS576 - BoCMS576 - BoCMS569 - BoCMS576 - B	$\begin{array}{c} 0 \\ 0 \\ -2.9 \\ 11.6 \\ -1.5 \\ 10.6 \\ 19.2 \\ 29.2 \\ -47 \\ -43.2 \\ -47 \\ -59.2 \\ -60.6 \\ -64.4 \end{array}$	5 - MR097 Na10-D11 - Bo CMS1423 Na12-C01 BnCMS396B - Bo CMS530 - Bo CMS530 - Bo CMS530 - Bo CMS530 - BnEMS 1002 - CNU_SSE293 NnEMS666 BrCMS778	C6 0 H 7.7 H 11.4 s 17 H 23.9 H 32.2 H 34 (39.6 C 53.3 H 60.1 H 67.7 H 69.7 H	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS610 D110-D03B D110-F09 31 GMS610 31 GMS64A 30 GMS64A 30 FEMS16A EB10211	C8 0 BrCMS8087 18.2 Bo CMS1166 29.2 BrGMS8087 38.4 Bo CMS515 42.2 BarCMS8087 45 Bo CMS979 52.1 CB10504 53.5 Bo CMS979 52.1 CB10504 53.5 Bo CMS979 53.5 Bo CMS979 53.5 Bo CMS979 53.5 Bo CMS979 53.5 Bo CMS979 53.5 Bo CMS978 53.5 Bo CMS978 54.5 Bo CMS978 55.5 Bo CMS978 5	C9 A 0 Bo GM5833 8.7 CNU_5SR372 10.4 Bb.GM5319 5.142 Bb.GM5772 20.1 NIAB_5SR129 22.6 CNU_5SR343 32.54 Bb.GM5762 30.7 Bb.EM5762 39.4 Bb.GM51382 50.1 Bb.EM5714 64.5 Bb.GM5809 67.9 Bb.GM518802 7.1 sc.Mb.GM5164 64.5 Bb.GM5164 64.5 Bb.GM5164 6
Cl 95 15.6 33.3 40.6 51.4 67.4 77.3 83.7	BrGMS116A BrGMS635 Bo GMS2348 BrGMS403 BrEMS859 BrEMS840 sN0425 Bo GMS1506 Bo GMS1506 Bo GMS1502 Bo GMS1275 BrGMS362	201.2 // Bo CMS 206.7 // Bo CMS 214.3 // Bo CM	892 447 1155B 0 327 5.7 141 - 17.2 7.7 - 31.7 325 365 - 43.4 55.1 - 60.7 - 64.4 71.2 -	3 - BnGMS2 - BnGMS274 - BoGMS274 - BoGMS76 - BoGMS141 - BnEMS986 - FITO 008B - Nal4-C06 - BnEMS1032 - BoGMS1421 - BRAS087 - BoGMS423 - BoGMS4558 - BoGMS576 - BoGMS51558 - BoGMS300	0 0 29 7 116- 15 7 15 7 15 7 15 7 15 7 15 7 15 7 15 7	 MR097 Na10-D11 Bo CMS1423 Na12-C01 BaCMS396B Bo CMS530 Bo CMS530 Bo CMS530 Bo CMS1397 BnEMS 1002 CNU_SSR293 BnEMS666 BrCM5778 OII0-D03A 	C6 0 H 7.7 H 114 s 17 H 23.9 H 32.2 H 34 (39.6 C 53.3 H 60.1 H 60.1 H 67.7 H 69.7 (75.4 H	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS610 D110-D03B D110-F09 31 GMS610 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A	C8 0 BrCMS8087 18.2 Bo GMS1166 29.2 BrGMS8088 38.4 Bo GMS515 42.2 BarCMS8088 38.4 Bo GMS979 52.1 CB10504 53.5 BorCMS979 52.1 CB10504 53.5 BorCMS979 70.9 BrGMS513 70.9 BrGMS513 70.9 BrGMS513	C9 A 0 Bo GMS833 8.7 CNU_SSR372 10.4 Bb.GMS319 14.2 Bb.GMS719 21.6 CNU_SSR343 22.6 CNU_SSR243 32.5.4 Bb.GMS70 30.7 Bb.EMS763 39.4 Bb.GMS182 50.1 Bb.EMS714 64.5 Bb.GMS809 67.9 Bb.GMS164 77.6 Bb.GMS148
Cl 9.5 +	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrEMS859 BrEMS1025 BrEMS840 sN0425 Bo GMS1506 Bo GMS1506 Bo GMS1502 Bo GMS1502 Bo GMS124	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CMS 214.3 -/ Bo CMS 214.3 -/ Bo CMS 214.3 -/ Bo CMS 214.5 -/ BrCMS369B 15.6 -/ BrCMS369B 15.6 -/ BrCMS369A 22.1 -/ Bo CMS1394B 25.2 -/ sN761 34.5 -/ BRA5083A 34.5 -/	892 447 155B 0 32,77 141 - 17,2 - 31,7 - 31,7 - 31,7 - 32,5 // 43,4 - 45,6 // 55,1 - 60,7 - 64,4 - 64,4 - 71,2 -	3 - BnGMS2 - BnGMS274 - Bo GMS274 - Bo GMS274 - Bo GMS274 - Bo GMS274 - Bo GMS274 - Bo GMS285 - BnEMS 1032 - Bo GMS287 - Bo GMS293 - Bo GMS295 - Bo GMS295 - Bo GMS2576 - Bo GMS300	0 0 2.9 7 11.6 15 7 15 7 15 7 15 7 15 7 15 7 15 7 15 7	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BaCMS131 B-CMS136B Bo GMS530 Bo GMS530 Bo GMS530 CNU_SSR293 BaEMS5666 B-GMS778 Oll0-D03A 	C6 0 H 7.7 H 114 s 17 H 23.9 H 32.2 H 34 (53.3 H 60.1 H 60.7 H 69.7 (75.4 H	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS610 D110-D03B D110-F09 31 GMS610 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A	C3 0 BrCMS8087 18.2 Bo GMS1166 29.2 BrCMS808E 38.4 Bo GMS515 42.2 BrEMS79 45 Bo GMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo GMS971 65.4 BrCMS505 70.9 BrCMS505 79.1 BrEMS1010	C9 A 0 Bo CMS833 8.7 CNU_SSR372 10.4 Bb.CMS319 14.2 Bb.CMS712 20.1 NIAB_SSR129 22.6 CMU_SSR343 32.5.4 Bb.CMS570 30.7 Bb.EMS762 36.3 CCNU_SSR343 50.1 Bb.EMS714 64.5 Br.CMS809 67.9 Bb.CMS809 67.9 Bb.CMS809 67.9 Bb.CMS808C 70.5 Bb.CMS148 81.2 Bb.EMS761
Cl 0 95 15.6 15.	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrEMS859 BrEMS859 BrEMS840 sN8425 Bo GMS1506 Bo GMS1506 Bo GMS1502 Bo GMS1575 BrGMS362 Bo GMS124 sS1867	201.2 // Bo CMS 206.7 // Bo CMS 214.3 // Bo CMS 214.3 // Bo CMS 214.3 // Bo CMS 214.3 // Bo CMS 214.5 // BrCMS369B 15.6 // BrCMS369B 15.6 // BrCMS369A 22.1 // Bo CMS1394B 25.2 // sN761 34.5 // BRAS083A 34.5 //	892 4447 155B 327 141- 17.2 7 31.7 7 31.5 1 325	3 BnGMS2 BnGMS274 BoGMS274 BoGMS274 BoGMS274 BoGMS274 BoGMS274 BoGMS274 BoGMS242 BoGMS242 BoGMS242 BoGMS26 BoGMS26 BoGMS276 BoGMS21558 BoGMS300	$\begin{array}{c} 0 \\ -2.9 \\ 11.6 \\ 15 \\ 19.2 \\ 29.2 \\ -47 \\ -47 \\ -59.2 \\ -60.6 \\ -644 \\ -78.3 \\ -\end{array}$	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BaCMS131 B-CMS396B Bo GMS530 Bo GMS530 Bo GMS530 CNU_SSR293 BnEMS666 BaCMS778 Oll0-D03A 	C6 0 H 7.7 H 114 s 17 H 23.9 H 32.2 H 34 (53.3 H 60.1 H 60.7 H 69.7 (75.4 H)	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS610 D110-D03B D110-F09 31 GMS610 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A	C3 0 BrCMS8087 18.2 Bo CMS1166 29.2 BrCMS808F 38.4 Bo CMS515 42.2 BrEMS79 45 Bo CMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo CMS971 65.4 BrCMS505 70.9 BrCMS61 82 BrGMS54 84 Bo CMS17 9.1 BrCMS505 79.1 BrCMS505 70.1 BrC	C9 A 0 Bo CMS833 8.7 CNU SSR372 10.4 Bb.CMS319 14.2 Bb.CMS712 20.1 NIAB SSR129 22.6 CNU SSR343 30.7 Bb.EMS762 36.3 CCNU SSR363 39.4 Bo GMS1382 50.1 Bb.EMS714 64.5 Br.CMS809 67.9 Bb.CMS809 67.9 Bb.CMS809 67.9 Bb.CMS809 67.9 Bb.CMS808C 70.6 Bb.CMS148 81.2 Bb.EMS761
C1 95	BrGMS116A BrGMS635 Bo CMS234B BrGMS403 BrEMS859 BrEMS1025 BrEMS840 sN9425 Bo GMS1506 Bo GMS1506 Bo GMS1502 Bo GMS1575 BrGMS362 Bo GMS124 s\$1867 BRA5123B GMS124	201.2 // Bo CMS 206.7 // Bo CMS 214.3 // Bo CMS 214.3 // Bo CMS 214.3 // Bo CMS 214.3 // Bo CMS 214.5 // BrCMS369B 15.6 // BrCMS369B 15.6 // BrCMS369B 15.6 // BrCMS369A 22.1 // Bo CMS1394B 25.2 // SN761 34.5 // BRAS083A 34.5 // BrCMS607 45 // Bo CMS496 13.7 // BrCMS407B 56.5 // Bo CMS496 70.1 // EST259 78.8 // EST258 89.1 // Bn EMS660	892 4447 155B 0 - 4 327 57 / 141 - 17.2 7 31.7 7 31.5 / 3.65 / 3.65 / 3.65 / 4.3.4 4.5.6 / 5.5.1 - 6.4.4 7 7.1.2 - 95.3 -	3 BnGMS2 BnGMS274 BoGMS274 BoGMS274 BoGMS274 BoGMS274 BoGMS274 NS142 BoGMS141 BnEMS1032 BoGMS142 BoGMS269 BOGMS269 BOGMS269 BOGMS269 BOGMS269 BOGMS26	$\begin{array}{c} 0 \\ 29 \\ 11.6 \\ 15 \\ 19.2 \\ 29.2 \\ 43.2 \\ 47 \\ 59.2 \\ 60.6 \\ 644 \\ 78.3 \\ \end{array}$	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BaCMS131 BaCMS131 BaCMS130 Bo GMS530 Bo GMS530 CNU_SSR293 BaEMS1666 BaCMS778 Oll0-D03A Ba GMS556 	C6 0	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS610 D10-D03B D10-F09 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A 21 D10211 31 GMS642	C3 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 42.2 BrEMS79 45 Bo GMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo GMS971 65.4 BrGMS505 70.9 BrGMS54 84 Bo GMS120 95.27 BrGMS1200 95.27 BrEMS20	C9 A 0 Bo CMS833 8.7 CNU SSR372 10.4 BaCMS319 11.2 BaCMS772 20.1 NIAB SSR129 22.6 CNU SSR136 32.5.4 Bo CMS270 30.7 BaEMS762 36.3 CCNU SSR263 50.1 BaEMS714 64.5 BaCMS809 67.9 BaCMS809 67.9 BaCMS808C 70.6 BaCMS148 81.2 BaEMS761
C1 95	BrGMS116A BrGMS636 Bo GMS234B BrGMS403 BrEMS89 BrEMS80 BrEMS80 sN9425 Bo GMS1506 Bo GMS1502 Bo GMS1502 Bo GMS1275 BrGMS362 Bo GMS124 es1867 BRAS123B CB10206A	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CMS 214.3 -/ Bo CMS 214.3 -/ Bo CMS 201.2 // Bo CM	892 4447 155B 0 4 3 22 5.7/ 141 17.2 7 30.5 7/ 30.5 7/ 30.5 7/ 30.5 7/ 30.5 7/ 30.5 7/ 30.5 7/ 30.5 7/ 30.5 7/ 55.1 - 60.7 - 64.4 7 71.2 - 95.3 -	3 BnGMS2 BnCMS274 BoCMS274 BoCMS274 BoCMS274 BoCMS26 FITO 008B Nal4-C06 BnEMS 1032 BoCMS142 BoCMS1421 BoCMS1452 BoCMS269 BoCMS569 BoCMS569 BoCMS556 BoCMS558 BoCMS558 BoCMS558	$\begin{array}{c} 0 \\ 2.9 \\ 11.6 \\ -15 \\ 16.6 \\ 19.2 \\ 29.2 \\ -47 \\ -59.2 \\ -60.6 \\ 64.4 \\ 78.3 \\ -78.3 \\ -98.1 \\ -\end{array}$	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BaCMS131 BrCMS396B Bo GMS530 Bo GMS530 BnEMS 1002 CNU_SSR293 BnEMS666 BrGMS778 Oll0-D03A Bo GMS556 	C6 0 1 1 11.4 s 17 1 1 23.9 1 32.2 1 34 0 53.3 1 60.1 1 60.1 1 60.7 1 60.7 1 60.7 0 75.4 0 1 0 1 8 7.7 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS610 D110-D03B D10-F09 31 GMS64A 31 GMS64A 31 GMS64A 32 GMS64A 12 H0211 31 GMS642 10 GMS642 10 GMS642	C8 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 42.2 BrEMS79 45 Bo GMS971 65.4 BrGMS40 82.7 BrGMS4505 70.9 BrGMS4 84 Bo GMS117 87.9 BrGMS40 BrGMS4 84 Bo GMS117 87.9 BrGMS40 BrGMS4 84 Bo GMS1200 95.2 BrEMS20	C9 A 0 Bo CMS833 8.7 CNU SSR372 10.4 BaCMS319 11.4 BaCMS772 20.1 NLAB SSR129 22.6 CNU SSR343 30.4 Bo GMS770 30.7 BaEMS762 36.3 CNU SSR363 50.1 BaEMS714 64.5 BaCMS809 67.9 BaCMS808C 70.6 Bo CMS164 77.6 BaCMS148 81.2 BaEMS761
C1 95	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrEMS269 BrEMS269 BrEMS260 sN8425 Bo GMS1506 Bo GMS1502 Bo GMS1275 BrGMS362 Bo GMS124 sS1867 BRAS123B CB10206A Bo GMS052A	201.2 // Bo CMS 206.7 // Bo CMS 214.3 → Bo CMS 214.3 → Bo CMS 214.3 → Bo CMS 200 // BrCMS369B 15.6 → BrCMS369A 22.1 → Bo CMS1394B 25.2 * N3761 34.5 → BRA5083A 38.8 → BnEMS1119 42.1 → BrCMS617 45.5 → Bo CMS1274 47.6 → BnCMS607 70.1 ← EST259 78.8 → EST258 89.1 → BnEMS660 C4 0 → BnCMS115	892 4447 155B C 0 4 322 57.7 141 17.2 7.7 36.5 19.7 7 17.2 36.5 1 43.4 7 12.5 1.5 55.1 - 64.4 7 12.2 - 95.3 - 95.3 -	3 BnCMS2 BnCMS274 BoCMS274 BoCMS274 BoCMS274 BoCMS141 BnEMS956 BnEMS1952 BoCMS1452 BoCMS1452 BoCMS1452 BoCMS1452 BoCMS1453 BoCMS5693 BoCMS576	$\begin{array}{c} 0 \\ 0 \\ 29 \\ 116 \\ 15 \\ 15 \\ 122 \\ 29.2 \\ 43.2 \\ 47 \\ 47 \\ 47 \\ 47 \\ 47 \\ 47 \\ 47 \\ 4$	5 MR097 Na10-D11 = Bo GMS1423 Na12-C01 Bn-GMS131 Br-GMS396B = Bo GMS130 = Bo GMS130 = Bo GMS1397 Bn-EMS1002 = CNU_SSR293 Bn-EMS666 Br-GMS778 = O110-D03A = Bo GMS256	C6 0 - II 7.7 II 11.4 s 17 II 23.9 II 32.2 II 33.4 C 53.3 II 60.1 II 60.7 C 75.4 C 0 - B 7.3 s 8.4 B 13.7 M B 13.7 M B	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS1448 30 GMS1610 D10-D03B D10-D03B D10-D03B D10-D03B D10-D03B 31 GMS614 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS64A 31 GMS644 31 GMS645 40 GMS61545 40 GMS61545 40 GMS1229	C8 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 42.2 BrEMS79 45 Bo GMS971 65.4 BrEMS1070 63.2 Bo GMS971 65.4 BrGMS613 79.1 BrEMS1070 63.2 Bo GMS971 65.4 Bo GMS971 6	C9 A 0 Bo CMS833 8.7 CNU SSR372 10.4 BaCMS319 11.4 BaCMS319 21.6 CNU SSR372 20.1 NLAB SSR129 22.6 CNU SSR363 30.4 Bo CMS176 30.7 BaEMS762 36.3 CCU SSR368 50.1 BaEMS762 50.1 BaEMS762 64.5 BaCMS188 50.1 BaEMS763 64.5 BaCMS188 81.2 BaEMS761
C1 95	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrEMS240 BrEMS240 sN9425 Bo GMS1506 Bo GMS1506 Bo GMS1502 Bo GMS1502 Bo GMS1275 BrGMS362 Bo GMS124 sS1867 BRAS123B CB10206A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS052A	201.2 Be CMS 206.7 Be CMS 214.3 Be CMS 214.3 Be CMS 214.3 Be CMS 206.7 Be CMS 206.7 Be CMS 206.7 Be CMS 206.7 Be CMS 207.8	892 4447 155B 577 141- 17:27 36.57 141- 17:27 36.57 36.57 43.47 55.1- 60.7- 64.47 71.2- 95.3- 110.5- 114.14	3 BnCMS2 BnCMS274 BoCMS274 BoCMS274 BoCMS276 BoCMS141 BnEMS596 BnTO 008B Nal 4 C06 BnEMS 1032 BoCMS1421 BRCMS169 BoCMS169	$\begin{array}{c} 0 \\ 0 \\ 2.9 \\ 11.6 \\ 15 \\ 15.2 \\ 29.2 \\ 43.2 \\ 47 \\ 47 \\ 47 \\ 59.2 \\ 60.6 \\ 64.4 \\ 78.3 \\ 98.1 \\ \end{array}$	- MR097 Na10-D11 - Bo GMS1423 Na12-C01 BnGMS131 BrGMS396B - Bo GMS530 - Bo GMS1397 - BnEMS1002 - CNU_5SR293 BnEMS666 BrGMS556 - Bo GMS556	C6 0 - II 7.7 - II 11.4 - s 17 - II 23.9 - II 32.2 - II 33.4 - (-) 33.4 - (-) 33.4 - (-) 33.4 - (-) 33.4 - (-) 53.3 - II 60.1 - II 60.7 - (-) 60.7	30 GMS162 30 GMS490 52 486 30 GM529 30 GM529 30 GM51448 30 GM5610 D10-D03B D10-F09 31 GM5610 31 GM564A 31 GM564A 31 GM5642 N0706 10 GM5645 10 GM51545 10 GM51545 10 GM5639	C8 0 BrGMS808/ 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 42.2 BnEMS79 45 Bo GMS971 52.1 CB10504 53.5 BnEMS1070 63.2 Bo GMS971 63.2 Bo GMS971 63.2 Bo GMS971 63.2 Bo GMS1025 95.2 BnEMS20 95.2 BnEMS20 95.2 BnEMS20 95.2 BnEMS20	C9 A 0 Bo GMS833 8.7 CNU_SSR372 10.4 BnGMS319 5.142 BnGMS712 20.1 NLAB SSR129 22.6 CNU_SSR343 25.4 Bo GMS270 30.7 BnEMS762 36.3 CNU_SSR263 39.4 Bo GMS1382 50.1 BnEMS714 64.5 BrGMS809 67.9 BrGMS809 60.0
C1 95	BrGMS116A BrGMS635 Bo GMS2348 BrCMS403 BrEMS89 BrEMS1025 BrEMS840 sr8425 Bo GMS1506 Bo GMS1506 Bo GMS1502 Bo GMS124 s\$1867 BrA5123B CB10206A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS052A	201.2 Bo CMS 206.7 Bo CMS 214.3 Bo CMS 214.3 Bo CMS 214.3 Bo CMS 206.7 Bo CMS 214.3 Bo CMS 206.7 Bo CMS 206.7 Bo CMS 207.8 Br CMS 209.8 Br CMS 209.8 Br CMS 209.8 Br CMS 209.8 CMS 209.8 CMS 200.8	892 4447 155B C 0 4 7 227 141 17:2 7 141 17:2 7 19:7 f 27.7 - 31.7 f 32.5 f 32.5 f 33.6 f 43.4 f 55.1 - 60.7 - 64.4 f 71.2 - 95.3 - 10.5 - 114.1 - 114.1 f 114.1 f 11	3 BnCMS2 BnCMS274 BoCMS274 BoCMS274 BoCMS276 BoCMS141 BnEMS956 HTTO 008B Nal 4 C06 BnHMS 1032 BoCMS1421 BRCMS1421 BRCMS1421 BRCMS1423 BoCMS456 BoCMS57	$\begin{array}{c} 0 \\ -2.9 \\ -1.5 $	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BnCMS1306 Bo GMS530 Bo GMS530 Bo GMS1397 BnEMS1002 CNU_SSR293 BnEMS666 BnCMS778 O110-D03A Bo GMS556 	C6 0 - II 7.7 - II 11.4 - s 17 - II 23.9 - II 32.2 - II 33.4 - C 33.6 - C 53.3 - II 60.1 - II 69.7 - C 75.4 - U C7 0 - B 7.3 - s 8.4 - B 13.7 - B 13.7 - B 21.9 - C 20 - S 21 - S	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS1448 30 GMS1448 30 GMS1448 30 GMS1448 30 GMS1448 30 GMS189 A 30 GMS164 31 GMS164 31 GMS164 32 GMS1545 30 G	C8 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS115 45 Bo GMS919 53.5 Bo GMS979 52.1 CB10504 53.5 Bo GMS979 53.2 Bo GMS979 63.2 Bo GMS979 53.2 Bo GMS970 53.4 Bo GMS10 84 Bo GMS117 87.9 Bo GMS1200 95.2 BrEMS20 113 Bo GMS1025	C9 A 0 B GMS833 8.7 CNU_SSR372 10.4 Br.GMS319 10.4 B.GMS712 20.1 VNLAB SSR129 22.6 CNU_SSR343 25.4 Bo.GMS1382 30.7 B.EMS762 36.3 CNU_SSR263 39.4 Bo.GMS1382 50.1 Br.EMS762 50.1 Br.EMS764 64.5 Br.GMS809 67.9 Br
CI 95	BrGMS116A BrGMS635 Bo GMS2348 BrCMS403 BrEMS89 BrEMS1025 BrEMS1025 BrGMS1506 Bo GMS1506 Bo GMS1502 Bo GMS124 s51867 BrGMS362 Bo GMS124 s51867 BrA5123B CB10206A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS052A	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CM	892 4447 155B 577 141- 17.2 p 577 141- 17.2 p 577 197 19	3 BnGMS2 BnGMS274 BoGMS274 BoGMS76 BoGMS76 BoGMS141 BnEMS966 FITO 008B Nal4 C06 BnHMS1032 BoGMS1421 BrAS087 BoGMS5421 BrGMS39 BoGMS576 BoGMS576 BoGMS576 BoGMS576 BoGMS576 BoGMS500 - CB10036 - BoGMS1464 BrGMS382 - BoGMS96	$\begin{array}{c} 0 \\ -2.9 \\ -15 \\ $	 MR097 Na10-D11 Bo CMS1423 Na12-C01 BnCMS1311 BrCMS1396B Bo CMS530 Bo CMS530 Bo CMS530 CNU_SSR293 BnEMS1666 BrCMS778 O110-D03A Bo CMS556 BnEMS1143B 	C6 0	30 GMS162 30 GMS162 30 GMS2486 30 GMS1448 30 GMS1448 30 GMS1448 30 GMS1448 30 GMS610 D10-003 31 GMS640 31 GMS642 10 GMS642 10 GMS642 10 GMS642 10 GMS645 10 GMS1545 10 GMS1545 10 GMS1545 10 GMS1545 10 GMS1545 10 GMS234C 10 GMS067	C8 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 42.2 BrEMS79 52.1 CB10504 53.5 BrGMS97 95.2 Bo GMS979 52.1 CB10504 53.4 Bo GMS979 95.2 BrGMS9613 79.1 BrEMS201 84 Bo GMS102 95.2 BrEMS20 113 Bo GMS1025	C9 A 0 Bo GM5833 8.7 CNU_SSR372 10.4 Bo.GM5319 1.42 B.GM5712 20.1 CNLB SSR343 25.4 Bo GM5270 30.7 B.EM5762 30.4 Bo GM51382 50.1 B.EM5761 64.5 B.CM5809 67.9 B.GM5164 7.6 Bo GM5164 7.6 Bo GM5164 7.6 Bo GM5164 81.2 B.GM5164
CI 95	BrGMS116A BrGMS346 BrGMS403 BrCMS403 BrEMS89 BrEMS1025 BrEMS840 sr0425 Bo GMS1506 Bo GMS1506 Bo GMS1502 Bo GMS124 s51867 BBRA5123B CB10206A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS055A	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CMS 214.4 -/ Bo CM	892 4447 155B 757 141- 17.2 ϕ 17.7 ϕ	3 BnCMS2 BnCMS74 BoCMS76 BoCMS76 BoCMS141 BnEMS956 FITO 008B Na14-C06 BnEMS1032 BoCMS1421 BrAS087 BoCMS1421 BrAS087 BoCMS569 BoCMS569 BoCMS569 BoCMS14558 BoCMS1558 BoCMS164 BrCMS382 BoCMS1464 BrCMS1823	$\begin{array}{c} 3\\ 0\\ -2.9 \\ 11.6 \\ -15 \\ 15.2 \\ 12.9 \\ -2.7 \\ 43.2 \\ -47 \\ -47 \\ -47 \\ -59.2 \\ -60.6^{+} \\ -64.4^{+} \\ 78.3 \\ -98.1 \\ -126 \\ -126 \\ -\end{array}$	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BnGMS131 BrGMS396B Bo GMS530 Bo GMS530 Bo GMS530 CNU_SSR293 BnEMS5666 BrGMS5778 O110-D03A Bo GMS556 BnEMS1143B 	C6 0	3o GMS162 3o GMS162 3o GMS29 3o GMS1448 3nGMS610 D10-D038 D110-F09 3rGMS189A 3nGMS64A 3nGMS64A 3nGMS64A 3rGMS16A CB10211 3rGMS212 4o GMS642 N0706 4o GMS1545 4o GMS129 4o GMS1246 4o GMS067	C8 0 BrGMS808/ 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 45 Bo GMS979 52.1 CB10504 53.4 BorGMS979 52.1 CB10504 53.4 BorGMS979 70.9 BrGMS979 70.9 BrGMS9613 70.9 BrGMS9613 70.9 BrGMS9613 70.9 BrGMS9613 70.9 BrGMS9505 70.9 BrGMS9505 71.9 BorGMS970 84 BorGMS9170 87.9 BorGMS9170 87.9 BorGMS9170 95.2 BnEMS20 113 BorGMS1025	C9 A 0 Bo GM5833 8.7 CNU_SSR372 10.4 Bo.GM5319 5.142 Bo.GM5712 20.1 CNLB SSR373 22.6 CNU_SSR343 32.54 Bo.GM5710 30.7 Bb.EM5762 36.3 CNU_SSR263 39.4 Bo.GM51382 50.1 Bb.CM5514 64.5 Bb.CM5809 67.9 Bb.CM5148 81.2 Bb.GM5148 81.2 Bb.CM5761
CI 0	BrGMS116A BrGMS346 BrGMS2348 BrCMS2348 BrCMS2403 BrEMS2403 BrEMS240 sN9425 BrGMS1506 BrGMS1506 BrGMS1502 BrGMS124 s51867 BrGMS24 BrGS524 BrGMS954 BrGMS394 BrGMS394 BrGMS594 BrGMS594 BrGMS694	2012 // Bo CMS 206.7 Bo CMS 214.3 Bo CMS 214.3 Bo CMS 214.3 Bo CMS 214.3 Bo CMS 214.3 Bo CMS 214.3 Bo CMS 252 sN3761 345 BrcMS303A 38.8 Bo EMS11394B 25.2 sN3761 345 BrcMS407B 51.5 Bo CMS1274 47.6 Nal2-A01 51.5 Bo CMS407B 51.5 Bo CMS408 61.3 Bo EMS406 61.3 Bo EMS408 61.3 Bo EMS408 61.4 Bo EMS408 61.3	892 4447 155B 0 4 7 325 577 141 17.2 7 19.7 7 32.5 7 43.4 7 43.4 7 45.6 7 19.7 7 33.65 7 43.4 7 45.6 7 19.7 7 19.7 7 19.7 7 19.7 7 43.4 7 45.6 7 19.7 7 19.7	3 BnGMS2 BnGMS74 BoGMS774 BoGMS774 BoGMS76 BoGMS76 PiTO 008B Na14-C06 BnFMS1032 BoGMS1421 BrAS087 BoGMS51421 BrAS087 BoGMS569 BoGMS569 BoGMS576 BoGMS577 BoGMS576 BoGMS577 BoGMS576 BoGMS576 BoGMS576 BoGMS576 BoGMS576 BoGMS576 BoGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS577 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS5777 BOGMS7777 BOGMS7777 BOGMS7777 BOGMS7777 BOGMS7777 BOGMS7777 BOGMS77777 BOGMS77777 BOGMS777777 BOGMS777777777777777777777777777777777777	$\begin{array}{c} 3\\ 0\\ -29 \\ 116 \\ -15 \\ 15 \\ 116 \\ 19.2 \\ 29.2 \\ -47 \\ -47 \\ -59.2 \\ -60.6 \\ -644 \\ -78.3 \\ -98.1 \\ -126 \\ -\end{array}$	- MR097 Na10-D11 Bo GMS1423 Na12-D11 BnGMS131 BnGMS130 BnGMS130 BnGMS130 BnEMS1002 - CNU_SSR293 BnEMS666 BrGMS778 - O110-D03A - Bo GMS556 - BnEMS1143B	C6 0	30 GMS162 30 GMS490 52 486 30 GMS29 30 GMS1448 30 GMS610 D110-D038 D110-F09 31 GMS640 31 GMS644 31 GMS644 32 GMS642 10 GMS642 10 GMS642 10 GMS645 10 GMS1239 10 GMS639 10 GMS067	C8 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 45.7 Bo GMS979 52.1 CB10504 53.5 BrGMS505 70.9 BrGMS613 79.1 BrGMS613 79.1 BrGMS613 79.1 BrGMS505 70.9 BrGMS613 79.1 BrGMS505 113 Bo GMS1025 113 Bo GMS1025	C9 A 0 Bo GM5833 3.7 CNU_5SR372 10.4 Bo.GM5319 5.142 Br.GM5712 20.1 WILAB SSR129 21.6 CNU_5SR343 32.54 Bo.GM5174 5.14 645 Br.GM5108 67.9 Br.GM5108 8.12 Br.GM5148 8.12 Br.GM5148
C1 95- 15.6 15.6 33.3 40.6 51.4 67.4 67.4 77.6 67.4 77.6 67.4 77.6 77.6	BrGMS116A BrGMS146A BrGMS234B BrGMS403 BrEMS240 BrEMS859 BrEMS1025 BrGMS1506 BoGMS1506 BoGMS1502 BoGMS1502 BoGMS1502 BoGMS124 s\$1867 BRA5123B CB10206A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS052A BoGMS0534	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CM	892 4447 155B 557 141 17.2 7 19.7 7 19.7 7 19.7 7 32.5 7 19.7 7 32.5 7 19.7 7 32.5 7 19.7 7 32.5 7 43.4 7 45.6 1 71.2 - 95.3 - 10.5 - 114.1 - 121.3 - 131.8 - 140.5 -	3 BnCMS2 BnCMS74 BoCMS774 BoCMS776 BoCMS76 BoCMS141 BnEMS956 FITO 008B Na14-C06 BnEMS1032 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1424 BoCMS1464 BrGMS382 BoCMS1464 BrGMS382 BoCMS1236 BnEMS178B	$\begin{array}{c} 3 \\ 0 \\ 29 \\ 116 \\ -15 \\ 15 \\ 116 \\ 19 \\ 29 \\ 29 \\ -47 \\ -47 \\ -47 \\ -59 \\ -47 \\ -59 \\ -47 \\ -8 \\ -126 \\ -1$	- MR097 Na10-D11 Bo GMS1423 Na12-O11 BnGMS131 BnGMS1397 BnEMS1002 - CNU_SSR293 BnEMS666 BrGMS778 - O110-D03A - Bo GMS256 - BnEMS1143B	C6 0 H 7.7 H 11.4 s 17 H 23.9 H 32.2 H 34 (0) 53.3 H 60.1 H 63.7 (0) 75.4 H 7.3 s 8.4 H 13.7 H 11.7 H 13.7 H 11.7 H 23.9 H 13.7 H 11.7 H 23.9 H 13.7 H 11.7 H 23.9 H 13.7 H 11.7 H 13.7 H	36 GMS162 36 GMS162 37 GMS29 36 GMS29 36 GMS1448 37 GMS610 D10-D038 D10-F09 37 GMS189A 37 GMS189A 37 GMS189A 37 GMS189A 37 GMS189A 37 GMS189A 37 GMS1229 38 GMS1545 39 GMS1229 30 GMS1229 30 GMS067	C8 0 BrGMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS815 45 Bo GMS979 52.1 CB10504 53.5 BrGMS979 52.1 CB10504 53.5 BrGMS979 53.5 BrGMS979 53.7 BrGMS97 95.2 BrGMS97 95.2 BrGMS97 95.2 BrGMS97 95.2 BrEMS20 113 Bo GMS1025	C9 A 0 Bo GM5833 8.7 CNU_5SR372 10.4 Bo.GM5319 5.142 Bo.GM5712 20.1 WILAB 5SR129 21.6 CNU_5SR343 32.54 Bo.GM5133 50.1 Bo.EM5761 64.5 Br.GM5809 67.9 Br.GM5808C 7.6 Bo.GM5148 81.2 Bo.EM5761
C1 95- 15.6 15.6 33.3 40.6 51.4 67.4 67.4 73.6 51.4 73.6 73.6 73.6 73.6 73.6 73.6 73.6 73.6	BrGMS116A BrGMS635 BoGMS234B BrGMS403 BrEMS240 BrEMS859 BrEMS840 sN8425 BoGMS1506 BoGMS1502 BoGMS1502 BoGMS1502 BoGMS124 sS1867 BRAS123B CB10206A BoGMS524 BoGMS524 BoGMS525 BrGMS594 BoGMS155A BoGMS594 BoGMS694	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CM	892 4447 155B 557 141 17.2 7 19.7 7 19.7 7 32.5 7 19.7 7 32.5 7 19.7 7 32.5 7 19.7 7 32.5 7 43.4 7 45.6 7 12.7 - 64.4 7 7 12.2 - 110.5 - 114.1 7 121.3 - 140.5 - 143.7 7	 BnCMS2 BnCMS274 BeCMS576 BeCMS576 BeCMS141 BnEMS956 FITO 008B Na14-C06 BnEMS1032 BeCMS1421 BRA5087 BeCMS1421 BRA5087 BeCMS5421 BrA5087 BeCMS545 BeCMS545 BeCMS545 BeCMS545 BeCMS545 BeCMS545 BeCMS546 BeCMS546 BeCMS546 BeCMS542 BeCMS542 BeCMS542 BeCMS542 BeCMS545 BeCMS545 BeCMS54 BeCMS54 BeCMS54 	$\begin{array}{c} 3 \\ 0 \\ -29 \\ 116 \\ -15 \\ 15 \\ 129 \\ 29 \\ -47 \\ -47 \\ -47 \\ -59 \\ -47 \\ -59 \\ -47 \\ -83 \\ -98 \\ 1-1 \\ 126 \\ -126 \\ -149 \\ -140 \\$	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BnGMS1397 BnEMS1002 CNU_SSR293 BnEMS566 BrGMS578 O110-D03A Bo GMS556 BnEMS1143B BnEMS1143C 	C6 0 H 7.7 H 11.4 s 17 H 23.9 H 32.2 H 34 (53.3 H 60.1 H 60.7 H 63.7 H 7.5 40 H 13.7 H 13.7 H 13.7 H 13.7 H 13.7 H 13.7 H 23.9 H 13.7 H 13.7 H 23.9 H 13.7 H 23.9 H 13.7 H 23.9 H 13.7 H 13.7 H 23.9 H 13.7 H 23.9 H 13.7 H 23.9 H 13.7 H	30 GMS162 30 GMS162 30 GMS29 30 GMS29 30 GMS1448 30 GMS610 D10-D038 D10-F09 31 GMS640 31 GMS644 31 GMS644 31 GMS644 31 GMS644 31 GMS642 32 GMS1545 30 GMS1545 30 GMS1545 30 GMS1239 30 GMS1245 30 GMS234C 30 GMS067 30 GMS067	C8 0 BrCMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS515 42.2 BrEMS79 45 Bo GMS979 52.1 CB0504 53.5 BrEMS1070 63.2 Bo GMS971 65.4 BrGMS450 79.1 BrEMS1010 82 BrGMS4 84 Bo GMS120 95.2 BrEMS20 113 Bo GMS1025	C9 A 0 Bo GM5833 8.7 CNU_5SR372 10.4 BnGM5319 5.142 BrGM5772 20.1 NIAB 55R129 21.6 CNU_5SR343 30.7 BnEM5762 30.7 BnEM5762 30.4 Bo GM51382 50.1 BnEM5714 64.5 BrGM5809 67.9 BrGM5808C 7.6 BnGM5148 81.2 BnEM5761
C1 95	BrGMS116A BrGMS635 Bo GMS234B BrGMS403 BrEMS859 BrEMS1025 BrEMS840 sN9425 Bo GMS1506 Bo GMS1506 Bo GMS1507 BrAS1238 GGNS124 s51867 BRAS1238 GGNS052A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS052A Bo GMS155A Bo GMS155A Bo GMS155A Bo GMS155A	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CM	892 4447 155B C 322 577 161 172 7577 3057 172 712 73 3057 1077 434 434 434 4367 712 - 953- 1055- 1141- 1213- 1318- 1435 1445- 1437 7 1257 -	 BnCMS2 BnCMS274 BoCMS274 BoCMS274 BoCMS174 BoCMS176 BoCMS161 BnEMS956 FITO 008B FITO 008B BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS162 BoCMS163 BoCMS164 BoCMS1658 BoCMS1658 BoCMS1644 BrGMS382 BoCMS1236 BoCMS1236 BoEMS1236 BoCMS1236 	CG 297 116- 157 156 192 292- 477 592 6067 644 783- 98.1- 126- 1537 126-	 MR097 Na10-D11 Bo GMS1423 Na12-C01 BaCMS396B Bo GMS530 Bo GMS530 Bo GMS530 Bo GMS530 CNU_SSR293 BaEMS5666 BrGMS778 O110-D03A Bo GMS256 BrEMS1143B BaEMS1143B BaEMS1143C Bo GMS1143C Bo GMS1143C Bo GMS1143C 	$\begin{array}{c} C6\\ 0 & -1\\ 7.7 & -1\\ 11.4 & s\\ 17 & -1\\ 23.9 & -1\\ 34 & (-)\\ 39.6 & (-)\\ 53.3 & -1\\ 60.1 & -1\\ 63.7 & $	Bo GMS162 Bo GMS162 Bo GMS29 Bo GMS29 Bo GMS29 Bo GMS1448 Bo GMS610 D10-D038 D10-F09 Br GMS640 Br GMS189A Br GMS164A Br EMS16A EB10211 Br GMS542 Br GMS1545 Bo GMS1545 Bo GMS1545 Bo GMS1545 Bo GMS1545 Bo GMS1545 Bo GMS1545 Bo GMS1545 Bo GMS1639 Bo GMS167 Bo GMS1309 Bo GMS1309 Bo GMS1309	C8 0 BrCMS8087 18.2 Bo CMS1166 29.2 BrGMS808F 38.4 Bo CMS515 42.2 BrEMS79 45 Bo CMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo CMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo CMS979 52.1 CB10504 53.5 BrEMS1070 82 BrEMS1070 82 BrEMS20 83.4 Bo CMS1202 84.4 Bo CMS1202 95.2 BrEMS20 113 Bo CMS1025	C9 A 0 Bo GMS833 8.7 CNU_SSR372 10.4 Bb.GMS319 14.2 Bb.GMS772 20.1 NIAB_SSR129 22.6 CNU_SSR343 32.5.4 Bb.GMS270 30.7 Bb.EMS762 39.4 Bb.GMS1382 50.1 Bb.GMS809 67.9 Bb.GMS148 81.2
C1 95	BrGMS116A BrGMS635 Bo CMS234B BrGMS403 BrEMS869 BrEMS1025 BrEMS840 sN9425 Bo GMS1506 Bo GMS1506 Bo GMS1506 Bo GMS162 Bo GMS124 s51867 BRA5123B GB1206A Bo GMS052A Bo GMS052A Bo GMS651 Ra3-E05 BrGMS794 Bo GMS155A Bo GMS694	201.2 // Bo CMS 206.7 // Bo CMS 204.3 // Bo CMS 205.4 // Bo CM	892 4447 155B 0 4 3 22 5.7/ 141 17.2 7 3.65 / 43.4 7.12 - 7.12 - 7.137 3.65 / 43.4 7.12 - 7.12 - 13.7 43.4 7.12 - 13.7 7.12 - 13.7 13.7 7.12 - 13.7 14.1 12.13 - 13.18 - 140.5 - 143.5 14.1 12.13 - 143.5 14.14 - 143.5 14.14 - 143.5 14.14 - 143.5 14.14 - 143.5 14.14 - 143.5 14.14 - 14.14	 BnCMS2 BnCMS274 BoCMS274 BoCMS274 BoCMS174 BoCMS176 BoCMS161 BnEMS956 FITO 008B FITO 008B BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1421 BRAS087 BoCMS1426 BoCMS1558 BoCMS100 CB10036 BoCMS1236 BoCMS1236 BoCMS1236 BoCMS1232 BoCMS1322 BoCMS1321 BoCMS1321 	C3 0	 MR097 Na10-D11 Bo CMS1423 Na12-C01 BaCMS396B Bo GMS530 Bo GMS530 Bo GMS530 Bo GMS530 CNU_SSR293 BaEMS666 BrGMS778 O110-D03A Bo GMS556 BrEM\$1143B BaEM\$1143B BaEM\$1143C Bo GMS197 	$\begin{array}{c} C6\\ 0 & F \\ 7.7 & F \\ 11.4 & s \\ 17 & F \\ 23.9 & F \\ 32.2 & F \\ 34 & (39.6 & C \\ 53.3 & F \\ 39.6 & C \\ 53.3 & F \\ 60.1 & F \\ 63.7 & F \\ 63.7 & F \\ 7.3 & s \\ 63.7 & F \\ 13.7 & F \\ 13.7 & F \\ 21.4 & F \\ 13.7 & F \\ 29 & F \\ 55.8 & F \\ 59.6 & F \\ 65.1 & s \\ 65.1 & s \\ \end{array}$	30 GMS162 30 GMS162 30 GMS29 30 GMS29 30 GMS29 30 GMS1448 30 GMS610 D10-D038 D10-F09 31 GMS64A 31 GMS64A 32 GMS64A 33 GMS64A 34 GM	C8 0 BrCMS8087 18.2 Bo GMS1166 29.2 BrGMS808F 38.4 Bo GMS515 42.2 BrEMS79 45 Bo GMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo GMS971 53.4 Bo GMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo GMS979 92.1 BrGMS4 84 Bo GMS910 82 BrGMS4 84 Bo GMS1002 85.2 BrEMS20 113 Bo GMS1025 113 Bo GMS1025	C9 A 0 Bo CMS833 8.7 CNU_SSR372 10.4 Bb.CMS772 20.1 NIAB_SSR129 22.6 CNU_SSR343 32.5.4 Bo CMS270 30.7 Bb.CMS70 30.3 CNU_SSR243 50.1 Bb.CMS50 64.5 Br.CMS809 67.9 Br.CMS808C 70.5 Bb.CMS148 81.2 B
C1 95	BrGMS116A BrGMS635 Bo CMS234B BrGMS403 BrEMS869 BrEMS80 sN9425 Bo CMS1506 Bo CMS1506 Bo CMS1502 Bo CMS1502 Bo CMS1502 Bo CMS1502 Bo CMS124 s51867 BRA5123B CB10206A Bo CMS052A Bo CMS651 Ra3-E05 BrGMS394 Bo CMS694	201.2 // Bo CMS 206.7 // Bo CMS 214.3 -/ Bo CMS 201.2 // Bo CM	892 4447 155B 0 4 3 22 5.7/ 141 17.2 7 3.65 / 43.4 7 3.65 / 43.4 7 17.2 7 3.65 / 43.4 7 17.2 7 3.65 / 43.4 7 10.7 / 10.7	3 BnCMS2 BnCMS274 BoCMS274 BoCMS274 BoCMS274 BoCMS276 BoCMS141 BnEMS956 BnCMS1452 BoCMS1452 BoCMS1452 BoCMS569 BoCMS569 BoCMS576 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS5776 BoCMS57777 BoCMS57777 BoCMS5777 BoCMS57777 BoCMS57777 BOCMS57777 BOCMS57777 BOCMS57777 BOCMS57777 BOCMS577777 BOCMS5777777777777777777777777777777777777	$\begin{array}{c} 0 & - \\ 2.9 & - \\ 15 & - \\ 15 & - \\ 15 & - \\ 15 & - \\ 15 & - \\ 15 & - \\ 15 & - \\ 15 & - \\ 15 & - \\ 126 & - \\ 126 & - \\ 160.4 & - \\ 160.4 & - \\ \end{array}$	 MR097 Na10-D11 Be GMS1423 Na12-C01 Be GMS131 Be GMS530 Be GMS530 Be GMS530 CNU_SSR293 Be GMS5778 O110-D03A Be GMS556 Be GMS556 Be GMS556 Be GMS556 Be GMS556 Be GMS557 	C6 0 - I I 11.4 * s 17 - I I 23.9 - I 32.2 - I 33.4 (33.4 (53.3 - I 60.1 - I 69.7 (75.4 + I C7 0 - B 73.4 - B 13.7 - B 13.7 - B 13.7 - B 13.7 - B 5.6 - B 5.7 - C 5.6 - B 5.7 - C 5.6 - B 5.6 - B 5.7 - B 5.6 - B 5.7 - C 5.7 -	30 GMS162 30 GMS162 30 GMS29 30 GMS29 30 GMS29 30 GMS1448 30 GMS610 D10-D038 D10-F09 31 GMS64A 31 GM	C8 0 BrCMS8087 18.2 Bo GMS1166 29.2 BrCMS808F 45 Bo GMS515 42.2 BrEMS79 45 Bo GMS979 52.1 CB10504 53.5 BrEMS1070 63.2 Bo GMS971 65.4 BrGMS40 82 BrGMS4 84 Bo GMS107 95.2 BrEMS200 95.2 BrEMS200 113 Bo GMS1025 113 Bo GMS1025 113 Bo GMS1025 114 Bo GMS1025 115 BrEMS200 115 BrCMS1025 116 GMS1025 117 BrCMS1025 118 Bo GMS1025 119 Bo GMS1025 119 Bo GMS1025 110 BrCMS1025 110 BrCMS1025 110 BrCMS1025 111 BrCMS1025 111 BrCMS1025 111 BrCMS1025 112 BrCMS1025 113 BrCMS1025 113 BrCMS1025 114 BrCMS1025 115 BrCMS105 115	C9 A 0 Bo CMS833 8.7 CNU 558372 10.4 Bo CMS319 14.2 Bo CMS319 21.6 CNU 558372 20.1 NIAB 558129 22.6 CNU 558243 33.7 Bo CMS170 30.7 Bo CMS170 33.7 Bo CMS170 33.4 Bo CMS1382 50.1 Bo CMS1382 50.1 Bo CMS1880 67.9 Bo CMS188 50.1 Bo CMS188 50.1 Bo CMS188 50.1 Bo CMS188 50.1 Bo CMS188 50.1 Bo CMS188 50.1 Bo CMS184 81.2 Bo CMS164 71.6 Bo CMS164 81.2 Bo CMS164 51.2

Population	Parents		F ₁	Segregating popu	$h_{\rm B}^2~(\%)$	
	P ₁	P ₂		Mean \pm SD	Range	
DH (2007)	3.04 ± 0.24 A	$2.11\pm0.14~\mathrm{B}$	2.54 ± 0.17	2.55 ± 0.33	1.90-3.80	82.9
DH (2008)	2.69 ± 0.18 a	$2.38\pm0.12~\mathrm{b}$	2.58 ± 0.09	2.74 ± 0.39	1.85-4.12	
F ₂ (2006)	$4.26\pm0.25~\mathrm{A}$	$2.68\pm0.06~\mathrm{B}$	3.44 ± 0.09	3.03 ± 0.37	2.18-4.35	76.5

Table 1 Descriptive statistics of 1,000-seed weight (g) in parents, F1 and segregating populations

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 \times JA177 (a) and F₂ population derived from the cross of J7046 \times 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. BACspecific SSR markers were then developed for mapping in SJ DH population (Table 3; Fig. 3). Two SSR markers, 10509 from KBrB084P16 and J0609 from KBrH001J06, were mapped close to TSWA7a and TSWA7b, respectively (Fig. 3). 10509 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 10509 and J0609 loci (Fig. 3).

Population	Year	QTL	Interval	Peak	Marker	LOD	А	$R^2 (\%)^{a}$
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

Table 2 The QTLs for seed weight detected in the SJ DH and F₂ populations

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 *10509* 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 *10509* 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
10509	SSR	5'-ATCATGATGACTTTTGCAATG-3'	5'-GCTCTTGGTAACATAAAATCG-3'
J0609	SSR	5'-GTTGGTTAAAATCGTGTATGC-3'	5'-CCTACAAAAAGCAATAACGTG-3'
MINI3a	CAPS (PstI)	5'-AGACCATAACAATCACCGAACC-3'	5'-ACACGATCAATCTCTGGTTCATT-3'
TTG2a	SNP	5'-CCGCGGGTGATTCATCTAAG-3'	5'-GGAAGCTAAAAAATAAAGAGTTAAA-3'

CAPS cleaved amplified polymorphic sequence, SNP single nucleotide polymorphism

Fig. 4 Alignment of putative protein sequences of TTG2 from <i>Brassica</i> and <i>Arabidopsis</i> . BrTTG2 is putative protein of <i>B. rapa</i> . WRKY domain defined by the conserved amino acid	BnTTG2a_P2 BrTTG2 BnTTG2a_P1 AtTTG2 BnTTG2a_P2 BrTTG2	MDVKESERNVVAKPVASRPSCSS-VRTFTDLLADSVTVSPQSNCHETVDASIIPKTERFKQPASASVSSPRVEGSGDVKSCDD-SESK 86 MDVKESERKVVAKPVASRPSCSS-VRTFTDLLADSVTVSPQSNCHETVDASIIPKTERFKQPASASVSSPRVEGSGDVKSCDD-SESK 86 MDVKESERKVVAKPVASRPSCSS-VRTFTDLLADSVTVSPQSNCHETVDASIIPKTERFKQPASASVSSPRVEGSGDVKSCDD-SESK 86 MEVNDGERVVIAKPVASRPSSSSGFRTFTELLTDSVTVSPQTTCHEIVDAAIRPKTLRFNQPVAASVSCPRAEVKGIGNGMSCDDDSDSR 90 *:*:::*******************************	3 5 5 0 69
with a C_2H_2 motif, is highlighted in <i>black boxes</i>	BnTIG2a_PI AtTTG2	SYVIYKEKAKLOSQAIVSALANNAWIKKEAVAYGKEVNQGIHKAVPNLVLKVEIPKESEISIGDKSYVDGYN NKKYCON 2VKGIE NYVVYKEKAKLOSKATUSALANNLQGNRQQTWRQSEAVSYQGKSVSQGTHRAGPNLVQKVPSFTESETSTGDRSSVDGYN NKKYCQK 2VKGIE .**:**********************************	59 80
	BnTTG2a_P2 BrTTG2 BnTTG2a_P1 AtTTG2	SDSPRGYYKUTHPKOPVKLKVERSSMGGHVSEIVYQGEINISKPSCPLPRRASSSSSGFQTPSEESMGQEPAPLWSDQ-24 SDSPRGYYKUTHPKOPVKLKVERSSMGGHVSEIVYQGEINISKPSCPLRRASSSSSGFQTPSEGSMGEEPAPLWSNQ-24 SDSPRGYYKUTHPKOPVKLKVERSSMGGHVSEIVYQGEINISKPSCPLPRRASSSSSGFQTPSEGSMGEEPAPLGSNQ-24 SECPRSYYKUTHPKOPVKLKVER-SVEGQVSEIVYQGEINISKPSCPLPRRASSSISSGFQKPPKSIASEGSMGQDPNNNLYSPLWNNQS 26 *:.**.********************************	18 48 48 69
	BnTTG2a_P2 BrTTG2 BnTTG2a_P1 AtTTG2	EKMNEGCVITPFEFAVPRTANSTGGTSDSGCRSSQCDERELDDPSRSKTSMKNETQSSEAGVSQSSGESDSLEDGFKWRKYG 33 EKMNEGCVITPFEFAVPRTANSTGGTSDSGCRSSQCDERELDDPSRSKTSMKNETQSSEAGVSQSSGESDSLEDGFKWRKYG 33 ERINEGCVIIPFEFAVLRTANSTGGTSDSGCRSGQCDERELDDPSRSKTSMKNEKQSSDGGVSQSSGESDSLEDGFKWRKYG 33 NDSTQNRTEKMSEGCVITPFEFAVPRSTNSNPGTSDSGCKSSQCDEGELDDPSRSKR-RKNEKQSSEAGVSQGSVESDSLEDGFRWRKYG 35 *::.***** ******* *::**. ******** ********	30 30 30 58
	BnTTG2a_P2 BrTTG2 BnTTG2a_P1 AtTTG2	MAVGGNAYPRSYYRTSVNORARKRVERASDDSRAFITYYEGKHNHHHLQLRPPTSSTLSFSSPQHSNQAI 402 MAVGGNAYPRSYYRTSVNORARKRVERANDDPKAFITYYEGKHNHHLQLRPPTSSTLPFSSPQHSNQAI 402 MAVGGNAYPRSYYRTSVNORARKRVERASDDSRAFITYYGKHNHHLLLRPPSSSTLPTTF 394 MVVGGNAYPRSYYRTSANGRARKHVERASDDPRAFITYYEGKHNHH-LLLSPPSSSTLPFNSPQLSKQTI 429 **. **********************************	
Fig. 5 Alignment of putative protein sequences of MINI3 from <i>Brassica</i> and <i>Arabidopsis</i> . BrMINI3 is putative protein of	BrMINI3 BnMINI3a_P2 BnMINI3a_P1 AtMINI3	MNAFDGTYRGVRTCWAAPSSPSPRSLLAMLNQGDNNDVVDQINEIFPQANHQPEQRSSLRERVAARVEFNLPPLETQNNR-PFA 83 MNAFDGTYRGVRTCWAAPSSPSPRSLLAMLNQGDNNDVVDQINEIFPQANHQPEQRSSLRERVAARVEFNLPPLETQNNR-PFA 83 MNAFDGTYRGVRTCWAAPSSPSPRSLLAMLNQGDNNDVVDQINEIFPQTNHQPEQRSSLRERVAARVEFNLPPLETQNNR-PFA 83 MSDFDENFIEMTSYWAPPSSPSPRTILAMLEQTDNGLNPISEIFPQESLPRDHTDQSGQRSGLRERLAARVGFNLPTLNTEENNSPLD 88 *. **.:::::***.*******::*****:****	3 3 3 8
<i>B. rapa.</i> WKKY domain defined by the conserved amino acid sequence WRKYGQK, together with a C_2H_2 motif, is highlighted in <i>black boxes</i>	BrMINI3 BnMINI3a_P2 BnMINI3a_P1 AtMINI3	AFFRNPSTTVP-SPLVLISPGFSPSAMLQFPNTFIDPSHMILPSPVANGGPPEAVESSGADHATMMISNNDPMHVALP16 AFFRNPSTTVP-SPLVLISPGFSPSAMLQFPNTFIDPSHMILPSPVANGGPPEAVESSGADHATMMISNNDPMHVALP16 AFFRNPSTTVP-SPLVLISPGFSPSAMLQFPNTFIDPSHMILPSPVANGGPPEAVESSGADHATMMISNNDPMHVALP16 AFFRNPSTTVP-SPLVLISPGFSPSAMLQFPNTFIDPSHMILPSPVANGGPPEAVESSGADHATMMISNNDPMHVALP16 AFFR-SSNVPNSPVVAISPGFSSALHTPNMVSDSSQIIPPSSATNYGPLEMVETSGEDNAAMMMFNNDLPYQPYNVDLPSLEVFDDI17 **** *:***	50 60 50 76
	BrMINI3 BnMINI3a_P2 BnMINI3a_P1 AtMINI3	PQQVAAFESGPALN-ETDLINMEIDRKNEDEEEYKEDEDEEHNIVDELDAE 21PQQVAAFESGPALN-ETDLINMEIDRKNEDEEEYKEDEDEEHNIVDELDAE 21PQQVAAFESGPALN-ETDLINMEIDRKNEDEEEYKEDEDEEHNIVDELDAE 21 ATEESFYIPSYEPHVDPIGTPLVTSFESELVDDAHTDIISIEDSESEDGNKDDDDDFQYEDEDEDQVDQDQDVDEDEEEEKDEDNVALD 26 . *::*** .: .**:*. :* .: ::**::::::::::	10 10 10 66
	BrMINI3 BnMINI3a_P2 BnMINI3a_P1 AtMINI3	PSSPKRRKPGESTMIGATRSCKSQRVILQMETEENNPDDGPFWRKYGGBVVKGNPNPRSYYKGTYTADVKKHVERGAEDVKFLLVT 22 PSSPKRRKFGESTMIGATRSCKSQRVILQMETEENNPDDGPFWRKYGGBVVKGNPNPRSYYKGTYTADVKKHVERGAEDVKFLLVT 23 PSSPKRRKFGESTMIGATRSCKSQRVILQMETEENNPDDGPFWRKYGGBVVKGNPNPRSYYKGTYTADVKKHVERGAEDVKFLLVT 23 DPQPPPPKRRRYEVSNMIGATRTSKTQRIILQMESDEDNPNDGYFWRKYGGBVVKGNPNPRSYFKGTNIFGRVKKHVERGAEDVKFLLVT 35 ******: *.******: *.******:*:*********	97 97 97 56
	BrMINI3 BnMINI3a_P2 BnMINI3a_P1 AtMINI3	YDGIIE DPPAARGSSSSGLKGQYSSSVSQDHNNHRTVPPSSSSASEALRFFPSSLDPPVDMTQFYMTGLAKLPSLPV 37 YDGIIE DPPAARGSSSSGLKGQYSSSVSQDHNNHRTVPPSSSSASEALRFFPSSLDPPVDMTQFYMTGLAKLQSLPV 37 YDGIIE DPPAARGSSSSGLKGQYSSSVSQDHNNHRTVPPSSSSASEALRFFPSSLDPPVDMTQFYMTGLAKLPSLPV 37 YDGIIN PSPPARRSNSSSRNRSAGATIPQNQNDRTSRLGRAPPTPTPPPPPSSYTPEEMRPF-SSLATEIDLTEVYMTGISMLPNIPV 44 ******: * .** * .** . : . :::::::::::::	75 75 75 45
	BrMINI3 BnMINI3a_P2 BnMINI3a_P1 AtMINI3	YQNHGLMNWNNEPEIDRVIPDGTEVFKGIRDRLNLNFGLNL 416 YQNHGLMNWNNEPEIDRVIPDGTEVFKGIRDRLNLNFGLNL 416 YQNHGLMNWNNEPEIDRVIPDGTEVFKGIRDRLNLNFGLNL 416 YENSGFMYQNDEPTNN-AMPDGSDVYDGIMERLYFKFGVDM 485 *:* *:* *:** :: ::*:::::::::::::::::::	

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

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 F₂ 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)

of *PstI*. **b** PCR products amplified from SW Hickory, JA177 and their hybrid (F_I) 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*

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 OTLs 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	Genot	ype		Ν	1,000-seed weight (g)			
	10509	J0609	A5		Mean ± SD (2007)	Mean ± SD (2008)		
Single loc	us effec	et ^a						
<i>I0509</i>	AA			84	$2.72\pm0.34~\mathrm{A}$	$2.94\pm0.36~A$		
	BB			101	$2.41\pm0.27~B$	$2.61\pm0.36~B$		
J0609		AA		92	$2.71\pm0.34~\mathrm{A}$	$2.91\pm0.39~A$		
		BB		98	$2.40\pm0.26~\mathrm{B}$	$2.61\pm0.34~B$		
MINI3a			AA	90	2.61 ± 0.36 a	2.80 ± 0.42 a		
			BB	90	$2.50\pm0.31~\text{b}$	2.73 ± 0.36 a		
TTG2a			AA	91	$2.62\pm0.36~\mathrm{A}$	2.79 ± 0.43 a		
			BB	99	$2.49\pm0.31~\mathrm{B}$	2.72 ± 0.35 a		
Multiple 1	ocus ef	fect ^b						
Ι	AA	AA	AA	19	2.97 ± 0.37 a	$3.18\pm0.43~a$		
II	AA	AA	BB	19	$2.76\pm0.29~b$	3.01 ± 0.32 ab		
III	BB	AA	AA	12	$2.60\pm0.26~bc$	$2.80\pm0.39~\text{bc}$		
IV	AA	BB	AA	13	$2.58\pm0.22~\mathrm{c}$	2.85 ± 0.26 bc		
V	BB	AA	BB	23	$2.54\pm0.24~cd$	$2.76\pm0.31~\mathrm{c}$		
VI	AA	BB	BB	14	$2.49\pm0.23~cd$	2.79 ± 0.28 bc		
VII	BB	BB	AA	23	2.38 ± 0.22 de	$2.52\pm0.34~d$		
VIII	BB	BB	BB	23	2.24 ± 0.25 e	$2.45\pm0.33~d$		

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 OTLs 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.

Acknowledgments The authors thank Drs. Jinling Meng, Jinxin Tu and Jianyi Zhao for providing SSR markers. The authors also appreciate three anonymous reviewers for their valuable comments and suggestions on the manuscript. This research was financially supported by National Basic Research Program (2006CB101604), National High-tech R&D Program (2006AA101A113) from Ministry of Science and Technology of China, National Natural Science Foundation of China (No. 30623012) and Science Foundation for the Youth Scholars of Ministry of Education of China (No. 20070504031).

References

- Adamskia NM, Anastasioub E, Erikssona S, O'Neillc CM, Lenharda M (2009) Local maternal control of seed size by KLUH/ CYP78A5-dependent growth signaling. Proc Natl Acad Sci USA 106:20115–20120
- Alonso-Blanco C, Blankestijn-De VH, Hanhart CJ, Koornneef M (1999) Natural allelic variation at seed size loci in relation to other life history traits of *Arabidopsis thaliana*. Proc Natl Acad Sci USA 96:4710–4717
- Basunanda P, Radoev M, Ecke W, Friedt W, Becker HC, Snowdon RJ (2010) Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (*Brassica* napus L.). Theor Appl Genet 120:271–281
- Butruille DV, Guries RP, Osborn TC (1999) Linkage analysis of molecular markers and quantitative trait loci in populations of inbred backcross lines of *Brassica napus* L. Genetics 153:949– 964
- Chen W, Zhang Y, Liu XP, Chen BY, Tu JX, Fu TD (2007) Detection of QTL for six yield-related traits in oilseed rape (*Brassica napus*) using DH and immortalized F₂ populations. Theor Appl Genet 115:849–858
- Cheng XM, Xu JS, Xia S, Gu JX, Yang Y, Fu J, Qian XJ, Zhang SC, Wu JS, Liu KD (2009) Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor Appl Genet 118:1121–1131
- Cheung WY, Champagne G, Hubert N, Landry BS (1997) Comparison of the genetic maps of *Brassica napus* and *Brassica oleracea*. Theor Appl Genet 94:569–582
- Clarke JM, Simpson GM (1978) Influence of irrigation and seeding rates on yield and yield components of *Brassica napus* cv. Tower. Can J Plant Sci 58:731–737
- Coventry SJ, Barr AR, Eglinton JK, McDonald GK (2003) The determinants and genome locations influencing grain weight and size in barley (*Hordeum vulgare* L.). Aust J Agric Res 54:1103– 1115
- Doebley J, Bacigalupo A, Stec A (1994) Inheritance of kernel weight in two maize-teosinte hybrid populations: implications for crop evolution. J Hered 85:191–195
- Doganlar S, Frary A, Tanksley SD (2000) The genetic basis of seed weight variation: tomato as a model system. Theor Appl Genet 100:1267–1273
- Fan CC, Xing YZ, Mao HL, Lu TT, Han B, Xu CG, Li XH, Zhang QF (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171
- Ferreira ME, Williams PH, Osborn TC (1994) RFLP mapping of Brassica napus using doubled haploid lines. Theor Appl Genet 89:615–621
- Garcia D, Jonathan N, Fitz Gerald, Berger F (2005) Maternal control of integument cell elongation and zygotic control of endosperm growth are coordinated to determine seed size in *Arabidopsis*. Plant Cell 17:52–60
- Geritz S, Meijdenb E, Metz J (1999) Evolutionary dynamics of seed size and seedling competitive ability. Theor Popul Biol 55:I324–I343
- Groos C, Robert N, Bervas E, Charmet G (2003) Genetics analysis of grain protein-content, grain yield and thousand-kernel weight in breed wheat. Theor Appl Genet 106:1032–1040

- Hyten DL, Pantalone VR, Sams CE, Saxton AM, Landau-Ellis D, Stefaniak TR, Schmidt ME (2004) Seed quality QTL in a prominent soybean population. Theor Appl Genet 109:552–561
- Iniguez-Luy FL, Voort AV, Osborn TC (2008) Development of a set of public SSR markers derived from genomic sequence of a rapid cycling *Brassica oleracea* L. genotype. Theor Appl Genet 117:977–985
- Jofuku KD, Omidyar PK, Gee Z, Okamuro JK (2005) Control of seed mass and seed yield by the floral homeotic gene *APETALA2*. Proc Natl Acad Sci USA 102:3123–3128
- Johnson CS, Kolevski B, Smyth DR (2002) TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of arabidopsis, encodes a WRKY transcription factor. Plant Cell 14:1359–1375
- Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175
- Lark KG, Chase K, Adelf F, Mansur LM, Orf JH (1995) Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proc Natl Acad Sci USA 92:4656–4660
- Lincoln S, Daly M, Lander E (1992) Constructing genetics maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report. Whitehead Institute, Cambridge
- Lionneton E, Aubert G, Ochatt S, Merah O (2004) Genetic analysis of agronomic and quality traits in mustard (*Brassica juncea*). Theor Appl Genet 109:792–799
- Liu DF, Liu HL (1987) Studies on genetic variation of quantitical traits in *Brassica napus* L. Acta Genet Sin 14:31–36
- Lowe A, Moule C, Trick M, Edwards K (2004) Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor Appl Genet 108:1103–1112
- Luo M, Dennis ES, Berger F, Peacock WJ, Chaudhury A (2005) MINISEED3 (MINI3), a WRKY family gene, and HAIKU2 (IKU2), a leucine-rich repeat (LRR) KINASE gene, are regulators of seed size in Arabidopsis. Proc Natl Acad Sci USA 102:17531– 17536
- Lysak MA, Koch MA, Pecinka A, Schubert I (2005) Chromosome triplication found across the tribe *Brassiceae*. Genome Res 15:516–525
- Maughan PJ, Saghai Maroof MA, Buss GR (1996) Molecular marker analysis of seed-weight: genomic locations, gene action, and evidence for orthologous evolution among three legume species. Theor Appl Genet 93:574–579
- Morgan CL, Arthur AE, Rawsthorne S (1998) Influence of testa colour and seed size on storage product composition in *Brassica juncea*. Plant Var Seeds 11:73–81
- Ohto MA, Fischer RL, Goldberg RB, Nakamura K, Harada JJ (2005) Control of seed mass by *APETALA2*. Proc Natl Acad Sci USA 102:3117–3122
- Parkin IAP, Sharpe AG, Keith DJ, Lydiate DJ (1995) Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome 38:1122–1131
- Paterson AH, Lin YR, Li ZK, Schertz KF, Doebley JF, Pinson SRM, Liu SC, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714–1718
- Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet I, Perret D, Villeger MJ, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523
- Plieske J, Struss D (2001) Microsatellite markers for genome analysis in *Brassica*. I. Development in *Brassica napus* and abundance in Brassicaceae species. Theor Appl Genet 102:689–694
- Qi CK, Gai JY, Fu SZ, Pu HM, Zhang JF, Chen XJ, Gao JQ (2004) Analysis of genetic system of 1,000 seed weight in *Brassica* napus L. Acta Agron Sin 30:1274–1277

- Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li J, Li D, Meng J, Bancroft I (2006) A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet 114:67–80
- Quijada PA, Udall JA, Lambert B, Osborn TC (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring oilseed rape (*Brassica napus* L.): 1. Identification of genomic regions from winter germplasm. Theor Appl Genet 113:549–561
- Schruff MC, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ (2005) The AUXIN RESPONSE FACTOR 2 gene of *Arabidopsis* links auxin signaling, cell division, and the size of seeds and other organs. Development 133:251–261
- Shi JQ, Li RY, Qiu D, Jiang CC, Long Y, Morgan C, Bancroft I, Zhao JY, Meng JL (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in *Brassica napus*. Genetics 182:851–861
- Sun Z, Wang Z, Tu J, Zhang J, Yu F, McVetty P, Li G (2007) An ultradense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers. Theor Appl Genet 114:1305–1317
- Suwabe K, Iketani H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 104:1092–1098
- Suwabe K, Morgan C, Bancroft I (2008) Integration of *Brassica* A genome genetic linkage map between *Brassica napus* and *B*. rapa. Genome 51:169–176

- Udall JA, Quijada PA, Lambert B, Osborn TC (2006) Quantitative trait analysis of seed yield and other complex traits in hybrid spring oilseed rape (*Brassica napus* L.): 2. Identification of alleles from unadapted germplasm. Theor Appl Genet 113:597– 609
- Uzunova M, Ecke W, Weissleder K, Röbbelen G (1995) Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor Appl Genet 90:194–204
- Wang S, Basten CJ, Zeng ZB (2004) Windows QTL Cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh
- Weng J, Gu S, Wan X, Gao H, Guo T, Su N, Lei C, Zhang X, Cheng Z, Guo X, Wang J, Jiang L, Zhai H, Wan J (2008) Isolation and initial characterization of *GW5*, a major QTL associated with rice grain width and weight. Cell Res 18:1199–1209
- Westermeier P, Wenzel G, Mohler V (2009) Development and evaluation of single-nucleotide polymorphism markers in allotetraploid rapeseed (*Brassica napus* L.). Theor Appl Genet 119:1301–1311
- Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:1527–1536
- Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231