Theor Appl Genet (2012) 124:407–421
DOI 10.1007/s00122-011-1716-3

ORIGINAL PAPER

Molecular mapping of *Arabidopsis thaliana* lipid-related orthologous genes in *Brassica napus*

Jianyi Zhao · Jixiang Huang · Fei Chen · Fei Xu · Xiyuan Ni · Haiming Xu · Yilong Wang · Chonchon Jiang · Hao Wang · Aixia Xu · Ruizhi Huang · Dianrong Li · Jinling Meng

Received: 5 July 2011 / Accepted: 24 September 2011 / Published online: 13 October 2011

Abstract Quantitative Trait Loci (QTL) for oil content has been previously analyzed in a SG-DH population from a cross between a Chinese cultivar and a European cultivar of *Brassica napus*. Eight QTL with additive and epistatic effects, and with environmental interactions were evaluated. Here we present an integrated linkage map of this population predominantly based on informative markers derived from *Brassica* sequences, including 249 orthologous *A. thaliana* genes, where nearly half (112) are acyl lipid metabolism related genes. Comparative genomic analysis between *B. napus* and *A. thaliana* revealed 33 colinearity regions. Each of the conserved *A. thaliana* segments is present two to six times in the *B. napus* genome. Approximately half of the mapped lipid-related orthologous gene loci (76/137) were assigned in these conserved colinearity regions. QTL analysis for seed oil content was performed using the new map and phenotypic data from 11 different field trials. Nine significant QTL were identified on linkage groups A1, A5, A7, A9, C2, C3, C6 and C8, together explaining 57.79% of the total phenotypic variation. A total of 14 lipid related candidate gene loci were located in the confidence intervals of six of these QTL, of which ten were assigned in the conserved colinearity regions and felled in the most frequently overlapped QTL intervals. The information obtained from this study demonstrates the potential role of the suggested candidate genes in rapeseed kernel oil accumulation.

Communicated by C. Quiros.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1716-3) contains supplementary material, which is available to authorized users.

J. Zhao (✉) · J. Huang · F. Chen · X. Ni
Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences, Hangzhou, China
e-mail: jyzhao3@yahoo.com

F. Xu · H. Xu
College of Agricultural and Biotechnology, Zhejiang University, Hangzhou, China

Y. Wang
College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, China

C. Jiang · J. Meng
National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China

H. Wang · D. Li
Hybrid Rapeseed Research Center of Shaanxi, Dali, China

A. Xu
College of Agronomy, Northwest Agricultural and forestry University, Yanglin, China

R. Huang
Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou, China

Introduction

Since the complete sequencing of *A. thaliana* genome, more than 600 candidate genes were found to be involved in the encoding of acyl lipid metabolism-related enzymes (Beisson et al. 2003). Revealed by the studies on transgenic plants, some of candidate genes originated from yeast have shown function to oil accumulation in the Brassicaceae

(Zou et al. 1997; Taylor et al. 2002; Vigeolas et al. 2007), while some orthologous candidate genes from *B. napus* exhibited close relations to the oil content in *A. thaliana* (Liu et al. 2009; Maisonneuve et al. 2010). However, it was reported that the up-regulation of single gene couldn't enhance the final oil accumulation in seeds (Thelen and Ohlrogge 2002). Investigations of transcription factors in regulating tissue specific oil accumulation attracted widespread interests. In particular, the *WRINKLED 1* (*WRI1*) gene in *A. thaliana* (Cernac and Benning 2004) and several other *WRI1*-related transcription factors, such as *LEAFY COTYLEDON2* (*LEC2*) (Baud et al. 2007), *LEAFY COTYLEDON1* (*LEC1*), *FUSCA3* (*FUS3*) and *ABA INSENSITIVE3* (*ABI3*) (Mu et al. 2008; Baud et al. 2009; Shen et al. 2010). Although numerous studies have provided important insights into the metabolic control of plant lipid formation (Ohlrogge and Browse 1995; Thelen and Ohlrogge 2002; Lung and Weselake 2006), there is lack of investigation in the seed oil content related genes in *B. napus.*

B. napus (AACC, 2n = 38) arises from spontaneous hybridization between the diploids turnip (*Brassica rapa;* AA, 2n = 20) and cabbage (*Brassica oleracea*; CC, 2n = 18). As the closest relative to *A. thaliana*, *B. napus* is considered as an ideal model crop in transferring information from the model species (Snowdon and Friedt 2004). Various comparative mapping studies were conducted, unraveling the extensive genome homology and microsynteny between *Brassica* species and *A. thaliana* (Schmidt et al. 2001; Parkin et al. 2005; Wang et al. 2011). Parkin et al. (2005) used sequenced RFLP probes to demonstrate 21 conserved syntenic genomic segments between *B. napus* and *A. thaliana*, which could be duplicated and rearranged to represent 90% of the *B. napus* genome. More recently, a genome-wide integration of *B. napus* maps identified 103 conserved collinearity blocks relative to *A. thaliana* (Wang et al. 2011). These findings provide valuable information for rapeseed research and enlighten us to construct an *A. thaliana* lipid-related orthologous gene map in *B. napus* based on *Brassica* sequence information, which might be interesting for identifying the candidate genes of oil content in combination with the quantitative trait loci (QTL) analysis in rapeseed.

In the past 20 years, a considerable number of linkage maps have been constructed for *B. napus* (see Cheng et al. 2009 and references therein), some of which were used for QTL analysis of seed oil content. The number of QTL identified in 19 linkage groups ranged from 3 to 14 in various reports (Ecke et al. 1995; Burns et al. 2003; Zhao et al. 2005, 2006; Qiu et al. 2006; Delourme et al. 2006; Yan et al. 2009; Chen et al. 2010). Delourme et al. (2006) observed four common QTL on A1, A3, A8 and C3 in at least three of the five analyzed populations. Some practical examples have proved the presence of collocations between collinear regions and QTL, covering various important traits in *B. napus* such as blackleg disease resistance (Mayerhofer et al. 2005; Kaur et al. 2009), heterosis (Basunanda et al. 2010) and flowering time (Long et al. 2007). These provided genetic information for the identification of candidate genes in *A. thaliana*, and subsequently clone and characterize the homologous genes in *B. napus*. However, most of the published linkage maps in rapeseed were based on molecular markers with little or no lipid related gene information, which induced difficulties in discovering candidate genes for oil content by performing a direct genome comparison between *B. napus* and *A. thaliana*.

Our aim of this study is to (1) refine the previous SSR map (Zhao et al. 2005) based on informative markers derived from *Brassica* expressed sequence tag (EST), genome survey sequences (GSS) and bacterial artificial chromosomes (BAC), which is sequence homologous to corresponding *A. thaliana* genes, especially those involved in the plant lipid biosynthesis or recycling pathways; (2) identify colinearity regions between *B. napus* and *A. thaliana* genome by comparative mapping; (3) re-evaluate QTL for seed oil content using the new map version and field experiments over 11 sites (locations); and (4) highlight new candidates that may exert impact on the variation of seed oil content in rapeseed on intersection points between QTL region and lipid-related gene loci.

Materials and methods

Plant materials and field experiments

The segregating DH population derived from the cross "Sollux" × "Gaoyou" was previously used for developing the SSR-based linkage map (SG map) and QTL evaluation for oil content in four locations (Zhao et al. 2005). In present study, the same population was tested in additional seven trials from 2003 to 2009. Except two experiments in Germany (2000–2001), all the other nine trials were conducted in China including 1 year in Wuhan, 2 years in Xi'an and 6 years in Hangzhou. All 282 DH lines together with parents "Sollux", "Gaoyou" and their F1's were employed in a randomized complete block design with two replications in seven experiments and three replications in the remaining four trials. Each plot consisted of 30–50 plants vary in different locations. The details of field experiments are described in Table S1.

Around 20 g of mature seeds were harvested from five to ten healthy plants of each line in each plot. The seed oil content was measured by Near Infrared Spectroscopy (NIRS) using standard methods (Mika et al. 2003).

Molecular marker analysis and map update

A total of 1,749 markers were used in present research, including 1,269 newly developed ones, 101 published EST-derived markers, 30 lipid or protein synthesis related orthologous gene markers (25 provided by Oil crops Institute, Chinese Academy of Agricultural Sciences (CAAS) and 5 obtained from Huazhong Agricultural University), 339 reference SSRs, 8 combinations of sequence-related amplified polymorphism (SRAP) primers and 2 sequence characterized amplified region (SCAR) markers. All primer pairs that showed polymorphisms in a screening with the two parents and F1's were applied to the population of 282 SG-lines. The genomic DNA was extracted in 1999, from young leaves by a modified cetyltrimethylammonium bromide (CTAB) method (Uzunova et al. 1995).

Informative marker development

Markers designated as ZAAS, ZAASA1, ZAASA7 and miRNA newly developed at Crop Research Institute, Zhejiang Academy of Agricultural Sciences (ZAAS), were originated from several different approaches. In the first step, *Brassica* EST/GSS sequences from *Brassica* Genome Gateway (http://brassica.bbsrc.ac.uk/BrassicaDB) and National Center of Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/est/) were randomly generated and assembled into consensus sequences. They were then aligned to the Arabidopsis genome ATH1v5 (http://www.tigr.org). The homologous contigs were selected to design the primer pairs using the software PRIMER 3 (http://frodo.wi.mit.edu/primer3/) and verified both in *Brassica* and *A. thaliana* genomes by electronic PCR (e-PCR; Schuler 1997). Finally, 331 primer pairs (corresponding to 242 homology genes including 15 candidate genes related to lipid synthesis) stably amplified in both genomes were selected for the development of STS (sequence tagged site) markers. The expected sizes of amplified fragments mostly ranged from 200 to 500 bp.

To further develop candidate gene markers for seed oil content, a reverse screening approach was employed. Two hundred and eight genes are known or suspected to be involved in *A. thaliana* acyl lipid metabolic pathways (http://www.plantbiology.msu.edu/lipids/genesurvey) including 95 key candidate genes and those expressed during seed development (Beisson et al. 2003) were selected. Besides, seven transcription factors including *BnWRI1* *BnLEC2*, *BnLEC1* and *LEC1*-like gene (*BnL1L*) and 22 orthologous genes involved in the protein and lignin biosynthetic pathways were also screened. Sequences from *Brassica* database were aligned to these candidate genes and produced in total of 689 primer pairs where homologous sequences were available, otherwise, primer pairs were developed from *A. thaliana* gene sequences.

Finally, in order to develop site specific markers on the linkage groups 1 (A1) and 7 (A7), 124 and 85 primer pairs corresponding to 38 and 66 deduced lipid-related or other informative genes were produced from 15 and 20 *B. rapa* BAC sequences of RaA1 and RaA7 (NCBI GenBank). Moreover, 40 siRNAs precursor (Wang et al. 2007) based primers with length of 100–200 bp from *B. napus* were developed.

Where possible, primers were designed from coding regions of genes flanking introns while avoiding splicing sites. One to four primer pairs per gene (or mRNA) were designed, depending on the length of deduced sequences. All primer pairs were synthesized by Sangon Biological and Engineering Co. (Shanghai, China)

CAPS markers

Four restriction enzymes *Hin*dIII, *Eco*RI, *Bam*HI and *Rsa*I were used to digest the PCR products around 1 kb fragment, from which no polymorphisms of fragment length could be detected. In total, around 100 primer pairs were tested by the method of cleaved amplified polymorphic sequences (CAPS) (Glazebrook et al. 1998).

SSR markers

A total of 339 references SSR markers were screened in SG-population of present study, while another 106 loci had previously been linked in SG-map (Zhao et al. 2005). The marker prefixes, source of primers and the No. of markers from different resources are summarized in Table S2a.

SRAP and SCAR markers

Based on the principles of Li and Quiros (2001), from 11 forward and 8 reverse primers (Li 2006), eight randomly combined primer pairs were used to test the polymorphisms of the SG-DH population. Two SCAR markers, which linked to the sterile gene *Bnms3* (He et al. 2008) and epistatic suppressor gene *Bnrf* (Xie et al. 2008) were included.

Marker detection

The PCR reaction was carried out in a total volume of 10 μl as described before (Zhao et al. 2005). PCR products were firstly separated by electrophoresis on 6% polyacrylamide gels in 1× TBE buffer to test the length polymorphism. Amplification products that showed length polymorphisms between the parents were directly used as STS markers. For monomorphic markers, single-strand conformational polymorphism (SSCP) technique was then

employed for testing the conformation differences caused by SNPs (single nucleotide polymorphism). Furthermore, a CAPS assay with restriction endonucleases (*Rsa*I and *Eco*RI) was also adapted. All polymorphic markers were examined in 282 SG-DH lines thereafter. Polyacrylamide gels with different concentrations (8 and 6%) were chosen for electrophoretic separation according to the size of the PCR products and visualized by a rapid silver-staining (Sanguinetti and Simpson 1994).

Marker names

In the case of a primer pair amplifying more than one polymorphic locus the names of the corresponding markers consisted of the primer pair name and a suffix of a, b, c, etc. The SRAP markers were named by forward primer "E" and reverse primer "M" followed by a suffix of a, b, c, etc. The markers designated with abbreviation of genes (e.g., NTP3 in A1) were EST-derived markers mapped by Li (2006).

Map update

All markers were checked for 1:1 segregation by the χ^2 test at a 5% significance level. Linkage analysis and map update were 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 "try" and "near" were used to assign new markers to the earlier published SG map (Zhao et al. 2005), the most probable marker order within each group was verified by the commands "three point", "order" and "ripple".

Genome alignment between *B. napus* and *A. thaliana*

Two hundred and eighty linked markers with known sequence information were aligned with *A. thaliana* genome sequences using the NCBI BLASTN program. The homologous loci ($E < 10^{-7}$) were located on a physical map of *A. thaliana* using the SeqViewer program from TAIR database (http://www.arabidopsis.org/). Regions with three or more markers showing continuous collinearity between *B. napus* and *A. thaliana* genomes were referred to conserved collinearity regions in the present study.

Genetic mapping and QTL analysis

Phenotypic data for oil content of the 282 DH lines over 11 trials were analyzed with SPSS 11.5 (SPSS Inc, Chicago, IL, USA). QTL detection at individual experiment was performed by the Composite Interval Mapping program (CIM) of WinQTL cartographer 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm; Zeng 1994; Wang et al. 2006) using the mean values of oil content of each line in each location. Significance levels for the LOD scores were determined by permutation analysis (Churchill and Doerge 1994) using 1,000 assortments and got critical values from 2.54 to 3.13 for individual experiment. QTL were considered to be identical if their confidence intervals overlapped each other within 1-LOD region in different environments.

Candidate gene nomination

Based on the genome alignment between *B. napus* and *A. thaliana* lipid-related genes described above, the QTL-candidate gene co-localization approach was then carried out to search for candidate genes. When physical positions of lipid-related genes fell into the overlapped confidence intervals of QTL, co-localization of QTL and orthologous candidate genes were assumed (Keurentjes et al. 2008).

Results

Informative gene Map

Marker development

1,269 PCR primer pairs were designed corresponding to 260 lipid-related and 311 other orthologous genes in *A. thaliana*, i.e. genes with sequence homology to *Brassica* EST or genomic DNA ($E < 10^{-7}$). After initial PCR amplification, 390 (31.0%) primers were found to be polymorphic in fragment length between parents and were subsequently genotyped in the SG-population. However, around half were mapped in the same position since they presented the identical gene. These reduplicated loci and a small part of markers, which were difficult to be scored, were deleted. Finally, 134 marker loci (STS marker) were integrated into the SG-map. Furthermore, SSCP and CAPS technology were adopted to screen the remainder of 879 primer pairs, which showed no polymorphisms in fragment size, and produced 74 and 10 additional loci. Totally, 218 gene-based marker loci were mapped, corresponding to 107 orthologous lipid related and 84 other functional genes (Table 1). In addition, another 50 marker loci from previously mapped EST-derived markers, 87 reference SSRs, 17 SRAP and two SCAR markers were also generated.

New version of the SG map

A total of 375 new marker loci were integrated into the framework of the previously SG map (Zhao et al. 2005). It is now composed of 481 marker loci including 106 previously mapped SSRs and spans 1,948.6 cM of the total

Table 1 The polymorphism survey of newly developed informative markers

Marker origin	No. of screened markers/genes			No. of polymorphic markers/genes			No. of polymorphic loci				
	Primer pairs	Total genes	Lipid genes	Total markers	Mapped genes	Lipid genes	STS	SSCP	CAPs	Total gene loci	Lipid gene loci
Brassica EST/GSS	1020	450	223	156	138	87	110	38	10	158	104
Brassica rape BACs	209	104	37	47	47	20	24	29	0	53	23
Brassica siRNAs precursors	40	17	0	7	6	0	0	7	0	7	0
Total	1269	571	260	210	191	107	134	74	10	218	127

genome with an average marker interval of 4.05 cM (Table 2). All the markers were assigned to 19 linkage groups, designating as A1-A10 and C1-C9 (Fig. 1), corresponding to the linkage groups N1-N10 and N11-N19 (Parkin et al. 1995).

All the mapped loci are listed in Table S2b with their marker names, types, fitness to 1:1 ratio and primer sequences. Details of the newly developed markers and those markers provided by others or obtained from published results are shown in Table S2c including the accession numbers, homology level to *A. thaliana* locus, origins and descriptions of putative function. Also, information regarding those reference SSRs that have been mapped in other *Brassica* populations are presented in Table S2d.

Nearly half of the mapped marker loci (48.0%; 229/481) showed distorted segregation ($P < 0.01$) in the SG population. Among those, 137 were skewed towards the female parent "Sollux" and 92 towards the male parent "Gaoyou" (Table S2b).

Comparative map between B. napus and A. thaliana

To identify the colinearity regions between *B. napus* and *A. thaliana*, SeqViewer was used to physically assign the 284 *Brassica* sequence based loci to the five *A. thaliana*

Table 2 Marker information with respect to individual linkage groups of the updated SG map

Linkage group	Size (cM)	No. of loci	Previous mapped SSR[a]	No. of new integrated marker loci						No. of gene markers	Marker density (cM/locus)
				SSR	STS	SSCP	CAPS	SRAP	SCAR		
A1	100.3	33	9	7	10	5		2		16	3.04
A2	91.6	24	5	3	12	3	1			17	3.82
A3	141.1	40	9	9	15	5		2		24	3.53
A4	80.1	23	2	3	10	7	1			19	3.48
A5	115.8	28	6	3	15	2	2			20	4.14
A6	118.5	19	1	3	7	6		2		13	6.24
A7	77.9	54	3	19	12	18		1	1	30	1.44
A8	87.1	12	4	2	2	4				7	7.26
A9	141.5	37	8	6	15	5		3		20	3.82
A10	79.9	22	3	4	9	5			1	15	3.63
C1	94.9	26	8	3	12	2	1			15	3.65
C2	94.8	19	5	4	5	4		1		10	4.99
C3	164.2	31	10	7	12	2				17	5.30
C4	110.3	17	7	4	1	1	1	3		4	6.49
C5	53.1	9	3	0	3	2	1			6	5.90
C6	63.2	23	4	1	10	7		1		18	2.75
C7	113.1	16	7	2	6		1			7	7.07
C8	100.1	22	5	3	9	2	2	1		12	4.55
C9	121.1	26	7	4	10	4	–	1		14	4.66
Total	1948.6	481	106	87	175	84	10	17	2	284[b]	4.05

[a] Previous mapped SSR marker loci (Zhao et al. 2005), Table S2d

[b] In total, 288 sequence-based markers were mapped (see Table S2c), 284 of them were physically positioned within the *A. thaliana* genome on the basis of sequence identity ($E < 10^{-7}$) and four loci were found no hits

chromosomes on the basis of sequence identity with the $E < 10^{-7}$. These orthologous *A. thaliana* loci were assembled into various segments and formed 135 homologous regions covering all 19 linkage groups. Of these regions, 45.9% (62/135) contained at least two closely linked homologous gene loci in the *A. thaliana* genome and the rest represented single gene loci (Fig. 1). Thirty-three collinearity regions were identified between *B. napus* and *A. thaliana* genomes in at least three or more continuous gene/marker loci (Fig. 1). The overall conserved colinearity regions covered 619.7 cM and represented 31.8% of the total length of SG-map, with an average length of 18.8 cM and 4.6 shared loci for each region. Notably, approximately half (76/137) of mapped lipid-related orthologous gene loci were assigned in the 33 conserved segments.

Genome duplications from conserved colinearity regions of *A. thaliana* were frequently presented two to six times in *B. napus* genome except segments from chromosome 4 of *A. thaliana* (AtC4) (Fig. 1). The collinear segment from 4.2 to 6.5 Mb of AtC3 was duplicated in linkage group A1, A3, A5, C1 and C3, that from 25.2 to 27.1 Mb of AtC1 was consistently identified in A2, A7, C3 and C6, that from 8.3 to 23.4 Mb in AtC5 was triplicated in A2, C2 and A6, and that from 14.3 to 16.8 Mb in AtC2 was doubled in A3 and A4. Moreover, obvious genome rearrangement events, such as inversion and insertion, were observed within the duplicated segments from different conserved colinearity regions (c.f. one gross chromosomal rearrangement was arisen in C1 from the identified conserved block in AtC3), indicating a certain level of genome reshuffle in *B. napus* genome during its evolution.

Lipid-related gene map

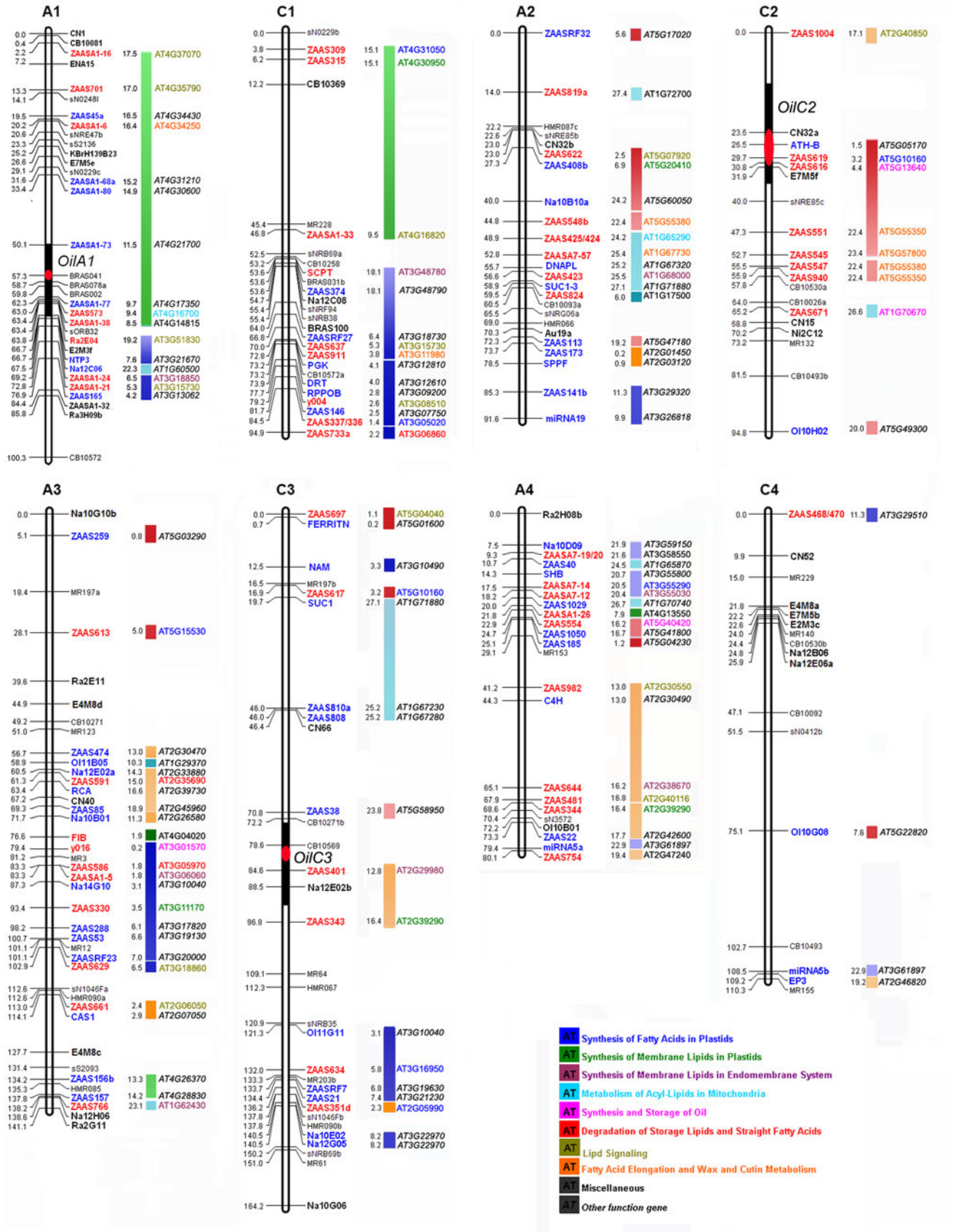
A total of 284 B*rassica* sequence based loci displayed homology to 249 orthologous *A. thaliana* genes, with an average sequence identity of 88.8% over all aligned segments (Fig. 1, Table S2c, S3a, and Table S3b). Two-thirds (188/284) of the loci were, for the first time localized on the genetic map of *B. napus.* Several duplicated copies of single gene were observed. For example, At3g55030 and At1g71880 allowed four and three loci to be mapped on A4, A7, A9, C6 and A2, A7, C3, respectively, and the other 30 homologous genes were mapped to only two loci. All the mapped homology genes were functionally analyzed based on Gene Ontology annotations (http://www.geneontology.org/) (Table S3a, b).

The most conspicuous characteristic of the present map is that approximately half of the mapped informative marker loci (137 of 284) corresponded to 112 orthologous *A. thaliana* genes involved in the acyl lipid metabolic pathways (Fig. 2a; Table S3a). Except seven homologous genes (corresponding to At1g24360, At1g62640, At2g05990, At2g29980, At3g05020, At4g30950, At5g13640) mapped previously (Wang et al. 2011; Parkin et al. 2005; Smooker et al. 2011), we mapped first, a major bulk of acyl lipid orthologous genes (105 genes/130 loci) and three transcriptional factors *BnWRI1*, *BnLEC1* and *BnL1L* on C8, A7 and A9 (TableS3b) in *B. napus* genome (Fig. 2b). Eighteen and seventeen corresponded to genes for fatty acid synthesis in plastids and synthesis of membrance lipids in endomembrane system, respectively, which accounted for 36.0% (18 out of 50) and 28.3% (17 out of 60) of total candidate genes in these two important synthesis pathways. Moreover, the mapped homologous gene loci included 8 of the 26 genes for synthesis of membrane lipids in plastids, 7 of the 38 genes for metabolism of acyl-lipids in mitochondria, six of the 20 genes for synthesis and storage of oil, 21 of the 164 genes for lipid signaling, as well as 23 genes encoding the enzymes involved in the lipid modification, processing or degradation processes. In addition, the functions of 12 orthologous genes were uncertain and classified as miscellaneous. These 137 putative lipid-related candidate gene loci were evenly distributed in all 19 linkage groups with the number of loci ranging from 2 to 13 for each chromosome except C4. Notably, over 30 functional loci were homologous genes involved in the acyl lipid metabolism pathways, which are preferentially expressed during seed development (Beisson et al. 2003).

Fig. 1 Informative gene map based on the Sollux/Gaoyou (F1) DH population and collinearity comparison with *A. thaliana*. The 19 linkage groups are represented by vertical bars. The numbers at the top of each linkage group, A1–A10 and C1–C9, indicate the internationally agreed chromosomal nomenclature of *B. napus*. The locus name and genetic distance are listed on the right and left side of the linkage groups, respectively. Markers that are homology to *A. thaliana* lipid-related genes are *highlighted in bold and red*; markers corresponding to other *A. thaliana* genes are presented in *bold and blue*; the rest of newly added SSR markers are given by *bold and black*; and the markers in *black* are originated from previous SSR map (Zhao et al. 2005). The collinearity regions with *A. thaliana* are shown to the right side of SG linkage groups as *colored vertical bars*, which represent five chromosomes of *A. thaliana*. The locus names and megabase distances of the homologous *A. thaliana* regions are respectively given on the right and left side of the *colored vertical bars*. The names of *A. thaliana* lipid-related gene loci are *highlighted in different colors* based on their different cellular functions (Beisson et al. 2003). The *black and red regions* on the linkage groups indicated the confidence intervals of QTL overlapped within 1-LOD region and the intervals between peak positions in different environments, respectively. QTL names are abbreviations of the trait followed by its respective linkage group's number. A number 1 or 2 was added if more than one QTL were detected in one linkage group

Mapping QTL for oil content in multiple experiments

The average oil content differed up to 8.4% between the highest and the lowest trials 01R (51.9%) and 04H (43.5%) (Table S4). Also, a difference of around 12% was found


A1
C1
A2
C2
OilA1
OilC2
A3
C3
A4
C4
OilC3
Synthesis of Fatty Acids in Plastids
Synthesis of Membrane Lipids in Plastids
Synthesis of Membrane Lipids in Endomembrane System
Metabolism of Acyl-Lipids in Mitochondria
Synthesis and Storage of Oil
Degradation of Storage Lipids and Straight Fatty Acids
Lipd Signaling
Fatty Acid Elongation and Wax and Cutin Metabolism
Miscellaneous
Other function gene

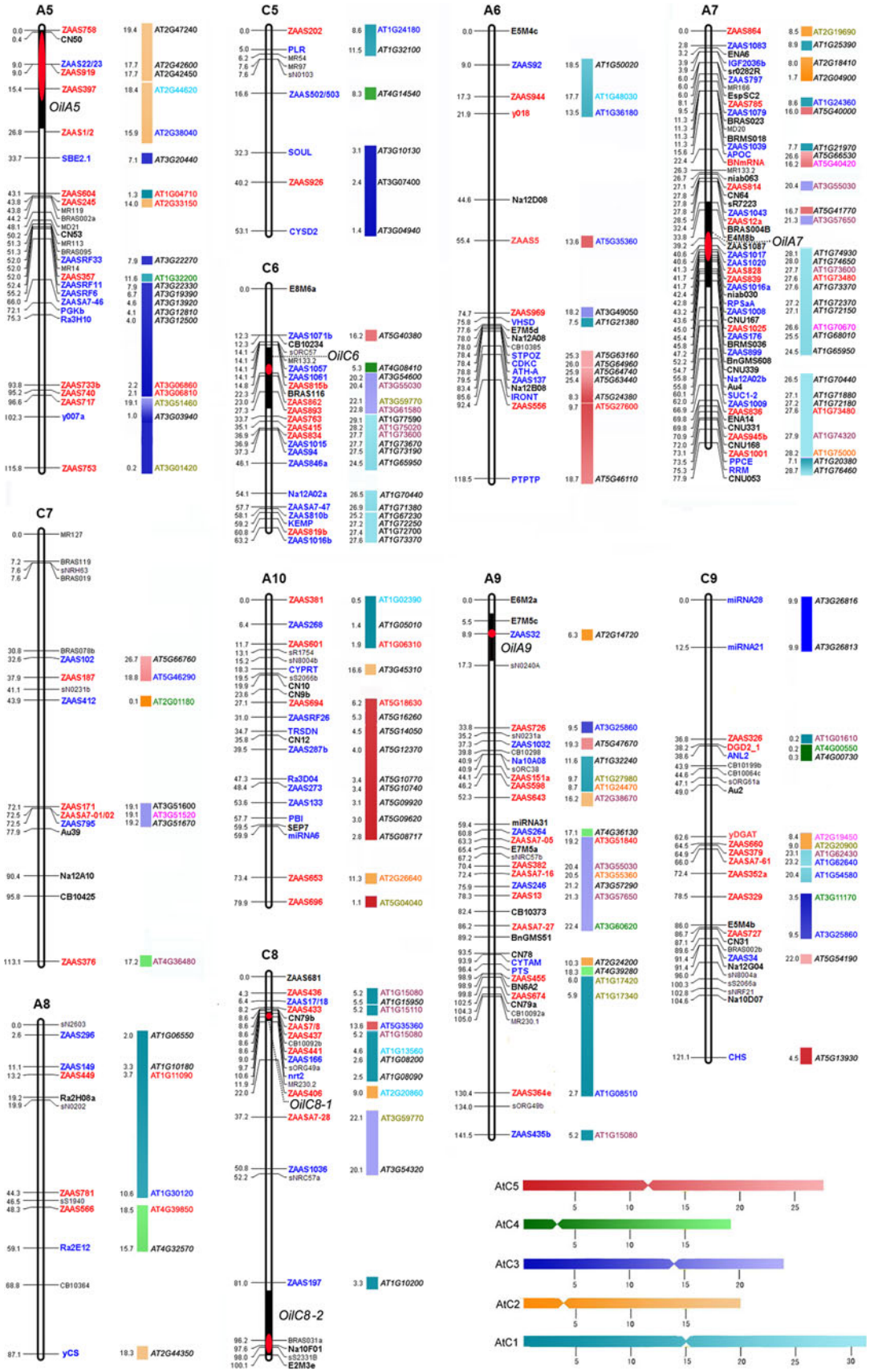


Fig. 1 continued

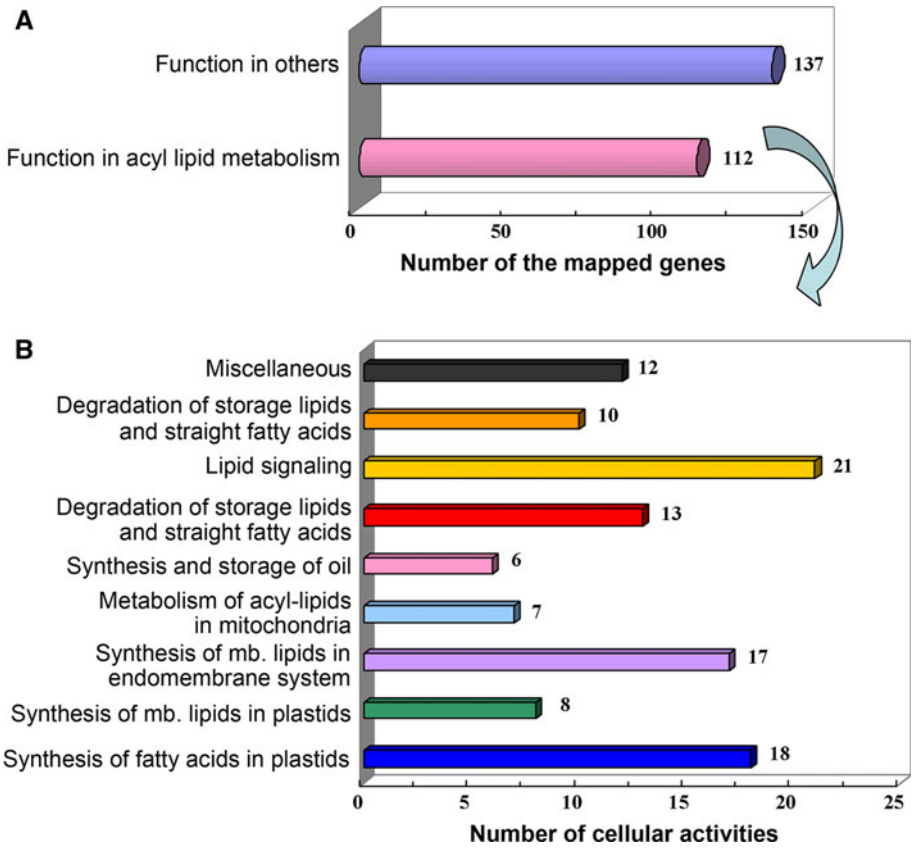


Fig. 2 Overview of homology genes mapped in SG-map (**a**) and functional classification of lipid-related orthologous genes (**b**). For a summary of the gene annotation, see supplement Table 3a and Table 3b

within the population (40.8–52.5%). QTL were analyzed in each experiment by CIM approach. The full mapping results are depicted in Table S5 and are summarized in Table 3. In total, nine significant QTL were identified in at least two field experiments. Those QTL were located on eight linkage groups, corresponding to nine genomic regions (Fig. 1). QTL are labeled with the prefix *Oil* followed by the chromosome Number. When more than one QTL were found on a chromosome, these are numbered consecutively by a suffix. Their additive effects ranged from 0.33 to 0.72, and mean R^2 value varied from 3.40 to 13.13%, together explaining 57.79% of the total phenotypic variation observed in SG population. Alleles from the Chinese parent "Gaoyou" increased seed oil content at five loci (*OilA5*, *OilA7*, *OilC3*, *OilC6* and *OilC8-2*), while the European parent "Sollux" contributed favorable alleles at the remaining four loci (*OilA1*, *OilA9*, *OilC2* and *OilC8-1*).

As shown in Fig. 3, the major QTL *OilA7* was significantly observed in all 11 experiments, explaining the phenotypic variation from 4.97 to 26.50% (13.13% on average, Table S5). Another QTL, *OilC8-2*, located at the bottom of the C8, was consistently detected in nine trials. In addition, *OilA1* and *OilC2,* were identified in three and four locations, and accounted on average of 5.49% and 7.49% of phenotypic variation in population. The other four QTL were detected in specifically geography regions. *OilA9* exhibited significant effect only in location of Xi'an (01X and 07X), *OilC8-1* was specially detected in Germany (01R and 01We), while *OilC3* (04H and 07H) and *OilC6* (05H and 07H) were identified in the experiments in Hangzhou (Table 3, Table S5).

The genomic regions of nine QTL were checked against distorted markers. It was shown that except three (*OilC2, OilC3* and *OilC8-1*), the other six QTL were located in undistorted marker intervals including two most significant QTL *OilA7* and *OilC8-2*.

Co-localizations between QTL and candidate genes

The colinearity map between *B. napus* and *A. thaliana* as well as confidence intervals of the nine QTL for oil content were shown in Fig. 1. A total of 14 lipid candidate gene loci were found in the regions of six QTL. Their putative functions and co-localizations with QTL are presented in Table 4. Out of the 14 gene loci, 3, 3 and 2 loci were localized in the three major QTL regions of *OilA1*, *OilA7* and *OilC2*, respectively. One candidate gene (At1g73600) was shown to be involved in synthesis of membrane lipids in endomembrane system, two (At1g70670 and At5g13640) were functional important in the formation and storage of oil. One gene for each was involved in the lipid signaling (At3g51830), synthesis of fatty acids in plastids

Table 3 Summarized QTL information of seed oil content in SG-DH population over 11 experiments

Name of QTL[a]	Linkage group	LOD value	Additive effect (%)[b]	R^2%[c]	Confidence interval[e]	Experiment[f]
OilA1	A1	4.48–5.25[d]	0.31–0.56	4.70–6.40	49.9–66.9	01R; 01We; 07Wu
OilA5	A5	2.83–4.64	−0.35–−0.45	2.85–5.40	0.0–25.5	05H; 07X; 09H
OilA7	A7	3.12–18.73	−0.42–−0.89	4.97–26.50	32.2–48.7	01X; 01H; 01R; 01We; 04H; 05H; 07H; 07X; 07Wu; 08H; 09H
OilA9	A9	4.65–5.09	0.39–0.40	5.97–6.12	3.5–15.3	01X; 07X
OilC2	C2	3.89–7.87	0.60–0.93	5.47–9.31	13.6–35.8	01R; 01We; 05H; 08H
OilC3	C3	3.29–6.33	−0.37–−0.74	3.75–8.25	73.8–92.1	04H; 07H
OilC6	C6	2.54–3.36	−0.32–−0.33	2.56–4.24	15.2–32.2	05H; 07H
OilC8-1	C8	4.14–5.18	0.47–0.61	5.01–6.00	9.5–11.0	01R; 01We
OilC8-2	C8	2.84–11.31	−0.27–−0.73	3.35–14.02	83.8–100.1	01X; 01R; 01We; 04H; 05H; 07H; 07X; 07Wu; 09H

[a] QTL names are abbreviations of the trait followed by its respective linkage group's number. A number 1 or 2 was added if more than one QTL were detected in one linkage group

[b] Additive effect with positive values indicated that QTL alleles increasing seed oil content originated from the European parent "Sollux" and negative values means favorable alleles from Chinese parent "Gaoyou"

[c] Proportion of the phenotypic variation explained by the QTL within SG-DH population

[d] The parameters were extracted from the smallest and largest value corresponding to data in Table S5 at individual experiments

[e] Indicating the confidence intervals of QTL overlapped within 1-LOD region over different environments

[f] X: Xi'an of China; H: Hangzhou of China; R: Reinshof of Germany; We: Weende of Germany; Wu: Wuhan of China; 01, 04, 05, 07, 08, 09: denote the year of 2001, 2004, 2005, 2007, 2008 and 2009, respectively

(At5g10160), metabolism of acyl-lipids in mitochondria (At4g16700), degradation of storage lipids (At1g73480) and miscellaneous (At4g14815). For the rest of six candidate loci, three co-localized with *OilA5*, two with *OilC6* and one linked to *OilC3*. Co-localization analysis further revealed that ten candidate genes were laid in the conserved colinearity regions and felled in the most frequently overlapped QTL intervals on A1, A5, A7 and C2 (Table S5), indicating their potentials in genetic control on seed oil content in *B. napus*.

Discussion

Informative functional map

The present SG map consisted of 481 marker loci, including 193 reference SSRs, which enabled its comparison with other published maps. In addition, 218 newly developed informative marker loci were combined into the map. It accounted for 17.2% of the total primer pairs screened (1,269; Table 1), suggesting the feasibility to develop functional markers directly based on *Brassica* EST or genomic contigs with sequence homology to known *A. thaliana* genes. Furthermore, blast analysis showed that 75.7% of the newly integrated marker loci (284/375) correspond to 249 orthologous *A. thaliana* genes (Fig. 1; Table S2c), in which nearly half (112) are genes functionally involved in the acyl lipid metabolism pathways (Table S3a), three are transcription factors that were previously reported as regulators of oil-accumulation genes (Table S3b). Two hundred and eighty-four orthologous *A. thaliana* gene loci in SG-map were compared to more recently published genome-wide integration of *Brassica* map (Wang et al. 2011), in which only 31 (seven lipid-related loci) were found consistently to be mapped in both maps and mostly (24 loci) located in the similar or collinearity A or C genomic regions. Therefore, this new SG-map should be remarkably useful to identify candidate genes for oil content and for other important agronomical traits in rapeseed by testing the associations between relevant QTLs and homologous genes with possible involvement in the traits of interest.

As known by all, *Brassica* and *A. thaliana* shared a common ancestor (Yang et al. 1999) and were separated into two lineages since 14–24 million years ago (Koch et al. 2000). As an amphidiploid, *B. napus* genome is much larger (~1,200 Mb, Niu et al. 2009) than that of Arabidopsis (~125 Mb, Meyerowitz 1992), so in accordance with previous studies, the frequent observation of complex rearrangements and duplications of the *A. thaliana* genome in genome of *Brassica* crops is expected in present study (Parkin et al. 2005; Suwabe et al. 2006; Wang et al. 2011). Each of 33 colinearity regions between *B. napus* and *A. thaliana* was present two to six times within the *B. napus* genome. In addition, our study further corroborated the

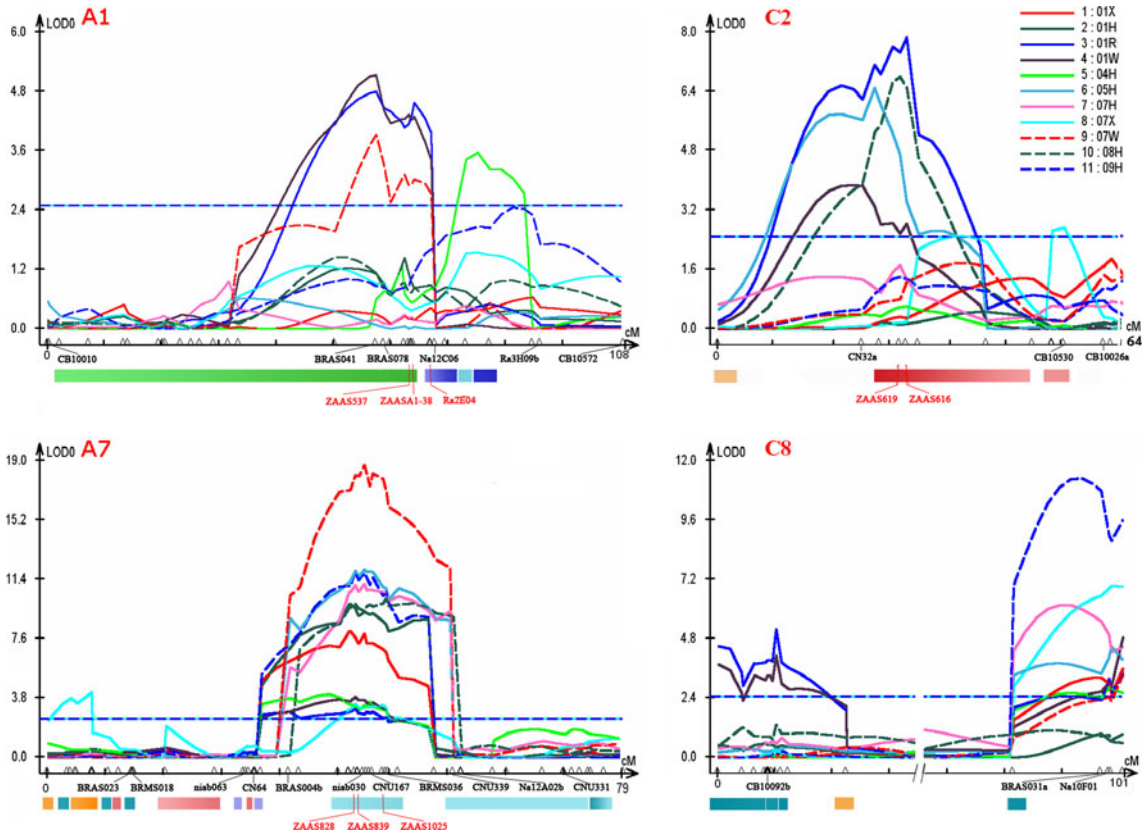


Fig. 3 Scanning results of four major QTL for oil content over 11 experiments in SG DH population. Curves of different colors represent QTL scanned from different experiments. The horizontal lines with LOD values 2.5 represent the standard of QTL detection. The triangles on *X*-axis show the positions of molecular markers in the corresponding linkage groups, and the markers with *black color* below the *X*-axis represent the public SSR markers. The *horizontal bars with different colors* under the *X*-axis represent the collinearity regions with *A. thaliana*. The markers with *red color* on the bottom represent the acyl lipid related candidate gene loci, which are described in Table 4

Table 4 Lipid-related orthologous candidate gene loci identified in the 1-LOD confidence intervals of QTL for oil content

QTL	Marker name	AGI gene index no.[a]	*Brassica* accession number	*E* value	Gene annotation
OilA1	Ra2E04	At3g51830	BZ518472	$4e^{-42}$	Sac domain-contain.
	ZAASA1-38	**At4g14815**	EV152375	$1e^{-42}$	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
	ZAAS573	**At4g16700**	CC951153	$1e^{-63}$	Encodes a mitochondrial phosphatidylserine decarboxylase
OilA5	ZAAS758	**At2g47240**	EU375561	0	Long-chain Acyl-CoA synthetase
	ZAAS919	**At2g42450**	CN830337	0	Lipase class 3 family protein
	ZAAS397	At2g44620	EE405725	$8e^{-93}$	Mitochondrial Acyl carrier protein
OilA7	ZAAS828	**At1g73600**	AC189598	$2e^{-57}$	Phosphoethanolamine N-methyltransferase
	ZAAS839	**At1g73480**	AC189598	$1e^{-98}$	Monoglyceride lipase
	ZAAS1025	**At1g70670**	AC189401	$2e^{-25}$	Caleosin-related family protein
OilC2	ZAAS619	**At5g10160**	DY022121/DY022512	0	Plastidial hydroxyacyl-ACP dehydrase
	ZAAS616	**At5g13640**	BH678617	$8e^{-73}$	Phosphatidylcholine : diacylglycerol acyltransferase (PDAT)
OilC3	ZAAS401	At2g29980	CX273864	e^{-165}	Omega-3 fatty acid desaturase, endoplasmic reticulum (FAD3)
OilC6	ZAAS862	**At3g59770**	AC189607	$1e^{-55}$	Sac domain-contain, phosphoinositide 5-phosphatase
	ZAAS893	At3g61580	AC189560	0	Delta-8 sphingolipid desaturase (SLD1)

[a] Candidate genes that were located in the conserved colinearity regions and fallen in the most frequently overlapped QTL intervals are highlighted in bold

previous finding on the close homology between the A genome with linkage groups A1–A10 and the corresponding linkage groups C1 to C9 of the C genome in *B. napus* (Parkin et al. 2005). For example, the linkage groups A1 and C1 showed almost entire-length homology, in which the upper half are homologous to the block segment (about 8.5–17.5 Mb) from AtC4, and the lower parts are homologous to the top arm of AtC3. Meanwhile, the segment from the top arm of AtC3 that was strongly conserved in A1/C1, co-linearing with the lower half of A3/C3 and A5/C5 (Fig. 1). Similar results were obtained by Parkin et al. (2005) and impelled the unraveling of the genome structure of *B. napus*. Besides, 76 mapped lipid-related orthologous genes located in the 33 conserved colinearity regions, exhibiting their relative high conservation in rapeseed genome, are more likely to be selectable as candidate genes for oil content.

Major QTL associated with seed oil content

Of nine significant QTL detected for oil content in our study, seven (A1, A7, A9, C2, C3, C6 and C8) were consistent over time (Zhao et al. 2005). Two additional QTL on A5 and C8 were newly discovered at the positions of extended genome regions under the inclusion of a high number of new markers (The length for A5 and C8 extended from 7.8 to 115.8 cM and from 2.2 to 100.1 cM, respectively). Apart from *OilA1*, *OilA5* and *OilC2*, the remaining QTL demonstrated two distinct patterns: one type as *OilC8-2* and *OilA7* showing steady occurrence over 9 and 11 trails, and was referred to stable QTL; while the other showed significant effect only in certain geographical regions, such as *OilA9* in Xi'an, *OilC3* and *OilC6* in Hangzhou, and *OilC8-1* in Göttingen (Germany), thus regarded as regional dependent QTL. The gene(s) linked to stable QTL is more structural important and less shaped by environment and epistatic interactions. This assumption has been confirmed by marginal QTL × environment interactions and none of any epistatic effects involved for *OilA7* (data not shown), and was validated through a set of substitution lines flanking *OilA7* by marker assistant backcross. The comparative analysis of oil content between homozygous BC4F2 sister sub-NILs (Near-isogenic lines) carrying "Gaoyou" fragment ($n = 65$, 46.4%) and NILs containing "Sollux" fragment ($n = 61$, 44.3%) in the QTL region showed significant difference of 2.1% ($p < 0.0001$) in oil content, strengthened our prediction over its stability. The isolation and characterization of regional dependent QTL would be relevant for breeding cultivars for specific geographic regions.

By alignment of mapped public SSRs, we yielded a comparison with those from five populations: "Darmorbzh" × "Yudal" (DY) and "Rapid" × "NSL96/25" (RNSL) DH population (Delourme et al., 2006), "Tapidor" × "Victor" (TV) substitution lines (Burns et al. 2003) "Tapidor" × "Ningyou 7" (TN) DH population (Qiu et al. 2006), and a recombinant DH population (RDH, Chen et al. 2010). Similarly, we found around half of the QTL detected in SG map were constantly identified in other populations. The QTL on A1 and C3 could be detected in all six maps. *OilA1* was potentially located in the same region as in DY and TN, and *OilC3* was observed at the similar position as in RNSL and in TN. *OilA7*, the most significant QTL in the SG map, was as well detected in RNSL and in TV, however at slightly different positions. QTL consistent across populations were also identified on C2, C6 and C8. The QTL on A5 and A9 were only detected in the SG population. The coincidence of QTL across different populations provides supplementary information for fine mapping and map-based cloning of genes contributing to seed oil content in *B. napus* .

Of three QTL (*OilC2, OilC3* and *OilC8-1*), where situated in the genomic regions with distorted segregation of marker loci, *OilC2* and *Oil8-1* showed highly *QE* interactions in 9 and 5 out of 11 mapping experiments. In addition, both *OilC2* and *Oil8-1* were significantly involved in the additive × additive epistatic interactions with six and three times, respectively (unpublished results). However, whether such phenomena are some relationships with distorted marker loci remain for further investigation.

Linkage of candidate genes to QTL for oil content

A manual scan of SG map was conducted to identify candidate genes for seed oil content in *B. napus,* specifically those homologous genes within the 1-LOD QTL confidence interval. Notably, ten candidate genes located on A1, A5, A7 and C2 might be more interesting because they felled in the most frequently overlapped QTL intervals over different environments and also located in the conserved colinearity regions (Table 4 and Table S5). However, the prevailing transcription factors *WRI1*, *LEC1* and their homologous genes in *B. napus*, *BnWRI1, BnLEC1* and *LEC1*-like gene *BnL1L*, were not found in the major QTL regions in present study. Here we suggest some candidate genes, showing both putatively functional important for oil content and closely co-localization with major QTL in SG-population.

Co-localized with *OilA1,* At4g14815 encodes lipid-transfer protein/seed storage 2S albumin superfamily protein, functions in lipid binding and involved in the lipid transport process. Its homologous locus ZAASA1-38 lies in the overlapped confidence interval of *OilA1*. The result from association mapping using 96 worldwide rapeseed cultivars containing different oil content gave a positive support to this candidate gene (date not show),

demonstrating its possible function for oil accumulation and therefore might be regarded as a candidate for this QTL.

It is interesting that *OilA5* was found to be similar collinearity with the most significant QTL observed in *A. thaliana*, which located in the bottom of chromosome 2 (AtC2) (Hobbs et al. 2004). A long chain acyl-CoA synthetase (At2g47240) lies in this collinearity region and was suggested as candidate gene for oil content in *A. thaliana* (Hobbs et al. 2004).

Three homologous candidate gene loci corresponding to the collinearity region of AtC1 (26.6–27.7 cM), ZAAS828 (At1g73600), ZAAS839 (At1g73480) and ZAAS1025 (At1g70670), were mapped to a position under the peak of the *OilA7* (Fig. 3). At1g73600 encoded phosphoethanolamine N-methyltransferase (PEAMT; EC 2.1.1.103), which catalyzes the key step in choline biosynthesis, is a vital precursor of the membrane phospholipid phosphatidylcholine, accounting for 40–60% of lipids in nonplastid plant membranes (BeGora et al. 2010). The other two loci encode *A. thaliana* monoglyceride lipase (MGL; EC 3.1.1.23; At1g73480) and caleosin-related family protein (At1g70670), respectively. The cellular function of At1g70670 is probably related with calcium-mediated fusion of oil bodies, which plays an important role in the maturation of seed oil bodies released from the endoplasmic reticulum (ER) (Frandsen et al. 2001; Hernandez-Pinzon et al. 2001).

Two closely linked loci of ZAAS616 and ZAAS619 showed high homology to At5g13640 and At5g10160 with an *E* value of $8e^{-73}$ and 0, respectively. Both were directly mapped within the highest peak position of *OilC2* and corresponded to the conserved collinearity region of AtC5. Along with diacylglycerol acyltransferase (DGAT1 and DGAT2), At5g13640 encoded diacylglycerol acyltransferase (PDAT; EC 2.3.1.158) catalyzes phospholipid and functions in the synthesis of triacylglycerol (TAG). PDAT also shows its activity in the selective accumulation of epoxy and hydroxy fatty acid in seed oil (Li et al. 2010; Van Erp et al. 2011). Another co-localized candidate gene At5g10160, encoding the thioesterase super family protein, is involved in the fatty acid biosynthetic process and functions in hydrolyase and 3-hydroxyacyl-ACP dehydratase activity (Beisson et al. 2003). These two highly homologous Arabidopsis gene loci might be selected as candidates for further investigations on *OilC2*.

Collectively, the information obtained from the present research demonstrates the potentially novel roles of candidate genes in rapeseed oil accumulation. The further research is mainly concentrated in the fine mapping of some important QTL as *OilA7* and *OilA1*, and Investigation for linked candidate genes suggested. Currently, QTL-NILs have been developed up to BC5F2 and BC4F3 by means of marker based advanced backcross. The cloning and characterization of some important candidate genes revealed in this study are underway. These continuing works will pose an excellent opportunity to identify genes that control seed oil accumulation in *B. napus*.

Acknowledgments The authors are grateful to Dr. Christian Moellers and Prof. Heiko Becker (University of Gottingen), and Dr Zhonghua Chen (University of Western Sydney) for their critical reading of the manuscript and to Dr. Wolfgang Ecke (University of Gottingen) for his providing the original SSR marker names from AAFC consortium. Thanks also to Drs. Liu Jing, Wang Lei and Shengyi Liu (CAAS) for offering marker primers, siRNAs precursor and BAC sequences. Prof. Weijun Zhou (Zhejiang University) for kindly providing the rapeseed line "Gaoyou". This research was financially supported by National Basic Research Programs (2006CB101601), National High-tech R&D Program (2011AA10A104), National Natural Science Foundations of China (No. 30871543, 31171180) and Zhejiang Provincial Natural Science Foundation of China (Z3100592).

References

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

Baud S, Mendoza MS, To A, Harscoet E, Lepiniec L, Dubreucq B (2007) *WRINKLED1* specifies the regulatory action of *LEAFY COTYLEDON2* towards fatty acid metabolism during seed maturation in Arabidopsis. Plant J 50:825–838

Baud S, Wuilleme S, To A, Rochat C, Lepiniec L (2009) Role of *WRINKLED1* in the transcriptional regulation of glycolytic and fatty acid biosynthetic genes in Arabidopsis. Plant J 60:933–947

BeGora MD, Macleod MJR, McCarry BE, Summers PS, Weretilnyk EA (2010) Identification of phosphomethylethanolamine N-methyltransferase from Arabidopsis and its role in choline and phospholipid metabolism. J Biol Chem 285:29147–29155

Beisson F, Koo AJK, Ruuska S, Schwender J, Pollard M (2003) Arabidopsis genes involved in acyl lipid metabolism: a census of the candidates, a 2003 study of the distribution of expressed sequence tags in organs, and a Web-based database. Plant Physiol 132:681–697

Burns MJ, Barnes SR, Bowman JG, Clarke MHE, Werner CP, Kearsey MJ (2003) QTL analysis of an intervarietal set of substitution lines in *Brassica napus*: (i) seed oil content and fatty acid composition. Heredity 90:39–48

Cernac A, Benning C (2004) *WRINKLED1* encodes an AP2/EREB domain protein involved in the control of storage compound biosynthesis in Arabidopsis. Plant J 40:575–585

Chen G, Geng JF, Rahman M, Liu XP, Tu JX, Fu TD, Li GY, McVetty PBE, Tahir M (2010) Identification of QTL for oil content, seed yield, and flowering time in oilseed rape (*Brassica napus*). Euphytica 175:161–174

Cheng XM, Xu JS, Xia S, Guo J, 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

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Delourme R, Falentin C, Huteau V, Clouet V, Horvais R, Gandon B, Specel S, Hanneton L, Dheu JE, Deschamps M et al (2006) Genetic control of oil content in oilseed rape (*Brassica napus* L.). Theor Appl Genet 113:1331–1345

Ecke W, Uzunova M, Wiessleder K (1995) Mapping the genome of rapeseed (*Brassica napus* L.). II. Localisation of genes controlling erucic acid synthesis and seed oil content. Theor Appl Genet 91:972–977

Frandsen GI, Mundy J, Tzen JTC (2001) Oil bodies and their associated proteins, oleosin and caleosin. Physiol Plantarum 112:301–307

Glazebrook J, Drenkarci E, Preuss D, Ausubel FM (1998) Use of cleaved amplified polymorphic sequences (CAPS) as genetic markers in *Arabidopsis thaliana*. Meth Mol Biol 82:173–182

He JP, Ke LP, Hong DF, Xie YZ, Wang GC, Liu PW, Yang GS (2008) Fine mapping of a recessive genic male sterility gene (*Bnms3*) in rapeseed (*Brassica napus*) with AFLP- and Arabidopsis-derived PCR markers. Theor Appl Genet 117:11–18

Hernandez-Pinzon I, Patel K, Murphy DJ (2001) The *Brassica napus* calcium-binding protein, caleosin, has distinct endoplasmic reticulum- and lipid body-associated isoforms. Plant Physiol Biochem 39:615–622

Hobbs DH, Flintham JE, Hills MJ (2004) Genetic control of storage oil synthesis in seeds of Arabidopsis. Plant Physiol 136:3341–3349

Kaur S, Cogan NOI, Ye G, Baillie RC, Hand ML, Ling AE, Mcgearey AK, Kaur J, Hopkins CJ, Todorovic M et al (2009) Genetic map construction and QTL mapping of resistance to blackleg (*Leptosphaeria maculans*) disease in Australian canola (*Brassica napus* L.) cultivars. Theor Appl Genet 120:71–83

Keurentjes JJ, Sulpice R, Gibon Y, Steinhauser MC, Fu J, Koornneef M, Stitt M, Vreugdenhil D (2008) Integrative analyses of genetic variation in enzyme activities of primary carbohydrate metabolism reveal distinct modes of regulation in *Arabidopsis thaliana*. Genome Biol 9:R129

Koch MA, Haubold B, Mitchell-Olds T (2000) Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). Mol Biol Evol 17:1483–1498

Li R, Yu K, Hildebrand DF (2010) *DGAT1*, *DGAT2* and *PDAT* expression in seeds and other tissues of epoxy and hydroxy fatty acid accumulating plants. Lipids 45(2):145–157

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103:455–461

Li YY (2006) Localization of QTLs for yield-related traits and heterosis dissection using functional markers in *Brassica napus* L. PhD Dissertation, Hauzhong Agricultural University, Wuhan (in Chinese)

Lincoln SE, Daly MJ, Lander ES (1993) Constructing genetic linkage maps with MAPMARKER/EXP 3.0. A tutorial and reference manual. Whitehead Institute for Biomedical Research, Cambridge

Liu J, Hua W, Zhan GM, Wei F, Wang XF, Liu GH, Wang HZ (2009) Increasing seed mass and oil content in transgenic Arabidopsis by the overexpression of *wri1*-like gene from *Brassica napus*. Plant Physiol Biochem 48:9–15

Long Y, Shi J, Qiu D, Li R, Zhang C, Wang J, Hou J, Zhao J, Shi L, Beom-Seok P (2007) Flowering time quantitative trait loci analysis of oilseed *Brassica* in multiple environments and genomewide alignment with Arabidopsis. Genetics 177:2433–2444

Lung SC, Weselake RJ (2006) Diacylglycerol acyltransferase: a key mediator of plant triacylglycerol synthesis. Lipids 41:1073–1088

Maisonneuve S, Bessoule JJ, Lessire R, Delseny M, Roscoe TJ (2010) Expression of rapeseed microsomal lysophosphatidic acid acyltransferase isozymes enhances seed oil content in Arabidopsis. Plant Physiol 152:670–684

Mayerhofer R, Wilde K, Mayerhofer M, Lydiate D, Bansal VK, Good AG, Parkin IAP (2005) Complexities of chromosome landing in a highly duplicated genome: toward map-based cloning of a gene controlling blackleg resistance in *Brassica napus*. Genetics 171:1977–1988

Meyerowitz E (1992) Introduction to the Arabidopsis genome. In: Koncz C, Chua N, Schell J (eds) Methods in Arabidopsis research. World Scientific, Singapore, pp 102–118

Mika V, Tillmann P, Koprna R, Nerusil P, Kucera V (2003) Fast prediction of quality parameters in whole seeds of oilseed rape (*Brassica napus* L.). Plant Soil Environ 49:141–145

Mu JY, Tan HL, Zheng Q, Fu FY, Liang Y, Zhang J, Yang XH, Wang T, Chong K, Wang XJ, Zuo JR (2008) *LEAFY COTYLEDON1* is a key regulator of fatty acid biosynthesis in Arabidopsis. Plant Physiol 148:1042–1054

Niu Y, Wu GZ, Ye R, Lin WH, Shi QM, Xue LJ, Xu XD, Li Y, Du YG, Xu HW (2009) Global analysis of gene expression profiles in *Brassica napus* developing seeds reveals a conserved lipid metabolism regulation with *Arabidopsis thaliana*. Mol Plant 2:1107–1122

Ohlrogge J, Browse J (1995) Lipid biosynthesis. Plant Cell 7:957–970

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

Parkin IAP, Gulden SM, Sharpe AG, Lukens L, Trick M, Osborn TC, Lydiate DJ (2005) Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics 171:765–781

Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E et al (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

Sanguinetti C, Simpson NE (1994) Arapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17:915–919

Schmidt R, Acarkan A, Boivin K (2001) Comparative structural genomics in the Brassicaceae family. Plant Physiol Biochem 39:253–262

Schuler GD (1997) Sequence mapping by electronic PCR. Genome Res 7:541–550

Shen B, Allen WB, Zheng PZ, Li CJ, Glassman K, Ranch J, Nubel D, Tarczynski MC (2010) Expression of *ZmLEC1* and *ZmWRI1* increases seed oil production in maize. Plant Physiol 153:980–987

Smooker AM, Wells R, Morgan C, Beaudoin F, Cho K, Fraser F, Bancroft I (2011) The identification and mapping of candidate genes and QTL involved in the fatty acid desaturation pathway in *Brassica napus*. Theor Appl Genet 122:1075–1090

Snowdon RJ, Friedt W (2004) Molecular markers in *Brassica* oilseed breeding: current status and future possibilities. Plant Breed 123:1–8

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Kondo M, Fujimura M, Nunome T, Fukuoka H, Hirai M, Matsumoto S (2006) Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics 173:309–319

Taylor DC, Katavic V, Zou JT, MacKenzie SL, Keller WA, An J, Friesen W, Barton DL, Pedersen KK, Giblin EM et al (2002) Field testing of transgenic rapeseed cv. Hero transformed with a yeast *sn-2* acyltransferase results in increased oil content, erucic acid content and seed yield. Mol Breed 8:317–322

Thelen JJ, Ohlrogge JB (2002) Metabolic engineering of fatty acid biosynthesis in plants. Metab Eng 4:12–21

Uzunova MI, Ecke W, Weissleder K, Rübbelen 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

Van Erp H, Bates PD, Burgal J, Shockey J, Browse J (2011) Castor phospholipid: diacylglycerol acyltransferase facilitates efficient metabolism of hydroxy fatty acids in transgenic Arabidopsis. Plant Physiol 155:683–693

Vigeolas H, Waldeck P, Zank T, Geigenberger P (2007) Increasing seed oil content in oil-seed rape (*Brassica napus* L.) by overexpression of a yeast glycerol-3-phosphate dehydrogenase under the control of a seed-specific promoter. Plant Biotechnol J 5:431–441

Wang SC, Bastern J, Zeng Z B (2006) Windows QTL Cartographer 2.5. North Carolina State University, Raleigh, NC (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wang L, Wang MB, Tu JX, Helliwell CA, Waterhouse PM, Dennis ES, Fu TD, Fan YL (2007) Cloning and characterization of microRNAs from *Brassica napus*. FEBS Lett 581:3848–3856

Wang J, Lydiate DJ, Parkin IAP, Falentin C, Delourme R, Carionl PWC, King GJ (2011) Integration of linkage maps for the amphidiploid *Brassica napus* and comparative mapping with Arabidopsis and *Brassica rapa*. BMC Genomics 12:101

Xie YZ, Hong DF, Xu ZH, Liu PW, Yang GS (2008) Identification of AFLP markers linked to the epistatic suppressor gene of a recessive genic male sterility in rapeseed and conversion to SCAR markers. Plant Breed 127:145–149

Yan XY, Li JN, Fu FY, Jin MY, Chen L, Liu LZ (2009) Co-location of seed oil content, seed hull content and seed coat color QTL in three different environments in *Brassica napus* L. Euphytica 170:355–364

Yang YW, Lai KN, Tai PY, Li WH (1999) Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between *Brassica* and other angiosperm lineages. J Mol Evol 48:597–604

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468

Zhao JY, Becker HC, Zhang DQ, Zhang YF, Ecke W (2005) Oil content in a European-Chinese rapeseed population: QTL with additive and epistatic effects and their genotype-environment interactions. Crop Sci 45:51–59

Zhao JY, Becker HC, Zhang DQ, Zhang YF, Ecke W (2006) Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theor Appl Genet 113:33–38

Zou JT, Katavic V, Giblin EM, Barton DL, MacKenzie SL, Keller WA, Hu X, Taylor DC (1997) Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast *sn-2* acyltransferase gene. Plant Cell 9:909–923