Clone and Function Verification of the OPR gene in Brassica napus Related to Linoleic Acid Synthesis

Min Tan¹, Juan Niu², Duo Zi Peng¹, Qian Cheng¹, Ming Bao Luan²* and Zhen Qian Zhang¹*

Abstract

Background: Fatty acid composition and content affect rapeseed oil quality. Fatty acid synthesis-related genes in rapeseed have been studied globally by researchers. Nevertheless, rapeseed oil is mainly composed of seven different fatty acids (FA), and each fatty acid was regulated by different genes. Furthermore, different FA affect each other, which needs continuous and in-depth research to obtain more clear results in Brassica napus.

Results: In this paper, broad-scale miRNA expression profiles were constructed and 21 differentially expressed miRNAs were detected. GO enrichment analysis showed that most up-regulated proteins were involved in transcription factor activity and catalytic activity. KEGG pathway enrichment analysis indicated that 20 pathways involving 36 target genes were enriched, of which the bna005920 pathway may be involved in fatty acid metabolism. The results were verified using a quantitative real-time PCR (RT-qPCR) analysis, we found that the target gene of bna-miR156b > c > g was the OPR (12-oxo-phytodienoic acid reductase). Four copies of OPR gene were found, and the over-expression vectors (pCAMBIA1300-35 s-OPR and pCAMBIA1300-RNAi-OPR) were constructed to verify their functions. In T₁ and T₂ generation, the content of linoleic acid (LA) increased significantly in OE but deceased in OPRi.

Conclusions: This is the first study to provide four copies of the OPR gene that regulates LA metabolism, can be used for the molecular mechanism of LA and optimizing fatty acid profiles in oilseed for breeding programs.

Keywords: Rapeseed, OPR gene, Linoleic acid, RNAi, Overexpression

Background

Brassica campestris L. (rapeseed) is one of the most important oil crops in the world [1, 2]. The quality of rapeseed oil mainly depends on its fatty acid composition, especially the proportion of three main unsaturated FA: oleic acid (OA, C₁₈:1), LA (C₁₈:2), and linolenic acid (C₁₈:₃) [3]. Many studies have shown that rapeseed oil with a high unsaturated fatty acid content will have better health effects and can prevent the occurrence of cardiovascular diseases [4]. LA can prevent or reduce the incidence of cardiovascular diseases and being used for the prevention and treatment of hypertension, hyperlipidemia, angina pectoris, coronary heart disease, atherosclerosis, and senile obesity [5, 6]. LA could be hydrogenated to produceα-linolenic acid (ALA) andγ-linolenic acid (GLA) [7].

miRNAs are short noncoding regulatory RNAs that regulate gene expression via post-transcriptional repression [8, 9]. In plants, miRNAs are involved in various biological processes, including the regulation of plant development [10], architecture formation [11], photosynthesis [9], tolerance to biotic and abiotic stresses [12–15]. In recent years, several new miRNAs have been found in oilseed rape [16–18]. The process of seed development
is a period that starts at embryo development and ends when dry seeds are mature; it is a key stage that affects the seed size, oil production, protein content, and anti-nutritional accumulation of rapeseed. Furthermore, gene expression changes during seed development [19–21].

Recently, miRNAs and their target transcripts involved in fatty acid and lipid metabolism have been studied in developing seed of *Brassica napus*, which is considered the third largest oil crop worldwide [22, 23]. The generation of acetyl-CoA and carbon chain desaturase were also observed; nevertheless, the total number of known miRNAs and their functions in *Brassica napus* are still unknown [23].

The development of molecular biology technology has greatly promoted the research on LA breeding in rape-seed. (I) In terms of mining the function of genes, a study showed significant correlation between *FAD3* and LA content [24], while another study revealed that the over-expression of *SaDGAT1* significantly affected LA content (about 16%) [25]. *McD6DES* generates a double bond at the carboxyl end of LA, which reduces the LA content [26]; *BJULFY* increases LA content (approximately 5%) [27]. The single nucleotide mutation of *FAD3* exon from G to A was screened from the rapeseed “Alboglabra”, which treated with an Ethyl Methyl Sulfone (EMS) solution, and the mutant materials with high LA content and low alanine content (about 2.0%) were obtained [28]. (II) In terms of screening the genes, a genome wide association study (GWAS) discovered 53 and 24 SNP related to LA in 2013Cq and 2014Cq rapeseed materials, respectively [29]; when combined with RT-qPCR, 95 candidate genes were found to be highly related to LA and other fatty acid metabolism [30]. (III) In terms of locating the genes, *FAD2* gene on chromosome A05 was found to be associated with LA content, and minor gene regulating LA content was found on chromosome A09 [31]; twenty QTLs related to LA were detected using two spring rapeseed varieties, “Polo” and “Topas”, and subsequently distributed in seven linkage groups: A01, A02, A03, A05, C01, C03, and C09 [32]. In fact, many studies indicate that OA metabolism depend on *FAD1* or *FAD2* activity [33, 34], and the have present the first evidence of Δ7 desaturation via the *FADS1* gene product by Hui et al.[35] LA are used as precursors of ultra-long chain polyunsaturated FA, such as α-linolenic acid (ALA) and γ-linolenic acid (GLA), which are hydrogenated by desaturation enzymes [36]. Correlations between oleic versus linoleic and oleic versus linolenic were negative and highly significant, and correlations between linoleic versus linolenic were of lower magnitude by Kondra et al. [37]. According to Belo et al. [38], the impact of presenting an interesting study focusing on environmental effects on oil quality of oil crops, who showed that the differential effects of climate conditions had an effect on fatty acid composition.

miRNA technology has been adopted to find several miRNAs in enzymes used for carbon chain desaturation, while being used in studies on fatty acid and lipid metabolism in rapeseed [22]. Employing this technology can be conducive to the breeding of rapeseed [23]. Therefore, we sequenced rapeseed material using miRNA technology. Then, we excavated four copies of *OPR* genes. The four copies of gene overexpression and RNAi vectors were transferred into *A. thaliana*. This is the first study to report that all copies of *OPR* gene could directly regulate the synthesis of LA. These findings will enhance the understanding of LA metabolism in rapeseed.

**Results**

**Overview of sRNA sequencing results**

We have already upload data and ensure the deposited data is made public (NCBI): https://www.ncbi.nlm.nih.gov/sra/PRJNA760803, accession number: PRJNA760803. A total of 22,744,964 (LOAR: low OA rapeseed materials, A) and 26,060,122 (HOAR: high OA rapeseed materials, B) raw reads were generated from the sequencing machine. After removing the adaptor sequences, filtering out low quality tags, and cleaning up sequences derived from adaptor ligation, 20,912,776 (A) and 23,710,938 (B) clean reads were obtained. Consequently, the bioinformatic analysis of these clean reads were carried out (Table 1). The size distribution patterns of the original and unique reads were displayed in Fig. 1A. Small RNAs (24 nt) were the most abundant in all the samples. In addition, the clean reads exhibited 87.45% (A) and 88.26% (B) homology with the reference genome sequences. The sRNA sequencing results were of high quality and reliable and can be used for further functional analysis.

**Differentially expressed miRNAs identification and their function analysis**

The clustering analysis method was used to investigate the similarity between samples by calculating the differential miRNA distance between LOAR (A) and HOAR (B) (Fig. 1B). As shown, three samples of high or low OA materials were found in a cluster, suggesting that these miRNAs might have similar biological functions.

After applying a *P*-value < 0.05 and an absolute value of log2 (treatment/control: LOAR/HOAR) greater than 1.5 to identify differentially expressed miRNAs, 21 differentially expressed miRNAs (8.39% of the total) were detected (Table 2; Supplemental Table 2); their frequencies were calculated using TPM (Table 3). Among them, nine genes (42.86%) were up-regulated and 12 (57.14%) were down-regulated in HOAR materials (Fig. 1C). Briefly, 21 miRNAs (including
21 miRNAs were found to target 358 genes. Subsequently, according to p-value < 0.05, 133 putative target genes were obtained by screening significantly enriched GO and KEGG pathways from 358 target genes.

The target genes were then subjected to GO functional and KEGG Pathway analyses. In many cases, multiple terms were assigned to the same miRNA. Thus, 133 putative target genes were associated with 21 differentially expressed miRNAs and distributed into the following subcategories: 62 “Biological process”, 32 “Cellular component”, and 39 “Molecular function” (Fig. 1D). Under “Biological process”, most of the target genes were related to “transcription”, and “regulation of transcription”. Within the “Cellular component” category, “nucleus” and “cytosol” were observed as much as “cytoplasm”. Among genes in the “Molecular function” category, most potential functions were related to “transcription factor activity”, “DNA binding”, and “Catalytic activity”. The distribution of target genes indicated that rapeseed underwent active metabolism.

The KEGG pathway enrichment analysis indicated that 20 pathways involving 36 target genes were enriched, including “alpha-Linolenic acid metabolism” (7 target genes), “Phagosome” (3 target genes), “Oxidative phosphorylation” (3 target genes), “Oxidative phosphorylation” (3 target genes), and “Protein processing in endoplasmic reticulum” (3 target genes). The fifteen target genes may be related to fatty acid metabolism: “alpha-linolenic acid metabolism” (7 genes), “Oxidative phosphorylation” (3 genes), “Carbon metabolism” (2 genes), “Citrate cycle (TCA cycle)” (1 gene), “Glycerolipid metabolism” (1 gene), and “Glycolysis / Gluconeogenesis” (1 gene). The top 20 KEGG enrichments (Fig. 2A) show that α-Linolenic acid metabolism is the most significant, suggesting that bna00592 KEGG pathway may be involved in fatty acid metabolism in rapeseed (Fig. 2B).

Expression pattern of bna-miR156b > c > g gene was detected by RT-qPCR

To confirm the results of the miRNA sequence analysis, 21 annotated differentially expressed miRNAs were compared to the Brassica napus genome using BLAST [39] (Fig. 2B).

Most expression trends of the RT-qPCR analysis results agreed with the miRNA sequencing data (NC_027757.1_266, NC_027760.1_5272, NC_027760.1_5272*, NC_027761.1_6665, NC_027761.1_5272*, NC_027761.1_17408, NC_027769.1_17408*, NC_027769.1_24533, NC_027774.1_24533, NW_013650328.1_26640*, bna-miR162a, bna-miR167a>b, bna-miR169m, bna-miR167d>b, bna-miR824, bna-miR396a, bna-miR824). In addition, among the 13 miRNAs with significant difference, 9 had
target genes (NC_027760.1_5272, NW_013650328.1_26640, bna-miR156b>c>g, bna-miR166f, bna-miR169n, bna-miR396a, bna-miR824, bna-miR156a, and bna-miR160a>b>c>d), which may be the novel miRNAs related to FA. Moreover, the expressions of fatty acid metabolism related to differential miRNAs, such as bna-miR396a, bna-miR156b>c>g, and their target genes, were studied in different developmental stages (Fig. 2C). The bna-miR396a has opposite expression pattern with its target gene, at first, the bna-miR396 had up-regulated expression, until the bud stage reached the peak, and the expression decreased with the growth stage; bna-miR156b>c>g had an opposite expression pattern with its target gene, it had down-regulated expression in the whole growth stages, and the expression decreased with the growth stages. Differentially expressed miRNAs and their target genes were related to fatty acid metabolism in bna-miR156b>c>g at different developmental stages (Fig. 1C). In contrast to the expression pattern of bna-miR156b>c>g and its target gene, the expression level was down-regulated throughout the whole growth stage of the plant, and the expression level gradually decreased with the growth process of rapeseed.

Cloning of OPR genes in rapeseed and bioinformatic analysis

Target gene: bna-miR156b>c>g was cloned by miRNA sequencing, and four copies were detected: GSBRNA2T0001242001, GSBRNA2T000135385001, GSBRNA2T00082938001, and GSBRNA2T00094910001, which were named OPR1, OPR2, OPR3, and OPR4, respectively. Both OPR1 and OPR3 were 1119 bp, OPR2 and OPR4 were 1125 bp and 1122 bp, respectively (Fig. 3A).
DNAMAN 7.0 software was used to compare the cloned target sequence with the raseepase sequence published on the B.napus Genome Browser website. Different base position (Fig. 3B), homology was more than 99% with the published sequences (Fig. 3C). OPR1, OPR3, OPR4 had no base difference with the published sequence, there were 10 base differences between OPR2 and published sequence and the homology was 99.11%. Preliminary identification of OPR2 and OPR3 were located in A genome and OPR4 and OPR1 in C genome was conducted.

The number of four copies of OPR gene amino acids ranged from 372 to 374 bp, with a molecular weight of about 41 kU; The encoded amino acids were acidic (<7), unstable (<40), exhibited a fat coefficient of about 75, and belonged to fat-soluble proteins. Predicted subcellular localization of proteins were encoded by different copies of OPR genes and we found that these four proteins were located in the cytoplasm. We found that the four copies were all extracellular proteins without a transmembrane structure. Predicted protein secondary structures were summarized in Table 4.

The predicted tertiary structure model of the protein shows that the tertiary structures of OPR1, OPR2, OPR3, and OPR4 were all adapted to the 12-O-plant dienoate reductase model (integrated with the crystal structure of At1g76680 protein in A. thaliana), but their
conformations were slightly different (Fig. 3D). It has been reported that the protein structure of A. thaliana of At1g76680 is similar to that of yeast ScOYE1 [40].

Vector construction and transformation of A. thaliana.
Using the synthesized cDNA as template, four target gene specific fragments were amplified by PCR with high fidelity, and the RNAi fragments with the same length were obtained (Fig. 3E). Then, the target genes were recombined with the overexpression vector pCAMBIA1300-35 s, positive strains were screened. After sequencing, the overexpression vectors pCAMBIA1300-35 s-OPR1 (OPR1-OE), pCAMBIA1300-35 s-OPR2 (OPR2-OE), pCAMBIA1300-35 s-OPR3 (OPR3-OE), and pCAMBIA1300-35 s-OPR4 (OPR4-OE) were obtained (Fig. 4A-B).

The RNAi fragment was recombined with the RNAi vector pCAMBIA1300-RNAi and the positive strains were screened. After sequencing, the RNAi vectors pCAMBIA1300-RNAi-OPR1 (OPR1i), pCAMBIA1300-RNAi-OPR2 (OPR2i), pCAMBIA1300-RNAi-OPR3 (OPR3i), and pCAMBIA1300-RNAi-OPR4 (OPR4i) were obtained (Fig. 4C).

The recombinant vector was transformed into A. thaliana and the obtained A. thaliana was detected. The T1 transgenic A. thaliana seeds were screened using hygromycin (Fig. 5A) and the results of hygromycin primer identification (Fig. 5B) showed that each copy of A. thaliana OPR had been successfully transformed, and the target plasmid T-DNA had been inserted into the genome of A. thaliana.

Analysis of fatty acid composition and fatty acid content
The transformation methods in A. thaliana, reference Clough and Bent (1998) [41]. fatty acid composition was detected by gas chromatography [42], we obtained fatty acid composition results of the T1 and T2 generations. Fatty acid composition of A. thaliana T-DNA insertion lines in Table 5, and transformation materials fatty acid composition in the contrast T1/T2. The contents of OA and stearic acid in OPR1i were significantly increased and the LA content decreased significantly; OPR1-OE will lead to a significant increase in palmitic acid and LA content; OPR2i significantly increased stearic acid content and decreased LA content; and OPR2-OE increased LA content.
The LA content in OPR3i decreased significantly; OPR3-OE significantly increased LA content; OPR4i significantly decreased LA content; and OPR4-OE increased LA content significantly.

Each copy of OPR-OE increased LA content, with an average increase of 12.56% in T1 generation and 7.185% in T2 generation. Subsequently, LA content in OPRi gene was significantly decreased, with an average decrease of 5.98% in T1 generation and 0.86% in T2 generation.

As shown in Table 5, oleic, linolenic, arachidonic, and erucic acids with the same variation trend as that of the fatty acid composition were selected for variance analysis. The results (Table 6) showed that the linolenic acid content in OPR1i significantly increased, while OPR2i significantly increased the linolenic acid content. Both OPR3-OE and OPR4-OE affected the content of arachidonic acid, which decreased significantly. In addition, OPR4i had no significant effect on the arachidonic acid content.

**Fig. 3** OPR genes PCR amplification, sequence and homology and the tertiary structure of protein analysis, RNAi fragment PCR amplification (A) Target gene PCR amplification (M: Trans 2000 bp; 1: OPR1; 2: OPR2; 3: OPR3; 4: OPR4); (B) Sequence alignment with the sequence published in Brassica Database; (C) Sequence homology analysis with Brassica Database; (D) Tertiary structure of protein (A. OPR1; B. OPR2; C. OPR3; D. OPR4); (E) Objective Gene RNAi fragment PCR electrophoresis map
We correlation analysis of FA in transgenic Arabidopsis seeds, according to the Table 7 shows that: In T1, the four OPR copies showed significant negative correlation between the OA, LA and linolenic acid content, OA mass fraction is higher, LA and linolenic acid mass fraction, the lower the relative; There was a positive correlation between LA and linolenic acid. Overall variation trend of LA and linolenic acid was basically the same. The mass fraction of linolenic acid was positively correlated with that of arachidonic acid. The stearic acid massed fraction and LA mass fraction had the same trend, and a larger trend with that of OA and linolenic acid. The mass fraction of palmitic acid was positively correlated with stearic acid, negatively correlated with OA (except OPR1), and positively correlated with LA and linolenic acid. The variation trend of FA in T1 and T2 generations is almost the same, there are also differences, and here was a significant negative correlation between LA and linolenic acid.

Table 4 Primary and secondary structure analysis of each copy

| structure                        | Analysis                                          |
|----------------------------------|---------------------------------------------------|
| Physical and chemical properties of primary structures | OPR1 | OPR2 | OPR3 | OPR4 |
| Number of amino acids            | 372     | 374  | 372  | 373  |
| Molecular weight                 | 41.4    | 41.57| 41.32| 41.28|
| Theoretical pi                   | 5.55    | 6.01 | 5.88 | 6.22 |
| Total number of positively charged residues(Arg + Lys) | 46     | 44   | 43   | 42   |
| Total number of negatively charged residues(Asp + Glu) | 37     | 38   | 37   | 38   |
| Formula                          | \(C_{1846}H_{3286}N_{80}\) \(O_{565}\) \(S_{15}\) | \(C_{1844}H_{3283}N_{80}\) \(O_{565}\) \(S_{16}\) | \(C_{1843}H_{3289}N_{80}\) \(O_{566}\) \(S_{15}\) | \(C_{1835}H_{3293}N_{80}\) \(O_{566}\) \(S_{16}\) |
| Instability index                | 38.82   | 35.76| 39.65| 38.57|
| Aliphatic index                  | 75.27   | 75.64| 75.27| 75.6 |
| Grand average of hydropathicity (GRAVY) | -0.355 | -0.359| -0.343| -0.325 |
| Secondary structure              | Alpha helix (Hh) 31.18  | 31.55  | 31.18  | 30.56  |
|                                 | Beta bridge (Bb) 0 | 0  | 0   | 0   |
|                                 | Extended strand (Ee) 12.63 | 12.03 | 12.63 | 12.87 |
|                                 | Beta turn (Tt) 6.72 | 6.42  | 7.26  | 8.04  |
|                                 | Random coil (Cc) 49.46 | 50  | 48.92 | 48.53 |

We correlation analysis of FA in transgenic Arabidopsis seeds, according to the Table 7 shows that: In T1, the four OPR copies showed significant negative correlation between the OA, LA and linolenic acid content, OA mass fraction is higher, LA and linolenic acid mass fraction, the lower the relative; There was a positive correlation between LA and linolenic acid. Overall variation trend of LA and linolenic acid was basically the same. The mass fraction of linolenic acid was positively correlated with that of arachidonic acid. The stearic acid massed fraction and LA mass fraction had the same trend, and a larger trend with that of OA and linolenic acid. The mass fraction of palmitic acid was positively correlated with stearic acid, negatively correlated with OA (except OPR1), and positively correlated with LA and linolenic acid. The variation trend of FA in T1 and T2 generations is almost the same, there are also differences, and here was a significant negative correlation between LA and linolenic acid.

Discussion

miRNA expression and enrichment analysis

In recent years, the number of known miRNAs has increased continually, such as A. thaliana [43] and Oryza sativa [44, 45]. Brassica napus L. has a relatively high genome size and complexity [39], and the number and function of miRNAs in Brassica napus L. have not been adequately studied; which suggests that many miRNAs have not yet been discovered, especially in seeds.

In this study, miRNA libraries were constructed from self-pollinated seeds that were collected 20–35 d after pollination of rapeseed with high OA content in the near-isogenic lines. Clean reads exhibited 87.45% (A) and 88.26% (B) homology with the referenced genome sequences (Table 1). The small RNAs (24 nt) were most abundant in all the samples (Fig. 1A). The clustering analysis results showed that three samples of high or low OA contents were found in a cluster, revealing that these miRNAs might have similar biological functions (Fig. 1B). A total of 21 differentially expressed miRNAs were detected (Table 2, Supplemental Table 2), including 9 (42.86%) up-regulated and 12 (57.14%) down-regulated genes (Fig. 1C) using GO and KEGG pathways. In our study, bna-miR156b>c>g may be involved in fatty acid metabolism. Genes related to Cd stress have previously been discovered using GO and KEGG pathways, such as BNPCS1, BNGSTU12, and BNGSTU5 [46]. Jian et al. (2018) found a total of 13 differentially expressed miRNAs were confirmed by RT-qPCR, and a hypothetical model of cadmium response mechanism in B.napus was proposed on this basis [47].

Multi copy phenomenon of OPR genes

We found that OPR gene has four copies in rapeseed, with OPR1 and OPR4 located in the C genome and OPR2 and OPR3 located in the A genome. The OPR gene has been identified in several species and there are often multiple copies. Three OPRs were found in A. thaliana and tomato [48], 6 in peas [49], and 8 in corn [50]. Meanwhile, rice comprised 13 OPRs [51] and wheat had 48 OPRs [52]. Multi-copy genes are ubiquitous in plants and play an important role in maintaining plant genetic
stability; However, they have hindered molecular breeding research. The loss of a few copies of gene function often does not cause phenotypic changes, and the probability of simultaneous mutation of multiple copy sites is too low to create a gene family or multiple copies of genes change simultaneously [53]. Rapeseed is an allotetraploid with many multi-copy genes. Conventional molecular breeding research methods are difficult to obtain phenotypic multi-copy gene mutants. Handa (2003) found that the main DNA sequence of the protein coding region was highly conserved between rapeseed and A. thaliana [54]. Transformation with A. thaliana as a receptor is helpful to study the function of multi-copy genes in rapeseed. In this study, we found four copies of OPR gene in rapeseed. Based on these multi-copy genes, we transformed four copies of OPR genes in A. thaliana.

Regulation function of OPR genes to fatty acid
In this study, we found that OPR genes may affect the metabolism of LA and each copy was transferred separately into A. thaliana. The LA content of OPR-OE transgenic plants was significantly increased (T1 12.56%, T2 7.185%), while the LA content of OPRi transgenic plants was significantly decreased (T1 5.98%, T2 0.86%). These results have rarely been reported before. However, it has been described that the CiOPR genes, particularly CiOPR2 and CiOPR4, significantly upregulated by exogenous jasmonic acid, salicylic acid, and ethylene.
treatments in watermelon [55]. Virus-induced gene silencing (VIGS) analysis suggested that knockdown of GhOPR9 could increase the susceptibility of cotton to V. dahliae infection [56]. OPR gene was cloned from Oryza sativa; the overexpression of OPR genes was found to enhance the stress resistance of tobacco to heavy metal Cd2+ [57]. Previous studies concluded that OPR genes were widely involved in abiotic stress processes [58]. In addition, OPR genes involved in fatty acid β oxidation, cinnamoyl acid reduction, and the octadecanoic acid metabolic pathway [59]. However, there were few reports on OPR genes regulating OA, LA, or saturated fatty acid synthesis. In our study, we found that OPR genes directly affect the synthesis of LA and indirectly affect the content of other FA (Fig. 6), which is consistent with the theoretical pathway wherein OPR genes regulate jasmonic acid synthesis using alpha-linolenic acid (18:3) [59, 60].

According to the Table 7, The four OPR copies showed significant negative correlation between the OA, LA and linolenic acid content, which was consistent with Zhao J (2008) [61] and Yan (2012) [62]: The stearic acid massed fraction and LA mass fraction had the same trend, and a larger trend with that of OA and linolenic acid. This is consistent with findings of Shang et al. [63]. Linolenic acid content is not only affected by OA and LA, but also affected by another independent gene system, which was consistent with Kondra (1975) [64]. The variation trend of FA in T1 and T2 generations is almost the same, there are also differences, and here was a significant negative correlation between LA and linolenic acid. In the meantime, there are many researchers believed that FA content of rapeseed was controlled by maternal genotype, and there were interaction, additive and non-additive gene effects between genotype and environment [65–67].

A. thaliana and rapeseed are both cruciferous plants and current studies have shown that their gene functions are basically the same, it is of practical significance to study in model plants [54]. We found the optimizing quality FA in A. thaliana can be changed by regulating OPR genes. OPR may be involved in regulating LA synthesis and improving fatty acid composition in rapeseed. This is the first study which discovered that the OPR gene can regulate LA metabolism. Therefore, this study is a good reference for studies researching the molecular mechanism of LA synthesis and molecular breeding in rapeseed.

In this study, 20 pathways were enriched using the KEGG pathway through high-throughput sequencing, of which 15 may be involved in the regulation of fatty acid metabolism. The reliability of the results was verified by performing the RT-qPCR analysis, which provided a basis for subsequent functional verification. We excavated a target gene OPR, bna-miR156b > c > g, from rapeseed that may be related to fatty acid synthesis and identified the function of OPR genes through transformation of A. thaliana. The LA content of OPR-OE transgenic plants significantly increased (T1 12.56%, T2 7.185%), and the LA content of OPRi transgenic plants significantly decreased
### Table 5: Analysis of fatty acid composition of T1 and T2 seeds

| Strain | Saturated fatty acids (%) | Unsaturated fatty acids (%) | Increase or decrease |
|--------|---------------------------|----------------------------|----------------------|
|        | Palmitic acid | Stearic acid | Total | Oleic acid | Linoleic acid | Linolenic acid | Arachidonic acid | Erucic acid | Total |
| **T1** |             |             |       |           |             |                |                      |             |       |
| Contrast T1 |          |             |       |           |             |                |                      |             |       |
| OPR1   | 7.85 ± 0.37 | 2.947 ± 0.14 | 10.798 | 15.145 ± 3.51 | 28.204 ± 3.11 | 19.115 ± 1.53 | 19.925 ± 2.05 | 0.0 ± 0.06 | 82.389 |
| OPR1i  | 6.8309 ± 0.277 | 3.3757 ± 0.221 | 10.2066 | 22.5651 ± 2.401 | 25.1403 ± 1.383 | 14.231 ± 1.057 | 18.7597 ± 1.975 | 21.414 ± 0.535 | 81.9102 |
| Increase or decrease | -12.99% | 14.55% | -5.48% | 48.99% | -10.86% | -25.55% | -5.85% | -0.58% |       |
| OPR1-OE | 9.5163 ± 0.1048 | 13.951 ± 0.438 | 10.9114 | 25.0872 ± 1.109 | 32.2019 ± 1.666 | 19.7963 ± 7.508 | 18.3739 ± 3.331 | 0.2149 ± 0.257 | 91.17303 |
| Increase or decrease | 21.21% | -6.26% | 1.09% | 35.90% | 14.17% | 3.58% | -1.77% | 1066% |       |
| **T2** |             |             |       |           |             |                |                      |             |       |
| Contrast T2 |          |             |       |           |             |                |                      |             |       |
| OPR2   | 7.2108 ± 0.384 | 3.2877 ± 0.183 | 10.4985 | 16.8825 ± 1.533 | 26.7364 ± 3.095 | 17.496 ± 1.08 | 19.9638 ± 0.183 | 0.0473 ± 0.100 | 81.0996 |
| OPR2i  | 9.1207 ± 0.584 | 1.1043 ± 0.523 | 10.225 | 21.4321 ± 3.32 | 31.029 ± 1.838 | 17.0149 ± 1.747 | 18.1661 ± 2.313 | 0.3125 ± 0.323 | 87.9546 |
| Increase or decrease | -8.15% | 11.56% | -2.77% | 114.7% | -5.20% | -8.61% | -0.19% | -1.57% |       |
| OPR2-OE | 10.2309 ± 1.924 | 1.351 ± 0.566 | 11.819 | 18.318 ± 2.432 | 27.7582 ± 1.057 | 17.5874 ± 1.266 | 19.3568 ± 0.831 | 0.1795 ± 0.384 | 83.1999 |
| Increase or decrease | -6.53% | -2.51% | -5.43% | 20.95% | -1.58% | -7.99% | -2.85% | -0.98% |       |
| OPR3   | 7.3383 ± 0.438 | 2.8731 ± 1.018 | 10.2114 | 18.318 ± 2.432 | 27.7582 ± 1.057 | 17.5874 ± 1.266 | 19.3568 ± 0.831 | 0.1795 ± 0.384 | 83.1999 |
| OPR3i  | 9.5163 ± 0.1048 | 13.951 ± 0.438 | 10.9114 | 25.0872 ± 1.109 | 32.2019 ± 1.666 | 19.7963 ± 7.508 | 18.3739 ± 3.331 | 0.2149 ± 0.257 | 91.17303 |
| Increase or decrease | 21.21% | -6.26% | 1.09% | 35.90% | 14.17% | 3.58% | -1.77% | 1066% |       |
| OPR3-OE | 10.2309 ± 1.924 | 1.351 ± 0.566 | 11.819 | 18.318 ± 2.432 | 27.7582 ± 1.057 | 17.5874 ± 1.266 | 19.3568 ± 0.831 | 0.1795 ± 0.384 | 83.1999 |
| Increase or decrease | -6.53% | -2.51% | -5.43% | 20.95% | -1.58% | -7.99% | -2.85% | -0.98% |       |
| OPR4   | 7.3477 ± 0.29 | 3.0894 ± 0.253 | 10.4371 | 19.9233 ± 5.850 | 26.4398 ± 3.586 | 16.0565 ± 2.200 | 18.3035 ± 2.300 | 1.5821 ± 0.399 | 82.2852 |
| OPR4i  | 9.5163 ± 0.1048 | 13.951 ± 0.438 | 10.9114 | 25.0872 ± 1.109 | 32.2019 ± 1.666 | 19.7963 ± 7.508 | 18.3739 ± 3.331 | 0.2149 ± 0.257 | 91.17303 |
| Increase or decrease | 21.21% | -6.26% | 1.09% | 35.90% | 14.17% | 3.58% | -1.77% | 1066% |       |
| OPR4-OE | 10.2309 ± 1.924 | 1.351 ± 0.566 | 11.819 | 18.318 ± 2.432 | 27.7582 ± 1.057 | 17.5874 ± 1.266 | 19.3568 ± 0.831 | 0.1795 ± 0.384 | 83.1999 |
| Increase or decrease | -6.53% | -2.51% | -5.43% | 20.95% | -1.58% | -7.99% | -2.85% | -0.98% |       |

Note: *: P < 0.05; **: 0.01 < P < 0.05. The same as Table 6.
In addition, by performing a bioinformatics analysis, we found four copies of the OPR gene in the cytoplasm that were located on chromosomes A and C. In this study, by detecting the fatty acid content of different generations of transgenic A. thaliana, OA and LA, linolenic acid content showed significant relationship, LA can be affected by FAD-related gene regulation and the environment. We found the four copies of OPR gene that can directly affect LA content and indirectly affect other high quality FA were discovered for the first time. These results can be used in breeding programs aimed at optimizing fatty acid profiles in rapeseed [68].

| Strain  | Oleic acid | Linolenic acid | Arachidonic acid | Erucic acid |
|---------|------------|----------------|------------------|-------------|
| T₁      | OPR1i      | 22.5651 ± 2.401| -                | 18.7597 ± 1.975 | - |
|         | OPR1-OE    | 20.5827 ± 1.609| -                | 18.3739 ± 3.331 | - |
|         | Mean square| 19.65           | -                | 0.744        | - |
|         | Mean square within group | 4.177          | -                | 7.496        | - |
|         | F value    | 4.705*         | -                | 0.099        | - |
|         | OPR2i      | 16.8825 ± 1.533| 17.4696 ± 1.08   | -            | - |
|         | OPR2-OE    | 21.4321 ± 3.32 | 17.0149 ± 1.747 | -            | - |
|         | Mean square| 103.494        | 1.034            | -            | - |
|         | Mean square within group | 6.685          | 2.109            | -            | - |
|         | F value    | 15.481**       | 0.49             | -            | - |
|         | OPR3i      | 18.318 ± 2.432 | 17.5874 ± 1.266 | 19.3568 ± 0.831 | - |
|         | OPR3-OE    | 22.9638 ± 1.382| 18.9167 ± 2.314 | 14.2964 ± 1.938 | - |
|         | Mean square| 5.259          | 8.835            | 128.038      | - |
|         | Mean square within group | 21.703        | 14.921           | 4.662        | - |
|         | F value    | 0.242          | 0.592            | 27.466*      | - |
|         | OPR4i      | 19.9233 ± 5.580| -                | 18.3035 ± 2.500 | - |
|         | OPR4-OE    | 20.9489 ± 3.030| -                | 15.3593 ± 1.484 | - |
|         | Mean square| 107.917        | -                | 43.342       | - |
|         | Mean square within group | 46.968        | -                | 4.225        | - |
|         | F value    | 2.298**        | 10.257**         | -            | - |
| T₂      | OPR1i      | 9.23 ± 1.546   | 23.574 ± 5.791   | -            | - |
|         | OPR1-OE    | 9.897 ± 2.364  | 21.122 ± 2.164   | -            | - |
|         | Mean square| 1.439          | 4.824            | -            | - |
|         | Mean square within group | 1.0266          | 1.993            | -            | - |
|         | F value    | 1.401          | 2.420*           | -            | - |
|         | OPR2i      | 8.578 ± 3.045  | 22.432 ± 1.578   | 17.839 ± 2.264 | 2.64 ± 4.165 |
|         | OPR2-OE    | 9.44 ± 1.231   | 21.467 ± 0.146   | 15.291 ± 1.91 | 0.841 |
|         | Mean square| 0.00002        | 11.601           | 1.559        | 0.047 |
|         | Mean square within group | 1.186          | 1.099           | 2.78         | 0.044 |
|         | F value    | 0.00002        | 10.560**         | 0.561        | 1.048 |
|         | OPR3i      | 8.259 ± 1.254  | -                | 17.029 ± 0.934 | 2.244 ± 0.771 |
|         | OPR3-OE    | 10.063 ± 0.187 | -                | 16.84 ± 0.943 | 2.147 ± 0.143 |
|         | Mean square| 3.855          | -                | 36.954       | 0.666 |
|         | Mean square within group | 1.931          | -                | 2.328        | 0.035 |
|         | F value    | 1.997          | -                | 15.877**     | 18.799** |
|         | OPR4i      | 7.517 ± 1.214  | -                | 17.791 ± 0.261 | - |
|         | OPR4-OE    | 11.382 ± 1.134 | -                | 16.674 ± 1.057 | - |
|         | Mean square| 3.968          | -                | 1.769        | - |
|         | Mean square within group | 1.654          | -                | 8.573        | - |
|         | F value    | 2.399*         | -                | 0.206        | - |
| Strain | Oleic acid / Linoleic acid | Oleic acid / Linolenic acid | Linoleic acid / Linolenic acid | Linolenic acid / Arachidonic acid | Palmitic acid / Oleic acid | Palmitic acid / Linoleic acid | Palmitic acid / Linolenic acid | Palmitic acid / Stearic acid | Stearic acid / Oleic acid | Stearic acid / Linoleic acid | Stearic acid / Linolenic acid |
|--------|-----------------------------|-----------------------------|-------------------------------|---------------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Contrast | -0.373**                   | -0.489**                   | -0.626**                     | 0.088                           | 0.963**                   | -0.754**                   | 0.891**                     | -0.203*                     | -0.549**                   | 0.980**                     | -0.460**                   |
| T1     | -0.788**                   | -0.837**                   | 0.719**                      | 0.866**                         | 0.225*                    | 0.878**                    | -0.690**                    | -0.570**                    | -0.448**                    | 0.676**                     | -0.704**                   |
| OPR1i  | -0.142                     | -0.331*                    | 0.501**                      | 0.110                           | 0.390*                    | 0.515**                    | 0.728**                     | 0.683**                     | 0.303**                     | 0.360**                     | -0.503**                   |
| OPR1-OE | -0.371*                    | -0.802**                   | -0.081                       | 0.819**                         | 0.526**                   | -0.974**                   | 0.502**                     | 0.894**                     | 0.829**                     | 0.050                       | -0.521**                   |
| OPR2i  | -0.852**                   | -0.916**                   | 0.850**                      | -0.219**                        | 0.778**                   | -0.427**                   | 0.593**                     | 0.611**                     | 0.047                       | 0.189                       | -0.188                     |
| OPR2-OE | -0.541**                   | -0.743**                   | 0.561**                      | 0.504**                         | 0.248**                   | -0.649**                   | -0.081**                    | 0.461**                     | -0.515**                    | -0.064                      | 0.039                      |
| OPR3i  | -0.550**                   | -0.579**                   | 0.431**                      | 0.184**                         | 0.866**                   | -0.437**                   | 0.888**                     | 0.218                       | 0.592**                     | 0.735**                     | 0.089                      |
| OPR3-OE | -0.967**                   | -0.970**                   | 0.884**                      | 0.901**                         | 0.746**                   | -0.613**                   | 0.596**                     | 0.624**                     | 0.265                       | 0.670**                     | -0.257*                    |
| OPR4i  | -0.772**                   | -0.505**                   | 0.600**                      | 0.053**                         | 0.208*                    | -0.092**                   | -0.330*                     | 0.216*                      | -0.263*                     | -0.510**                    |
| OPR4-OE | -0.830**                   | -0.728**                   | -0.179*                      | 0.234*                          | 0.690**                   | -0.188                     | 0.481**                     | -0.235*                     | -0.893**                    | 0.328*                      | -0.642**                   |
| T2     | -0.692**                   | -0.488**                   | -0.292*                      | 0.120                           | 0.811**                   | -0.669**                   | 0.825**                     | -0.147                      | -0.810**                    | 0.478**                     | -0.180                     |
| OPR1i  | -0.216                     | -0.081**                   | -0.556**                     | 0.484**                         | 0.938**                   | -0.322*                    | 0.344**                     | -0.729**                    | -0.956**                    | 0.260**                     | -0.838**                   |
| OPR1-OE | -0.375*                    | -0.654**                   | -0.125                       | 0.296*                          | 0.553**                   | -0.429**                   | 0.971**                     | -0.272*                     | -0.328*                     | 0.746**                     | -0.270**                   |
| OPR2i  | -0.416**                   | -0.271*                    | -0.405**                     | 0.704**                         | 0.885**                   | -0.053*                    | 0.726**                     | 0.719**                     | 0.074                       | 0.859**                     |
| OPR2-OE | -0.500**                   | -0.660**                   | -0.633**                     | 0.149                           | 0.350**                   | -0.744**                   | 0.305**                     | -0.007                      | -0.202                      | 0.283**                     | -0.341*                    |
| OPR3i  | -0.122                     | -0.689**                   | -0.197*                      | 0.132                           | 0.330                    | -0.713**                   | 0.682**                     | -0.038**                    | -0.378**                    | 0.166                       | -0.330*                    |
| OPR3-OE | -0.785**                   | -0.611**                   | -0.551*                      | 0.069                           | 0.662**                   | -0.324**                   | 0.790**                     | -0.180                      | -0.898**                    | 0.771**                     | -0.180                     |

Note: Lower triangle is r, upper triangle is p; ** highly significant correlation (P < 0.01); * significant correlation (P < 0.05)
Methods

Plant materials and growth conditions

The near-isogenic rapeseed lines with high (81.4%) and low (56.2%) OA contents were provided by Rapeseed Molecular Breeding of Hunan Agricultural University, high OA rapeseed materials became known as HOAR and low OA rapeseed materials became known as LOAR, the strain was originally cultivated by National Oil Improvement Center of Hunan Agricultural University. The materials exhibited stable traits and were planted in the

Fig. 6  Bra00592 KEGG pathway network
experimental field of Hunan Agricultural University, China (Changsha, China) with standard agronomic methods [69]. The seeds 20 d after pollination were quickly frozen in liquid nitrogen and stored at -80 °C for subsequent studies [70]. The sample treatment method adopted in this study is the same with our previous study [71].

The seeds of wild-type (WT) A. thaliana (ecotype: Columbia) were bought from Think Gene Biotechnical Technology Co., LTD, Shanghai, China. Plants were grown under greenhouse conditions: 24 °C with a photoperiod of 18.5 h-light/5.5 h-dark, with a light intensity of 6500 lx.

**Methods**

**sRNA library construction and high throughput sequencing**

Total RNA was extracted from frozen seeds using the Trizol reagent (Sigma Aldrich, St. Louis, MO, USA), according to the manufacturer's instructions. The quality and quantity of the purified RNA were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, Santa Clara, USA) and RNA 6000 nanokit (Agilent Technologies, Palo Alto, Santa Clara, USA). sRNAs with lengths of 18–30 nt were separated and purified using 15% denaturing polyacrylamide gel electrophoresis. Consequently, sRNAs fractions were ligated to the 5′ lectrophoresters using T4 RNA ligase (Epicentre, America). The adapter-ligated fragments were then reverse transcribed and amplified by performing PCR with a pair of adapter complementary primers. These PCR products were purified and sequenced using IlluminaHiseq XTEN (Illumina, USA). Construction of the sRNA libraries and deep sequencing were carried out by Oebiotech Genomics (Shanghai, China).

**Bioinformatic analysis of the sRNAs sequencing data**

Clean reads were generated after eliminating the low-quality reads, poly As, reads smaller than 18 nt, and gener adaptor contaminants and subsequently inserting nulls. Using bowtie software [72], the clean reads were aligned against the NCBI Gen Bank [73], Brassica napus oilseed genome [39], and Rfam databases (version 10.0). Reads annotated into the noncoding RNA categories, including rRNA, tRNA, snoRNA, and snoRNA were filtered. The remaining sRNA sequences were aligned against the miRBase21.0 [74]. The nearly matched sequences (less than two mismatches) were considered to be the known miRNAs. Target miRAN will computationally predict small RNA binding sites on target transcripts from a sequence database, this is done by aligning the input small RNA sequence against all transcripts, followed by site scoring using a position-weighted scoring matrix [75]. First, used ‘targetfinder’ software, set threshold -C = 4, predicting the miRNA target genes, we got 64 target genes with miRNA156; and then, to GO and KEGG analysis (P-value ≤ 0.05) of 64 target genes, we found there are six of them related to the metabolism of FA. The unannotated sRNAs were further analyzed to predict the novel miRNAs using the Mirdeep 2 software. The secondary structures of premiRNAs were also predicted using the RNAfold software.

To reveal the continuous changes in expression of miRNAs during the biosynthesis process, the variation in expression was analyzed in immature seed libraries of HOAR. The frequency of miRNAs was normalized as transcripts per million (TPM) for further analysis. In addition, miRNAs were assessed using the negative binomial distribution test, with P-value ≤ 0.05 and absolute value of log2 (treatment/control: LOAR/ HOAR) > 1.5 being considered as differentially expressed. Moreover, the similarity between samples was investigated by the clustering method. The Blast 2 GO software with default parameters was applied to determine the functional annotation and categorization of the target genes [76]. The KEGG (Kyoto Encyclopedia of Genes and Genomes) and InterPro databases were also searched with an evalue of 1e−10 [77–79].

**Real time quantitative polymerase chain reaction (RT-qPCR) validation of differentially expressed miRNAs**

To validate the expression of differentially expressed miRNAs, all annotated miRNAs were selected for RT-qPCR validation using the poly (T) adaptor RT-qPCR method [80]. The RT-qPCR amplifications were performed according to an established procedure [81]. 5S RNA was used as an internal reference gene of miRNA and two primers were used: F: 5′-CTCGGCAACCGGA TATCTCG-3′ and R: 5′-CTAATGGCTTGGGGCG-3′. The internal reference gene of the miRNA target gene was UBC9 and two primers were used: F: 5′-TCCATC CGACAGCCCTTACTCT-3′ and R: 5′-ACACTTTGG TCTAAAAGCCACC-3′. All reactions were performed in triplicate. The RT-qPCR was conducted on the Step One Plus RT-qPCR System (ABI, America). The relative expression was calculated using the 2 − ΔΔCT method [82]. Statistical analyses consisted of analysis of variance and Fisher’s post-hoc tests. A p-value < 0.05 was considered statistically significant. Details of the RT-qPCR primers were provided in Table 2.

**Cloning of OPR genes in rapeseed and bioinformatic analysis**

Four copies of OPR genes were found using miRNA sequencing homologous cloning. We used material...
HOAR (high OA rapeseed materials, B) to clone, and the total RNA was extracted from the rapeseed leaf samples using the CTAB method adopted by Niu et al. (2018) and Meng et al. (2006) [83, 84], with minor modifications. cDNA was produced by implementing reverse transcription, which was followed by PCR amplification (Table 8). According to each copy sequence of OPR, the physical and chemical properties of corresponding proteins were analyzed by using online websites such as the ExPASY-Protparam Tool and modifying the methods described by Li et al. (2019) and Sun et al. (2016) [85, 86].

Bioinformatic analysis of OPRs: The ExPASy-Protparam tool website (https://web.expasy.org/protparam/) predicted the primary structure of the protein. The subcellular localization of OPR proteins were performed using the PredictProtein SOPMA website (http://www.predictedprotein.org/). TMHMM Server, v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) was used to analyze the transmembrane helix region of proteins. The SOPMA website (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automation.pl?Page=npsa_sopma.html) predicted the secondary protein structure. The tertiary structure model of each copy of OPR protein was constructed using the SWISS-MODEL website (https://swissmodel.expasy.org/).

### Vector construction and transformation of *A. thaliana*
Specific RNAi primer (Table 9) cDNA was used as a template. The base before the 5'-end enzyme digestion site was used for the construction of recombinant plasmids (the same below), according to the method adopted by Li et al. (2021) with minor modifications [87]. The cloned OPR sequence was digested with BamHI and SalI (Table 8) and recombinant with pCAMBIA1300-35 s vector (Kangyan Corporation, China) to construct pCAMBIA1300-35 s-OPR vector.

Similarly, the pCAMBIA1300-RNAi-OPR vector (Kangyan Corporation, China) was reconstructed using a double digestion process in vitro (Table 9) by following the methods of Yang et al. (2012) and Qu et al. (2017) with minor modifications [6, 29]. pCAMBIA1300-35 s-OPR and pCAMBIA1300-RNAi-OPR vectors were transformed into *A. thaliana* because of inflorescence infection to explore the function of each copy by using the inflorescence infection method of Clough and Bent (1998) [41], with minor modifications.

### Fatty acid detection and statistical methods
Using Agilent Technologies 7890B gas chromatograph, MSD detector, 5977A chromatographic workstation, and DB-23 capillary chromatographic column, sample size

### Table 8 Primers for OPR genes amplification

| Target ID | Primer name | Primer sequence (5'-3') |
|-----------|-------------|-------------------------|
| GSBRNA2T00012422001 (OPR1) | OPR1 BamHI-F | TCT TGA TCA AGA GAC ACA GAG TGA GAA AAT GCA GTA GCG AAAG |
|          | OPR1 Sall-R | CAT CCG TGC ACT AGT GTC GAC TTA ACT AGC TGT TGA TTC AAG |
| GSBRNA2T00135385001 (OPR2) | OPR2 BamHI-F | TCT TGA TCA AGA GAC ACA GAG TGA GAA AAC GTA GTC AAAC |
|          | OPR2 Sall-R | CAT CCG TGC ACT AGT GTC GAC TTA ACT AGC TGT TGA ATC AAG |
| GSBRNA2T00082938001 (OPR3) | OPR3 BamHI-F | TCT TGA TCA AGA GAC ACA GAG TGA GAA AAT GCA GTA GCG AAAG |
|          | OPR3 Sall-R | CAT CCG TGC ACT AGT GTC GAC TTA ACT AGC TGT TGA TTC AAG |
| GSBRNA2T00094910001 (OPR4) | OPR4 BamHI-F | TCT TGA TCA AGA GAC ACA GAG TGA GAA AAC GTA GTC AAAC |
|          | OPR4 Sall-R | CAT CCG TGC ACT AGT GTC GAC TTA ACT AGC TGT TGA TTC AAG |

Note: the underline indicates the restriction site. the same as Table 9

### Table 9 RNAi primers

| Gene name | Primer name | Primer sequence (5'-3') |
|-----------|-------------|-------------------------|
| OPR1      | OPR1 Ri-PBF | GAG TCT CTC TGC AGG GAAG TGG AAATG AGT GAG GCG |
|           | OPR1 Ri-SXR | TTT CAG TGC TGC ACT CGA CTG AAG TGG AAATG AGT GAG GCG |
| OPR2      | OPR2 Ri-PBF | GAG TCT CTC TGC AGG GAAG TGG AAATG AGT GAG GCG |
|           | OPR2 Ri-SXR | TTT CAG TGC TGC ACT CGA CTG AAG TGG AAATG AGT GAG GCG |
| OPR3      | OPR3 Ri-PBF | GAG TCT CTC TGC AGG GAAG TGG AAATG AGT GAG GCG |
|           | OPR3 Ri-SXR | TTT CAG TGC TGC ACT CGA CTG AAG TGG AAATG AGT GAG GCG |
| OPR4      | OPR4 Ri-PBF | GAG TCT CTC TGC AGG GAAG TGG AAATG AGT GAG GCG |
|           | OPR4 Ri-SXR | TTT CAG TGC TGC ACT CGA CTG AAG TGG AAATG AGT GAG GCG |
1μ, Heating procedure: initial holding time 10 min, initial temperature 180 °C, 20 °C / min, rising to 250 °C, according to the fatty acid detection method of Mao et al. (2020) [42], with minor modifications. Microsoft Excel 2010 was used to collate the data and one-way analysis of variance was used for analysis [88].

Abbreviations
OPR: 12-Oxo-phytodienoic acid reductase; LOAR (A): Low Oleic Acid Rapseseed (81.4%) materials; HOAR (B): High Oleic Acid Rapseseed (56.2%) materials; RT-qPCR: Quantitative Real-time PCR; OPR:RNA-interference strain; FA: Fatty acids; OA: Oleic acid; LA: Linoleic acid; ALA: α-Linolenic acid; GLA: γ-Linolenic acid.

Supplementary Information
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Additional file 1.

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Not applicable.

Author contributions
ZQ Z and MB L designed the experiments, analyzed the data, M T and DZ P performed the experiments and wrote the manuscript, Q C and J N performed the bioinformatics analysis. All authors read and approved of the manuscript.

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Availability of data and materials
Data of RNA-seq in this study is available in NCBI with accession number PRJNA760803 that are publicly accessible at https://www.ncbi.nlm.nih.gov/sra/PRJNA760803.

Declarations

Ethics approval and consent to participate
Brassica napus L. were collected in Yunyuan, Chongshana, Hunan Province in 2021, and then planted in the Hunan Agricultural University in Yuyuan. Brassica napus L. used in this experiment were all collected from the Yunyuan of Hunan Agricultural University, and the seeds of wild-type (WT) A. thaliana (ecotype: Columbia) were bought from Think Gene Biological Technology Co., LTD, Shanghai, China. Permission was not necessary for collecting these species, which have not been included in the list of national key protected plants. Experimental research on Brassica napus L. and A. thaliana complies with Hunan Agricultural University guidelines (https://amd.hunau.edu.cn/). The authors have complied with all relevant institutional and national guidelines and legislation in experimental research and field studies on plants, including the collection of plant materials for this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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