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RESEARCH

Inheritance of cellulose, hemicellulose and lignin content in relation to seed oil and protein content in oilseed rape

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Abstract Oilseed rape is worldwide an important oil and protein crop. Its oil is valued because of its excellent quality. The oil extracted meal is marketed as a lower value by-product for feeding livestock. Recently, interest in vegetable proteins has increased to use the oilseed rape protein as an alternative vegetable source for human consumption. However, the use of the protein rich meal for food production is greatly limited by the presence of residual glucosinolate, phenolic acid esters and crude fibre contents which affect its techno-functional properties, taste and colour. Further reducing contents of glucosinolates, cellulose, hemicellulose and indigestible lignin, is expected to enhance protein content and quality. To this end, two half-sib DH populations were tested in replicated field experiments. Inheritance of individual seed fibre components in relation to each other and to oil, protein and glucosinolate content were investigated. The DH populations were genotyped with Brassica 15K SNP Illumina chip, QTL were mapped and candidate genes were identified using the high quality long read reference genome of Express 617. Novel QTL for fibre components were identified that co-located to each other, with QTL for oil, protein and glucosinolate content, and with opposite direction of additive effects. The parallel investigation of two half-sib DH populations gave insight into the direction of the additive effects which depended on the indvidual parents. The results provide additional understanding of genetic loci underlying the seed quality traits which may help achieving the breeding goals in oilseed rape.

Keywords Hemicellulose · Cellulose · Lignin · Glucosinolate

Introduction

Oilseed rape (*Brassica napus* L.) is one of the major sources of vegetable oil in the world. The oil extracted meal with about 40% protein serves as a good source for feeding livestock. Recently, interest has increased in European countries in using plant-based protein for human consumption. Vegetable protein is more environmentally friendly compared to animal-based protein (So and Duncan 2021). However, the use of the protein rich vegetable meal for food production is greatly limited by the presence of residual glucosinolate (GSL), phenolic acid esters and crude fibre contents which affect its techno-functional properties, taste and colour (Zum Felde et al. 2006; Wittkop et al. 2009; Hald et al. 2019). Their biosynthesis compete with synthesis of oil and protein and can reduce their

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value (Gacek et al. 2018, 2021). Hence, a genetic reduction of the negatively associated constituents is attempted to enhance seed protein content (SPC) and quality. Oilseed rape protein content and quality has been under intensive studies over the years and a number of QTL for SPC on different chromosomes has been identified in diverse bi-parental populations (Schatzki et al 2014; Behnke et al. 2018; Chao et al. 2017; Gacek et al. 2021; Stolte et al. 2022). Schilbert et al. (2022) identified 15 genomic regions on 7 chromosomes associated with SPC in which many overlapped with regions associated with seed oil content (OC).

Glucosinolate (GSL) content in modern canola rapeseed has been reduced to 15 µmol per gram of seed and less from the original level in traditional cultivars with 60–100 µmol per gram of seed (Nesi et al. 2008; Rahman et al. 2014). Because of their antinutritive effects, breeding aims at a further reduction of GSL content (Chao et al. 2022a). The genetic loci involved in control of GSL have been broadly studied in *Brassica napus* and major loci identified are mostly on chromosome A04, A06, A09, C02, C07 and C09 (He et al. 2018; Liu et al. 2020; Chao et al. 2022a; Gacek et al. 2021; Kittipol et al. 2019; Schilbert et al. 2022).

As an oil and protein crop, oilseed rape has a comparatively high crude fibre content. Crude fibre consists of cellulose (CC), hemicellulose (HC) and lignin (LC) content. Van Soest et al. (1991) developed a method that allowed quantification of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL = LC). Subtraction of ADF from NDF and ADL from ADF yields HC and CC, respectively. Previous work reported QTL for lignin content (LC) on different chromosomes and candidate genes (Liu et al. 2012, 2013; Stein et al. 2017; Miao et al. 2019). Negative correlations between fibre content and OC and SPC in oilseed rape have been reported (Dimov et al. 2012; Behnke et al. 2018; Miao et al. 2019). In a transcriptome- and genome-wide association study, Zhang et al. (2022) identified genes significantly associated with seed coat content and negatively affecting OC during seed development. In an attempt to further reducing fibre content in oilseed rape, more detailed investigations of the genes involved in the biosynthesis of LC, HC and CC, and their individual effects on each other and on OC and SPC is required. In a doubled haploid population Miao et al. (2019) found that LC was significantly positively correlated with CC, but negatively correlated with HC. Furthermore, CC was positively correlated with HC. In addition, co-localized QTL for individual fibre components and OC with opposite additive effects were detected. Candidate genes were identified based on the alignment of SNP marker sequences with the ZS11 reference genome (Song et al. 2020; Sun et al. 2017). The objective of this project was to study the inheritance of individual seed fibre components in relation to OC, SPC and GSL content and to identify QTL in two half-sib DH populations. Since one of the parental genotypes was derived from a cross with Express 617, candidate genes were identified based on the high quality long read reference genome of this genotype (Lee et al. 2020).

Materials and methods

Plant material

The study material consisted of two half-sib DH populations. The first ASG population (henceforth referred to as population 1) consisted of 170 F_1 derived doubled haploid (DH) lines from a cross between the canola cultivar Adriana and the DH line SGEDH13. Adriana is a German winter rapeseed line cultivar (00 double low (canola) quality). SGEDH13 is a DH line derived from the cross between DH line SGDH14 (Zhao et al. 2005) and inbred line 617 of the German winter rapeseed cultivar Express (Behnke et al. 2018). SGEDH13 is characterized by high oil content, low GSL content and intermediate erucic acid content caused by the presence of one fae1 gene (Ecke et al. 1995). The second AZH DH population (henceforth referred to as population 2) consisted of 95 F1 derived doubled haploid lines derived by microspore culture from a cross between Adriana and Zheyou 50. Zheyou 50 is a canola quality semi-winter cultivar from China. Both DH populations were developed at the Division of Crop Plant Genetics, Georg-August University, Göttingen, Germany.

Field experiments

DH lines of population 1 and the parents were tested in three growing seasons (2015/16, 2016/17, and

2017/18) in five field environments located in north-western Germany and Poland. The DH population 2 was evaluated in four consecutive seasons in one environment in north-western Germany. The field experiments were conducted in small plots as a Randomized Complete Block design without replication. Each genotype was sown with 100 seeds in a row of five meters length; distance between the rows was 75 to 90 cm. At maturity, open pollinated seeds were bulk harvested from each genotype from the terminal raceme and three upper most primary branches of ten healthy plants. The harvested seeds were de-husked and cleaned and stored at room temperature for seed quality trait analysis using near-infrared reflectance spectroscopy (NIRS).

Phenotyping using near infrared reflectance spectroscopy (NIRS)

In order to measure the seed oil and quality traits contents, about 3 g of bulked harvested seed samples for each genotype were scanned with NIRS monochromatic as described in Behnke et al. (2018). The seed oil, seed protein and GSL content measured were expressed on basis of 91% dry matter content. The fibre components of the Neutral detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL = LC) in the defatted meal were estimated using the calibration equation developed by Dimov et al. (2012). The HC and CC contents were calculated by subtracting ADF from NDF and LC from ADF contents, respectively. The protein (PidM) in the defatted meal was calculated from the estimated OC, SPC using the following equation: %Protein in the defatted meal (PidM) = %SPC/(100 − %seed oil content) × 100.

Statistical analysis

Analysis of Variance (ANOVA) was calculated for the data using Restricted maximum likelihood (REML) using *lme4* package (Bates et al. 2015) and *lmer* test (Kuznetsova et al. 2017) in R (R core team 2022). Both the genotype and the environment were considered as random factors using the following simple linear model:

$$Y_{ij} = \mu + g_i + e_j + ge_{ij}$$

where *Yij* is the trait value of *ith* genotype in *jth* environment and μ is the overall mean, g_i is the effect of the *ith* genotype ($i = 1,2\ldots$), while e_j is the effect of *j* environment and ge_{ij} is the interaction between *ith* genotype and *jth* environment and the random error. Broad sense heritability (H^2) was calculated for each trait using

$$H^2 = \frac{\sigma_g^2}{\left(\sigma_g^2 + \frac{\sigma_{ge}^2}{E}\right)}$$

where σ_g^2 and σ_{ge}^2 are variance components for the genotype and random error and E is the number of environments. The mean values across the environments were used to calculate the spearman rank correlation coefficient using R 4.0.3 Package (R Core Team 2022).

Linkage map construction and QTL mapping

Details and results on linkage map construction and QTL mapping procedure for both DH populations are provided in Yusuf et al. (2022). Mean phenotypic data from the different field experiments were used for QTL mapping.

SNP marker sequence alignments to reference genomes and candidate gene identification

To identify the potential candidate genes of QTL, the positions of the SNP markers on the genetic map were aligned with their physical position by blasting the sequence of each SNP against the *Brassica napus* Express 617 reference genome (Lee et al. 2020). The SNP sequences were provided by Isobel Parking (Agriculture and Agri-Food Canada). The physical position of each SNP locus was located by blasting the sequence of each SNP against the high quality Express 617 *Brassica napus* reference genome (Lee et al. 2020). The position was recorded based on genetic map data information, as well as on the best matching and the lowest E-value. *Arabidopsis thaliana* related functional genes were annotated on *A. thaliana* Araport 11 (TAIR; https://www.arabidopsis.org/index.jsp). The assignment of *A. thaliana* annotation to the *Brassica napus* Express 617 gene models

was based on Schilbert et al. (2021). The QTL interval spanned over several Kbp and many potential candidate genes were found within each QTL region (Table Suppl. S3 and S4). The available literature was scrutinized for candidate genes involved in biosynthesis of cellulose, hemicellulose, lignin, oil and SPC, and genes identified within QTL confidence intervals were mentioned in the discussion.

Results

Phenotypic analysis

The genotypic and environmental variance components were statistically significant for all traits studied in both DH populations (Table 1). The heritabilities for seed quality traits in both populations ranged from 66% for GSL content to 95% for LC. Although parental lines of both populations had similar seed quality characters, there was a large range and transgressive segregation in both populations. For most traits, including HC and CC, a normal frequency distribution was found in both populations (Suppl. Figs. S1 and S2). However, LC content showed a bimodal distribution and a similar large variation in both populations.

Seed quality correlations in the two half-sib populations

In population 1, the three fibre fractions NDF, ADF and LC were closely correlated to each other based on their overlapping contents of HC and CC (Table 2). However, NDF was more closely correlated with LC followed by CC and was not correlated with HC. OC was negatively correlated with LC and was more strongly positive correlated with HC than with CC. SPC was more strongly negative correlated with CC than with HC, followed by LC. LC was negatively correlated with HC and positively correlated with CC. HC was weakly positive correlated with CC.

Table 1 Descriptive statistics for the quality traits and variance components in two doubled haploid populations for hemicellulose (HC), cellulose (CC), seed oil content (OC), lignin content (LC), seed protein content (SPC), protein in the defatted meal (PidM), glucosinolate content (GSL), neutral detergent fibre (NDF), acid detergent fibre (ADF)

Traits	POP	Mean [%]	Range	SD	CV	P1	P2	G	E	GE	H^2 [%]
CC	1	15.5	13.9–16.9	0.6	3.54	15.6	15.6	0.24**	0.34**	0.24	82.9
CC	2	15.5	14.1–17.0	0.6	3.70	15.8	15.6	0.24**	0.44**	0.32	74.6
HC	1	3.77	1.64–5.39	0.7	18.3	4.16	4.73	0.36**	0.28**	0.44	80.1
HC	2	3.51	1.78–5.27	0.9	24.1	4.01	4.32	0.45**	0.37**	0.88	67.0
LC	1	12.5	9.79–15.8	1.6	12.6	12.5	10.5	2.31**	0.07**	0.59	95.1
LC	2	13.3	10.1–17.8	2.1	16.0	12.4	11.5	4.30**	0.10**	0.85	95.3
OC	1	44.7	39.1–47.1	1.0	2.33	44.4	46.1	0.96**	0.13**	0.38	92.7
OC	2	43.8	41.5–46.2	0.9	2.14	44.6	44.6	0.64**	0.25**	0.94	72.9
SPC	1	17.9	16.7–20.8	0.5	2.78	17.5	17.9	0.18**	0.08**	0.19	82.4
SPC	2	17.8	16.4–19.0	0.6	3.19	17.3	17.4	0.22**	0.30**	0.36	71.3
PidM	1	32.4	30.2–34.2	0.8	2.38	31.5	33.4	0.52**	0.15**	0.29	90.0
PidM	2	31.6	29.8–33.2	0.7	2.26	31.2	31.3	0.40**	0.50**	0.38	80.6
GSL	1	15.8	11.4–33.8	3.1	19.4	13.0	14.5	5.41**	1.89**	10.7	71.7
GSL	2	16.3	10.3–25.0	2.9	18.3	13.7	14.2	4.91**	36.4**	10.9	66.4
NDF	1	31.8	25.2–35.9	1.8	5.68	32.2	30.8	2.71**	1.00**	1.70	88.8
NDF	2	32.3	27.8–37.1	2.5	7.74	32.2	31.5	5.32**	1.84**	3.28	86.6
ADF	1	28.0	24.1–32.5	1.8	6.49	28.0	26.1	3.04**	0.68**	1.18	92.8
ADF	2	28.8	24.7–33.1	2.1	7.31	28.2	27.2	4.04**	0.79**	1.47	91.6

POP population, *SD* standard deviation, *CV* coefficient of variation (%), *P1* parent Adriana, *P2* parent SGEDH13 in pop 1 and Zheyou 50 in pop 2, *G* genotypic variance, *E* environmental variance, *GE* genotype by environment variance, H^2 broad sense heritability

**Significant at $p \leq 0.01$

Table 2 Correlations among seed quality traits in population 1 for hemicellulose (HC), cellulose (CC), lignin (LC), seed oil content (OC), seed protein content (SPC), protein in the defatted meal (PidM), glucosinolate content (GSL), neutral detergent fibre (NDF) and acid detergent fibre (ADF)

Trait	HC	CC	LC	OC	SPC	PidM	GSL	NDF
CC	0.11							
LC	−0.44**	0.35**						
OC	0.48**	0.33**	−0.24*					
SPC	−0.45**	−0.68**	−0.34**	−0.42**				
PidM	−0.11	−0.47**	−0.54**	0.31**	0.68**			
GSL	−0.38**	−0.32**	−0.01	−0.02	0.38**	0.43**		
NDF	−0.02	0.60**	0.86**	0.04	−0.65**	−0.68**	−0.23*	
ADF	−0.35**	0.55**	0.96**	−0.12	−0.46**	−0.59**	−0.10	0.93**

*Significant at $p \leq 0.05$

**Significant at $p \leq 0.01$

Interestingly, GSL content was not correlated with LC but was negatively correlated with HC and CC. GSL content was in addition not correlated with OC but was positively correlated with SPC (Table 2). As for population 2, all three fibre fractions were closely correlated to each other as in population 1. In contrast to the population 1, NDF was positively correlated with HC and was not correlated with CC (Table 3). OC was again weak negatively correlated with LC and positively correlated with HC and CC. In contrast to the population 1, SPC was much stronger negatively correlated with HC than with CC, followed by LC. Furthermore, LC was in contrast positively correlated with HC and weak negatively correlated with CC. HC was weak positively correlated with CC. As for population 1, GSL content was not significantly correlated with LC but was negatively correlated with HC and CC. GSL content was negatively correlated with OC and was positively correlated with SPC (Table 3).

QTL analysis and identification of candidate genes in the two half-sib DH populations

The SNP positions on the genetic map (in cM) in each linkage group were aligned with physical position based on the reference genome. The genetic marker position was predominant linearly correlated with the physical marker position in all linkage groups in both populations (Suppl. Tables S1 and S2; Suppl. Figs. S3 and S4). The alignment of the SNP marker sequences to the Express 617 reference genome allowed the comparison of their physical positions with those of candidate genes. The main interest was to identify

Table 3 Correlations among seed quality traits in population 2 for hemicellulose (HC), cellulose (CC), lignin (LC), seed oil content (OC), seed protein content (SPC), protein in the defatted meal (PidM), glucosinolate content (GSL), neutral detergent fibre (NDF) and acid detergent fibre (ADF)

Trait	HC	CC	LC	OC	SPC	PidM	GSL	NDF
CC	0.22*							
LC	0.25*	−0.17						
OC	0.52**	0.55**	−0.23*					
SPC	−0.78**	−0.52**	−0.28*	−0.74**				
PidM	−0.71**	−0.33**	−0.57**	−0.30*	0.87**			
GSL	−0.27*	−0.32**	0.15	−0.22*	0.25*	0.20		
NDF	0.60**	0.16	0.90**	0.11	−0.62**	−0.80**	−0.03	
ADF	0.31**	0.10	0.96**	−0.08	−0.43**	−0.67**	0.07	0.95**

*Significant at $p \leq 0.05$

**Significant at $p \leq 0.01$

co-locating QTL for individual fibre components with the same or opposite direction of the additive effects to each other and as well as to QTL for oil, protein and GSL content. This finding could facilitate understanding connections between the different biosynthetic pathways and identifying genes reducing fibre and simultaneously enhancing oil and protein content.

Population 1 Transgressive segregation for oil and protein content in the DH population is caused by a number of different QTL for OC and SPC with alleles from both parents either increasing oil or protein content. The majority of QTL alleles with negative additive effects increasing OC were derived from SGEDH13. SGEDH13 contributed with the QTL 1Oil-3 on chromosome C03 the fae1 allele for erucic acid biosynthesis leading to enhanced oil content (Table 4). Candidate is the well-known 3-ketoacyl-CoA synthase (KCS) gene (C03p062840.1; Table 6). This QTL 1Oil-3 allele did not lead to an enhanced SPC. However, the QTL 1Oil-3 allele collocated with QTL 1Pidm-4 and led to enhanced protein content in the defatted meal, indicating that fibre content in the meal is reduced by the erucic acid allele. However, there was no significant QTL at the same position on C03 with an opposite additive effect neither for NDF nor for CC, HC or LC. The confidence interval of the QTL 1Oil-1 overlapped with the QTL 1CC-1 with an opposite additive effect, suggesting that an increase in OC led to a reduction of CC or vice versa (Table 4). Candidate for QTL 1Oil-1 is the lysophosphatidyl acyltransferase gene (LPAT 5; Table 6). However, co-location of QTL 1Oil-1 with QTL 1ADF-1 and 1NDF-1 specifically confirmed the presence of a cellulose biosynthesis gene as a causal factor. Candidates for QTL 1CC-1 are two NAC domain transcription factors (Table 6). Candidates for QTL 1Oil-2 on A02 are the 3-ketoacyl-CoA synthase gene (KCS21) and the MYB96 transcription factor gene (Table 6). The two QTL 1oilpro-1 and 1oilpro-2 both with a negative additive effect increased contents of the sum of oil and protein in the seed. However, this was only due to their effects on OC on C03 and C05. QTL 1oil-4 and 1oil-pro-2 on C05 co-located with QTL 1LC-3 with an opposite additive effect, suggesting that a reduction of LC led to an enhanced OC. There are a number of candidates for QTL 1LC-3 which include phenylalanine ammonia-lyase 4 (PAL4), laccase (LAC7), cellulose synthase (CEV1), cinnamoyl-CoA reductase (CCR1), MYB83 gene, SEC8 and a MYB5 gene (Table 6, Suppl. Fig. S5). Notably, QTL 1LC-3 also co-located with QTL 1HC-3 with a negative additive effect, indicating that a LC reduction leads to an increase in HC. There was no corresponding QTL effect on CC. The second QTL 1CC-2 on A07 was detected at a similar position as QTL 1LC-2 with opposite additive effects, suggesting competing biosynthetic pathways. It also mapped at the same position as 1SPC-2 with the same direction of the effect (Table 4, Suppl. Fig. S5). Candidates for QTL 1LC-2 are a cellulose synthase-like gene and a PAL2 gene (Table 6). Furthermore, QTL 1CC-2 mapped with overlapping confidence intervals with QTL 1SPC-2, 1PidM-1 and 1LC-2 implying that a reduction of CC led to an increase in SPC, PidM and LC. Candidate for QTL 1CC-2 is a cellulose synthase-like gene (CSLA10; Table 6). Otherwise, candidates for QTL 1SPC-2 are LEC1 and LEC2 genes. QTL 1PidM-3 was identified at a very similar position as QTL 1CC-3 with opposite additive effects. QTL 1HC-1 mapped at a very similar position as QTL 1SPC-3 on C01 with opposite additive effects. Candidate for QTL 1HC-1 is a COBRA like protein gene. QTL 1HC-1 and 1HC-2 did not show co-locating positions with QTL for LC and CC. QTL positions of NDF and ADF confirmed individual QTL positions for HC, CC and LC (Table 4). There was no significant QTL for GSL content detected in this population, indicating that parental lines had identical or similar alleles at relevant loci.

Population 2 The half-sib DH population 2 shared with population 1 the QTL 2Oil-1 for oil content on A01 (Table 5). In both populations, the flanking markers were located between 20 and 28 Mbp with the same LPAT5 candidate gene (Table 7). However, in population 1, the SGEDH13 allele increased the OC whereas the Adriana allele increased the OC in population 2. The increase in OC in population 2 was accompanied by lower protein content at QTL 2SPC-1 and by an increase in CC at QTL 2CC-1. For the second QTL 2Oil-2 on A04, the Zheyou 50 allele led to an increase in OC and LC (QTL 2LC-1; Table 5; Suppl. Fig. S6). On the other hand, the effect of QTL 2Oil-2 is accompanied by a decrease in SPC at QTL 2SPC-2 and 2PidM-1. Candidate is an acetyl CoA synthetase (ACS; Table 7; Suppl. Fig. S6). With almost 20% the largest fraction of variance for oil content is explained by QTL 2Oil-4 on C02 with the Adriana allele increasing

Table 4 QTL mapped for oil content (OC), seed protein (SPC), protein in the defatted meal (PidM), NDF, ADF, lignin content (LC), hemicellulose content (HC) and cellulose content (CC) in population 1 (Adriana X SGEDH13)

Trait	QTL name	LG	Peak (cM)	CI[a] (cM)	[b]Additive effect	LOD	[c]R^2	[d]TR^2	P-value
OC	1Oil-1	A01	106.6	94–106	−0.23	3.40	4.50	53.2	0.000104
	1Oil-2	A02	9.00	5–14	0.22	3.10	4.10		0.000217
	1Oil-3	C03	23.8	22–25	−0.67	22.5	39.4		<2e−16
	1Oil-4	C05	68.0	60–73	−0.31	5.90	8.20		2.72E−07
SPC	1SPC-1	A02	39.2	32–47	0.13	3.39	7.27	24.9	9.62E−05
	1SPC-2	A07	6.10	0–15	0.15	4.24	9.18		1.31E−05
	1SPC-3	C01	10.0	0–16	−0.15	3.76	8.09		4.04E−05
PidM	1PidM-1	A07	12.0	1–20	0.21	5.05	6.33	47.1	2.43E−06
	1PidM-2	A10	14.6	6–18	−0.15	3.09	3.77		2.26E−04
	1PidM-3	C01	56.8	50–67	−0.19	4.84	6.05		3.96E−06
	1PidM-4	C03	26.2	21–31	−0.36	14.7	21.1		9.99E−16
	1PidM-5	C05	64.0	56–71	−0.27	7.32	9.47		1.39E−08
OC + SPC	1oilpro-1	C03	23.3	22–24	−0.65	33.6	51.2	65.9	<2e−16
	1oilpro-2	C05	66.0	61–69	−0.36	12.5	13.8		6.68E−11
NDF	1NDF-1	A01	93.4	86–106	0.40	3.23	4.73	48.6	1.48E−04
	1NDF-2	C01	23.0	16–32	0.58	6.59	10.1		6.05E−08
	1NDF-3	C04	80.0	76–88	0.41	2.98	4.35		2.69E−04
	1NDF-4	C05	68.6	64–69	1.05	18.2	32.9		2.00E−16
ADF	1ADF-1	A01	93.4	85–101	0.38	5.12	4.14	72.4	2.22E−06
	1ADF-2	A04	38.0	30–53	−0.25	2.38	1.85		0.00124
	1ADF-3	A07	2.40	0–14	−0.37	4.69	3.76		5.95E−06
	1ADF-4	A10	14.6	6–19.5	0.37	4.73	3.80		5.38E−06
	1ADF-5	C01	69.0	60–80	0.34	4.28	3.41		1.53E−05
	1ADF-6	C05	68.0	65–69	1.35	37.9	49.8		2.00E−16
LC	1LC-1	A04	38.0	30–43	0.22	2.80	2.00	74.5	0.000447
	1LC-2	A07	2.40	0–20	0.24	2.90	2.10		0.00031
	1LC-3	A10	15.0	8–19	−0.24	5.20	3.90		9.18E−06
	1LC-4	C05	68.6	66–70	1.28	44.9	61.3		2.00E−16
HC	1HC-1	C01	8.50	0–22	0.18	3.29	6.35	32.2	0.000124
	1HC-2	C04	75.1	69–88	0.21	3.90	7.61		2.87E−05
	1HC-3	C05	68.6	63–69	−0.31	9.37	19.7		9.05E−11
CC	1CC-1	A01	96.0	86–106	0.19	5.71	11.1	33.8	4.53E−07
	1CC-2	A07	7.00	0–15	−0.14	3.54	6.71		7.10E−05
	1CC-3	C01	69.0	57–80	0.17	5.23	10.1		1.38E−06

[a]QTL confidence interval at $p \leq 0.01$

[b]Negative sign indicates alleles from SGEDH13 increase trait values

[c]R^2 percentage of the phenotypic variation explained by a QTL

[d]TR^2 percentage of the phenotypic variation explained by all the QTL for that trait

trait value. Candidate is a 3-ketoacyl-CoA synthase gene (KCS19; Table 7). Confidence interval of QTL 2Oil-3 on A05 overlapped with QTL 2LC-2 with opposite direction of additive effects, suggesting that an increase in oil content led to a reduction in lignin content. Candidate is a glycerol-3-phosphate acyltransferase gene (GPAT6). The QTL 2Oil-4 and 2Oil-3 were not identified in population 1. Population 2 shared the QTL 2Oil-5 on C05 with population 1. In both populations, the SGEDH13 and the Zheyou

Table 5 QTL mapped for oil content (OC), seed protein (SPC), protein in the defatted meal (PidM), NDF, ADF, lignin content (LC), hemicellulose content (HC) and cellulose content (CC) in population 2 (Adriana X Zheyou 50)

Trait	QTL name	LG	Peak (cM)	CI[a] (cM)	[b]Additive effect	LOD	[c]R^2	[d]TR2	P-value
OC	2Oil-1	A01	18.3	8–32	0.32	5.90	11.7	65.2	6.58e−07
	2Oil-2	A04	25.0	13–27	− 0.23	5.88	11.6		4.21e−06
	2Oil-3	A05	31.0	26–42	0.20	6.84	13.9		5.55e−07
	2Oil-4	C02	2.10	0–6	0.06	9.24	19.9		1.62e−08
	2Oil-5	C05	31.0	25–41	− 0.42	8.27	17.4		3.96e−09
	A04:C02	A04:C02			0.23	3.00	5.51		0.00039
	A05:C02	A05:C02			0.32	5.57	10.9		1.36e−06
SPC	2SPC-1	A01	23.4	15–33	− 0.16	2.32	7.17	40.4	0.001433
	2SPC-2	A04	24.0	21–27	0.31	7.84	27.9		4.57e−09
	2SPC-3	C05	80.0	75–85	− 0.18	3.03	9.55		0.000266
PidM	2PidM-1	A04	23.0	19–26	0.33	6.27	20.1	44.2	1.34e−07
	2PidM-2	C05	66.0	62–81	− 0.48	9.58	33.4		7.12e−11
GSL	2GSL-1	A02	101	97–109	1.63	6.80	26.1	33.9	4.03E−08
	2GSL-2	A09	73.0	65–80	− 0.80	3.16	7.02		0.00251
NDF	2NDF-1	A01	23.4	18–31	0.58	3.71	5.38	73.0	6.02E−05
	2NDF-2	A04	23.0	18–27	− 1.20	11.6	20.6		1.41E−12
	2NDF-3	C05	33.0	26–39	1.17	7.01	11.1		3.49E−08
	2NDF-4	C05	79.3	75–85	0.99	5.53	8.39		9.71E−07
ADF	2ADF-1	A01	28.0	20–34	1.04	4.29	5.60	76.1	2.23e−05
	2ADF-2	A01	39.0	29–58	− 0.72	2.07	2.55		0.00325
	2ADF-3	A04	23.0	17–30	− 0.68	6.55	9.06		1.62e−07
	2ADF-4	A09	39.0	31–48	− 0.30	1.20	1.45		0.02497
	2ADF-5	C05	33.0	31–38	1.48	19.4	37.8		< 2e−16
	2ADF-6	C06	50.4	40–50	0.21	2.26	2.81		0.00849
LC	2LC-1	A04	23.0	21–26	− 0.48	4.02	4.26	67.3	3.27e−05
	2LC-2	A05	45.0	41–48	− 0.56	5.82	6.44		5.84e−07
	2LC-3	A09	60.4	54–66	− 0.54	5.36	5.87		1.63e−06
	2LC-4	C03	121	0–195	0.26	1.45	1.44		0.0127
	2LC-5	C05	36.0	31–39	1.96	30.0	65.4		< 2e−16
HC	2HC-1	A04	32.0	29–47	− 0.47	6.93	26.8	33.8	2.28e−07
	2HC-2	C05	61.0	54–89	0.42	4.90	17.9		2.04e−05
CC	2CC-1	A01	11.6	0–82	0.17	2.54	7.62	42.5	0.000904
	2CC-2	A02	103.1	85–112	− 0.17	2.29	6.82		0.001632
	2CC-3	A10	56.2	52–68	0.14	1.86	5.47		0.004554
	2CC-4	C01	93.0	84–99	− 0.24	4.05	12.6		2.73e−05

[a]QTL confidence interval at $p \leq 0.01$

[b]Negative sign indicates alleles from Zheyou 50 increasing trait values

[c]R^2 percentage of the phenotypic variation explained by a QTL

[d]TR^2 percentage of the phenotypic variation explained by all the QTL for that trait

50 alleles were increasing the OC. Likewise, in both populations these QTL co-located with a QTL for LC content with an opposite direction of the effect and with the Adriana allele increasing trait values (1LC-3 and 2LC-5). There are a number of candidate genes for this QTL, e.g. the PAL4 gene and the LAC7 gene (cf. population 1 and Table 7). Epistatic interactions were found between QTL 2Oil-2, 2Oil-3 and

Table 6 QTL and candidate genes based on Express 617 for population 1 (Adriana X SGEDH13)

QTL	Chr	Flanking SNPs	Physical position (bp)	Candidate gene (Express 617)	Physical position (bp)	Functional annotation araport11	Express 617 candidate gene
1Oil-1	A01	Bn-A01-p26426004	28,704,088	A01p031950.1	26,077,758	AT3G18850	BnaC01g33800D; LPAT5; lysophosphatidyl acyltransferase 5
		Bn-A01-p27954294	29,819,772				
1Oil-2	A02	Bn-A02-p24971607	28,645,783	A02p035450.1	27,530,583	AT5G49070	BnaA02g30560D; KCS21; 3-ketoacyl-CoA synthase 21
		Bn-A02-p25615285	29,351,916	A02p039110.2	30,032,315	AT5G62470	BnaA02g33410D; MYB96; Myb domain protein 96
1Oil-3	C03	Bn-scaff_15794_3-p154541	55,932,529	C03p062840.1	56,197,015	AT4G34520	BnaC03g65980D; KCS18;3-ketoacyl-CoA synthase 18
		Bn-A08-p13019549	56,641,328	C03p062850.1	56,213,052	AT4G34510	BnaC03g66040D; KCS17;3-ketoacyl-CoA synthase 17
1Oil-4	C05	Bn-scaff_21369_1-p1212906	39,180,456	C05p040780.1			See QTL 1LC-3
		Bn-scaff_20219_1-p50816	42,540,453	C05p041260.1			
1SPC-1	A02	Bn-A02-p7853194	7,161,946	A02p015980.1	9,109,411	AT1G66160	BnaA02g12360D; CMPG1; CYS, MET, PRO, and GLY protein 1
		Bn-A02-p13123802	12,690,164	A02p020660.1	12,902,711	AT1G18570	BnaA02g16690D; MYB51; myb domain protein 51
1SPC-2	A07	Bn-A07-p187342	206,199	A07p008230.1	9,868,724	AT1G28300	BnaA07g08500D; LEC2; AP2/B3-like transcriptional factor family protein
		Bn-A07-p10554944	13,777,886	A07p011110.1	11,946,345	AT1G21970	BnaA07g10770D; LEC1; Histone superfamily protein
				A07p010610.1	11,598,637	AT1G22710	BnaA07g10320D; SUC2; Sucrose-proton symporter 2
1SPC-3	C01	Bn-scaff_19168_1-p109723	38,651,892				
		Bn-A01-p27774666	41,762,974				
1PidM- 1	A07	Bn-A07-p187342	206,199				See 1SPC-2
		Bn-A07-p11512981	15,833,275				
1PidM- 2	A10	Bn-A10-p7278102	10,939,905	A10p015840.1	14,165,784	AT5G20900	BnaA10g14660D; JAZ12; jasmonate-zim-domain protein 12
		Bn-A10-p11059267	15,008,230	A10p011370.1	11,199,820	AT5G56750	BnaA10g10880D; NDL1; N-MYC downregulated-like 1

Table 6 (Continued)

QTL	Chr	Flanking SNPs	Physical position (bp)	Candidate gene (Express 617)	Physical position (bp)	Functional annotation araport11	Express 617 candidate gene
1PidM- 3	C01	Bn-scaff_20210_1-p166012	10,020,034				
		Bn-scaff_15803_1-p729620	17,384,422				
1PidM- 4	C03	Bn-scaff_15794_3-p154541	55,932,529				See QTL 1Oil-3
		Bn-A08-p13019549	56,641,328				
1PidM- 5	C05	Bn-scaff_18826_1-p232573	36,047,584	C05p043140.1	42,591,158	AT3G07450	BnaC05g44510D; Bifunct. Inhibit./lipid-transfer prot./ seed storage 2S albumin
		Bn-scaff_20219_1-p50816	42,540,453				See QTL 1LC-3
1oilpro1	C03	Bn-scaff_15794_3-p154541	55,932,529				See QTL 1Oil-3
		Bn-A08-p13019549	56,641,328				
1oilpro2	C05	Bn-scaff_21369_1-p1212906	39,180,456				See QTL 1Oil-4 and 1LC-3
		Bn-scaff_20219_1-p50816	42,540,453				
1NDF-1	A01	Bn-A01-p18306617	26,704,088				See QTL 1CC-1
		Bn-A01-p27954294	29,819,772				
1NDF-2	C01	Bn-scaff_17217_1-p4769	36,966,399				See QTL 1ADF-5 and 1HC-1
		Bn-scaff_19168_1-p109723	38,651,892				
1NDF-3	C04	Bn-scaff_27914_1-p74694	30,664,328				See QTL 1HC-2
		Bn-scaff_21956_1-p232420	44,985,085				
1NDF-4	C05	Bn-scaff_21369_1-p1212906	39,180,456				See QTL 1LC-3, 1Oil-4, 1PidM-5, 1ADF-6, 1Oil-4, 1HC-3
		Bn-scaff_17441_3-p37334	42,001,429				
1ADF-1	A01	Bn-A01-p18306617	26,704,088				See QTL 1NDF-1 and 1CC-1
		Bn-A01-p27954294	29,819,772				
1ADF-2	A04	Bn-A04-p14315336	16,932,263				See QTL 1LC-1
		Bn-A04-p16627363	19,433,149				
1ADF-3	A07	Bn-A07-p187342	206,199				See QTL 1LC-2
		Bn-A07-p10554944	13,777,886				
1ADF-4	A10	Bn-A10-p7644362	11,362,272				See QTL 1PidM-2
		Bn-A10-p11059267	15,008,230				
1ADF-5	C01	Bn-scaff_17217_1-p4769	36,966,399				See QTL 1HC-1
		Bn-scaff_19168_1-p109723	38,651,892				
1ADF-6	C05	Bn-scaff_21369_1-p1212906	39,180,456				See QTL 1LC-3, 1Oil-4, 1NDF-4, 1ADF-6, 1HC-3
		Bn-scaff_17441_3-p37334	42,001,429				

Table 6 (Continued)

QTL	Chr	Flanking SNPs	Physical position (bp)	Candidate gene (Express 617)	Physical position (bp)	Functional annotation araport11	Express 617 candidate gene
1LC-1	A04	Bn-A04-p14410667	17,053,407	A04p018830.1	16,245,744	AT2G30490	BnaA04g17570D; C4H; cinnamate-4-hydroxylase
		Bn-A04-p16368852	19,181,647	A04p023010.1	18,786,242	AT2G37040	BnaA04g21230D; PAL1; phenylalanine ammonialyase 1
1LC-2	A07	Bn-A07-p187342	206,199	A07p009890.1	11,020,886	AT1G24070	BnaA07g09050D; CSLA10; cellulose synthase-like A10
		Bn-A07-p10554944	13,777,886	A07p016620.1	15,797,092	AT3G53260	BnaA07g16060D; PAL2; phenylalanine ammonia-lyase 2
1LC-3	A10	Bn-A10-p7644362	11,362,272				See QTL 1PidM-2
		Bn-A10-p11059267	15,008,230				
1LC-4	C05	Bn-scaff_21369_1-p1212906	39,180,456	C05p038330.1	39,547,901	AT3G13540	BnaA05g37010D; MYB5; myb domain protein 5
		Bn-scaff_17441_3-p37334	42,001,429	C05p040780.1	41,326,930	AT5G05170	BnaA05g28060D; CEV1; Cellulose synthase family protein
				C05p041220.1	4,15,62,723	AT3G10380	BnaC05g42720D; SEC8; subunit of exocyst complex 8
				C05p041260.1	41,588,681	AT3G10340	BnaC05g42780D; PAL4; phenylalanine ammonia-lyase 4
				C05p041720.1	41,852,113	AT3G09780	BnaC05g43230D; CCR1; CRINKLY4 related 1
				C05p042120.1	42,094,161	AT3G09220	BnaCnng24340D; LAC7; laccase 7
				C05p042650.1	42,319,659	AT3G08500	BnaC05g44010D; MYB83; myb domain protein 83
1HC-1	C01	Bn-scaff_19168_1-p109723	38,651,892	C01p037480.1	42,680,552	AT3G16860	BnaC01g44070D; COBL8; COBRA-like protein 8 precursor
		Bn-A01-p27774666	41,762,974				
1HC-2	C04	Bn-scaff_27914_1-p74694	30,664,328	C04p034430.1	40,652,520	AT5G04230	BnaC04g33780D; PAL3; phenyl alanine ammonia-lyase 3
		Bn-scaff_21956_1-p232420	44,985,085	C04p025700.1	30,335,686	AT3G53260	BnaCnng52250D; PAL2; phenylalanine ammonia-lyase 2

Table 6 (Continued)

QTL	Chr	Flanking SNPs	Physical position (bp)	Candidate gene (Express 617)	Physical position (bp)	Functional annotation araport11	Express 617 candidate gene
1HC-3	C05	Bn-scaff_21369_1-p1212906	39,180,456				See QTL 1LC-3
		Bn-scaff_17441_3-p37334	42,001,429				
1CC-1	A01	Bn-A01-p18306617	26,704,088	A01p032330.1	26,469,580	AT3G18400	BnaCnng63190D; NAC058; NAC domain containing protein 58
		Bn-A01-p27954294	29,819,772	A01p034370.2	28,355,575	AT3G12910	BnaA01g30220D; NAC (No Apical Meristem) domain transcript. regulator
1CC-2	A07	Bn-A07-p187342	206,199	A07p009890.1	11,020,886	AT1G24070	BnaA07g09050D; CSLA10; cellulose synthase-like A10
		Bn-A07-p10554944	13,777,886				
1CC-3	C01	Bn-scaff_17731_1-p590773	6,407,584				
		Bn-scaff_19193_1-p875889	8,040,678				

2Oil-4, respectively (Table 5). On chromosome C05 there was in addition QTL 2HC-2 at 61 cM with the Zheyou 50 allele decreasing HC and increasing SPC and protein content in the defatted meal (QTL 2SPC-2 and 2PidM-2). These three QTL mapped in addition at the same position as QTL 2NDF-4 specifying the hemicellulose effect of this QTL. Candidates are a number of NAC domain transcriptional regulator genes (Table 7). The major GSL QTL 2GSL-1 on A02 explained 26% of the phenotypic variance with the Adriana allele increasing trait value. This QTL mapped at the same position as QTL 2CC-2 with an opposite effect, indicating that a reduction in GSL content led to an increase in CC or vice versa. Candidates for GSL content are GTR2, TGG1, TGG2, the MBY28 and MYB34 transcription factor genes. Candidates for 2CC-2 are transparent testa genes TT4 and TT10 (Table 7; Suppl. Fig. S6). On the contrary to this, an increase of LC with QTL 2LC-3 appears to result in an increase of GSL content at QTL 2GSL-2 on A09. As for population 1 most of the QTL for composite traits NDF and ADF confirmed individual QTL positions for HC, CC and LC.

Discussion

The main objective of this study was to map QTL for the three seed fibre components HC, CC and LC and to determine individual interactions among them and with OC, SPC and GSL content. There are only few studies addressing this detailed question. Miao et al. (2019) identified in the KN DH population between 21 and 35 QTL for LC, HC, and CC. They found a significant positive correlation between LC and CC and a negative correlation to HC and OC. In population 1 also a positive correlation between LC and CC was found, whereas in the population 2 a weak negative correlation was found (Table 2). In an earlier work, Liu et al. (2012) also reported a somewhat lower positive correlation between LC and CC. Furthermore, in population 1 a negative correlation between LC and HC and OC was identified, whereas in the population 2 a positive correlation to HC and a negative correlation to OC was detected. As in the KN DH population a slightly positive correlation between CC and HC content was found for both populations. In contrast to the results of Miao et al. (2019) a positive

Table 7 QTL and candidate genes based on Express 617 for population 2 (Adriana x Zheyou 50)

QTL	Chr	Flanking SNPs	Physical position (bp)	Candidate gene (Express 617)	Physical position (bp)	Functional annotation araport11	Express candidate gene
1Oil-1	A01	Bn-A01-p18230629	20,247,938	A01p031950.1	26,077,758	AT3G18850	BnaC01g33800D; LPAT5; lysophosphatidyl acyltransferase 5
		Bn-A01-p26426004	28,704,088				See QTL 2CC-1
2Oil-2	A04	Bn-A04-p8481222	9,621,091	A04p012950.1	11,892,091	AT5G36880	BnaA04g12330D; ACS; acetyl-CoA synthetase
		Bn-A04-p9296314	11,834,759				
2Oil-3	A05	Bn-A05-p2645307	2,928,767	A05p007180.1	3,981,157	AT2G38110	BnaA05g06580D; GPAT6; glycerol-3-phosphate acyltransferase 6
		Bn-A05-p3414540	3,721,186				
2Oil-4	C02	Bn-scaff_17752_1-p76499	1,136,711	C02p001490.1	1,317,027	AT5G04530	BnaC02g02670D; KCS19; 3-ketoacyl-CoA synthase 19
		Bn-scaff_22970_1-p335296	1,577,208				
2Oil-5	C05	Bn-A05-p22111286	41,608,831				See QTL, 1Oil-4, 1NDF-4, 1ADF-6, 1HC-3, 2NDF-3, 2ADF-5, 2LC-4
		Bn-scaff_17441_3-p156691	42,138,047				
2SPC-1	A01	Bn-A01-p20990218	9,735,795	A01p021570.1	14,651,829	AT3G51810	BnaA01g19290D; EM1; Stress induced protein
		Bn-A01-p10026020	22,435,826				
2SPC-2	A04	Bn-A04-p7373248	9,621,091				
		Bn-A04-p9296314	11,834,759				
2SPC-3	C05	Bn-scaff_16770_1-p107862	34,645,234				See QTL 2NDF-4, 2PidM-2, 2HC-2
		Bn-scaff_18826_1-p76018	36,181,332				
2PidM-1	A04	Bn-A04-p7373248	9,621,091				See QTL 2Oil-2, 2SPC-2, 2ADF-3, 2LC-1
		Bn-A04-p9296314	11,834,759				
2PidM-2	C05	Bn-scaff_16770_1-p107862	34,645,234				See QTL 2NDF-4, 2HC-2
		Bn-scaff_18826_1-p76018	36,181,332				
2GSL-1	A02	Bn-A02-p24345550	28,231,787	A02p036570.1	28,280,336	AT5G26000	BnaCnng53320D; TGG1; thioglucoside glucohydrolase 1
		Bn-A02-p25615285	29,351,916	A02p036570.3	29,450,406	AT5G25980	BnaA09g26590D; TGG2; glucoside glucohydrolase 2
				A02p038170.1	29,440,826	AT5G60890	BnaAnng06640D; MYB34; myb domain protein 34
				A02p038110.1	29,371,538	AT5G23010	BnaA02g33040D; MAM1; methylthioalkylmalate synthase 1
				A02p038540.1	29,647,030	AT5G61420	BnaC09g05300D; MYB28; myb domain protein 28
				A02p038990.2	29,973,314	AT5G62680	BnaC02g42260D; GTR2; Major facilitator superfamily protein
							See QTL 2CC_2

Table 7 (Continued)

QTL	Chr	Flanking SNPs	Physical position (bp)	Candidate gene (Express 617)	Physical position (bp)	Functional annotation araport11	Express candidate gene
2GSL-2	A09	Bn-A09-p24854950	30,201,881	A09p036110.2	32,059,393	AT3G53260	BnaA09g33560D; PAL2; Phenylalanine ammonia-lyase 2
		Bn-A09-p28988381	34,547,984				
2NDF-1	A01	Bn-A01-p10945930	10,392,745				See QTL 2ADF-1
		Bn-A01-p11306490	10,739,014				
2NDF-2	A04	Bn-A04-p7373248	9,621,091				See QTL 2Oil-2, 2SPC-2, 2PidM-1, 2ADF-3, 2LC-1
		Bn-A04-p9296314	11,834,759				
2NDF-3	C05	Bn-scaff_17441_1-p958418	41,704,005				See QTL 1Oil-4, 1NDF-4, 1ADF-6, 1HC-3, 2ADF-5, 2LC-4
		Bn-scaff_20219_1-p143569	42,594,324				
2NDF-4	C05	Bn-scaff_16770_1-p107862	34,645,234				See QTL 2HC-2
		Bn-scaff_18826_1-p76018	36,181,332				
2ADF-1	A01	Bn-A01-p10026020	9,735,795				See QTL 2NDF-2
		Bn-A01-p11306490	10,739,014				
2ADF-2	A01	Bn-A01-p6825673	6,945,733				
		Bn-A01-p10026020	9,735,795				
2ADF-3	A04	Bn-A04-p7373248	9,621,091				See QTL 2Oil-2, 2SPC-2, 2PidM-1, 2LC-1
		Bn-A04-p9296314	11,834,759				
2ADF-4	A09	Bn-A09-p4905766	5,816,951				
		Bn-A09-p5476558	6,284,902				
2ADF-5	C05	Bn-scaff_17441_1-p958418	41,704,005				See QTL 1Oil-4, 1NDF-4, 1ADF-6, 1HC-3, 2NDF-3, 2LC-4
		Bn-scaff_20219_1-p143569	42,594,324				
2LC-1	A04	Bn-A04-p7373248	9,621,091	A04p012590.1	11,661,554	AT5G04230	BnaA04g11940D; PAL3; phenylalanine ammonia-lyase 3
		Bn-A04-p9296314	11,834,759				
2LC-2	A05	Bn-A05-p3414540	3,721,186				See QTL 2Oil-3
		Bn-A05-p4093306	4,394,132				
2LC-3	A09	Bn-A09-p11110730	25,154,269				
		Bn-A09-p23928417	28,902,308				

Table 7 (Continued)

QTL	Chr	Flanking SNPs	Physical position (bp)	Candidate gene (Express 617)	Physical position (bp)	Functional annotation araport11	Express candidate gene
2LC-5	C05	Bn-scaff_17441_1-p958418	41,704,005	C05p038330.1	39,547,901	AT3G13540	BnaA05g37010D; MYB5; Myb domain protein 5
		Bn-scaff_20219_1-p143569	42,594,324	C05p040780.1	41,326,930	AT5G05170	BnaA05g28060D; CEV1; Cellulose synthase family protein
				C05p041220.1	41,562,723	AT3G10380	BnaC05g42720D; SEC8; Subunit of exocyst complex 8
				C05p041260.1	41,588,681	AT3G10340	BnaC05g42780D; PAL4; Phenylalanine ammonia-lyase 4
				C05p041720.1	41,852,113	AT3G09780	BnaC05g43230D; CCR1; CRINKLY4 related 1
				C05p042120.1	42,094,161	AT3G09220	BnaCnng24340D; LAC7; Laccase 7
				C05p042650.1	42,319,659	AT3G08500	BnaC05g44010D; MYB83; Myb domain protein 83
2HC-1	A04	Bn-A04-p9510750	12,105,311	A04p018820.1	16,240,675	AT2G30490	BnaA04g17560D; C4H; Cinnamate-4-hydroxylase
		Bn-A04-p13119516	15,827,404				
2HC-2	C05	Bn-scaff_16770_1-p107862	34,645,234	C05p035490.1	35,754,463	AT3G15510	BnaC05g38130D; NAC2; NAC domain containing protein 2
		Bn-scaff_18826_1-p76018	36,181,332	C05p035510.1	35,779,863	AT3G15500	BnaC05g38150D; NAC3; NAC domain containing protein 3
				C05p035510.1	35,779,981	AT3G15500	BnaC05g38150D; NAC3; NAC domain containing protein 3
				C05p035900.1	36,067,006	AT3G15170	CUC1; NAC (No Apical Meristem) domain transcript. regulator superfam. protein
2CC-1	A01	Bn-A01-p20990218	22,435,826	A01p029500.1	23,785,201	AT3G18400	BnaA01g26760D; NAC058; NAC domain containing protein 58
		Bn-A01-p26426004	28,704,088	A01p032330.1	26,469,580	AT3G18400	BnaCnng63190D; NAC058; NAC domain containing protein 58
				A01p034370.2	28,355,575	AT3G12910	BnaA01g30220D; NAC (No Apical Meristem) domain transcriptional regulator
2CC-2	A02	Bn-A02-p23792703	27,449,577	A02p035210.1	27,390,029	AT5G13930	BnaA02g30320D; TT4; Chalcone and stilbene synthase family protein
		Bn-A02-p24971607	28,645,783	A02p034830.1	27,137,641	AT5G48100	BnaAnng08030D; TT10; Laccase/Diphenol oxidase family protein
2CC-3	A10	Bn-A10-p10149557	14,104,587				
		Bn-A10-p13008713	15,747,237				
2CC-4	C01	Bn-scaff_15712_3-p88356	41,758,234				
		Bn-A01-p23642494	43,461,421				

correlation for both CC and HC to OC was found for population 1 and 2. All three fibre components were negatively correlated with SPC in both of the present DH populations. Liu et al. (2012) found a positive correlation between HC and SPC but negative correlation between CC and SPC. GSL content was negatively correlated with HC and CC. In this study, in both populations there was a positive correlation between the GSL content and the SPC as was reported earlier for other populations (Schatzki et al. 2014; Gacek et al. 2021). Correlations of GSL to SPC were not reported by Miao et al. (2019).

QTL mapping in the KN DH population (Miao et al. 2019) allowed the identification of 13 co-localized QTL with pleiotropic effects on at least two of the above mentioned four traits. These pleiotropic unique QTL for seed fibre components and OC were located on chromosomes A08, A09, A10, C03, C05 and C06. Interestingly, the QTL flanking markers on these chromosomes were either not located on the same chromosome as in the present two populations or they mapped at a very large distance based on the Express 617 reference genome. Obviously, different co-locating QTL with opposite additive effects for LC, CC, HC and OC were identified in the different populations. The comparative analysis of the two half-sib DH populations including the semi-winter Chinese cultivar Zheyou 50 allows direct comparison of QTL positions and of the direction of their allelic effects. In an updated analysis of the same KN DH population of Miao et al. (2019), Chao et al. (2022b) reported that a major QTL for seed colour on A09 led to a reduction in LC and CC, and pleiotropic to an increase in OC. Chao et al. (2022b) reported three candidate genes for LC (JAZ1, GH3, LOX3); they mapped however far away from the QTL 2LC-3 in population 2. Liu et al. (2012; 2013) mapped a major QTL for LC on A09 which collocated with seed colour. In this study, only a minor QTL for LC was mapped on A09.

In both of the present DH populations there was a close negative correlation between SPC and OC. This is in line with previous earlier results (Zum Felde et al. 2006; Liu et al. 2012; Chao et al. 2017; Gacek et al. 2021; Schilbert et al. 2022). Chao et al. (2017) mapped in the same above mentioned KN DH population the fae1 gene as a QTL for OC on C03 as in the present population 1 (cf. Table 6). Furthermore, Chao et al. (2017) reported QTL for OC on C05 in the same region (39–43 Mbp) as in the present two populations. However, co-location of QTL for fibre components were not investigated in the work of Chao et al. (2017) as in the present study. Therefore, it remains unclear if the QTL for OC or the QTL for fibre components are causal for the increase in OC. Schilbert et al. (2022) in a mapping by sequencing approach identified in different oilseed rape material chromosomes for seed quality traits, but none of regions for SPC overlapped with regions for OC or SPC as in the present DH populations. Regulation of seed storage protein synthesis has been reviewed by Yang et al. (2022a). Some of the candidate genes listed for *Arabidopsis* were located within the flanking markers of QTL 1SPC-2 and 2SPC-1 (Tables 6 and 7). Some of the key structural genes of fatty acid and triglyceride biosynthesis listed by Yang et al. (2022a) were identified within the oil QTL confidence intervals (cf. Tables 6 and 7). This includes the acetyl-CoA synthase (ACS), the lysophosphatidyl acyltransferase (LPAAT), the glycerol-3-phosphate acyltransferase (GPAT), and the 3-ketoacyl-CoA synthase (KCS) genes in both populations. Except MYB96, none of the other key regulators of seed oil accumulation (e.g. LEC1, LEC2, ABI3, FUS3, LTL) and of the two epigenetic regulators (PICKLE, CLF) were found within the oil QTL confidence intervals (Yang et al. 2022b). The effect of the fatty acid elongase gene (fae1) in population 1 confirms for a new population the earlier observed positive effect of fae1 gene on the protein content in the defatted meal (Behnke et al. 2018).

In a multi-omics study a negative correlation between seed coat content and OC was found by Zhang et al. (2022). In line with this, a negative correlation between LC and OC was found in both DH populations. In both DH populations co-locating QTL positions were detected for LC and OC on C05. In population 1 a reduction of LC led to an increase in OC and SPC in defatted meal (PidM), whereas in the population 2 only OC increased. Furthermore, in population 1 the reduction of LC led to an increase in HC, whereas in the population 2 there was no co-locating QTL for HC. Obviously, the effect of the QTL 1LC-3 and QTL 2LC-5 on C05 depends on the cross. Surprisingly, both populations carried the same QTL on C05. Furthermore, the same QTL on C05 was already mentioned by Behnke et al. (2018) for a different population and the BnPAL4 gene on C05 was reported as a likely candidate. Yusuf et al. (2022) speculated

that the Chinese cultivar Zheyou 50 may be derived from the same ancestor cross as SGEDH13. Genome sequencing and read mapping of SGDH14 (Behnke et al. 2018) against the Express 617 genome confirmed chromosomal structural rearrangement as the cause for the reported major QTL for low lignin content (Schilbert et al. 2023). This confirmed the accurate position of the major QTL for low LC on C05 (Behnke et al. 2018; Yusuf et al. 2022). Based on the Express 617 reference genome, in addition to the BnPAL4 gene, the CEV1, the CCR1, the SEC8 and the LAC7 were identified as candidate genes in both populations. Phenylalanine Ammonia Lyase is the key enzyme in phenylpropanoid pathway, which leads to the biosynthesis of a wide array of secondary metabolites including phenolic acid esters and lignin (Zhang et al. 2022). Members of the laccase (LAC) gene family catalyzes lignification and relatively high expressions have been found in seed coats (Ping et al. 2019). Cinnamoyl-CoA reductase (CCR1) and Cellulose synthase family genes (CEV1, CESA3) are associated with the phenylpropanoid-lignin pathways and seed coat development (Miao et al. 2019). SEC8 is involved in post-golgi trafficking of mucilage components to the plasma membrane (Kulich et al. 2010) and was mentioned as candidate in the multi-omics study of Zhang et al. (2022). Furthermore, the transcription factor genes MYB83 and MYB5 are known as regulator of phenylpropanoid metabolism in plants (Liu et al. 2015; Wang et al. 2015) and of mucilage differentiation (Xu et al. 2018), respectively. All these genes were located between the flanking SNP markers in both populations. However, also individual QTL for CC and HC co-located with QTL for OC. Overlapping QTL positions for OC and CC were detected on chromosome A01, at which in population 1 a reduction in CC led to an increase in OC (QTL 1CC-1 and 1Oil-1), whereas in population 2 the same QTL led to an increase in CC and OC. Zhang et al. (2022) and Pedersen et al. (2022) provided a comprehensive list of candidate genes involved in the biosynthesis of HC, CC and LC. A COBRA like protein gene (BnaC01g44070D) has been identified nearby the flanking markers of QTL 1HC-1 on C01 (Ben-Tov et al. 2015). Among many others, phenylalanine ammonium-lyase (PAL), cinnamate-4-hydroxylase (C4H), cinnamoyl-CoA reductase (CCR1), laccase (LAC7), transparent testa genes TT4 and TT10, NAC (No Apical Meristem) transcriptional regulator genes were found as candidate genes between flanking markers of QTL for individual seed fibre traits (cf. Tables 6 and 7). Transparent testa (TT) are key enzymes in proanthocyanidin and lignin biosynthesis.

In population 2, the major QTL 2GSL-1 on A02 has not yet been reported by others. Candidate genes for QTL 2GSL-1 on A02 are *GTR2*, *MYB34*, *TGG1, TGG2* and *MYB28*. All four genes were reported as candidate genes (Seo and Kim 2017; Kittipol et al. 2019; Wei et al. 2017; Schilbert et al. 2022). QTL 2GSL-1 mapped with an opposite effect nearby QTL 2CL-2, suggesting competing biosynthetic pathways. Wei et al. (2017) found that GSL metabolic processes affected lignin biosynthesis and Vanholme et al. (2012) reported that transcripts involved in GSL biosynthesis were more abundant in low lignin mutants. Recently, Gacek et al. (2021) also reported in oilseed rape negative correlations between GSL and ADF and NDF contents, respectively. Additional evidence on crosstalk of the glucosinolate pathway with the phenylpropanoid pathway is provided by Yin et al. (2022) and references given therein. A second QTL for GSL content was located on A09. None of the genomic intervals for GSL content identified by Schilbert et al. (2022) in their mapping-by-sequencing study overlapped with the A09 GSL region identified in this study. This points to an additional minor GSL locus on A09.

Conclusions

In two half-sib DH populations a large number of novel diverse QTL for seed fibre components on different chromosomes were identified. The effect of a major QTL for low LC on C05 on contents of CC, HC, OC, SPC and GSL were determined. Some of the fibre components related QTL co-located to each other and with QTL for OC and SPC with opposite direction of additive effects. This suggests that individual QTL alleles for fibre components can be used to further reduce overall fibre content and to increase oil and protein content in oilseed rape. The parallel investigation of two half-sib DH populations gave insight into the direction of the additive effects which depended on the indvidual parental lines of the two crosses. This complicates breeding for improved seed quality traits in oilseed rape.

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Authors contributions CM designed the experiment and developed the DH populations. AOY and CM performed the field experiments. AOY did the NIRS and Chromatography analysis and analyszed the data. AOY wrote the initial draft of the manuscript. CM revised the manuscript and all authors agreed on the final manuscript.

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Declarations

Conflict of interest The authors have no competeing interest.

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