

# Multi-trait and multi-environment QTL analysis reveals the impact of seed colour on seed composition traits in *Brassica napus*

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**Abstract** *Brassica napus* seed composition traits (fibre, protein, oil and fatty acid profiles), seed colour and yield-associated traits are regulated by a complex network of genetic factors. Although previous studies have attempted to dissect the underlying genetic basis for these traits, a more complete picture of the available quantitative trait loci (QTL) variation and any interaction between the different traits is required. In this study, QTL mapping for eleven seed composition traits, seed colour and a yield-related trait (TSW) was conducted in a spring-type canola-quality

*B. napus* doubled haploid (DH) population from a cross between black-seeded (DH12075) and yellow-seeded (YN01-429) lines across five environments. A major QTL associated with fibre traits (acid detergent fibre, acid detergent lignin and neutral detergent fibre) and seed colour (whiteness index) was mapped on chromosome N9 across the five environments. Multi-trait analysis identified QTL which had pleiotropic effect for seed colour and other composition traits. Multi-environment analysis revealed genetic (QTL) × environment effects on most QTL. These findings provide a more detailed insight into the complex QTL networks controlling seed composition and yield-associated traits in canola-quality *B. napus*.

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## Introduction

Oilseed rape (*Brassica napus*, genome AACC,  $2n = 38$ ) is one of most important oilseed crops for edible oil (canola) worldwide. It is also suitable for biofuel and bioproduct applications, and is being used in European biodiesel production (Tahir et al. 2012). The amino acid composition of the meal is well suited for animal feed. However, the inclusion rate of rapeseed meal in animal feed is still limited due to the high fibre content, in particular for feeding

monogastrics (pigs and poultry) (Rakow et al. 2007). The fibre fraction includes neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Acid detergent lignin, consisting largely of lignin-related phenolic compounds, is the most nutritionally relevant fibre fraction (Wittkop et al. 2012). It has been reported that meal from yellow-seeded lines has much lower fibre content than meal from black-seeded lines due to their thinner seed (Rakow et al. 2007). Reducing the seed coat thickness also resulted in an increased proportion of oil and protein in the seed, leading to an improvement in overall seed value (Slominski et al. 1999).

To date, substantial efforts have been made to reduce fibre content in oilseed rape meal by using the yellow seed trait as a selection marker for reduced seed coat thickness (Wittkop et al. 2012). However, seed colour is known to be environmentally sensitive (Boesewinkel and Bouman 1995). Due to the complexity of inheritance and environmental instability of the trait, various major effect QTL in addition to minor QTL were detected in different genetic backgrounds of *Brassica* species tested in different environments (Liu et al. 2005; Badani et al. 2006; Liu et al. 2006; Fu et al. 2007; Snowdon et al. 2010; Liu et al. 2012; Xiao et al. 2012). Badani et al. (2006) observed a highly positive correlation between seed colour and ADF, and detected a major QTL on N18 with a large effect on both traits in multiple environments. A major QTL influencing seed colour, fibre content and phenolic compounds was mapped to the same position on *B. napus* chromosome A9 (N9) in populations derived from two different yellow- by black-seeded *B. napus* crosses (Snowdon et al. 2010). Furthermore, a strong positive correlation was observed between ADL content and seed colour (Liu et al. 2012).

Seed oil content is the most important agronomic trait in oilseed crops, with fatty acid composition being one of the most critical determinants of oil quality (Zhao et al. 2008). Seed oil content and fatty acid composition are typical quantitative traits controlled by multiple QTL and influenced by genotype–environment interactions. Several studies have detected QTL associated with oil content in different rapeseed populations (Ecke et al. 1995; Burns et al. 2003; Qiu et al. 2006; Delourme et al. 2006; Zhao et al. 2006; Chen et al. 2010). QTL controlling fatty acid composition and a possible relationship between those QTL and oil content QTL have been investigated in *B.*

*napus* (Ecke et al. 1995; Qiu et al. 2006; Zhao et al. 2008; Cao et al. 2010; Smooker et al. 2011). Multiple QTL were detected, and QTL specificity was inferred for particular combinations of substrate and product of fatty acid synthesis and modification pathway in *B. oleracea* (Barker et al. 2007). Furthermore, allele-specific markers were developed for *FAD2* and *FAD3*, major genes for the control of C18:1 and C18:3 content in *B. napus* (Yang et al. 2012).

Genetic improvement of seed yield has been the primary objective of canola breeders for many years. Seed yield is a complex quantitative trait, which is largely influenced by the environment. The seed yield per plant is directly determined by three yield-component traits, specifically, siliques per plant, seeds per silique and seed weight. Therefore, yield-associated traits (yield-components and yield-related traits such as plant height) are known as important factors for improving rapeseed yield. Chen et al. (2010) detected 18 QTL associated with seed yield, but only one of them was reproducible in at least two environments. A number of QTL detected were associated with silique traits (silique length and seeds per silique) (Chen et al. 2007; Zhang et al. 2011a, b, 2012).

The increasing demands for high-quality vegetable oil and biodiesel have resulted in a dramatic increase in production of rapeseed worldwide. Developing varieties with high seed yield and desired seed composition have been targets of rapeseed breeding, and a genetic interrelationship has been noted among the desired traits. A strong negative correlation between oil and protein content was reported (Zhao et al. 2006), and a co-localized QTL was identified for lower oil content and higher seed yield alleles (Chen et al. 2010). Although some studies detected QTL responsible for individual composition (fibre, protein, oil and fatty acids) and yield-associated traits, and a few studies have looked at a combination of a small number of these traits, no study has investigated and mapped QTL for all these traits in one population. The main objectives of this study are to unravel a more complete genetic architecture of seed colour and seed quality-related traits such as fibre content and fatty acid composition. In the current study, QTL were mapped for all these traits in a *B. napus* doubled haploid (DH) population (BnaYB) derived from a cross between a black- (DH12075) and a yellow-seeded (YN01-429) line, assessed across five environments. DH12075 and YN01-429 have distinct seed

colour and fibre content, which might lead to the identification of novel loci/alleles for these traits. Furthermore, multi-trait and multi-environment QTL analysis were performed to dissect the genetic correlation between traits and QTL  $\times$  environment interaction. This established an integrated map of QTL for seed-related traits in *B. napus* and refined the genomic regions conferring these seed features, which will accelerate breeding of cultivars carrying not only one desirable trait, but a combination of various improved traits.

## Materials and methods

### Plant material

A segregating doubled haploid (DH) population developed by Dr. G. Séguin-Schwartz (Agriculture and Agri-Food Canada, Saskatoon Research Centre) was derived from reciprocal crosses between two canola-quality spring-type *B. napus* lines. One parent (YN01-429) is yellow-seeded, has low fibre, and has high oil content. YN01-429 originates from complex crosses involving the black-seeded, canola-quality *B. napus* variety Regent and yellow-seeded *B. rapa* yellow sarson, *B. carinata* and *B. juncea* (Rashid et al. 1994). YN01-429 is an  $F_8$  inbred selection that displays normal meiotic behaviour and genetically stable yellow-seeded trait (Rakow and Relf-Eckstein, 2005). The second parent (DH12075) is black-seeded, has high fibre, and has relatively low oil content. It is a DH from the  $F_1$  progeny of a cross between the French variety Cresor and the Canadian variety Westar (Dr. G. Séguin-Schwartz and Dr. G. Rakow, Agriculture and Agri-Food Canada, Saskatoon Research Centre). The resulting population, called BnaYB, consists of >500 DH lines and has been evaluated for seed traits under field conditions. One hundred and ninety-two of these lines were genotyped using SSR markers to identify the most diverse individuals, from which the core set of 96 lines used in this study was selected, with the exception that additional selections were required when seed was limiting.

### Field experiments

The DH lines together with parents and two yellow seed check lines YN97-262 and YN90-1016 were

evaluated in the field over 4 years (2008, 2009, 2010, 2011) at two locations near Saskatoon, SK [AAFC farms in Saskatoon (S) and Llewellyn (L)]. A randomized complete block design was used for field trial with two replicates in 2008 and 2009, and three replicates in 2010 and 2011. The parental lines and two yellow seed check lines were present at least once in every range, as well as being replicated throughout the trial. The DH lines and checks were planted in ten-foot rows with 24-inch row spacing for all years. The granular pre-emergent herbicide Edge was applied to the site prior to seeding. Corn grits treated with Helix insecticide were side-banded with the seed for flea beetle control. Fertilizer was applied prior to seeding. Due to inclement weather, insufficient materials could be harvested from trials at Llewellyn in 2009–2011 to produce reliable data and were not analysed.

Once combined, seed samples were dried to bring the moisture content to approximately 10 %. Excess chaff was cleaned from the samples using a Clipper Seed Separator. Subsamples weighing approximately 65 grams were removed from the bulk sample and further cleaned using a ASC-3 Seed Cleaner (Agricullex, Guelph, ON) prior to analysis. The subsamples were dried at 40 °C for 48 h, before analysis.

### Trait measurement

Oil content, iodine value, protein and fibre content were determined by near-infrared reflectance (NIR Systems Model 6500, FOSS, Eden Prairie, MN) according to AOCS standard procedure Am 1-92: determination of oil, moisture and volatile matter, and protein by near-infrared reflectance. For oil content, the system was calibrated with oilseed samples extracted with hexane as described by Raney et al. (1987). Results are reported as a percentage of whole seed dry matter (zero moisture). For iodine value (IV), a measurement of fatty acid unsaturation, the system was calibrated with oilseed samples whose iodine values were determined according to AOCS Recommended Practice Cd 1c-85 reapproved (1997): calculated iodine value.

Fatty acid composition was analysed using an Agilent 6890 GC-FID (Agilent Technologies, Santa Clara, CA) following preparation of fatty acid methyl esters by base-catalysed methanolysis (Thies 1971), and individual fatty acids were reported as a per cent of total fatty acids by mass. Very long chain fatty acid

(VLCFA) is the sum of fatty acid molecules with carbon chains of 20 or longer. C16:018:0 is the sum of palmitic acid (C16:0) and stearic acid (C18:0).

For protein, the system was calibrated with oilseed samples whose protein contents were determined by the AOCS Official Method Ba 4e-93, revised 2003: generic combustion method for determination of crude protein using a LECO FP-528 protein analyser (LECO, St. Joseph, MI). Results were reported as a percentage on a whole seed dry matter (zero moisture) basis.

For fibre, the system was calibrated with oilseed samples whose total fibre contents were determined by a modified AOCS Approved Procedure Ba 6a-05, approved 2005: crude fibre analysis in feeds by filter bag technique.

For seed colour, whiteness index (WIE) was determined by Hunter Lab reflectance (Hunter Associates Laboratory, Reston, VA) (Rakow et al. 1999) where high negative values indicate light-coloured seed.

Thousand seed weight (TSW) was the weight of 1000 randomly chosen seeds from one line.

#### Molecular markers and genetic map construction

Three classes of molecular markers, 265 simple sequence repeat (SSR), 17 single-nucleotide polymorphism (SNP) (prefixed with “BSNP”) and 38 gene expression markers (GEM) (prefixed with “pp” and fibre), were used for genotyping the BnaYB DH population. The SSR primer sequences were developed at the Saskatoon Research Centre, Agriculture and Agri-Food Canada (<http://aaafc-aac.usask.ca/BrassicaMAST/>). These were analysed using fluorescently labelled tail PCR amplifications in 384-well format using the 5-dye system on an Applied Biosystems 3730xl. Genographer (available at <http://www.genographer.com/>) was used to produce a gel-like image of the genotype data, and polymorphic loci were scored visually. Polymorphic loci were named according to the marker used and markers detecting multiple loci were affixed with alphabetic letters.

Since the two parents of the BnaYB DH population share a common ancestry, a limitation of polymorphic markers and consequent gaps in the genetic map was expected. To reduce gaps in the SSR genetic map, SNP markers were identified using a 5509 Illumina Infinium

SNP array (<http://aaafc-aac.usask.ca/ASSYST/>) with a subset of the BnaYB DH lines. SNPs were chosen if they appeared polymorphic in the BnaYB subpopulation and potentially mapped to regions containing large gaps. SNP genotyping was performed using KBiosciences (<http://www.kbioscience.co.uk/>) fluorescence-based allele-specific competitive PCR (KASPar).

In addition, GEMs characterized by large parental differences in transcript levels causing a distinctly bimodal distribution of expression phenotypes in the population (West et al. 2006) were added to the map. GEMs selection was performed as described in (Potokina et al. 2008) based on gene expression analysis performed with Agilent two-colour 4 × 44 K *B. napus* arrays (unpublished data; <https://www.ebi.ac.uk/arrayexpress/arrays>). Normalized expression values of each probe from all DH lines and parents were divided into two clusters using k-means clustering, and the probes with two non-overlapping clusters were identified.

Genetic maps were constructed using Mapmaker v3.0b (Lincoln et al. 1992) with a total of 320 markers using a minimum logarithm of odds ratio (LOD) linkage threshold of 3.0 and a maximum likelihood distance of 40 cM. SSR marker order was determined based on initial screening of a population of 192 BnaYB individuals, and these markers were used as a framework for ordering the remaining markers. Genetic distances in cM were estimated using Kosambi's mapping function (Kosambi 1943). The markers were distributed over 21 linkage groups (LGs) that were named according to the Brassica chromosome nomenclature with N1–N10 representing the A genome (*B. rapa*) and N11–N19 the C genome (*B. oleracea*) of *B. napus*, respectively (Parkin et al. 1995). Each Brassica chromosome is represented by a single LG except for N12 and N14 which were divided into top and bottom (bot) fragments.

Although most lines were tested in all environments, a few were only represented in a subset of environments. Therefore, genetic maps were constructed for each year individually; these have slightly different marker distances, and MergeMap (Wu et al. 2011) was used to construct a consensus map that was used for all QTL mapping.

## Statistical analysis

Estimates of components of variance and covariance were conducted using the R package lme4 (Bates et al. 2011) (<http://lme4.r-forge.r-project.org/>). The environments corresponded to compounded effects of location and year. Environments S8, S9, S10 and S11 corresponded to field trials conducted in Saskatoon [S] between 2008 and 2011, and L8 corresponded to the field trial conducted in Llewellyn [L] in 2008. Environment (Env), genotype (DH lines), genotype by environment (GE) interactions and replication (Rep) within each environment (block effect) were treated as random effects (<http://articles.extension.org/pages/61006/estimating-heritability-and-blups-for-traits-using-tomato-phenotypic-data>). The components of variance were estimated using a linear mixed model fit by REML in R package lme4 (<http://cran.r-project.org/web/packages/lme4/index.html>) (`lmer(Trait ~ (1|DH) + (1|Env) + (1|DH:Env) + (1|Rep%in%Env))`) and used for broad-sense heritability ( $H^2$ ) estimate for each trait (Xu et al. 2013).

ANOVA was performed in R (R Development Core Team 2012, <http://www.R-project.org/>). Pairwise phenotypic correlations between traits were calculated using the Pearson correlation coefficient in the R package Hmisc (<http://cran.r-project.org/web/packages/Hmisc/index.html>). The correlations were grouped into three classes based on the magnitude of absolute value of correlation coefficient: (1) strong: correlation coefficient  $\geq 0.5$ ; (2) moderate: correlation coefficient between 0.3 and 0.5; and (3) weak: correlation coefficient  $< 0.3$  (Hill et al. 2015).

## QTL mapping

Mean values for each trait from two or three replicates in each environment were used for the detection of QTL. Outliers of trait values were detected and removed using a Z-score transformation with a threshold of 3. QTL detection was performed using composite interval mapping (CIM) in WinQTL Cartographer v.2.5 software (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>; Wang et al. 2012). A walking speed of 2 cM was used. The forward and backward stepwise regression was used for the selection of the markers to control the genetic background (control markers or cofactors) with up to five control markers. A window size of 10 cM was used to exclude closely

linked control markers at the testing site. The logarithm of odds (LOD) thresholds at a significance level of 0.05 for identifying statistical significant QTL for each trait in each environment was calculated by 1000 permutations. The additive effect (a) and phenotypic variance explained by each QTL ( $R^2$ ) were estimated by CIM for each individual trait–environment combination. QTL detected in different environments for each trait were considered to be the same if the confidence intervals overlapped and the additive effect was contributed by the same parent.

The digenic epistatic interactions among all pairwise combinations of QTL were analysed with multiple interval mapping (MIM) in WinQTL Cartographer v.2.5 software. The initial QTL model was set using the CIM results obtained for each trait in each environment. The QTL model was progressively refined by searching and testing QTLs or epistasis, and re-estimating. Both main additive effects of QTL and their epistatic interactions were tested for significance using the Bayesian information criterion (BIC). Not only main QTL (QTL with statistically significant main effect) and interactions among main QTL, but epistatic QTL (QTL that has no or small main effect but statistically significant interaction effect with another QTL position) interacting with main QTL were searched.

Multiple-trait composite interval mapping (Mt-CIM) implemented in WinQTL Cart was used to test for the presence of QTL  $\times$  environment (Q  $\times$  E) interaction at the main chromosome regions affecting the target traits (Maccaferri et al. 2008; Wang et al. 2012). The value of the trait in each environment was treated as a separate trait for the common genotypes. The G  $\times$  E (H4) hypothesis was tested. Mt-CIM was also used for joint analysis of various traits in each environment.

## QTL nomenclature

QTL nomenclature followed the one used in McCouch et al. (1997) for rice, with modifications. Each QTL starts with a lowercase “q” followed by 2–5 letters in capital abbreviated from the trait name to designate the trait measured then an underscore followed by the chromosome where the QTL is found and an additional underscore and final numerical code make it possible to uniquely identify QTLs for



the same trait on the same chromosome. For example, qNDF\_N16\_1 stands for QTL of trait NDF identified on chromosome N16 and is the first QTL mapped on this chromosome.

## Results

### Phenotypic variation and correlation between traits

The two parents, DH12075 and YN01-429, showed significant variation for most traits investigated in the five environments (Supplementary Table S1, S2), making them suitable for uncovering the underlying genetic basis for seed quality through a bi-parental cross. The BnaYB population displayed continuous variation at all environments for each trait, especially obvious for fibre traits (ADF, ADL and NDF), as well as wide transgressive segregation. Individual DH lines displayed bidirectional transgressive segregation for most traits, as shown by the maximum and minimum values in Supplementary Table S2. However, the mean BnaYB DH population values of most traits were closer to the parent DH12075 value than to the parent YN01-429. High broad-sense heritability was observed for most traits across five environments (Table 1), varying from 0.44 (Protein) to 0.91(ADL), indicating the phenotypic variation was attributable mainly to genetic variation.

Pairwise correlations between traits across environments were examined (Table 1). In general, both positive and negative correlations were observed. Three fibre traits (ADF, ADL and NDF) and seed colour whiteness index (WIE) had strong significant positive correlations. These four traits showed moderate to strong significant negative correlations with the fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) and with iodine value (IV). There was a strong negative correlation between protein and oil, which is suggestive of competition among these sinks for assimilates. Oil content also displayed a significant negative correlation (weak to moderate) with VLCFA, C16:018:0 (sum of C16:0 and C18:0), C18:3 and fibre traits. Oil content had a significant but weak positive correlation with oleic acid (C18:1) and C18:2. Oleic acid, a precursor for desaturation, showed a highly negative correlation with C18:2 and C18:3 and a moderate positive correlation with

C16:0C18:0, implying that there could be common genetic factors affecting these components. A moderate but significant positive correlation was observed between thousand seed weight (TSW) and protein.

### QTL mapping of single traits in single environments

Due to significant genotype  $\times$  environment interactions revealed by ANOVA (Supplementary Table S3), QTL analysis was first performed for individual trait in each environment. Variable numbers of significant QTL, from 2 to 8, were detected for each trait over the five environments (Table 2; Supplementary Fig. S1). Globally, the largest number of QTL (8) was detected for VLCFA and oil. The highest LOD scores were seen for QTL associated with fibre traits (ADF, ADL and NDF) and WIE in all environments. Clustering of QTL was observed on N9. A QTL mapping to the same interval was found on N9 for all traits investigated, except C183, protein and TSW. The additive effect in relation to parental contribution was also estimated. The relative contribution of the two parents for most of the traits followed expectations, with QTL having a positive contribution from the predicted donor parent. In the case of fibre traits, inheritance of the DH12075 alleles at the identified QTL increased fibre traits (ADF, ADL and NDF) and WIE in most instances, but decreased the oil content and IV, coinciding with the parental performance for these traits (Supplementary Table S1, S2).

Seed colour is a prominent phenotype segregating in the BnaYB population. A major QTL for WIE was mapped on chromosome N9 with  $R^2$  (phenotypic variance explained by QTL) varying from 56.3 to 74.8 % in each environment. This region on N9 is rich in genetic variation, with detected QTL for several other traits, as described in the previous paragraph. All fibre traits (ADF, ADL and NDF) had a major QTL mapped on N9 with intervals overlapping with qWIE\_N9 across all five environments, coinciding with the strong positive correlations between these traits (Table 1). This major QTL had trait value increasing allele from DH12075. A minor QTL, qADL\_N19, detected for ADL has an allele from YN01-429 contributing to higher ADL content. Three minor QTL for NDF identified in a single environment, qNDF\_N13, qNDF\_N16\_2 and qNDF\_N4,

**Table 1** Pairwise Pearson correlations between traits across environments and broad-sense heritability of traits

	ADF	ADL	NDF	WIE	Oil	C160180	C181	C182	C183	VLCFA	IV	Protein	TSW
ADF													
ADL	0.983***												
NDF	0.962***	0.944***											
WIE	0.815***	0.858***	0.806***										
Oil	-0.110***	-0.217***	-0.070*	-0.345***									
C160180	0.536***	0.535***	0.524***	0.449***	-0.386***								
C181	0.679***	0.649***	0.660***	0.443***	0.128***	0.386***							
C182	-0.705***	-0.691***	-0.655***	-0.531***	0.075*	-0.554***	-0.869***						
C183	-0.384***	-0.354***	-0.409***	-0.184***	-0.183***	-0.199***	-0.700***	0.288***					
VLCFA	0.193***	0.271***	0.147***	0.337***	-0.499***	0.181***	0.088*	-0.121***	-0.166***				
IV	-0.592***	-0.561***	-0.595***	-0.385***	-0.104***	-0.459***	-0.885***	0.712***	0.764***	-0.135***			
Protein	-0.191***	-0.072*	-0.249***	0.066*	-0.803***	0.104**	-0.287***	0.05	0.395***	0.380***	0.200***		
TSW	-0.270***	-0.192***	-0.245***	-0.119***	-0.188***	-0.184***	-0.353***	0.271***	0.303***	0.187***	0.131***	0.464***	
H <sup>2</sup>	0.89	0.91	0.83	0.76	0.59	0.8	0.79	0.88	0.64	0.69	0.66	0.44	0.89

H<sup>2</sup>: broad-sense heritability

Significance level: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ , \*  $p < 0.05$

**Table 2** Overview of QTL identified for all traits across five environments

Trait	QTL	Environment	Chromosome	Peak position (cM)	LOD	Additive <sup>a</sup>	R <sup>2</sup> (%) <sup>b</sup>	1 LOD interval (cM)	2 LOD interval (cM)
ADF	qADF_N16	L8	N16	34.51	8.10	1.14	8.59	30.2–38	28.1–39.2
ADF	qADF_N9	L8	N9	159.41	28.28	3.03	70.77	156.1–163.6	153.3–163.6
ADF	qADF_N9	S8	N9	159.41	34.87	3.09	81.09	156.3–163.6	153.7–163.6
ADF	qADF_N9	S9	N9	161.11	52.67	3.89	76.66	160.3–162.8	159.6–163.1
ADF	qADF_N9	S10	N9	156.91	50.26	3.39	82.78	153.8–158.9	151.8–159.1
ADF	qADF_N9	S11	N9	163.11	50.62	3.40	68.01	161.8–163.2	161.1–163.3
ADL	qADL_N19	S10	N19	36.31	5.95	-2.68	4.91	34.3–38.3	34.3–38.3
ADL	qADL_N9	L8	N9	159.41	22.82	2.02	70.72	155.8–163.6	153.0–163.6
ADL	qADL_N9	S8	N9	159.41	33.82	2.18	82.29	156.3–163.6	153.8–163.6
ADL	qADL_N9	S9	N9	161.11	50.42	3.10	78.50	160.7–163.1	160.3–163.1
ADL	qADL_N9	S10	N9	156.91	60.32	2.75	83.41	153.8–158.9	151.8–159.1
ADL	qADL_N9	S11	N9	163.11	52.98	2.61	81.95	161.8–163.2	161.1–163.3
NDF	qNDF_N13	S8	N13	117.01	5.39	-0.54	6.94	108.3–127.8	105.4–129.6
NDF	qNDF_N16_1	L8	N16	33.01	4.75	0.58	6.36	26.1–44.6	26.1–44.8
NDF	qNDF_N16_2	S9	N16	106.01	3.42	-0.47	2.45	89.8–116.1	84.8–117
NDF	qNDF_N4	L8	N4	40.11	3.09	-1.26	4.18	39.9–42.5	37–44.9
NDF	qNDF_N9	L8	N9	159.41	22.79	1.94	62.96	156–163.6	153.2–163.6
NDF	qNDF_N9	S8	N9	159.41	26.34	1.97	70.92	156.1–163.6	153.3–163.6
NDF	qNDF_N9	S9	N9	161.11	39.09	2.26	70.08	159.4–162.8	159.4–163.1
NDF	qNDF_N9	S10	N9	156.91	44.47	2.72	76.70	153.8–158.9	151.8–159.1
NDF	qNDF_N9	S11	N9	159.41	44.64	2.55	73.13	157–161.1	154.9–161.8
WIE	qWIE_N11	S9	N11	0.01	7.72	2.36	3.23	0–3.5	0–12.2
WIE	qWIE_N11	S10	N11	0.01	4.80	2.89	3.48	0–6.9	0–11.1
WIE	qWIE_N19	S10	N19	55.11	5.05	2.60	4.05	51.3–62.5	46.2–63.1
WIE	qWIE_N9	L8	N9	159.41	22.14	3.23	71.56	155.7–163.6	152.8–163.6
WIE	qWIE_N9	S8	N9	159.41	19.56	2.37	56.34	155.8–163.6	152.9–163.6
WIE	qWIE_N9	S9	N9	160.41	48.67	4.92	74.80	159.8–160.8	159.4–161.1
WIE	qWIE_N9	S10	N9	158.91	38.03	6.08	64.95	154.9–160.7	152.9–159.4
WIE	qWIE_N9	S11	N9	161.11	44.26	4.80	72.44	160.6–162.2	160.1–163.3
Oil	qOil_N10	S8	N10	33.01	4.16	-0.71	15.04	18.9–35	11.2–36.8
Oil	qOil_N10	S10	N10	37.71	4.05	-0.76	10.25	31.4–41.5	24.8–41.5
Oil	qOil_N16	S11	N16	112.01	4.36	-0.98	11.71	102–116.5	102–117.1
Oil	qOil_N16	S9	N16	116.01	4.45	-0.90	10.96	86.8–118.4	84.5–120.8
Oil	qOil_N19	L8	N19	14.01	3.50	-0.89	16.05	4.9–34.5	0–38.3
Oil	qOil_N2_1	L8	N2	11.31	4.03	-0.86	14.16	0–17.4	0–21.3
Oil	qOil_N2_2	S10	N2	81.11	3.38	-0.70	9.58	74.2–87.1	74.2–87.1
Oil	qOil_N2_2	S9	N2	72.31	3.55	-0.76	8.41	57.5–81.6	49.1–87.2
Oil	qOil_N4	S11	N4	36.11	3.65	-0.82	8.51	29.8–40.6	21.3–40.6
Oil	qOil_N9	S11	N9	159.41	3.49	-0.88	9.34	152.4–161.1	147.2–161.1
Oil	qOil_N9	S9	N9	163.61	5.99	-1.00	15.13	162.1–165.6	161–165.6
Oil	qOil_N8	S9	N8	36.71	4.13	0.83	10.61	32.8–46.9	29.7–50.7
IV	qIV_N19_1	S10	N19	24.21	4.18	0.82	9.64	16.4–30.5	11.2–34.4
IV	qIV_N19_1	S9	N19	24.21	3.95	0.89	6.84	11.3–32.7	4.8–38.5



**Table 2** continued

Trait	QTL	Environment	Chromosome	Peak position (cM)	LOD	Additive <sup>a</sup>	R <sup>2</sup> (%) <sup>b</sup>	1 LOD interval (cM)	2 LOD interval (cM)
IV	qIV_N19_2	S11	N19	55.11	5.22	1.02	13.55	44.3–60.9	40.1–65.1
IV	qIV_N2	L8	N2	92.01	4.22	1.13	12.41	86–97.9	80.5–97.9
IV	qIV_N5	S9	N5	50.41	3.74	−0.88	5.97	47.9–58.3	47.9–67.3
IV	qIV_N9	L8	N9	154.91	7.39	−1.67	27.65	148.2–159.4	145.2–159.4
IV	qIV_N9	S8	N9	159.41	12.84	−1.79	40.40	154.5–163.6	151.1–163.6
IV	qIV_N9	S9	N9	161.11	16.70	−2.14	40.49	159.4–163.1	156.4–163.4
IV	qIV_N9	S10	N9	161.11	15.74	−1.95	50.58	159.4–163.3	159.4–163.6
IV	qIV_N9	S11	N9	161.11	11.46	−1.69	39.59	154.9–163.8	150.2–163.1
IV	qIV_N10	S9	N10	36.81	4.22	−0.99	7.35	32.1–40.4	27.1–45.9
C16:018:0	qC160180_N10	S9	N10	4.01	3.29	0.10	5.64	0–17.1	0–23.2
C16:018:0	qC160180_N10	S11	N10	0.01	4.00	0.14	13.51	0–18	0–23.7
C16:018:0	qC160180_N16	S9	N16	108.01	7.36	0.15	13.38	92.3–116	86.9–116.4
C16:018:0	qC160180_N17	S11	N17	48.41	4.26	0.14	14.45	46.8–54.1	45.4–57.3
C16:018:0	qC160180_N2	L8	N2	13.31	4.48	0.15	15.75	1.5–21.5	0–23.4
C16:018:0	qC160180_N9	L8	N9	163.11	6.12	0.17	21.04	153.7–163.6	149.7–163.6
C16:018:0	qC160180_N9	S9	N9	163.11	16.25	0.24	34.10	159.3–163.6	153.7–163.6
C16:018:0	qC160180_N9	S11	N9	163.61	7.18	0.21	30.27	161.9–165.6	156.3–165.6
C18:1	qC181_N2_1	L8	N2	92.01	4.81	−0.87	14.72	83.9–96	80.3–97.9
C18:1	qC181_N2_1	S11	N2	91.11	4.85	−0.59	12.07	80.6–95.8	76.7–97.8
C18:1	qC181_N2_2	S8	N2	105.91	7.64	−1.07	24.46	99.1–111.9	105.4–121.3
C18:1	qC181_N9	L8	N9	156.91	9.51	1.44	36.59	151.4–163.6	148.4–163.6
C18:1	qC181_N9	S8	N9	159.41	9.55	1.29	32.08	152.5–163.6	149.2–163.6
C18:1	qC181_N9	S9	N9	159.41	20.30	1.66	50.65	154.4–162.3	151.7–163.2
C18:1	qC181_N9	S11	N9	163.61	12.60	1.20	38.78	161.5–165.6	159.4–1645.6
C18:1	qC181_N19	S11	N19	26.21	3.37	−0.48	8.16	12.6–37.5	5.5–38.3
C18:2	qC182_N10	S9	N10	6.01	4.86	−0.63	6.43	0–21.3	0–23.2
C18:2	qC182_N11	S9	N11	0.01	3.33	0.49	4.14	0–5.1	0–12.2
C18:2	qC182_N18	S8	N18	100.91	3.52	0.62	7.05	84.6–108.4	79.8–113.9
C18:2	qC182_N19	S9	N19	63.21	3.92	0.55	4.92	56.8–69.1	53.3–72.5
C18:2	qC182_N2	L8	N2	107.91	5.40	0.82	17.80	101.4–112.1	99.2–113.3
C18:2	qC182_N2	S8	N2	111.01	5.10	0.80	13.96	101.4–113.3	98.1–113.3
C18:2	qC182_N9	L8	N9	159.41	11.23	−1.30	43.34	152.3–163.6	148.4–163.6
C18:2	qC182_N9	S8	N9	159.41	11.70	−1.46	41.80	155.2–163.6	152–163.6
C18:2	qC182_N9	S9	N9	161.11	22.57	−1.55	49.38	158.8–163.8	155–163.2
C18:2	qC182_N9	S11	N9	152.91	5.03	−1.09	20.77	144–158.6	140.8–161.1
C18:3	qC183_N15	S11	N15	0.01	3.66	0.50	13.80	0–6.7	0–11
C18:3	qC183_N16	L8	N16	143.21	4.10	0.64	23.01	133.1–145.2	129.1–145.2
C18:3	qC183_N18	S8	N18	2.01	3.48	0.31	11.05	0–16.1	0–24.3
C18:3	qC183_N18	S11	N18	13.21	3.93	0.43	12.99	6.1–23.2	1.3–27.3
C18:3	qC183_N19	S11	N19	24.21	4.75	0.49	18.57	12.8–31	6.4–33.7
C18:3	qC183_N2	L8	N2	0.01	3.45	−0.51	15.90	0–9	0–9
C18:3	qC183_N6	S11	N6	46.61	5.96	0.67	25.53	39.8–51.3	34.5–52.7
C18:3	qC183_N8	S8	N8	0.01	4.90	−0.45	20.68	0–0.2	0–2.1

**Table 2** continued

Trait	QTL	Environment	Chromosome	Peak position (cM)	LOD	Additive <sup>a</sup>	R <sup>2</sup> (%) <sup>b</sup>	1 LOD interval (cM)	2 LOD interval (cM)
VLCFA	qVLCFA_N10	L8	N10	83.51	3.44	0.06	11.29	80.1–88.8	75.3–92.2
VLCFA	qVLCFA_N13	L8	N13	142.01	4.38	0.06	10.73	138.5–156.6	137.4–160.9
VLCFA	qVLCFA_N18	S9	N18	15.21	3.09	−0.07	9.65	3.2–29.6	3.2–35.8
VLCFA	qVLCFA_N4	S8	N4	64.51	3.60	−0.05	9.37	59.6–77	57.1–77.3
VLCFA	qVLCFA_N7_1	L8	N7	82.11	6.66	0.09	24.89	72.5–84.7	69.2–95
VLCFA	qVLCFA_N7_1	S8	N7	85.21	7.54	0.08	20.72	82.1–93.7	82.1–96.9
VLCFA	qVLCFA_N7_1	S9	N7	72.51	4.03	0.07	10.00	64.4–80.8	64.4–81.4
VLCFA	qVLCFA_N7_2	S9	N7	5.71	3.07	0.06	6.18	0–14.4	0–15.5
VLCFA	qVLCFA_N8	L8	N8	8.81	5.84	0.08	20.60	0.2–18	0.1–21.9
VLCFA	qVLCFA_N8	S8	N8	12.11	6.00	0.07	16.01	10.7–18.1	8.8–21.4
VLCFA	qVLCFA_N8	S9	N8	13.81	3.89	0.06	7.89	12.9–22.3	12.9–26.9
VLCFA	qVLCFA_N8	S11	N8	13.81	3.91	0.10	16.55	12.9–19.2	12.9–22.9
VLCFA	qVLCFA_N9	S8	N9	163.61	5.17	0.06	13.40	148.3–165.6	142.7–165.6
VLCFA	qVLCFA_N9	S9	N9	163.61	8.33	0.10	18.70	161–165.4	159.4–165.6
Protein	qProtein_N16	S11	N16	104.01	6.43	0.89	19.54	95.7–114.1	91.8–116.2
Protein	qProtein_N18	S9	N18	74.01	2.81	2.11	9.33	67.5–79.4	50.8–91.8
Protein	qProtein_N3	S8	N3	62.51	4.69	0.52	16.95	55.7–69.7	55.6–72.2
TSW	qTSW_N11	S11	N11	8.71	3.93	−0.15	13.48	0–17.1	0–18.7
TSW	qTSW_N14top	S10	N14top	63.81	3.54	0.14	10.21	58.4–67.7	45.4–69.2
TSW	qTSW_N16_1	S8	N16	34.51	4.89	−0.15	19.89	30.9–40	28.6–40.6
TSW	qTSW_N16_2	S8	N16	110.01	3.76	0.14	15.87	102–116.4	102–117.2
TSW	qTSW_N16_2	S10	N16	102.01	3.21	0.14	10.68	92–111	85.7–116.1
TSW	qTSW_N16_2	S11	N16	83.51	4.24	0.16	13.80	72.4–91.3	66.3–95.1
TSW	qTSW_N16_3	S9	N16	125.21	7.55	0.22	24.81	120.5–131.5	122.2–134.8
TSW	qTSW_N3	L8	N3	48.11	3.55	0.17	15.98	42.8–59.1	42.8–60
TSW	qTSW_N6	S8	N6	18.51	4.66	−0.17	29.22	0.4–26.9	0–31.1

<sup>a</sup> Additive effect; the positive values indicate that the alleles from DH12075 have the effect of increasing the trait value

<sup>b</sup> Percentage of variation explained by each QTL

have the alleles from YN01-429 contributing to higher NDF content.

A total of eight QTL were identified for oil, with 8.4–16.1 % phenotypic variance explained by each QTL. For all QTL except qOIL\_N8, the alleles from YN01-429 contributed higher oil content. Five QTL were detected for IV, with one QTL on N9 co-localized with fibre QTL and repeatedly mapped in five environments. A total of 30 QTL associated with fatty acid components were detected, with one QTL on N9 co-localized with the major fibre QTL identified for all fatty acid components except C18:3. YN01-429 was the source of alleles on N9 for increasing oil, IV

and C18:2, while DH12075 contributed to a higher value of C16:018:0, C18:1 and VLCFA. Two QTL identified for oil on N2 (qOil\_N2\_1) and N16 (qOil\_N16), respectively, coincided with QTL for C16:018:0 (qC160180\_N2 and qC160180\_N16), with alleles from YN01-429 increasing oil and alleles from DH12075 increasing C16:018:0. Another QTL for C18:1 on N2 (qC181\_N2\_2) was also associated with C18:2 (qC182\_N2), with the trait-enhancing allele for C18:1 inherited from YN01-429 and a higher proportion of C18:2 conferred by the allele from DH12075. Furthermore, this QTL had an opposite effect for these traits relative to the QTL on N9. The QTL on N19 for

C18:1 (qC181\_N19) and C18:3 (qC183\_N19) had overlapping intervals. The co-localization and opposite additive effect observed for QTL associated with these fatty acids could explain the strong negative correlation between C18:1 and C18:2/C18:3 (Table 1). These results suggest that enhancing alleles for various traits originated from the different parents. Iodine value (IV), used to determine the amount of unsaturation of fats and oils, had one QTL on N19 (qIV\_N19\_1) co-localized with qC181\_N19 as well. For all QTL of VLCFA except two on N4 and N18, respectively, the alleles from DH12075 contributed to higher VLCFA levels. The QTL qVLCFA\_N7\_1 and qVLCFA\_N8 was repeatedly detected in 3 and 4 environments, respectively.

Three QTL were identified for protein with increasing alleles from DH12075. However, no QTL was repeatedly detected in two or more environments. In terms of QTL for TSW, an equal number of trait-enhancing alleles were inherited from both parents. Co-localization of QTL on N16 underlying protein (qProtein\_N16) and TSW (qTSW\_N16\_2) accorded with the significant positive correlation between these two traits (Table 1).

#### Identification of epistatic interaction of QTL

The epistatic interactions detected by MIM together with their average effects are reported in Table 3. A total of seven pairwise QTL–QTL interactions were detected for six traits at a significance level  $\alpha = 0.05$ . Compared to the QTL effects, the epistatic interactions showed less consistency across environments. All of the interactions were detected in a single environment. Most of the epistatic effects detected with the MIM procedure and retained in the MIM models were due to the interaction between a major QTL on N9 and QTL on other chromosomes. For WIE, C16:0/18:0 and IV, both loci involved in epistatic interactions were detected as significant QTL in CIM scans. For ADL, C18:1 and C18:3, the additive effect of the second locus was too small to reach genome-wide significance in CIM scans. However, the QTL–QTL interactions between these loci were significant.

#### Multi-trait QTL analysis in single environments

Multi-trait QTL was performed on fibre traits and WIE in each environment (Fig. 1). A significant QTL on N9

was detected for all traits, and joint analysis was significant as well. For this QTL, all individual traits had a similar trend as well as the joint trait along the chromosome. In addition, in four environments L8, S8, S10 and S11, individual traits had a similar pattern as to the influence on the joint profile. The trait most influencing the joint profile was ADL, which had a profile very similar to the joint profile. It was observed that the additive effects of QTL located on other chromosomes for the individual traits were relatively high, such as qADF\_N16, qADL\_N19 and qNDF\_N13, and the additive effects were too small to be detected as significant for the joint analysis. In all environments, trait ADL had the most influence on the joint trait. The least influencing trait was WIE while NDF in S9. Notably, in all environments except S9 (the peak position of NDF was slightly shifted from one of other traits), the QTL peak of all individual traits was located at the same position as the one for the joint trait.

Multi-trait analysis was also performed on all traits except protein and TSW to investigate the genetic correlation of seed colour with oil and fatty acid compositions (Supplementary Fig. S2). In general, fibre traits and WIE are more influencing for joint profile. In all environments except S10 where no fatty acid compositions were measured, no significant QTL was detected for joint trait on any chromosomes. A QTL on chromosome N9 was significant for fibre traits, WIE, IV, C18:1 and C18:2 in all environments tested, which was in agreement with the results of single-trait CIM analysis. Other linked or pleiotropic QTL were inconsistent across environments, such as the QTL on N2 detected for both IV and C18:1 (Supplementary Fig. S2d). Notably, a significant QTL on N10 (close to marker sR12317) was detected for oil in the joint analysis (Supplementary Fig. S2b) in environment L8. However, this QTL was not identified in environment L8 using single-trait CIM analysis, but detected in environment S8 and S10. A QTL on N7 was detected for C18:2, although not detected using single-trait CIM analysis in any environment (Supplementary Fig. S2c). On N2, a significant QTL was identified for IV and C18:1 which coincided with the results of single-trait CIM analysis (Supplementary Fig. S2d).

Surprisingly, qVLCFA\_N9 detected on N9, using single-trait CIM analysis in environment S8 (Supplementary Fig. S2e), was not significant in the joint

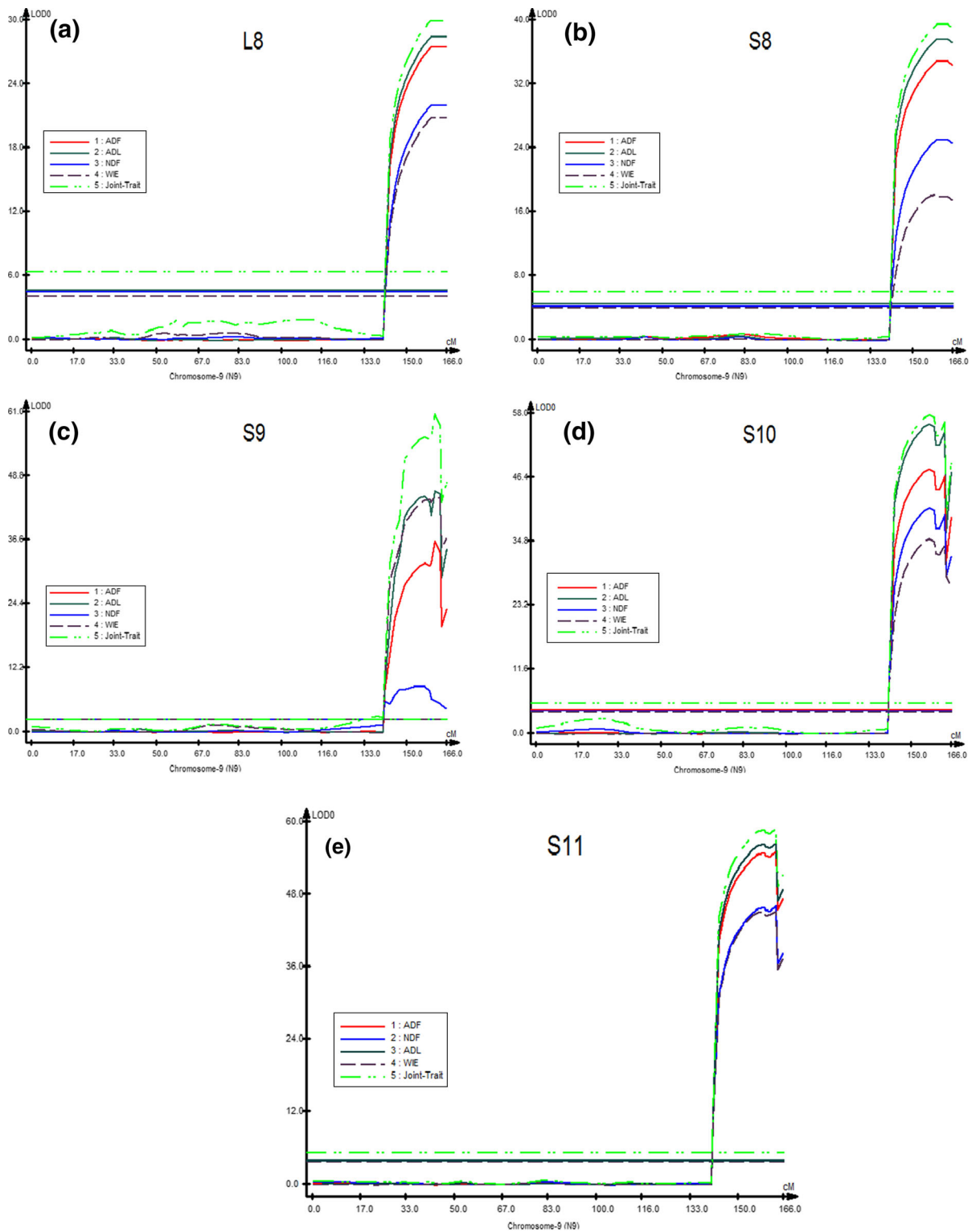
**Table 3** Epistatic interaction between QTL

Trait	Environment	Number of QTL	QTL1	QTL2	Type	Chromosome	Position (cM)	Effect	LOD	Score statistics
ADL	S9	1	1	X	Additive	N4	36.1	-0.25	1.07	6.60
ADL	S9	2	2	X	Additive	N9	161.1	2.87	41.65	82.03
ADL	S9	3	3	X	Additive	N11	0.1	0.22	0.83	7.51
ADL	S9	4	1	2	A × A			-0.68	1.78	14.77
ADL	S9	5	1	3	A × A			0.70	1.96	15.53
WIE	S10	1	1	X	Additive	N9	157.9	6.08	34.07	80.58
WIE	S10	2	2	X	Additive	N11	0	2.89	3.46	17.13
WIE	S10	3	3	X	Additive	N19	51.1	2.60	2.24	11.70
WIE	S10	4	1	3	A × A			4.54	1.73	9.50
C16:018:0	S9	1	1	X	Additive	N9	161.1	0.25	13.94	57.22
C16:018:0	S9	2	2	X	Additive	N10	9.1	0.12	3.92	19.65
C16:018:0	S9	3	3	X	Additive	N16	108.1	0.13	4.64	24.42
C16:018:0	S9	4	1	3	A × A			0.15	1.69	8.38
C18:1	S11	1	1	X	Additive	N9	160.1	-1.84	13.39	58.82
C18:1	S11	2	2	X	Additive	N19	55.1	0.93	3.75	14.96
C18:1	S11	3	1	2	A × A			-0.91	1.08	5.13
C18:3	L8	1	1	X	Additive	N2	0.1	-0.45	2.46	13.00
C18:3	L8	2	2	X	Additive	N14 <sup>top</sup>	58.1	0.14	0.26	1.29
C18:3	L8	3	3	X	Additive	N16	146.1	0.53	2.82	10.42
C18:3	L8	4	1	2	A × A			-1.04	3.25	15.77
IV	S11	1	1	X	Additive	N9	160.1	-1.84	13.39	58.82
IV	S11	2	2	X	Additive	N19	55.1	0.93	3.75	14.96
IV	S11	3	1	2	A × A			-0.91	1.08	5.13

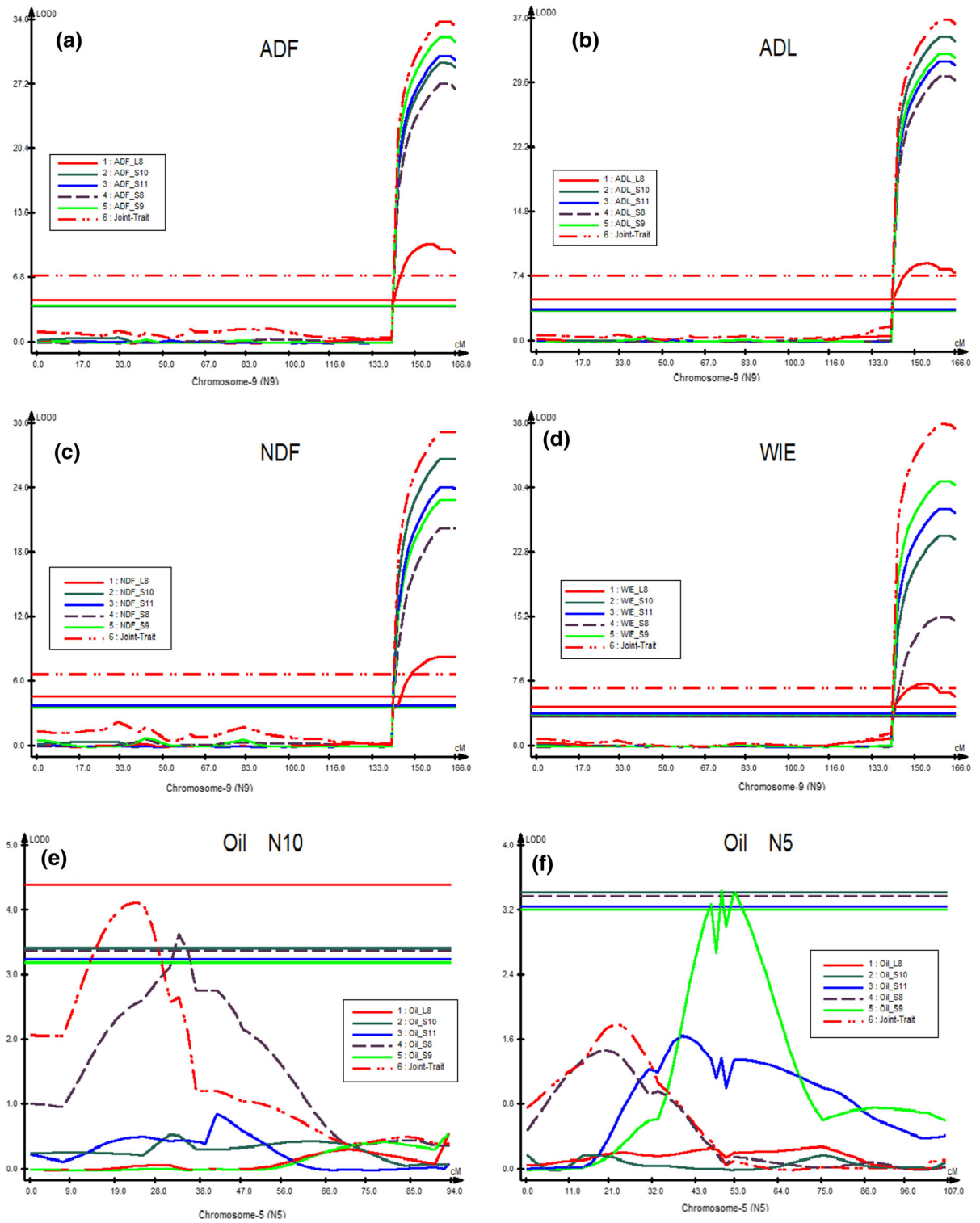
analysis. In environment S9, no QTL on N9 was detected for oil, C16:018:0 and VLCFA while identified using single-trait CIM analysis (Supplementary Fig. S2f). In environment S10, where no fatty acid compositions were measured, joint analysis was performed on fibre traits, WIE, oil and IV. The QTL on N9 was significant for all individual traits except oil and joint trait (Supplementary Fig. S2g). A significant QTL was detected for oil and joint trait on N10 which agreed with single-trait analysis (Supplementary Fig. S2h). A significant QTL was detected on N11 and N19 for joint trait, respectively, while not for individual trait (Supplementary Fig. S2i, j). In environment S11, the QTL identified for oil on N9 (close to marker pP078731) was not significant in joint analysis (Supplementary Fig. S2k).

### Multi-environment QTL analysis

Multi-environment QTL analysis was performed to detect the significant QTL across environments and genetic (QTL) × environment effect (Fig. 2). In general, environment S9 influenced joint analysis the most with highest additive effect for traits ADF and WIE, while environment S10 influenced joint analysis the most for traits ADL and NDF (Fig. 2a–d). The environment L8 had the least influence with the lowest additive effect for all fibre traits and WIE. A significant QTL on N9 was detected for all fibre traits and WIE across all environments. This QTL was also statistically significant for multi-environment joint analysis. However, the Q × E effect was significant. The QTL mapped on other chromosomes in only one

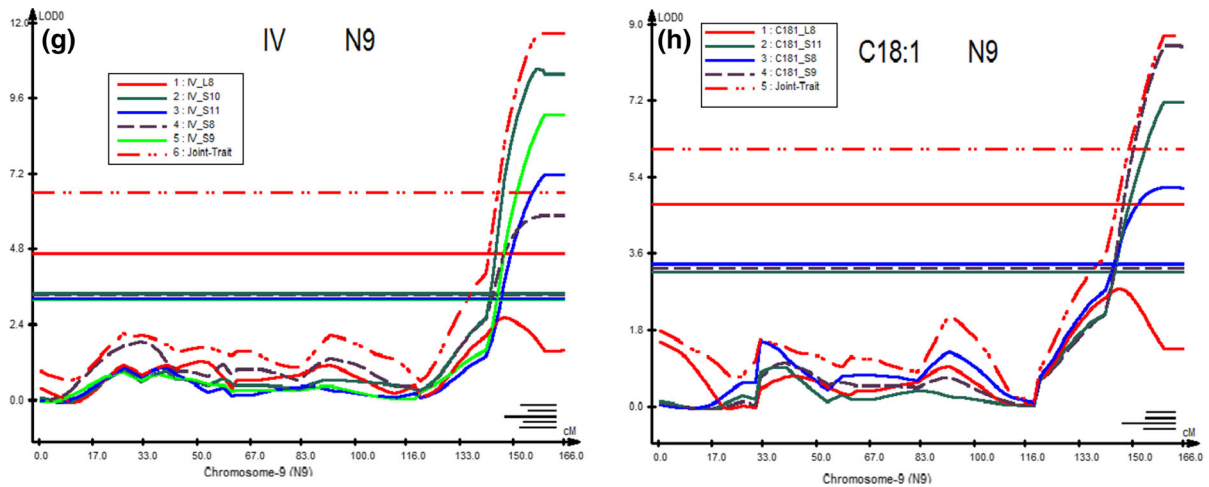


**Fig. 1** Multi-trait QTL analysis for fibre traits and WIE across five environments. The *horizontal lines* indicate LOD significance threshold



**Fig. 2** Multi-environment QTL analysis. The horizontal lines indicate LOD significance threshold





**Fig. 2** continued

or two environments did not reach the significance threshold value in the multi-environment QTL analysis, such as qADF\_N16, qADL\_N19 and qWIE\_N11.

For the oil QTL detected on N10, environment S8 was more influential on the joint profile. However, environment S9 showed the most influence on oil QTL on N5 which was not identified in single-trait QTL mapping (Fig. 2e, f). For the QTL on N9 identified for IV, environment S10 influenced joint analysis the most, while L8 was not significant (Fig. 2g). The QTL on N9 detected for C18:1 was significant for joint analysis and all environments except L8 (Fig. 2h). Surprisingly, no significant QTL was detected for C16:018:0, C18:2, C18:3 and VLCFA.

## Discussion

In the present study, a major QTL qWIE\_N9 located on N9 was stably detected over five environments, indicating it could harbour a causal gene/gene cluster for regulating seed pigment biosynthesis and/or storage. In other studies, a major QTL with a large effect on seed colour was mapped to the two homoeologous *B. napus* chromosomes A9 and C8 along with other contributing QTL (Badani et al. 2006; Fu et al. 2007; Snowdon et al. 2010; Zhang et al. 2011a, b; Liu et al. 2012). It has also been reported that a major QTL on N9 (A9) for seed colour explained 64 % of phenotypic variance in *B. rapa* (Kebede et al. 2012). The loss in

function of a bHLH transcription factor *BrTT8* on A9, which regulates the late biosynthetic genes of flavonoid pathway, has been reported to result in yellow seed coat in *B. rapa* (Li et al. 2012). In this study, parental line YN01-429 has the genetic background of yellow-seeded *B. rapa*, *B. carinata* and *B. juncea*. Further investigation is required to determine whether *BrTT8* is causal gene underlying the QTL qWIE\_N9.

Due to the strong positive correlation between seed colour and fibre content observed in this study and previous research (Badani et al. 2006; Liu et al. 2012; Wittkop et al. 2012), it is not unexpected that the major QTL on N9 influencing both traits was mapped to the same position. Our study not only detected the most reported chromosome (N9/A9) associated with fibre content and seed colour, but also identified new significant QTL on other chromosomes. Snowdon et al. (2010) reported a major QTL influencing both seed colour and fibre content mapped on A9 (N9). A major QTL on A9 for ADL content was explained by a loss-of-function allele of the *Bna.CCR1* locus on chromosome C8 caused by a homoeologous non-reciprocal translocation (Liu et al. 2012). Whether the fibre-related QTL on N9 (A9) reported in the present study is related to mutation of the *CCR1* locus is still under investigation. Another major QTL with large effect on both seed colour and ADF was reported on chromosome N18 in three *B. napus* populations across multiple environments (Badani et al. 2006).

Depending on the population and environments studied, different QTL for oil content have been reported (Burns et al. 2003; Zhao et al. 2005; Delourme et al. 2006; Qiu et al. 2006; Zhao et al. 2006; Chen et al. 2010; Zhao et al. 2012). Delourme et al. (2006) consistently detected one common QTL for oil content on N3 in two *B. napus* populations and in addition, located QTL on N2, N4, N9, N10, N16 and N17 in the same two populations. However, in the present study, no oil content QTL was identified on N3, but QTL were identified on chromosomes N2, N4, N8, N9, N10, N16 and N19. These QTL were more environment specific than the fibre QTL. Only four QTL (on N2, N9, N10 and N16) were repeatedly identified in two out of five environments in the present study. It has been reported that additive  $\times$  environment interactions was a major factor contributing to variation of oil content in *B. napus* (Zhao et al. 2005). Likewise, Chen et al. (2010) identified 23 environment-specific QTL associated with oil content and only four reproducible QTL in at least two environments. Notably, Zhao et al. (2008) detected QTL for oil content on N2, N9 and N19 in a *B. napus* DH population derived from a cross between parents both having a high erucic acid (C22:1) and a very high oil content. QTL were also identified on these chromosomes in the present study. Similarly, pleiotropic QTL for oil content and fatty acid composition were reported by Zhao et al. (2008).

The fatty acid composition is the major determinant of edible and processing quality of rapeseed oil (Yang et al. 2012). A number of independent and pleiotropic QTL were identified for different fatty acid species in this study, indicating that complex interactions exist between individual fatty acid biochemical pathways. Acting in parallel with desaturation of 18-carbon fatty acid, elongation of the fatty acid chain produces VLCFA (Millar and Kunst 1997). Since these two pathways compete for the same precursor, it is not unexpected to see coincident QTL for C18:1 and C18:2 on N9. Similarly, the presence of coincident QTL for C18:1 and C18:2 with the major QTL for C22:1 on A8 and C3 was reported by Smooker et al. (2011). Interestingly, one locus associated with C18:2 at the top of N11 in this study is most likely the same as the one identified for C18:1 and C18:2 on C1 by Smooker et al. (2011) which coincided with an orthologue of *FAD2*. Six copies of the *FAD3* gene are distributed on the A and C genomes (A3, A4, A5,

C3 and two copies on C4) in *B. napus* (Yang et al. 2012). Two major QTL for C18:3 identified on A4 and C4 corresponded with two *FAD3* copies in a *B. napus* DH population of high oleic acid  $\times$  low oleic acid (Hu et al. 2006). Surprisingly, no QTL was identified for C18:3 on the chromosomes harbouring *FAD3* gene in this study. In this study, the co-localization of QTL with contrasting parental effects for C18:1 and C18:2 on chromosome N19 would be indicative of one copy of the *FAD2* gene.

Of the traits analysed in the present study, seed protein content appears to be the most affected by QTL  $\times$  E, since no QTL were reproducible in two or more environments. The co-localization of one QTL on N16 for both oil and protein with opposite additive effects agrees with the strong negative correlation between these two traits in the present and previous studies (Zhao et al. 2006 and references therein), as a result of the competition for the common basic substrates during the biosynthesis of oil and protein. The strong negative correlation between oil and protein contents and their linked QTL with opposite source of favourable alleles represents a potential challenge for improving both oil and protein simultaneously in *B. napus*. However, apart from the linked QTL for seed oil and protein, independent QTL (seven for oil and two for protein) identified in this study are suitable for simultaneous improvement of these two traits.

Epistatic interaction between QTL have been shown to be important for unravelling the genetic basis for complex traits in plants (Xing et al. 2002; Malmberg et al. 2005; Xu and Jia 2007; Yang et al. 2010). The digenic epistatic interactions observed in this study are environment specific. No significant epistatic interaction was observed for the QTL of oil content in the present study. However, a number of pairs of loci with additive  $\times$  additive epistatic interactions associated with oil content were reported in a *B. napus* population derived from a cross of parents both with high erucic acid, glucosinolate and oil contents (Zhao et al. 2005, 2006). This discrepancy, at least in part, could result from the different genetic background of the populations studied. Another possible reason could be the use of different statistical models, as demonstrated by previous studies performed in *B. napus* (Delourme et al. 2006).

In several cases, the QTL intervals for multiple traits overlapped. The most noticeable one is the co-localization of individual QTL on N9 for various

traits. Likewise, a seed-related QTL cluster on A9 was reported in *B. rapa* (Bagheri et al. 2013). One major QTL for seed colour was co-localized with other seed-related QTL for seed size, seed weight, seed oil content, number of siliques and number of seeds per silique. These common chromosome regions with QTL for different traits indicate either linkage or pleiotropic effects. There could be pleiotropic effects of single genes or certain tightly linked trait-specific genes affecting multiple traits. Some of the pleiotropy could be the consequence of correlation between traits, such as fatty acid components, which are subject to autocorrelation since fatty acid components are expressed as a percentage of the total identified fatty acids (Smooker et al. 2011). There are reports of QTL having pleiotropic effects in *B. napus* and other crops (Shi et al. 2009 and references therein).

To dissect the genetic correlation between traits studied, multi-trait joint analysis was performed. Multiple-trait analysis takes into account the structure of genetic correlations between traits. It can test the nature of genetic correlations between traits, such as pleiotropy versus close linkage. Furthermore, multiple-trait analysis allows the estimation of genetic variance–covariance matrix between traits and its decomposition in terms of individual QTL (Da Costa et al. 2012). The change in QTL effect size of the individual trait was observed using multi-trait mapping compared to single-trait analysis. Some QTL profiles from single-trait and multi-trait analyses were very similar, such as joint analysis of fibre traits and WIE. However, inconsistency of QTL profiles from single-trait and multi-trait analysis were also observed. For instance, a significant QTL on N10 (close to marker sR12317) was detected for oil in the multi-trait analysis in environment L8 while not identified using single-trait CIM analysis. The opposite behaviour of inconsistency of QTL profiles from single-trait and multi-trait analysis was evidenced by some QTL such as qVLCFA\_N9, which was detected as significant on N9 using single-trait CIM analysis in environment S8, but not significant in the joint analysis. The similar inconsistency of QTL profiles was also observed in other multi-trait QTL analysis (Singh et al. 2012). The joint effect of multi-trait analysis on the additive effect of each trait reflects the correlation structure between traits. Multi-trait analysis should provide more useful information than individual QTL mapping analyses due to the

systematic nature of physiological processes in plants (Singh et al. 2012). Multi-trait analysis showed that the QTL on N9 was significant for individual and joint trait when WIE and fibre traits were combined, which was indicative of pleiotropy of this QTL. Similarly, pleiotropy of this QTL was also observed for IV, C18:1 and C18:2. The strong correlations between these traits were mostly caused by this pleiotropic QTL. For other traits, the QTL on N9 might be closely linked rather than pleiotropic.

Multi-environment QTL analysis is more powerful in detecting significant QTL for complex traits controlled by a number of QTL of smaller effects that might not be detectable with single-environment analysis (Alimi et al. 2013), which accounts for  $G(QTL) \times E$  and tests all detected QTL in all environments. This was demonstrated by the detection of additional QTL for oil in the present study.  $QTL \times E$  is significant if the QTL effects are strongly expressed in one environment but weakly in another (MacMillan et al. 2006). Of the QTL examined here,  $QTL \times E$  was found for all of them.  $QTL \times E$  observed was due to the difference of magnitude and not the direction of QTL. This was also evidenced by the QTL analysis in single environments. No crossover interaction was observed as QTL effects were consistently positive or negative across environments. The major QTL on N9 pleiotropic for WIE and fibre traits was the most stable across environments but with different magnitude of effect. The marker linked to this QTL is valuable for selection in breeding. Environment-specific QTL for seed colour was also observed in the present study and other study (Fu et al. 2007).

As the two parents in this study have some common ancestry, it is not surprising that some regions of the genome lack polymorphic markers. Therefore, given our marker density and population size, this study has sufficient power to detect QTL of large effect that are common across environments and environment specific. A larger population size coupled with higher density of markers would empower us to identify more QTL of small effects. Nevertheless, this study has led to the identification of numerous genomic regions involved in the control of seed-related traits across multiple environments with a few novel QTL being identified. To the best of our knowledge, this was the first study of multi-trait QTL analysis by taking into account the correlated structure of seed colour and seed composition in *B. napus*. These results identified

the critical genomic regions governing seed features and will facilitate the development of strategies for integration of favourable alleles to produce *B. napus* cultivars with desirable seed quality characteristics.

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