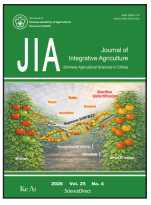
Journal of Integrative Agriculture 2026, 25(4): 1373–1383

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

High-resolution mapping through whole-genome resequencing identifies two novel QTLs controlling oil content in peanut

Nian Liu, Huaiyong Luo, Li Huang, Xiaojing Zhou, Weigang Chen, Bei Wu, Jianbin Guo, Dongxin Huai, Yuning Chen, Yong Lei, Boshou Liao, Huifang Jiang#

Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture and Rural Affairs/Oil Crops Research Institute, Chinese Academy of Agricultural Sciences, Wuhan 430062, China

Highlights

- Two novel stable QTLs with two candidate genes each were identified for oil content.
- Markers developed from two QTLs were verified to increase 1.6% of peanut oil content.

Abstract

Increasing the oil content is a key objective in peanut breeding programs. Accurate identification of quantitative trait loci (QTLs) with linked markers for oil content can facilitate marker-assisted selection for high-oil breeding. In this study, a high-density bin map was constructed by resequencing a recombinant inbred line (RIL) population (ZH16×J11) consisting of 295 lines. The bin map contained 4,212 loci and had a total length of 1,162.3 cM. Ten QTLs for oil content were identified in six linkage groups. Notably, two of these QTLs, *qOCB03.1* and *qOCB06.1*, were consistently detected in a minimum of three environments and explained up to 13.62% of the phenotypic variation. They have not been reported in previous studies and thus are novel QTLs. The combination of favorable alleles from *qOCB03.1* and *qOCB06* in the RIL population could increase oil content across multiple environments from 1.50 to 2.46%. Two insertions/deletions (InDels) markers linked to *qOCB03.1* and *qOCB06.1* were developed, and their association with oil content was validated in another RIL population (ZH10×ICG12625) with diverse phenotypes. In addition, the high-resolution map allowed for the precise positioning of *qOCB03.1* and *qOCB06.1* within a 1.77 Mb interval on chromosome B03 and a 1.51 Mb interval on chromosome B06, respectively. The annotation of genomic variants, analysis of transcriptome sequencing, and evaluation of the allelic effects in 292 peanut varieties revealed two candidate genes associated with oil content for each of the two QTLs. The candidate genes identified in this study can enable the map-based cloning of key genes controlling oil content in peanut. Furthermore, these novel and stable QTLs and their tightly linked markers are valuable for marker-assisted breeding for greater oil content in peanut.

Keywords: peanut, oil content, QTL mapping, whole genome resequencing

1. Introduction

The peanut (*Arachis hypogaea* L.) is an economically important oilseed crop worldwide. It is cultivated on over 32.7 million hectares of land, resulting in an annual production of approximately 53.9 million tons (FAOSTAT 2021). Globally, over 50% of peanut production is designated for oil extraction, with the remainder consumed as raw or processed food. Notably, a 1% increase in the oil content of peanuts can lead to a substantial 7% boost in economic benefits for both farmers and oil processing units (Shasidhar *et al.* 2017). Furthermore, the extensive variation in oil content observed in peanut germplasm can greatly contribute to the breeding of cultivars with high oil content (Yol *et al.* 2017). Thus, enhancing peanut oil content is a primary, long-term goal of peanut breeding programs.

The peanut oil content is a polygenic trait significantly impacted by environmental conditions (Baring *et al.* 2013; Wilson *et al.* 2013). Conventional breeding methods are known to be inefficient and time-consuming for enhancing oil content in crops. However, marker-assisted selection

Received 8 April 2024; Received in revised form 4 June 2024; Accepted 1 July 2024; Available online 30 August 2024
Nian Liu, E-mail: lnian0531@163.com; #Correspondence Huifang Jiang, Tel: +86-28-87283955, E-mail: peanutlab@oilcrops.cn

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2024.08.028

(MAS) has emerged as a more efficient and targeted approach for the genetic improvement of various crops, including peanut (Janila *et al.* 2016; Varshney 2016; Pandey *et al.* 2020). Before deploying MAS in breeding programs for high oil content, well-validated genomic loci with linked markers must be identified. The biosynthetic pathway and the corresponding genes involved in the accumulation of oil in seeds have been extensively investigated in *Arabidopsis* (Beisson 2003; Manan *et al.* 2017). However, the genetic variation of functional genes involved in oil biosynthesis in peanut remains understudied compared to other oil crops, such as soybean. The identification of favorable alleles and further fine mapping of high-oil-content related genes in peanut would provide valuable genetic resources for marker-assisted breeding.

To achieve the MAS breeding objective, several previous efforts for the identification of QTLs for oil content have been reported (Sarvamangala *et al.* 2011; Pandey *et al.* 2014; Huang *et al.* 2015; Wilson *et al.* 2017). Based on the early mapping of QTLs on SSR-based genetic maps, the limited and sparse markers have significantly restricted the application of those QTLs in MAS. Precise QTL mapping with dense markers can dramatically improve the efficiency of MAS. Genomic variants, such as single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels), are pervasive and extensively distributed throughout the chromosomes. The reference genomes of diploid progenitors and cultivated peanuts have recently been released (Bertioli *et al.* 2016, 2019; Chen *et al.* 2019; Zhuang *et al.* 2019), opening up new opportunities for using genomic sequencing technologies in the development of high-density genetic maps. Several studies have reported high-resolution mapping of complex traits in peanut, including oil content (Gangurde *et al.* 2020; Liu N *et al.* 2020a; Jadhav *et al.* 2021; Jiang *et al.* 2021; Huang *et al.* 2025). For example, a double-digest restriction-site-associated DNA sequencing was performed to construct a genetic map containing 2,595 loci, which enabled the precise positioning of the major QTL for oil content (*qOCA08.1*) within a 0.8 Mb genomic interval. Sun *et al.* (2022) recently developed a high-density map with 4,561 bin markers using whole-genome resequencing. A stable QTL across multiple environments (*qA05.1*) was located within a ~1.5 Mb genomic interval, where two SNP sites were used to develop KASP markers tightly linked with the oil content.

In this study, we conducted whole-genome resequencing to genotype a population of recombinant inbred lines (RILs) to achieve the following three objectives: (1) develop a high-resolution bin map in peanut, (2) fine map stable QTLs for oil content and develop linked markers that can be implemented in MAS breeding, and (3) identify the favorable alleles of candidate genes that improve oil content in peanut.

2. Materials and methods

2.1. Plant materials

Zhonghua 16 (ZH16, zh.h7720) was the female parent of the population, developed by the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences (OCRI-CAAS) in Wuhan, Hubei Province, China. J11 (W.h3729) was the male parent introduced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India. F_1 hybrids were obtained from the cross between ZH16 and J11. A RIL population named ZJ_RIL and consisting of 295 lines was developed from the cross *via* the single seed descent method. Phenotypic data for ZJ_RIL were obtained through four generations, F_8 (2018) to F_{11} (2021).

Another RIL population, named ZI_RIL and consisting of 140 lines, was previously developed from a cross between Zhonghua 10 (ZH10, zh.h7714) and ICG12625 (W.h7154) (Guo *et al.* 2021), and it was used to validate the novel QTLs. The oil content of the ZI_RIL population was measured in 2019 and 2020. An additional 292 peanut varieties with varying oil content were collected from 17 provinces across China (Liu N *et al.* 2020b). The phenotypic data of these cultivars was based on the average oil content measured from 2015 to 2017.

2.2. Field trials and phenotypic evaluation

The ZJ_RIL population and its parental lines were planted in an experimental field of OCRI-CAAS, using a completely randomized block design with three replications. In each replicate, each line was planted individually in a single row with a row length of 2.50 m and row spacing of 0.30 m. Standard agricultural practices were implemented for managing the field operations.

Seed oil content was quantified using nuclear magnetic resonance (PQ001, Niumag, China). The mature seeds of 8–10 individual plants of each line were harvested, and ~10 g of mature seeds from each line were randomly selected for measurement. The average of three measurements was used to define the oil content of each line. The oil content percentage (%) was calculated by employing the previously reported formula: Oil content percentage (%)=[Oil/(100−H_2O)]×100 (Liu N *et al.* 2020a).

2.3. Statistical analysis

The data obtained from the field trials were analyzed using the IBM SPSS Statistics software (version 22; IBM, USA). The normal distributions of phenotypic values were assessed using the Shapiro-Wilk test. Student's *t*-test was used to determine significant phenotypic differences between the two groups. The broad-sense heritability of the target traits was calculated using a previously described method (Liu N *et al.* 2020a): $H^2=\sigma_g^2/(\sigma_g^2+\sigma_{g\times e}^2/r+\sigma_\varepsilon^2/rn)$.

2.4. Resequencing and identification of genomic variants

Genomic DNA was isolated from fresh leaves of the RILs and their parents using the DNeasy Plant Maxi Kit (Qiagen, Germany). After quality assessment, the high-quality DNA was fragmented into 300–500 bp fragments using sonication. These fragments were then ligated with adapters to construct the libraries with the NEBNext® Ultra™ II DNA Library Prep Kit (NEB, Ipswich, MA, USA). Finally, the libraries were subjected to sequencing on the Illumina HiSeq platform (Illumina, San Diego, CA, USA).

The collected raw data were processed using the

Trimmomatic software (Bolger *et al.* 2014) to eliminate low-quality reads and adaptor sequences. The Burrow-Wheeler Aligner (BWA) software was used to map the clean reads to the reference genome of peanut, employing the MEM algorithms (Li and Durbin 2009). The mapped reads were then sorted and used to identify genomic variants (SNPs and InDels) with the GATK pipeline (version 3.7). The variants that satisfied the following criteria were retained: 1) minor allele frequency (MAF)≥20%; (2) missing rate≤50%; (3) relative heterozygosity rate≤20%; and (4) occurrence of polymorphism between parents. The ANNOVAR software was used to determine the genomic regions where the variants occurred and predict their effects on gene function (Wang K *et al.* 2010).

2.5. Construction of a bin map and QTL mapping

A hidden Markov model was used to impute the genotypes of recombinant chromosome fragments and to convert the variants (SNPs and InDel) into bin markers (Xie *et al.* 2010). The MSTMap software was used to evaluate the recombination rate and calculate the genetic distances between bin markers using the Kosambi mapping function (Wu *et al.* 2008). The bin map and phenotypic data across four years were used to analyze the QTLs for oil content. The QTL IciMapping software, employing the inclusive composite interval mapping (ICIM) function, was applied to map the QTLs. The mapping parameters were established as follows: a 0.1 cM step, a probability threshold of 0.001, and a minimum LOD score of 2.5.

2.6. Transcriptome analysis

Total RNA was isolated from the developing seeds of ZH16 and J11 using the RNAprep Pure Plant Kit (Tiangen, Beijing, China). Construction and sequencing of 12 libraries were performed on the DNBSEQ-T7 platform at Beijing Genomics Institute (https://www.genomics.cn/). After filtering out low-quality data, the clean reads were aligned to the peanut genome (Bertioli *et al.* 2019) using the HISAT2 software with default parameters (Kim *et al.* 2015). The gene expression levels were quantified and normalized as fragments per kilobase of transcript per million mapped reads (FPKM) using the RSEM package (Li and Dewey 2011). Differentially expressed genes (DEGs) were identified using the DEGseq2 package (Wang L *et al.* 2010). DEGs were defined based on the following criteria: a minimum FPKM value of 1 in at least one sample, a fold change of at least 2, and an adjusted *P*-value of ≤0.001.

2.7. Targeted sequencing

Multiple pairs of primers were designed based on the positions of targeted genomic variants, and they were employed to amplify all target sequences simultaneously. Then, the targeted amplicons were ligated with barcodes to construct DNA libraries. Finally, the Illumina HiSeq platform (Illumina, San Diego, CA, USA) was used to perform paired-end 150 bp sequencing. After the raw data were obtained, the Trimmomatic software (Bolger *et al.* 2014) was used to filter out low-quality reads and adaptors. The clean reads were then mapped to the reference genome using the BWA software (Li and Durbin 2009). The genotypes of targeted loci were analyzed using an in-house Python script. Detailed information on the genotypes for targeted loci is listed in Appendix A.

3. Results

3.1. Construction of a high-density genetic map using whole genome resequencing

In this study, all 295 lines and the two parents (ZH16 and J11) of the ZJ_RIL population were subjected to whole-genome resequencing. The parents were sequenced at a 10× depth, while the RILs were sequenced at 2.48× to 6.83× depths, with an average sequencing depth of ~3.45×. A total of 7.64 billion clean reads with an average of 25.03 million reads per line were obtained. Over 80% of the clean reads from each line were uniquely mapped to the reference genome. A total of 306,924 genomic variations, including 282,083 SNPs and 24,841 InDels, were identified in the RIL population.

The data set for variations was converted into 4,212 recombination bins with an average bin length of 535.04 kb. A bin map consisting of 20 linkage groups (LGs) was constructed (Fig. 1-A). The total length of the genetic map was 1,162.30 cM, covering 2,520.03 Mb of the genomic regions (Table 1). The average bin interval for the map was 0.28 cM, and the fraction of large intervals (>1 cM) was less than 1.90% throughout the entire genetic map (Table 1). The number of bin markers on individual LGs ranged from 134 (B02) to 351 (A08), and the average distance between bins ranged from 0.22 to 0.38 cM. The genetic map within each bin showed good collinearity with the reference genome (Fig. 1-B).

3.2. Oil content of the RIL population in multiple environments

The seed oil contents of the two parents and the 295 RILs were measured across four years (2018–2021). The oil content percentage of the female parent (ZH16) ranged from 49.39 to 54.54%, while that of the male parent (J11) ranged from 46.89 to 51.46% (Table 2). The oil contents of the RILs displayed a normal distribution with transgressive segregation (Fig. 2), varying from 43.44 to 53.11% in 2018, from 47.04 to 57.88% in 2019, from 45.33 to 57.58% in 2020, and from 49.11 to 57.39% in 2021. The oil content was positively correlated between the different environments. Correlation coefficients between the four environments ranged from 0.427 to 0.543 at $P<0.001$ (Appendix B). The continuous distribution of the oil content values, exhibiting transgressive segregation, is shown in Fig. 2. The normality of the phenotypic data in the RIL population over four years was confirmed by conducting the Shapiro-Wilk (w) normality test (Table 2). Variance analysis indicated that genotypes, environments, and the interaction between genotypes and environments had significant effects on oil content ($P<0.001$). The broad sense heritability of the target trait was estimated to be 0.75 in the RIL population (Appendix C).

3.3. Bin mapping-based QTL analysis for oil content

We used the ICIM method of the QTL IciMapping software

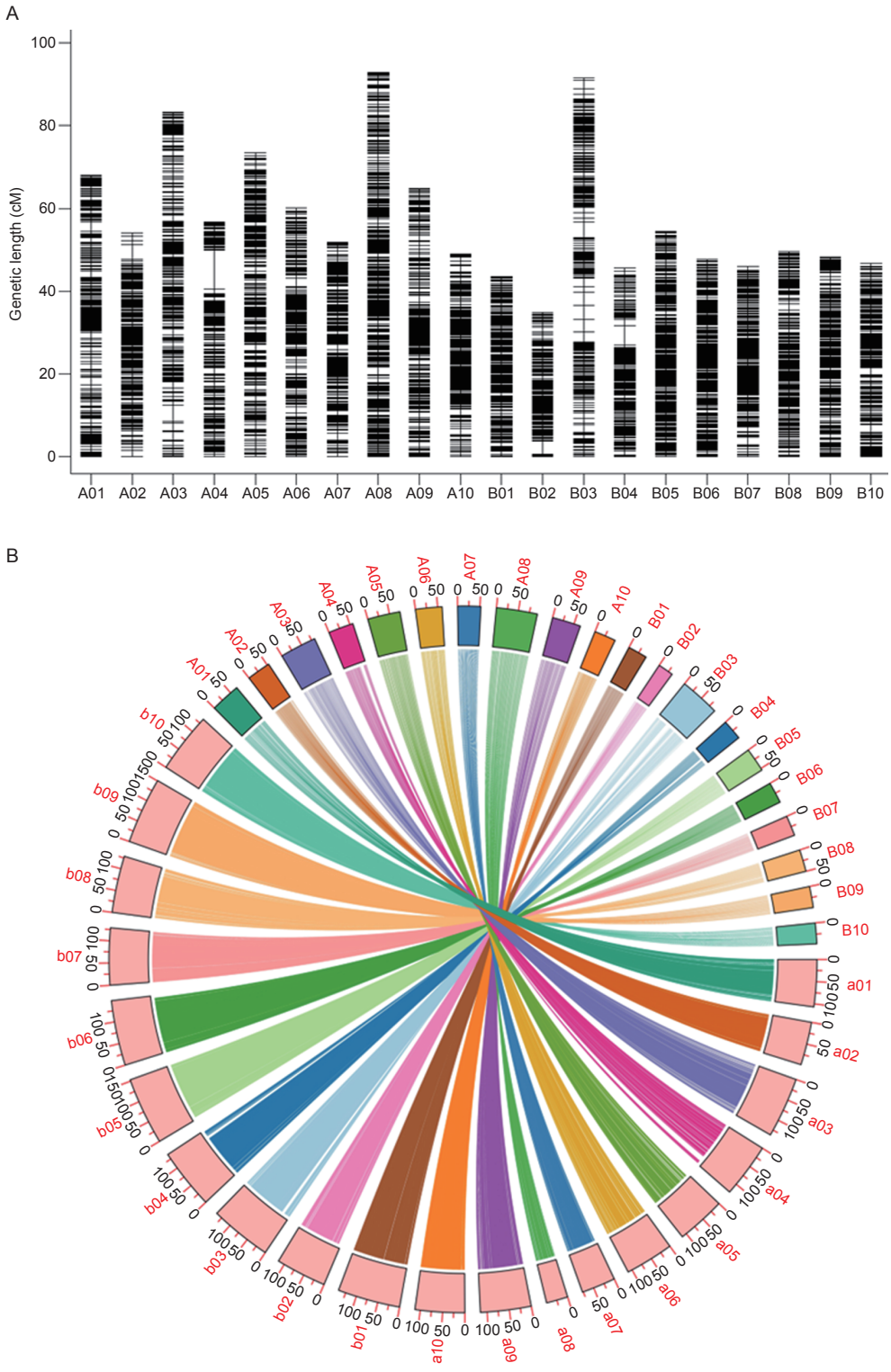


Fig. 1 Overview of the high-resolution bin map. A, the genetic map consisting of 20 linkage groups based on 4,212 bin markers. B, collinearity analysis between linkage groups and their corresponding chromosomes. a01–a10 and b01–b10 denote the chromosomes of *Arachis hypogaea*; A01–A10 and B01–B10 indicate linkage groups of the genetic map.

to conduct a genome-wide QTL analysis of the phenotypic data collected during the four years. A total of 10 QTLs with logarithm of odds (LODs) ranging from 2.89 to 10.40 were identified, with two on linkage group A05 (*qOCA05.1* and *qOCA05.2*), two on B03 (*qOCB03.1* and *qOCB03.2*), two on B06 (*qOCB06.1* and *qOCB06.2*), and one each on A08 (*qOCA08.1*), A10 (*qOCA10.1*), B08 (*qOCB08.1*), and B10 (*qOCB10.1*). These QTLs individually explained 3.01–13.62% of the phenotypic variation for oil content (Table 3), with the additive effect values ranging from –0.67 to 0.49. Five, three, three, and two QTLs were identified in the 2018–2021, respectively. Of these QTLs, *qOCB06.1* was

Table 1 Information on the high-resolution bin map

Linkage group	Length (cM)	Number of bins	Bin interval (cM)	Gaps>1 (%)[1)]	Physical length (Mb)
A01	68.07	218	0.31	3.67	112.40
A02	54.17	198	0.28	2.02	100.91
A03	83.17	237	0.35	4.22	143.10
A04	56.76	177	0.32	2.26	126.02
A05	73.52	248	0.30	2.82	115.91
A06	60.05	216	0.28	1.39	116.44
A07	51.82	197	0.26	2.03	80.62
A08	92.78	351	0.27	1.42	51.52
A09	64.75	219	0.30	2.74	120.45
A10	48.95	186	0.27	1.61	117.01
B01	43.61	188	0.23	0.00	149.29
B02	34.91	134	0.26	1.49	118.08
B03	91.48	244	0.38	6.15	145.93
B04	45.63	162	0.28	1.85	140.47
B05	54.39	245	0.22	0.00	160.00
B06	47.76	222	0.22	0.00	148.72
B07	46.03	191	0.24	1.05	134.17
B08	49.64	197	0.25	0.51	135.02
B09	48.18	199	0.24	0.00	159.32
B10	46.65	183	0.26	1.09	144.65
Whole	1,162.30	4,212	0.28	1.88	2,520.03

[1)] Percentage of gaps in which the interval between adjacent markers exceeds 1 cM.

Table 2 Phenotypic description of oil content in the RIL population[1)]

Year	P1	P2	Range (%)	Mean (%)	SD	Kurt	Skew	W (Sig)
2018	49.39	46.89	43.44–53.11	49.09	1.38	–0.21	0.80	0.99 (0.96)
2019	54.34	49.79	47.04–57.88	53.32	1.99	–0.22	–0.23	0.98 (0.13)
2020	53.46	47.72	45.33–57.58	52.38	1.94	–0.30	0.22	0.99 (0.78)
2021	54.54	51.46	49.11–57.39	53.79	1.46	–0.18	0.12	0.98 (0.66)

[1)] P1, female parent; P2, male parent; SD, standard deviation; Kurt, kurtosis; Skew, skewness; W, Shariro-Wilk statistical value; Sig significance.

consistently detected in all four years and explained up to 13.62% of the phenotypic variation. In addition, *qOCB03.1* was repeatedly detected in three of the four years (2019–2021) and explained up to 7.22% of the phenotypic variation for oil content. The favorable allele of *qOCB03.1* originated from the high-oil content parent (ZH16) with an additive effect greater than 0.47. In contrast, the favorable allele of *qOCB06.1* came from the low-oil content parent (J11) with an additive effect of up to 0.67.

The physical positions of the 10 QTLs were determined by

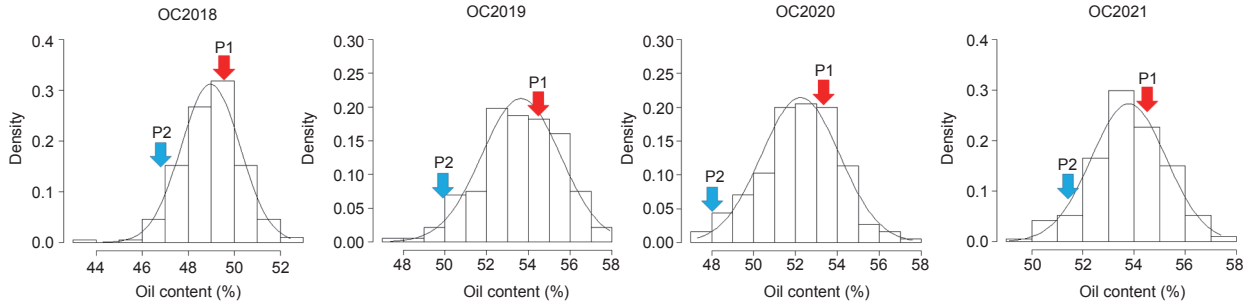


Fig. 2 Distribution of oil content (OC) in the recombinant inbred line (RIL) population in each of the four years (2018–2021). P1 and P2 represent the female and male parents, respectively.

Table 3 Locations of QTLs for oil content in the high-resolution bin map[1)]

QTL	Environment	LG	Marker interval	LOD	PVE (%)	Additive effect	Physical length (Mb) (number of genes)
qOCA05.1	OC2018	A05	c05b107–c05b108	5.96	6.37	0.35	2.57 (64)
qOCA05.2	OC2018	A05	c05b194–c05b195	4.31	4.53	–0.30	0.11 (11)
qOCA08.1	OC2018	A08	c08b173–c08b174	2.89	3.01	–0.24	0.99 (55)
qOCA10.1	OC2020	A10	c10b173–c10b174	2.55	3.04	0.35	3.07 (91)
qOCB03.1	OC2019	B03	c13b082–c13b084	4.30	5.52	0.47	1.77 (99)
	OC2020			4.90	6.47	0.49	
	OC2021			5.71	7.22	0.47	
qOCB03.2	OC2018	B03	c13b149–c13b150	6.42	6.87	0.36	3.21 (80)
qOCB06.1	OC2018	B06	c16b073–c16b075	5.89	6.27	–0.35	1.51 (66)
	OC2019			9.00	11.12	–0.67	
	OC2020			6.43	8.55	–0.57	
	OC2021			10.40	13.62	–0.65	
qOCB06.2	OC2018	B06	c16b210–c16b211	3.05	3.20	–0.25	0.21 (12)
qOCB08.1	OC2018	B08	c18b020–c18b021	3.57	3.74	0.27	0.65 (46)
qOCB10.1	OC2019	B10	c20b099–c20b100	3.19	3.77	0.39	6.51 (224)

[1)] LG, linkage group; LOD, logarithm of odds; PVE, phenotypic variation explained.

mapping bin markers on the reference genomes of *Arachis hypogea* (Bertioli *et al.* 2019). The physical length ranged from 0.11 Mb for *qOCA05.2* to 6.51 Mb for *qOCB10.1*, and the mean distance of the QTL intervals was no more than 2 Mb (Table 3). *qOCB03.1* was located in a genomic region of 1.77 Mb between c13b082 and c13b084, which includes 99 annotated gene models. *qOCB06.1* was located in a genomic region of 1.51 Mb between c16b073 and c16b075, which includes 66 annotated gene models (Fig. 3). The two consistent QTLs, *qOCB03.1* and *qOCB06.1*, have not been reported in previous studies and are therefore considered novel.

To assess the combined effect of *qOCB03.1* and *qOCB06.1*, we analyzed the genotypes of the bin markers (c13b083 and c16b074) closest to the two QTLs. The genotypes of c13b083 and c16b074 derived from ZH16 were denoted as "AA" and "BB", and those from J11 were designated as "aa" and "bb", respectively. The two loci associated with oil content were present in four genotype combinations (AABB, aaBB, AAbb, and aabb). The oil contents in each of the four years were (49.20±1.21)%, (53.12±1.50)%, (52.52±1.98)%, and (53.68±1.60)% for the aabb genotype; (48.45±1.30)%, (51.82±1.63)%, (51.50±1.85)%, and (52.26±1.83)% for the aaBB genotype; (49.96±1.27)%, (54.13±1.25)%, (53.62±1.41)%, and (54.72±1.60)% for the AAbb genotype; and (49.06±1.31)%, (52.72±1.65)%, (52.25±1.86)%, and (53.13±2.00)% for the AABB genotype (Table 4). A multiple comparison test indicated that the AAbb genotype resulted in the highest oil content compared to the other three genotype combinations (aabb, aaBB, and AABB). Specifically, the oil contents of the AAbb genotype were 1.50, 2.31, 2.12, and 2.46% higher, respectively, in each year compared to the corresponding values of the AAbb genotype. Thus, we concluded that simultaneously harboring the two favorable alleles of *qOCB03.1* and *qOCB06.1* could improve oil content in peanut.

3.4. Validation of markers linked to *qOCB03.1* and *qOCB06.1* in the ZI_RIL population

Since *qOCB03.1* and *qOCB06.1* exhibited the largest and most stable additive effects among the 10 QTLs detected in ZJ_RIL, we attempted to develop linked markers that could detect the favorable genotypes of the two QTLs in another population (ZI_RIL), which was derived from a cross between ZH10 and ICG12625. The oil contents of the ZI_RIL population ranged from 48.83 to 59.30% in 2019 and 48.34 to 59.69% in 2020. Based on the genetic variation data between Z16 and J11, a 7 bp InDel polymorphism in the *qOCB03.1* interval and a 123 bp InDel polymorphism in the *qOCB06.1* interval were chosen to design markers InDel_B03 and InDel_B06, respectively. These two markers were polymorphic between ZH10 and ICG12625 and between ZH16 and J11 (Appendix D). A total of 140 lines in the ZI_RILs were genotyped with the InDel_B03 and InDel_B06 markers. The alleles from ZH16 and ZH10 at the *qOCB03.1* locus were designated as "AA", while those from J11 and ICG12625 were designated as "aa". For *qOCB06.1*, the alleles from ZH16 and ZH10 were designated as "BB", and those from J11 and ICG12625 were designated as "bb". The two markers produced four genotypes (aabb, aaBB, AAbb, and AABB) in

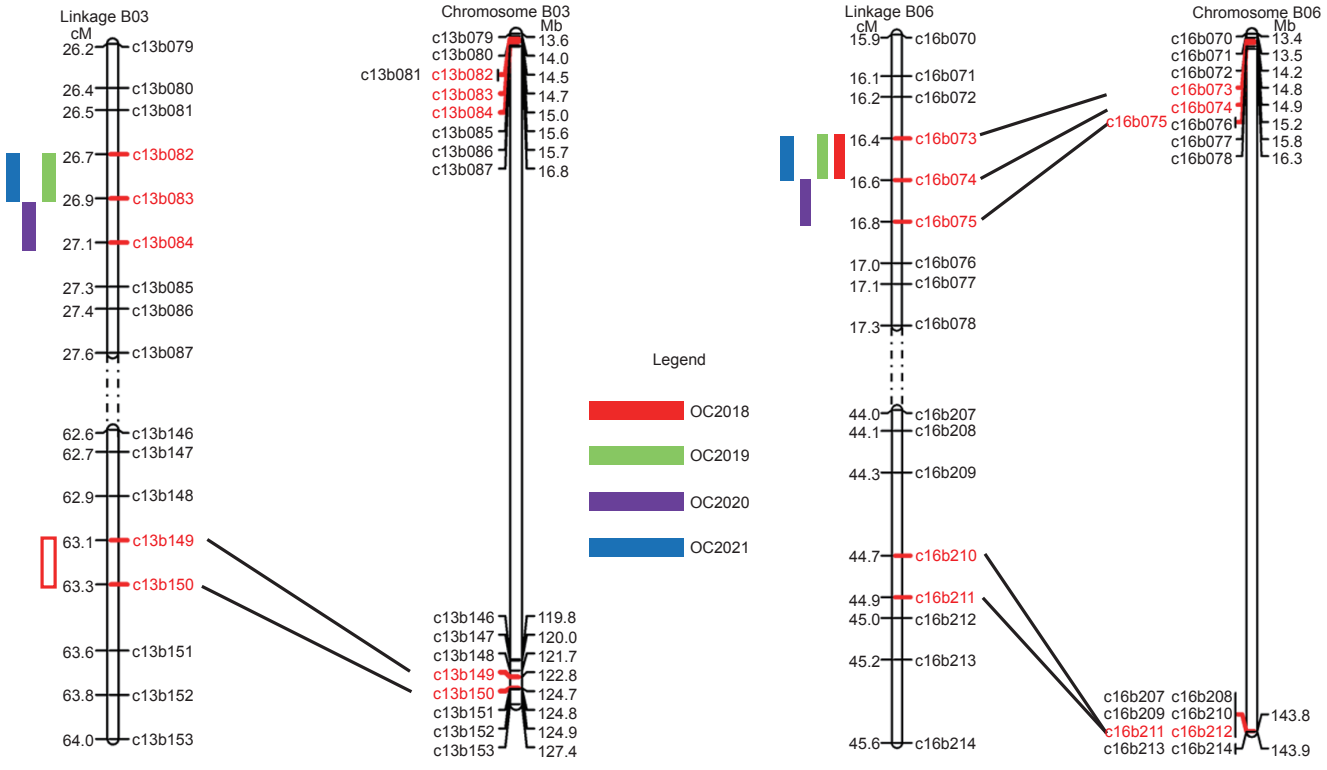


Fig. 3 Mapping the QTLs on two linkage groups (B03 and B06) and their corresponding physical map. QTLs detected across different years are denoted by the different colored boxes. The markers linked to QTLs are highlighted in red.

Table 4 Joint effect of the two QTLs (*qOCB03.1* and *qOCB06.1*) in the recombinant inbred line population from across between ZH16 and J11 (ZJ_RIL) populations

Genotype[1)]	2018	2019	2020	2021
aabb	49.20±1.21 b	53.12±1.50 b	52.52±1.98 b	53.68±1.60 b
aaBB	48.45±1.30 c	51.82±1.63 c	51.50±1.85 c	52.26±1.83 c
AAbb	49.96±1.27 a	54.13±1.25 a	53.62±1.41 a	54.72±1.60 a
AABB	49.06±1.31 b	52.72±1.65 b	52.25±1.86 b	53.13±2.00 b

[1)] AA and aa represent recombinant inbred line (RILs) with homozygous alleles in the c13b083 locus (*qOCB03.1*) from ZH16 and J11, respectively; BB and bb represent RILs with homozygous alleles in the c16b074 locus (*qOCB06.1*) from ZH16 and J11, respectively.

Values are mean±SD. Different letters within each column are significantly different at the 0.05 probability level.

the ZI_RIL population. In 2019, the average oil content was (54.06±2.14)% for the aabb genotype, (53.82±1.94)% for the aaBB genotype, (55.40±1.86)% for the AAbb genotype, and (53.90±1.85)% for the AABB genotype (Fig. 4-A). Therefore, the AAbb genotype resulted in a significantly higher oil content accumulation than the other three genotypes (aabb, aaBB, and AABB). In 2020, the average oil contents were (54.52±2.03)% for the aabb genotype, (53.97±1.78)% for the aaBB genotype, (55.49±2.03)% for the AAbb genotype, and (54.33±1.85)% for the AABB genotype (Fig. 4-B). A multiple comparison test indicated that the oil content of the AAbb genotype was significantly higher than that of the aaBB genotype. These results indicated that the two markers are potentially valuable for the MAS of peanut genotypes associated with high oil content.

3.5. Prediction of candidate genes within the two stable QTLs

The 1.77 Mb genomic region of *qOCB03.1* included 242 sequence variants between ZH16 and J11, consisting of 209 SNPs and 33 InDels (Appendix E). Among these variants, 189 were in intergenic regions, eight were in exons, 20 were in introns, one was in the 5′ UTR, one was in the 3′ UTR, and 12 were in the 2 kb-upstream or 2 kb-downstream regions of gene bodies. In addition, six SNPs resulted in nonsynonymous mutations affecting six candidate genes. The 1.51 Mb genomic region corresponding to *qOCB06.1* included 216 variants, consisting of 196 SNPs and 20 InDels (Appendix F). Among these variants, 191 were in intergenic regions, six were in exons, 10 were in introns, one was in the 5′ UTR, two were in the 3′ UTR, and six were in the 2 kb-upstream or 2 kb-downstream regions of gene bodies. Of these variants, five SNPs resulted in nonsynonymous mutations, and an InDel caused a non-frameshift insertion.

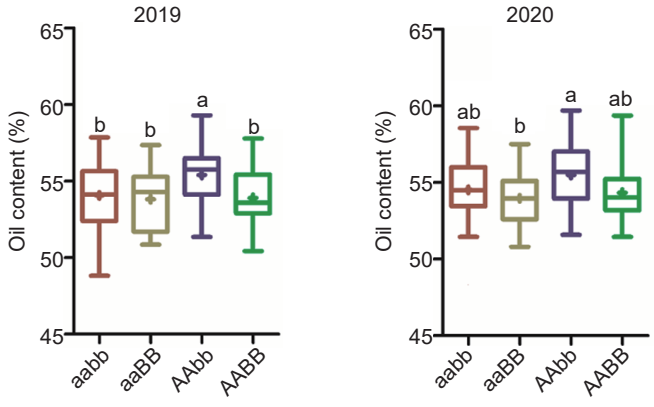


Fig. 4 Phenotypic effect of four genotype combinations of InDel_B03 and InDel_B06. Central bold line within each box represents the median value, + within each box represents the mean value. Bars mean SD. Different letters (a and b) in the plots denote values that are significantly different at the 0.05 probability level.

To detect DEGs between the two parents, four materials (JR6, JR8, ZR6, and ZR8) representing developing seeds of ZH16 and J11 at the early lipid accumulation stage (R6) and late lipid accumulation stage (R8) were selected and used to perform transcriptome sequencing. Three biological replications of each material were used to construct 12 RNA-seq libraries and 10.79–10.83 Gb of clean data per library was obtained (Appendix G). An average of 98.41% of clean reads were mapped on the reference genome (Bertioli *et al.* 2019), and 41,670 to 44,704 genes were identified in each library, resulting in a total of 67,124 genes identified among all the samples. A comparative transcriptomic analysis was performed, and 3,273 and 4,306 DEGs were identified at the R6 (JR6 *vs.* ZR6) and R8 (JR8 *vs.* ZR8) stages, respectively.

A combined analysis of the transcriptome and genomic variation data was performed to further screen candidate genes in the target regions. In the physical interval of *qOCB03.1*, 45 genes were found to be expressed in the developing seeds (R6 or R8), and 17 of 45 expressed genes harbored SNP or InDel polymorphisms between the two parents (Fig. 5-A; Appendix E). SNPs resulting in nonsynonymous mutations were found on the exons of four genes (*Arahy.D7EPYG*, *Arahy.N5TJ4W*, *Arahy.2RX12T*, and *Arahy.F4SVGY*), and they were considered as potential candidate genes. In addition, seven DEGs were identified in the region of *qOCB03.1*, but none of them exhibited genomic variation in their UTR regions or upstream (promoter) region of the genes (Fig. 5-A; Appendices E, H and I). Within the physical interval of *qOCB06.1*, 36 expressed genes were identified, eight of which exhibited genomic variations (Fig. 5-B). SNPs resulting in nonsynonymous mutations were found on the exons of *Arahy.AJ9Z2K* and *Arahy.T7DX97*, and an InDel resulting in a non-frameshift insertion was detected on the exon of *Arahy.S8NMKG* (Fig. 5-B; Appendices F, H, and I). Therefore, *Arahy.S8NMKG*, *Arahy.AJ9Z2K* and *Arahy.T7DX97* were designated as potential candidates. Furthermore, five DEGs were identified in the physical interval of *qOCB06.1*. However, only one of them (*Arahy.8LB7BZ*) harbored an SNP in its 3′ UTR region. Since the UTR is a *cis*-element regulating gene expression, *Arahy.8LB7BZ* was also designated as a candidate gene (Fig. 5-B; Appendix F). In total, eight genes were identified as candidate genes underlying the stable QTLs *qOCB03.1* (*Arahy.D7EPYG*, *Arahy.N5TJ4W*, *Arahy.2RX12T*, and *Arahy.F4SVGY*) and *qOCB06.1* (*Arahy.S8NMKG*, *Arahy.AJ9Z2K*, *Arahy.T7DX97*, and *Arahy.8LB7BZ*).

3.6. Association analysis of oil content in 292 Chinese peanut cultivars

To confirm the allelic effect of candidate genes on oil content in peanut germplasm, targeted sequencing was performed to analyze the genomic variants of the eight candidate genes in 292 Chinese peanut cultivars. Seven SNP sites (B03_14220687 for *Arahy.N5TJ4W*, B03_14939955 for *Arahy.F4SVGY*, B03_15268865 for *Arahy.D7EPYG*, B03_15638227 for *Arahy.2RX12T*, B06_14312679 for *Arahy.AJ9Z2K*, B06_14994747 for *Arahy.8LB7BZ*, and B06_15632871 for *Arahy.T7DX97*) and one InDel site (B06_14367717 for *Arahy.S8NMKG*) were selected and genotyped in Chinese peanut cultivars. The oil contents of these Chinese peanut cultivars were obtained from a previously published study and represented the average of oil content in the four environments (Liu N *et al.* 2020b). Three sites (B03_14220687, B03_14939955, and B06_14312679) were not polymorphic in the 292 Chinese peanut cultivars, and they were consistent with ZH16 (Appendix A). In the remaining five sites, allelic variations of B03_15268865, B03_15638227, B06_14994747, and B06_14367717 significantly affected oil content (Fig. 5-C; Appendix A). However, no significant difference in oil content was observed between accessions carrying the two alleles

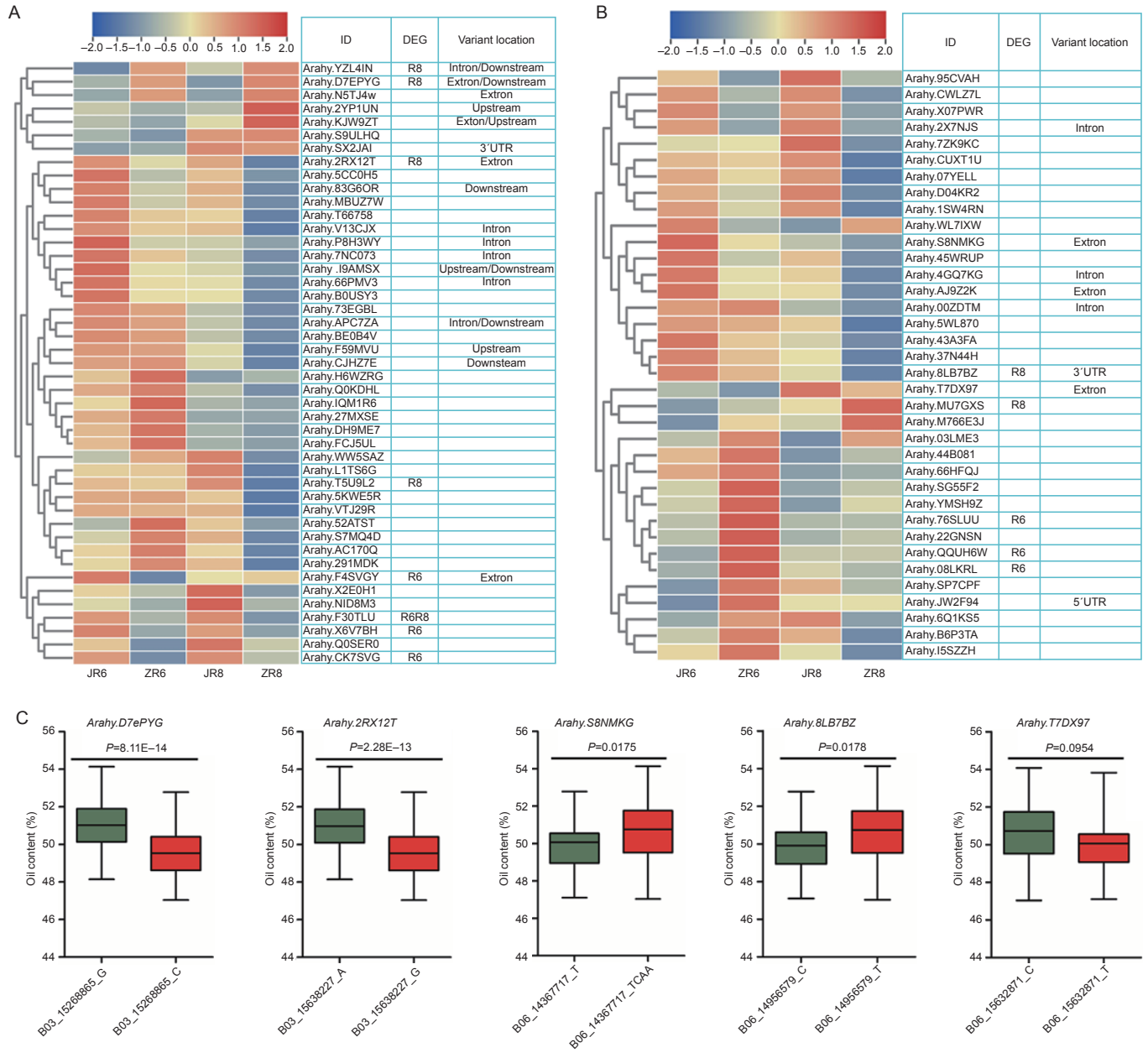


Fig. 5 Screening of candidate genes located within the intervals of the stable QTLs through transcriptome, genomic variation, and allelic effect analyses. A and B, heatmaps of candidate gene expression in developing seeds of Z16 and J11 for *qOCB03.1* (A) and *qOCB06.1* (B). Details of the differentially expressed genes (DEG) and variant locations are indicated with the gene ID. JR6 and ZR6 indicate developing seeds of J11 and ZH16 at the R6 stage, respectively. JR8 and ZR8 indicate developing seeds of J11 and ZH16 at the R8 stage, respectively. C, comparison of seed oil contents between accessions carrying different alleles of the five candidate genes. *P*-values were determined by a two-tailed Student's *t*-test. The central bold line within each box represents the median. Bars mean SD.

of B06_15632871 (*P*=0.0954). These results suggested that *Arahy.D7EPYG* and *Arahy.2RX12T* for *qOCB03.1* and *Arahy.S8NMKG* and *Arahy.8LB7BZ* for *qOCB06.1* are strong candidates affecting oil content in peanut.

4. Discussion

4.1. A high-density genetic map can increase the efficacy of QTL mapping

The complex genome and narrow genetic base of the allotetraploid peanut present challenges in constructing dense genetic maps for precise QTL mapping. The application of whole-genome resequencing is an effective strategy for genome-wide marker mining and high-resolution genotyping (Pandey *et al.* 2016). Thus, it has been widely deployed in the construction of dense genetic maps for several crops with complex genomes (Gu *et al.* 2020; Dong *et al.* 2021; Sun *et al.* 2022; Tian *et al.* 2022). In this study, whole-genome resequencing of 295 RILs and their two parents was performed to construct a high-density genetic map. The map, consisting of 4,212 bins/markers and spanning 1,162.30 cM, exhibits an average genetic distance of 0.28 cM between each marker (Table 1). To our knowledge, the density of this map matches or even surpasses those of other recently published genetic maps of peanut (Gangurde *et al.* 2020; Liu H *et al.* 2020; Sun *et al.* 2022). A genetic map with a marker interval of 0.58 cM/locus was previously constructed using 200 lines from the same RIL population (Jiang *et al.* 2021). With the addition of 195 inbred lines, a greater number of recombination events within the population were identified through the whole-genome resequencing performed in this study. The greater frequency of genomic fragment exchanges led to a notable reduction in the average genetic distance between adjacent markers and a reduced total length of the new genetic map. As a result, the density of the new linkage map developed in the current study has been markedly improved compared to the previous map. The two linkage groups (LGs) with the most notable increases in the number of loci were A05 (from 116 to 248) and A08 (from 181 to 351). Intriguingly, three additional QTLs in the newly developed map were located on LGs A05 and A08. These results suggest that the high-density genetic map developed in this study can enhance the efficacy of QTL mapping.

4.2. Mapping two novel and stable QTLs on chromosomes B03 and B06

The oil content is a crucial trait in peanut breeding, but it exhibits polygenic inheritance and is greatly affected by environmental influences. Mapping and pyramiding stable QTLs across different environments can guide MAS toward breeding for high-oil content. In this study, a total of 10 QTLs were identified and found to be widely distributed on chromosomes A05, A08, A10, B03, B06, B08, and B10 (Table 3). Several research groups have also mapped QTLs with linked markers for oil content to chromosomes A05, A08, A10, B08, and B10 (Pandey *et al.* 2014; Liu N *et al.* 2020a; Guo *et al.* 2021; Jadhav *et al.* 2021; Sun *et al.* 2022; Yang *et al.* 2023). For instance, *qOCA05.1* and *qOCA05.2* in this study were located in the 87.4–89.9 and 105.2–105.3 Mb intervals on the A05 chromosome, respectively. A comparison with the physical positions of linked markers from prior studies revealed three QTLs located on chromosome A05 in the 102.0–102.6, 99.15–108.3, and 99.2–115.1 Mb intervals (Liu N *et al.* 2020a; Guo *et al.* 2021; Jadhav *et al.* 2021). It is unclear whether the *qOCA05.1* and *qOCA05.2* identified in this study are identical to the previously detected QTLs, but the physical region of 87.4–115.1 Mb on chromosome A05 appears to be a hotspot for genes controlling oil content. Likewise, the 30.9–46.8 Mb interval on chromosome A08 is another hotspot that harbors multiple oil content QTLs identified in this and previous studies (Pandey *et al.* 2014; Liu *et al.* 2020b; Sun *et al.* 2022; Yang *et al.* 2023). Interestingly, the QTLs on chromosome B03 have not been previously reported, suggesting that the stable QTL *qOCB03.1* identified in this study is novel. Another stable QTL, *qOCB06.1*, has up to 13.62% PVE and is located in the 14.8–15.2 Mb interval on chromosome B06 (Fig. 3). Stable QTLs in this region of B06 have not been identified in earlier studies. Thus, the stable and large-effect QTL *qOCB06.1* in the present study also appears to be novel. These two novel and stable QTLs (*qOCB03.1* and *qOCB06.1*) can enable the identification of additional elite genetic materials and allelic variants for MAS breeding. To assess the phenotypic effect of QTLs across different genetic backgrounds, we developed linked markers for the two stable QTLs, and assessed them in another RIL population (ZI). A combination of two allelic variants could increase the oil content by ~1.5% within the offspring of the ZI population (Fig. 4). These findings suggest that the genotyping of these two stable QTLs with linked markers can guide MAS breeding to enhance the oil content in peanut.

4.3. Prediction of candidate genes associated with oil content

The high-resolution bin map guided the demarcation of *qOCB03.1* and *qOCB06.1* to 1.77 and 1.51 Mb intervalswhich contain only 99 and 66 genes, respectively. A three-step procedure was used to narrow down the number of candidate genes. First, genomic variants of the candidate genes between the parents (ZH16 and J11) were annotated (Appendices D and E). Second, transcriptome sequencing was performed to analyze their expression levels in developing seeds (Fig. 5-A and B). Third, their allelic effects were estimated in peanut germplasm (Fig. 5-C). Finally, two out of 99 genes (*Arahy.D7EPYG* and *Arahy.2RX12T*) were deduced to be candidate genes for *qOCB03.1*. In addition, *Arahy.S8NMKG* and *Arahy.8LB7BZ* were inferred as candidate genes from the 66 genes within the *qOCB06.1* interval. One SNP (B03_15268865_G/C) was located in the exon of *Arahy.D7EPYG*, which led to a nonsynonymous mutation (Appendix E). The gene encodes a patellin-3-like protein, which can bind phospholipids and play a role in embryonic development (Zhou *et al.* 2019). However, the potential effect of the patellin-3-like protein on lipid metabolism, either directly or indirectly, remains unclear. *Arahy.2RX12T* encodes a zinc ion binding protein that belongs to the zinc-finger TF superfamily, which includes *GmZF392* and *GmZF351*. These genes (*GmZF392* and

GmZF351) have been shown to play important roles in the regulation of oil accumulation (Li *et al.* 2017; Lu *et al.* 2021). The nonsynonymous mutation in *Arahy.2RX12T* was a result of the SNP B03_15638227_A/G (Appendix E). Within the interval of *qOCB06.1*, a three bp insertion was found in the exon of *Arahy.S8NMKG* (Appendix F). This gene encodes a member of the MYB transcription factor family, which is involved in various plant developmental processes, including lipid accumulation in the seeds (Stracke *et al.* 2001; Liu *et al.* 2014). *Arahy.8LB7BZ* encodes a peptide/nitrate transporter protein, which is involved in nitrate uptake and sugar transport (Tsay *et al.* 2007; Yang *et al.* 2022). It was differentially expressed between the two parents, and one SNP (B06_14994747_G/A) was detected on the 3′ UTR of the gene (Fig. 5-B). However, further evidence is needed to support the claim that this variant in the 3′ UTR region of *Arahy.8LB7BZ* influences transcript abundance between the parents. Despite indications from QTL mapping, genomic variant annotation, and gene expression analysis that these putative genes within the QTL intervals could be candidate genes for oil content, further studies, including functional assessment in transgenic plants, are required to verify their functions.

5. Conclusion

A high-density bin map of peanut covering 4,212 loci with an average genetic length of 0.28 cM/locus was constructed in this study. Using the high-resolution map, we identified and mapped two stable and novel QTLs (*qOCB03.1* and *qOCB06.1*) associated with seed oil content into 1.77 and 1.51 Mb intervals, respectively. Developing linked markers could enable pyramiding of the favorable alleles of two QTLs to increase the oil content by 1.5% in the test RIL population. Through a combined analysis of genomic variants and transcriptome sequencing, the number of candidate genes for *qOCB03.1* and *qOCB06.1* was narrowed down to two candidates each. The results of this study could further reveal essential genes affecting oil content and may also provide valuable loci with linked markers in MAS for breeding high seed oil content in peanut.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2022YFD1200400), the National Natural Science Foundation of China (32161143006 and 31971903), the National Peanut Industry Technology System Construction, China (CARS13), the National Crop Germplasm Resources Center, China (NCGRC-2022-036), the National Program for Crop Germplasm Protection of China (19210163), the Agricultural Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences (CAAS-ASTIP-2021-OCRI), and the Guangdong Provincial Key Research and Development Program-Modern Seed Industry, China (2022B0202060004).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT 3.5 to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2024.08.028

References

Baring M R, Wilson J N, Burow M D, Simpson C E, Ayers J L, Cason J M. 2013. Variability of total oil content in peanut across the state of texas. *Journal of Crop Improvement*, **27**, 125–136.

Beisson F, Koo A J K, Ruuska S, Schwender J, Pollard M, Thelen J J, Paddock T, Salas J J, Savage L, Milcamps A, Mhaske V B, Cho Y, Ohlrogge J B. 2003. *Arabidopsis* genes involved in acyl lipid metabolism. A 2003 Census of the candidates, a study of the distribution of expressed sequence tags in organs, and a web-based database. *Plant Physiology*, **132**, 681–697.

Bertioli D J, Cannon S B, Froenicke L, Huang G, Farmer A D, Cannon E K S, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn M C, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth C E, Umale P, Araújo A C G, *et al.* 2016. The genome sequences of *Arachis duranensis* and *Arachis ipaensis*, the diploid ancestors of cultivated peanut. *Nature Genetics*, **48**, 438–446.

Bertioli D J, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli S C M, Ren L, Farmer A D, Pandey M K, Samoluk S S, Abernathy B, Agarwal G, Ballén-Taborda C, Cameron C, Campbell J, Chavarro C, Chitikineni A, Chu Y, Dash S, *et al.* 2019. The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nature Genetics*, **51**, 877–884.

Bolger A M, Lohse M, Usadel B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, **30**, 2114–2120.

Chen X, Lu Q, Liu H, Zhang J, Hong Y, Lan H, Li H, Wang J, Liu H, Li S, Pandey M K, Zhang Z, Zhou G, Yu J, Zhang G, Yuan J, Li X, Wen S, Meng F, Yu S, *et al.* 2019. Sequencing of cultivated peanut, *Arachis hypogaea*, yields insights into genome evolution and oil improvement. *Molecular Plant*, **12**, 920–934.

Dong Z, Alam M K, Xie M, Yang L, Liu J, Helal M M U, Huang J, Cheng X, Liu Y, Tong C, Zhao C, Liu S. 2021. Mapping of a major QTL controlling plant height using a high-density genetic map and QTL-seq methods based on whole-genome resequencing in *Brassica napus*. *G3-Genes Genomes Genetics*, **11**, jkab118.

FAOSTAT. 2021. Statistical database FAOSTAT. [2023-9-12]. http://faostat3.fao.org

Gangurde S S, Wang H, Yaduru S, Pandey M K, Fountain J C, Chu Y, Isleib T, Holbrook C C, Xavier A, Culbreath A K, Ozias-Akins P, Varshney R K, Guo B. 2020. Nested-association mapping (NAM)-based genetic dissection uncovers candidate genes for seed and pod weights in peanut (*Arachis hypogaea*). *Plant Biotechnology Journal*, **18**, 1457–1471.

Gu Q, Ke H, Liu Z, Lv X, Sun Z, Zhang M, Chen L, Yang J, Zhang Y, Wu L, Li Z, Wu J, Wang G, Meng C, Zhang G, Wang X, Ma Z. 2020. A high-density genetic map and multiple environmental tests reveal novel quantitative trait loci and candidate genes for fibre quality and yield in cotton. *Theoretical and Applied Genetics*, **133**, 3395–3408.

Guo J, Liu N, Li W, Wu B, Chen H, Huang L, Chen W, Luo H, Zhou X, Jiang H. 2021. Identification of two major loci and linked marker for oil content in peanut (*Arachis hypogaea* L.). *Euphytica*, **217**, 29.

Huang B Y, Liu H, Fang Y J, Miao L J, Qin L, Sun Z Q, Qi F Y, Chen L, Zhang F Y, Li S Z, Zheng Q H, Shi L, Wu J H, Dong W Z, Zhang X Y. 2025. Identification of oil content QTL on Arahy12 and Arahy16 and development of KASP markers in cultivated peanut (*Arachis hypogaea* L.). *Journal of Integrative Agriculture*, **24**, 2096–2105.

Huang L, He H, Chen W, Ren X, Chen Y, Zhou X, Xia Y, Wang X, Jiang X, Liao B. 2015. Quantitative trait locus analysis of agronomic and quality-related traits in cultivated peanut (*Arachis hypogaea* L.). *Theoretical and Applied Genetics*, **128**, 1103–1115.

Jadhav M P, Gangurde S S, Hake A A, Yadawad A, Mahadevaiah S S, Pattanashetti S K, Gowda M V C, Shirasawa K, Varshney R K, Pandey M K, Bhat R S. 2021. Genotyping-by-sequencing based genetic mapping identified major and consistent genomic regions for productivity and quality traits in peanut. *Frontiers in Plant Science*, **12**, 668020.

Janila P, Variath M T, Pandey M K, Desmae H, Motagi B N, Okori P, Manohar S S, Rathnakumar A L, Radhakrishnan T, Liao B, Varshney R K. 2016. Genomic tools in groundnut breeding program: Status and Perspectives. *Frontiers in Plant Science*, **7**, 289.

Jiang Y, Luo H, Yu B, Ding Y, Kang Y, Huang L, Zhou X, Liu N, Chen W, Guo J, Huai D, Lei Y, Jiang H, Yan L, Liao B. 2021. High-density genetic linkage map construction using whole-genome resequencing for mapping QTLs of resistance to *Aspergillus flavus* infection in peanut. *Frontiers in Plant Science*, **12**, 745408.

Kim D, Langmead B, Salzberg S L. 2015. HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, **12**, 357–360.

Li B, Dewey C N. 2011. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*, **12**, 323.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, **25**, 1754–1760.

Li Q T, Lu X, Song Q X, Chen H W, Wei W, Tao J J, Bian X H, Shen M, Ma B, Zhang W K, Bi Y D, Li W, Lai Y C, Lam S M, Shui G H, Chen S Y, Zhang J S. 2017. Selection for a Zinc-Finger protein contributes to seed oil increase during soybean domestication. *Plant Physiology*, **173**, 2208–2224.

Liu H, Sun Z, Zhang X, Qin L, Qi F, Wang Z, Du P, Xu J, Zhang Z, Han S, Li S, Gao M, Zhang L, Cheng Y, Zheng Z, Huang B, Dong W. 2020. QTL mapping of web blotch resistance in peanut by high-throughput genome-wide sequencing. *BMC Plant Biology*, **20**, 249.

Liu N, Guo J, Zhou X, Wu B, Huang L, Luo H, Chen Y, Chen W, Lei Y, Huang Y, Liao B, Jiang H. 2020a. High-resolution mapping of a major and consensus quantitative trait locus for oil content to a ~0.8-Mb region on chromosome A08 in peanut (*Arachis hypogaea* L.). *Theoretical and Applied Genetics*, **133**, 37–49.

Liu N, Huang L, Chen W, Wu B, Pandey M K, Luo H, Zhou X, Guo J, Chen H, Huai D, Chen Y, Lei Y, Liao B, Ren X, Varshney R K, Jiang H. 2020b. Dissection of the genetic basis of oil content in Chinese peanut cultivars through association mapping. *BMC Genetics*, **21**, 60.

Liu Y F, Li Q T, Lu X, Song Q X, Lam S M, Zhang W K, Ma B, Lin Q, Man W Q, Du W G, Shui G H, Chen S Y, Zhang J S. 2014. Soybean GmMYB73 promotes lipid accumulation in transgenic plants. *BMC Plant Biology*, **14**, 73.

Lu L, Wei W, Li Q, Bian X, Lu X, Hu Y, Cheng T, Wang Z, Jin M, Tao J, Yin C, He S, Man W, Li W, Lai Y, Zhang W, Chen S, Zhang J. 2021. A transcriptional regulatory module controls lipid accumulation in soybean. *New Phytologist*, **231**, 661–678.

Manan S, Chen B, She G, Wan X, Zhao J. 2017. Transport and transcriptional regulation of oil production in plants. *Critical Reviews in Biotechnology*, **37**, 641–655.

Pandey M K, Pandey A K, Kumar R, Bhat R S, Liao B. 2020. Translational genomics for achieving higher genetic gains in groundnut. *Theoretical and Applied Genetics*, **133**, 1679–1702.

Pandey M K, Roorkiwal M, Singh V K, Ramalingam A, Kudapa H, Thudi M, Chitikineni A, Rathore A, Varshney R K. 2016. Emerging genomic tools for legume breeding: Current status and future prospects. *Frontiers in Plant Science*, **7**, 455.

Pandey M K, Wang M L, Qiao L, Feng S, Khera P, Wang H, Tonnis B, Barkley N A, Wang J, Holbrook C C, Culbreath A K, Varshney R K, Guo B. 2014. Identification of QTLs associated with oil content and mapping FAD2 genes and their relative contribution to oil quality in peanut (*Arachis hypogaeaL.*). *BMC Genetics*, **15**, 133.

Sarvamangala C, Gowda M V C, Varshney R K. 2011. Identification of quantitative trait loci for protein content, oil content and oil quality for groundnut (*Arachis hypogaea* L.). *Field Crops Research*, **122**, 49–59.

Shasidhar Y, Vishwakarma M K, Pandey M K, Janila P, Variath M T, Manohar S S, Nigam S N, Guo B, Varshney R K. 2017. Molecular mapping of oil content and fatty acids using dense genetic maps in groundnut (*Arachis hypogaea* L.). *Frontiers in Plant Science*, **8**, 794.

Stracke R, Werber M, Weisshaar B. 2001. The *R2R3-MYB* gene family in *Arabidopsis thaliana*. *Current Opinion in Plant Biology*, **4**, 447–456.

Sun Z, Qi F, Liu H, Qin L, Xu J, Shi L, Zhang Z, Miao L, Huang B, Dong W, Wang X, Tian M, Feng J, Zhao R, Zheng Z, Zhang X. 2022. QTL mapping of quality traits in peanut using whole-genome resequencing. *The Crop Journal*, **10**, 177–184.

Tian Y, Yang L, Lu H F, Zhang B, Li Y F, Liu C, Ge T L, Liu Y L, Han J N, Li Y H, Qiu L J. 2022. QTL analysis for plant height and fine mapping of two environmentally stable QTLs with major effects in. *Journal of Integrative Agriculture*, **21**, 933–946.

Tsay Y F, Chiu C C, Tsai C B, Ho C H, Hsu P K. 2007. Nitrate transporters and peptide transporters. *FEBS Letters*, **581**, 2290–2300.

Varshney R K. 2016. Exciting journey of 10 years from genomes to fields and markets: Some success stories of genomics-assisted breeding in chickpea, pigeonpea and groundnut. *Plant Science*, **242**, 98–107.

Wang K, Li M, Hakonarson H. 2010. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*, **38**, e164.

Wang L, Feng Z, Wang X, Wang X, Zhang X. 2010. DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics*, **26**, 136–138.

Wilson J N, Baring M, Burow M, Rooney W, Simpson C. 2013. Generation means analysis of oil concentration in peanut. *Journal of Crop Improvement*, **27**, 85–95.

Wilson J N, Chopra R, Baring M R, Selvaraj M G, Simpson C E, Chagoya J, Burow M D. 2017. Advanced backcross quantitative trait loci (QTL) analysis of oil concentration and oil quality traits in peanut (*Arachis hypogaea* L.). *Tropical Plant Biology*, **10**, 1–17.

Wu Y, Bhat P R, Close T J, Lonardi S. 2008. Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. *PLoS Genetics*, **4**, e1000212.

Xie W, Feng Q, Yu H, Huang X, Zhao Q, Xing Y, Yu S, Han B, Zhang Q. 2010. Parent-independent genotyping for constructing an ultrahigh-density linkage map based on population sequencing. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 10578–10583.

Yang B, Wang J, Yu M, Zhang M, Zhong Y, Wang T, Liu P, Song W, Zhao H, Fastner A, Suter M, Rentsch D, Ludewig U, Jin W, Geiger D, Hedrich R, Braun D M, Koch K E, McCarty D R, Wu W, *et al.* 2022. The sugar transporter ZmSUGCAR1 of the nitrate transporter 1/peptide transporter family is critical for maize grain filling. *The Plant Cell*, **34**, 4232–4254.

Yang Y, Li Y, Cheng Z, Su Q, Jin X, Song Y, Wang J. 2023. Genetic analysis and exploration of major effect QTLs underlying oil content in peanut. *Theoretical and Applied Genetics*, **136**, 97.

Yol E, Ustun R, Golukcu M, Uzun B. 2017. Oil content, oil yield and fatty acid profile of groundnut germplasm in mediterranean climates. *Journal of the American Oil Chemists Society*, **94**, 1–18.

Zhou H, Duan H, Liu Y, Sun X, Zhao J, Lin H. 2019. Patellin protein family functions in plant development and stress response. *Journal of Plant Physiology*, **234–235**, 94–97.

Zhuang W, Chen H, Yang M, Wang J, Pandey M K, Zhang C, Chang W C, Zhang L, Zhang X, Tang R, Garg V, Wang X, Tang H, Chow C N, Wang J, Deng Y, Wang D, Khan A W, Yang Q, Cai T, *et al.* 2019. The genome of cultivated peanut provides insight into legume karyotypes, polyploid evolution and crop domestication. *Nature Genetics*, **51**, 865–876.

Executive Editor-in-Chief Xueyong Zhang
Managing Editor Ning Wang