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ORIGINAL PAPER

Characterization of the quantitative trait locus *OilA1* for oil content in *Brassica napus*

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Abstract Increasing seed oil content has become one of the most important breeding criteria in rapeseed (*Brassica napus*). However, oil content is a complex quantitative trait. QTL mapping in a double haploid population (SG population) emerging from a cross between a German (Sollux) and Chinese (Gaoyou) cultivars revealed one QTL for oil content on linkage group A1 (*OilA1),* which was mapped to a 17 cM genetic interval. To further validate and characterize the *OilA1*, we constructed a high-resolution map using *B. rapa* sequence resources and developed a set of near-isogenic lines (NILs) by employing a DH line SG-DH267 as donor and Chinese parent Gaoyou as recurrent background. The results showed highly conserved synteny order between *B. rapa* and *B. napus* within the linkage group A1 and revealed a possible centromere region between two markers ZAASA1-38 and NTP3 (2.5 cM). *OilA1* was firstly validated by 250 BC_5F_2 plants and was confirmed in a 10.6 cM interval between the markers ZAASA1-47 and ZAASA1-77. Further substitution mapping was conducted by using two generations of QTL-NILs, 283 lines from eight $BC_5F_{3:4}$ families and 428 plants from six BC_5F_4 sub-NILs and thus narrowed the *OilA1* interval to 6.9 cM and 4.3 cM (1.4 Mb), respectively. Field investigations with two replications using homozygous $BC_5F_{3:4}$ sister sub-NILs indicated that NILs, which carry a Sollux chromosome segment across the target region showed significant higher oil content (1.26 %, $p < 0.001$) than their sister NILs containing Gaoyou chromosome. The *OilA1* locus is of particular interest for breeding purpose in China because 80 % of Chinese cultivars do not carry this desirable allele.

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Introduction

In the past two decades, many studies focusing on QTL identification of oil content in *Brassica napus* (*B. napus*) have been carried out. A large body of QTL information is therefore presently available. Oil QTL were found in almost all the 19 linkage groups, ranging from 3 to 14 in different reports (Ecke et al. 1995; Burns et al. 2003; Zhao et al. 2005, 2006, 2012; Qiu et al. 2006; Delourme et al. 2006; Yan et al. 2009; Chen et al. 2010; Sun et al. 2012a).

However, it seems difficult to find a major QTL, which exhibits a predominant genetic effect over different environments. In most cases, the identified QTL depend largely on their genetic backgrounds and environmental conditions. Previously, we have identified nine oil QTL in the SG population, which were evaluated across 11 environments (Zhao et al. 2012). Among them, one locus on linkage group A1 (*OilA1*) was mapped to a 17.0 cM genomic region (near the public SSR markers Bras041, Bras078 and Ra2E04), demonstrating an increase in oil content by Sollux allele, a parent from Germany ($a = 0.31$–0.56 %). Although only 4.70–6.40 % of phenotypic variation could be explained by the *OilA1* locus in SG population, it was frequently detected in several other populations, such as RNSL, SD (Delourme et al. 2006, near the marker Bras041), and TN (Qiu et al. 2006, common marker Ra2E04). Moreover, recent association mapping studies also strongly supported the marker-trait association for oil content at this locus (Zou et al. 2010; Sun et al. 2012a). Notably, almost all alleles that were discovered for increasing oil content in different populations consistently originate from European winter rapeseed (Delourme et al. 2006; Qiu et al. 2006; Zhao et al. 2012).

Although DH populations could be a suitable material for primary QTL analysis, it is difficult, however, to use them for fine mapping and map-based gene cloning purposes (Zamir 2001). Indeed, limitation in using this type of bi-parental populations is mainly due to the genetic background noise (Xing et al. 2008; Zamir 2001). In addition, the confidence interval of a QTL region determined in primary QTL survey is usually too large (10–20 cM) and has to be narrowed down before further characterization. To resolve these problems, a marker based backcross strategy was proposed by developing near-isogenic lines (NILs) (Tanksley and Nelson 1996). This has been successfully used in fine mapping and cloning genes underlying QTL in many other crops such as tomato (Xu et al. 2008; Zhang et al. 2012b), wheat (Alfares et al. 2009; Jakobson et al. 2012), maize (Liu et al. 2012) and rice (Jiao et al. 2010; Zhang et al. 2012c). In contrast, despite a large number of available QTL information for oil content in rapeseed, fine mapping and map-based gene cloning remain rarely explored. Nevertheless, a recent report on complete sequencing from *B. rapa* (Cheng et al. 2011; http://brassicadb.org/brad/) not only promotes the study on vegetable *B. rapa* crops, but also shed lights on research in *B. napus* (Zhang et al. 2012a; Sun et al. 2012a).

In the present study, we developed near-isogenic lines (NILs) containing introgressions from a donor parent "Sollux", using advanced backcross approach to establish BC_5F_2, BC_5F_3 and BC_5F_4 progenies. The objectives of the study were to (1) construct a high-resolution map for *OilA1*, (2) validate and reduce the QTL region by fine mapping with advanced backcross generations and thus (3) characterize the genetic effect of this novel locus.

Materials and methods

Plant materials and NILs construction

Using the previously developed SG-DH population (Zhao et al. 2005), *OilA1* was mapped in the interval between flanking markers ZASSA1-73 and NTP3 in linkage group A1 (Zhao et al. 2012). To define this QTL, one DH line SGDH-267 was chosen as the donor for successive backcrosses with recurrent parent Gaoyou, since it contains a Sollux introgression in the target region and exhibits higher oil content than both parents (Fig. 1). As shown in Fig. 2, after four backcross generations based on linked markers, three plants (HI3, PQ4 and PQ5) from two BC_4F_1 lines (HI and PQ) were selected. HI and PQ were derived from selected BC_3F_1 plants N5-4 and N9-3, which showed 96 and 93 % genetic background of Gaoyou and evaluated by 250 SSR/STS markers distributed evenly in 19 SG-linkage groups. Both plants contained the heterozygous alleles Sollux/Gaoyou in target region. The three lines (HI3, PQ4 and PQ5) were further backcrossed with Gaoyou to generate BC_5F_1 offsprings and followed by self-pollination to eliminate Sollux fragments in non-target genomic regions. In 2010, four BC_5F_2 lines, HI3-14, PQ4-16, PQ5-2 and PQ5-12 were sown in the field and 93 individual plants per each line were genotyped by target markers. Mature seeds were harvested from self-pollinated plants. In 2011, 726 plants from eight BC_5F_3 lines of PQ family (PQ4-16-42, PQ4-16-44, PQ5-2-42, PQ5-2-76, PQ5-2-78, PQ5-6-17, PQ5-6-18

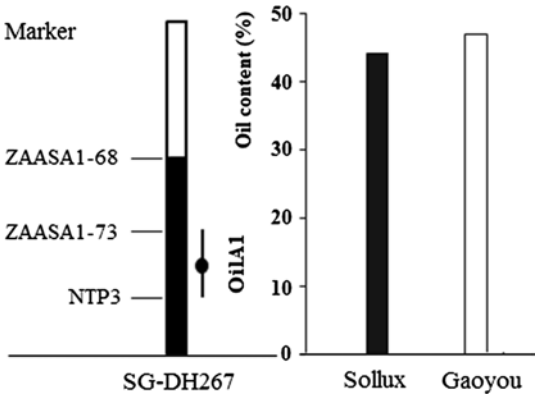


Fig. 1 Oil content of parents (Sollux and Gaoyou) and donor line SG-DH267 (average over 11 environments), *black* and *white* portions indicate Sollux and Gaoyou genomic regions, respectively. The *left* column also presents the linkage group A1 in SG population. Position of symbol indicates peak of *OilA1*. *Line* indicates interval of *OilA1* significant at $P < 0.05$

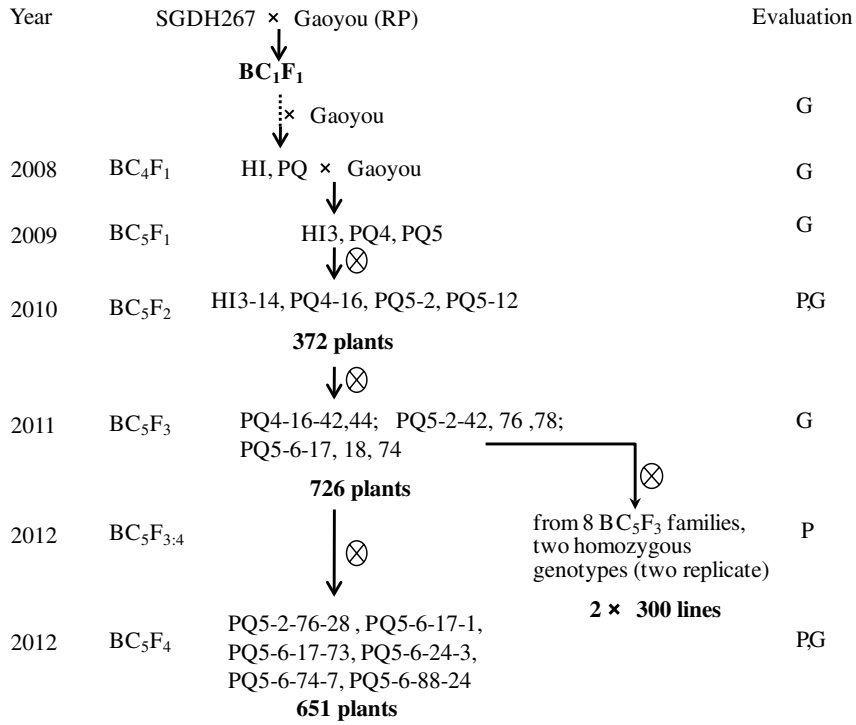


Fig. 2 Procedure of QTL-NILs development. *P* phenotyping, *G* genotyping

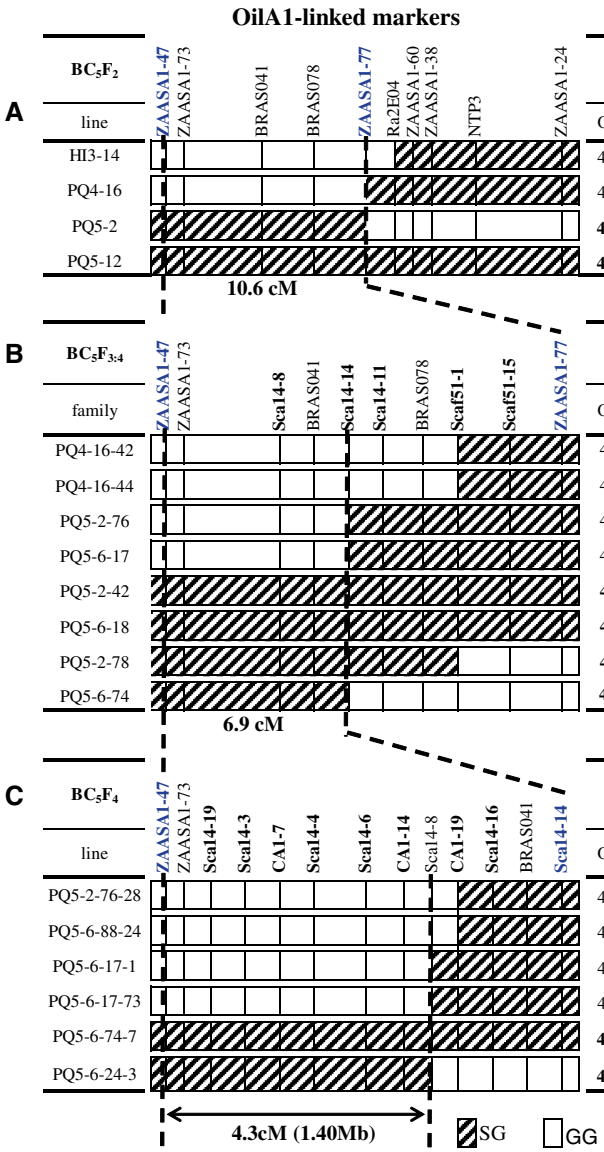
and PQ5-6-74) were further analyzed by newly developed locus specific markers. Among them, 300 $BC_5F_{3:4}$ NILs carrying either homozygous, Sollux or Gaoyou, genomic fragments overlapped in *OilA1* region were further characterized under field conditions. Six BC_5F_3 plants (PQ5-2-76-28, PQ5-6-17-1, PQ5-6-17-73, PQ5-6-24-3, PQ5-6-74-7 and PQ5-6-88-24) showing recombination in the target region were selected to generate 651 BC_5F_4 plants (186 plants for PQ5-6-88-24 and 93 for each of the rest five lines) for marker analysis and phenotypic evaluation.

Field trials and trait evaluation

To evaluate the NILs for their oil content in seeds, the two parents (Sollux and Gaoyou) and three NIL generations (BC_5F_2, $BC_5F_{3:4}$ and BC_5F_4) were grown in the field of Hangzhou, China from 2010 to 2012. For BC_5F_2 and BC_5F_4 progenies, the seeds were sown in four rows per plot with 0.33 m between rows and 0.15 m between plants within rows. All the plants were self-pollinated by plastic bags from the beginning to the end of flowering. Experimental data for each individual plant were collected for both marker genotyping with young leaves and phenotyping for seed oil content. Moreover, a subset of 300 lines derived from eight $BC_5F_{3:4}$ families were evaluated in the growing period 2011–2012. For this experiment, a randomized complete block design with two replicates was used. The seeds were sown in double rows for each plot, with rows of 2.0 m length and a spacing of 0.4 m between rows and 0.12 m between plants within rows, consisting of 26 plants per line. At maturity, around 50 g seeds for each plot were bulk harvested from the terminal raceme and the two uppermost primary branches of healthy plants. The seed oil content was measured by Near Infrared Spectroscopy (NIRS) using standard methods (Mika et al. 2003). Each sample was measured three times, and the mean value was taken for statistic analysis. The number of plants obtained from BC_5F_2 and BC_5F_4 progenies for mapping analysis were 250 and 428, respectively, excluding those plants which showed recombination in the target region, those with too small amount of seeds for measuring oil content and those with diseases or lodging occurrence. In the case of $BC_5F_{3:4}$ families, 283 lines were used for analysis (Fig. 3).

Development of locus specific markers

Genomic DNA of BC_5F_2, BC_5F_3, BC_5F_4 plants and their parents were extracted from young leaves individually using a modified cetyltrimethyl ammonium bromide method (Zhao et al. 2005). A total of 108 primer pairs were tested with the two parents. Among them, 45 were obtained from 20 BAC sequences (NCBI GenBank) assigned to the whole *B. rapa* A1 genome, 44 derived from three Scaffold resources (Scaffold 000011, Scaffold 000014 and Scaffold000051) were focused on *OilA1* and 19 primer pairs flanking the reduced QTL region between ZAASA1-47 and Sca14-14 (6.9 cM) were based on the full sequence information of *B. rapa* (BRAD,



A BC_5F_2

line	ZAASA1-47	ZAASA1-73	BRAS041	BRAS078	ZAASA1-77	Ra2E04	ZAASA1-60	ZAASA1-38	NTP3	ZAASA1-24	SS Oil (%)±SD	SS n	SG Oil (%)±SD	SG n	GG Oil (%)±SD	GG n	P	No. of plants n	No. of plants total
HI3-14											47.31±3.27[a]	17	47.16±2.52[a]	28	47.37±1.98[a]	20	0.956	65	
PQ4-16											47.44±2.76[a]	13	46.69±3.48[a]	31	47.58±2.81[a]	20	0.567	64	**250**
PQ5-2											**48.94±1.76[a]**	**20**	**47.97±2.44[b]**	**36**	**47.24±3.43[c]**	**21**	**0.040**	**77**	
PQ5-12											**49.30±1.68[a]**	**12**	**48.55±1.88[b]**	**22**	**47.27±1.14[c]**	**10**	**0.023**	**44**	

B $BC_5F_{3:4}$

family	ZAASA1-47	ZAASA1-73	Sca14-8	BRAS041	Sca14-14	Sca14-11	BRAS078	Scaf51-1	Scaf51-15	ZAASA1-77	SS Oil (%)±SD	SS n	GG Oil (%)±SD	GG n	P	No. of lines n	No. of lines total
PQ4-16-42											47.46±0.63	11	47.39±0.64	10	0.791	21	
PQ4-16-44											47.47±1.06	17	47.58±0.79	21	0.707	38	
PQ5-2-76											46.90±0.73	11	46.85±0.89	15	0.882	26	
PQ5-6-17											46.23±0.91	22	46.33±0.98	33	0.716	55	**283**
PQ5-2-42											**47.41±0.95**	**18**	**46.04±0.79**	**15**	**0.004**	**33**	
PQ5-6-18											**48.31±0.52**	**16**	**47.00±0.94**	**13**	**0.000**	**29**	
PQ5-2-78											**47.51±0.78**	**13**	**46.35±0.60**	**16**	**0.001**	**29**	
PQ5-6-74											**48.02±0.47**	**26**	**46.83±0.82**	**26**	**0.000**	**52**	

C BC_5F_4

line	ZAASA1-47	ZAASA1-73	Sca14-19	Sca14-3	CA1-7	Sca14-4	Sca14-6	CA1-14	Sca14-8	CA1-19	Sca14-16	BRAS041	Sca14-14	SS Oil (%)±SD	SS n	SG Oil (%)±SD	SG n	GG Oil (%)±SD	GG n	P	No. of plants n	No. of plants total
PQ5-2-76-28														42.74±1.53[a]	12	42.55±1.52[a]	20	43.08±1.06[a]	12	0.589	44	
PQ5-6-88-24														42.70±1.73[a]	29	42.50±1.59[a]	56	42.64±1.60[a]	27	0.858	112	
PQ5-6-17-1														44.24±1.93[a]	21	44.23±2.21[a]	37	44.62±1.88[a]	20	0.772	78	**428**
PQ5-6-17-73														42.42±1.39[a]	19	42.53±1.37[a]	40	42.82±1.78[a]	19	0.680	78	
PQ5-6-74-7														**45.46±1.06[a]**	**12**	**44.14±1.43[b]**	**24**	**44.37±1.02[b]**	**12**	**0.016**	**48**	
PQ5-6-24-3														**43.45±1.34[a]**	**21**	**43.16±1.72[b]**	**28**	**42.20±1.39[c]**	**19**	**0.030**	**68**	

Fig. 3 Fine mapping of *OilA1* from F_2 to F_4 generations of BC_5 populations, *White* portions of the graph indicate homozygous Gaoyou/Gaoyou segment, *diagonal slashes* present heterozygous regions Gaoyou/Sollux. *SS* homozygous for Sollux alleles, *GG* homozygous for Gaoyou alleles and *SG* heterozygote of Sollux/Gaoyou alleles. The table to the *right* of the graphical genotypes indicate mean oil content and standard deviation (SD) for each of the three (SS, SG, GG) or two (SS, GG) genotypic classes. *P* values and the superscript letters (**a**, **b** and **c**) on the *right* panel indicate significant difference in oil content among three or two marker genotypes within each line at $P = 0.05$ based on the *t*-test. **a** BC_5F_2 QTL-NILs: population size, $n = 250$ plants, the *broken vertical lines* indicate mapped OilA1 location between ZAASA1-47 and ZAASA1-77. **b** $BC_5F_{3:4}$ families: population size, $n = 283$ lines from 8 $BC_5F_{3:4}$ families; trait performance obtained from field test by two replications; the *broken vertical lines* indicate mapped *OilA1* location in a 6.9 cM region between ZAASA1-47 and Sca14-14. *Five markers* with *bold* and *black* are newly integrated with generation of BC_5F_3 **c** BC_5F_4 QTL-NILs: population size, $n = 428$ plants; the *broken vertical lines* indicate finally mapped *OilA1* region between ZAASA1-47 and Sca14-8, corresponding to a 1.4 Mb physical distance of *Brassica rapa*. *Eight markers* with *bold* and *black* are newly developed by generation of BC_5F_4

http://brassicadb.org/brad). These markers were designated as ZAASA1, Sca and CA1, respectively. The process of primer production was the same as described in our previous study (Zhao et al. 2012). All primer pairs that showed polymorphisms between the two parents were applied to the population of SG-lines first, and those located in the *OilA1* target region were used for genotyping the NILs of BC_5F_2, BC_5F_3 and BC_5F_4 plants.

The PCR reactions were carried out in a total volume of 10 μl as previously described (Zhao et al. 2005). Two types of markers, STS (sequence tagged site) and SSCP (single-strand conformational polymorphism), were employed for

testing the length polymorphism and conformation differences (Zhao et al. 2012). Polyacrylamide gels with different concentrations (6 and 8 %) were chosen for electrophoretic separation according to the size of the PCR products and visualized by a rapid silver staining (Sanguinetti et al. 1994).

Construction of linkage group A1

Linkage analysis was performed by MAPMAKER/EXP version 3.0 (Lincoln et al. 1993), using a maximum recombination fraction of 30 cM (Kosambi function) and a minimum LOD threshold of 3.0. Commands "near" and "try" were used to assign newly developed markers to linkage group 1 (A1) of the SG-map (Zhao et al. 2012).

Genome alignment among *B. napus*, *B. rapa* and *A. thaliana*

Forty-nine markers designed from *B. rapa*-A1 genome sequences were aligned to the physical map of *B. rapa*-A (Brapa_sequence_v1.1.fa) and *A. thaliana*, using the BLASTN program from BRAD and NCBI database, respectively. The homologous loci ($E < 10^{-30}$) were located on a physical map of *A. thaliana,* using the SeqViewer program from TAIR (http://www.arabidopsis.org/).

Data analysis

Analyses of variances were performed using SPSS Version 17 (Statistical Product and Service Solutions) as described previously (Bryman 2012). Phenotypic means were compared using *t*-test to test variation of oil content among three allelic combinations within each BC_5F_2 and BC_5F_4 lines, and estimate the difference of oil content between two homozygous genotypes within each of eight $BC_5F_{3:4}$ families. All families were tested in open field condition.

Results

Construction of high-resolution map of SG-A1

From a total of 108 STS/SSCP markers derived from *B. rapa* A1 genome sequences that were screened for polymorphism between the two parents Sollux and Gaoyou, 15 (13.9 %) markers exhibited polymorphism in fragment length and 40 (37 %) showed a clear difference in single-strand conformation. These informative markers were subsequently used to genotype the SG population. Twenty-nine of them could be integrated into the linkage group A1 (Fig. 4; Table 1), while the remaining markers were mapped to C1 and other linkage groups. Among these 29 newly mapped loci, 16 fell into the confidence interval (17.0 cM) of *OilA1*, including 8 from *B. rapa* Scaffold 000014, 4 from Scaffold 000051, 1 from BAC KBrB036M22, and another 3 locus specific markers were based on complete *B. rapa* genome sequence in the reduced QTL region between marker ZAASA1-47 and Sca14-14.

The present SG-A1 is composed of 60 marker loci. As shown in Fig. 4 and supplemental Table 1 (Table S1), 49 could be physically aligned with *B. rapa* A1, which covers almost the whole *B. rapa* A1 genome. Notably, except for two markers on the top position (CN1 and CB10081), all the other 47 sequence-based loci exhibited completely consistent collinearity with the physical map of *B. rapa.* However, the average physical distance covered in the *B. rapa* A1 genome per 1 cM of genetic distance in the *B. napus* showed skewed distribution across the whole *B. rapa* A1 genome. The top part of 62.5 cM (CN1- ZAASA1-38) and lower part of 19.1 cM (NTP3- Ra3H09b) in SG-A1 map are homologous to 11.85 and 7.44 Mb in *B. rapa* genome, giving a ratio per 1 cM of the *B. napus* to 189 and 389 kb of *B. rapa* sequences, respectively. It is worth noting that the 2.5 cM interval between ZAASA1-38 and NTP3 corresponds to a large physical distance of 8.45 Mb, with a ratio of 1 cM in SG-A1 to 3,380 kb in *B. rapa* physical map.

A collinearity between SG-A1 and *A. thaliana* genome was determined by alignment (Fig. 4). Among 49 *B. rapa* sequence based loci, 38 could be physically assigned to *A. thaliana* chromosome 3 (AtC3) and chromosome 4 (AtC4) on the basis of sequence identity of $E < 10^{-30}$. These loci harboured 71.8 cM genetic distance (85.4 % of total linkage group A1) and exhibited significant homology (with four or more markers showing collinearity between two species) to four conserved blocks of *A. thaliana*, with one in chromosome 3 (2.14–7.63 Mb) and three in chromosome 4 (14.13–18.56 Mb, 10.95–13.42 Mb and 8.51–9.70 Mb), respectively.

Validation and characterization of *OilA1* by QTL-NILs

HI and PQ, the BC_4F_1 source lines for NIL development, contained Sollux introgression across the entire 17.0 cM interval of *OilA1*. They were backcrossed with Gaoyou to generate BC_5F_1 lines. The lines were genotyped with five markers between ZAASA1-47 and ZAASA1-24 (ZAASA1-73, BRAS078, Ra2E04, NTP3 and ZAASA1-24) mapping to *OilA1*.Three informative recombinants (HI3-14, PQ4-16 and PQ5-2) carrying heterozygous introgression in part of *OilA1* interval, and one non-recombinant heterozygous in target region (PQ5-12) were self-pollinated to produce four BC_5F_2 sub-NILs (Fig. 2, 3a). Subsequently, 250 individuals derived from these sub-NILs were assayed by all the ten markers between ZAASA1-47 and ZAASA1-24, and their corresponding phenotypic variations were

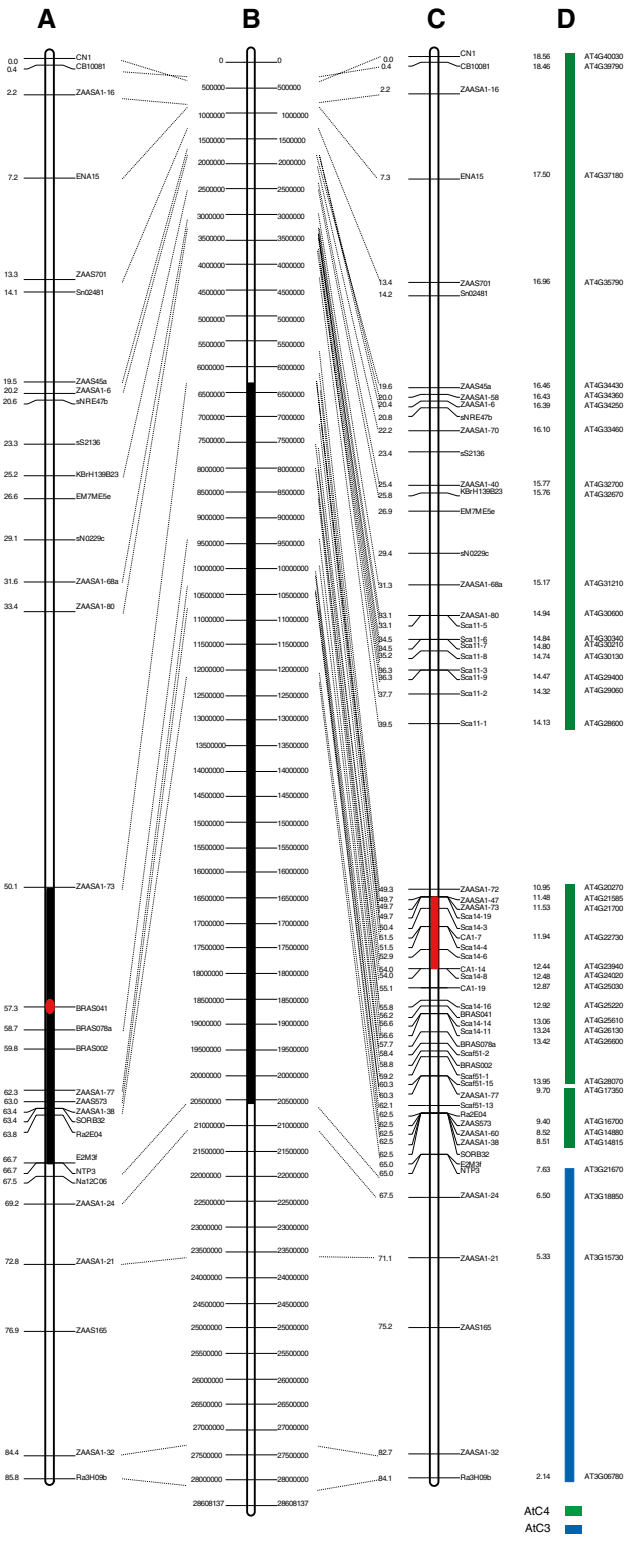


Fig. 4 Comparative alignment between SG-A1 and physical map of *B. rapa* A1, and collinearity with *A. thaliana*. **a** Previously published linkage group A1 in SG population (Zhao et al. 2012). **b** Physical map of *B. rapa* A1 genome (Chiifu-401). The base distances are listed on the *right* and *left* side of physical map. **c** The high-resolution map focusing on *OilA1*, the locus name and genetic distance (cM) are listed on the *right* and *left* side of the linkage group. Marker loci in SG-A1 and their corresponding physical position in *B. rapa* genome are connected by *dotted lines*. **d** Co-linearity regions in *A. thaliana* genome. The locus name and mega base distance of homologous *A. thaliana* regions are, respectively given on the *right* and *left* side of the *colored vertical bars*, which represent the genomic regions from chromosome 3 and chromosome 4 of *A. thaliana* (AtC3 and AtC4). The *black* and *red* regions on the Fig. 4a indicate the intervals of oil QTL overlapped within 1-LOD region and the intervals between peak positions in different environments (Zhao et al. 2012), while the *red* region in Fig. 4c present the final interval of *OilA1* (4.3 cM) by substitution mapping with BC_5F_4 QTL-NILs, respectively

evaluated based on three marker genotypes Sollux/Sollux (SS), Sollux/Gaoyou (SG) and Gaoyou/Gaoyou (GG). As shown in Fig. 3a, no significant associations for oil content with three marker genotypes (SS, SG and GG) were observed in populations HI3-14 ($n = 65$) and PQ4-16 ($n = 64$), while significant differences of oil content were detected among three genotypic classes (SS, SG and GG) in another two sub-populations PQ5-2 ($n = 77$) and PQ5-12 ($n = 44$) (ANOVA, $P < 0.05$). It is noteworthy that the genetic region between ZAASA1-47 and ZAASA1-77 are homozygous for Gaoyou fragment in the first two NILs (HI3-14 and PQ4-16), while a clear 1:2:1 (SS:SG:GG) segregation pattern in PQ5-2 and PQ5-12 populations were presented (date not show). Thus, *OilA1* was confirmed in this region (ZAASA1-47 to ZAASA1-77) with a genetic distance of 10.6 cM (Fig. 3a), which overlaps with the original *OilA1* mapping interval (Zhao et al. 2012).

To be able to have a more precise position of this QTL, five locus specific markers were developed to further narrow down the ZAASA1-47 and ZAASA1-77 interval. Using ten markers (including five newly developed markers), a total of 726 plants derived from six BC_5F_3 lines (from PQ family) that showed recombination between ZAASA1-47 and ZAASA1-77 were genotyped together with two non-recombinant heterozygous PQ5-6-18 and PQ5-2-42. Six informative recombinants were further selected to generate BC_5F_4 sub-NILs by self-pollination. Meanwhile, 283 lines derived from eight $BC_5F_{3:4}$ families, which were supposed to carry on either homozygous Sollux or Gaoyou segments overlapping within 10.6 cM target region, were phenotyped by two replications in the next year. Phenotypic means of oil content were then compared among the two extreme genotypic classes in the interval ZAASA1-47 and ZAASA1-77 (Fig. 3b). The results demonstrated that the first four $BC_5F_{3:4}$ families (PQ4-16-42, PQ4-16-44, PQ5-2-76 and PQ5-6-17) showed consistent Gaoyou background in the interval between ZAASA1-47 and Sca14-14 and segregation in other regions. Two homozygous genotypes (SS and GG) showed no reliability to oil content in this case ($P = 0.716$–0.882). However, highly significant marker—trait associations for oil content between SS and GG genotypes were observed

Table 1 Characterization of new developed markers in the SG-A1 and their homologous regions in *Brassica rapa* A1

Marker	Assay type	Homologous to *Brassica rapa* A1		Primer fwd (5′–3′)	Primer rev (5′–3′)
		Corresponding region	Gene		
ZAASA1-58	STS	1834370–1834669	Bra011523	aggatgtactgtttgtggatgct	gaatccatcgccaaaagtgt
ZAASA1-70	STS	2153707–2154006	Bra011442	gagctcatccaacaacagca	tagaagctgatgtagcgtttgg
ZAASA1-40	STS	2489600–2489931	Bra011372	agttgaagttgcccataccg	tgtacctttgcgtgctcaag
Sca11-5	SSCP	3374364–3374672	No hits found	cagaaccagcttccctttca	ttgcatagcacaacatccaaa
Sca11-6	SSCP	3442790–3443093	Bra011184	caaattcaattccaattgctga	ttgtcatgaaaaaggcagaaa
Sca11-7	SSCP	3482740–3483043	Bra011174	catctacgaggaagagctcca	cctgttcttgagctatggtgtg
Sca11-8	SSCP	3562048–3562361	Bra011166	aaggagacaactcttccgca	gcaagtaatcgcttggcttc
Sca11-3	SSCP	3757213–3757334	No hits found	ttcgatcgtgtgtgtgtgtg	cattctcgtttcctacatgagc
Sca11-9	SSCP	3850279–3850580	Bra011105	cctgtggataaaaggccaaa	agctgatttgtgtcaatgcg
Sca11-2	SSCP	4051855–4052043	Bra011081	gcacaagggattcaaatgct	ggcccagtttcctctcagat
Sca11-1	SSCP	4280647–4280830	Bra011041	tgtctctgcgtgagacaagc	ctcaatgccgcctctatgtt
ZAASA1-72	STS	5751413–5751775	Bra013451	cgtgactctcgacgcttaca	tccaagttcttgagatgccc
ZAASA1-47	STS	6201229–6201532	Bra013541	tacagaaacgctaccccagg	aatcccaccttgtgcaagtc
Sca14-19	SSCP	6311746–6312051	Bra013554	gaagatggctcgtgcttcag	tctttcccaatgacacacca
Sca14-3	SSCP	6572260–6572409	No hits found	cgctgcatcgcctataaatc	gaggattgcatgatggagaga
CA1-7	SSCP	6777272–6777571	Bra013636	tcctccatgtcacacgaaaa	gatcaacgggttgaactgct
Sca14-4	SSCP	6789806–6790014	No hits found	tggctgatggttacacacaaa	catttagatgcaatatggcaaag
Sca14-6	SSCP	7141540–7141711	No hits found	caactcgggcaagaaagtgt	tcaaccaaaccgatgtgaaa
CA1-14	SSCP	7554582–7554892	Bra013747	tcacgtgtagaatcgttggc	atcatgacccaaccgctatc
Sca14-8	SSCP	7600326–7600534	Bra013754	agcctcgccgaactttaact	cagccactgatggtgaagaa
CA1-19	SSCP	8087886–8088215	Bra013856	gcctctaggggtttggagag	acaacactagctgctcccgt
Sca14-16	SSCP	8181387–8181699	Bra013874	ggaacagccttgctaggaca	gagcactccaaacacccataa
Sca14-14	SSCP	8379596–8379911	Bra013903	agcattctcgatgatgcaag	tctcagagcttccgcatctt
Sca14-11	SSCP	8673429–8673563	Bra013955	tgaagaaaatggtgccaatg	Ttgttcaccgttcactctgc
Scaf51-2	SSCP	9975713–9976124	No hits found	ttgtcgtcggattgttcttg	ccaacttcctccaaaattgc
Scaf51-1	SSCP	10042129–10042431	No hits found	attaatgcaatcgccaaaaa	tgtcggtcttgataggaagca
Scaf51-15	SSCP	10222217–10222518	Bra026286	tcctccagttaccgacatga	atccacttgaagtcgttggc
Scaf51-13	SSCP	10460387–10460711	Bra026239	gttcagcttctctggcaacc	gcttttgtcttggaagcctg
ZAASA1-60	STS	12148044–12148400	Bra036910	ccatcagtgcctttccaaat	gggagttgggttagcattca

in another four $BC_5F_{3:4}$ families (PQ5-2-42, $P = 0.004$; PQ5-6-18, $P = 0.000$; PQ5-2-78, $P = 0.001$ and PQ5-6-74, $P = 0.000$), which exhibit 1:2:1 (SS:SG:GG) marker segregation in the region from ZAASA1-47 to Sca14-14 (data not shown). When combining the two situations of eight $BC_5F_{3:4}$ families together, *OilA1* was reduced to the 6.9 cM interval between markers ZAASA1-47 and Sca14-14.

To further refine the position of *OilA1*, eight new markers were further added in the defined region and used to genotype 428 plants from six BC_5F_4 sub-NILs, which contained recombinant points between ZAASA1-47 and Sca14-14 (Fig. 3c). Phenotypic comparisons among the three genotypic classes in PQ5-6-74-7 and PQ5-6-24-3 suggested that *OilA1* could be narrowed down to the interval between ZAASA1-47 and Sca14-8, because significant differences in oil content between two homozygous genotypes were observed. This demonstrated the dominant role of Sollux allele over Gaoyou allele. The data also illustrate that there was no *OilA1* in the interval between Sca14-8 and Sca14-14, since the segregation of three marker genotypes from this interval did not correspond to the phenotypic performance. Therefore, the *OilA1* was finally mapped into a 4.3 cM genetic region. On the basis of sequencing information from *B. rapa* (http://brassicadb.org/brad), the physical distance between these two markers was estimated to be 1.4 Mb (A01: 6201229–7600534).

Allelic differentiation of *OilA1* effect revealed by replicated field test in $BC_5F_{3:4}$ families

The phenotypic performance, using four $BC_5F_{3:4}$ families (showing significant difference in oil content between two

Table 2 Genetic effect of *OilA1* in replicated progeny test derived from four $BC_5F_{3:4}$ families

$BC_5F_{3:4}$	Oil content (%)				Mean oil content (%) ± SD[a]				SS–GG[b] (%)	R^2(%)[c]
	N	Max	Min	Max–Min	*N*	SS	*N*	GG		
PQ5-2-42	33	48.60	44.62	3.98	18	47.41 ± 0.95	15	46.04 ± 0.79	1.37**	34.4
PQ5-6-18	29	48.69	44.96	3.73	16	48.31 ± 0.52	13	47.00 ± 0.94	1.31**	35.1
PQ5-2-78	29	48.93	45.94	2.99	13	47.51 ± 0.78	16	46.35 ± 0.60	1.16**	38.8
PQ5-6-74	52	49.09	45.17	3.92	26	48.02 ± 0.47	26	46.83 ± 0.82	1.19**	30.4
Mean	–	–	–	–	–	47.84 ± 0.67	–	46.58 ± 0.74	1.26**	34.7

** $P < 0.01$

[a] Standard deviation

[b] Presents the difference of oil content between two homozygous genotypes Sollux/Sollux (SS) and Gaoyou/Gaoyou (GG) in OilA1 locus

[c] Percentage of phenotypic variance explained by OilA1 in the four BC5F3:4 families as expressed by the difference between lines with highest and lowest mean values

homozygous marker genotypes SS and GG) in the field experiment by two replications (Table 2) demonstrated significantly higher mean oil contents of homozygous Sollux classes (47.41–48.31 %) compared to homozygous Gaoyou groups (46.04–47.00 %). The differences of oil content between the two classes ranged from 1.16 to 1.37 % and explained 30.4 to 38.8 % of the phenotypic variations observed in the four $BC_5F_{3:4}$ families as expressed by the difference between lines with highest and lowest mean values (Table 2). Furthermore, from the distribution of the oil content based on the two homozygous genotypes Sollux and Gaoyou over four $BC_5F_{3:4}$ families, only one line with an oil content <46 % but 29 lines with an oil content ≥48 % were found in the Sollux class, while in the Gaoyou group, 31 lines showed an oil content <46 % and only five lines had an oil content exceeding 48 % (Fig. 5). The mean difference in oil content between genotypes carrying the homozygous Sollux and Gaoyou *OilA1* alleles is 1.26 % (significant at $P < 0.001$).

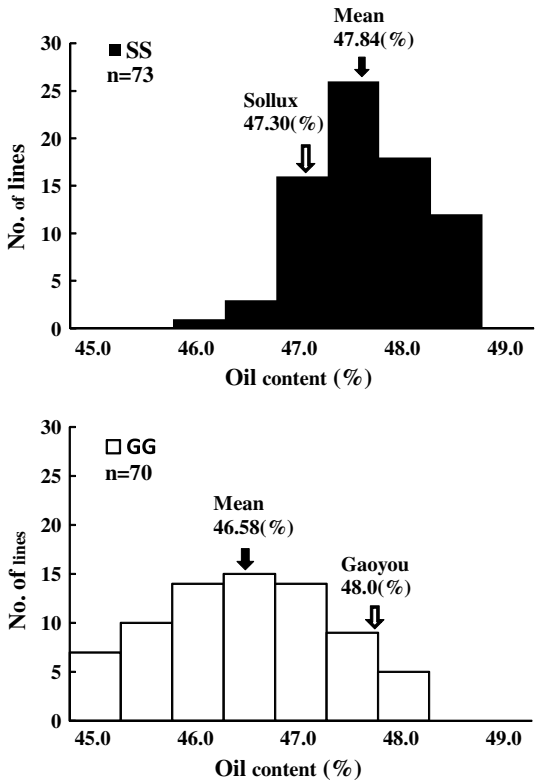


Fig. 5 Frequency distribution of oil content for two genotypic groups based on a two replicated progeny test by 143 lines from four $BC_5F_{3:4}$ families (PQ5-2-42, PQ5-2-78, PQ5-6-18 and PQ5-6-74). *SS* Sollux/Sollux, *GG* Gaoyou/Gaoyou, *solid arrows* show the mean oil content of each group and *hollow arrows* show the mean oil content of two parents Sollux and Gaoyou averaged by six replications

Discussion

Co-linearization among *B. napus*, *B. rapa* and *A. thaliana*

Many comparative genetic mapping studies have been conducted among various *Brassica* species and *Arabidopsis*, unraveling the extensive genome homology and microsynteny between *Brassica* species and *A. thaliana* and between the A, B, and C genomes of *Brassica* species (Parkin et al. 2005; Babula et al. 2003; Panjabi et al. 2008; Wang et al. 2011). Moreover, the recent sequencing of the *B. rapa* genome (Cheng et al. 2011; http://brassicadb.org/brad/) generated large amounts of genetic information for comparative study of the "A" genome in many other *Brassica* crops like rapeseed.

The present linkage group SG-A1 consists of 60 marker loci including 49 *B. rapa* sequences-based markers, which allows comparison with the *B. rapa* A1 genome sequence. Results showed a highly conserved marker order between the two *Brassica* species and a conserved synteny between *B. rapa* and *A. thaliana* (Fig. 4). In addition, of the 108

tested primer pairs based on *B. rapa* A1 sequences, half of them could be mapped in the SG-map and 29 were located on SG-A1, indicating high efficiency of *B. rapa* sequence-based markers in generating comparative relationships between *B. napus* and *B. rapa* and between *B. napus* and *Arabidopsis*. We compared SG-A1 with the high-resolution map of Parkin et al. (2005) and the newly published integrated *Brassica* maps by Wang et al. (2011). We found that the three *B. napus* A1 maps showed consistent collinearity along the entire length of the *Arabidopsis* genome. As evidenced by Parkin et al. (2005) and Wang et al. (2011), the top half of A1 shows significant homology to the long arm of *Arabidopsis* chromosome 4 (block C4B) and the lower half is homologous to the top arm of *Arabidopsis* chromosome 3 (block C3A). The present SG-A1 spans 84.1 cM in total length, which is 10–15 cM shorter than that revealed by Parkin et al. (2005) or Wang et al. (2011). The top of the 52.7 cM length is well aligned with three conserved blocks of At4 (block C4B) with one inversion in the middle, showing similar linearization with previous studies (Parkin et al. 2005; Wang et al. 2011). However, in the lower part, only around 20 cM exhibited collinearity with the block of AtC3A (Fig. 4). It is clearly shorter than that reported by Parkin et al. (2005) and Wang et al. (2011), who presented linkage group A1 of *B. napus* with 93.8 and 101 cM, respectively. The missing part is at the end of the chromosome corresponding to the top region of *Arabidopsis* C3A.

Comparative alignment between two *Brassica* species indicated that every one cM of genetic distance in *B. napus* is equivalent to an average of 334 kb *B. rapa* genomic sequence across the whole SG-A1. The ratio is a slightly higher than that in *Arabidopsis* (285 kb/cM, Parkin et al. 2002) but lower than that in *B. napus* (494 kb/cM, Lombard and Delourme 2001). Notably, the distribution between genetic distance of SG-A1 and the physical map of *B. rapa* A1 was strongly skewed in different genomic regions, as it was reported by Parkin et al. (2005). Particularly, the region between ZAASA1-38 and NTP3 covers 2.5 cM genetic distance, while this interval corresponds to 8.45 Mb in *B. rapa* A1 genome (A01:12175000-20631028), resulting in a ratio of 3,380 kb/cM; tenfold higher than that throughout the whole SG-A1 genome. Therefore, one can speculate that this region contains the centromere because recombination is clearly suppressed. A similar situation was observed in centromere regions of rice (Wu et al. 2002) and tomato (Ganal et al. 1989), and supports this assumption.

Strategy for fine mapping QTL with small magnitude effect

In *B. napus,* the large number of identified QTL for oil content did not lead to subsequent success in cloning functional genes. This could be due to two reasons. Firstly, most of the detected QTL display a minor genetic effect, and there is no major QTL identified so far which could explain over 20 % of the phenotypic variation. Secondly, the common existence of QTL × environment effects and epitasis among QTL result in complex genetic interactions, which are hard to explore. Therefore, the development of a strategy for better characterization of QTL for oil content remains a challenge. In this regard, our current study might provide a first evidence for achieving molecular dissection of QTL with small magnitude effects, using high-resolution NILs.

A QTL associated with oil content located in the linkage group 1 (A1) near the public SSR markers BRAS041 or Ra2E04 of *B. napus* has been frequently reported (Zhao et al. 2005, 2012; Delourme et al. 2006; Qiu et al. 2006), and was regarded as the same locus for oil content (Delourme et al. 2006; Zhao et al. 2012). In all cases, the European winter parent was associated with a favorable allele for oil content. The additive effect was small to medium as reported by Delourme et al. (2006) ($a = 0.78$ %, $R^2 = 8.4$ %), Qiu et al. (2006) ($a = 0.62$ %, $R^2 = 9.7$ %) and Zhao et al. (2012) ($a = 0.46$ %, $R^2 = 5.34$ %), but remained constant across diverse genetic backgrounds and different environments. To validate and fine map *OilA1*, a substitution mapping strategy was applied based on the advanced backcross populations and high-resolution SG-A1 map. We attempted to conduct substitution mapping by using the populations from selfing progenies of BC_4 generations (BC_4F_2 to BC_4F_4). The result showed no significant difference in oil content among three marker genotypes of *OilA1,* even though the Sollux allele has more favorable effect for oil content than the Gaoyou allele (data not shown). However, when back-crossing advanced generation to BC_5 and more markers were added to the target region, significant marker-trait associations were visualized in all three successive selfing progenies (F_2 to F_4). *OilA1* was finally fixed in a reduced genomic region (from 17.0 to 4.3 cM), which overlaps with the initial mapping study (Zhao et al. 2012).

Our present study demonstrated that it is possible to fine map QTL, which were of minor additive effect but stably expressed over diverse genetic backgrounds/environments. The key lays in developing advanced backcross NILs in combination with accurate phenotypic evaluation. Indeed, a small magnitude of QTL effect is difficult to be estimated under background noise with lower backcross generations. This issue was also frequently mentioned in rice. The decrease of donor's number (i.e., Sollux) of introgressions in the genetic background, combined with the increase in linkage between markers and the target gene(s) could give rise to the final enhancement of the percentage of

phenotypic variation explained by markers (Li et al. 2004; Xie et al. 2006).

Potential application of *OilA1* in breeding programs

In the present study, *OilA1* was confirmed to influence oil content in rapeseed by a significant allelic difference of 1.26 % ($P = 0.004$–0.000) between two homozygous genotypes (SS–GG), and explains 34.7 % of the total variation across four $BC_5F_{3:4}$ sub-NILs (Table 2). The genetic effect of *OilA1* may not be very large, yet it is important for breeding purpose, because oil content in rapeseed is governed by a number of genes, which show a typical quantitative trait feature. To date, no QTL with additive effect (a) over 2 % were identified. Apart from *OilA7* (Zhao et al. 2011), *OilA1* is another important QTL being isolated from an initial QTL study by means of advanced QTL-NILs. The genetic value of 1.26 % is generally in line with the previously assayed additive effect (Zhao et al. 2012, $2a = 2 \times 0.46\ \% = 0.92\ \%$). In addition, in our more recent research (Sun et al. 2012b), a significant marker-trait association in *OilA1* locus was observed in a breeding population consisting of 81 world-wide cultivars. 32 accessions with Sollux alleles showed statistically higher oil content than that of 49 varieties carrying the Gaoyou alleles. The difference of oil content between the two allelic groups was 1.33 % ($P < 0.05$), which is close to the value revealed in the present study. These results support previous findings, where *OilA1* can be stably expressed across diverse genetic background populations. According to our mapping study under 11 environments (unpublished results), *OilA1* interacted less with environments (one *ae* interaction, data not show) and with few epistatic interactions (involved only in one epistatic pair, data not show). Furthermore, allelic distribution of *OilA1* in 81 genetically different cultivars demonstrated that the majority of European cultivars (>80 %) have carried positive alleles for oil content in *OilA1* locus, while 80 % of Chinese materials have not, suggesting that the integration of favorable *OilA1* alleles into Chinese cultivars by marker assistant selection would be highly interesting for rapeseed breeding in China.

Conceived and designed the experiments: JZ. Performed the experiments: YC LQ XZ JH JW HC XN YD. Analyzed the data: YC LQ, Primers design: FX, Bioinformatics analysis: HX. Wrote the paper: JZ YC.

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