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Identification of QTL influencing seed oil content, fatty acid profile and days to flowering in *Brassica napus* L.

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Abstract The objective of this study was to identify quantitative trait loci (QTL) controlling oil content, fatty acid profile and flowering time in *Brassica napus* L. This research was conducted using a doubled haploid mapping population derived from a cross of Polo × Topas. The population was phenotyped in four environments. The composite interval method of QTL analysis was performed with a previously available genetic map that consisted of mainly simple sequence repeat markers with an average genetic distance of 3.7 cM. The markers were assembled and anchored to 19 chromosomes with a map coverage of 2244.1 cM. Fourteen QTL were identified for oil content, 131 QTL were found to be associated with six fatty acids and 14 QTL were associated with flowering time. A QTL, *qOIL-A10a* with a positive Topas-allele explained 26.99 % of the variation in oil content. Additionally, transgressive segregation for oil content was observed beyond the parental phenotypes (31.5–55.5 %). Two genomic regions on C3, at 147.83 and 154.55 cM were associated with QTL for all six fatty acids studied. We hypothesize this genomic region on C3 modulates the correlations between fatty acids and further investigation of this region could provide insight into the genes determining total seed oil content in *B. napus.* An early flowering QTL, *qFLRa-A2c* containing a Polo-allele was detected in the vicinity of a known Brassica vernalization gene that explained 43.22 % of the trait variation. The phenotypic correlation between traits and collocation of different QTL on thirty-four genomic regions suggests that the traits studied have genetic dependencies on each other.

Keywords *Brassica napus* · Canola · Correlation · Fatty acids · Flowering time · Genetic map · Oil content · QTL

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Introduction

Oil content and quality are the most valuable components of *Brassica napus* L. seed and important traits for future Brassica breeding endeavours (Abbadi and Leckband 2011; Gupta and Pratap 2007). Based on 1716 harvest survey samples collected from the western provinces of Canada in 2013, the top grade

B. napus seed samples contained on average 43.5 % oil (Canadian Grain Commission 2013). This reflects a slight reduction from the 10-year average of 43.8 % oil content. Increases in seed oil content using the same seed yield per hectare will result in greater oil yield per unit of land (Weselake et al. 2009). Actually, a one percent increase in seed oil content is equal to an increase of 2.5 % in seed yield (Wang 2004).

Variable numbers of QTL have been identified that control oil content in *B. napus*. Earlier studies (Ecke et al. 1995; Cheung et al. 1998) reported that a small number of QTL control oil content. In recent studies, oil content has been reported to be controlled by 14–63 QTL (Zhao et al. 2005; Delourme et al. 2006; Chen et al. 2010; Wang et al. 2013). The former two studies mainly reported QTL that appeared in one environment only. However, Chen et al. (2010) identified four oil content QTL that were common in two out of six environments. Whereas, Delourme et al. (2006) reported five QTL in different environments while only one QTL on N3 (A3) was potentially common between the two populations studied. Consistent oil content QTL appearing across environments and genetic backgrounds are important targets to develop cultivars for improved oil production.

The oil profile composed of constituent fatty acids determines the end use of Brassica oil (Voelker and Kinney 2001). Several studies have suggested that the interaction among fatty acids limits the extent of oil accumulation in the seed (Voelker and Kinney 2001; Barker et al. 2007; Zhao et al. 2007). Zhao et al. (2007) identified several QTL that are likely affecting the correlations of different fatty acids. For example, a QTL on N6 (A6) had a negative effect on palmitic acid and a positive effect for stearic acid. However, few such loci with pleiotropic effects on several fatty acids for the regulation of total seed oil content have been reported in *B. napus* (Lionneton et al. 2002; Burns et al. 2003).

Seed yield and seed oil content determine total oil yield. Seed yield is affected by many agronomic traits of which, flowering is one of the most critical affecting seed oil content (Diepenbrock 2000). A non-significant but negative correlation of days to flowering with seed oil content in *Brassica* spp. has been reported by Engqvist and Becker (1993) and Chen et al. (2010). However, Wurschum et al. (2012) speculated that flowering time had no relationship with oil accumulation/content and proposed that metabolic efficiencies of a plant are responsible. Simultaneous improvement of early maturity and increased seed oil content remain important *B. napus* breeding objectives.

The effective utilization of previously identified QTL depends upon the reliability of the QTL position and the degree of impact the QTL has on the expression of the trait (Dudley 1994; Charmet et al. 1999). To obtain this kind of information, it is critical that the effect of the QTL is verified by multiple year and multiple location experiments. Additionally, the development of high-density genetic maps for reliably locating the QTL with associated markers is critical. The current study focuses on identifying *B. napus* (canola) QTL that control total oil content, fatty acid profile [palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and arachidic acid (C20:0)] and flowering time.

Materials and methods

Plant material

Two spring canola varieties, Polo (maternal parent) and Topas, were selected to develop mapping populations for detection of QTL influencing seed oil content, fatty acid profile and flowering time. The variety Polo was registered by Mycogen Canada, Inc. (Dow AgroSciences) in 1994 for cultivation in western Canada. The variety Topas was developed by Svalof A.B., Sweden and was registered in 1987 for cultivation in eastern Canada. According to the registration data of these varieties, Polo matures in 93 days and contains on average 46.2 % seed oil content. The variety Topas matures in 101 days and contains seed oil content of 42 %. Registered cultivars contain a suite of favorable alleles that are already selected for elite performance. To further understand the differing oil content levels, fatty acid profiles and maturities of Polo and Topas, the two parents were selected to develop a mapping population.

For the production of DH lines, the F_1 from Polo and Topas were grown in a growth chamber with the growing conditions of 15 °C and a 15 h day length. Before anthesis, the buds of varying sizes were macerated and examined for uninucleate microspores. Buds ranging from 3 to 4 mm had the highest frequency of uninucleate microspores and were selected for microspore embryogenesis. Haploid

plantlets were produced using standard microspore culture techniques as described by Coventry et al. (1988). The chromosome number of haploid line plants was doubled by submerging the plantlets in 0.02 % (w/v) colchicine solution for 5 h. Plantlets were grown in the greenhouse in pots containing a soil, sand and peat ratio of 2:2:1 and fertilized twice until flowering at the approximate rate of 3.3 ml/L (NPK: 20:20:20). One hundred fifty-six pollen producing DH plants were selected to produce DH lines for trait evaluation in the field.

Field evaluation and seed oil analysis

The Polo × Topas DH line population was evaluated in the field for 2 years (2009 and 2010) at the University of Manitoba, Winnipeg (Win) and near Portage La Prairie (PlaP), approximately 100 km west of Winnipeg, Manitoba. The mapping population at each location along with respective parents was evaluated in a randomized complete block design with two replications. Each DH line was seeded in a single row that was 3 m long with 0.4 m spacing between rows. Days to flowering was recorded when 50 % of the plants in a row had flowered. Each DH line row was harvested separately at physiological maturity, dried in bundles in the field and threshed with a small plot combine (Wintersteiger, Salt Lake City, UT). A 30 g seed sample from each DH line row was collected to determine seed oil content at zero percent moisture using American Oil Chemist's Society's approved methods using a FOSS 6500 near-infrared-reflectance-spectroscopy (Daun et al. 1994).

Fatty acid methyl esters were prepared according to Liu (1984) and the fatty acid profile of the oil including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and arachidic acid (C20:0) was determined using gas chromatography (Christie 1989; Hougen and Bodo 1973) using the apparatus, Varian Model 3900, California, USA. The proportion of each fatty acid was expressed as a percentage of the total fatty acids identified.

Statistical analysis of field data

The analysis of variance (ANOVA; *PROC MIXED* of Statistical Analysis System, SAS ver. 9.1; SAS Institute, Cary, NC) partitioned source of variation into effects of lines, location, year, replicates, and error ($T_{ijkq} = \mu + g_i + l_j + y_k + ly_{jk} + rep_{jkq} + gl_{ij} + gy_{ik} + gly_{ijk} + e_{ijkq}$) where T_{ijkq} is the mean trait value of the ith line of the kth year at jth location in qth replication, and μ population mean, g_i line effect, l_j location effect, Y_k year effect, ly location × year effect, rep_{jkq} effect of replication, gl line × location effect, gy line × year effect, gly line × location × year effect, and e_{ijkq} the residual. Phenotypic correlations between all pairs of traits were determined through the CORR procedure of SAS.

Genetic map

The genetic map used for QTL identification was developed by Geng et al. (2012). This saturated genetic map covers a map distance of 2241.1 cM of the *B. napus* genome with average marker-to-marker interval of 3.71 cM. This linkage map consists of 620 polymorphic loci, mostly SSR (383), Inter-Simple Sequence Repeats (ISSR, [29]), Sequence Related Amplified Polymorphism (SRAP, [191]) and Sequence Characterized Amplified Region (SCAR, [17]) molecular markers. These polymorphic loci form 19 linkage groups (LG), which are anchored to chromosomes through common SSR markers (Cheng et al. 2009; Piquemal et al. 2005).

QTL mapping

The QTL analyses for the traits under study were performed using Windows QTL Cartographer version 2.5 with default settings (walking speed 1 cM, Model 6: Standard model) and the composite interval mapping method (Wang et al. 2011). For each trait data set, a threshold-likelihood ratio (LR) was determined by selecting 1,000 fold permutations test ($\alpha = 0.05$) using ZMapQtl program built within the QTL software (Wang et al. 2011; Chen et al. 2010). A QTL was considered significant if detected above the threshold LR or LOD value (logarithm of odds) (Churchill and Doerge 1994). The LOD peak (higher than the threshold-LOD value) corresponding to the genetic interval along the LG was taken as the likely position of a QTL (Liu 1998). The confidence interval of a QTL was determined from a LOD difference from either side of the QTL peak (Hackett 2002; Lander and Botstein 1989). If present, multiple QTL peaks

exceeding one-half of the LOD difference were considered separate QTL (Chen et al. 2010; Flint-Garcia et al. 2003). A QTL was considered common if it appeared in more than one environment at the same map position or within the same QTL interval on a LG. A QTL appearing in only one environment was considered an independent QTL.

QTL were designated according to Raman et al. (2012), where the '*q*' indicates a QTL identified for a trait (abbreviated), followed by the related chromosome number (hyphenated). A suffix (a, b, c etc.) to a chromosome number was added if multiple QTL were identified on a chromosome. For example, *qOIL-A10b* indicates detection of more than one QTL for oil content on chromosome A10.

Results

Trait statistics and correlation

Highly significant ($P < 0.001$) interaction effect (line × Year × Loc) indicated that all the traits studied with the exception of palmitic acid were affected by the year and location of evaluation Table S1. Therefore, the data set from each environment was analyzed separately for the identification of QTL controlling each trait. The Spearman's rank correlations based on means of DH lines for the respective traits revealed correlations of variable magnitude and direction (Table S2). Oil content was positively correlated with oleic acid content ($r = 0.32$, $P < 0.0001$) and flowering time ($r = 0.30$, $P < 0.0001$). The correlation of stearic acid content with arachidic acid content was positive ($r = 0.85$, $P < 0.0001$). Oleic acid content was negatively correlated with linoleic acid content ($r = -0.77, P < 0.0001$) and linolenic acid content ($r = -0.71, P < 0.0001$).

The histograms for all traits display continuous variation, suggesting that all of these traits are quantitative traits (data not shown). Additionally, transgressive segregation for all traits was observed. This was evident for seed oil content (Fig. 1), because a maximum seed oil content of 55.84 % was observed in one DH line which was 9.34 % higher than the average oil content of the high oil parent Polo. This significant transgressive segregation for oil content in the segregating DH line mapping population is a manifestation of the presence of substantial allelic variation for seed oil content in the parents.

QTL identification for oil content, fatty acids and flowering time

QTL detected for the field trials combined over four environments are given in Tables 1, 2, 3, 4, 5, 6, 7 and 8. The LG locations of major QTL (explaining more than 10 % variation), collocating QTL and select QTL of traits identified in four environments of the study are given in Fig. 2.

Seed oil content

In total, 14 QTL were found to influence seed oil content in the four environments (Table 1). The oil content QTL were scattered on five linkage groups namely, A3, A10, C1, C5, and C6. QTL *qOIL-A10c* was the only common QTL identified in all four environments. Three QTL, *qOIL-A10b*, *qOIL-C5a* and *qOIL-C5b* were found to be common in three environments. Five QTL were detected in two environments, of which two QTL each appeared on A3 (*qOIL-A3a* and *qOIL-A3b*) and C1 (*qOIL-C1b* and *qOIL-C1c)* and one QTL (*qOIL-C6b*) on C6. Five QTL were identified in a single environment (independent); two were present on A10 (*qOIL-A10a* and *qOIL-A10d*), and one each on A3 (*qOIL-A3c*), C1 (*qOIL-C1a*) and C6 (*qOIL-C6a*). The proportion of phenotypic variation (R^2) in oil content explained by individual QTL ranged from 5.23 to 26.99 %; where QTL *qOIL-A10a* (independent) had the largest effect. The additive effect of oil content QTL ranged from −2.32 to 2.38 %.

Both parents contributed positive as well as negative alleles for seed oil content. Topas contributed negative alleles for four QTL (*qOIL-A3a*, *qOIL-A3b*, *qOIL-A10b* and *qOIL-A10d*) whereas Polo contributed negative alleles for five QTL (*qOIL-C1a, qOIL-C1b, qOIL-C1c*, *qOIL-C5a* and *qOIL-C6b*) (Table 1). The low oil content parent Topas contributed positive alleles for oil content for two QTL (*qOIL-A10a* and *qOIL-C5b*) whereas, the high oil content parent Polo contributed positive alleles for three oil content QTL (*qOIL-A3c*, *qOIL-A10c* and *qOIL-C6a*). The QTL *qOIL-A10b* had the strongest association with seed oil content according to its LOD value of 4.72.

Palmitic acid content

The variation in palmitic acid was controlled by 33 QTL across the four environments studied (Table 2).

The QTL were scattered on ten linkage groups namely A3, A4, A5, A8, A10, C2, C3, C5, C8 and C9. Seven QTL, one on A8 (*qC16:0-A8a*), two on C8 (*qC16:0-C8b* and *qC16:0-C8c*) and four on A10 (*qC16:0-A10a, qC16:0-A10b, qC16:0-A10c* and *qC16:0-A10d*) were discovered to be common QTL in two environments. A majority (26 QTL) of the palmitic acid content QTL appeared in only one environment. The phenotypic variation explained by individual palmitic acid QTL across four environments ranged from 5.21 to 32.41 %. The palmitic acid content QTL identified in this study carried minor additive effects and their contribution towards the variation in the palmitic acid content ranged from −0.33 to 0.12 %.

Stearic acid content

Eighteen QTL controlling the content of stearic acid in the mapping population of *B. napus* were distributed on six LG namely A1, A3, A8, C1, C3 and C8 (Table 3). Three common QTL on C3 were identified in all four environments. Two common QTL were found in two environments and both were identified on A1. The majority of stearic acid content QTL (13) were detected only in one environment. The individual stearic acid QTL explained a phenotypic variance ranging from 4.68 to 22.71 % across four environments of the study. Seven QTL explained more than 10 % of the phenotypic variation in stearic acid. The most significant linkage of marker with a stearic acid phenotype was determined for the QTL *qC18:0-C3e*. This QTL identified in all four environments had a LOD value of 11.27 and explained 22.71 % of the phenotypic variation for stearic acid content.

Oleic acid content

Twenty-one QTL determined the content of oleic acid in the mapping population grown in four environments (Table 4). These QTL were located on five linkage groups A1, A2, A3, C3 and C8. Four common oleic acid content QTL, three on C3, and one on A3 were discovered in all four environments. Two oleic acid content QTL on A2 were found common in three environments. Three oleic acid content QTL, one on A1 and two on A2 were common in two environments. Twelve oleic acid content QTL, five on A1, three respectively on A2 and A3 and one on C8 were detected in one environment. The phenotypic variance

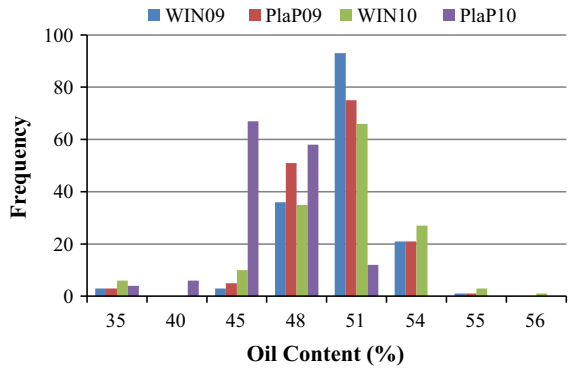


Fig. 1 Frequency distribution of oil content (%) in the Polo × Topas DH line population grown in four environments (*WIN* Winnipeg, *PlaP* Portage la Prairie) during 2009 and 2010

explained by identified oleic acid content QTL ranged from 4.57 to 28.47 %. QTL *qC18:1-C3c* was the most significant QTL having the highest LOD value of 16.84 and it also explained the most phenotypic variation (28.47 %) for oleic acid content across four environments of the study.

Linoleic acid content

A total of 20 QTL for linoleic acid content were identified on seven linkage groups, A1, A2, A3, A5, C1, C3 and C9 (Table 5). Eight QTL were common in three environments, two on A1, three on A2 and three on C3. Three common linoleic acid content QTL, two on A2 and one on A3 were identified in two environments. Nine linoleic acid content QTL, one on A1, two on A3, one respectively on A5, C1 and C3 and three on C9 were identified in only one of the four environments studied. Individually, linoleic acid content QTL explained phenotypic variance in the range of 3.61–22.09 %. The most significant marker linkage with linoleic acid content was determined for QTL *qC18:2-C3c*. This QTL, among the linoleic acid content QTL identified in three environments, had the highest LOD value of 13.21 and explained the most phenotypic variation (22.09 %) for linoleic acid content.

Linolenic acid content

Across the four environments studied, 23 QTL were associated with linolenic acid content (Table 6). These QTL were scattered on eight linkage groups: A1, A2, A3, A5, A9, A10, C3 and C6. Seven linolenic acid

Table 1 Summarized list of QTL detected for seed oil content using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^2*	ADD	Allele	Flanked markers
qOIL-A3a	Win10, PlaP09	A3	129.23	2.85	5.87	−0.48	Topas	BnGMS265-181/odd3/bg4-275
qOIL-A3b	Win10, PlaP09	A3	141.43	3.23	6.55	−0.51	Topas	sa12/pm18-388/pm88/pm46-388
qOIL-A3c	PlaP09	A3	149.11	2.54	5.23	0.47	Polo	em1/pm4-238/bg23/pm60-125
qOIL-A10a	Win09	A10	4.01	3.81	26.99	0.99	Topas	BnGMS288-311/CB10536-163
qOIL-A10b	Win10, PlaP10, PlaP09	A10	10.12	4.72	11.45	−2.32	Topas	CB10124-169/BnGMS288-283
qOIL-A10c	Win10, PlaP10, Win09, PlaP09	A10	11.64	3.61	12.65	2.38	Polo	BnGMS288-283/Na12E09-315
qOIL-A10d	PlaP09	A10	17.70	2.50	5.46	−0.47	Topas	Na12E09-315/sa12/pm7-345
qOIL-C1a	Win09	C1	42.09	2.73	6.17	−0.53	Polo	CB10369-155/CB10369-176
qOIL-C1b	Win10, PlaP09	C1	68.69	2.92	6.54	−0.48	Polo	BnGMS299-375/SR084-223
qOIL-C1c	Win10, PLaP09	C1	77.73	2.86	5.84	−0.49	Polo	sa7/pm55-461/SR076-200
qOIL-C5a	Win10, Win09, PlaP09	C5	62.98	2.67	6.34	−0.51	Polo	ISSR140b/sa12/pm7-171
qOIL-C5b	Win10, Win09,PlaP09	C5	67.12	4.15	9.26	0.62	Topas	sa12/pm7-171/sa7/pm55-170
qOIL-C6a	Win10	C6	45.54	3.87	12.69	0.71	Polo	pm88/pm45-463/sa7/pm55-414
qOIL-C6b	Win10, PlaP09	C6	53.76	3.97	8.14	−0.57	Polo	em1/bg19-478/sa7/pm63-147

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (*OIL*, seed oil content), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

content QTL were common in all four environments studied. Out of the common linolenic acid content QTL, four were detected on A3 and three on C3. Only one linolenic acid content QTL (on A10) was common in three environments. Five linolenic acid content QTL on A2 were common in two environments. Ten linolenic acid content QTL appeared only in one environment. Two of the independent linolenic acid content QTL were on A1, one QTL each on A3, A5 and C6, two QTL on A9 and three QTL on A10. The individual linolenic acid content QTL explained phenotypic variation varying from 4.82 to 35.21 %. The most significant marker linkage with linolenic acid content was determined for QTL *qC18:3-C3c*. This QTL, common in all environments, had a LOD value of 18.06 and explained 35.21 % of the phenotypic variation for linolenic acid content.

Arachidic acid content

Sixteen QTL related to arachidic acid content were identified on A1, A10, C1, C3, C5, C8 and C9 (Table 7). Among arachidic acid content QTL, three QTL on C3 were common in all four environments studied. Three arachidic acid content QTL were common in two environments with two on A1 and one on C1, respectively. Ten arachidic acid content QTL were independent; two were identified respectively on A1, C3, C8 and one QTL each on A10, C1, C5 and C9. The individual arachidic acid content QTL explained a phenotypic variance between 4.63 and 26.51 % across four environments. The most significant marker linkage was determined for the QTL *qC20:0-C3d*. This QTL had a LOD value of 11.85 and explained a phenotypic variation of 26.51 % in arachidic acid content. The positive allele for this QTL was contributed by Topas.

Flowering time

In this study, 14 QTL were found that were associated with flowering time (Table 8). They were scattered on five linkage groups, A1, A2, A10, C3, and C5 across all four environments studied. Three common QTL, two on A2 and one on C3 were found in three environments of the study. Three common QTL, two

Table 2 Summarized list of QTL detected for palmitic acid content using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^2*	ADD	Allele	Flanked markers
qC16:0-A3	Win10	A3	73.69	3.30	6.25	0.06	Topas	ISSR055/Na10D03-164
qC16:0-A4	PlaP09	A4	19.14	2.81	5.21	0.06	Polo	Ol10B06-234/Na10C01-276
qC16:0-A5a	Win10	A5	1.01	2.94	5.45	−0.06	Polo	SR115-401/SR044-437
qC16:0-A5b	Win09	A5	82.89	3.01	7.11	−0.20	Topas	BRMS047-151/SR119-176
qC16:0-A5c	PlaP09	A5	100.62	3.01	6.16	0.07	Polo	sa7/pm52-387/BRMS196-274
qC16:0-A5d	Win10	A5	113.94	5.02	11.12	0.08	Topas	ISSR139/ISSR140
qC16:0-A8a	Win09, PlaP10	A8	90.96	2.87	5.92	−0.05	Polo	ISSR124/CB10364-237
qC16:0-A8b	PlaP10	A8	94.43	3.06	6.91	0.06	Topas	BnGMS003-378/SR092-131
qC16:0-A8c	Win10	A8	119.35	3.60	14.52	−0.10	Polo	BnGMS177-354/SR050-291
qC16:0-A10a	PlaP10, PlaP09	A10	11.64	5.50	10.33	−0.09	Polo	BnGMS288-283/Na12E09-315
qC16:0-A10b	PlaP10, PlaP09	A10	17.70	3.89	7.56	0.08	Topas	Na12E09-315/sa12/pm7-345
qC16:0-A10c	PlaP10, Win10	A10	22.85	4.52	10.91	−0.08	Polo	odd3/bg38-305/me2/pm79-481
qC16:0-A10d	PlaP10, PlaP09	A10	26.19	4.88	10.62	0.08	Topas	MR156-209/BnGMS625-360
qC16:0-A10e	PlaP09	A10	32.94	4.60	8.75	−0.09	Polo	EST001-435/em1/pm4-157
qC16:0-C2a	Win10	C2	35.36	2.96	6.24	0.06	Polo	SR117-408/odd20/pm18-311
qC16:0-C2b	Win10	C2	38.16	3.89	9.68	0.08	Polo	odd20/pm18-311/sa7/bg29-177
qC16:0-C2c	Win10	C2	42.65	6.19	21.73	0.12	Topas	Ol13G05-141/em1/pm4-141
qC16:0-C2d	Win10	C2	45.41	3.59	8.26	−0.07	Topas	CB10093-284/CB10026-145
qC16:0-C2e	Win10	C2	46.86	3.90	8.11	0.07	Polo	CB10093-299/BRAS011-256
qC16:0-C2f	Win10	C2	48.72	2.58	5.43	−0.06	Topas	BRAS011-256/Na12E09-369
qC16:0-C2 g	Win10	C2	51.33	3.36	6.97	0.07	Polo	Na12E09-369/odd20/bg2-314
qC16:0-C3a	PlaP09	C3	144.42	4.01	7.04	0.07	Polo	sa7/pm52-252/CB10427-166
qC16:0-C3b	PlaP09	C3	147.83	3.41	5.91	0.07	Polo	me2/bg33-245/odd3/bg2-466
qC16:0-C3c	PlaP09	C3	154.55	4.66	7.98	−0.07	Topas	pm88/pm45-177/odd3/pm3-399
qC16:0-C3d	PlaP09	C3	160.56	4.83	8.16	−0.08	Polo	Ol10B08-150/BRMS269-329
qC16:0-C3e	Win10	C3	188.15	3.96	7.54	0.07	Polo	odd3/bg38-199/Na10G10-152
qC16:0-C5a	PlaP09	C5	62.98	2.76	6.13	0.07	Topas	ISSR140b/sa12/pm7-171
qC16:0-C5b	PlaP09	C5	67.20	4.37	7.38	−0.07	Topas	sa12/pm7-171/sa7/pm55-170
qC16:0-C8a	PlaP09	C8	43.78	3.29	5.94	0.07	Polo	em1/bg9-434/bg23/pm59-285
qC16:0-C8b	PlaP09, PlaP10	C8	46.78	4.55	32.41	−0.12	Topas	em1/bg9-434/bg23/pm59-285
qC16:0-C8c	PlaP09, PlaP10	C8	54.90	4.98	10.43	0.07	Topas	sa12/bg8-230/CB10028-151
qC16:0-C9a	Win09	C9	19.05	3.68	12.43	−0.33	Topas	BnFUS3#2-1-91/CB10355-218
qC16:0-C9b	Win09	C9	87.77	2.87	9.79	−0.26	Polo	Na12G05-118/BRMS054-148

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C16:0), palmitic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

on A10 and one on A1 were found in two environments. Eight QTL were detected only in one environment. Of the independent flowering time QTL, two were present respectively on A1, A10 and C3 and one each on A2 and C5. Individual, flowering time QTL explained a phenotypic variance varying from 3.59 to

Table 3 List of stearic acid content QTL identified using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^2*	ADD	Allele	Flanked markers
qC18:0-A1a	Win10	A1	62.58	3.89	8.02	−0.11	Polo	sa7/pm52-150/BRAS111-195
qC18:0-A1b	Win09	A1	70.83	2.84	5.51	0.06	Polo	D11-194/pm88/pm17-312
qC18:0-A1c	Win10, PlaP09	A1	75.83	5.37	15.04	0.17	Polo	odd3/pm3-384/em1/pm11-409
qC18:0-A1d	Win10, PlaP09	A1	80.01	5.94	14.77	0.15	Polo	SR012-236/SR117-442
qC18:0-A3a	PlaP10	A3	60.95	2.82	6.18	0.07	Polo	sa12/bg18-224/D21-420
qC18:0-A3b	PlaP09	A3	64.12	2.67	5.76	−0.07	Topas	D21-420/Ra2F11-416
qC18:0-A3c	PlaP09	A3	67.14	4.69	14.62	0.10	Polo	Ra2F11-416/odd3/bg2-532
qC18:0-A8	Win10	A8	84.24	3.82	7.51	0.09	Polo	CB10578-251/P26-118
qC18:0-C1a	PlaP09	C1	42.09	3.54	6.47	0.07	Polo	CB10369-155/CB10369-176
qC18:0-C1b	Win09	C1	50.29	2.64	4.68	0.07	Polo	Na14G06-204/CB10355-538
qC18:0-C1c	PlaP09	C1	56.93	2.58	5.27	−0.06	Polo	CB10355-538/BnGMS299a
qC18:0-C3a	PlaP10	C3	126.47	2.54	5.80	0.08	Polo	sa12/bg8-340/BRAS087-165
qC18:0-C3b	PlaP10	C3	135.16	3.63	7.38	0.09	Polo	KSA1-175/ISSR158
qC18:0-C3c	Win10, PlaP10, Win09, PlaP09	C3	145.28	6.20	14.86	0.13	Topas	CB10427-166/odd20/pm117-468
qC18:0-C3d	Win10, PlaP10, Win09, PlaP09	C3	147.83	7.58	16.90	−0.13	Polo	me2/bg33-245/odd3/bg2-466
qC18:0-C3e	Win10, PlaP10, Win09, PlaP09	C3	154.55	11.27	22.71	0.12	Topas	pm88/pm45-177/odd3/pm3-399
qC18:0-C8a	PlaP10	C8	0.01	4.87	10.07	0.09	Topas	BnGMS004-383/CB10106-247
qC18:0-C8b	PlaP10	C8	7.42	2.66	6.11	−0.0	Polo	em1/bg19-311/BnGMS375-281

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:0), stearic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

43.22 %. The most significant marker linkage with the early flowering phenotype was determined for the QTL *qFLR-A2c*. This QTL had a LOD value of 21.69 and explained 43.22 % of the phenotypic variation in three environments. Moreover, the positive allele for this QTL promoted earliness in *B. napus* and was contributed by the high oil parent Polo. In addition, the negative allele for QTL, *qFLR-C5* promoted lateness and was contributed by Topas.

Colocalization of QTL

Thirty-four genomic regions were associated with QTL for more than one trait (Fig. 2; Table S3). These colocalized genomic regions i.e. a locus related with QTL for more than one trait, were distributed on 10 linkage groups: nine colocalizations were on A1, five each on A2 and A10, four each on A3 and C3, two each on C5 and C8, and one each on A5, C1 and C9. Four QTL, one each on A1, A2, A10 and C5 colocalized for flowering time and fatty acid profile (Table S3). Six genomic regions, three on A10, one on C1 and two on C5 were found to colocalize for oil content, palmitic acid content, stearic acid content, linolenic acid content, arachidic acid content and flowering time QTL. However, no colocalization of oil content QTL with any of the oleic acid content QTL or linoleic acid QTL were found. Twenty-four QTL colocalization sites were identified for the fatty acids studied (Fig. 2). Two genomic regions on C3 (147.8 and 154 cM) colocalized with the QTL for all fatty acids studied (Table 9).

Discussion

Oil content

The alleles increasing seed oil content as identified by the positive additive effect of a QTL were inherited from both parents (Table 1). The low oil content

Table 4 Summarized list of QTL detected for oleic acid content using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^2*	ADD	Allele	Flanked markers
qC18:1-A1a	PlaP10	A1	44.40	2.52	4.92	−0.43	Topas	CB10099-203/BRMS056-221
qC18:1-A1b	PlaP10	A1	46.13	2.70	5.21	0.45	Polo	BRMS056-227/BRMS196-229
qC18:1-A1c	Win09	A1	66.32	4.14	5.96	−0.44	Topas	BRAS084-131/BRAS111-190
qC18:1-A1d	Win09	A1	73.48	3.21	10.37	0.58	Topas	pm88/pm17-312/ISSR008
qC18:1-A1e	Win10, PlaP09	A1	104.51	3.28	5.13	0.56	Polo	CB10597-133/SR083-245
qC18:1-A1f	PlaP09	A1	112.78	3.02	4.57	−0.45	Topas	BRAS029-232/odd3/bg4-249
qC18:1-A2a	Win10, PlaP09, Win09	A2	20.16	7.20	14.10	−0.73	Topas	WRI1-3-170/ISSR059
qC18:1-A2b	Win09, PlaP09	A2	23.41	6.70	10.73	0.61	Polo	ISSR061/BnGMS067-231
qC18:1-A2c	Win10	A2	26.18	5.21	8.89	−0.58	Topas	BnGMS067-231/em1/bg19-251
qC18:1-A2d	Win09, PlaP09, Win10	A2	29.06	7.90	13.41	0.68	Polo	me2/pm45-262/SR058-298
qC18:1-A2e	Win09, PlaP09	A2	33.54	7.10	12.29	0.65	Polo	sa7/pm55-252/ISSR048
qC18:1-A2f	PlaP09	A2	40.21	2.50	6.18	0.46	Topas	odd20/pm18-333/sa12/pm18-132
qC18:1-A2 g	PlaP10	A2	71.73	2.61	12.89	0.70	Polo	CB10172-239/C20-135
qC18:1-A3a	Win10	A3	43.68	5.88	9.34	−0.56	Topas	D03-217/BnGMS079-353
qC18:1-A3b	Win09	A3	51.53	5.16	8.71	0.56	Polo	BnGMS079-353/BnGMS079-333
qC18:1-A3c	Win10, PlaP10, Win09, PlaP09	A3	55.95	10.04	14.99	0.70	Polo	sa12/bg18-224/D21-420
qC18:1-A3d	Win09	A3	67.14	3.56	7.55	−0.50	Polo	Ra2F11-416/odd3/bg2-532
qC18:1-C3a	Win10, PlaP10, Win09, PlaP09	C3	144.42	9.09	19.40	−0.87	Polo	sa7/pm52-252/CB10427-166
qC18:1-C3b	Win10, PlaP10, Win09, PlaP09	C3	147.83	8.51	17.66	−0.83	Polo	me2/bg33-245/odd3/bg2-466
qC18:1-C3c	Win10, PlaP10, Win09, PlaP09	C3	154.55	16.84	28.47	1.05	Topas	pm88/pm45-177/odd3/pm3-399
qC18:1-C8	Win10	C8	54.47	2.70	6.26	−0.49	Polo	PROM008-229/sa12/bg8-230

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:1), palmitic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

parent, Topas, contributed an oil increasing allele for the QTL *qOIL-A10a* that explained 26.99 % of the variation for seed oil content in a single environment. This phenotypic variation in oil content is higher than found in previous studies (Delourme et al. 2006; Yan et al. 2009; Sun et al. 2012; Wang et al. 2013). The increased oil content (Fig. 1) in some DH lines observed in the current study can be attributed to inheritance of positive and negative alleles contributed by both parents. Delourme et al. (2006) mapped seed oil content in a *B. napus* population designated as RNSL. The parents of the RNSL population contained seed oil content of 47.7 and 41.7 % respectively. The alleles increasing oil content in the RNSL population were mostly inherited from the high oil parent. Consequently, less transgressive segregation for seed oil content was observed for the RNSL population in comparison to the current study.

In the current study, the region between 4.01 and 17.70 cM on A10 was associated with the oil content QTL and explained the greatest phenotypic variation in the study. This region on A10 has also been reported to contain a linoleic acid QTL (Smooker et al. 2011), erucic acid and oil content QTL (Qiu et al. 2006). The study by Delourme et al. (2006) reported an oil content QTL in multiple environments on A10 that was located less than 1 cM from an SSR marker, MR156 in a *DY* designated population. The marker MR156 in our study is located approximately 10 cM from the

Table 5 Summarized list of QTL detected for linoleic acid content using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^{2*}	ADD	Allele	Flanked markers
qC18:2-A1a	PlaP10	A1	92.73	3.15	7.51	0.56	Topas	SR083-251/SR027-338
qC18:2-A1b	Win10, PlaP10, Win09	A1	104.51	6.04	10.32	−0.52	Polo	CB10597-133/SR083-245
qC18:2-A1c	Win10, PlaP10, Win09	A1	112.78	4.32	10.17	0.70	Topas	BRAS029-232/odd3/bg4-249
qC18:2-A2a	Win10, Win09, PlaP09	A2	20.16	6.22	12.10	0.47	Topas	WRI1-3-170/ISSR059
qC18:2-A2b	Win10, PlaP09	A2	23.41	5.46	9.84	−0.42	Polo	ISSR061/BnGMS067-231
qC18:2-A2c	Win10, PlaP09	A2	26.18	5.55	10.06	0.42	Topas	BnGMS067-231/em1/bg19-251
qC18:2-A2d	Win10, Win09, PlaP09	A2	29.06	7.59	14.07	−0.50	Polo	me2/pm45-262/SR058-298
qC18:2-A2e	Win10, Win09, PlaP09	A2	33.54	6.05	11.56	−0.46	Polo	sa7/pm55-252/ISSR048
qC18:2-A3a	Win09	A3	43.68	3.17	4.49	0.29	Topas	D03-217/BnGMS079-353
qC18:2-A3b	Win09	A3	52.24	2.80	4.00	0.27	Polo	BnGMS079-333/sa12/bg18-224
qC18:2-A3c	Win10, Win09	A3	55.24	3.06	9.30	−0.41	Polo	BnGMS079-333/sa12/bg18-224
qC18:2-A5	Win10	A5	125.01	3.08	5.00	−0.30	Topas	BRAS072-199/BRAS072-212
qC18:2-C1	PlaP10	C1	133.91	3.78	8.16	−0.59	Topas	BRAS067-139/BRAS074-143
qC18:2-C3a	Win10, Win09, PlaP09	C3	144.42	5.03	9.76	0.42	Polo	sa7/pm52-252/CB10427-166
qC18:2-C3b	Win10, Win09, PlaP09	C3	147.83	3.32	6.39	0.34	Polo	me2/bg33-245/odd3/bg2-466
qC18:2-C3c	Win10, Win09, PlaP09	C3	154.55	13.21	22.09	−0.64	Topas	pm88/pm45-177/odd3/pm3-399
qC18:2-C3d	PlaP09	C3	157.36	5.70	12.56	0.72	Polo	odd3/pm3-399/Ol10B08-144
qC18:2-C9a	PlaP09	C9	0.01	3.56	7.60	−0.90	Topas	CB10344-202/SR043-342
qC18:2-C9b	PlaP09	C9	8.40	2.92	7.15	−0.82	Topas	CB10350-220/BnFUS3#2-1-91
qC18:2-C9c	Win09	C9	41.75	2.59	3.61	−0.25	Topas	SR090-182/BnFUS3#2-5-329

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:2), palmitic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

confidence intervals of the three oil content QTL identified on A10. Thus, A10 is an interesting target to increase seed oil content.

Identification of the oil content QTL on LG A3, C1 and C5 is consistent with earlier reports. The two oil content QTL reported by Wang et al. (2013) on A3 are likely to be similar to our study because QTL exist in both studies that are close to a common SSR marker (BRAS050). This marker is positioned 25.30 cM away from the A3 QTL in the study by Wang et al. (2013) and only 7.99 cM from the A3 QTL (*qOIL-A3c*) in the current study. Another QTL found in our study on C5, *qOIL.C5a* was located 17 cM from a SSR marker, Na10D11. Delourme et al. (2006), also identified a QTL for oil content on C5 (LG DY-N15) at a distance of 23.40 cM from Na10D11.

Palmitic acid content

The detection of seven QTL for palmitic acid on C2 may be linked to the *B. napus* response to increased environmental temperatures. These QTL were identified in one environment (Win10) when the growing conditions at the seed filling stage experienced environmental temperatures peaking around 30 °C for 12 days in the month of August, 2010 (weather data not shown). Increased temperature during seed maturation has been reported to reduce oil content in *B. napus*. Zhu et al. (2012), explained the effect of increased temperature on oil content at a molecular level. They developed two near isogenic lines (*B. napus*), NIL-9 and NIL-1 that differed for a QTL, *qOC.C2.2* and further analyzed the developing seeds

Table 6 Summarized list of QTL detected for linolenic acid content using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^2*	ADD	Allele	Flanked markers
qC18:3-A1a	PlaP10	A1	44.40	3.45	6.23	0.22	Topas	CB10099-203/BRMS056-221
qC18:3-A1b	PlaP10	A1	46.13	3.97	7.04	−0.24	Polo	BRMS056-227/BRMS196-229
qC18:3-A2a	Win10, PlaP09	A2	19.16	4.07	7.60	0.22	Topas	WRI1-3-170/ISSR059
qC18:3-A2b	Win10, PlaP09	A2	23.41	3.68	4.22	−0.19	Polo	ISSR061/BnGMS067-231
qC18:3-A2c	Win10, PlaP09	A2	25.41	3.87	17.81	0.33	Polo	ISSR061/BnGMS067-231
qC18:3-A2d	Win10, PlaP09	A2	29.06	5.07	8.39	−0.23	Polo	me2/pm45-262/SR058-298
qC18:3-A2e	Win10, PlaP09	A2	33.54	4.27	7.51	−0.22	Polo	sa7/pm55-252/ISSR048
qC18:3-A3a	PlaP10	A3	22.00	4.03	8.00	0.26	Topas	sa12/bg38-422/BRMS071-278
qC18:3-A3b	Win10, PlaP10, Win09, PlaP09	A3	37.58	5.91	11.48	−0.30	Polo	BRMS071-278/SR075a
qC18:3-A3c	Win10, PlaP10, Win09, PlaP09	A3	42.29	7.82	14.69	−0.34	Polo	BRMS303-217/D03-203
qC18:3-A3d	Win10, PlaP10, Win09, PlaP09	A3	51.53	5.83	11.85	−0.31	Polo	BnGMS079-353/BnGMS079-333
qC18:3-A3e	Win10, PlaP10, Win09, PlaP09	A3	56.95	6.57	18.81	−0.34	Polo	sa12/bg18-224/D21-420
qC18:3-A5	Win10	A5	130.32	2.78	15.23	−0.31	Topas	BRAS072-212/ISSR160
qC18:3-A9a	PlaP10	A9	23.90	3.61	6.43	−0.23	Topas	Na10D09-300/em1/bg19-299
qC18:3-A9b	PlaP10	A9	32.27	2.69	4.82	0.20	Polo	BnGMS313-169/CB10347-190
qC18:3-A10a	Win10, Win09, PlaP09	A10	8.50	4.36	6.92	−0.21	Polo	CB10124-155/CB10124-169
qC18:3-A10b	Win10	A10	11.64	4.67	7.26	−0.22	Polo	BnGMS288-283/Na12E09-315
qC18:3-A10c	Win10	A10	19.59	3.32	5.24	−0.20	Polo	sa12/pm7-345/MR156-195
qC18:3-A10d	Win10	A10	47.91	2.52	5.25	−0.20	Topas	ISSR031/bg23/pm52-216
qC18:3-C3a	Win10, PlaP10, Win09, PlaP09	C3	145.28	12.59	26.61	−0.42	Topas	CB10427-166/odd20/pm117-468
qC18:3-C3b	Win10, PlaP10, Win09, PlaP09	C3	147.83	10.61	22.90	0.38	Polo	me2/bg33-245/odd3/bg2-466
qC18:3-C3c	Win10, PlaP10, Win09, PlaP09	C3	154.55	18.06	35.21	−0.47	Topas	pm88/pm45-177/odd3/pm3-399
qC18:3-C6	PlaP10	C6	59.12	2.69	13.32	0.33	Polo	me2/bg33-133/sa7/pm52-518

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C18:3), palmitic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

of the isogenic lines for transcriptome variation under different growing temperatures. This QTL region was only detected at high temperatures (30 °C) and enhanced the oil content. This temperature sensitive oil content QTL, *qOC.C2.2* was flanked by SSR markers, OI13G05 and CB10530 that were mapped in our study with multiple C16:0 QTL (Table 2; Fig. 2 [C2]). The former marker was linked with a major QTL, *qC16:0-C2c* that explained 21.73 % variation in palmitic acid content.

Stearic acid content

Wang et al. (2013), combined QTL information from eight populations of different genetic backgrounds or gene pools to perform a meta QTL analysis to identify

Table 7 Summarized list of QTL detected for arachidic acid content using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^2*	ADD	Allele	Flanked markers
qC20:0-A1a	Win10	A1	61.97	4.89	10.21	−0.02	Polo	Ol12F11-236/sa7/pm52-150
qC20:0-A1b	Win09	A1	72.48	2.81	5.00	−0.01	Topas	pm88/pm17-312/ISSR008
qC20:0-A1c	Win09, Win10	A1	76.19	5.62	11.33	−0.03	Topas	em1/pm11-409/me2/pm86-158
qC20:0-A1d	Win10, Win09	A1	80.01	7.21	13.21	0.03	Polo	SR012-236/SR117-442
qC20:0-A10	PlaP10	A10	24.08	2.80	15.43	−0.03	Topas	me2/pm79-481/Na12C05-351
qC20:0-C1a	Win10, Win09	C1	40.91	2.97	5.05	0.01	Polo	BRMS096-179/CB10369-155
qC20:0-C1b	Win10	C1	147.91	2.88	4.63	0.01	Topas	BRAS067-139/BRAS074-143
qC20:0-C3a	PlaP10	C3	115.20	3.21	13.02	−0.03	Polo	Na10D03-153/Na10D03-179
qC20:0-C3b	Win10, PlaP10, Win09, PlaP09	C3	145.28	4.76	16.79	0.05	Topas	CB10427-166/odd20/pm117-468
qC20:0-C3c	Win10, PlaP10, Win09, PlaP09	C3	147.83	5.57	12.87	−0.03	Polo	me2/bg33-245/odd3/bg2-466
qC20:0-C3d	Win10, PlaP10, Win09, PlaP09	C3	154.55	11.85	26.51	0.04	Topas	pm88/pm45-177/odd3/pm3-399
qC20:0-C3e	PlaP09	C3	157.36	4.77	11.72	−0.03	Polo	odd3/pm3-399/Ol10B08-144
qC20:0-C5	Win10	C5	23.81	2.52	12.79	−0.02	Topas	SR112-370/SR112-340
qC20:0-C8a	PlaP10	C8	0.01	3.47	6.63	0.02	Topas	BnGMS004-383/CB10106-247
qC20:0-C8b	PlaP09	C8	18.78	2.50	5.52	−0.02	Polo	BnGMS004-349/em1/bg1-409
qC20:0-C9	PlaP09	C9	17.40	3.87	8.73	−0.03	Topas	BnFUS3#2-1-91/CB10355-218

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (C20:0), palmitic acid), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

consensus oil content QTL in *B. napus*. Seven consensus QTL on the consensus LG A1 were projected to have a map distance of 9.20 cM between common SSR markers, BRAS111 and BRAS026/CB10206. The QTL peaks of four stearic acid content QTL on A1 (Fig. 2, A1) in our study spanned a region of 17.43 cM and were located in the vicinity of previously mentioned common SSR markers (BRAS111, BRAS026 and CB10206). Therefore, the stearic acid content QTL on A1 in our study represent the consensus oil content QTL projected on A1 by Wang et al. (2013).

In the current study, the stearic acid content QTL on A3 were detected on different map positions than the oil content QTL, suggesting these QTL may have involvement either in the desaturation or elongation pathways. The QTL peaks of all three stearic acid content QTL on A3 fall within the QTL span of oleic acid and linoleic acid content QTL identified by Smooker et al. (2011) on N3 (A3). Both studies detected these QTL in close proximity to a common marker, Na10D03-164. These stearic acid content QTL are most likely involved in substrate supply for the elongation and desaturation activities.

Based on QTL mapping results of the previous studies, C3 consistently harbors oil content QTL across different gene pools of *B. napus* (Zhao et al. 2012; Wang et al. 2013). Examining the five stearic acid content QTL on C3 in the current study, two QTL, *qC18:0-C3b* and *qC18:0-C3c* were flanked by common SSR markers, CB10057 and CB10427 (Wang et al. 2013). The genomic region between these markers reported to contain QTL intervals of two consensus oil content QTL (*DY-qOC-3* and *GS/12-qOC-3*) on C3 (Wang et al. 2013). In our study, *qC18:0-C3b* showed environmental sensitivity as it was detected only in one environment and was also linked to a SCAR marker (KSAI-175). Whereas, the

Table 8 Summarized list of QTL detected for flowering time using composite interval mapping in a doubled haploid line population—Polo × Topas grown in four environments in 2009 and 2010

QTL[a]	Location	LG	Position	LOD	R^2*	ADD	Allele	Flanked markers
qFLR-A1a	Win10, Win09	A1	56.75	2.80	11.14	−0.57	Polo	bg23/pm117-444/odd20/bg2-248
qFLR-A1b	Win10	A1	60.97	2.51	3.59	0.34	Polo	Ol12F11-236/sa7/pm52-150
qFLR-A1c	Win10	A1	66.32	2.65	4.01	−0.36	Topas	BRAS084-131/BRAS111-190
qFLR-A2a	Win10	A2	17.16	2.70	4.05	−0.34	Topas	WRI1-3-170/ISSR059
qFLR-A2b	Win10, Win09, PlaP09	A2	67.73	6.41	15.17	−0.70	Polo	CB10172-239/C20-135
qFLR-A2c	Win10, Win09, PlaP09	A2	83.73	21.69	43.22	1.13	Polo	CB10172-239/C20-135
qFLR-A10a	Win09	A10	42.53	2.49	4.50	−0.39	Topas	bg23/pm60-157/ISSR031
qFLR-A10b	Win10, PlaP09	A10	47.91	5.49	9.99	−0.58	Topas	ISSR031/bg23/pm52-216
qFLR-A10c	Win09, Win10	A10	53.06	3.51	7.14	0.48	Polo	sa7/bg37-293/OlAJ120-360
qFLR-A10d	Win10	A10	60.85	2.78	4.32	−0.36	Polo	OlAJ120-360/sa7/bg37-430
qFLR-C3a	PlaP10	C3	168.71	3.05	8.33	1.77	Polo	BRMS269-341/ISSR065
qFLR-C3b	PlaP10	C3	185.71	2.97	8.31	−1.18	Polo	BRMS269-341/ISSR065
qFLR-C3c	Win10, Win09, PlaP09	C3	206.02	5.28	9.84	−0.54	Topas	Na10G10-165/pm88/bg1-275
qFLR-C5	Win10	C5	66.12	2.82	23.64	−0.83	Topas	sa12/pm7-171/sa7/pm55-170

Winnipeg 2009, Portage la Prairie 2009, Winnipeg 2010, Portage la Prairie 2010

[a] QTL nomenclature: *q* indicate a QTL that is identified for a trait which is indicated by an abbreviation (FLR, flowering time), followed by a chromosome number and a suffix if multiple QTL are identified on a chromosome

LG linkage groups, *LOD* estimation of LOD score at the QTL peak, *ADD* additive effect of QTL, *Allele* the source of allele inheritance for the respective QTL

* % of phenotypic variation explained by the QTL

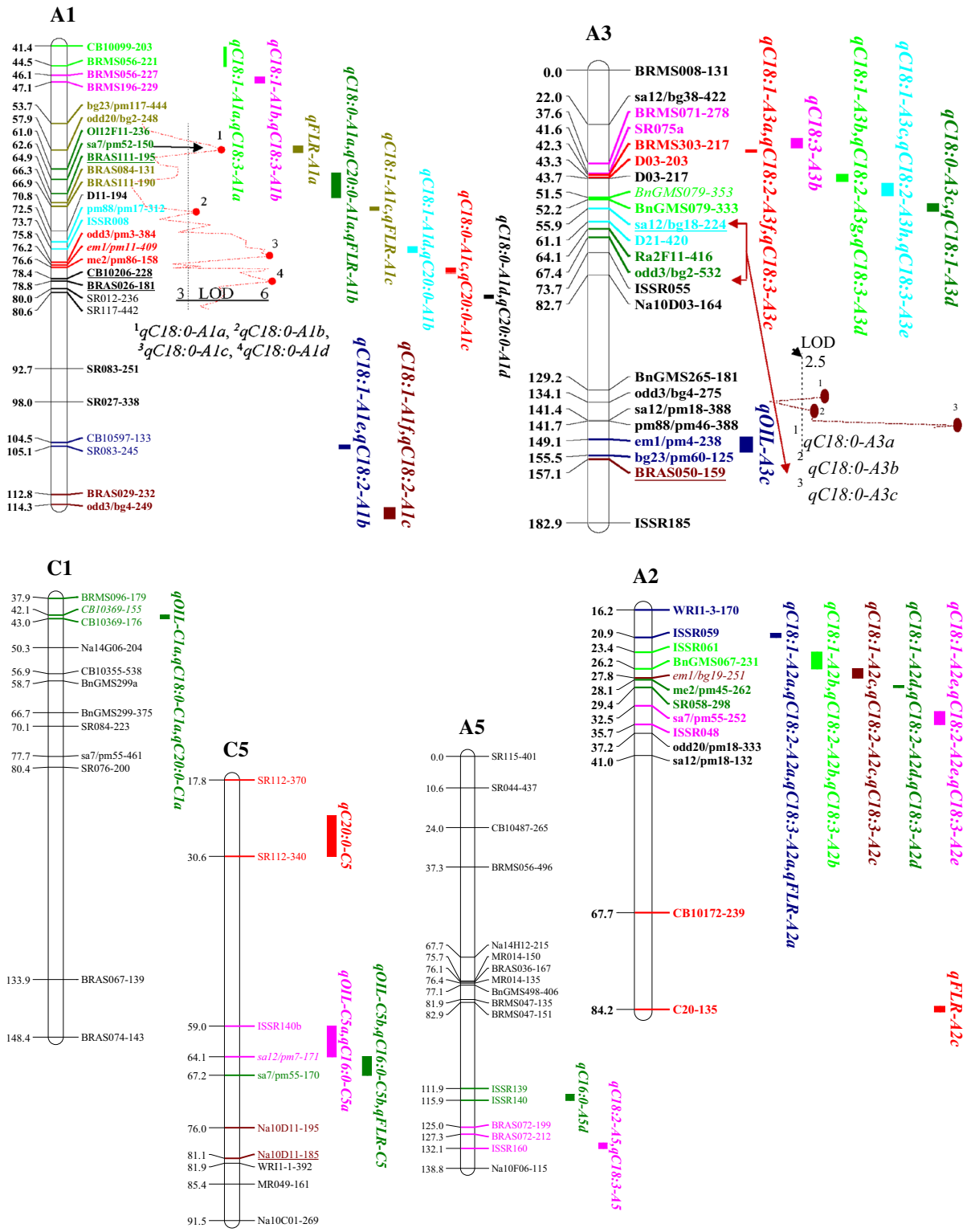
QTL, *qC18:0-C3c* explained major phenotypic variation for stearic acid and was detected in all four environments of our study.

Oleic acid content

With respect to the current information available on the location of *FAD2* genes in *B. napus*, we identified six oleic acid content QTL on A1 in this study. The location of QTL *qC18:1-A1d* in this study is in close proximity to a *FAD2* locus that was mapped by Scheffler et al. (1997) with an Restriction Fragment Length Polymorphisms (RFLP) marker, pO12e. The QTL *qc18:1-A1d* is not mapped with a common marker but since RFLP and SSR markers have a strong correlation in estimating the genetic distance, it is possible that *qC18:1-A1d* represents a *FAD2* locus. Similarly, Hu et al. (2006) using independent markers (SSR and RFLP) identified two oleic acid content QTL, one with a larger LOD (3.5) and the other with a lower LOD value (2.5) respectively on the top and bottom of LG N1 (A1). Identification of stronger oleic acid content QTL (*qC18:1-A1c* and *qC18:1-A1f*) with larger LOD value is in agreement with Wang et al. (2013) who reported that A1 could contain oil content QTL in most genetic backgrounds of *B. napus*. No oleic acid content QTL on A5, C1 or C5 were detected in the current study which is in contradiction to other known locations of *FAD2* genes. This could be due to lack of segregation for these *FAD2* loci in our mapping population (Zhao et al. 2008).

Linoleic acid content

The LG A5 in the current study contained a QTL for linoleic acid content and linolenic acid content that was linked with a SSR marker, BRAS072. This marker was also mapped 9.10 cM away from the QTL, *qC16:0-A5d* that positively affected the content of palmitic acid and decreased both contents of linoleic acid and linolenic acid in our study. The QTL, *qC18:2-A5* and *qC18:3-A5* are in repulsion which suggests an interaction that might be affecting another phenotype (seed coat color). The marker BRAS072 has been previously been reported to be linked to a QTL, *qSCBB05A-7-2* (Fu et al. 2007) that control variation in the yellow seed color in *B. napus*. The QTL flanked by the marker BRAS072 can be useful to develop


A1
41.4 CB10099-203
44.5 BRMS056-221
46.1 BRMS056-227
47.1 BRMS196-229
53.7 bg23/pm117-444
57.9 odd20/bg2-248
61.0 OI12F11-236
62.6 sa7/pm52-150
64.9 BRAS111-195
66.3 BRAS084-131
66.9 BRAS111-190
70.8 D11-194
72.5 pm88/pm17-312
73.7 ISSR008
75.8 odd3/pm3-384
76.2 em1/pm11-409
76.6 me2/pm86-158
78.4 CB10206-228
78.8 BRAS026-181
80.0 SR012-236
80.6 SR117-442
92.7 SR083-251
98.0 SR027-338
104.5 CB10597-133
105.1 SR083-245
112.8 BRAS029-232
114.3 odd3/bg4-249
LOD 3 6
qC18:1-A1a,qC18:3-A1a
qC18:1-A1b,qC18:3-A1b
qFLR-A1a
qC18:0-A1a,qC20:0-A1a,qFLR-A1b
qC18:1-A1c,qFLR-A1c
qC18:1-A1d,qC20:0-A1b
qC18:0-A1c,qC20:0-A1c
qC18:0-A1d,qC20:0-A1d
qC18:1-A1e,qC18:2-A1b
qC18:1-A1f,qC18:2-A1c
[1]qC18:0-A1a, [2]qC18:0-A1b,
[3]qC18:0-A1c, [4]qC18:0-A1d
A3
0.0 BRMS008-131
22.0 sa12/bg38-422
37.6 BRMS071-278
41.6 SR075a
42.3 BRMS303-217
43.3 D03-203
43.7 D03-217
51.5 BnGMS079-353
52.2 BnGMS079-333
55.9 sa12/bg18-224
61.1 D21-420
64.1 Ra2F11-416
67.4 odd3/bg2-532
73.7 ISSR055
82.7 Na10D03-164
129.2 BnGMS265-181
134.1 odd3/bg4-275
141.4 sa12/pm18-388
141.7 pm88/pm46-388
149.1 em1/pm4-238
155.5 bg23/pm60-125
157.1 BRAS050-159
182.9 ISSR185
qC18:1-A3a,qC18:2-A3f,qC18:3-A3c
qC18:3-A3b
qC18:1-A3b,qC18:2-A3g,qC18:3-A3d
qC18:1-A3c,qC18:2-A3h,qC18:3-A3e
qC18:0-A3c,qC18:1-A3d
qOIL-A3c
LOD 2.5
qC18:0-A3a
qC18:0-A3b
qC18:0-A3c
C1
37.9 BRMS096-179
42.1 CB10369-155
43.0 CB10369-176
50.3 Na14G06-204
56.9 CB10355-538
58.7 BnGMS299a
66.7 BnGMS299-375
70.1 SR084-223
77.7 sa7/pm55-461
80.4 SR076-200
133.9 BRAS067-139
148.4 BRAS074-143
qOIL-C1a,qC18:0-C1a,qC20:0-C1a
C5
17.8 SR112-370
30.6 SR112-340
59.0 ISSR140b
64.1 sa12/pm7-171
67.2 sa7/pm55-170
76.0 Na10D11-195
81.1 Na10D11-185
81.9 WRI1-1-392
85.4 MR049-161
91.5 Na10C01-269
qC20:0-C5
qOIL-C5a,qC16:0-C5a
qOIL-C5b,qC16:0-C5b,qFLR-C5
A5
0.0 SR115-401
10.6 SR044-437
24.0 CB10487-265
37.3 BRMS056-496
67.7 Na14H12-215
75.7 MR014-150
76.1 BRAS036-167
76.4 MR014-135
77.1 BnGMS498-406
81.9 BRMS047-135
82.9 BRMS047-151
111.9 ISSR139
115.9 ISSR140
125.0 BRAS072-199
127.3 BRAS072-212
132.1 ISSR160
138.8 Na10F06-115
qC16:0-A5d
qC18:2-A5,qC18:3-A5
A2
16.2 WRI1-3-170
20.9 ISSR059
23.4 ISSR061
26.2 BnGMS067-231
27.8 em1/bg19-251
28.1 me2/pm45-262
29.4 SR058-298
32.5 sa7/pm55-252
35.7 ISSR048
37.2 odd20/pm18-333
41.0 sa12/pm18-132
67.7 CB10172-239
84.2 C20-135
qC18:1-A2a,qC18:2-A2a,qC18:3-A2a,qFLR-A2a
qC18:1-A2b,qC18:2-A2b,qC18:3-A2b
qC18:1-A2c,qC18:2-A2c,qC18:3-A2c
qC18:1-A2d,qC18:2-A2d,qC18:3-A2d
qC18:1-A2e,qC18:2-A2e,qC18:3-A2e
qFLR-A2c

◀**Fig. 2** The QTL illustrations were produced using the genetic map developed by Geng et al. (2012) and Mapchart 2.2 software. Different outputs of MapChart 2.2 were combined to show collocating, major and select QTL [with log of odd (LOD) values' graph] that were identified in the doubled haploid line population—Polo × Topas. The flanking markers (the italicized marker locus; flanks with the neighboring loci) and the respective QTL have matching color. Genetic distance between markers is given on the *left side* of the LG. The underlined marker locus on a LG has been reported to be associated with QTL for oil content and composition in previous studies (and discussed elsewhere in the current study)

canola cultivars with improved oil content and reduced linoleic acid and linolenic acid content that can enhances shelf life of canola oil.

Linolenic acid content

The content of linolenic acid in our study was controlled by 23 QTL of which six QTL were independent and 17 QTL collocated with QTL each for oil content, saturated and unsaturated fatty acids content and flowering time (Fig. 2). This redundancy of genetic control highlights the difficulty of reducing the content of linolenic acid to improve the shelf life of canola oil. However, the QTL, *qC18:3-C3b* explained 22.90 % variation in the content of linolenic acid and positively affected the phenotype. Avoiding this QTL integration improves the possibility of reduction in linolenic acid content in *B. napus*.

Arachidic acid content

QTL of saturated fatty acids and unsaturated fatty acids have been reported to colocalize (Burns et al. 2003; Lionneton et al. 2002). Stepwise elongation from C16 to C18 is required for the synthesis of very long chain fatty acids (VLCFA) (Baud et al. 2003). Oleic acid is a preferred substrate for the activity of fatty acid elongase (FAE) for the synthesis VLCFA such as erucic acid (Bao et al. 1998) or arachidic acid. The arachidic acid content QTL, *qC20:0-A1b* and oleic acid content QTL, *qC18:1-A1d* colocalized to the same position and likely represent a *FAE* locus in our study.

Flowering time

There are inconsistent reports on the effects of flowering time on oil content. In some reports, days to flowering was negatively correlated to oil content (Chen et al. 2010). In contrast, Delourme et al. (2006) reported two genomic regions where both early and late flowering QTL collocated with high oil content, however, most of the QTL for these two traits were independent of each other. In the current study, out of 14 flowering time QTL, nine QTL are also independent. The phenotypic correlation between flowering time and seed oil content is positive and significantly correlated ($r = 0.30$, $P < .0001$, Table S2). However, the magnitude of the correlation is small and can be supported in our study by colocation of two late flowering time QTL, *qFLR-C5* and *qFLR- A2a* respectively with an oil content QTL, *qOIL-C5b* and linoleic acid and linolenic acid content QTL (*qC18:2-A2a* and *qC18:3-A2a*). Both of these QTL inherited the lateness and the respective phenotype enhancing allele from Topas, indicating that late flowering increases oil content (or affects oil profile). In contrast, the parent Polo is high oil and flowers early. The Polo could have been bred through selection or pyramiding of early flowering alleles that promoted oil content.

Transition to flowering in *Arabidopsis* is regulated by five pathways: photoperiod, vernalization, gibberellic acid (GA), autonomous pathway and thermal clock (Putterill et al. 2004). There are two growth types of *B. napus*; the annual (spring type) and biennial (winter types) types (Teutonico and Osborn 1995). The biennial types require vernalization to induce flowering. A region on LG 9 reported to contain a vernalization QTL (Ferreira et al. 1995) aligned with N2 (A02) of *B. napus* (Teutonico and Osborn 1995). This segment of A2 in our study also contains a major flowering time QTL, *qFLR-A2c*. This QTL explained 43.22 % of the variation in flowering time and the allele for earliness for this QTL was contributed by the early flowering parent (Polo). Presence of flowering time QTL in this study at the reported location of a vernalization QTL may be due to the fact that these are related traits. Additionally, vernalization in annual growth types of *B. napus* can promote earliness (Medham and Scott 1975). The QTL *qFLR-A2c* on A2 is most likely a candidate for a vernalization gene that has lost function in spring types of *B. napus*.

Early flowering and early maturing varieties in *B. napus* have value in agro-climatic zones where the growing period is short. The flowering time QTL identified in this study on A2 can be used to develop early flowering and early maturing *B. napus* lines with

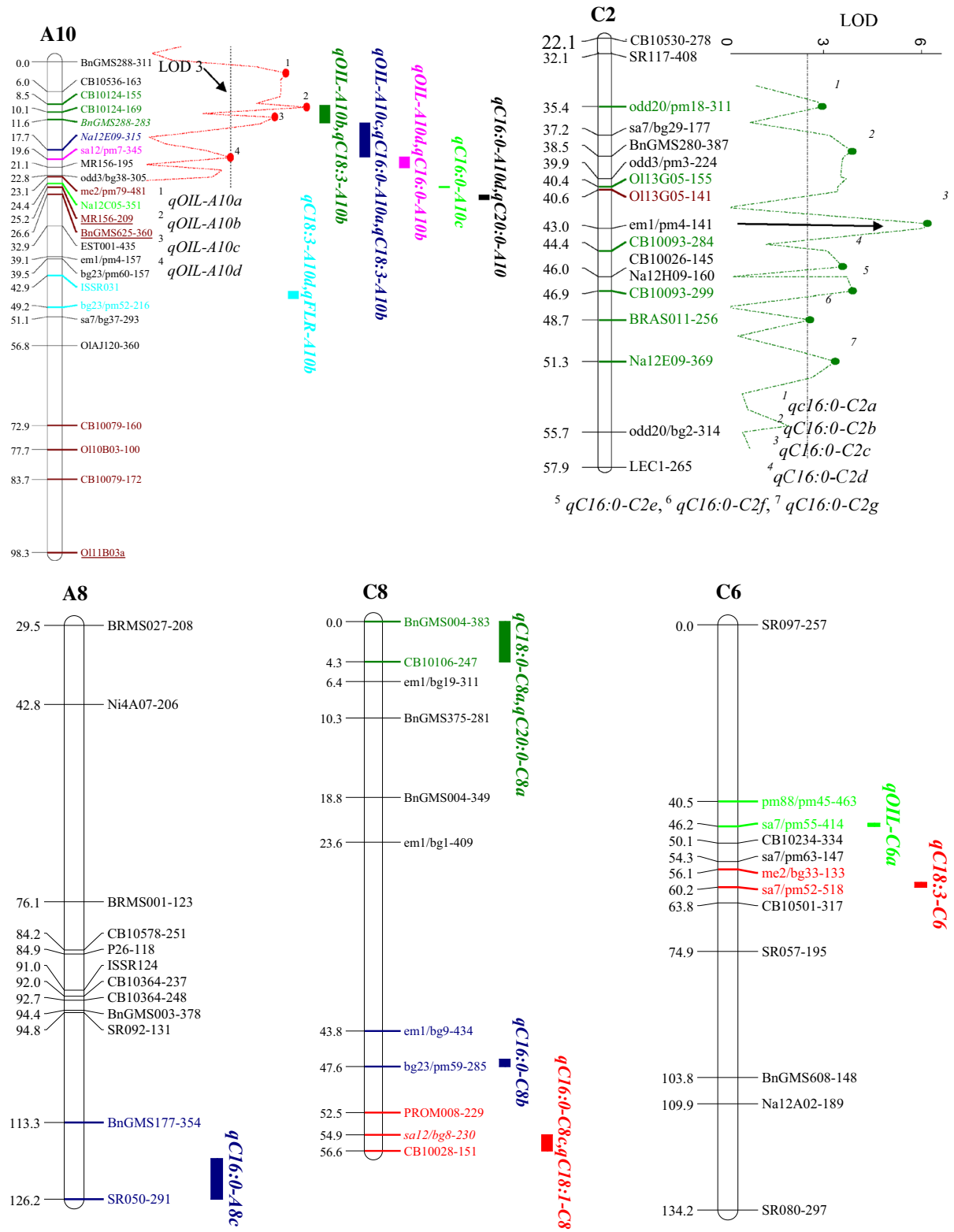


Fig. 2 continued

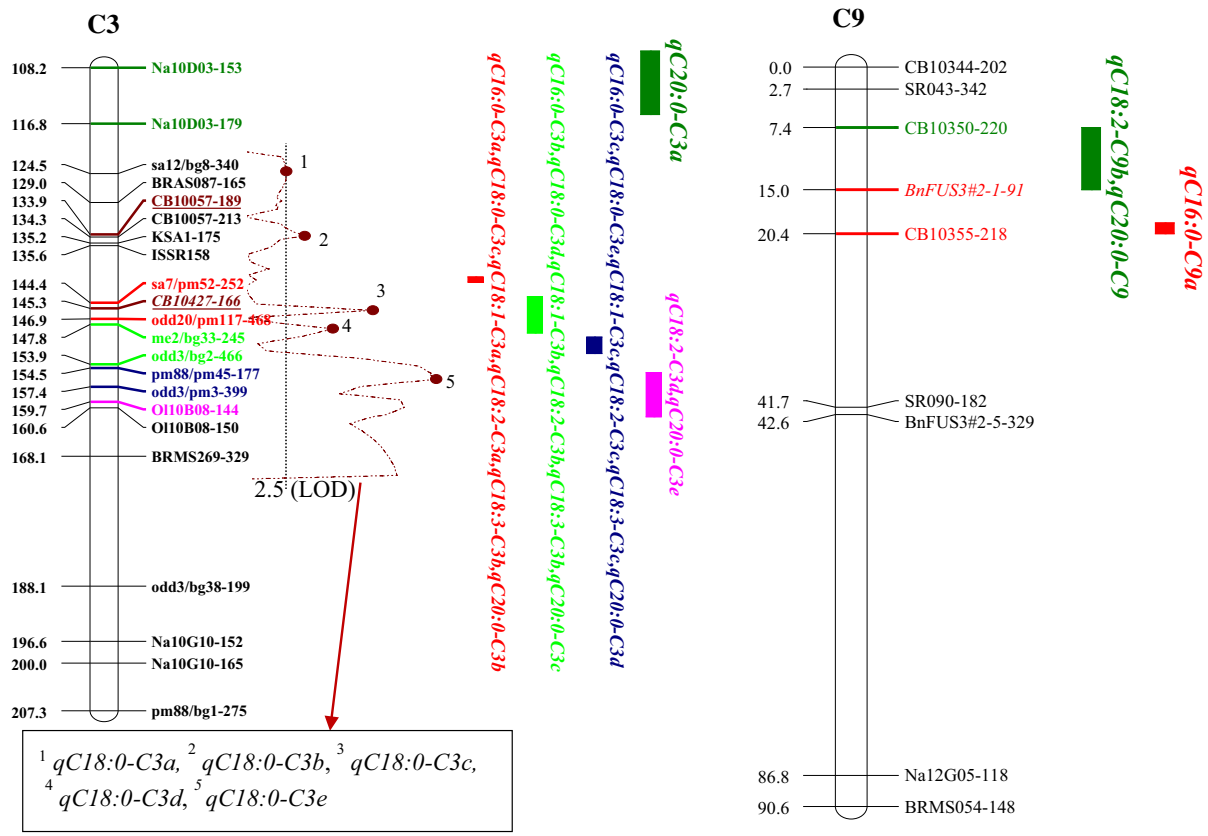


Fig. 2 continued

increased oil content. The QTL *qFLR-A2c* explained 43.22 % of the variation and the high oil parent Polo contributed the alleles for earliness. Another QTL, *qFLR-A2b* explained 15.17 % variation in flowering. However, this late flowering allele was also contributed by the high oil and early flowering parent (Polo). This is consistent with the phenotypic correlation observed between days to flowering and oil content (Table S2).

Colocalization of QTL

Multiple colocalization sites for QTL controlling variation in the mono and polyunsaturated fatty acid contents were identified on LG A2 and A3. We found that the upper part of LG A2 (20.16–33.54 cM) contained QTL for oleic acid content, linoleic acid content and linolenic acid content. The clustering of QTL on A2 can be explained with three pieces of evidence. First, the QTL of polyunsaturated fatty acids are linked with a transcription factor (marker WRI1-3-170) that affects multiple pathways. Secondly, the newly developed and integrated SSR marker on A2 in the current study, SR058-298 corresponds to a *B. rapa* gene, Bra008247 (data not shown) that encodes a protein for pollen development. Since linolenic acid content of pollen has strong correlation (r = 87) with seed linolenic acid content (Jourdren et al. 1996) this collocation of QTL is expected. Thirdly, independent studies have reported oil content QTL on this part of A2 (Wang et al. 2013; Delourme et al. 2006) in particular by Zhao et al. (2012) who has mapped QTL *OilC2* that represent a diacylglycerol acyltransferase/phospholipid: diacylglycerol acyltransferase (*DGAT/PDAT*) locus (*At5g136040* and *At5g10160*) that functions in fatty acid biosynthesis (Li-Beisson et al. 2010). The clustering of QTL for different fatty acids on the upper region of A2 is therefore likely related to DGAT activity, which has broad specificity for fatty acids biosynthesis (Topfer et al. 1995).

Mono unsaturated fatty acid (C18:1) is produced through the activity of the *Fatty Acid Biosynthesis 2*

Table 9 C3 genomic regions of a doubled haploid line population—Polo × Topas harboring two QTL (*rQTL)* for fatty acids which are modulating correlation between different fatty acids through their respective additive effects

Map position (cM)	C16:0[a]	C18:0[b]	C18:1[c]	C18:2[d]	C18:3[e]	C20:0[f]
147.83	+	–	–	+	+	–
154.55	–	+	+	–	–	+
		*				
			**			

At any of these map positions of N13, the appearing QTL if have alternate direction of polarity of additive effects between two fatty acids, it will result in a negative correlation between those fatty acids otherwise positive

* Negative correlation

** Positive correlation

[a] C16:0: palmitic acid

[b] C18:0: stearic acid

[c] C18:1: oleic acid

[d] C18:2: linoleic acid

[e] C18:3: linolenic acid

[f] C20:0: arachidic acid

(*FAB2*) (Kachroo et al. 2007) that encodes a stearoyl-ACP desaturase (SAD). Using locus specific markers, Smooker et al. (2011) mapped a *FAB2* QTL on A3; 10 cM away from a SSR marker (Na10D03-155). In our study, *qC18:1-A3d* and *qC18:0-A3c* were identified 15.30 cM away from the same marker (Na10D03-164). The colocalization of the QTL, *qC18:1-A3d* and *qC18:0-A3c* also indicate that they are involved in the desaturation pathway.

An oleic acid content (*qC18:1-A1e*) and a linoleic acid content QTL (*qC18:2-A1b*) colocalized at map position, 104.51 cM on A1. The alleles of both of these QTL that increase the oleic acid content or decrease the content of linoleic acid were contributed by Polo (Tables 4, 5, Table S3). Considering the opposing effects of QTL where the two traits are both contributed by the same parent highlights the difficulties of introgressing multiple favorable alleles from one parent (Ramchiary et al. 2007).

We report two genomic regions (QTL) on C3 that are likely controlling the mutual correlations of fatty acids in *B. napus*. QTL associated with all fatty acids studied in this research clustered at two genomic regions, 147.83 and 154.55 cM of C3 referred as Correlation QTL (*rQTL*) (Table 9). The *rQTL* explain a high proportion of the phenotypic variance. The additive effects of *rQTL* either positive or negative affect multiple traits. The interaction of *rQTL* for fatty acids results in a pattern (Table 9) that coincides with the phenotypic correlations observed between the fatty acids (Table S2). These findings are consistent with the study by Zhao et al. (2007) who found opposing additive effects for oleic acid content (negative) and erucic acid content QTL (positive) which indicates their genetic correlation. The *rQTL* had higher LOD values highlighting the possibility that they contain pleiotropic loci affecting multiple traits. Zhao et al. (2012) mapped *Arabidopsis* lipid orthologous genes in *B. napus* and reported genes at the bottom of C3 that are related to plastidial fatty acid synthesis, fatty acid elongation, and wax and cutin metabolism. Further fine mapping and comparative genomic studies at these two genomic positions of C3 may provide insight into genes controlling seed oil content, which is influenced in part by the correlations between fatty acids (Sanyal and Linder 2012).

This study has presented QTL identification results that were obtained using a saturated linkage map derived mainly of SSR markers. The relatively large population size and reduced marker interval of 3.77 cM for this linkage map allowed detection of QTL with minor effects. Common/stable QTL for oil content, fatty acid profile and flowering time have been identified and compared with published results where common markers permitted such comparison. Several of the fatty acid QTL reported here are also

associated with transcription factors for lipid biosynthesis. Most importantly, QTL explaining major phenotypic variations in oil content (27 %), 35 % linolenic acid content, and flowering time (43 %) have been reported in this study. These QTL can possibly be introgressed into elite germplasm of *B. napus* using genome-wide selection.

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Compliance with ethical standards

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

References

Abbadi A, Leckband G (2011) Rapeseed breeding for oil content, quality, and sustainability. Eur J Lipid Sci Technol 113:1198–1206. doi:10.1002/ejlt.201100063

Bao XM, Pollard M, Ohlrogge J (1998) The biosynthesis of erucic acid in developing embryos of *Brassica rapa*. Plant Physiol 118:183–190. doi:10.1104/pp.118.1.183

Barker GC, Larson TR, Graham IA, Lynn JR, King GJ (2007) Novel insights into seed fatty acid synthesis and modification pathways from genetic diversity and quantitative trait loci analysis of the *Brassica* C genome. Plant Physiol 144:1827–1842. doi:10.1104/pp.107.096172

Baud S, Guyon V, Kronenberger J, Wuilleme S, Miquel M, Caboche M, Lepiniec L, Rochat C (2003) Multifunctional acetyl-CoA carboxylase 1 is essential for very long chain fatty acid elongation and embryo development in *Arabidopsis*. Plant J 33:75–86. doi:10.1046/j.1365-313X.2003.016010.x

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. doi:10.1038/sj.hdy.6800176

Canadian Grain Commission (2013) Western Canadian canola, export quality data, December 2013. http://www.grainscanada.gc.ca/canola/export-exportation/ceqd-dqec-eng.htm

Charmet G, Robert N, Perretant MR, Gay G, Sourdille P, Groos C, Bernard S, Bernard M (1999) Marker-assisted recurrent selection for cumulating additive and interactive QTLs in recombinant inbred lines. Theor Appl Genet 99:1143–1148. doi:10.1007/s001220051318

Chen G, Geng J, Rahman M, Liu X, Tu J, Fu T, Li G, 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. doi:10.1007/s10681-010-0144-9

Cheung WY, Landry BS, Raney P, Rakow GFW (1998) Molecular mapping of seed quality traits in *Brassica juncea* L. Czern and Coss. In: Thomas G, Monteiro AA (eds) Brassica 97: international symposium on *Brassicas*. Acta Horticulturae 459:139–147

Cheng X, Xu J, Xia S, Gu J, Yang Y, Fu J, Qian X, Zhang S, Wu J, Liu K (2009) Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor Appl Genet 118:1121–1131. doi:10.1007/s00122-009-0967-8

Christie WW (1989) Gas chromatography and lipids. A Practical guide. The Oily Press Ltd, Dundee

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

Coventry J, Kott LS, Beversdorf WD (1988) Manual for microspore culture technique for *Brassica napus*. Technical Bulletin, OAC Publ. 0489. Department Crop Science, University of Guelph, Guelph

Daun JK, Clear KM, Williams P (1994) Comparison Of 3 whole seed near-infrared analyzers for measuring quality components of canola seed. J Am Oil Chem Soc 71:1063–1068. doi:10.1007/bf02675897

Delourme R, Falentin C, Huteau V, Clouet V, Horvais R, Gandon B, Specel S, Hanneton L, Dheu JE, Deschamps M, Margale E, Vincourt P, Renard M (2006) Genetic control of oil content in oilseed rape (*Brassica napus* L.). Theor Appl Genet 113:1331–1345. doi:10.1007/s00122-006-0386-z

Diepenbrock W (2000) Yield analysis of winter oilseed rape (*Brassica napus* L.): a review. Field Crops Res 67:35–49. doi:10.1016/s0378-4290(00)00082-4

Dudley JW (1994) Molecular markers in plant improvement—manipulation of genes affecting quantitative traits. Crop Sci 34:322

Ecke W, Uzunova M, Weissleder K (1995) Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. Theor Appl Genet 91:972–977. doi:10.1007/BF00223908

Engqvist GM, Becker HC (1993) Correlation studies for agronomic characters in segregating families of spring oilseed rape (*Brassica napus*). Hereditas 118:211–216. doi:10.1111/j.1601-5223.1993.00211.x

Ferreira ME, Satagopan J, Yandell BS, Williams PH, Osborn TC (1995) Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor Appl Genet 90:727–732

Flint-Garcia SA, Jampatong C, Darrah LL, McMullen MD (2003) Quantitative trait locus analysis of stalk strength in four maize populations. Crop Sci 43:13–22

Fu F-Y, Liu L-Z, Chai Y-R, Chen L, Yang T, Jin M-Y, Ma A-F, Yan X-Y, Zhang Z-S, Li J-N (2007) Localization of QTLs for seed color using recombinant inbred lines of *Brassica napus* in different environments. Genome 50:840–854. doi:10.1139/G07-068

Geng J, Javed N, McVetty PBE, Li G, Tahir M (2012) An integrated genetic map for *Brassica napus* derived from double haploid and recombinant inbred populations. Hered Genet 1(1):103. doi:10.4172/2161-1041.1000103

Gupta SK, Pratap A (2007) History, origin, and evolution. Adv Bot Res 45:1–20. doi:10.1016/S0065-2296(07)45001-7

Hackett C (2002) Statistical methods for QTL mapping in cereals. Plant Mol Biol 48:585–599. doi:10.1023/A:1014896712447

Hougen FW, Bodo V (1973) Extraction and methanolysis of oil from whole or crushed rapeseed for fatty acid analysis. J Am Oil Chem Soc 50:230–234

Hu X, Sullivan-Gilbert M, Gupta M, Sa Thompson (2006) Mapping of the loci controlling oleic and linolenic acid contents and development of fad2 and fad3 allele-specific markers in canola (*Brassica napus* L.). Theor Appl Genet 113:497–507. doi:10.1007/s00122-006-0315-1

Jourdren C, Simonneaux D, Renard M (1996) Selection on pollen for linolenic acid content in rapeseed, *Brassica napus* L. Plant Breeding 115:11–15. doi:10.1111/j.1439-0523.1996.tb00863.x

Kachroo A, Shanklin J, Whittle E, Lapchyk L, Hildebrand D, Kachroo P (2007) The Arabidopsis stearoyl-acyl carrier protein-desaturase family and the contribution of leaf isoforms to oleic acid synthesis. Plant Mol Biol 63:257–271. doi:10.1007/s11103-006-9086-y

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Li-Beisson Y, Shorrosh B, Beisson F, Andersson MX, Arondel V, Bates PD, Bird D, Debono A, Durrett TP, Franke RB, Graham IA, Katayama K, Kelly A, Larson T, Markham JE, Miquel M, Molina I, Nishida I, Rowland O, Schmid KM, Wada H, Welti R, Xu C, Zallot Re, Ohlrogge J (2010) Acyl-lipid metabolism. In: The *Arabidopsis* book, vol book, whole. American Society of Plant Biologists, Rockville, pp 1–65. doi:10.1199/tab.0133

Lionneton E, Ravera S, Sanchez L, Aubert G, Delourme R, Ochatt S (2002) Development of an AFLP-based linkage map and localization of QTLs for seed fatty acid content in condiment mustard (*Brassica juncea*). Genome 45:1203–1215. doi:10.1139/G02-095

Liu K-S (1984) Review-preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. J Am Oil Chem Soc 71:1179–1187

Liu B (1998) Statistical genomics: linkage, mapping and QTL analysis. vol Book, whole. CRC Press, Boca Raton

Medham NJ, Scott RK (1975) The limiting effect of plant size at flower initiation on subsequent growth and yield of oilseed rape (*Brassica napus* L.). J Agric Sci 84:487–500

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet I, Perret D, Villeger MJ, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523. doi:10.1007/s00122-005-0080-6

Putterill J, Laurie R, Macknight R (2004) It's time to flower: the genetic control of flowering time. BioEssays 26:363–373. doi:10.1002/bies.20021

Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li J, Li D, Meng J, Bancroft I (2006) A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet 114:67–80. doi:10.1007/s00122-006-0411-2

Raman R, Taylor B, Marcroft S, Stiller J, Eckermann P, Coombes N, Rehman A, Lindbeck K, Luckett D, Wratten N, Batley J, Edwards D, Wang X, Raman H (2012) Molecular mapping of qualitative and quantitative loci for resistance to *Leptosphaeria maculans* causing blackleg disease in canola (*Brassica napus* L.). Theor Appl Genet 125:405–418. doi:10.1007/s00122-012-1842-6

Ramchiary N, Padmaja KL, Sharma S, Gupta V, Sodhi YS, Mukhopadhyay A, Arumugam N, Pental D, Pradhan AK (2007) Mapping of yield influencing QTL in *Brassica juncea*: implications for breeding of a major oilseed crop of dryland areas. Theor Appl Genet 115:807–817. doi:10.1007/s00122-007-0610-5

Sanyal A, Linder CR (2012) Quantitative trait loci involved in regulating seed oil composition in *Arabidopsis thaliana* and their evolutionary implications. Theor Appl Genet 124:723–738. doi:10.1007/s00122-011-1742-1

Scheffler JA, Sharpe AG, Schmidt H, Sperling P, Parkin IAP, Luhs W, Lydiate DJ, Heinz E (1997) Desaturase multigene families of *Brassica napus* arose through genome duplication. Theor Appl Genet 94:583–591. doi:10.1007/s001220050454

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. doi:10.1007/s00122-010-1512-5

Sun M, Hua W, Liu J, Huang S, Wang X, Liu G, Wang H (2012) Design of new genome and gene-sourced primers and identification of QTL for seed oil content in a specially high-oil *Brassica napus* cultivar. PLoS ONE 7(10):e47037. doi:10.1371/journal.pone.0047037

Teutonico RA, Osborn T (1995) Mapping loci controlling vernalization requirement in *Brassica rapa*. Theor Appl Genet 91:1279–1283

Topfer R, Martini N, Schell J (1995) Modification of plant lipid synthesis. Science 268:681–686. doi:10.1126/science.268.5211.681

Voelker T, Kinney AT (2001) Variations in the biosynthesis of seed-storage lipids. Annu Rev Plant Physiol Plant Mol Biol 52:335–361. doi:10.1146/annurev.arplant.52.1.335

Wang HZ (2004) Strategy for rapeseed genetic improvement in China in the coming fifteen years. Chin J Oil Crop Sci 26:98–101

Wang S, Basten CJ, Zeng ZB (2011) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh

Wang X, Wang H, Long Y, Li D, Yin Y, Tian J, Chen L, Liu L, Zhao W, Zhao Y, Yu L, Li M (2013) Identification of QTLs associated with oil content in a high-oil *Brassica napus* cultivar and construction of a high-density consensus map for QTLs comparison in *B. napus*. PLoS ONE 8(12):e80569. doi:10.1371/journal.pone.0080569

Weselake RJ, Taylor DC, Rahman MH, Shah S, Laroche A, McVetty PBE, Harwood JL (2009) Increasing the flow of carbon into seed oil. Biotechnol Adv 27:866–878. doi:10.1016/j.biotechadv.2009.07.001

Wurschum T, Liu W, Maurer HP, Abel S, Reif JC (2012) Dissecting the genetic architecture of agronomic traits in multiple segregating populations in rapeseed (*Brassica napus* L.). Theor Appl Genet 124:153–161

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. doi:10.1007/s10681-009-0006-5

Zhao J, Becker HC, Zhang D, Zhang Y, Ecke W (2005) Oil content in a European X Chinese rapeseed population : qTL with additive and epistatic effects and their genotype–environment interactions. Crop Sci 45:51–59

Zhao J, Dimov Z, Becker HC, Ecke W, Moellers C (2007) Mapping QTL controlling fatty acid composition in a doubled haploid rapeseed population segregating for oil content. Mol Breed 21:115–125. doi:10.1007/s11032-007-9113-y

Zhao J, Dimov Z, Becker HC, Ecke W, Moellers C (2008) Mapping QTL controlling fatty acid composition in a doubled haploid rapeseed population segregating for oil content. Mol Breed 21:115–125. doi:10.1007/s11032-007-9113-y

Zhao J, Huang J, Chen F, Xu F, Ni X, Xu H, Wang Y, Jiang C, Wang H, Xu A, Huang R, Li D, Meng J (2012) Molecular mapping of *Arabidopsis thaliana* lipid-related orthologous genes in *Brassica napus*. Theor Appl Genet 124:407–421. doi:10.1007/s00122-011-1716-3

Zhu Y, Cao Z, Xu F, Huang Y, Chen M, Guo W, Zhou W, Zhu J, Meng J, Zou J, Jiang L (2012) Analysis of gene expression profiles of two near-isogenic lines differing at a QTL region affecting oil content at high temperatures during seed maturation in oilseed rape (*Brassica napus* L.). Theor Appl Genet 124:515–531. doi:10.1007/s00122-011-1725-2