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RESEARCH

# QTL Mapping of Seed Fatty Acid Contents in *Camelina sativa* Under Heat Stress

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## Abstract

Heat stress alters oil quality in oilseed crops, yet its genetic underpinnings in *Camelina sativa* remain unclear. This study investigated the genetic basis of heat-induced changes in seed fatty acids using a recombinant inbred line (RIL) population derived from a cross between two camelina varieties, Suneson and Pryzeth. Exposure to high temperature during reproductive growth led to increased proportions of saturated (C16:0, C18:0) and monounsaturated (C18:1) fatty acids, whereas polyunsaturated C18:3, total unsaturated fatty acids (UFA) and the PUFA/MUFA ratio were decreased, suggesting an inhibition of the C18:1 → C18:2 → C18:3 desaturation pathway. A high-density linkage map (4981 bins across 20 chromosomes) was built, and 25 QTLs for fatty acids were detected, with hotspots on chromosomes 1, 9, 12, 13, 16, and 20. A major QTL on chromosome 1 (~ 80 cM) explained the largest variance component for PUFA/MUFA under heat. Three desaturase genes (*FAD2*, *FAD7*, *FAD8*) were located within key QTL intervals, nominating them as candidates for modulating unsaturation under elevated temperature. These results provide a genetic basis for fine mapping and functional validation, supporting future molecular and breeding efforts to stabilize oil quality under warming conditions.

**Keywords** Camelina · Heat stress · Fatty acid composition · Oilseed · QTL

## Introduction

Rising temperatures are of great concern for agriculture. Heat stress often accompanying drought is among the most impactful stressors for most plant species, including agricultural crops (Brás et al. 2021). It was estimated that, globally, 1.4% of cereal production, 0.5% of oil crops, 0.6% of pulses, 0.2% of fruits, and 0.09% of vegetables were lost due to heat and drought disasters from 1961 to 2014 (Mehrabi and Ramankutty 2017). High daytime temperatures negatively affect plant growth but impact the most during reproduction coinciding with summer heat and drought. Heat stress disrupts plant homeostasis, impairing essential metabolic and physiological processes, restricting energy production, and threatening cellular integrity (Rivero et al. 2022). Heat stress during flowering and seed development significantly compromises plant reproductive success by reducing pollen viability, disrupting fertilization, causing flower or pod abortion, and ultimately diminishing seed set and yield (Smith and Lu 2024). To withstand these adverse conditions, plants must swiftly reprogram their metabolism and physiology to establish a new functional balance, a process known as acclimation (Taiz and Zeiger 2014). Under heat stress, plants experience increased production of reactive oxygen species (ROS), which disrupt cellular metabolism by damaging membranes, proteins, and photosynthetic machinery. In response, plants activate heat shock proteins (HSPs) and a network of antioxidant defenses often regulated by signaling hormones like abscisic acid (ABA) and salicylic acid (SA)

Nicusor Nastase and Dayu Yang contributed equally to this study.

**Key Message** We identified 25 QTLs for heat-responsive variation in camelina seed fatty acids using a high-density bin map. Several chromosome regions harbored clusters of QTLs for multiple fatty acid traits. Three desaturase genes within major QTL intervals were identified as candidates influencing fatty acid composition under high temperature.

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to stabilize proteins, scavenge ROS, and re-establish cellular homeostasis (Wahid et al. 2007; Bita and Gerats 2013).

Major oilseed crops such as soybean, canola (*Brassica napus*) and sunflower are vital sources of edible oils, protein-rich feed, and renewable biofuels (Berti et al. 2016). Heat stress during seed development in oilseed crops significantly reduces seed weight and total yield, impedes oil accumulation and alters fatty acid composition, often resulting in increased saturation levels due to disrupted desaturation activities (Nadakuduti et al. 2023; Markie et al. 2025). Producers and breeders are under increasing pressure to develop climate-resilient varieties that can maintain yield and quality under high temperature stress (Ahmad et al. 2021). *Camelina sativa* (L.) Crantz, also known as false flax, is an oilseed crop valued for its short lifecycle, low input needs, and adaptability to marginal soils. Camelina oil, primarily used for biofuel production, is rich in polyunsaturated fatty acids, making it useful for nutrition and feed. These traits, along with pest and disease tolerance, position camelina as a promising alternative to conventional oilseeds and a candidate for heat- and drought-resilient cropping systems (Zubr 2003; Berti et al. 2016). Camelina is considered relatively resilient to abiotic stresses, yet heat stress during reproductive and seed-filling stages substantially reduces yields of seed and oil with altered fatty acid composition particularly reduced levels of polyunsaturated fatty acids, such as α-linolenic acid (C18:3), and increased oleic acid (Smith and Lu 2024; Nadakuduti et al. 2023). Similar changes were also observed in a study on oilseed rape (*Brassica napus*) that affects both the quality of the oil and its market value (Markie et al. 2025; Aksouh-Harradj et al. 2006). Developing camelina genotypes that can withstand heat stress is essential for maintaining oil yield and quality in a warming climate. Therefore, understanding the genetic and genomic architecture underlying fatty acid biosynthesis is critical for effective breeding in camelina.

*Camelina sativa* is a recent allohexaploid species ($2n = 6x = 40$) that originated from hybridization events involving three ancestral genomes (Mandáková and Lysak 2022). As a consequence of whole-genome triplication, extensive gene retention has occurred across subgenomes, including genes involved in lipid metabolism. Genome-wide analyses have identified a total of 62 fatty acid desaturase (*FAD*) genes in camelina, with major desaturases such as *FAD2*, *FAD3*, *FAD7*, and *FAD8* typically present as three homeologous copies located on different chromosomes (Raboanatahiry et al. 2021). Phylogenetic and gene structure analyses revealed intron gain and loss events as well as variation in conserved histidine motifs among FAD copies, while expression profiling showed differential accumulation across tissues such as leaves, flowers, and developing seeds and variation in response to environmental stress rather than strict functional redundancy (Ahmadizadeh et al. 2020; Sun et al. 2022). Functional analyses in transgenic Arabidopsis demonstrated that although both *CsaFAD7* and *CsaFAD8* increased C18:3 levels, overexpression of *CsaFAD7* led to reduced seed oil content, decreased thousand-seed weight, and wrinkled seed phenotypes, whereas *CsaFAD8* did not cause significant alterations in seed morphology or oil accumulation (Raboanatahiry et al. 2021), indicating distinct phenotypic consequences despite their similar enzymatic roles. Together, these findings highlight the complexity of the *FAD* gene family in camelina and underscore the importance of integrating polyploid genomic context into genetic analyses of fatty acid traits.

Recent advancements in genomic science have resulted in the development of camelina genome sequences and molecular markers that are associated with important agronomic traits (Decker et al. 2025; Bird et al. 2025; Huang Li et al. 2021; Brock et al. 2024). Combining genetic mapping in a recombinant inbred line (RIL) population and transcriptomic profiling of heat response contrasting lines, we were able to identify quantitative trait loci (QTLs) and candidate genes that affect reproductive fertility during heat stress (Smith et al. 2024). Here, we focused on the seed-filling stage and examined the fatty acid composition influenced by high growth temperatures. Using a high-density molecular map comprising markers derived from an improved camelina reference genome, we identified QTLs for major fatty acids in camelina seeds under heat stress. Several genes for fatty acid desaturases were found in the QTLs. Our findings contribute to a deeper understanding of the genetic basis of thermotolerance and provide genetic tools for developing heat-resilient camelina by marker-assisted selection.

## Materials and Methods

### Plant Materials and Growth Conditions

A population consisting of 257 recombinant inbred lines (RILs) was previously developed by single-seed descent (SSD) from a cross between two contrasting genotypes of *Camelina sativa* Suneson (Montana State University variety) and Pryzeth (MSU collection accession number: CS251) (King et al. 2019). The two parental genotypes are different in multiple traits such as seed size, oil content and seeds per pod, and heat sensitivity during reproductive growth (Smith and Lu 2024; King et al. 2019; Huang Li et al. 2021). The plants were grown in 4-inch pots filled with a 1:1 mix of topsoil and Sunshine Mix (Clinton, OK, USA) at Montana State University's Plant Growth Center. The experiment evaluated the RILs at the F6-7 generation, using a Randomized Complete Block Design (RCBD) with 3 blocks. Each RIL was represented by one pot per block under each treatment, resulting in 3 independent pots per RIL per treatment. Two

plants were grown in each pot in a growth chamber with a 16 h light (6:00–22:00) and 8 h dark (22:00–6:00) photoperiod at 21–22 °C and a light intensity of 325 µmol $m^{-2}$ $s^{-1}$. The pot was considered the experimental unit, and fatty acid measurements were averaged at the pot level prior to statistical and QTL analyses. For the heat treatment batch, we used a similar design by growing plants in a controlled growth chamber. The treatment was initiated shortly after flowering when six pods were visible on the main stem and tagged with color strings. The temperature was ramped up from 22 to 35 °C from 6:00 to 22:00, maintained at 35 °C until 16:00, and ramped down to 22 °C from 16:00 to 18:00, and maintained at 22 °C until 6:00 (Smith and Lu 2024). Plants were grown in the chamber for a period of 14 days and then transferred back to the control chamber. Throughout the experiment, all plants were regularly watered and fertilized to maintain optimal nutrient availability and prevent confounding effects due to drought or nutrient deficiency.

### Fatty Acid Analysis

At full physiological maturity, we collected the tagged pods within each pot from the two plants, dried and stored them. The analysis of fatty acid composition and the determination of total oil content were conducted by converting fatty acids into fatty acid methyl esters (FAME), which were subsequently analyzed using gas chromatography (Agilent Technologies 7890 A) as previously described (Snapp et al. 2014). The susceptibility index was calculated according to the formula given by Fisher and Maurer (1978).

$$\mathrm{HSI} = \frac{(1 - (Y_D / Y_P)}{D}$$

where

$Y_D$ = mean of the genotypes in stress environment
$Y_P$ = mean of the genotypes under non-stress environment
D = 1 - (mean $Y_D$ of all genotypes/mean $Y_D$ of all genotypes)

Mean values of each fatty acid trait for each RIL under each treatment were used for QTL mapping and Pearson's correlation analysis. All statistical analyses were performed in R software (R Core Team 2025).

### Genotyping and Marker Development

Whole-genome resequencing of the RILs was performed previously (Huang Li et al. 2021). Reads with more than 95% simple sequence content or shorter than 50 bp after trimming adapters and low-quality bases ($q < 20$) were removed. The filtered reads were then aligned to the Suneson reference genome (Decker et al. 2025) using BWA-MEM (v0.7.18) (Heng Li and Durbin 2010). The single nucleotide polymorphism (SNP) variation of the population was detected using GATK4 (4.6.1.0). SNPs and indels were filtered based on standard GATK hard-filtering parameters. Further filtering was performed using bcftools (Danecek et al. 2021) and VCFtools (Danecek et al. 2011) to retain only biallelic SNPs with a minor allele frequency (MAF) greater than 0.05 and missing rate less than 10%. Individuals with high heterozygosity were also removed.

### Genetic Mapping and Identification of QTL

The high-quality SNPs were binned into bin markers using SNPbinner (Gonda et al. 2019) with a minimum bin size of 1000 bp. The bin genotype matrices of each chromosome were then used to construct genetic linkage maps with LepMap3 (Rastas 2017). QTL mapping was performed in R/qtl (Broman et al. 2003). Genotype probabilities were computed every 1 cM using the Kosambi map. Stepwise multiple-QTL mapping (stepwiseqtl) was performed using main-effect penalties derived from 100 two-dimensional permutations (scantwo, $\alpha = 0.05$).

## Results

### Heat Conditions Influence Seed Fatty Acid Composition

The fatty acid composition of camelina seeds was determined by gas chromatography (GC). Major fatty acids were analyzed, including saturated fatty acids (SFAs, e.g., C16:0 and C18:0) and unsaturated fatty acids (UFAs, e.g., C18:1, C18:2, C18:3, and C20:1). These fatty acids were further grouped into monounsaturated fatty acids (MUFA: C18:1, C20:1) and polyunsaturated fatty acids (PUFA: 18:2, 18:3). Under heat stress, the contents of SFA, C18:1, C18:2, and MUFA increased significantly compared to the control, while C18:3, total UFA, PUFA, and the PUFA/MUFA ratio decreased significantly (Fig. 1, Supplementary Table 1). Specifically, the mean UFA decreased from 90.1% to 86.6% whereas SFA increased from 9.9% to 13. 5% and PUFA/MUFA ratio decreased from 1.8 to 1.3 (Supplementary Table 1). This shift suggests that fatty acid desaturation or subsequent metabolic conversion may be altered under heat stress, resulting in the observation of increased accumulation of saturated and monounsaturated fatty acids and a reduction in polyunsaturated fatty acids.

Correlation analysis of the reconstituted fatty acid composition may reflect the altered lipid metabolic network in plants under heat stress. Under control conditions, C16:0 and

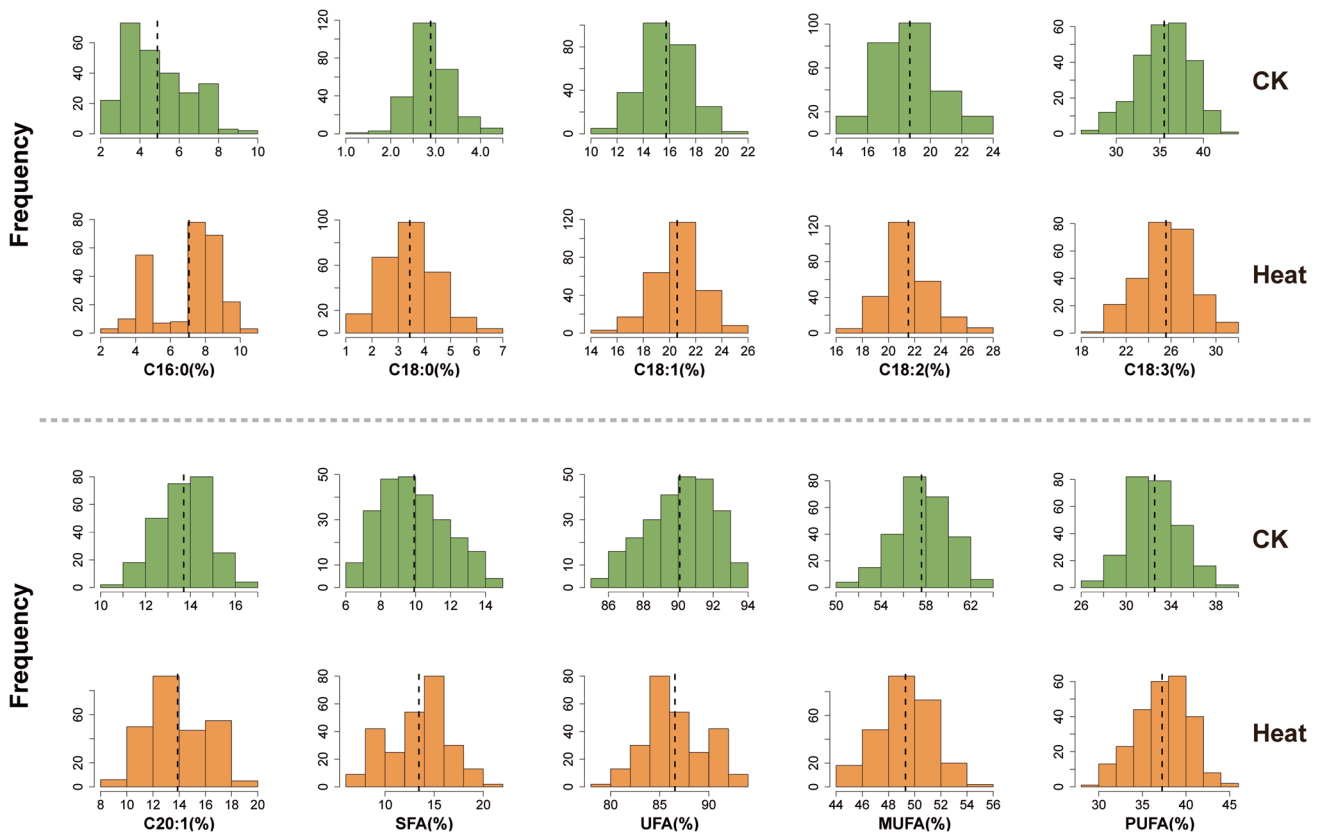



**Fig. 1** Frequency distribution of major fatty acids in *Camelina sativa* RIL population under different temperatures. Vertical dashed lines represent the mean values. *SFA* saturated fatty acids, *UFA* unsaturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

C18:0 were not correlated ($r = -0.097$, $P > 0.05$); however, heat stress dramatically altered this relationship showing a strong positive correlation ($r = 0.737$, $P < 0.01$) (Fig. 2, Supplementary Table S2). The relationship of C18:3 with other fatty acids (e.g., C18:1 and C18:2) was also changed slightly under heat stress. Its negative correlation with linoleic acid (C18:2) decreased from $r = -0.667$ to $r = -0.580$ ($P < 0.01$), while its negative correlation with oleic acid (C18:1) strengthened from $r = -0.413$ to $r = -0.527$ ($P < 0.01$).

Heat stress also reshaped the PUFA/MUFA ratio. Its association with PUFA declined from $r = 0.854$ to $r = 0.683$ ($P < 0.01$), whereas the strong negative association with

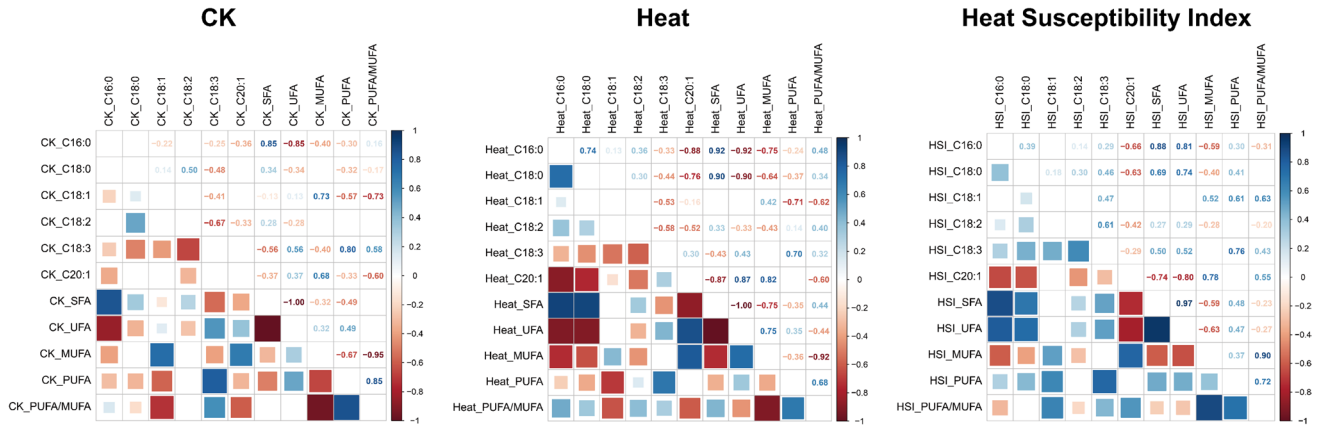



**Fig. 2** Trait correlation matrices for fatty acid profiles under control, heat stress, and heat susceptibility index. The color and size of each square indicate the strength and direction of the Pearson correlation coefficients. Only statistically significant correlations are shown

MUFA was largely preserved ($r = -0.952$ vs $-0.917$, $P < 0.01$) (Fig. 2, Supplementary Table S2). These relationships suggest that variation in the ratio aligns more closely with MUFA and less with PUFA, reflecting increased instability of the latter under heat stress.

To assess the variation of heat tolerance in the population, heat susceptibility indices (HSI) of the RILs were calculated. Correlation analysis of HSI revealed coordinated responses among fatty acid traits (Fig. 2). Strong positive correlations were observed among saturated fatty acids, such as HSI_C16:0 and HSI_C18:0 ($r = 0.39$, $P < 0.01$), indicating coordinated sensitivity among saturated fatty acids. HSI_C18:3 was positively correlated with HSI_C18:1 and HSI_C18:2 ($r = 0.46$–$0.61$, $P < 0.01$), suggesting that the magnitude of response across the C18:1 → C18:2 → C18:3 desaturation pathway is coordinated. In addition, heat-tolerant lines could be identified based on low HSI values for key traits like C18:3 and PUFA/MUFA ratio. Lines with lower C18:3 levels appeared to have greater stability under heat stress conditions.

## Construction of a High-Density Genetic Map

We previously performed QTL studies in the RIL population based on markers derived from the DH55 reference genome generated in 2014 (Kagale et al. 2014). To improve the genetic map, we reanalyzed the whole-genome resequencing data of the RIL population (257 individuals) and their parents (Suneson and Pryzeth) using a newly assembled Suneson genome (Decker et al. 2025; Brock et al. 2024). After a series of strict filtering steps, 228,858 high-quality SNPs were retained and subsequently binned into 4981 bin markers using SNPbinner. Most bin sizes fall within 0–500 bp (Fig. 3B). The 4981 bins were assigned to 20 linkage groups (LGs) corresponding to the 20 chromosomes (Fig. 3C). Per-chromosome bin-marker counts ranged from 68 to 416 (Fig. 3A; Supplementary Table S3).

The resulting genetic map spanned 2834.13 cM in total. This map was significantly improved over the previous map comprising 2376 SNPs spanning 2034.6 cM. Individual LGs ranged from 42.51 cM (Chr10; 68 markers) to 222.34 cM (Chr11; 416 markers), with a median LG length of 147.99 cM (Supplementary Table S3). The overall mean inter-marker spacing was 0.57 cM, while the per-LG median of mean spacing was 0.57 cM. The largest single inter-marker gap occurred on Chr4 (12.55 cM); across LGs, 4 had a maximum gap > 5 cM, and 2 had a gap > 10 cM (Supplementary Table S4). Together, these statistics indicate a dense, well-ordered linkage map providing sufficient resolution for robust QTL detection.

## QTL Analysis of Fatty Acids Under Control and Heat Treatments

QTL mapping identified 25 QTLs for eight fatty acid traits in seeds harvested from control and heat stress conditions (Table 1). The LOD scores ranged from 3.07 to 6.53, with phenotypic variance explained (PVE) values between 5.01% and 10.74%. Under control conditions, 9 QTLs were identified on LGs 9, 12, 13, 17, and 19. Heat stress yielded 16 QTLs on LGs 1, 3, 7, 9, 10, 11, 13, 16, and 20. The strongest signal (SNP01@80.216) was detected under heat stress, controlling the PUFA/MUFA ratio with a LOD score of 6.53 and explaining 10.74% of the phenotypic variance.

In this study, all QTLs exhibited strong condition-specific effects, with no locus showing consistent effects between control and heat stress conditions. Several chromosomal regions emerged as hotspots harboring multiple QTLs for different fatty acids. Under control conditions, Chr09 (115—155 cM) contained three QTLs, including a noticeable co-localization at 128.14 cM where SFA and UFA QTLs showed opposite additive effects (+0.51 vs −0.51), reflecting their inverse relationship in fatty acid synthesis. Similarly, Chr12 (103 −134.8 cM) harbored QTLs for C18:1 and PUFA/MUFA ratio within overlapping confidence intervals. Under heat stress, Chromosome 1 became a major hotspot with multiple QTLs clustering around 80 cM, controlling C18:1, C18:2, and PUFA/MUFA ratio, while Chr13 (99.4—120.6 cM) showed co-localized SFA and UFA QTLs with opposite effects. Additionally, Chr16 (100–112.8 cM) harbored co-localized QTLs for C18:2 and C18:3 under heat stress, and Chr20 (108.1–128.6 cM) contained QTLs for C18:0, SFA, and UFA under heat stress. These hotspot regions might suggest the presence of either pleiotropic genes or tightly linked gene clusters that coordinately regulate multiple aspects of fatty acid biosynthesis under specific environmental conditions.

## Candidate Genes Within Major QTLs

To search for candidate genes that influence fatty acid composition, we focused on hotspot QTL regions identified under heat conditions. The regions contain about 6751 putative genes. Pinpointing candidate genes would require further fine mapping and testing. Interestingly, we identified three fatty acid desaturase (*FAD*) genes within the hotspot regions (Table 2). *FAD2* and *FAD7* resided in Chr01 (around 80 cM), and *FAD8* was found in Chr20 (108.1–128.6 cM). These candidate genes are functionally relevant to the observed QTL effects, as *FAD2* catalyzes the conversion of C18:1 to C18:2 on the endoplasmic reticulum, while *FAD7* and *FAD8* are responsible for the desaturation of C18:2 to C18:3 in the plastids. The co-localization of these desaturase genes with their corresponding QTLs strongly suggests their

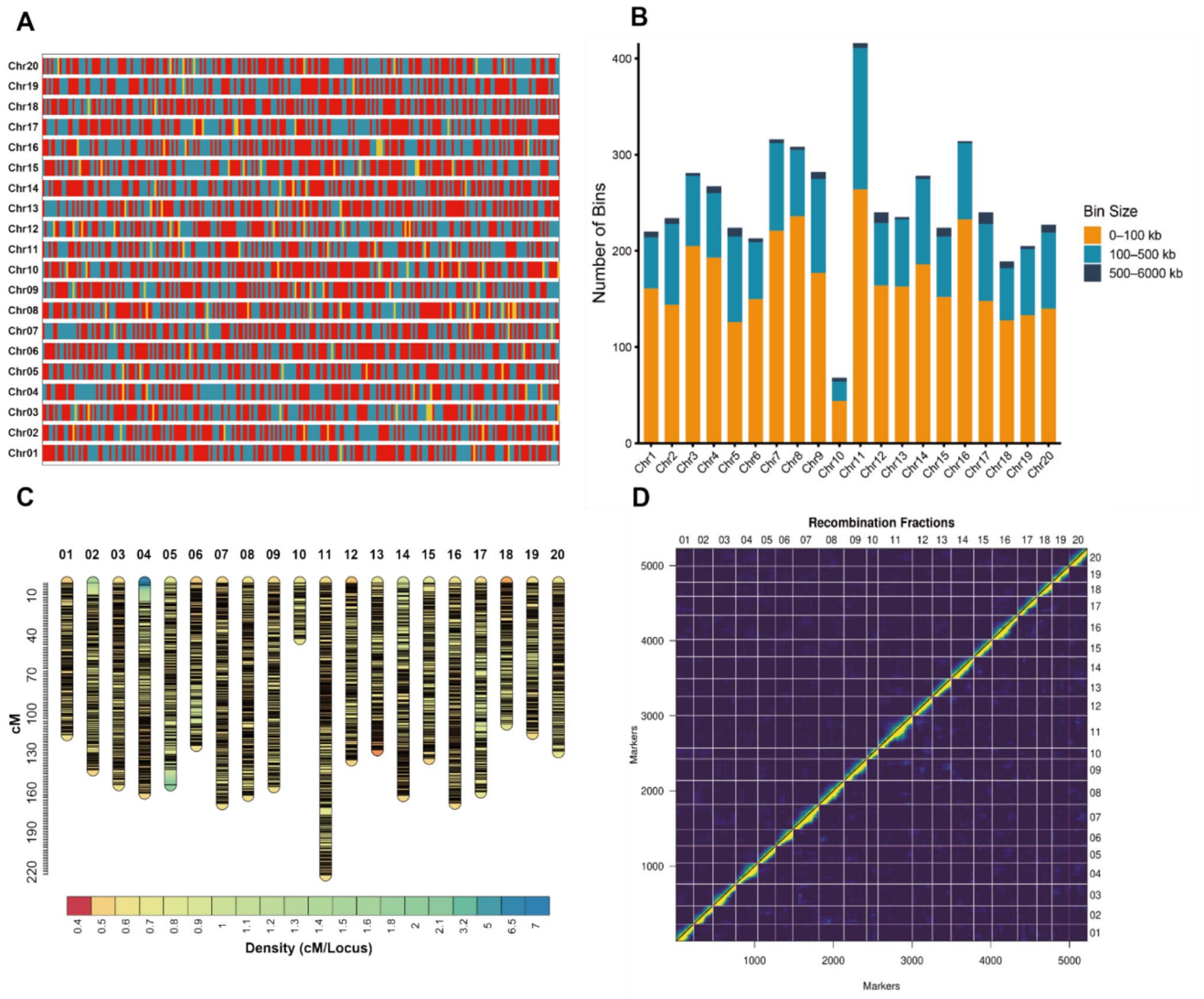



**Fig. 3** **A** The bin map of the RIL population. Each column represents an individual RIL. Blue indicates genomic regions derived from the Pryzeth parent. Red indicates regions from the Suneson parent, and yellow represents heterozygous regions. **B** Distribution of bin length across 20 chromosomes. **C** Genetic linkage map of *C. sativa*. **D** Recombination fraction matrix for the RIL population

roles in controlling fatty acid composition under heat stress conditions.

## Discussion

Fatty acids as major components of cellular membranes play critical roles in plants facing different environments such as temperature fluctuations. Polyunsaturated fatty acids enhance membrane fluidity, which is beneficial under normal and cool conditions but a lower level may enhance the fitness under high temperatures by preventing excessive membrane fluidity and maintaining structural stability (Murakami et al. 2000). Fatty acids in storage oils may also be changed by the growth temperatures. This study demonstrates that in the seed of *Camelina sativa*, saturated and monounsaturated fatty acids increased their concentrations under high temperatures, while the polyunsaturated forms, especially linolenic acid (C18:3), decreased. This shift is not unique to camelina; it has also been described in oilseeds like *Brassica napus* and in Arabidopsis (Baux et al. 2013; Kourani et al. 2022).

The correlation between different fatty acids or groups of fatty acids in a camelina population revealed coordinated modification of lipid composition responding to different growth temperatures. For example, a strong positive link was observed between palmitic acid (C16:0) and stearic acid (C18:0) under stress pointing to a linear route

**Table 1** QTL analysis for fatty acids under control and heat stress

| QTL | Trait | Group | Chr | Position (cM) | LOD | Genetic region (cM) | Physical region (bp) | Add | PVE (%) |
|---|---|---|---|---|---|---|---|---|---|
| SNP09@140.435 | C16:0 | Control | 9 | 140.44 | 3.31 | 98.138–155 | 1,827,047–22,233,307 | 0.40 | 5.80 |
| SNP09@55.632 | C16:0 | Heat | 9 | 55.63 | 3.12 | 50–72 | 27,428,293–31,342,526 | 0.44 | 5.47 |
| SNP12@76 | C18:0 | Control | 12 | 76.00 | 3.17 | 69–93 | 5,252,068–7,717,400 | −0.10 | 5.83 |
| SNP07@166.477 | C18:0 | Heat | 7 | 166.48 | 3.57 | 148.349–168.144 | 30,628,536–33,456,892 | −0.24 | 6.04 |
| SNP20@118.345 | C18:0 | Heat | 20 | 118.35 | 3.28 | 109.177–128.556 | 428,269–3,138,590 | 0.23 | 5.53 |
| SNP12@120.642 | C18:1 | Control | 12 | 120.64 | 3.81 | 103–134.81 | 578,814–4,603,131 | 0.47 | 6.15 |
| SNP13@32.087 | C18:1 | Control | 13 | 32.09 | 3.73 | 2.01–38 | 1,417,584–5,419,266 | −0.46 | 6.01 |
| SNP01@79.383 | C18:1 | Heat | 1 | 79.38 | 3.07 | 21.878–93 | 3,466,608–11,635,139 | −0.43 | 5.01 |
| SNP10@20.84 | C18:1 | Heat | 10 | 20.84 | 3.08 | 0–34.592 | 6,837,980–15,114,457 | −0.44 | 5.04 |
| SNP19@45.424 | C18:2 | Control | 19 | 45.42 | 3.36 | 0–75 | 793,864–11,981,620 | −0.4 | 6.32 |
| SNP01@80.633 | C18:2 | Heat | 1 | 80.63 | 3.39 | 74.174–115.637 | 0–5,506,428 | 0.45 | 5.37 |
| SNP11@202.55 | C18:2 | Heat | 11 | 202.55 | 4.01 | 189.838–212 | 5,324,342–7,706,011 | 0.48 | 6.37 |
| SNP16@128.765 | C18:2 | Heat | 16 | 128.77 | 3.24 | 100.013–145 | 4,555,706–18,393,598 | 0.43 | 5.11 |
| SNP17@121.295 | C18:3 | Control | 17 | 121.30 | 3.78 | 94.819–127 | 7,083,661–11,154,717 | 0.73 | 6.91 |
| SNP01@26.461 | C18:3 | Heat | 1 | 26.46 | 4.68 | 21.044–36 | 9,647,501–11,668,626 | 0.70 | 7.79 |
| SNP16@92.512 | C18:3 | Heat | 16 | 92.51 | 3.21 | 78.136–112.722 | 13,554,033–21,329,394 | −0.58 | 5.26 |
| SNP12@123 | PUFA/MUFA | Control | 12 | 123.00 | 4.31 | 103–134.81 | 578,814–4,603,131 | −0.05 | 7.52 |
| SNP01@80.216 | PUFA/MUFA | Heat | 1 | 80.22 | 6.53 | 70–83.55 | 4,441,455–6,050,325 | 0.05 | 10.74 |
| SNP03@28.127 | PUFA/MUFA | Heat | 3 | 28.13 | 3.87 | 22.502–48.337 | 14,072,804–24,997,636 | −0.04 | 6.20 |
| SNP09@128.141 | SFA | Control | 9 | 128.14 | 4.29 | 115.015–145 | 3,165,995–18,671,892 | 0.51 | 7.66 |
| SNP13@115.223 | SFA | Heat | 13 | 115.22 | 3.72 | 99.389–120.64 | 12,771,214–22,656,980 | −0.78 | 6.18 |
| SNP20@121.054 | SFA | Heat | 20 | 121.05 | 3.22 | 108.136–128.556 | 428,269–3,253,385 | 0.73 | 5.32 |
| SNP09@128.141 | UFA | Control | 9 | 128.14 | 4.29 | 115.015–145 | 3,165,995–18,671,892 | −0.51 | 7.66 |
| SNP13@115.223 | UFA | Heat | 13 | 115.22 | 3.72 | 99.389–120.64 | 12,771,214–22,656,980 | 0.78 | 6.18 |
| SNP20@121.054 | UFA | Heat | 20 | 121.05 | 3.22 | 108.136–128.556 | 428,269–3,253,385 | −0.73 | 5.32 |

**Table 2** Candidate fatty acid desaturase genes within major QTL regions

| Gene ID | Start | End | Description |
|---|---|---|---|
| CS01g001250 | 4,779,159 | 4,782,425 | FAD2: Delta (12)-fatty-acid desaturase |
| CS01g001140 | 4,323,160 | 4,326,163 | FAD7: sn-2 acyl-lipid omega-3 desaturase |
| CS20g000549 | 2,187,043 | 2,189,536 | FAD8: Temperature-sensitive sn-2 acyl-lipid omega-3 desaturase |

of biosynthesis; while the increased negative relationship between C18:3 and C18:1 indicates a reduction in desaturation efficiency. This suggests that certain enzymes, particularly the plastidial desaturases *FAD7* and *FAD8*, are highly sensitive to temperature and may slow down or shut off when the heat rises (Wickramanayake et al. 2020). To support this, candidate genes encoding *FAD2*, *FAD7*, and *FAD8* were found in QTL regions. Similar effects have been reported in other species such as canola (Lohani et al. 2022), oilseed rape (Brunel-Muguet et al. 2015) and wheat (Chen et al. 2025), reinforcing the idea that trienoic fatty acids are the most sensitive to temperature fluctuations and are among the first to be sacrificed in the interest of stability. Given that each of these genes is represented by multiple homeologous copies in the hexaploid camelina genome, the detected QTLs likely reflect allelic variation at specific subgenome loci rather than uniform effects of the entire gene family.

The QTL mapping results provide further insight into the genetic architecture underlying these responses. We identified 25 QTLs for fatty acid traits across control and heat conditions, with most loci exhibiting strong environment specificity and minimal overlap between treatments (control vs. heat conditions). This underlines the complexity of the genotype × environment interactions. Such environment-specific QTLs are consistent with differential regulation of lipid biosynthesis genes under heat stress,

rather than constitutive genetic effects. QTL hotspots were found on chromosomes 1, 9, 12, 13, and 20, which often contained multiple QTLs for biosynthetically related fatty acids. The strongest signal was on chromosome 1, where a single region explained more than 10% of the variation in the PUFA/MUFA ratio under stress. The moderate variance explained by individual QTLs reflects the polygenic control of fatty acid metabolism. Further analysis of such loci may lead to discoveries of novel alleles besides fatty acid desaturases for fatty acid metabolism and molecular markers for selection in breeding programs.

Given the allohexaploidy of camelina, the identification of *FAD2*, *FAD7*, and *FAD8* within major QTL intervals requires consideration of its polyploid genome structure. Each of these genes is represented by three homeologous copies distributed across different chromosomes as a consequence of polyploidization (Mandáková and Lysak 2022). Previous studies have demonstrated intron gain and loss events, variation in conserved domains, differential expression across tissues, and distinct phenotypic consequences among members of the *FAD* gene family (Blume et al. 2024; Sun et al. 2022). Therefore, the QTL intervals identified here likely reflect allelic variation in specific homeologs rather than uniform contributions of all copies. Subgenome-specific regulatory effects may explain why only particular loci were detected as credible candidates and why most QTLs were environment specific. These findings underscore the importance of considering polyploid genome structure when interpreting candidate gene loci for complex metabolic traits.

From a breeding perspective, the observed variation in heat sensitivity index (HSI) across the RIL population revealed valuable genetic diversity for improving temperature resilience. Lines that maintained relatively stable C18:3 levels and balanced PUFA/MUFA ratios under heat stress may provide useful parental material for breeding programs aimed at stabilizing oil quality under warming climates. Integrating QTL information with HSI and fatty acid stability profiles could facilitate marker-assisted selection for alleles that buffer lipid composition against temperature fluctuations.

Despite the robust QTL signals detected in this study, several limitations should be acknowledged. Candidate genes were nominated based on positional overlap and known biological functions, but functional validation through homeolog-specific expression analysis, allelic sequencing, or reverse genetics approaches was not performed. Moreover, heat treatments were conducted under controlled conditions, and multi-environment field validation will be necessary to determine the stability and breeding utility of these loci under agronomically relevant conditions. Future studies integrating fine mapping, transcriptomics, and genome editing approaches will further elucidate the molecular basis of heat-responsive fatty acid regulation and accelerate the development of temperature-resilient camelina varieties. Integrating QTL information with breeding strategies that effectively utilize existing genetic diversity will facilitate the enhancement of oil quality stability under changing climatic conditions (Ghidoli et al. 2024).

## Conclusion

This study demonstrated the variation of seed fatty acid composition of *Camelina sativa* in a recombinant inbred line population. Exposure to high temperature during seed-filling stages significantly increased the proportions of C16:0, C18:0, and C18:1, while reducing C18:3, UFA, and the PUFA/MUFA ratio, indicating inhibition of the C18:1 → C18:2 → C18:3 desaturation pathway. A high-density linkage map (4981 bin markers across 20 chromosomes) resolved 25 QTLs, including hotspots on chromosomes 1, 9, 12, 13, 16, and 20, for various seed fatty acids. A major QTL on chromosome 1 (~ 80 cM) explained the greatest proportion of variance for PUFA/MUFA under heat stress. Three fatty acid desaturase genes (*FAD2*, *FAD7*, and *FAD8*) were located within key QTL intervals, suggesting potential roles in mediating lipid desaturation under elevated temperatures. Collectively, the findings of this study, including identified molecular markers and potential heat-tolerant genotypes, provide valuable resources to understand the genetic control of thermal modulation of seed oil composition in *Camelina sativa* and for the future development of heat-tolerant varieties with improved oil quality.

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**Data Availability** All data supporting the findings of this study are available from the corresponding author upon request.

## Declarations

**Competing interests** The authors declare no competing interests.

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