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ORIGINAL ARTICLE

A major yellow-seed QTL on chromosome A09 significantly increases the oil content and reduces the fiber content of seed in *Brassica napus*

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Abstract

Key message **A major yellow-seed QTL on chromosome A09 significantly increases the oil content and reduces the fiber content of seed in *Brassica napus*.**

Abstract The yellow-seed trait (YST) has always been a main breeding objective for rapeseed because yellow-seeded *B. napus* generally contains higher oil contents, fewer pigments and polyphenols and lower fiber content than black-seeded *B. napus*, although the mechanism controlling this correlation remains unclear. In this study, QTL mapping was implemented for YST based on a KN double haploid population derived from the hybridization of yellow-seeded *B. napus* N53-2 with a high oil content and black-seeded Ken-C8 with a relatively low oil content. Ten QTLs were identified, including four stable QTLs that could be detected in multiple environments. A major QTL, *cqSC-A09*, on chromosome A09 was identified by both QTL mapping and BSR-Seq technology, and explained more than 41% of the phenotypic variance. The major QTL *cqSC-A09* for YST not only controls the seed color but also affects the oil and fiber contents in seeds. More importantly, the advantageous allele could increase the oil content and reduce the pigment and fiber content at the same time. This is the first QTL reported to control seed color, oil content and fiber content simultaneously with a large effect and has great application value for breeding high oil varieties with high seed quality. Important candidate genes, including *BnaA09. JAZ1*, *BnaA09. GH3.3* and *BnaA09. LOX3*, were identified for *cqSC-A09* by combining sequence variation annotation, expression differences and an interaction network, which lays a foundation for further cloning and breeding applications in the future.

Introduction

Brassica napus. L, an important oil crop, is planted to produce edible oils, biofuels and animal feed. Increasing the oil content (OC) and yield and improving seed quality have been the main breeding objectives in *B. napus* in recent decades (Abbadi and Leckband 2011). In addition, yellow-seeded rapeseed has many advantages, such as being highly conducive to breeding and producing high-quality seeds requiring less processing during pressing (Wang et al. 2017). To data, most yellow-seeded rapeseed has generally been selected from interspecific hybridization of *Brassica* species (Rahman et al. 2001; Warwick et al. 2003; Wen et al. 2012).

The seed color trait is a quantitative trait, and studies, including quantitative trait loci (QTL) mapping and candidate gene cloning, have been performed to understand the mechanisms involved in yellow-seed rapeseed. For example, a major QTL for seed coat color with a large effect was identified on chromosome C08 by Badani et al. (2006) and Yan et al. (2011) in multiple environments. In addition, a major seed coat color QTL explaining much of the phenotypic variation (PV) was identified on the homologous fragments of chromosome A09 in different studies (Liu et al. 2012a, 2013; Stein et al. 2013). Wang et al. (2017) also detected two homologous loci on C08 and A09 through genome-wide association mapping (GWAS). In addition, many QTLs with minor effects have also been detected on other chromosomes, such as C02, C05, C06, C07, A01, A04, A07, and A08 (Fu et al. 2007; Gacek et al. 2021; Wang et al. 2017).

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Generally, yellow-seeded rapeseed has a higher OC, a higher protein content, fewer pigments and polyphenols, and a lower fiber content than black-seeded rapeseed (Snowdon et al. 2010; Zhou et al. 2016). However, the mechanism behind the association of seed color with other traits, including protein, oil and fiber contents, is rarely reported. Badani et al. (2006) reported that a major QTL for seed color detected on C08 was colocalized with a major QTL controlling acid detergent fiber (ADF). Similarly, a major QTL located on homologous chromosome A09 was also reported to affect seed color and fiber content simultaneously (Liu et al. 2012a, 2013; Stein et al. 2013). Due to the complexity of the *B. napus* genome (Chalhoub et al. 2014) and yellow-seed trait (YST), which is sensitive to harvesting time, temperature and fertilizers (Jiang et al. 2019; Niu et al. 2020), the molecular mechanisms controlling this phenotype and its connection with other important traits need to be elucidated for further utilization.

The newly developed BSR-Seq technique, which combines bulked segregant analysis (BSA) and transcriptome sequencing, not only provides global gene expression information but can also provide genetic mapping results and has become one of the most promising methods for mapping mono- or multigenic traits (Du et al. 2017; Gu et al. 2017; Liu et al. 2012b). The combination of QTL mapping and BSR-Seq could be used to identify several differentially expressed genes within QTL intervals and refine the gene candidate search; these methods have been used to comprehensively dissect the genetic mechanism controlling the important agricultural traits (Cubillos et al. 2017; Liu et al. 2016).

In this study, the major QTL for the yellow-seed trait was identified by QTL mapping and confirmed by BSR-Seq technology, and the present results are the first to show that a yellow-seed QTL not only controlled seed color and fiber content but also controlled oil content in the seeds. Sufficiently dissecting the major QTL could help to deeply understand the regulatory mechanism controlling seed color and its association with oil and fiber biosynthesis, and breed varieties with high-quality seeds and a high oil content.

Materials and methods

Plant materials and field experiments

The KN double haploid (DH) population derived from the hybridization of yellow-seeded 'N53-2' and black-seeded 'KenC-8' (Wang et al. 2013) was employed to survey the seed color and detect QTLs for the yellow-seed trait in this study. The female parent 'N53-2' has a high oil content (approximately 50%) and a relatively low fiber component content (approximately 3.1% lignin and 4.8% cellulose), and the male parent 'KenC-8' has a low oil content (approximately 40%) and relatively high fiber component content (approximately 7.0% lignin and 6.2% cellulose).

A total of 300 DH lines from the KN population and the parents were cultivated in two experimental plots under natural growing conditions in Wuhan (WH, a semi-winter-type *B. napus* planting area), Hubei Province, and Dali (DL, a winter-type *B. napus* planting area), Shaanxi Province, for several consecutive years (Chao et al. 2017; Miao et al. 2019). The field trials were implemented using a randomized block design based on Chao et al. (2019) with three replications. Open-pollinated seeds were collected at maturity from five randomly chosen plants for each line for phenotypic analysis.

Seed color measurement and trait analysis

The same amount of seeds from each line was spread on a plastic dish and scanned in real color at 600 dpi. The yellow-seeded degree (YSD) was calculated and used to evaluate the seed color phenotype according to the method described by Fu et al. (2007).

QTL mapping

The KN high-density genetic map, constructed using the Brassica 60 K SNP Array with 3207 markers, was used for QTL mapping of the yellow-seeded phenotype (Chao et al. 2017). QTL mapping was performed using WinQTLCart 2.5 software according to Chao et al. (2017). When the significant QTLs that were identified consistently across different environments had overlapping confidence intervals (CIs), they were integrated into a consensus QTL. This process was performed by BioMercator 4.2 software with default parameters (Arcade et al. 2004). The method described by Mccouch et al. (1997) was modified and applied to the nomenclature of yellow-seeded QTLs, e.g., "*qSC-C05-1*" represents the first significant QTL identified on linkage group C05, and "*cqSC-C05-2*" represents the second consensus QTL on C05 after integration by meta-analysis. Consensus QTLs that were detected in at least two trials and had a PV explained of > 10% in each trial were considered as major QTLs.

Correlation of YST with other traits and QTL colocalization analysis

The phenotype data for OC, CC and LC were surveyed and QTL mapping for these traits was performed by Chao et al. (2017) and Miao et al. (2019). Correlations between YST with other traits were analyzed using SPSS software. The QTLs detected for YST, OC, and fiber content were aligned to the "ZS11" reference genome (http://cbi.hzau.edu.cn/

cgi-bin/rape/download_ext) based on closely linked markers for QTL colocalization analysis. Alignment was performed according to the method described by Cai et al. (2014).

BSR-Seq for seed color and differentially expressed gene (DEG) analysis

Sixteen extreme yellow- and black-seeded lines from the KN DH population were selected for BSR-Seq. At the seed-coloration stage (45–50 days after flowering), the seed coat from the 32 lines was stripped and immediately placed in liquid nitrogen. Subsequently, total RNA was extracted separately from 16 extreme yellow- and 16 black-seeded lines, and an equal amount of the RNA from each line was mixed into two RNA bulks of extreme yellow and black seeds for RNA sequencing. The resequencing results of 'N53-2' and 'KenC-8' have been deposited in the NCBI database with the SRA accession SRP156346 (Chao et al. 2017; Li et al. 2018).

Differentially expressed genes (DEGs) identification between the two bulks was performed using the calculation |log2 (fold change)|≥ 1 with a false discovery rate (FDR) < 0.05. The fragments per kilobase of exon model per million mapped reads (FPKM) value was calculated to determine the gene expression level. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed by the online software Blast2GO and KOBAS2.0 (http://kobas.cbi.pku.edu.cn/home.do), respectively.

QTL alignment to the reference genome and candidate gene analysis

QTLs identified in previous reports and in this study were aligned to the "ZS11" reference genome (http://cbi.hzau.edu.cn/cgi-bin/rape/download_ext) according to Raboanatahiry et al. (2017). The closely linked markers and their sequences acquired from corresponding papers were submitted to BLASTn (for SNP markers) or e-PCR (for SSR markers) for physical position identification and projected onto the reference genome. The genes lying in the genomic interval of the QTLs were identified as candidate genes. The interaction network was generated by the String website (https://string-db.org/) and visualized using Cytoscape software (Shannon et al. 2003).

Validating the different expression levels of DEGs using qRT-PCR

RNA samples from yellow and black seed coat bulks were used to synthesize cDNA for qRT-PCR analysis. The qRT-PCR experiment was implemented using SYBR qPCR Mix (Bio-Rad) according to the manufacturer's specification. Three technical replicates were implemented to analyze the relative expression levels. *BnActin7* was used as an internal control. The normalized expression levels were calculated according to the $-\Delta\Delta$Ct method reported by Livak and Schmittgen (2001). The specific primers for *BnActin7* and the target genes are listed in Table S1.

Result

QTL mapping for YST

The parental line N53-2 showed a distinct seed coat color compared to the black-seeded Ken-C8, and the color difference varied considerably between the two parents in different environments (Fig. 1a and Table S2). Seed color exhibited a near-normal distribution and obvious transgressive segregation in KN DH population in each environment (Fig. 1b and Table S2), and the differences in seed color of the two parents and frequency distribution in the KN DH population in the different environments suggested that seed color was also influenced by the environments.

QTL mapping was implemented for YST based on the seed color phenotypic data and KN high-density genetic map. Twenty QTLs were identified with R^2 values of

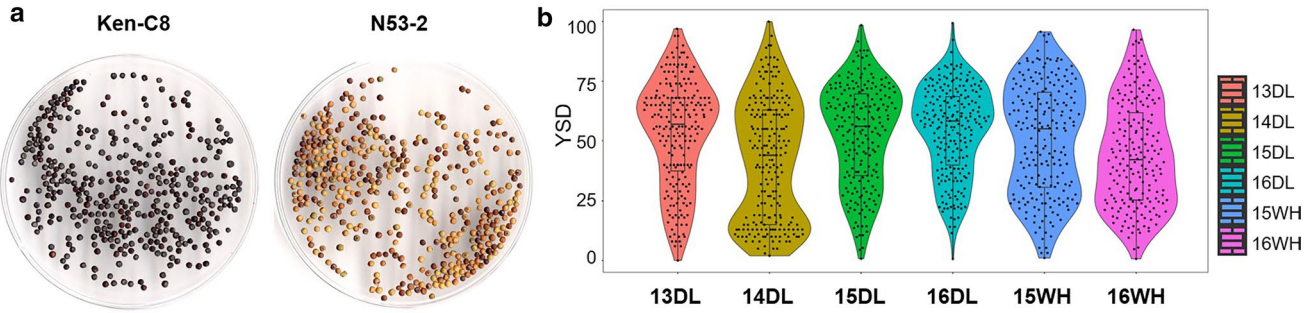

Fig. 1 Phenotypic analysis of seed color in the parents and KN double haploid population. (**a**) The seed color difference between the two parents, and (**b**) the phenotypic distribution of the yellow-seed trait in the KN double haploid population

2.60–41.11% in six environments (Fig. 2 and Table S3) and were distributed on chromosomes A09, A10, C01, C03, C05, and C08. Almost all of the QTLs showed a positive additive effect (AE), except for *cqSC-C03*. Through meta-analysis, ten consensus QTLs were obtained from twenty identified QTLs (Table 1). Four consensus QTLs distributed on A09, C05 and C08, *cqSC-A09*, *cqSC-C05-2*, *cqSC-C08-1,* and *cqSC-C08-2*, could be detected in two or more environments. The QTL *cqSC-A09* was considered a major QTL because it could be detected in six environments and explain R^2 of up to 41.11%. Through collinearity analysis between the KN high-density genetic map and the reference genome, *cqSC-A09* corresponded to the 58.17–63.18 Mb (5.01 Mb) region at the end of chromosome A09.

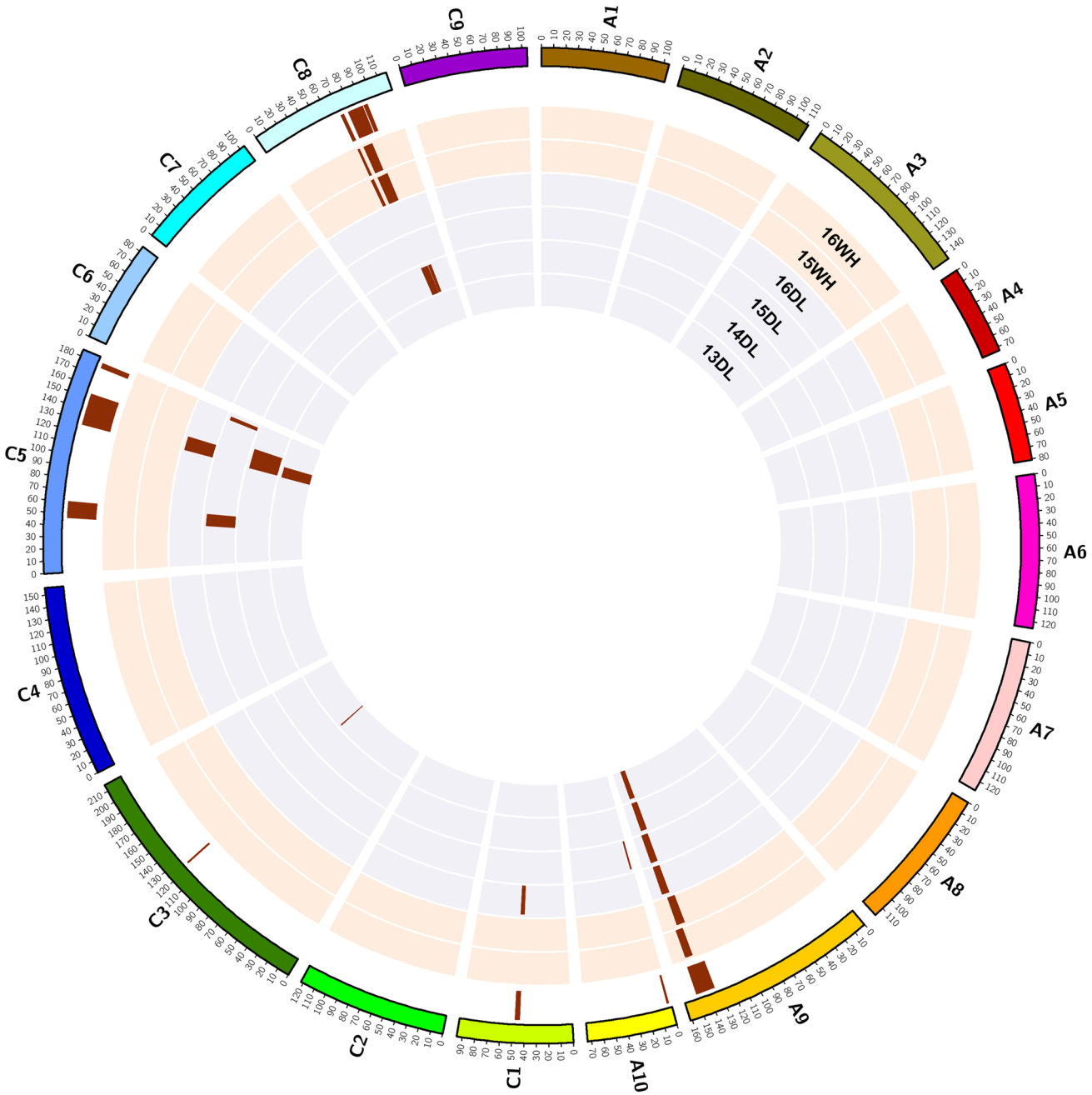

Fig. 2 Locations of QTLs mapped for the yellow-seed trait in the KN population. The 19 linkage groups are shown on the outermost circle with a scale that represents the genetic position. Six different natural environments are indicated by the background circles. The bars on the background circles represent the significant QTLs identified in the corresponding environments, and the bars near the linkage groups represent consensus QTLs

Table 1 Consensus QTL detected for the yellow-seed trait in the KN double haploid population

Consensus QTL	Chr.[a]	Marker interval	LOD	R^{2} [b]	Add[c]	Environment[d]
cqSC-A09	A09	Bn-A09-p5977032 // Bn-A09-p35691478	12.18–30.90	17.49–41.11	10.46–17.08	13DL/14DL/15DL /16DL/15WH/1 6WH
cqSC-A10	A10	Bn-A10-p2295027 // BnGMS9	3.02	3.89	4.74	15DL
cqSC-C01	C01	Bn-A01-p3179292 // Bn-scaff_19193_1-p1250152	3.07	4.48	3.96	16DL
cqSC-C03	C03	Bn-scaff_17298_1-p1159391 // Bn-scaff_17298_1-p1249353	4.20	4.61	−6.27	13DL
cqSC-C05-1	C05	Bn-scaff_15712_10-p598383 // Bn-scaff_22183_1-p41540	4.05	5.13	4.83	15DL
cqSC-C05-2	C05	e4m5-289 // Bn-A05-p23512396	3.01–6.38	3.86–8.93	4.06–6.98	13DL/14DL/16DL
cqSC-C05-3	C05	Bn-A05-p22320477 // Bn-A05-p21973951	2.61	3.29	3.96	15DL
cqSC-C08-1	C08	Bn-scaff_16770_1-p4204848 // e17m15-470	2.59–4.45	3.05–7.09	4.35–6.30	15WH/16WH
cqSC-C08-2	C08	Bn-scaff_16361_1-p330276 // Bn-scaff_16021_1-p585766	2.65–4.98	2.60–7.86	4.36–6.59	14DL/15WH/16WH
cqSC-C08-3	C08	e10m19-312 // Bn-scaff_16197_1-p567002	3.31	3.29	4.99	14DL

[a]Chromosome

[b]Percentage of the phenotypic variance

[c]Additive effect, the direction of additive effect is from the allele of 'N53-2', while a negative additive effect indicates an allelic contribution from 'Ken-C8'

[d]Environments that QTL could be detected. WH represents Wuhan in Hubei Province (a *B. napus* semi-winter-type planting area) and DL represents Dali in Shaanxi Province (a winter-type *B. napus* planting area). Combination of numbers and locations represents plant environment, for example, "13DL" represents the QTL that could be detected in the population planted in WH in 2013 year

BSR analysis for seed color

Two extreme bulks were prepared using equal amounts of RNA from the seed coats of lines with extreme seed color phenotypes, yellow (YSD: 68–100) and black (YSD: 2–16). Total RNA pools from the two extreme bulks were sequenced based on next-generation high-throughput sequencing technology, and 20.41 Gb and 21.64 Gb of clean bases were obtained for the yellow and black bulks after removing the low-quality data. A total of 108.41 Mb and 113.86 Mb of clean reads from the two extreme bulks were mapped to the 'ZS11' reference genome and accounted for 79.66% and 78.91% of the total clean reads, respectively. The high-quality sequencing data ensured that the subsequent analysis was accurate (Table S4). The resequencing results of the two parents were used to obtain credible SNPs for calculating the SNP-index for the two extreme bulks. Associated genomic regions were surveyed by the Δ(SNP-index), which was calculated by subtracting the SNP-index values from the yellow bulk from those of the black bulk (Fig. 3). A significantly associated region (SAR) was detected within a 3.76 Mb genomic region (57.70–61.46 Mb) on chromosome A09 (Δ(SNP-index) > 0.5 and p-value < 0.01). By combining the QTL mapping results, we concluded that the locus *cqSC-A09*, which controls seed color, was located in the chromosomal region at 58.17–61.64 Mb (3.29 Mb) on chromosome A09.

The major YST-QTL controls both oil content and seed fiber

The correlations between YST and other traits, including OC and two fiber components, lignin content (LC) and cellulose content (CC), were analyzed using SPSS software. YST showed a significant correlation with OC, LC and CC (Table 2). The OC, LC and CC traits were evaluated for the two extreme bulks for BSR analysis, and the yellow-seed bulk showed a higher OC and lower LC and CC than the black seed bulk (Fig. 4a).

QTL mapping for seed fibers and OC has been performed on the KN DH population (Chao et al. 2017; Miao et al. 2019). Compared to the QTLs for fiber components and OC, the major YST-QTL *cqSC-A09* was colocalized with the QTLs for OC and two fiber components (Fig. 4b), *cqOC-A09* for OC (controlling OC with an AE of 0.89, and an R^2 of up to 11.98%), *cqLC-A9-1* for LC (controlling seed lignin content with an AE of -1.12, and an R^2 of up to 48.50%) and *cqCC-A9-2* for CC (controlling seed cellulose content with an AE of -0.32, and an R^2 of up to 16.21%), on chromosome A09. Significantly, all three QTLs, *cqOC-A9-3*, *cqLC-A9-1* and *cqCC-A9-2*, were major QTLs and explained the highest PV for seed OC, LC and CC, respectively. The results from QTL mapping and BSR confirmed that *cqSC-A09* was a pleiotropic QTL that increased the oil content and reduced the contents of antinutrients, flavonoids and fiber. Compared

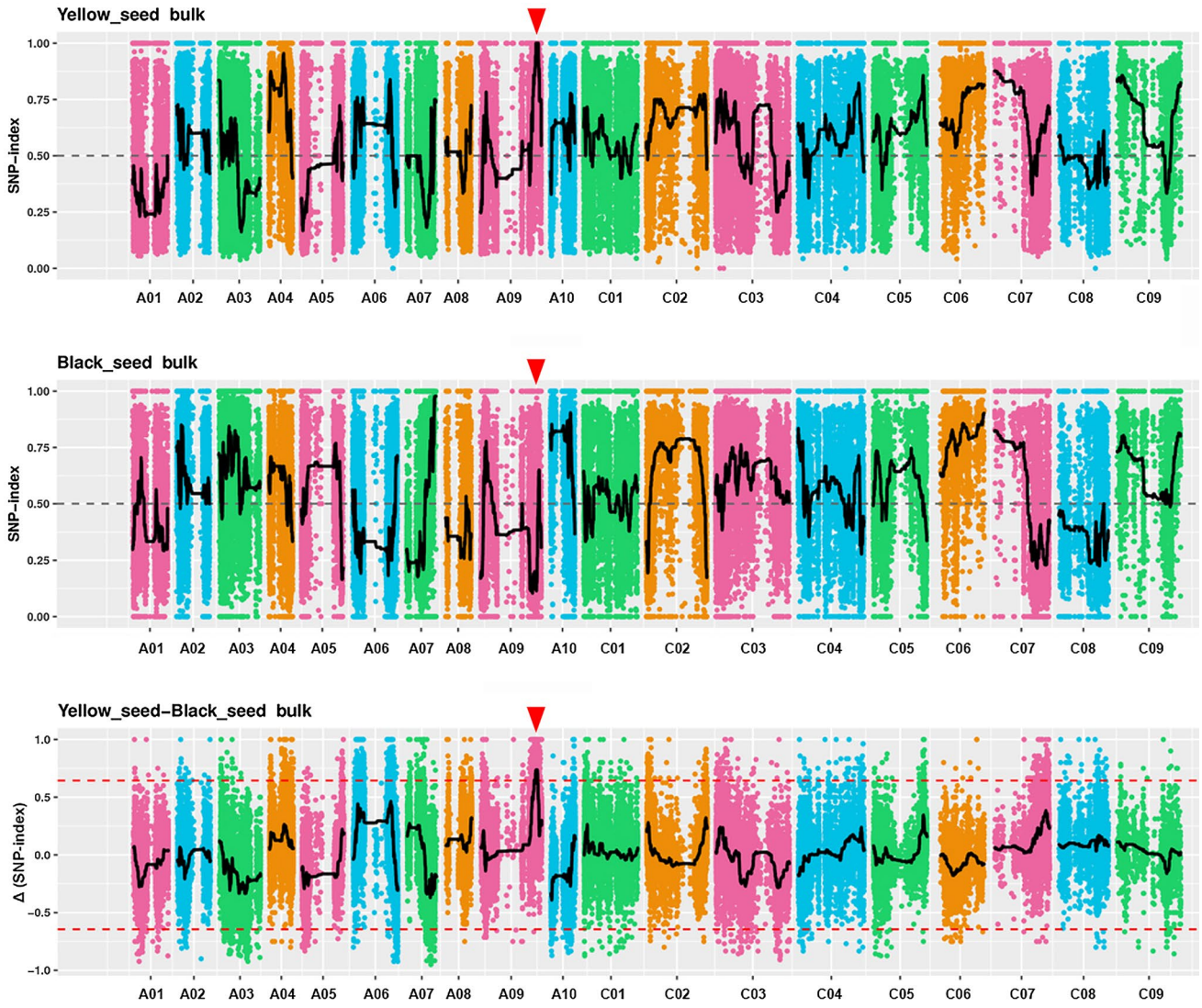

Fig. 3 Manhattan plot of the bulked segregant RNA-Seq (BSR) analysis. The red inverted triangles indicate the location of the significant locus

Table 2 Correlation between the yellow-seed trait (YST) and oil content (OC), YST and lignin content (LC), and YST and cellulose content (CC)

Trait	OC	LC	CC
YST	0.453**	−0.790**	−0.471**

**Represents significance at the level of 0.01

to the reported QTLs for seed color, fiber content and OC in previous studies, the major QTL *cqSC-A09* was found to colocalize with the major QTL detected for seed color and fiber content on chromosome A09 (Liu et al. 2012a, 2013; Stein et al. 2013; Wang et al. 2015, 2017), a major QTL detected in the TN DH population (Jiang et al. 2014) and a significantly associated locus identified by GWAS (Wang et al. 2018) for OC (Figure S1). However, there are no reports that the locus could simultaneously control oil content, seed color and fiber content.

DEG analysis between the yellow and black seed coat

To identify potential candidates controlling seed color and dissect the mechanism controlling the correlation between OC and seed color, gene expression analysis of the two bulks was performed based on RNA-Seq data from the BSR analysis. DEGs were determined by their |log2 (fold change)| value, and then the differential gene expression from RNA-Seq analysis was validated by qRT-PCR using six randomly selected DEGs (Figure S2). In total, 5643 DEGs were identified in the black bulk vs. yellow bulk, including 889

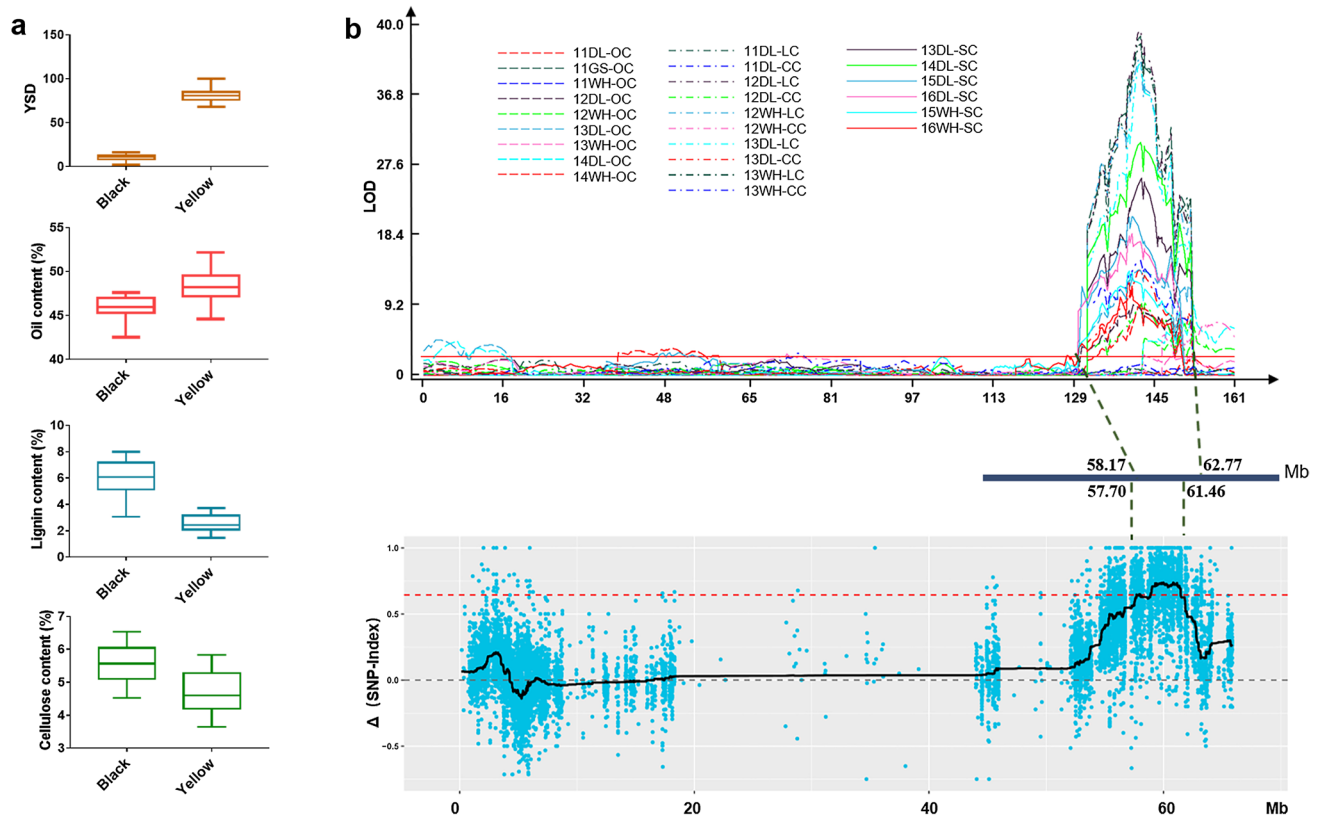

Fig. 4 The major QTL *cqSC-A09* simultaneously controls oil content and fiber components. (**a**) Seed color (SC), oil content (OC), lignin content (LC) and cellulose content (CC) of two extreme bulks for bulked segregant RNA-Seq (BSR) analysis. Boxes represent the phenotypic variation of the 16 extreme lines used to construct the two extreme bulks. (**b**) The result of QTL scanning for SC, OC, LC, and CC on A09. QTL scanning for seed color, OC, LC, and CC in different environments is shown by the colored curves. The Δ (SNP-index) plot from the SC-BSR analysis is shown below. The associated regions with statistical significance (red dotted line, $P < 0.01$) and the region corresponding to the CI of the major QTL *cqSC-A09* are connected to the bar in the middle representing part of A09 chromosome. The upper and lower X-axes mark the A09 linkage group and A09 chromosome, respectively

upregulated and 4754 downregulated genes (Fig. 5a). In addition, 571 differentially expressed transcription factors were detected, including numerous MYB, NAC, bHLH and WRKY family members (Fig. 5b).

GO and KEGG analyses of the DEGs were used to explore the potential metabolic mechanism leading to the difference in seed coat color. The changed biological processes were identified by subjecting DEGs to GO enrichment analysis. Secondary metabolic process, phenylpropanoid biosynthetic process, and phenylpropanoid metabolic process were included in the top 20 significantly enriched GO in terms of the biological process category (Fig. 5c), secondary metabolite biosynthetic process. In addition, the lipid metabolic process and fatty acid metabolic process involved in oil accumulation were also significantly enriched. To further determine the metabolic pathways associated with seed coat color, KEGG enrichment analysis was performed (Fig. 5d). Biosynthesis of secondary metabolites, flavonoid biosynthesis, phenylpropanoid biosynthesis, and phenylalanine metabolism were found in the top 20 significantly enriched pathways. In addition, cutin, suberine and wax biosynthesis, fatty acid biosynthesis, fatty acid metabolism, and α-linolenic acid metabolism pathways were significantly enriched. The results indicated that flavonoid metabolism was associated with lipid metabolism in the seed coat.

Candidate gene identification

The 3.29 Mb mapping interval on chromosome A09 contained 648 genes, 55 of which carried a significant SNP associated with seed color within their coding region (Table S5), and 65 genes showed expression differences between yellow and black bulks (Table S6), including seven transcription factors (*BnaA09. CMTA3*, *BnaA09. JAZ5*, *BnaA09. LRL1, BnaA09. MYB47*, *BnaA09G0632400ZS*, *BnaA09. NAC038,* and *BnaA09. JAZ1*). In addition, ten genes with significantly associated SNPs showed a significant expression difference (Table S6). The TFs *BnaA09. MYB47* and *BnaA09.LRL1* belongs to the R2R3-MYB and bHLH transcript factor families, respectively, which play important roles in flavonoid

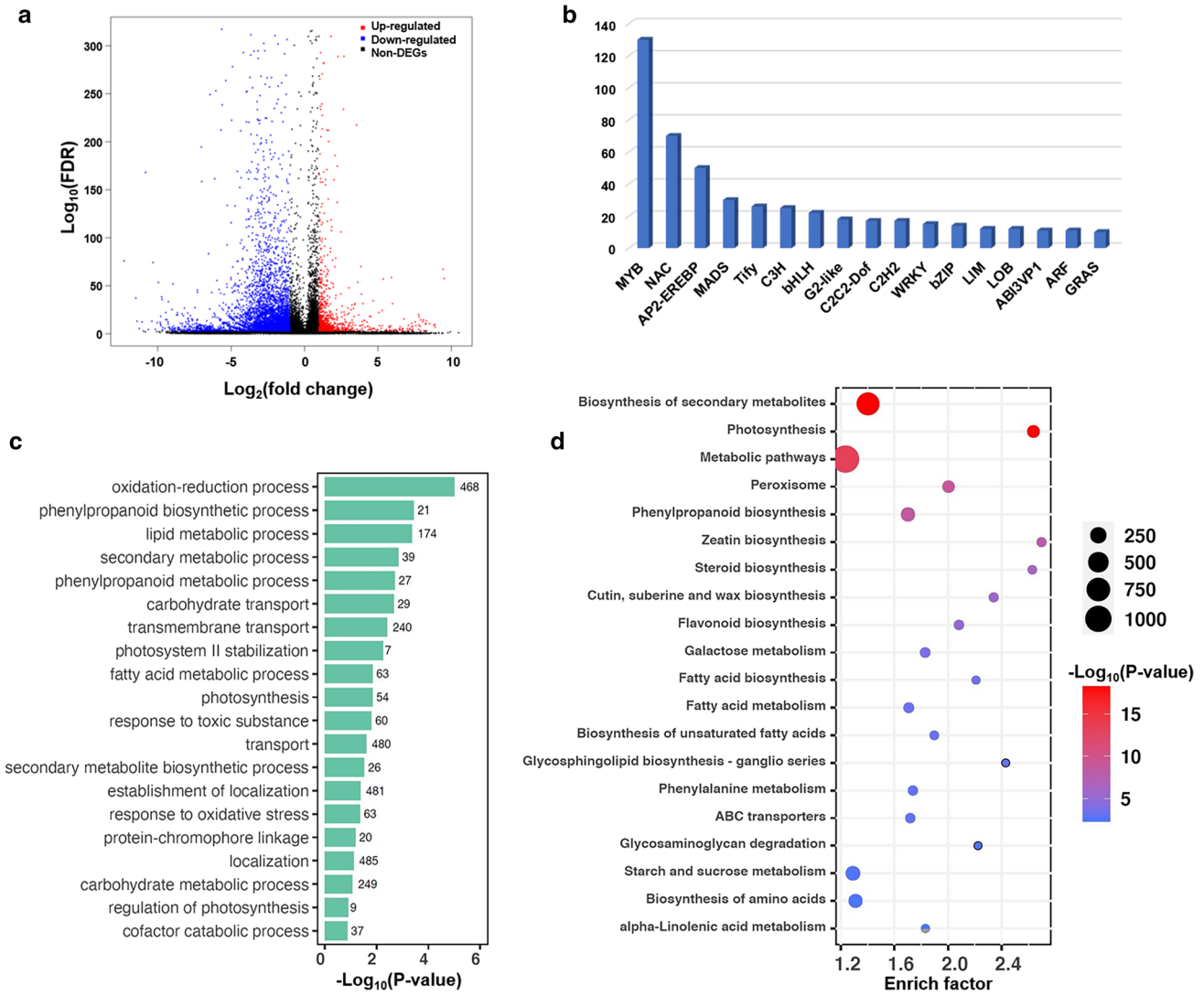

Fig. 5 Differentially expressed gene (DEG) analysis between the yellow and black seed extreme bulks. (**a**) Position distribution of DEGs. (**b**) The categories of TFs show expression differences. (**c**) and (**d**) show the top 20 significantly enriched GO biologic processes and KEGG pathways, respectively

biosynthesis, thus, these TFs may be important candidates. *BnaA09. JAZ1*, orthologous to the *Arabidopsis JAZ1* gene involved in jasmonate signaling (Huang et al. 2018), and carried three significantly associated SNPs (including a nonsynonymous coding mutation) and showed a significant expression difference.

To further and more comprehensively screen for key candidate genes, an interaction network was constructed based on the candidate genes carrying significantly associated SNPs and/or showing significant expression differences in the 3.29 Mb mapping interval on chromosome A09 and all differentially expressed genes enriched in the terms lipid metabolism, flavonoid biosynthesis and phenylpropanoid biosynthesis process (Fig. 6). Sixteen genes acted as a bridge to connect lipid metabolism and flavonoid and phenylpropanoid pathways. In the network, the *JAZ1* gene affects lipid metabolism and flavonoid metabolism by interacting with the genes involved in the corresponding biological processes. *BnaA09. JAZ1* was located near the peak of *cqSC-A09*, carried three significantly associated SNPs (including a nonsynonymous coding mutation) and showed a significant expression difference between the extreme yellow and black bulks (Table S5 and Table S6); hence, it was considered a valuable candidate. *BnaA09. GH3.3*, orthologous to the *Arabidopsis GH3.3* gene that encodes an IAA-amido synthase, was an important candidate because of its connection to both lipid metabolism processes and flavonoid metabolism, and the BSR results showed that *BnaA09. GH3.3* has a significant expression difference and three significant SNPs associated with seed color (Table S5

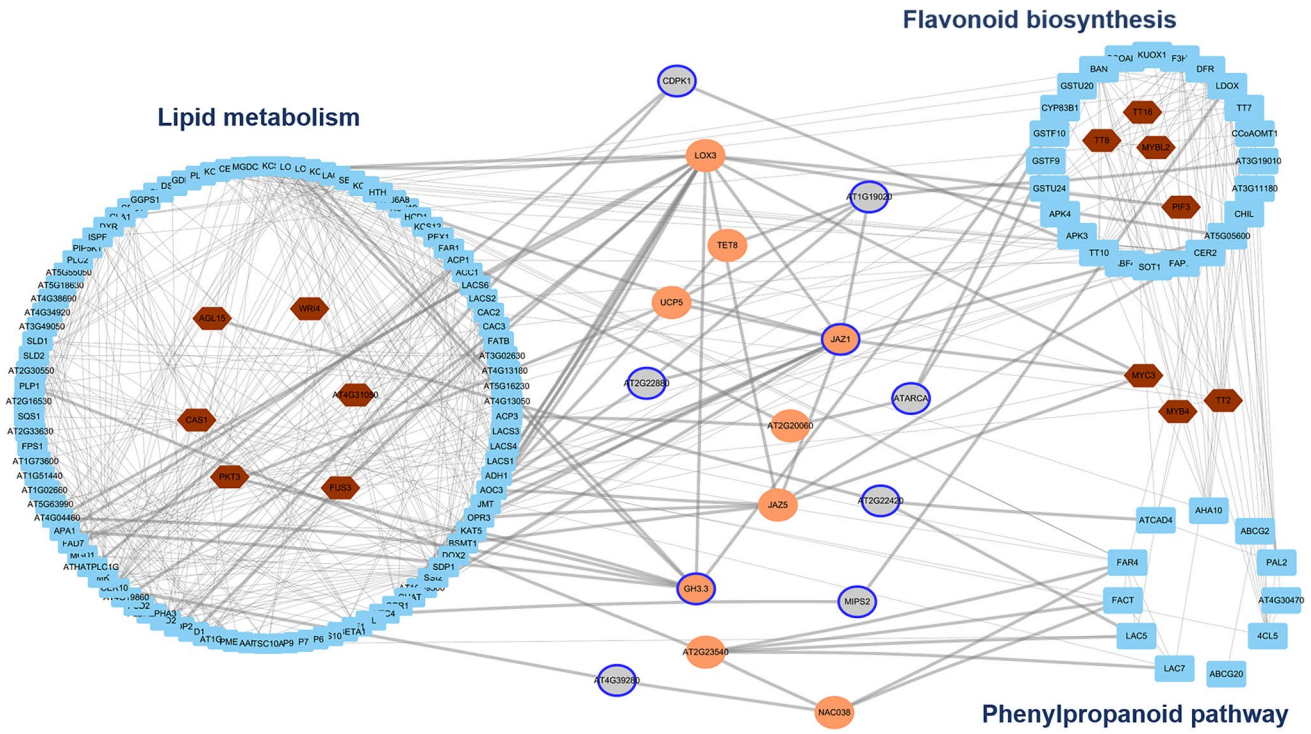

Fig. 6 The interaction network constructed based on differentially expressed genes (DEGs) and candidate genes. DEGs involved in lipid metabolism, flavonoid biosynthesis and phenylpropanoid pathway are grouped into circles, and TFs are represented by an oxblood hexagon. The candidate genes within the interval of *cqSC-A09* are represented by ellipses and are shown in the middle; those with differential expression in the two bulks are painted orange, and those with significantly associated SNPs are edged in blue

and Table S6). In addition, *BnaA09. LOX3*, orthologous to the *Arabidopsis* gene *LOX3*, which encodes a lipoxygenase catalyzing the oxygenation of fatty acids, interacts with *phytochrome-interacting factor 3* (*PIF3*), which positively regulates anthocyanin metabolism (Shin et al. 2007). *BnaA09. LOX3* was located near the peak of *cqSC-A09* and showed significantly different expression between the two bulks; it was also considered an important candidate gene.

Discussion

Seed color is an important agronomic trait related to seed quality because pigment deposits interfere with industrial processing in *B. napus*. In recent decades, many attempts have been made to discover genes controlling yellow seeds and conduct parallel genetic research for breeding in rapeseed. However, currently, few genes controlling seed color have been cloned and appropriately applied to production, which might be because the yellow-seed trait is influenced by several factors with minor effects and environment × genotype interactions (G × E). In this study, six of the 10 QTLs detected for YST were identified in only a single environment, which indicated that they could influence seed color by interacting with the environment to a certain extent. Three QTLs, *cqSC-C08-1* (detected in two environments), *cqSC-C05-2* and *cqSC-C08-2* (detected in three environments), showed relatively stable effects on seed color, which suggested that these QTLs were affected by the environment to lesser extent. Although it is difficult to utilize and fine-map environment-specific QTLs under G × E, such findings would be helpful to dissect the complex genetic mechanism controlling seed color in rapeseed.

Although influenced by the environment, YST has been reported to be controlled mainly by a few major QTLs on chromosome A09 (Liu et al. 2012a, 2013; Stein et al. 2013; Wang et al. 2017) and C08 (Badani et al. 2006; Wang et al. 2017). In this study, the QTL *cqSC-C08-1* identified on C08 colocalized with the major QTL with an R^2 of 51.6% that Badani et al. (2006) identified by linkage mapping and the significantly associated locus Wang et al. (2017) identified by GWAS. The major QTL *cqSC-A09* with the highest R^2 (41.11%) in this study was found to colocalize with the major QTL on chromosome A09 detected in several other studies (Liu et al. 2012a, 2013; Stein et al. 2013; Wang et al. 2017) (Figure S1). Notably, the significant region containing the major yellow-seed QTL on C08 was homologous to the region containing the major YST-QTL on A09, as previously demonstrated (Liu et al. 2012a; Wang et al. 2017). The major QTL *cqSC-A09*, which is more stable and has a large

effect, is of great value to yellow-seeded rapeseed breeding by molecular marker-assisted selection and has attracted much attention.

Yellow-seeded rapeseed has a lower pigment content, lower fiber content, and higher oil content than black-seeded varieties, which has been widely recognized. In this study, we found the same correlation between the seed color and OC and identified a major QTL, *cqSC-A09*, by QTL mapping and BSR that not only controls YST with positive AE but also affects oil content with positive AE and a relatively large R^2 and fiber (cellulose and lignin) content with negative AE and a large R^2. These results indicated that the advantageous allele of the major QTL reduced pigment accumulation, increased oil content and decreased the fiber content in seeds. In previous studies, *cqSC-A09* was found to be colocalized with a seed fiber QTL (Badani et al. 2006; Liu et al. 2012a; Stein et al. 2013), but was never reported to be related to OC, and we first uncovered that *cqSC-A09* simultaneously controlled seed color and fiber and oil contents herein. Behnke et al. (2018) reported a major pleiotropic QTL on chromosome C05 that reduced acid detergent lignin content and increased oil and protein contents in rapeseed but did not affect seed color. The discovery that *cqSC-A09* simultaneously controlled YST and OC partially explained why yellow-seed color could be used as a visible phenotypic marker for high OC selection. In addition, *cqSC-A09* colocalized with a major QTL detected in the TN DH population (Jiang et al. 2014) and a significantly associated locus identified by GWAS (Wang et al. 2018) (Figure S1). These findings suggested that the yellow-seed QTL *cqSC-A09* is stable and reliable in affecting oil and fiber contents, and would have great application value in developing high OC varieties with high seed quality.

The seed coat color of rapeseed is mainly determined by the content of the phenolic compounds cyanidin and procyanidins (Akhov et al. 2009; Auger et al. 2010), which are synthesized as end products of the flavonoid biosynthesis pathway. Phenolic compounds were correlated with lignin through the phenylpropanoid metabolism, flavonoid biosynthesis and phenylalanine pathways via common substrates, such as coumaroyl CoA and caffeoyl CoA (Lepiniec et al. 2006; Mittasch et al. 2013). However, the reasons for the correlation between flavonoid and oil contents remain unclear, although several studies have provided many clues. For example, Xuan et al. (2018) reported that *AtTT4* regulates carbon source redistribution, which affects fatty acid biosynthesis by mediating flavonoids to regulate WRI1 and auxin transport (Xuan et al., 2018). Additionally, the key regulatory factors that regulate flavonoid biosynthetic pathways, such as TT2 and TT8, inhibit the accumulation of fatty acids by targeting TFs related to lipid metabolism in seeds (such as FUS3, LEC1 and LEC2) (Chen et al. 2014; Wang et al. 2014). In addition, the *B. napus* mutants *tt2* and *tt8* created by CRISPR/Cas9 recently showed yellow-seed color and higher OC (Xie et al. 2020; Zhai et al. 2020). The pleiotropic major QTL *cqSC-A09* identified herein may provide new insights into the correlations between flavonoid and oil metabolism. Through the analysis of DEGs identified between extreme yellow and black seed bulks from BSR analyses, the enrichment of phenylpropanoid metabolic, phenylpropanoid biosynthetic and lipid metabolic processes indicated that flavonoid pathways and oil biosynthesis pathways were simultaneously regulated. A mature seed comprises the seed coat, endosperm and embryo in *Arabidopsis* and *B. napus*. The endosperm supplies nutrients to the embryo and degrades into a monolayer in mature seeds, and the embryo is the major organ for the synthesis and accumulation of storage compounds (Buer and Djordjevic 2009; Stone et al. 2008). Unlike the embryo and endosperm, which originated from both parents, the seed coat has a maternal origin (Debeaujon et al. 2000). Sugars are first delivered to the maternal seed coat via the funicular phloem, which is symplistically connected to the outer integument and then reaches the embryo (Chen et al. 2015; Xuan et al. 2018). Rhamnose and glucose are substrates for flavonoid synthesis, and rhamnose is also a substrate for mucilage, which is mainly composed of cell wall polysaccharides and is deposited in the outer integument layer attached to the seed coat (Western et al. 2000). More sugars are intercepted to synthesize flavonols and PAs in the seed coats of dark-colored seeds (starch and sucrose metabolism and flavonoid related pathways were enriched for DEGs between the yellow and black seed coat bulks, as shown in Fig. 5d), reducing the flow to the embryo for matter storage, which might be one of the reasons for the correlation between the seed color and OC.

A knockout mutation in the *CCR1* gene that participated in the lignin biosynthesis was considered to explain the major QTL for seed color and lignin content (Liu et al. 2012a); however, Stein et al. (2013) thought that *CAD2* was also the cause of the major QTL for both fiber content and seed color. In this study, *CCR1* and *CAD2* were located within the interval of QTL *cqSC-A09*, but no sequence variation or expression difference was found between the two parents. In addition, there is no evidence that the variation in *CCR1* and *CAD2* resulted in changes in the oil and cellulose contents. Therefore, there may be other causal genes underlying the major QTL. In this study, some important candidates were identified by combining sequence variation annotation, expression differences and an interaction network constructed with enriched DEGs related to lipid, flavonoid and phenylpropanoid metabolism and genes within QTL intervals. *BnaA09. JAZ1, BnaA09. LOX3* and *BnaA09. GH3.3* that was identified to affect lipid and flavonoid metabolism were considered as the most important candidates underlying the QTL *cqSC-A09*. However, the reason for the link between seed color and oil and fiber contents needs to be determined

by further fine mapping to clone the causal gene, which could promote the locus utility for breeding new varieties.

Conclusion

Yellow-seeded *B. napus* generally has a high oil content and high seed quality with few pigments and a low fiber content; however, the genetic mechanism behind this trait remains unclear. A major yellow-seed QTL on chromosome A09 was identified by QTL mapping and BSR in this study. This QTL not only controlled seed color but also affected seed oil and fiber contents with a large effect, and the advantageous allele increased the oil content and reduced the pigment and fiber content. This is the first QTL that was reported to control seed color, oil content and fiber content simultaneously with a large effect, and it may be of great application value for breeding high oil varieties with high seed quality. The related candidate genes were identified by combining sequence variation annotation, expression differences and an interaction network. This study provides a theoretical basis for the application of yellow-seeded rapeseed resources for breeding high oil varieties.

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Author's contribution statement HC carried out QTL mapping and BSR analysis and wrote the manuscript. LG, WZ and HL participated in the field experiment and surveyed and analyzed the phenotypic data. ML designed the overall study and provided guidelines for writing the paper.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards The authors declare that the experiments comply with the current laws of the country in which they were performed.

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