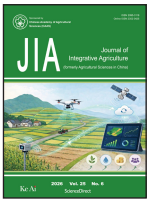
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RESEARCH ARTICLE

Inheritance and QTL mapping identify multi-effect loci for fatty acid related traits in peanut (*Arachis hypogaea* L.)

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Highlights

- A high-density peanut genetic linkage map containing 3,141 SNP markers and covering 3,051.81 cM was constructed.
- Sixty QTLs associated with oil content and fatty acid composition were identified, among which two major pleiotropic loci, *qFAT_A05.1* and *qFAT_A08.1*, were included.
- Haplotype combinations *A05.1_H004* and *A08.1_H002* were identified, and the pyramiding of these elite haplotypes contributes to the increase in peanut oil content.

Abstract

Peanut (*Arachis hypogaea* L.) is an important oil and edible protein crop. Its fatty acid composition not only influences the quality of peanut oil but also impacts flavor, shelf life, and consumer health. Peanut oil is comprised of approximately 80% oleic acid (C18:1) and linoleic acid (C18:2), 10% palmitic acid (C16:0), and the remaining 10% includes stearic acid (C18:0), arachidic acid (C20:0), gadoleic acid (C20:1), behenic acid (C22:0), and lignoceric acid (C24:0). To unravel the genetic foundation of fatty acid content and delve into QTL localization, high-density SNP microarrays were used to genotype the RIL population of 'SunOleic 97R' × 'NC94022'. A genetic linkage map was constructed with 3,141 SNP markers, covering a total genetic distance of 3,051.81 cM. Sixty quantitative trait loci (QTLs) associated with fatty acids were distributed in 11 linkage groups, with phenotypic variance explained (PVE) ranging from 1.37 to 44.92%. Notably, the QTLs *qFAT_A05.1* and *qFAT_A08.1* are multiple-effect loci contributing to various fatty acid compositions. Moreover, 15 haplotypes for the QTLs *qFAT_A05.1* and *qFAT_A08.1* were identified through genotyping 178 peanut germplasms. Haplotype analysis in a natural population confirmed the close relationship of the QTLs with the contents of oil, oleic acid, lignoceric acid, palmitic acid and behenic acid. This study serves as a valuable reference for selecting improved peanut genotypes with superior oil quality and desirable fatty acid composition.

Keywords: peanut, fatty acid, QTL, genetic map

1. Introduction

Peanut (*Arachis hypogaea* L.) is a crucial oil and edible protein crop cultivated across the tropical, subtropical, and warm temperate regions of the world. It holds the distinction of being the sole domesticated species within the genus *Arachis* (Bertioli *et al.* 2019). China has taken the lead as the largest peanut producer, closely followed by India. Peanuts are used in many applications, such as the extraction of edible oil, direct consumption as seeds, inclusion in processed food products, and use as both livestock fodder and green manure. Notably, peanut oil constitutes 45–56% of the dry seed weight (Dean *et al.* 2009; Chen *et al.* 2016), with

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the fatty acid composition playing a pivotal role in determining the final oil quality. Changing the fatty acid composition of peanut oil is a key objective in cultivar breeding efforts.

Plant traits can be broadly categorized into qualitative and quantitative traits, each displaying distinct performance and inheritance patterns. Gai and Wang (1998) proposed that most quantitative traits exhibit uneven effects across multiple genes, encompassing both main effects and micro effects. They introduced the major gene and polygenic inheritance theory, coupling it with relevant statistical methods to formulate a comprehensive framework for plant quantitative trait inheritance (Zhang *et al.* 2001). Analysis employing a mixed inheritance model involving major genes and polygenes allows for the exploration of the genetic characteristics of quantitative traits. This approach aids in estimating the effects and variances attributed to major genes, thereby offering preliminary insights into the genetic composition of breeding traits. In recent years, the major gene plus polygene genetic model has gained widespread application in scrutinizing diverse agronomic traits in many plants. Examples include hull color in foxtail millet (Guo *et al.* 2023), internode length in tomato (Sun *et al.* 2019), architecture traits in crape myrtle (Ye *et al.* 2017), and root traits in soybeans (Zhang *et al.* 2022).

The predominant fatty acids in peanut oil are oleic acid (C18:1) and linoleic acid (C18:2), comprising roughly 80% of the total (Davis *et al.* 2016; Neelakandan *et al.* 2022; Zhou *et al.* 2022). The emphasis on breeding high oleic peanuts has been driven by several advantages associated with oleic acid. Notably, oleic acid exhibits a 10-fold higher auto-oxidative stability than linoleic acid (Nile *et al.* 2013), resulting in an extended shelf life for high oleic acid and low linoleic acid peanuts compared to their conventional counterparts (Isleib *et al.* 2006). Furthermore, oleic acid has demonstrated benefits such as reducing low-density lipoprotein (LDL) levels, suppressing tumorigenesis, and alleviating inflammatory diseases (O'Byrne *et al.* 1997; Yamaki *et al.* 2005; García *et al.* 2006). The first and most renowned high oleic acid peanut mutant, *F435*, has a G to A mutation in the *AhFAD2A* coding region (López *et al.* 2000), leading to the development of numerous high oleic acid peanut cultivars centered around the *AhFAD2A* gene. Given the intricacies of gene regulation, identifying new loci is crucial for offering more avenues for enhancing the oleic acid content in peanut seeds (Pattee *et al.* 2002; Clevenger *et al.* 2017). The remaining six peanut fatty acid components, palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0), and gadoleic acid (C20:1), constitute only 20% of the total fatty acids. Five of these six are saturated fatty acids, except for C20:1, and a high intake of saturated fatty acids is often associated with increased cholesterol, triglyceride, and LDL-C levels, heightening the risk of cardiovascular disease. However, not all saturated fatty acids are universally deemed unhealthy. Studies indicate that stearic acid can serve as a healthful substitute for trans fatty acids in food manufacturing, potentially reducing the risk of atrial fibrillation (Crupkin and Zambelli 2008; Fretts *et al.* 2014; Amico *et al.* 2021).

As quantitative characters, fatty acid components are notably susceptible to environmental influences. Currently, research on the mapping of quantitative trait loci (QTLs) associated with fatty acids in peanuts is limited, and most relevant QTLs have been identified through linkage mapping with hybrid segregating populations (Pandey *et al.* 2014; Wang *et al.* 2015; Liu *et al.* 2020a; Yang *et al.* 2023; Zhang H *et al.* 2023). Huang *et al*. (2025) constructed a genetic map using RAD-seq technology and identified two QTLs associated with oil content in peanut seeds, *qOCAh12.1* and *qOCAh16.1*. Liu *et al*. (2026) mapped two QTLs, *qOCB03.1* and *qOCB06*, in a recombinant inbred line (RIL) population using a genetic map, and they increased the peanut oil content by 1.50 to 2.46%. However, typical linkage maps consist of only a few hundred markers, except for a few cases that surpass 1,000 markers (Shasidhar *et al.* 2017; Hu *et al.* 2018). The sparse densities of these linkage maps hinder the provision of precise information regarding the number and locations of QTLs governing fatty acid traits. Furthermore, the reported genome sequence resources for *A. duranensis* (Chen *et al.* 2016), *A. ipaensis* (Bertioli *et al.* 2016; Lu *et al.* 2018), and *A. hypogaea* (Bertioli *et al.* 2019; Chen *et al.* 2019; Zhuang *et al.* 2019) have laid essential groundwork for exploring the molecular underpinnings of phenotypic variations in peanuts.

In this study, we delved into the genetic patterns of peanut fatty acids by analyzing two years of phenotypic data from a RIL population. We crafted genetic linkage maps employing high-density single nucleotide polymorphism (SNP) microarrays and systematically pinpointed QTLs for fatty acid components across diverse environments. The quest for candidate genes with substantial PVE was a pivotal aspect of our investigation. A more detailed analysis of these findings will not only enhance our understanding of the genetic framework governing fatty acids but will also furnish valuable insights for advancing peanut breeding strategies.

2. Materials and methods

2.1. Plant materials, phenotypic evaluation, and DNA extraction

In this study, we used a RIL population consisting of 350 individuals derived from the cross 'SunOleic 97R'×'NC94022' (Pandey *et al.* 2014; Wang *et al.* 2015). This population was developed following the single seed decent (SSD) method at the Crop Protection and Management Research Unit, USDA-ARS, Tifton, GA, USA. 'SunOleic 97R' is a high oleic runner market-type peanut harboring two recessive mutant genes (*FAD2A* and *FAD2B*) inherited from the original high oleic line F435 (Gorbet and Knauft 2000), while 'NC94022' is a breeding selection characterized by Virginia type and hirsute features with wild-type *FAD2* alleles. Each fatty acid fraction was examined following established protocols. Phenotypic and correlation analyses were conducted using the R package corrplot (https://github.com/taiyun/corrplot).

For genotyping analysis *via* SNP array, leaf samples from the parental lines and RILs were collected for genomic DNA extraction, which was performed using the GeneJET™ Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). The quality and concentration of DNA samples were assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Tech, Wilmington, DE, USA).

2.2. Genetic modeling of major gene and polygene interactions

The genetic modeling of the peanut oil score employed a comprehensive approach combining P_1, P_2, and DH/RIL population analyses, as proposed by Zhang *et al.* (2001). To estimate the genetic parameters and component distribution in each generation, the segregation analysis software SEA V2.0 was used. Algorithms such as maximum likelihood estimation and IECM were applied, and the selection of the most suitable genetic model involved the Akaike's information criterion (AIC value) minimum criterion, along with fitness tests, including the AIC. The fitness tests comprised uniformity tests (U_1^2, U_2^2, and U_3^2), Smirnov's test ($_nW^2$), and Kolmogorov's test (D_n). The determination of the optimal genetic model also considered effect values and variance, estimated by combining the first-order genetic parameters with the least squares method. In addition, the least squares method, along with the first-order genetic parameters of the optimal genetic model, were employed to estimate second-order parameters, including the effect and variance of each gene.

2.3. Genotyping using a SNP array

For genotyping using the Version II SNP Array featuring 48K probes, DNAs from both parents and 350 RIL lines were genotyped at Affymetrix, following the workflows detailed in our prior studies (Zhao *et al.* 2022). In essence, the raw results obtained from Affymetrix were stored as .CEL files and analyzed using Axiom Analysis Suite (Thermo Fisher Scientific, Waltham, MA, USA) to ensure quality control (QC) and extract high-resolution SNPs. Subsequently, polymorphic SNPs between the parental lines were singled out for further QTL analysis. The distribution and density of SNP markers across different chromosomes were visualized using the R package CMplot (https://github.com/YinLiLin/R-CMplot).

2.4. Linkage map construction and QTL analysis

The linkage map was constructed using JoinMap 5.0 software (https://www.kyazma.nl/index.php/JoinMap/). Chi-squared (χ^2) tests were conducted to eliminate markers with distorted segregation (P>0.001). The Kosambi map function was applied to convert the recombinant ratios into genetic distances (centimorgans, cM), with all linkage groups calculated at a minimum LOD score of 2.5. Linkage maps were drawn using MapChart 2.3 software (Voorrips 2002).

For QTL analysis, the inclusive composite interval mapping of additive (ICIM-ADD) was executed using QTL IciMapping V4.1 software (Meng *et al.* 2015). The LOD score of 2.5 was set to identify potential QTLs associated with the target trait. Following established standards, QTLs with a phenotypic variance explained (PVE) greater than 10% were classified as major QTLs, while others were considered minor QTLs (Luo *et al.* 2017; Li *et al.* 2019).

2.5. Physical mapping, haplotype analysis and prediction of candidate genes

To determine the physical positions of the SNP markers, the sequences of SNP probes were aligned with the recently released Tifrunner genome sequences (https://peanutbase.org/) through a BLAST search. The BLAST was executed with criteria requiring an E-value of $<10^{-5}$ and a minimum similarity cutoff exceeding 95% between the query and database sequences. In cases where multiple locations were mapped with the same SNP probes, only the best-matched one was considered. An integrated analysis aligning the genetic map with the physical map was conducted based on the positions of SNP markers on the chromosomes. Circa software (http://omgenomics.com/circa/) was used to assess the collinearity of SNP markers between the genetic and physical positions. The natural population used for haplotype analysis is described in a paper published by our research group (Zhao *et al.* 2022), and geneHapR (Zhang R *et al.* 2023) software was used for the mapping analysis. Functional annotation of candidate genes, along with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, were performed using the annotation files in PeanutBase (https://peanutbase.org/). The peanut transcriptome data was downloaded from PeanutBase (https://peanutbase.org/), the primers used for qRT-PCR are listed in Appendix A, and *AhEF1A* (*arahy.Tifrunner.gnm2.ann1.PSFB2E*) was used as an internal reference gene.

3. Results

3.1. Genetic modeling of oil-associated master genes and polygenes

The RIL population of (SunOleic 97R×NC94022) was used to study the inheritance of QTLs related to the fatty acid compositions of peanut. The female parent and male parent showed significant phenotypic differences in oleic acid (Oa), palmitic acid (Pa), linoleic acid (La), gadoleic acid (Ga) and oil content (Oc). SunOleic 97R showed higher contents of Oa and Oc, whereas NC94022 showed higher contents of Pa, La and Ga (Table 1). The RIL population exhibited a range of oil contents from 41.59 to 53.96%. The oleic acid contents varied between 36.42 and 83.23%, while linoleic acid ranged from 2.17 to 41.69%, and the palmitic acid contents were between 5.72 and 6.14%. Stearic acid contents spanned from 1.17 to 4.21%, arachidic acid ranged from 0.69 to 2.09%, gadoleic acid varied from 0.55 to 2.57%, and the behenic acid contents were between 1.58 and 4.24%. Lignoceric acid content ranged from 0.72 to 1.92%, with the oleic/linoleic ratios spanning from 0.90 to 38.76%. In addition, the contents of very long-chain fatty acids ranged from 3.99 to 9.27%. The mean values of the phenotypes within the RIL population fell between those of the two parents. Three phenotypes Pa (10.76%), La (24.14%), and Oc (47.62%) exhibited means surpassing the mid-parental values (Pa=9.42%, La=16.64%, and Oc=46.52%) and favoring the higher-value parent, while the remaining eight phenotypes had means below the mid-parental value and favoring the lower-value parent. Furthermore, the RIL population demonstrated considerable variation and heterobeltiosis inheritance for each trait, rendering it suitable for principal gene-polygenic genetic analysis. The oleic acid/linoleic acid ratio (OLR) exhibited the highest coefficient of variation among all phenotypes at 149.15%, followed by linoleic acid at 47.71%. In contrast,

the smallest coefficient of variation was observed for total oil content, standing at a mere 4.95% (Table 1). The coefficients of variation for the other eight fatty acid phenotypes ranged from 14.67 to 30.19%. This suggests that fatty acids exhibit greater compositional variation than total oil content, which will aid in the selection of high-quality lines with elevated oil content, high oleic acid, lower harmful fatty acid content, and higher beneficial fatty acid content.

The Pearson's correlation coefficient analysis of the RIL population phenotypes (Fig. 1) revealed significant positive correlations between Oc and various traits, predominantly very long-chain fatty acids (VLCFA2010, 0.50; VLCFA2011, 0.47), Lia (Lia2010, 0.38; Lia2011, 0.34), Aa (Aa2010, 0.43; Aa2011, 0.45), and Ba (Ba2010, 0.52; Ba2011, 0.51). The VLCFA, derived from the sum of three fatty acids, exhibited significant positive correlations mainly with Lia (Lia2010, 0.93; Lia 2011, 0.93), Ba (Ba2010, 0.89; Ba2011, 0.88), and Ga (Ga2010, 0.59; Ga2011, 0.64). Pa demonstrated significant negative correlations with many fatty acids, except for La, which exhibited notable positive correlations with Oa (Oa2010, –0.93; Oa2011, –0.89), OLR (OLR2010, –0.81; OLR2011, –0.77), Ga (Ga2010, –0.67; Ga2011, –0.68), and VLCFA (VLCFA2010, –0.36; VLCFA2011, –0.45).

The remaining traits, excluding Oa and La which exhibited bimodal distributions, demonstrated single-peaked continuous distributions. Their kurtosis and skewness values were below 1, indicating approximately normal distributions. Notably, OLR displayed a skewed single-peak distribution, with kurtosis and skewness absolute values surpassing 1 (Table 1). This aligns with the characteristics of the analysis involving the main gene plus multiple genes.

Through a mixed model analysis of main gene+polygenes, a total of 38 genetic models were obtained for each of the 11 phenotypes detected, and the group of models with the

Table 1 Statistical parameters of fatty acids, the oleic/linoleic acid ratio and oil content in the parents and recombinant inbred line (RIL) population[1)]

Character[2)]	P_1 (%)	P_2 (%)	Mid-parent (%)	Min (%)	Max (%)	Mean (%)	SD	CV (%)	Kurtosis	Skewness
Pa (16:0)	5.98	12.87	9.42	5.72	16.14	10.76	2.31	21.50	–0.62	–0.43
Sa (18:0)	2.70	2.51	2.60	1.17	4.21	2.34	0.53	22.85	–0.42	0.25
Oa (18:1)	80.82	48.27	64.55	36.42	83.23	56.61	13.63	24.07	–0.65	0.76
La (18:2)	2.89	30.38	16.64	2.17	41.69	24.14	11.52	47.71	–0.66	–0.75
Aa (20:0)	1.38	1.27	1.33	0.69	2.09	1.17	0.19	16.58	0.34	0.24
Ga (20:1)	1.77	1.00	1.39	0.55	2.57	1.22	0.37	30.19	0.39	0.72
Ba (22:0)	2.70	2.50	2.60	1.58	4.24	2.45	0.42	17.37	–0.02	0.38
Lia (24:0)	1.77	1.21	1.49	0.72	1.92	1.30	0.27	20.47	–0.96	0.18
OLR	30.20	1.60	15.90	0.90	38.76	6.42	9.57	149.15	2.26	1.91
VLCFA	7.62	5.98	6.80	3.99	9.27	6.15	0.90	14.67	–0.79	0.18
Oc	49.42	43.61	46.52	41.59	53.96	47.62	2.36	4.95	–0.50	–0.12

[1)] P_1, SunOleic 97R; P_2, NC94022.
[2)] Pa, palmitic acid, C16:0; Sa, stearic acid, C18:0; Oa, oleic acid, C18:1; La, linoleic acid, C18:2; Aa, arachidic acid, C20:0; Ga, gadoleic acid, C20:1; Ba, behenic acid, C22:0; Lia, lignoceric acid, C24:0; OLR, oleic/linoleic ratio; VLCFA, very long-chain fatty acids; Oc, oil content.

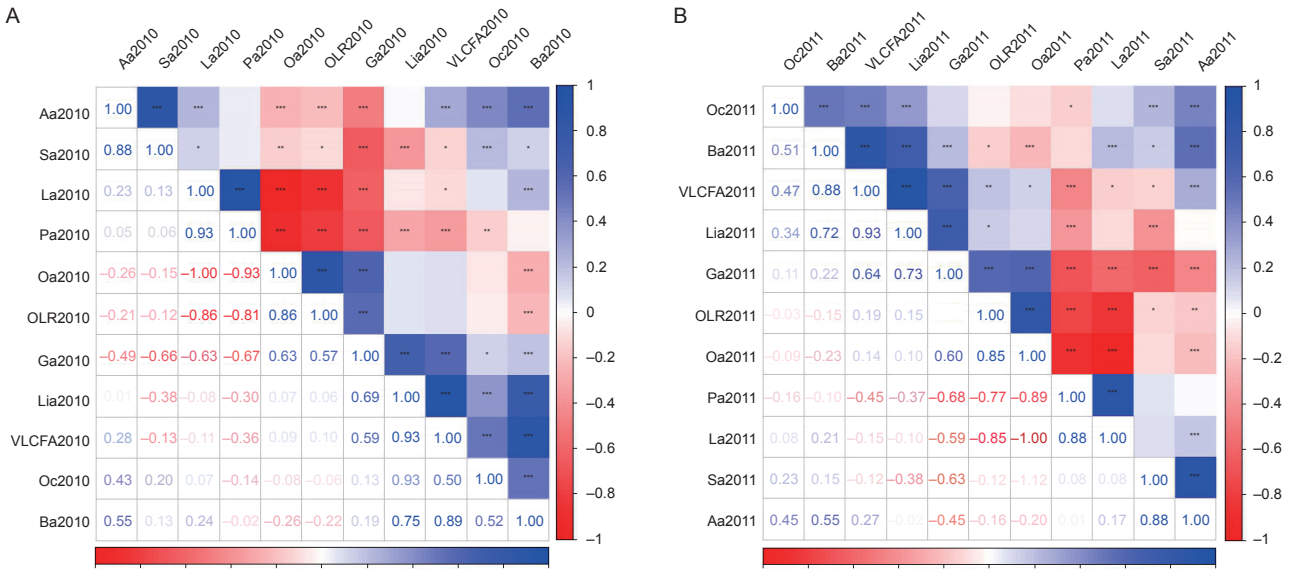


Fig. 1 Phenotypic pairwise correlations of fatty acid related traits in the recombinant inbred line (RIL) population. A, phenotypic data in 2010. B, phenotypic data in 2011. Aa, arachidic acid, C20:0; Sa, stearic acid, C18:0; La, linoleic acid, C18:2; Pa, palmitic acid, C16:0; Oa, oleic acid, C18:1; OLR, oleic/linoleic; Ga, gadoleic acid, C20:1; Lia, lignoceric acid, C24:0; VLCFA, very long-chain fatty acids; Oc, oil content; Ba, behenic acid, C22:0.

smallest AIC value was selected as the alternative model (Table 2). The genetic models obtained can be classified into five categories, i.e., four pairs of additive-superior principal genes 4MG-AI (Pa, Oa, La, and OLR), two pairs of cumulative-interacting principal genes 2MG-AE (Sa, Aa), two pairs of overlapping-interacting principal genes and additive polygenes MX2-DE-A (Ga, Ba), two pairs of cumulative-interacting principal genes and additive polygenes MX2-AE-A (Lia, VLCFA), and two pairs of additive-superior main genes and additive polygenes MX2-AI-A (Oc).

Estimation of the first- and second-order parameters based on the optimal genetic model revealed that the primary gene heritability for Sa was 59% (Appendix B), except that Aa, Pa, Oa, La, and OLR exhibited main gene heritabilities ranging from 95.1 to 98.6%. Oc demonstrated a main gene heritability of 99.84% and a polygenic heritability of 0.15%. Lia and VLCFA displayed main gene heritabilities of 78.7 and 75%, respectively, along with polygenic heritabilities of 11.1 and 14.9%. Ga and Ba showed main gene heritabilities of 47.8 and 44.6%, with polygenic heritabilities of 50.5 and 48.0%, respectively.

3.2. Genome-wide SNP scanning using the SNP array

To further refine the QTL region map, genotyping was conducted on the parental lines and a total of 350 RILs using the Version II SNP Array (Affymetrix '*Axiom_Arachis2*' 48K SNP array) (Clevenger *et al.* 2018). Among the 47,837 SNPs, 5,706 displayed polymorphisms between the parents (Fig. 2). Interestingly, 5,035 (88.24%) and 5,125 (89.82%) of these were homozygous (AA or BB) in SunOleic 97R and NC94022, respectively (Fig. 2-A). Notably, 4,457 (78.06%) markers were homozygous in both parental lines

Table 2 Fitness test results of the optimization models for fatty acids, the oleic/linoleic acid ratio and oil content of the RIL population[1)]

Trait[2)]	Model	Generation[3)]	U_1^2	U_2^2	U_3^2	${}_nW^2$	D_n
Pa (16:0)	4MG-AI	P_1	4.0613(+)	3.5437(–)	0.0757(–)	0.5050(+)	0.6902(+)
		P_2	0.2310(–)	0.1774(–)	0.0311(–)	0.0486(–)	0.2661(–)
		DH	0.0007(–)	0.0001(–)	0.0049(–)	0.0130(–)	0.0194(–)
Sa (18:0)	2MG-AE	P_1	0.5824(–)	0.1588(–)	1.8542(–)	0.1547(–)	0.4475(–)
		P_2	0.1934(–)	0.6997(–)	2.6988(–)	0.1596(–)	0.3571(–)
		DH	0.0105(–)	0.0018(–)	0.0508(–)	0.0402(–)	0.0298(–)
Oa (18:1)	4MG-AI	P_1	0.0026(–)	0.0005(–)	0.0113(–)	0.0430(–)	0.2425(–)
		P_2	1.5402(–)	0.5301(–)	3.5882(–)	0.2106(–)	0.4883(–)
		DH	0.0184(–)	0.0217(–)	0.0040(–)	0.0226(–)	0.0276(–)
La (18:2)	4MG-AI	P_1	0.1227(–)	0.3383(–)	0.9405(–)	0.0649(–)	0.2403(–)
		P_2	1.9872(–)	3.2171(–)	2.9407(–)	0.2359(–)	0.4869(–)
		DH	0.0252(–)	0.0292(–)	0.0048(–)	0.0287(–)	0.0323(–)
Aa (20:0)	2MG-AE	P_1	0.1214(–)	0.5938(–)	3.0027(–)	0.1452(–)	0.4154(–)
		P_2	0.0769(–)	0.3829(–)	1.9627(–)	0.1324(–)	0.3582(–)
		DH	0.0302(–)	0.0732(–)	0.1677(–)	0.1020(–)	0.0391(–)
Ga (20:1)	MX2-DE-A	P_1	0.1521(–)	0.0325(–)	0.6228(–)	0.0865(–)	0.3293(–)
		P_2	0.0002(–)	0.0431(–)	0.7918(–)	0.0471(–)	0.2495(–)
		DH	0.1809(–)	0.1257(–)	0.0526(–)	0.1411(–)	0.0586(–)
Ba (22:0)	MX2-DE-A	P_1	0.0001(–)	0.0306(–)	0.5349(–)	0.0336(–)	0.2039(–)
		P_2	0.0177(–)	0.0544(–)	0.1740(–)	0.1250(–)	0.3870(–)
		DH	0.0167(–)	0.0111(–)	0.8499(–)	0.1375(–)	0.0454(–)
Lia (24:0)	MX2-AE-A	P_1	0.0890(–)	0.0453(–)	4.0298(+)	0.1473(–)	0.4038(–)
		P_2	0.5007(–)	1.0558(–)	1.8758(–)	0.1511(–)	0.3769(–)
		DH	0.0039(–)	0.0047(–)	0.0010(–)	0.0214(–)	0.0248(–)
OLR	4MG-AI	P_1	0.0147(–)	0.7934(–)	16.2647(+)	0.2859(–)	0.4999(–)
		P_2	0.2428(–)	0.9869(–)	4.2658(+)	0.2795(–)	0.5212(–)
		DH	0.0696(–)	4.8470(+)	60.5977(+)	5.4329(+)	0.2512(+)
Oc	MX2-AI-A	P_1	0.0003(–)	0.0664(–)	1.2087(–)	0.0493(–)	0.2628(–)
		P_2	0.0183(–)	0.0526(–)	2.0752(–)	0.0763(–)	0.3185(–)
		DH	0.0002(–)	0.0012(–)	0.0388(–)	0.0232(–)	0.0229(–)
VLCFA	MX2-AE-A	P_1	0.1159(–)	0.0002(–)	1.8727(–)	0.0872(–)	0.3410(–)
		P_2	0.4687(–)	0.6793(–)	0.4162(–)	0.1170(–)	0.3811(–)
		DH	0(–)	0.0003(–)	0.0063(–)	0.0473(–)	0.0321(–)

[1)] U_1^2, U_2^2 and U_3^2 are uniformity test statistics; ${}_nW^2$ is the Smirnov test statistic; D_n is the Kolmogorov test statistic. + indicates a significant difference at the 0.05 level; – indicates no significant difference.

[2)] Pa, palmitic acid, C16:0; Sa, stearic acid, C18:0; Oa, oleic acid, C18:1; La, linoleic acid, C18:2; Aa, arachidic acid, C20:0; Ga, gadoleic acid, C20:1; Ba, behenic acid, C22:0; Lia, lignoceric acid, C24:0; OLR, oleic/linoleic ratio; Oc, oil content; VLCFA, very long-chain fatty acids.

[3)] P_1, SunOleic 97R; P_2, NC94022; DH, double haploid.

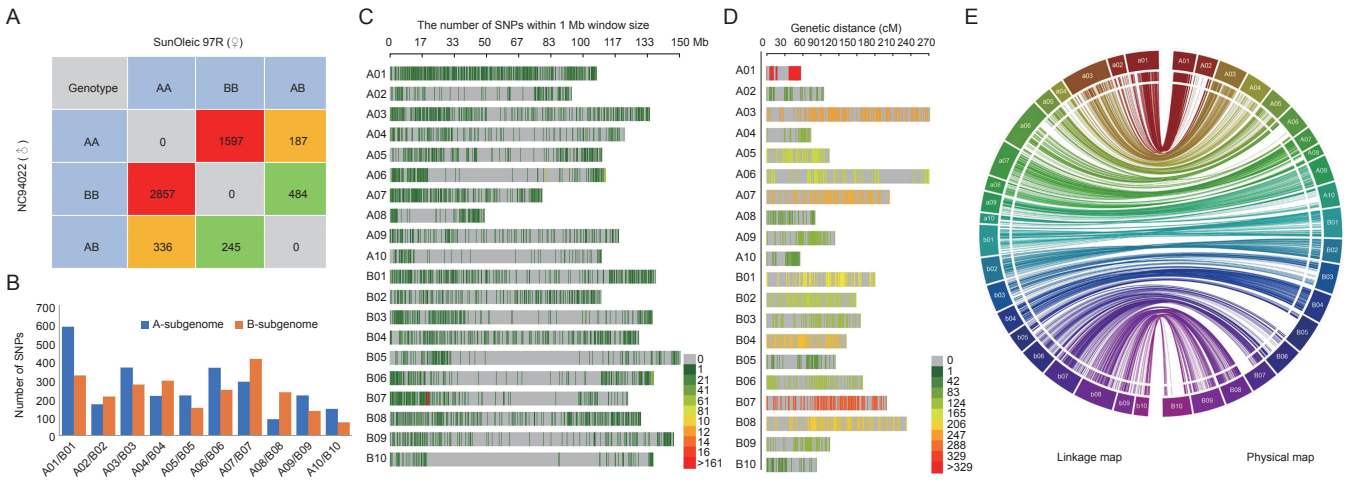


Fig. 2 Polymorphic SNP markers and genetic map identified using the version II SNP array. A, number of different types of SNPs in the parents. B, number of polymorphic SNPs in the chromosomes of peanut. C, distribution and density of polymorphic SNPs in the chromosomes of peanut. D, distribution of SNP markers on 20 linkage groups. The bars denote SNP markers. Different colors show the density of the linkage map. The *x*-axis indicates the length of the linkage map; the *y*-axis indicates the 20 linkage groups named as A01–A10 and B01–B10 corresponding to the chromosomes of A and B subgenomes of peanut. E, collinearity analysis between the genetic map and the physical map. a01–a10 and b01–b10 represent the 20 linkage groups. A01–A10 and B01–B10 indicate the physical maps.

(Fig. 2-A). The polymorphic SNPs were distributed across all 20 chromosomes of peanut, with chromosome A01 (581) having the most SNPs and A08 (91) the fewest (Fig. 2-B). In addition, the SNPs on chromosome A01 exhibited high density and a more even distribution compared to the other chromosomes (Fig. 2-C).

3.3. SNP array based high-density linkage map construction

After eliminating SNPs with significant segregation distortion (P<0.001), more than 10% missing data, and unlinked SNPs, a total of 3,141 SNPs were successfully mapped onto 20 linkage groups, corresponding to the 20 chromosomes of the peanut genome. This effort resulted in the construction of a high-density linkage map. The final linkage map encompassed 3,141 SNPs and spanned 1,425.33 and 1,626.49 cM for genomes A and B of the peanut, respectively (Appendix C). The overall length of the linkage map amounted to 3,051.81 cM, with a density of 1.03 SNPs cM^{-1} (Appendix C). Notably, A01 emerged as the largest linkage group, housing 367 loci and exhibiting the highest density, with an average distance of only 0.17 cM between two loci (Fig. 2-D; Appendix C). Collinearity analysis between the genetic and physical positions of the SNP loci indicated robust coverage across the peanut genome. Furthermore, most of the markers on the genetic map maintained the same order as those on the corresponding chromosomes of the reference genome (Fig. 2-E).

3.4. QTL mapping for fatty acids

Following the successful construction of the genetic map, QTL localization was conducted using the IciMapping software. A total of 60 QTLs for oil content and fatty acid composition were mapped on 11 peanut chromosomes (Appendix D). Five QTLs for Aa were identified with PVE values ranging from 3.147 to 44.925%, while six QTLs for Ba were obtained with PVE values ranging from 3.570 to 26.199%. Ga displayed seven QTLs with PVE values ranging from 4.857 to 13.351%. La had two QTLs with PVE values in the range of 24.710 to 27.663%, while Lia showed seven QTLs with PVE values ranging from 3.361 to 22.794%, and Oa had two QTLs with PVE values in the range of 24.870 to 27.455%. Oc featured 16 QTLs with PVE values in the range of 1.374 to 24.687%, and OLR had two QTLs with PVE values in the range of 16.322 to 17.195%. Pa displayed five QTLs with PVE values in the range of 2.988 to 25.936%, Sa had three QTLs with PVE values in the range of 2.205 to 7.284%, and VLCFA exhibited five QTLs with PVE values ranging from 4.109 to 28.512%.

Interestingly, many QTLs were found to contribute to multiple fatty acid-related traits (FATs). The data in Fig. 3 clearly show that chromosomes A05, A08, A09 and B04 possess multiple fatty acid QTL traits. Five of these QTLs corresponded to at least three FATs. Among them, three QTLs were located on the A05 chromosome (Table 3; Fig. 4), namely *qFAT_A05.1*, *qFAT_A05.2*, and *qFAT_A05.3*. The *qFAT_A05.1* QTL contributed to ten FATs, exhibiting the highest PVE contribution ranging from 5.532 to 28.512%, and consistently showing positive additive effects. Furthermore, *qFAT_A05.2* and *qFAT_A05.3* were identified across six and three FATs, respectively. On chromosome A08, *qFAT_A08.1* corresponded to four FATs, with PVE contributions ranging from 3.147 to 10.039%. Moreover, *qFAT_A09.1* on chromosome A09 was identified as controlling nine FATs, with PVE contributions ranging from 11.509 to 27.663%.

3.5. Haplotype analysis of QTLs for multiple fatty acid traits in natural populations

In our previous studies, a natural population including 178 peanut varieties was genotyped by the Version II SNP Array, and more than 10,000 SNPs were identified (Zhao

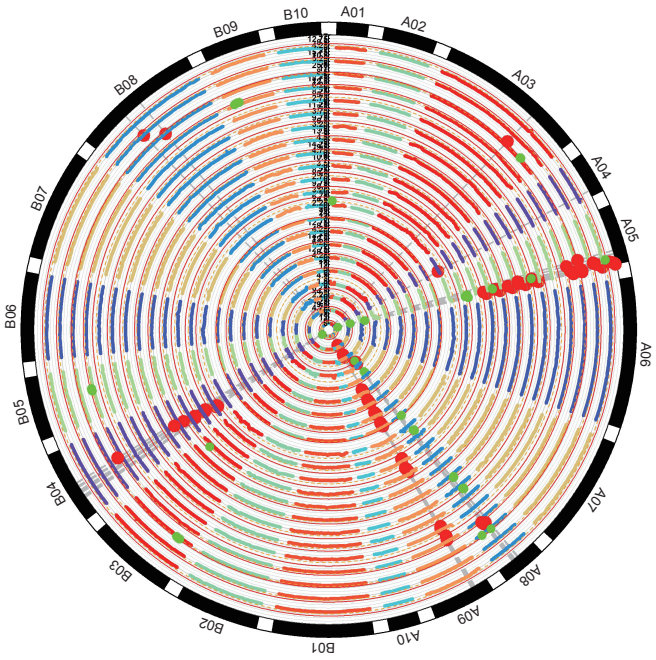


Fig. 3 Circular Manhattan plots of the genome-wide association scan for 22 traits. The green (6>LOD>3) and red points (LOD>6) indicate the significance. The 23 circles from outside to inside the threshold represent the chromosome and 22 traits (Pa2010, Pa2011, Sa2010, Sa2011, Oa2010, Oa2011, La2010, La2011, Aa2010, Aa2011, Ga2010, Ga2011, Ba2010, Ba2011, Lia2010, Lia2011, OLR2010, OLR2011, Oc2010, Oc2011, VLCFA2010, and VLCFA2011).

et al. 2022). To further investigate the relationship between QTL loci and fatty acids, the SNPs within the intervals of *qA05.1*, *qA05.2*, *qA05.3*, and *qA08.1* were used for haplotype analysis.

The physical interval of *qA05.1* includes nine SNPs. In all the 178 peanut varieties of the natural population, these SNPs could be arranged into eight haplotype combinations (Appendix E). Among them, two haplotype combinations existed in no more than five varieties and are known as incredible haplotypes. The other six haplotypes are known as credible haplotypes and were used for further analysis. Under the above cutoff, totals of two, four and three haplotypes were identified within the intervals of *qA05.2* (Appendix F), *qA05.3* (Appendix G), and *qA08.1* (Appendix H), respectively. The different haplotypes showed significant variations in Oc, Oa, Lia, Pa, and Ba contents. For example, the 15 varieties with haplotype A05.1_H004 exhibit higher Oc content (53.74%, *n*=15) than A05.1_H001 (51.44%, *n*=54) (Fig. 5-A and E), while Lia, Pa, and Ba show significantly lower contents than A05.1_H001 (Appendix E). For *qA05.3*, the median oil content of haplotype A05.3_H003 is 55.28% (*n*=9), which is significantly higher than that of haplotype A05.3_H004 (49.43%, *n*=6) (Fig. 5-C and G). For *qA08.1*, haplotype A08.1_H002 (53.98%, *n*=24) shows significantly higher Oc content than A08.1_H003 (49.06%, *n*=5) (Fig. 5-D and H), with the Oa, Lia, and Pa contents significantly lower than in A08.1_H003 and A08.1_H003 (Appendix H). The above results confirmed that *qA05.1*, *qA05.2*, *qA05.3*, and *qA08.1* are closely related with the contents of Oc, Oa, Lia, Pa, and Ba, so these haplotypes can used as the references for screening peanut varieties with desired fatty acid compositions.

3.6. Candidate gene prediction

To further predict the candidate genes for the QTLs controlling multiple FATs, GO enrichment and KEGG pathway analyses were performed for the genomic regions of these QTLs. GO enrichment analysis of the genes within these 60 QTLs revealed enrichment in molecular functions such as transcription factor activity, transcription regulator activity, and oxidoreductase activity (Fig. 6-A). In terms of cellular components, enrichment was observed in lytic vacuole and transcription repressor complex. In addition, the analysis highlighted enrichment in various biological processes including carbohydrate metabolic processes and glycosinolate biosynthetic processes.

The KEGG pathway analysis indicated enrichment in

Table 3 Summary of the QTLs identified by multiple fatty acid traits or phenotypic variance explained (PVE)>10 in at least one year

QTL[1)]	Position (bp)	Chr	LOD	PVE (%)	Add	No. trait/Trait name
qFAT_A05.1	4,282,992–7,266,706	A05	5.771–20.148	5.532–28.512	0.087–0.983	10
qFAT_A05.2	8,246,469–8,859,373	A05	3.441–7.296	2.205–8.858	–0.084–0.229	6
qFAT_A05.3	18,124,006–18,602,224	A05	4.238–7.197	4.265–5.548	–0.561–0.726	3
qFAT_A08.1	43,512,165–43,744,767	A08	4.623–5.886	3.147–10.039	–0.134–0.070	4
qFAT_A09.1	114,692,575–115,064,096	A09	10.300–24.183	11.500–27.663	–6.198–7.340	9
qGAT_B04.1	38,317,965–76,451,600	B04	9.038	10.375	–0.134	Ga2010
qGAT_B04.1	38,317,965–76,451,600	B04	12.203	12.714	–0.132	Ga2011
qAAT_A04.1	45,363,969–66,678,224	A04	47.216	44.925	0.136	Aa2010
qAAT_B04.1	108,730,358–109,618,577	B04	8.473	15.086	–0.056	Aa2011
qOCT_A08.1	45,682,761–45,992,613	A08	3.307	1.789	–0.472	Oc2011
qOCT_A08.2	45,992,613–46,449,452	A08	13.163	7.617	–0.677	Oc2010
qOCT_B08.1	29,200,985–29,879,112	B08	33.932	24.687	1.757	Oc2011
qOCT_B08.2	34,321,986–42,878,614	B08	24.455	14.731	–0.942	Oc2010

[1)] *qFAT_A05.1* is the first QTL by multiple fatty acid traits on A05; *qGAT_B04.1* is the first QTL by multiple gadoleic acid traits on B04; *qAAT_A04.1* is the first QTL by multiple arachidic acid traits on A04; *qOCT_A08.1* is the first QTL by multiple oil content traits on A08.

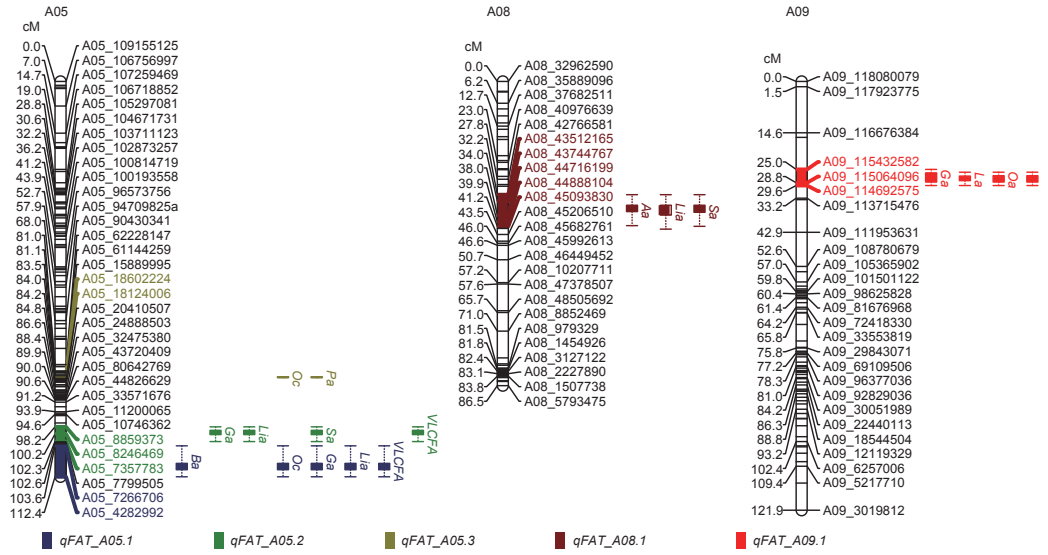


Fig. 4 Genetic map of multiple fatty acid trait QTLs. Blue, *qFAT_A05.1*; green, *qFAT_A05.2*; olive, *qFAT_A05.3*; brown, *qFAT_A08.1*; red, *qFAT_A09.1*.

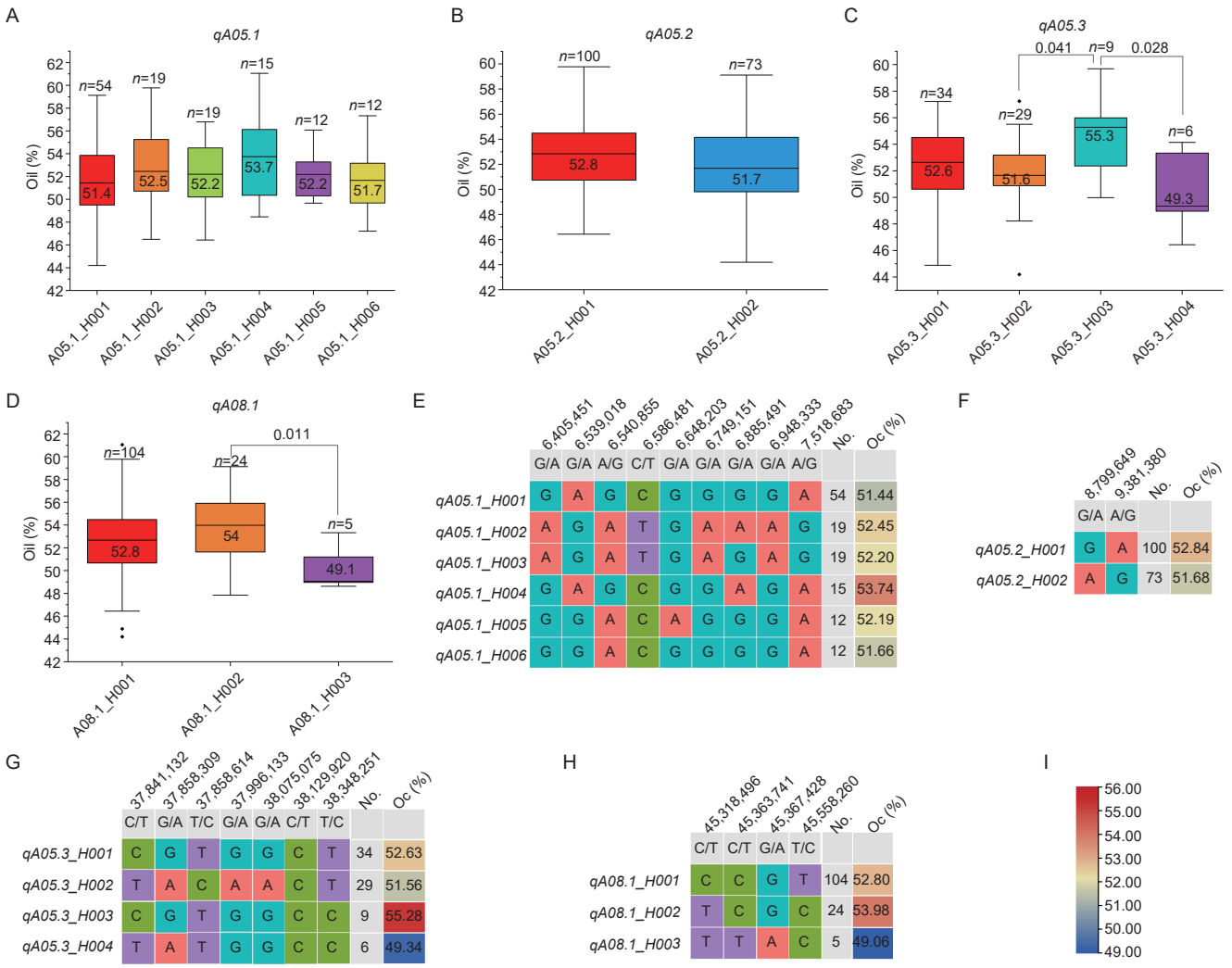


Fig. 5 Haplotype analysis of quantitative trait loci (QTLs) for multiple fatty acid traits in the natural populations. A–D, haplotype analysis of oil content (Oc) (%) traits in *qA05.1*, *qA05.2*, *qA05.3*, and *qA08.1*. E–H, haplotype analysis of Oc (%) traits in *qA05.1*, *qA05.2*, *qA05.3*, and *qA08.1*. The median of Oc (%) for each haplotype is represented by the heatmap and number, and E shows the legend of the heatmap. No. means the number of peanut germplasms.

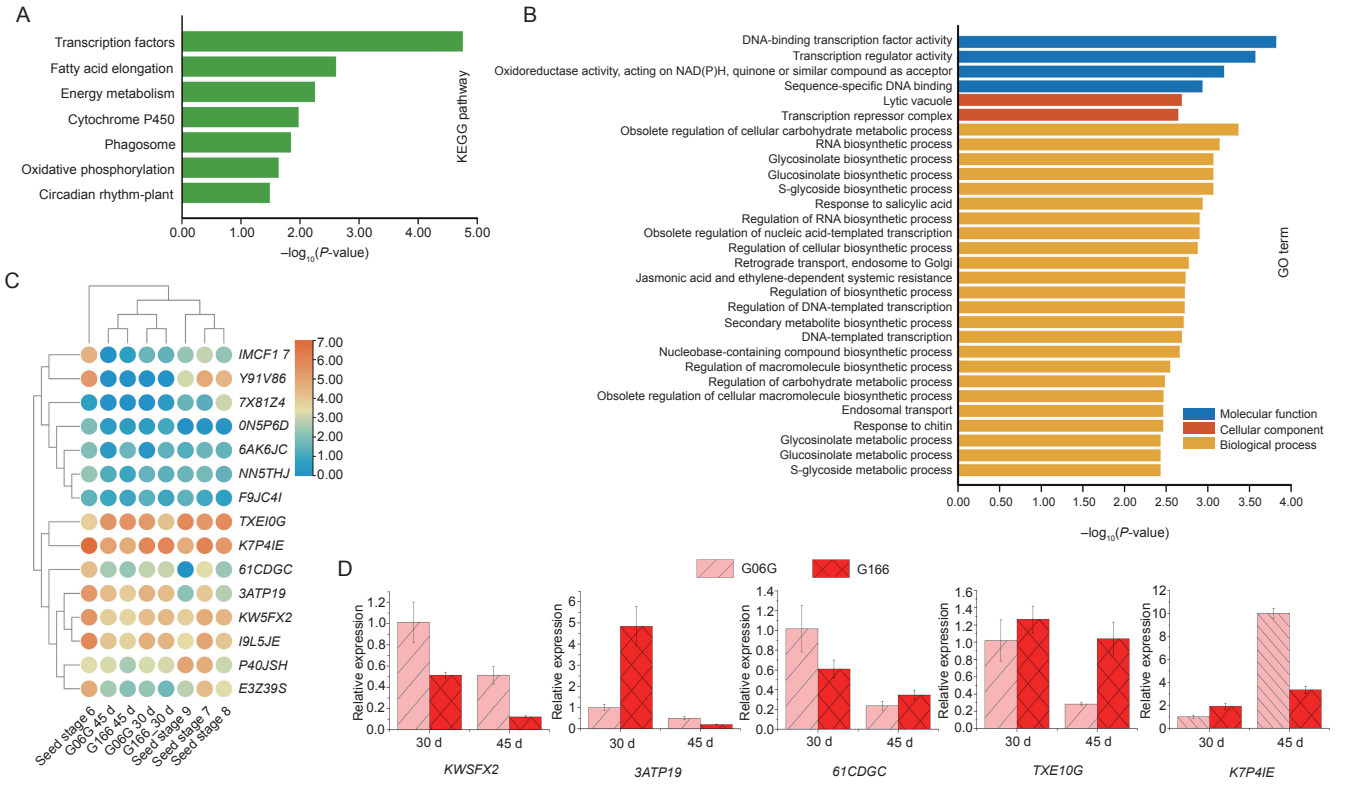


Fig. 6 Enrichment and expression analysis of fatty acid-related candidate genes. A, Gene Ontology (GO) enrichment analysis of genes within all localized QTL intervals. B, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of genes within all localized QTL intervals. C, transcriptome expression analysis of genes during peanut seed development. D, candidate genes of the qRT-PCR analysis. G06G, Geogia-06g; G166, Guihuahong 166. Bars mean SD (*n*=3).

transcription factors, fatty acid elongation, energy metabolism, and cytochrome P450 (Fig. 6-B). Specifically, there were five genes related to fatty acid elongation and 24 genes related to energy metabolism. Examining the RNA-seq data revealed that 15 of the 29 genes were expressed during seed development (Appendix I). Notably, the genes *Arahy.K7P4IE* and *Arahy.KW5FX2* displayed consistently high expression across all stages of seed development, while gradually decreasing over time (Fig. 6-C and D; Appendix I). Most other genes exhibited a similar decreasing expression trend, while the *Arahy.TXEI0G* gene demonstrated consistently high expression over time (Fig. 6-C and D; Appendix I).

Furthermore, it is noteworthy that four QTLs with PVE>10 were identified, each occurring uniquely: *qAAT_A04.1*, *qAAT_B04.1*, *qOCT_B08.1*, and *qOCT_B08.2* (Table 3). Within the *qOCT_B08.2* interval, the genes *Araip.0LA9D* and *Araip.CQ3BI* are notable as they encode long-chain acyl-CoA synthetases belonging to the ACS family. These enzymes play a role in metabolizing fatty acids with lengths of C12 to C20. In cultivated peanuts, *Arahy.F0YWKB* and *Arahy.4DL9G8* are homologous to these two genes. The *qAAT_A04.1* interval contains the *Aradu.FV2AZ* gene, which encodes glucose-6-phosphate isomerase. This enzyme catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate, thus initiating a series of enzymatic reactions that lead to the production of fatty acids. *Arahy.GIX57Y* serves as a homologue in cultivated peanuts.

4. Discussion

4.1. Fatty acid-related genetic analysis helps QTL localization

Enhancing peanut quality traits stands as a crucial task for expediting the peanut breeding process, particularly focusing on the development of high-quality varieties characterized by elevated oil content, high oleic acid, and reduced very long-chain fatty acids. This study delineates the conspicuous phenomenon of transgressive inheritance in oil content and fatty acid traits, indicating their quantitative nature. The use of a stable and unaffected permanent RIL population, which is less susceptible to environmental influences and devoid of dominant interactions, facilitates a more accurate estimation of additive effects, additive interactions, and main gene contributions. This enhances the authenticity and credibility of the main gene plus multigene genetic analyses.

Quantitative traits are governed by a set of genes with varying effects, in which major genes exert more substantial influences and perceptible performances, while polygenes contribute with smaller effects and less conspicuous impacts. Effective breeding strategies can be devised based on the heritability of primary and polygenic genes governing quantitative traits (Wang and Gai 1997; Gai and Wang 1998). This study underscores that the main gene heritability of Sa and Aa is 59%, whereas the heritabilities of other trait genes range from 89.8 to 99.8% (Appendix B). This suggests that while Aa and Sa are significantly influenced by genetic

factors, they are also considerably affected by environmental factors, whereas the remaining phenotypes remain nearly unaffected or less affected by environmental factors.

Both Aa and Sa exhibit positive additive effect values for their main genes, signifying positive genetic inheritance (Appendix B). Conversely, OLR and Oa demonstrate three main genes with positive genetic effects and one main gene with a negative genetic effect, but the positive genetic effect far surpasses the negative genetic effect. For Pa, all four pairs of main genes exhibit negative additive effects, indicating an overall negative genetic effect (Appendix B). La shows two high-effect negative genetic genes and two low-effect positive genetic genes. This information is valuable for breeders, as it can aid in preliminary assessments of the genetic composition of breeding traits in segregating generations of hybrids. It also helps with formulating appropriate breeding strategies and refining the genetic composition of traits identified through QTL localization.

4.2. Substantial genetic distances among markers can impede the localization of QTLs

The distribution of DNA markers exhibits non-random patterns, with certain genomic regions densely marked while others remain only sparsely marked. In the genetic profiles presented, the inter-marker distances varied, and 90% of them are less than or equal to 2.0 cM in A01, A04, A05, A10, B04, and B07. Distances exceeding this threshold accounted for less than 90% on the remaining chromosomes. Each cluster encompasses 53–367 markers, with eight clusters having an average distance between markers less than or equal to 1 cM, 11 clusters having an average distance between markers greater than 1 but less than 2 cM, and the B05 cluster having an average distance between markers exceeding 2 cM. However, certain markers show considerable genetic distances between them, such as the A06 markers with a maximum distance of 57.99 cM.

The markers with large genetic distances could potentially hinder the practical application of localized DNA markers. For instance, genomic regions lacking DNA markers might impede the detection of QTLs. In addition, the peanut genome is highly complex, with AA and BB subgenomes that exhibit high similarity. The genome assembly version 2 for *Arachis hypogaea* cultivar Tifrunner is similar to version 1 at the base pair level, but it features significant structural rearrangements based on HiC assessments. Key changes include an ~8 Mb translocation in the pericentromere of A02, rearrangements in the pericentromere of A03, a swap in the telomeric regions of A06 and B06, and an increase in the number of pericentromeres in the pericentromere of A03. These structural complexities may pose challenges in assigning SNP markers to the two subgenomes. Consequently, more comparable markers are needed to fill inter-marker distances across the different peanut maps to ensure a more comprehensive coverage of the peanut genome.

4.3. New genetic map helps pinpoint fatty acid-related QTLs

Oleic acid (C18:1) and linoleic acid (C18:2) constitute the predominant fatty acids, representing approximately 80% of peanut oil, with palmitic acid contributing about 10%. Consequently, obtaining valuable genetic insights is crucial for breeding novel peanut varieties characterized by elevated oleic acid levels and outstanding agronomic traits. In normal peanut lines, oleic acid (C18:1) comprises about 47%, while high oleic peanut lines can reach up to 80%. One study used RIL populations from normal and high oleic peanut varieties to pinpoint the C18:1, C18:2, and *FAD2* genes responsible for the oleic/linoleic acid ratio (OLR) (Pandey *et al.* 2014). The *AhFAD2A* (subgenome A) and *AhFAD2B* (subgenome B) genes were localized to the A09 and B09 linkage groups, and *AhFAD2B* exhibited PVE values of 26.54, 25.59, and 41.02% for C18:1, C18:2, and OLR, respectively. The PVEs of *AhFAD2A* against C18:1, C18:2, and OLR were 8.08, 6.86, and 3.78%, respectively (Pandey *et al.* 2014). In this study, the major QTLs for Oa, La, and OLR were identified in A09. The PVEs of the Oa, La, and OLR QTLs in A09 ranged from 24.870 to 27.455%, 24.710 to 27.455%, and 16.322 to 17.195%, respectively, highlighting significant genetic contributions (Appendix D).

QTL mapping forms the basis of crop molecular breeding and gene discovery. In recent years, significant strides have been made in identifying the QTLs related to oil content in peanuts. Numerous major QTLs for oil content have been pinpointed on chromosomes A05 and A08. The QTL on chromosome A08 is situated within the 30–50 Mb interval, making it a hotspot for oil content QTLs, and the *qFAT_A08.1* gene investigated in this study also falls within this region. Liu *et al.* (2020b) mapped *qOCA08.1* to the 42.21–44.53 Mb range on A08, while *A05.1* and *qA08.4* are positioned within the 6.34–7.83 Mb range on A05 and the 37.11–37.99 Mb range on A08, respectively (Sun *et al.* 2022). Three stable primary QTLs were identified through single-marker QTL interval mapping, with linkage markers found at 23.97 Mb on A03, 21.79 Mb on A05, and 11.87 Mb on A08 (Wilson *et al.* 2016). Guo *et al.* (2024) developed genetic maps using SSR markers and identified two QTLs, *qOCA05* and *qOCA08*, with PVE values of 13.44 and 10.03%, respectively.

In this study, we found that traits with high fatty acid correlation coefficients often share common loci. Five QTL loci were identified that could be localized for different fatty acid species. This phenomenon may arise from upstream and downstream relationships in metabolic pathways, where a single locus influences the compositions of different fatty acids simultaneously. For instance, oleic acid serves as a direct substrate for linoleic acid synthesis, and mutations in the *FAD2A*/*2B* genes lead to an increase in oleic acid and a reduction in linoleic acid.

Several of the loci identified in this study align with QTLs observed in segregating populations. The primary loci linked to oleic acid and linoleic acid on A09, as identified in this study, coincide with QTL regions identified in three segregation populations (Table 3): *S_mqOA_a09* and *S_mqLA_a09* in the S population (SunOleic 97R×NC94022) (Wang *et al.* 2015); *T_mqOA_a09* and *T_mqLA_a09* in the T population (Trifrunner×GT-C20) (Wang *et al.* 2015), as well as *qOle-A09-1* and *qLin-A09-1* in the FA population (ICGV 06420×SunOleic 95R) (Shasidhar *et al.* 2017). However,

the QTL locus on B09 associated with oleic acid and linoleic acid was not localized in this paper. This discrepancy might be attributed to the fact that *AhFAD2B* is situated at 154048543–154050975 of B09, whereas the terminal marker of the genetic linkage group of B09 constructed in this paper, B09_146444849, is physically distant from *AhFAD2B* by 7.6 Mb (Fig. 2-D).

In this study, four novel QTL loci were identified on chromosomes A05 and A08 within this population. These findings underscore the significance of the newly constructed genetic map based on SNP microarrays in uncovering previously unknown QTLs associated with fatty acids in peanuts.

5. Conclusion

In this study, we developed a high-resolution genetic map comprising 3,141 polymorphic SNP markers within the RIL population and examined the fatty acid inheritance patterns. We successfully identified five QTLs, *qFAT_A05.1*, *qFAT_A05.2*, *qFAT_A05.3*, *qFAT_A08.1*, and *qFAT_A09.1*, across different environments and conducted validation through haplotype analysis in a natural population of 178 peanut varieties. These findings hold significant utility for enhancing the quality of peanuts through targeted breeding efforts.

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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 4.0 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.

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