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Effect of Different Types of Organic Manure on Oil and Fatty Acid Accumulation and Desaturase Gene Expression of Oilseed Flax in the Dry Areas of the Loess Plateau of China

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Abstract: In order to understand the mechanism of action of oil and fatty acid accumulation and desaturase gene expression in how oilseed flax responds to different fertilization conditions, a three-factor split-plot experiment was conducted to investigate the accumulation trends of oil and fatty acids. The results revealed that soluble sugar (SS) and sucrose (SUC) contents decreased, and the starch (ST) content increased gradually with the grain development and maturity of oilseed flax. The application of sheep manure promoted the accumulation of nonstructural carbohydrates in the grains. Soluble sugar (SS) and sucrose (SUC) contents were negatively correlated with the oil content. Compared with chemical fertilizer, organic manure decreased the total saturated fatty acid but increased the unsaturated fatty acid. Organic manure significantly upregulated the expression of various genes, and *fad2a* gene expression was higher with the 5.8 t ha^{-1} chicken manure treatment. The 25 t ha^{-1} sheep manure treatment was more conducive to the expression of *fad3a* and *fad3b* genes and promoted the accumulation of linolenic acid (LIN), and the LIN content increased by 0.64–3.90% compared with other treatments. Consequently, an ongoing anthropogenic sheep manure input may impact the regulation of grain oil quality and yield per unit area.

Keywords: oilseed flax; organic manure; nonstructural carbohydrate; oil and fatty acid accumulation; desaturase gene

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1. Introduction

Oilseed flax (*Linum usitatisimum* L.) is an annual herb and the main oil crop and economic crop in arid and semi-arid areas of the Loess Plateau of China. Flaxseed is widely used in the food, medicine, and chemical industries [1]. It is rich in C 18:3 polyunsaturated fatty acids, which are a group of fatty acids in which the human body cannot synthesize the essential fatty acids (alpha-linolenic acid (ALA) or LIN), and their content can be as high as 45–60%. Oilseed flax also contains dietary fiber, vitamin E, lignan, and other nutrients, which can reduce blood lipids and blood pressure and improve human immunity [2]. People are paying increasing attention to a reasonable and healthy diet with improvements in living standards. The oil of oilseed flax has attracted special attention because of its preferable fatty acid composition ratio, causing the gap between production and demand to continue to widen. Therefore, it is urgent to improve the grain yield and the quality of oilseed flax [3,4]. Yield and quality are mainly controlled by genotypes, but they are also regulated by external environmental conditions [5]. Fertilization is an important agricultural measure that affects crop yield and quality [6]. Livestock and poultry manure is a high-quality organic fertilizer, which is rich in organic matter and a variety of trace elements. It can not only improve the physical and chemical properties of soil but also promote growth and improve crop quality on the basis of a stable yield [7,8].

Carbohydrates provide energy for plant growth and development and can be divided into structural carbohydrates (SCs) and nonstructural carbohydrates (NSCs) [9]. NSCs are the main form of energy storage in plant metabolism and mainly comprise soluble sugar (SS) and starch (ST). The high or low contents of these photosynthates can better reflect the plant's carbon budget and response and inform adaptation strategies to the changes in the external environment. SS (mainly sucrose) is the main substance in which carbohydrates in plants can be transformed and reused and is closely related to ST accumulation [10,11]. As temporary storage materials, SS and ST can be redistributed during the grain-filling stage [12]. It has been reported that organic fertilizers can promote carbon metabolism in crop seedlings and increase the NSC content of crops [13]. Therefore, optimizing fertilization measures to improve the accumulation and transport of NSCs is an effective way to promote grain filling.

Organic fertilizers have a significant effect on the oil content and fatty acid composition of oil crops [14]. The application of farmyard manure can significantly increase the oil yield and unsaturated fatty acid content of rape in a rotation system [15]. Mohammadi reported that the combined application of farmyard manure and chemical fertilizer increased the rate of nitrogen uptake but decreased the oil content, while the application of organic manure alone increased the oil yield of soybean [16]. Another study showed that earthworm composting regulates the ratio of omega-3 and omega-6 essential fatty acids, provides a lower LA/ALA (linoleic acid/linolenic acid) ratio, and increases the P/S ratio (polyunsaturated fatty acids/saturated fatty acids), which helps to reduce blood cholesterol [17]. NSCs are closely related to the synthesis of fatty acids. The seed protein and ST content are higher in the early stage, while the SS content fluctuates, and the oil content of seeds increases significantly during the ripening process, indicating that the synthesis and accumulation of oils and fatty acids require carbohydrate hydrolysates synthesized by photosynthesis [18]. Additionally, SS is the main raw material for oil synthesis. Lin et al. [19] also proved that sugars first accumulate during oilseed development, while the accumulation of oil occurs slightly later than that of sugars. Therefore, it is of great significance to further reveal the causes of oil and fatty acid formation by studying the changes in oil and sugar during the seed development of oil crops and clarifying the metabolic transformation relationship between them.

The desaturation of fatty acids is an important biochemical process in fatty acid biosynthesis. Fatty acid desaturase inserts double bonds into the hydrocarbon chain to determine the ratio of saturated fatty acids to unsaturated fatty acids and thus the direction of oil use [20]. Some studies have reported that fatty acid desaturase 2 (*fad2*) and fatty acid desaturase 3 (*fad3*) are the key enzymes for desaturating oleic acid (18:2, cis-$\triangle^{9,12}$, OLE) to polyunsaturated fatty acids, namely linoleic acid (18:2, cis-$\triangle^{9,12}$, LIO) and linolenic acid (18:3, cis - $\triangle^{9,12,15}$, LIN), respectively [21]. However, currently, research on the effects of organic fertilizer on the accumulation of oil and unsaturated fatty acids in oilseed flax is very limited. The mechanism through which organic fertilizers regulate the key enzyme genes in fatty acid desaturation has not been reported. Therefore, we hypothesized that the application of farmyard manure instead of chemical fertilizer could improve the grain oil quality of oilseed flax and increase the content of unsaturated fatty acids and the expression of key genes in the biosynthesis process. To verify this hypothesis, a two-year field experiment was conducted to analyze the effects of different types and amounts of organic manure on the changes in nonstructural carbohydrate content, oil, and fatty acid accumulation, as well as the desaturase gene expression of different oilseed flax varieties after anthesis from 2021 to 2022 in dry areas of the Loess Plateau of China. The main objectives of this study were to explore the effects of the molecular biological regulation mechanism of organic manure on the grain oil formation of oilseed flax and provide a theoretical basis and technical guidance for the production of high-quality oilseed flax and its quality optimization using green production technology in the dry areas of the Loess Plateau of China.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in 2021 and 2022 at the Oil Crops Institute of Dingxi Academy of Agricultural Sciences (104°49′ E, 35°48′ N), Gansu Province, China. The site is located in a hilly and gully semi-arid area of the Loess Plateau, with an average altitude of 2050 m, an average annual rainfall of approximately 392 mm, an average annual temperature of 7.3 °C, and an annual sunshine duration of 2472 h. The soil type is yellow cotton soil, and the basic soil nutrients are shown in Table 1.

Table 1. Basic physical and chemical properties of tested soil.

Year	Organic Matter (g kg^{-1})	Total N (g N kg^{-1})	Available N (mg N kg^{-1})	Total P (g P kg^{-1})	Available P (mg P kg^{-1})	Total K (g K kg^{-1})	Available K (mg K kg^{-1})	pH
2021	10.37	0.81	48.91	0.69	27.43	29.33	108.30	8.14
2022	9.21	0.75	46.87	0.68	27.11	26.87	176.00	8.31

2.2. Materials

Three oilseed flax varieties with different fatty acid contents were selected for the experiment, namely Zhangya 2 (V3), Longya 11 (V1), and Dingya 26 (V2), considered the oilseed flax varieties with relatively high (≥51%), medium (49–51%), and low (≤49%) linolenic acid content, respectively.

2.3. Experimental Design

A three-factor split-plot, randomized block experimental design was used in the field experiment, with different varieties of oilseed flax as well as different types and application amounts of fertilizer considering the main plot, split-plot, and split–split-plot designs. The types of fertilizer selected were C, rotten chicken manure (N, 1.94%; P_2O_5, 1.19%; and K_2O, 0.85%); S, rotten sheep manure (N, 0.9%; P_2O_5, 0.5%; and K_2O, 0.45%); F, chemical fertilizer; and CK, no fertilization. Chemical fertilizer and organic manure had the same nitrogen content, but the content of phosphorus and potassium in organic manure was lower than that in chemical fertilizer. Therefore, calcium superphosphate and potassium sulfate fertilizer were used to supplement the insufficient phosphorus and potassium content in organic manure to ensure the equality of nitrogen, phosphorus, and potassium in all treatments. The specific fertilizer amounts are shown in Table 2. A total of 21 treatments were performed with 3 replications; a total of 63 plots with a plot area of 2 m × 4 m = 8 m^2 were used, and each main plot was 19 m long and 5 m wide. The interval between each plot was 1 m, and a 60 cm deep plastic film was used to prevent fertilizer diffusion. Two rows of adjacent treatment areas were not investigated as protective rows, and 2 m protective rows were planted around the experimental site. The sowing rate was 7.5 million seeds·ha^{-1}, sowing was carried out in rows at a depth of 3 cm and a row spacing of 20 cm. The previous two years' crop stubble was wheat, and the basic soil fertility was essentially the same in both growing seasons. The seeds were sown on 8 April 2021 and 27 April 2022, and both were harvested on 11 August of the same year. The plants were grown without irrigation in both growing seasons. Crops were kept free of weeds by hand hoeing when necessary.

The amounts of nitrogen, phosphorus, and potassium used for chemical fertilizers were the locally recommended amounts. Urea (46% N) was used for nitrogen, calcium superphosphate (16% P_2O_5) was used for phosphate, and potassium sulfate (51% K_2O) was used for potash; these were all produced by Gansu Liuhua (Group) Co., Linxia, China. The types of organic manure were determined according to the investigation results concerning commonly used local manure and based on many years of experimentation. The application amounts were determined through the equal conversion of total N content and chemical fertilizer according to the nutrient analysis of organic manure; the fertilizers were produced by Shijiazhuang Fengdi Fertilizer Co., Shijiazhuang, China. All fertilizers were buried a week before sowing. Other fields were managed in the same way as the local general field.

Table 2. Different fertilizer types, organic manure application amounts, and nutrient contents.

Fertilizer Types	Fertilization Treatments	Amounts of Organic Manure Application ($t\ ha^{-1}$)	N ($kg\ ha^{-1}$)	P_2O_5 ($kg\ ha^{-1}$)	K_2O ($kg\ ha^{-1}$)
Chicken manure	C_1	5.8	0	6	18.2
	C_2	11.6	0	12	36.4
Chemical fertilizer	F_1	0	112.5	75	67.5
	F_2	0	225	150	135
Sheep manure	S_1	12.5	0	12.5	11.2
	S_2	25	0	25	22.4
CK	No fertilizer	0	0	0	0

2.4. Measurement and Calculation

2.4.1. Grain oil Content

The plants with basically the same growth rate and strong growth on the same day were selected from the first day of oilseed flax anthesis in each plot, and different color ropes were used to bind and mark the plants and indicate different sampling days, from anthesis to maturity (0, 7, 14, 21, 28, 35, and 42 days) (Figure 1).

Figure 1. Field markers of test materials.

The oil content of each sample at days 0 and 7 was very low, so the plant capsule after the 14th day was taken for the determination of seed oil content, and the seeds were naturally air-dried for use. The air-dried seeds were placed in an oven (90 °C) for 30 min, and then 1 g was weighed, fully ground, and put into a folded filter paper package. The filter paper package was placed in an aluminum foil cup, an amount of no more than 2/3 of the aluminum foil cup of petroleum ether was added, and a Soxhlet extractor was used for extraction. The aluminum foil was weighed at the end of leaching, and the grain oil content was calculated using the following formula:

$$\text{Grain oil content} = (\mathrm{B} - \mathrm{C}/\mathrm{B} - \mathrm{A}) \times 100\%$$

where A represents the filter paper package weight (g), B represents the filter paper package weight plus sample weight (g), and C represents the filter paper bag weight plus sample weight after extraction (g).

2.4.2. Fatty Acids

The extracted oil samples were pretreated via methyl esterification, and then the fatty acid content was determined using gas chromatography (Agilent 7890B, Agilent Technologies Inc., Wilmington, DE, USA). Figure 2 shows the gas chromatographic conditions for fatty acid determination and the gas chromatogram of standard fatty acids in oilseed flax. The contents of different fatty acid components in the test samples were calculated using the standard fatty acid curve, which was drawn according to Figure 2. The obtained data were calculated using the peak area normalization method, and the fatty acid content was expressed as the relative ratio of a single fatty acid to the total fatty acid (%).

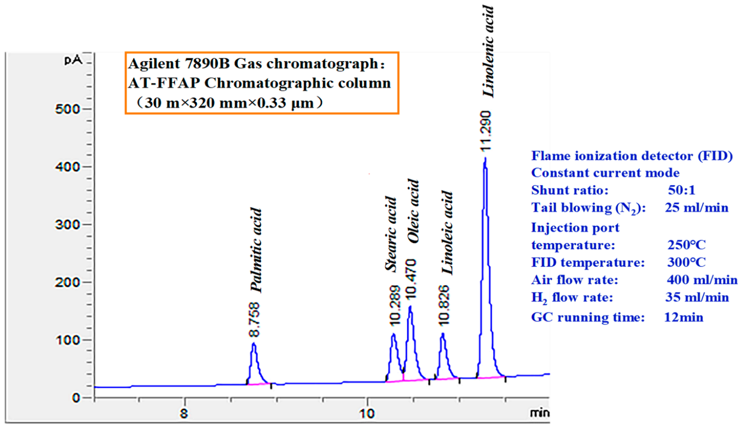

Figure 2. Gas chromatogram of standard fatty acids.

The carbon element of fatty acid biosynthesis is derived from the sucrose produced via plant photosynthesis. Sucrose is transported from photosynthetic organs such as leaves to seed cells, where hexose is formed through glycolysis, and then oxidizes to form the substrate of fatty acid biosynthesis, i.e., acetyl-CoA (Figure 3). Acetyl-CoA forms different fatty acid components through dehydration, condensation, reduction, desaturation, and other enzymatic reactions. Therefore, in order to further clarify the cause of oil and fatty acid accumulation, we also determined nonstructural carbohydrate content and *fad* desaturase gene expression.

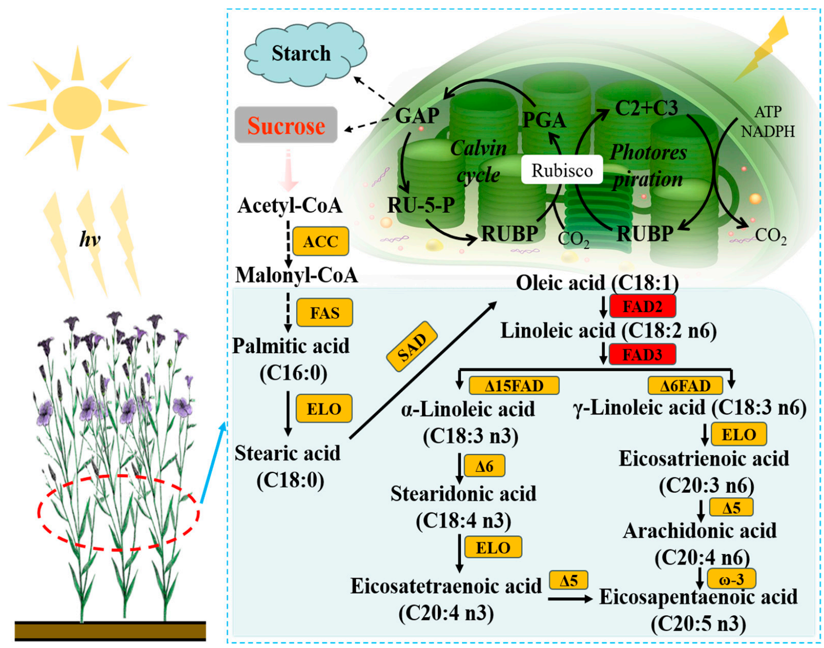

Figure 3. Schematic diagram of fatty acid synthesis [22].

2.4.3. Nonstructural Carbohydrates

The method established by Chen et al. for the determination of nonstructural carbohydrate content [23] has been widely used in China. Soluble sugar in the capsule was determined via anthrone colorimetry, starch was determined via perchloric acid hydrolysis-anthrone colorimetry, and sucrose was determined via the resorcinol method.

2.4.4. Gene Expression

(1) Sample collection

Capsule samples of each oilseed flax variety were taken at 0, 7, 21, and 35 days after anthesis, frozen in liquid nitrogen, and then transferred to a cryogenic refrigerator at −80 °C for storage.

(2) Primer design

Primers were designed using Primer 3 software (version 0.4.0, Applied Biosystems Inc., Foster City, CA, USA) and were synthesized by Shenggong Bioengineering Co., Ltd. (Shanghai, China) according to the sequence provided on the NCBI website and the principles of real-time fluorescent quantitative PCR primer design (Table 3).

Table 3. Sequence of specific primers (5′→3′) used for target gene expression analyses via real-time fluorescent quantitative PCR.

Gene	Forward Primer	Reverse Primer	Method
apt1	GTTTATGAATGCGCTTGTCTCA	TAGAGCTGACCAGGACAAACA	qRT-PCR
fad2a	CGTGGATCGAGACTACGGGTTA	ATGGTGCGCGACATGTGT	
fad3a	GACTTCAAAACTGTGGCTCT	GATAGCCACACCATTGGTGC	
fad3b	GCAGCGGTCTTGATTTCAACA	ATTTTGAGGACCGGAGCGAA	

(3) Total RNA extraction and cDNA reverse transcription

The specific steps for the extraction of total RNA from oilseed flax capsules were carried out according to the instructions of the RNAsimple Total RNA Kit (centrifugal column type), and 50–100 mg capsule samples were needed for each extraction. In order to ensure the quality of the sample, the integrity of RNA was assessed using agarose gel electrophoresis. The concentration, purity, and quality of the samples were determined using a micro-ultraviolet spectrophotometer, and the total RNA concentration was ≥200 ng/μL, OD 260/280 ≥ 1.8, and OD 260/230 ≥ 1.5. The results show that the quality of the extracted RNA was good [24]. The genomic cDNA was removed using the FastKing one-step method, and the premixed reagent FastKing gDNA Dispelling RT SuperMix (first-chain synthesis kit (de-genomic)) was used to reverse-transcribe RNA into cDNA, which was stored at −20 °C.

(4) Real-time fluorescence quantitative PCR

The PCR reaction was carried out using LightCycler96 (Roche, Basel, Switzerland) real-time fluorescence quantitative PCR. The qRT-reaction system had a 20 μL reaction volume, containing 2 × SuperReal PreMix Plus at 10 μL and 10 μM, forward and reverse primers at 0.6 μL each, and cDNA at 3.3 μL, to which RNase Free dd H_2O was added to reach a final volume of 20 μL. The PCR reactions were first predenatured at 95 °C for 10 min, followed by 40 cycles consisting of denaturation at 95 °C for 15 s, primer annealing at 62 °C, and extension for 32 s. The procedure for each sample was repeated 3 times. The adenine phosphoribosyltransferase 1 gene (*apt1*) in oilseed flax was used as an internal reference gene to calculate the relative expression of the fatty acid desaturase 2a gene (*fad2a*), fatty acid desaturase 3a gene (*fad3a*), and fatty acid desaturase 3a gene (*fad3a*) in three varieties at four stages after anthesis. The relative value of gene expression was calculated using the following formula:

$$Y = 2^{-\triangle\triangle Ct}$$

where Ct represents the number of cycles in which the gene amplification product can be initially detected by fluorescence. ΔCt represents the difference between the number of *apt1* cycles of the target gene and the internal reference gene; that is, $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{internal reference gene}}$. $\Delta\Delta Ct = (Ct_{\text{target gene}} - Ct_{\text{internal reference gene}})$ experimental group $- (Ct_{\text{target gene}} - Ct_{\text{internal reference gene}})$ control group and $2^{-\Delta\Delta Ct}$ represents the change times of gene expression in the treatment group compared with the control group. The expression of corresponding genes in the CK group was set to 1, and the data were based on the average and standard deviation of three sets of biological repeats.

2.5. Statistical Analyses

Data preprocessing was carried out using Excel 2016. The figures were plotted using Origin 2021b (OriginLab Corp., Northampton, MA, USA). All data were submitted for ANOVA using the SPSS package (SPSS, 20.0 Software, Inc., Chicago, IL, USA); all data for each index passed the normality test, and all pairwise multiple comparisons of the treatment means were performed using the least significant difference (LSD) test, with significance determined at the 5% level.

3. Results

3.1. Dynamic Changes in NSC Content

3.1.1. Soluble Sugar Content

The content of soluble sugar (SS) in flaxseed showed a decreasing trend, and it was significantly higher at the kernel stage than at the maturity stage (Figure 4). In both stages, the flaxseed SS content of the Dingya 26 (V2) variety was 4.85% and 11.42% higher than that of the Longya 11 (V1) and Zhangya 2 (V3) varieties, respectively. The average SS content of flaxseed with the sheep manure treatment (S) was 12.39%, 7.63%, and 33.97% higher than that with chicken manure (C), chemical fertilizer (F), and no fertilizer (CK) treatments, respectively. At the kernel stage, the SS content of flaxseed decreased with the increase in the amount of fertilizer, and with 12.5 t ha^{-1} sheep manure (S1), it was significantly higher, by 5.22–38.77%, than other treatments. The SS content also increased with the increase in fertilizer application amount at the maturity stage, and the application of 25 t ha^{-1} sheep manure (S2) was conducive to the transport and accumulation of SS to grains; the decrease in SS content under a low fertilization level may be due to more oil transformation.

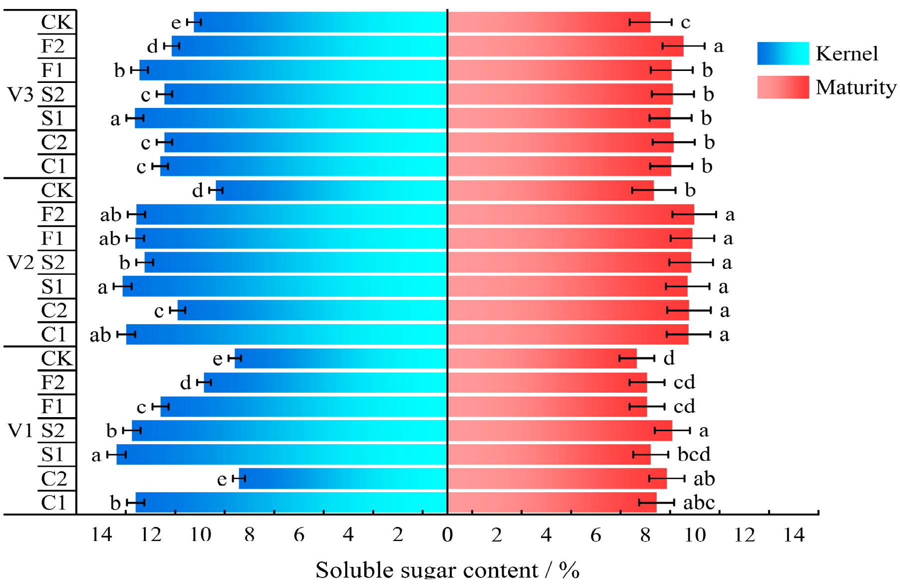

Figure 4. Dynamic changes in SS content in flaxseed with different fertilization treatments. Different lowercase letters indicate significant differences among all treatments at the same variety ($p < 0.05$).

3.1.2. Starch Content

The content of starch (ST) in flaxseed showed an increasing trend from the kernel stage to the maturity stage (Figure 5). The ST content was higher in the flaxseed of the V2 variety than that of the other varieties. Fertilization significantly increased the ST content in flaxseed, and the ST content of all varieties with the sheep manure treatment was on average 5.44% and 15.26% higher than that of the chemical fertilizer and CK treatments, respectively. There was no significant difference between the chicken manure and chemical fertilizer treatments. The ST content of all varieties decreased with the increase in fertilizer application amount, and the S1 treatment resulted in significantly higher ST content, namely by 6.58–24.72% (V1), 2.73–18.60% (V2), and 6.33–18.56% (V3), than those of other treatments, respectively. This indicates that the 12.5 t ha^{-1} sheep manure treatment is beneficial in promoting starch accumulation in flaxseed.

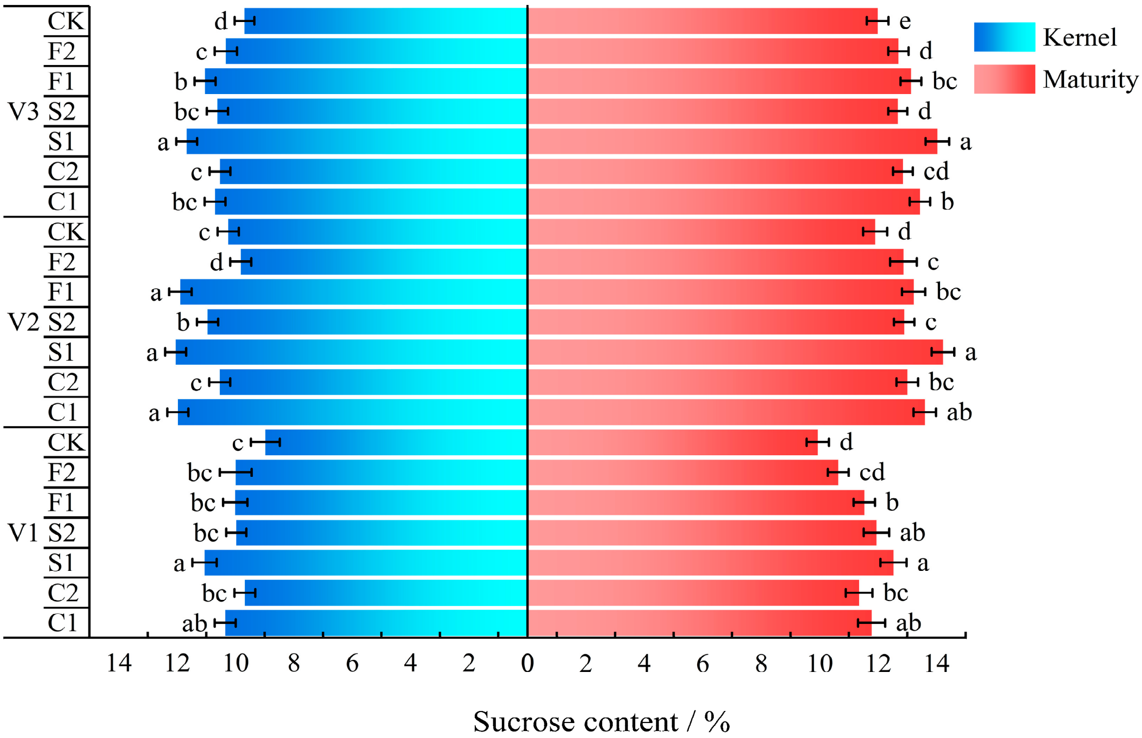

Figure 5. Dynamic changes in ST content in flaxseed with different fertilization treatments. Different lowercase letters indicate significant differences among all treatments at the same variety ($p < 0.05$).

3.1.3. Sucrose Content

The changing trend of the sucrose (SUC) content in flaxseed was consistent with that of SS and decreased with the progression of the oilseed flax growth period (Figure 6). The SUC content of the V2 variety was 16.83% and 10.85% higher than that of the V1 and V3 varieties at the kernel stage, respectively. However, there was no significant difference between the V2 and V3 varieties at the maturity stage. The SUC content of each variety with the S treatment in the kernel stage was significantly higher than that of the F and CK treatments, on average by 13.25% and 19.74%, respectively. The SUC content gradually decreased with the increase in fertilizer application amount, and in the 12.5 t ha^{-1} sheep manure (S1) treatment, it was significantly higher than in other treatments. At the maturity stage, the average SUC content under different fertilizer treatments was F > S > C > CK, and high-fertilizer application amounts yielded a higher average SUC content than low-treatment amounts. The SUC content under the 25 t ha^{-1} sheep manure treatment (S2) was significantly higher than that of other treatments, namely by 1.34–11.31%. In summary, it can be seen that sucrose, as the main form of carbohydrate transport, is affected by the type of fertilizer and the amount of fertilization, and the application of sheep manure can significantly increase the sucrose content in flaxseed.

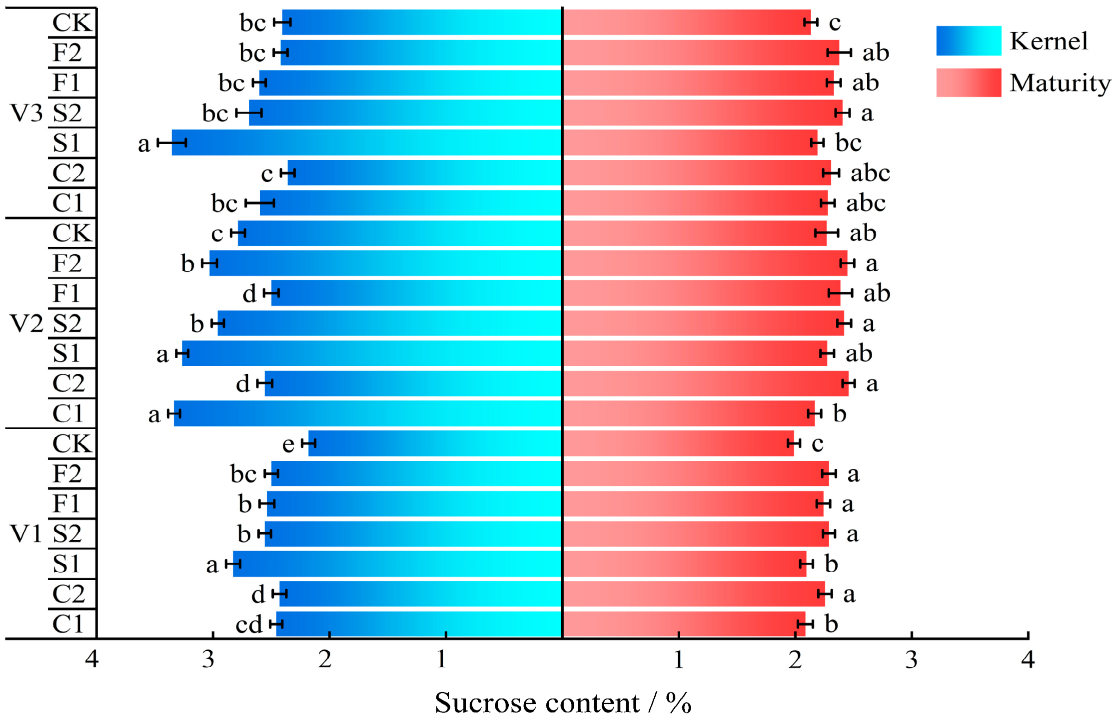

Figure 6. Dynamic changes in SUC content in flaxseed with different fertilization treatments. Different lowercase letters indicate significant differences among all treatments at the same variety ($p < 0.05$).

3.2. Oil and Fatty Acid Accumulation Trend

3.2.1. Grain Oil Content

The grain oil content of different oilseed flax varieties gradually increased after anthesis, reaching the maximum at 42 days after anthesis (Figure 7). The average oil content of the V3 variety was higher than other varieties in both growing seasons. The application of organic manure significantly increased the oil content. In 2021, the oil content with the sheep manure treatment was on average 0.08–1.15% (V2) and 0.65–1.60% (V3) higher than that of the other treatments. In the V1 variety, the chicken manure treatment resulted in a higher oil content than other treatments. In 2022, the oil content of all varieties subjected to the S treatment was on average 0.48–1.49% higher than that of the other treatments. In both years, the oil content of all varieties decreased with the increase in fertilizer application amount. The average increase for the S1 treatment was 0.61% (2021) and 0.90% (2022) compared to that of the S2 treatment, and it was significantly higher than the other treatments, i.e., by 0.38–1.62% (2021) and 0.56–1.97% (2022). Additionally, there were significant differences among all the treatments ($p < 0.05$). Therefore, the continuous application of organic manure could increase the oil content of oilseed flax, and the oil content with the 12.5 t ha^{-1} sheep manure treatment was highest compared with other treatments.

There was a correlation between the oil content and NSCs of oilseed flax (Figure 8). In the two growing seasons, the oil content was slightly or negatively correlated with the contents of SS and SUC in flaxseed at the maturity stage but positively correlated with ST. It was found that SS and SUC could be converted into oil and fatty acids at the maturity stage of oilseed flax, which decreased with the increase in oil content, but there was a nonsignificant correlation between ST and oil synthesis.

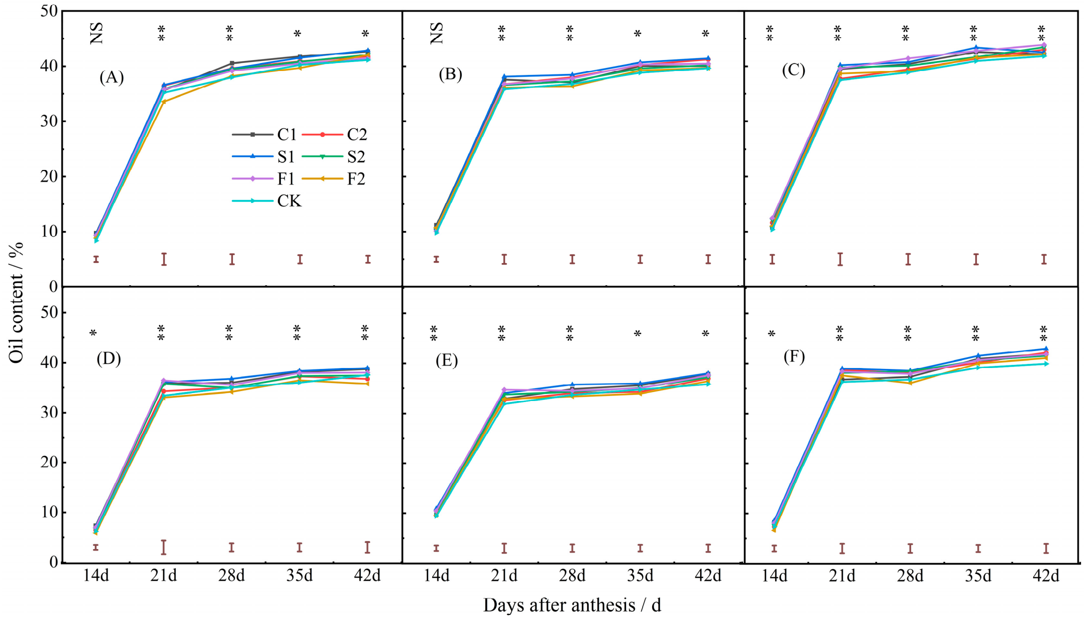

Figure 7. Dynamic changes in grain oil content with different fertilization treatments: (**A**–**C**) represent V1, V2, and V3 varieties in 2021; (**D**–**F**) represent those of 2022, respectively. NS indicates nonsignificant values; * and ** indicate significant and extremely significant differences between treatments at $p < 0.05$ and $p < 0.01$, respectively.

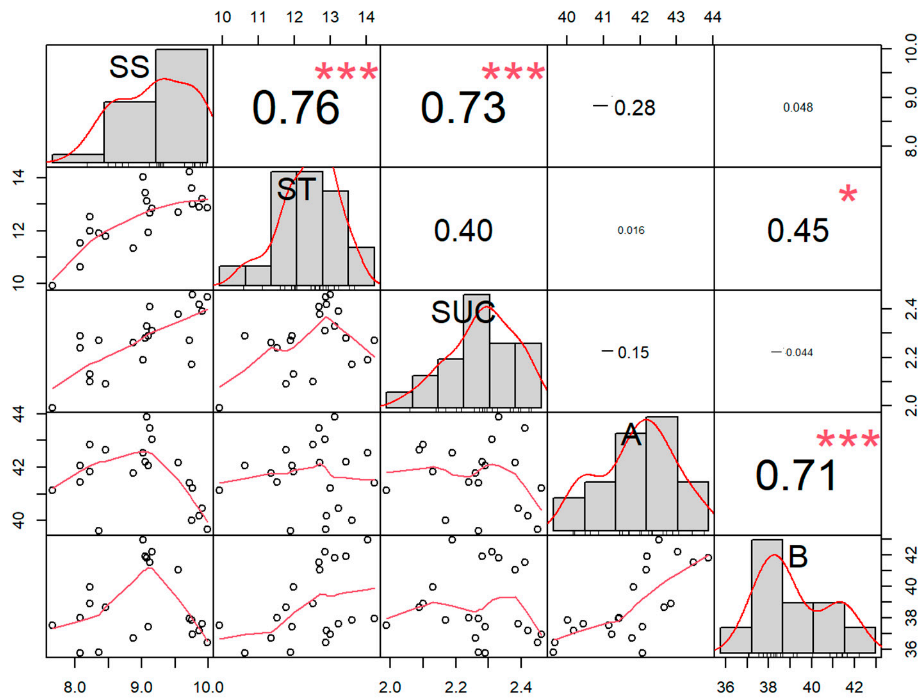

Figure 8. Analysis of correlations between NSCs and oil content in 2021 (A) and 2022 (B). SS indicates soluble sugar, ST indicates starch, and SUC indicates sucrose sugar. The panels with vertical bars (diagonal from top left to bottom right) show the distribution of each variable. The values of the X and Y axes indicate the data size range of the corresponding index. The triangular panel (below the diagonal of the histogram) is a scatter plot of the two variables of interest. The upper triangular panel (above the diagonal of the histogram) provides the numbers representing the correlation coefficients between any two variables, and * and *** represent significant correlations at the 0.05 and 0.001 probability levels, respectively.

3.2.2. Grain Oil Yield

The oil yield in the oilseed flax of the V2 variety was on average 9.67% and 24.34% higher than that of the V1 and V3 varieties in the two growing seasons, respectively (Figure 9). In both growing seasons, except for V3, other varieties had higher oil yield with the treatment of sheep manure and increased with the increase in fertilizer application. The oil yield with the S2 treatment was significantly increased, by 6.79–45.86% (V1), 5.53–42.17% (V2), and 4.29–42.98% (V3), compared with other treatments. This indicates that the application of 25 t ha^{-1} sheep manure can increase the oil yield per unit area of oilseed flax.

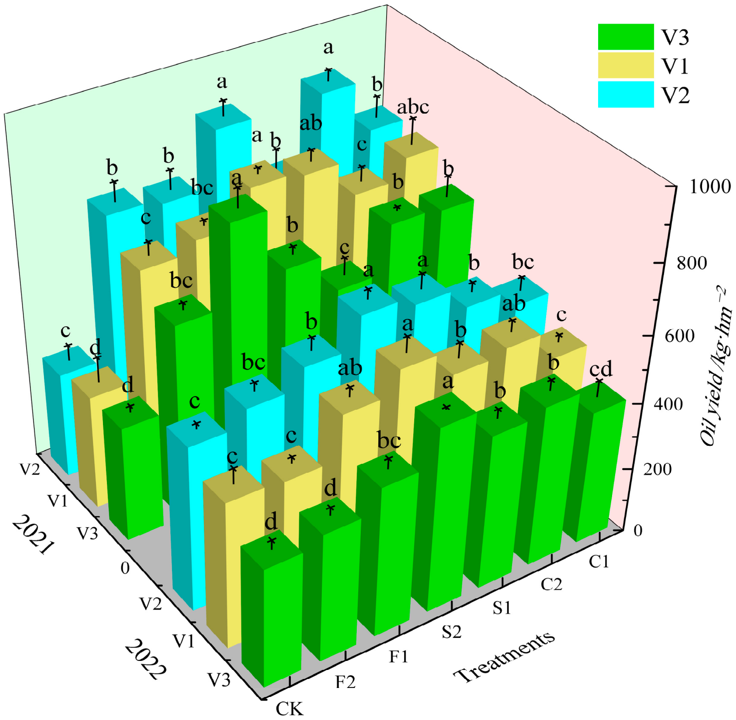

Figure 9. Effects of different fertilization treatments on grain oil yield of oilseed flax in the two growing seasons. Different lowercase letters indicate that the difference was significant at $p < 0.05$ under LSD testing.

3.2.3. Accumulation Rules of Different FA Components

The fatty acid (FA) compositions of different genotypes of oilseed flax at different stages after anthesis were determined using gas chromatography. The linolenic acid (LIN) content was highest in different FA components, which increased with the change in days after anthesis and gradually decreased at the maturity stage. Palmitic acid (PAL), stearic acid (STE), and linoleic acid (LIO) all showed a decreasing trend, while oleic acid (OLE) showed an unstable trend (Figure 10). Different fertilization treatments had significant effects on the accumulation of different fatty acid components in the two growing seasons (Tables 4 and 5). The content of FA markedly changed 21 days after anthesis, which indicated that this was the peak of oil synthesis and accumulation. The contents of LIN and LIO were significantly different among the different genotypes of oilseed flax. We observed that in oilseed flax samples with a higher relative LIN content, i.e., the V3 and V1 varieties, the LIN content was 4.56% and 3.24% higher than that of the V2 variety in the two growing seasons, respectively, while the contents of STE, OLE, and LIO were higher in oilseed flax with a relatively low LIN content, i.e., the V2 variety. There was no significant difference among the different varieties in terms of PAL. The application of organic manure seemed to increase the contents of OLE, LIO, and LIN, while PAL and STE concentrations improved with chemical fertilizer treatments.

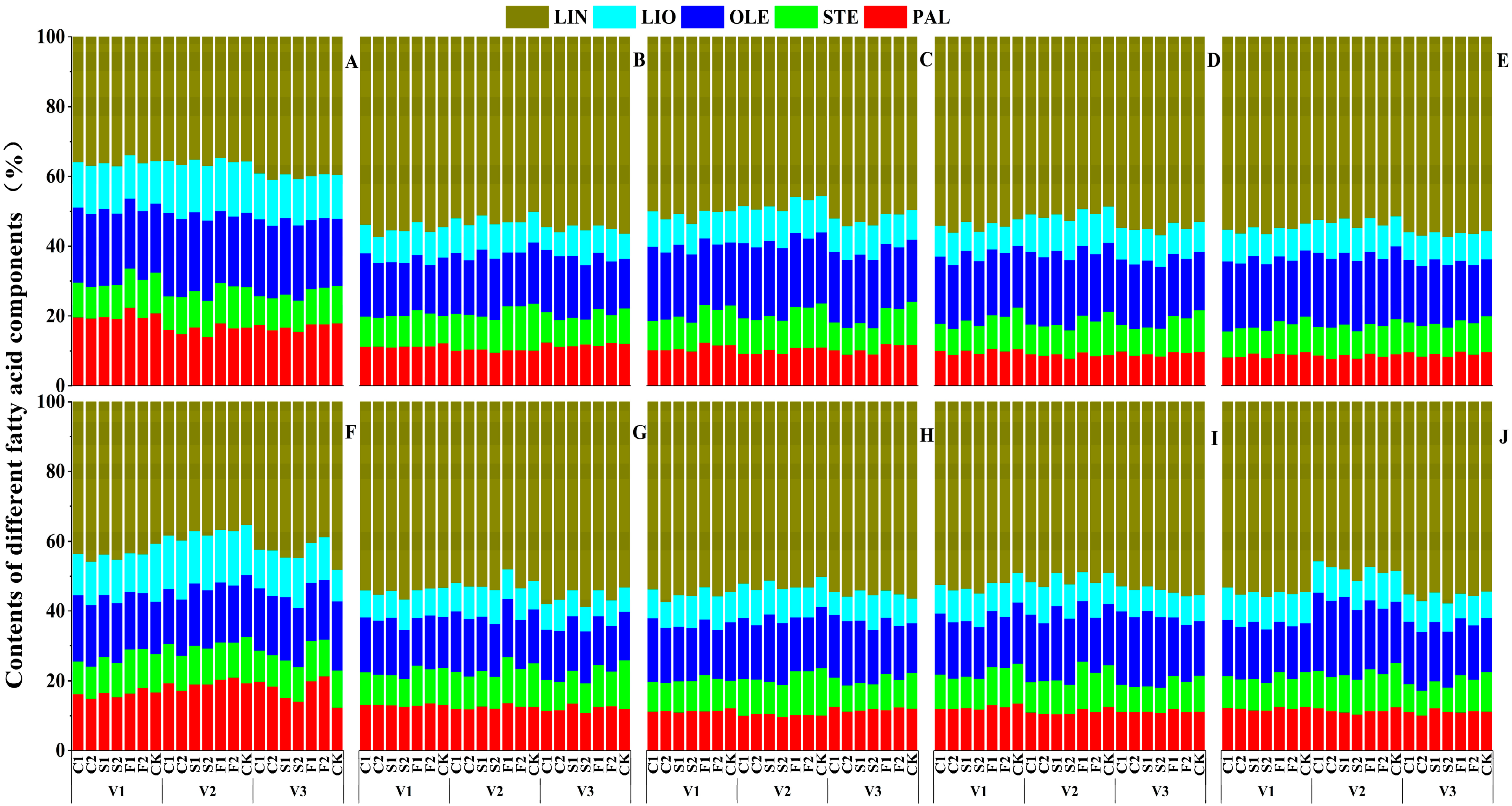

Figure 10. Changes in fatty acid accumulation in different varieties: (**A**–**E**) represent the changes in different FA components at 14, 21, 28, 35, and 42 days after anthesis in 2022; (**F**–**J**) represent the changes in 2021.

Table 4. ANOVA results concerning the accumulation of different fatty acid components in 2021. Different lowercase letters indicate that the difference was significant at $p < 0.05$ in LSD testing. NS indicates a nonsignificant value; * and ** indicate significant and extremely significant differences between treatments at $p < 0.05$ and $p < 0.01$, respectively.

Treatments		14 Days after Anthesis					21 Days after Anthesis					28 Days after Anthesis					35 Days after Anthesis					42 Days after Anthesis				
		PAL	**STE**	**OLE**	**LIO**	**LIN**	**PAL**	**STE**	**OLE**	**LIO**	**LIN**	**PAL**	**STE**	**OLE**	**LIO**	**LIN**	**PAL**	**STE**	**OLE**	**LIO**	**LIN**	**PAL**	**STE**	**OLE**	**LIO**	**LIN**
V1	C1	b	e	a	c	b	a	cd	ab	c	ab	bc	bcd	a	c	c	c	bc	a	cd	b	ab	b	a	a	b
	C2	c	e	abc	b	a	a	de	abc	c	ab	b	cd	bc	a	a	c	d	b	b	ab	bc	bc	bc	a	ab
	S1	b	cd	ab	c	b	a	de	a	c	ab	c	bc	bc	abc	b	bc	cd	b	b	ab	d	b	a	b	ab
	S2	c	de	bc	b	a	a	e	cd	a	a	bc	bcd	c	ab	b	c	cd	c	a	a	d	c	b	ab	a
	F1	b	a	bcd	d	b	a	a	d	bc	ab	bc	a	bc	ab	c	ab	ab	b	d	b	b	a	cd	b	ab
	F2	a	b	cd	d	b	a	bc	abc	c	b	b	b	d	a	b	bc	a	c	a	b	c	bc	bc	ab	ab
	CK	b	bc	d	a	c	a	b	bcd	ab	b	a	d	b	bc	bc	a	a	a	c	c	a	a	d	ab	ab
V2	C1	b	b	d	b	ab	b	b	a	c	ab	ab	c	b	a	abc	b	de	b	bc	abc	ab	b	a	bc	b
	C2	a	cd	cd	a	a	b	cd	ab	a	a	a	cd	c	a	a	b	cd	d	a	a	bc	b	a	ab	ab
	S1	b	bc	a	b	ab	ab	bc	bc	bc	a	a	d	a	a	bc	b	c	a	bc	bc	c	b	a	d	ab
	S2	b	cd	abcd	ab	ab	b	d	cd	a	a	b	d	b	a	ab	b	e	bc	ab	a	c	b	b	cd	a
	F1	ab	bcd	abc	b	ab	a	a	ab	bc	b	ab	b	c	b	ab	a	a	cd	d	c	bc	a	b	ab	ab
	F2	a	d	bcd	ab	ab	ab	b	d	ab	a	ab	b	c	b	ab	b	b	d	ab	ab	bc	b	bc	a	ab
	CK	b	a	ab	b	b	ab	a	bcd	c	ab	ab	a	b	b	c	a	b	cd	cd	bc	a	a	c	c	ab
V3	C1	ab	a	b	d	bcd	cd	de	ab	b	ab	a	b	a	d	ab	a	c	a	bc	b	bc	c	a	bc	abc
	C2	b	a	b	b	bc	cd	e	ab	a	ab	b	cd	a	d	ab	a	c	a	abc	b	c	d	ab	a	ab
	S1	c	ab	b	cd	ab	a	cd	a	b	ab	ab	bc	a	b	b	a	c	a	bc	ab	a	cd	ab	ab	bc
	S2	c	b	b	a	ab	d	e	ab	b	a	ab	d	bc	a	ab	a	c	a	ab	ab	bc	d	b	bc	a
	F1	ab	a	b	cd	cd	abc	b	bc	b	ab	ab	a	b	c	b	a	ab	b	c	ab	bc	a	b	d	bc
	F2	a	b	b	bc	d	ab	c	c	b	ab	ab	bcd	bc	b	ab	a	b	b	a	a	ab	b	b	abc	abc
	CK	d	ab	a	e	a	bcd	a	bc	b	b	ab	a	c	cd	a	a	a	b	abc	a	ab	a	b	cd	c
Variety		**	**	**	**	**	NS	NS	**	**	**	**	**	**	**	**	**	NS	**	**	**	**	NS	**	**	**
Fertilization		**	**	**	NS	*	**	**	**	**	**	**	**	**	*	**	*	**	**	*	**	**	**	*	*	NS

Table 5. ANOVA results concerning the accumulation of different fatty acid components in 2022. Different lowercase letters indicate that the difference was significant at $p < 0.05$ in LSD testing. NS indicates a nonsignificant value; * and ** indicate significant or extremely significant differences between treatments at $p < 0.05$ and $p < 0.01$, respectively.

Treatments		14 Days after Anthesis					21 Days after Anthesis					28 Days after Anthesis					35 Days after Anthesis					42 Days after Anthesis				
		PAL	STE	OLE	LIO	LIN	PAL	STE	OLE	LIO	LIN	PAL	STE	OLE	LIO	LIN	PAL	STE	OLE	LIO	LIN	PAL	STE	OLE	LIO	LIN
V1	C1	bc	bcd	ab	a	a	ab	bc	a	ab	bc	b	c	a	a	b	ab	cd	ab	ab	abc	bc	b	a	a	ab
	C2	c	d	ab	a	a	b	d	a	a	ab	b	bc	abc	ab	ab	c	d	ab	a	a	bc	ab	b	ab	a
	S1	bc	d	a	a	a	ab	cd	a	ab	abc	b	abc	ab	bc	ab	ab	c	a	ab	bc	ab	b	a	ab	bc
	S2	c	cd	ab	a	a	b	b	ab	bc	a	b	c	abc	bc	a	bc	cd	ab	ab	ab	c	ab	b	ab	a
	F1	a	ab	ab	a	b	a	ab	b	c	c	a	ab	bc	c	b	a	b	ab	b	abc	abc	ab	b	ab	bc
	F2	bc	abc	b	a	a	ab	a	b	c	abc	a	abc	bc	ab	b	ab	b	ab	b	abc	abc	ab	b	a	abc
	CK	b	a	b	a	a	ab	ab	ab	bc	c	a	a	c	abc	b	a	a	b	b	c	a	a	b	b	c
V2	C1	ab	a	a	a	abc	bc	b	a	ab	ab	b	b	a	a	ab	ab	cd	ab	bc	abc	bc	bc	a	b	ab
	C2	b	a	abc	a	ab	bc	b	a	a	a	b	b	ab	a	a	b	cd	bc	ab	ab	d	b	a	a	ab
	S1	ab	a	abc	a	bc	bc	b	a	a	ab	a	b	a	a	ab	ab	cd	a	c	abc	ab	bc	a	ab	ab
	S2	b	a	ab	a	a	c	b	a	a	a	b	b	ab	a	a	c	d	abc	ab	a	d	c	a	ab	a
	F1	a	a	bc	a	c	a	a	ab	c	b	a	a	ab	a	b	a	b	bc	bc	bc	a	bc	a	ab	ab
	F2	ab	a	c	a	abc	ab	a	ab	bc	ab	a	a	b	a	ab	b	bc	c	a	abc	c	bc	a	ab	ab
	CK	ab	a	abc	a	abc	abc	a	b	c	a	a	a	ab	a	b	b	a	bc	c	c	ab	a	a	c	b
V3	C1	a	b	a	ab	a	c	abc	a	abc	c	ab	c	a	ab	ab	a	c	a	a	bcd	ab	b	ab	ab	ab
	C2	b	ab	abc	a	a	c	bc	ab	a	ab	b	c	ab	ab	a	a	c	ab	a	ab	b	b	ab	ab	ab
	S1	ab	ab	ab	bc	a	bc	abc	a	ab	c	ab	c	a	ab	ab	a	c	a	a	abc	ab	b	a	b	ab
	S2	b	ab	ab	a	a	c	c	ab	ab	a	b	c	a	a	a	a	c	ab	a	a	b	b	ab	ab	a
	F1	a	a	bc	c	a	a	ab	ab	d	c	a	b	bc	b	ab	a	ab	ab	a	cd	a	b	ab	ab	ab
	F2	a	a	abc	bc	a	ab	ab	b	bcd	bc	a	b	c	ab	ab	a	b	ab	a	abc	ab	b	b	a	ab
	CK	a	a	c	bc	a	ab	a	ab	cd	c	a	a	c	b	b	a	a	b	a	d	ab	a	b	ab	b
Variety		**	**	**	**	**	**	**	**	**	**	NS	**	**	**	NS	**	**	**	**	**	**	**	**	**	**
Fertilization		**	**	*	**	**	**	**	**	NS	NS	NS	**	**	**	NS	**	**	**	NS	NS	*	**	**	**	NS

We further studied the characteristic responses of FA components to different fertilizer types in different oilseed flax varieties (Figure 11). The characteristic values of PAL and STE for different oilseed flax varieties were higher with the treatments using a lower fertilizer application amount, and chemical fertilizer promoted the accumulation of more PAL and STE than organic manure. The contents of PAL and STE were increased on average by 1.14–9.60% and 6.70–19.39%, respectively, in the low-fertilizer treatment (F1) compared with other treatments. The characteristic intensity of OLE for all varieties was the highest in the treatment with the low-fertilizer application of sheep manure (S1). There were significant differences in LIO among the different oilseed flax varieties. The characteristic values of the V1 variety were higher under low-fertilizer treatments, and the characteristic values of the V2 and V3 varieties increased with the increase in fertilizer application amount; therefore, LIO was more likely to accumulate with the treatments using high-fertilizer application amount. However, on average, the LIO content of each variety increased by 0.56–17.55% compared with other treatments with the low-fertilizer (C1) chicken manure treatment. Linolenic acid is the most important index to evaluate the quality of oilseed flax among these fatty acid components. The characteristic intensity of LIN for all varieties increased with the increase in fertilizer application amount, and the LIN content of the S2 treatment was 0.24–4.88% (V1), 0.42–4.86% (V2), and 1.18–3.25% (V3) higher than other fertilizer treatments, respectively, indicating that the application of sheep manure is an effective way to improve the quality of oilseed flax.

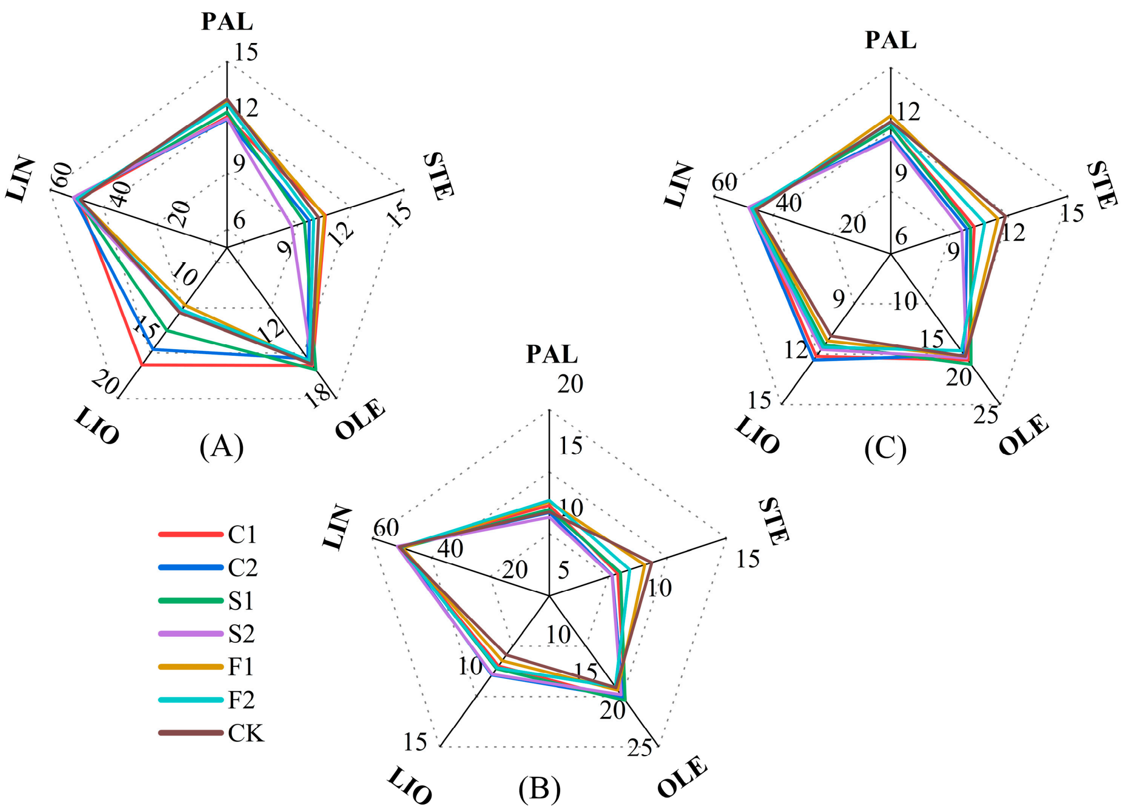

Figure 11. Characteristic responses of different FA components to fertilizer treatments: (**A**) Longya 11 (V1) variety; (**B**) Dingya 26 (V2) variety; (**C**) Zhangya 2 (V3) variety.

Correlation analysis among the FA components showed that PAL in the grains of oilseed flax was significantly negatively correlated with OLE and LIO and positively correlated with STE and LIN (Figure 12). OLE was positively correlated with LIO but negatively correlated with the other fatty acids. The correlation between LIO and other fatty acids was basically the same as OLE. LIN was positively correlated with PAL but not significantly, while it was negatively correlated with STE, OLE, and LIO, indicating that the increase in the LIN content was accompanied by a decrease in the OLE and LIO content.

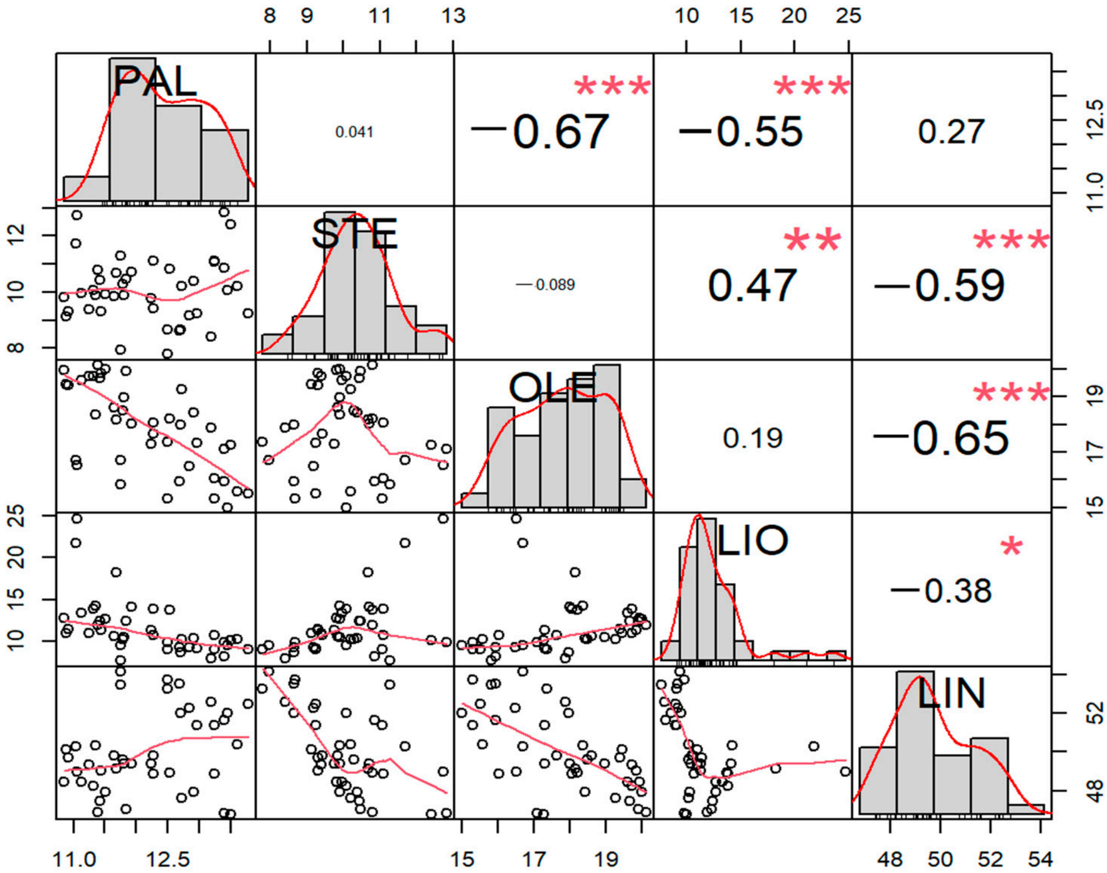

Figure 12. Correlation analysis of different FA components. The panels with vertical bars (diagonal from top left to bottom right) show the distribution of each variable. The values of the X and Y axes indicate the data size range of the corresponding index. The triangular panel (below the diagonal of the histogram) is a scatter plot of the two variables of interest. The upper triangular panel (above the diagonal of the histogram) provides the numbers representing the correlation coefficients between any two variables, and *, **, and *** represent significant correlations at the 0.05, 0.01, and 0.001 probability levels, respectively.

3.3. Desaturase Gene Expression

3.3.1. Analysis of Gene Expression Pattern

The expression profiles of three genes in the different oilseed flax varieties with different fertilization treatments in different periods were drawn as thermograms after normalization (Figure 13). The expression of the *fad2a* gene was significantly upregulated at 0 and 7 days after anthesis but significantly downregulated at 21 days after anthesis. The expression of the *fad3a* gene was upregulated at 21 and 35 days after anthesis, while the expression was low in the early stage of seed development. The expression patterns of the *fad3b* and *fad3a* genes were similar. Considering organic manure, the gene expression of the different varieties on different days after anthesis was upregulated compared to that of the chemical fertilizer treatment, which was beneficial to the process of fatty acid desaturation.

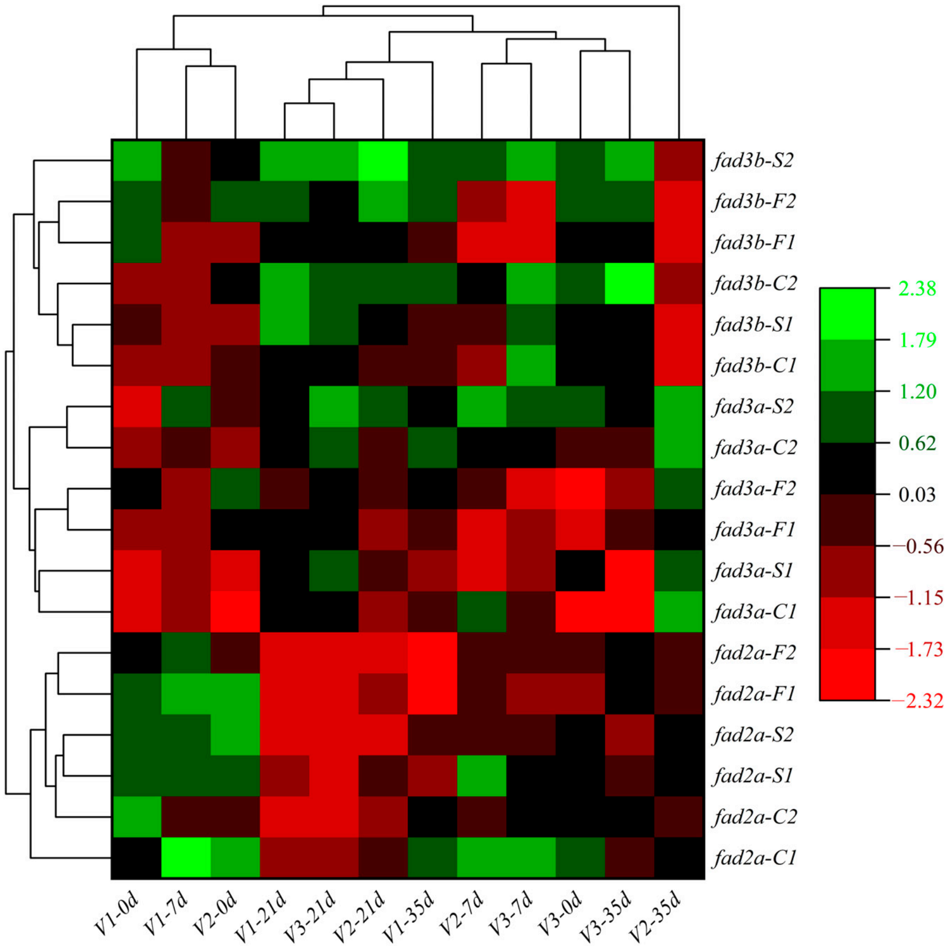

Figure 13. Expression patterns of target genes with different fertilization treatments. Green represents upregulated gene expression, and red represents downregulated gene expression.

3.3.2. fad2a Expression

The fatty acid desaturase 2a gene (*fad2a*) was expressed in the capsules of each oilseed flax variety. The expression of the *fad2a* gene at 0 days after anthesis was lower than that of the internal reference gene, which was the adenine phosphate ribose transferase 1 gene (*apt1*), and then it gradually increased, reaching a peak at about 21 days, and significantly decreased at 35 days (Figure 14). The relative expression of *fad2a* in the V2 variety was the highest; its expression levels at 0, 7, 21, and 35 days increased by 1.49, 4.36, 49.05, and 1.68 times, respectively, compared with the internal reference gene. The expression of the V3 variety was the lowest. With no fertilization (CK) as the control, there was no significant difference in *fad2a* expression among the different fertilization treatments at 0 days. The expression of *fad2a* was higher under organic manure treatments at 7 and 21 days and increased by 56.42 times (V2), 45.56 times (V1), and 37.36 times (V3) at 21 days under the C treatment; these values were 12.88, 14.15, and 3.93 times higher, respectively, than those for chemical fertilizer. A comparison of different fertilizer application amounts showed that the *fad2a* gene expression of the 5.8 t ha^{-1} chicken manure (C1) treatment was 0.55–1.33 times and 5.38–20.32 times higher than that of other treatments at 7 and 21 days after anthesis. It was found that *fad2a* gene expression was higher in low-linolenic acid varieties, and the 5.8 t ha^{-1} chicken manure treatment significantly increased its relative expression.

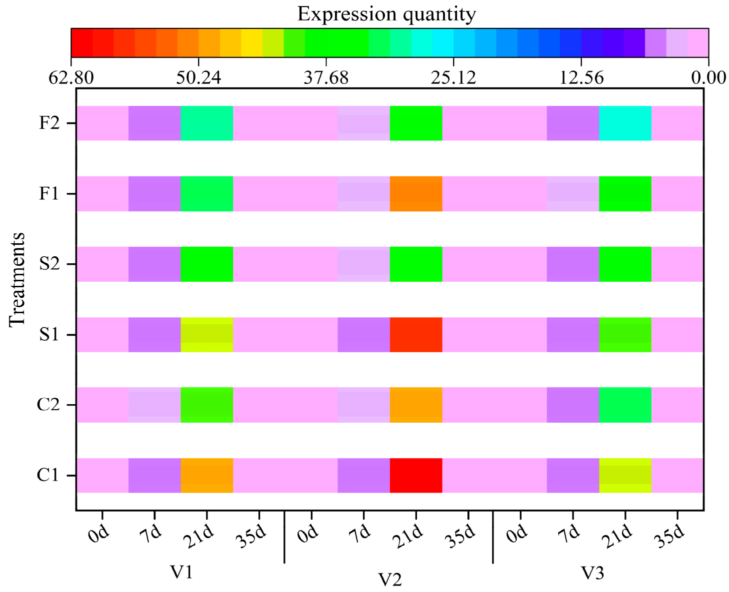

Figure 14. Expression of *fad2a* gene in different varieties at different days after anthesis.

3.3.3. fad3a Expression

With the increase in days after anthesis, the expression of the fatty acid desaturase 3a gene (*fad3a*) increased at first and then decreased, reaching a peak at 21 days (Figure 15). The expression of the *fad3a* gene in each variety was lower in the early stage of capsule development. The expression of the *fad3a* gene in the V3, V2, and V1 varieties increased by 92.94, 66.69, and 79.48 times compared with that of *apt1* at 21 days, respectively. The highest expression was in the V1 variety and the lowest was in the V2 variety at 35 d. The relative expression of *fad3a* subjected to the S treatment was the highest at 7 and 21 days. The relative expression of *fad3a* in capsules of all oilseed flax varieties increased with the increase in fertilizer application amount. At 21 days after anthesis, the relative expression of *fad3a* undergoing the S2 treatment was 9.03–29.02 times higher than that of the other treatments. It was observed that the expression of the *fad3a* gene in high-linolenic acid oilseed flax varieties was higher, and treatment with 25 t ha^{-1} sheep manure was beneficial for the upregulation of the expression of this gene.

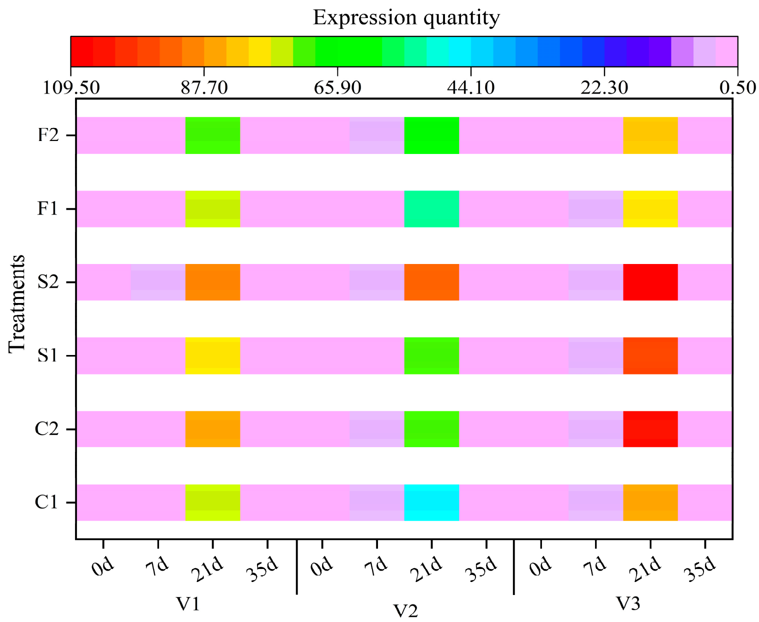

Figure 15. Expression of *fad3a* gene in different varieties at different days after anthesis.

3.3.4. fad3b Expression

The expression patterns of *fad3b* and *fad3a* in oilseed flax capsules were similar (Figure 16). The expression of the *fad3b* gene in oilseed flax capsules subjected to the organic manure treatment was significantly higher than that with the chemical fertilizer treatment. The expression of *fad3b* in capsules of each oilseed flax variety increased with the increase in fertilizer application amount, and the S2 treatment was 0.01–0.34, 0.30–1.40, and 16.64–38.08 times higher than other treatments at 0, 7, and 21 days, respectively. It can be seen that the 25 t ha^{-1} sheep manure treatment promoted the expression of the *fad3b* gene in oilseed flax capsules, and the Zhangya 2 variety had a higher peak expression of the *fad3b* gene.

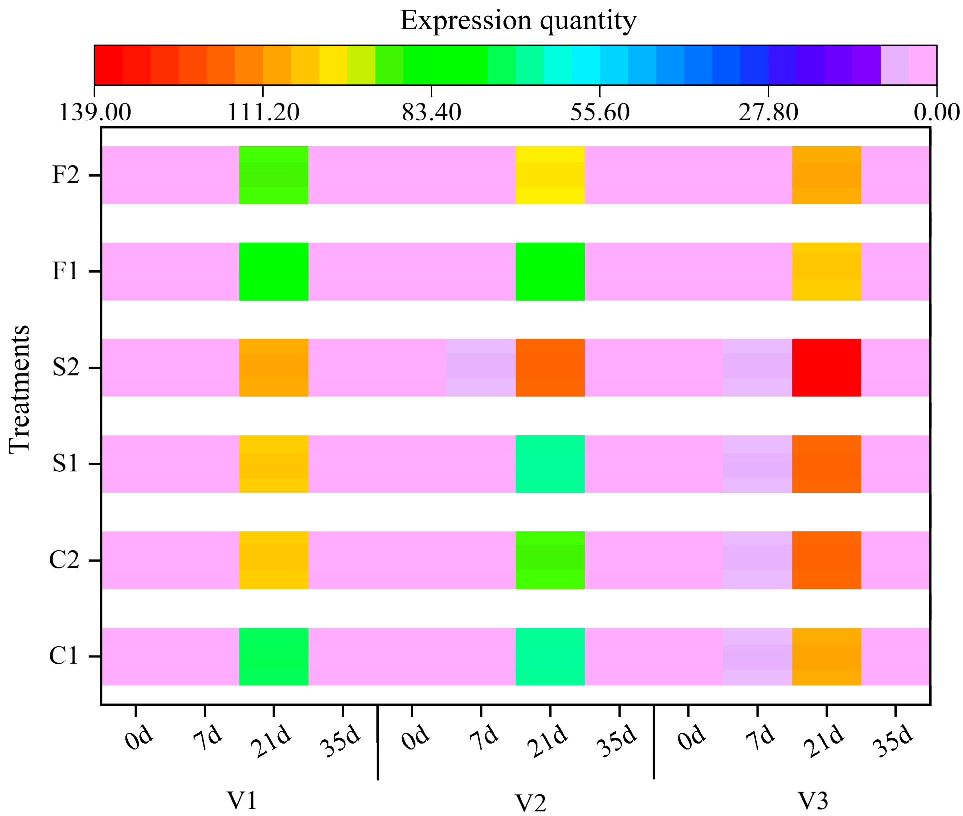

Figure 16. Expression of *fad3b* gene in different varieties at different days after anthesis.

4. Discussion

NSCs are the material basis for the formation of crop yield, and changes in their content represent the synthesis, transport, and utilization of plant carbohydrates. In the current study, it was found that the SS content in flaxseed decreased from the kernel stage, which may have been caused by the transformation of SS to oil and fatty acid, and the changing trend of SUC was similar to that of SS. Grain filling primarily involves starch synthesis and accumulation. ST is an important energy storage material for plants. When crop production decreases, more photosynthate is stored in the plant in the form of ST [25]. The current study results show that the ST content in the capsules increased, and the ST content in the maturity stage was significantly higher than that in the kernel stage, indicating that the assimilates synthesized from oilseed flax leaves were transported to the grain and stored in the form of ST.

The application of organic manure can increase the content of soil organic matter, and the transformation of soil carbon is closely related to it. Therefore, the application of organic fertilizer provides a more enzymatic matrix for invertase, increases the activity of sucrose invertase, and increases the accumulation of sugar [26,27]. It has been reported that sheep manure can reduce the content of soluble protein but significantly increases the content of soluble sugar compared with chicken manure [28]. The current study results show that the SS content of oilseed flax capsules subjected to sheep manure treatment was significantly higher than that of other treatments, and the application of 12.5 t ha^{-1} yielded significantly higher levels than the other treatments at the kernel stage, namely by 5.22–38.77%, while the 25 t ha^{-1}sheep manure treatment yielded higher levels than the other treatments at the maturity stage. The changing trend of the SUC content was basically the same as that of SS. Gebbing et al. reported that higher nitrogen fertilizer levels enhance soil microbial activity and promote the transformation of soil inorganic nitrogen into organic nitrogen, which requires photosynthesis to form additional carbon skeletons. Therefore, when there is enough nitrogen in plants, carbon is essential for nitrogen metabolism, which leads to the conversion of a large amount of soluble sugar into carbon skeletons through the plant metabolic process, thus reducing the carbohydrate content [29]. However, a lower amount of nitrogen fertilizer will cause more carbohydrates to accumulate in leaves and a higher level of carbon to be distributed to roots. This increases the root-shoot ratio and strengthens the osmotic regulation ability of plants, allowing them to rapidly accumulate a large amount of carbon for their growth and maintain the balance of carbon and nitrogen metabolism [30,31]. In the current study, the contents of SS and SUC in grains decreased with the increase in fertilizer application amount at the kernel stage, which is consistent with the results of previous studies. However, SS and SUC showed higher contents after high-fertilizer application at the maturity stage, which may be due to the enhancement of sugar metabolism and the sink strength of roots under low nitrogen stress, which causes more sugar to be transported to the root, and therefore the sugar output becomes greater than the accumulated sugar due to oil accumulation, resulting in a decrease in sugar content under low-fertilizer treatment [32]. The application of organic fertilizer can increase the activity of enzymes related to starch synthesis and decomposition and thus regulate starch content [33]. Wang et al. suggested that chicken manure, despite promoting plant growth, weakens the storage nutrients of plants, and the contents of soluble sugar and starch are significantly lower in these plants than in those subjected to sheep manure treatment [34]. The results of the current study show that organic manure significantly promoted the accumulation of ST in oilseed flax capsules. With the sheep manure treatment at 12.5 t ha^{-1}, ST accumulated more in the capsules, while an excessive amount of nitrogen would have caused most of the starch to remain in the vegetative organs, which would limit its transfer to the grain to a certain extent. It may be that the vegetative organs of oilseed flax maintain vigorous growth in the later growth stage with high-fertilizer application amount, and a large amount of energy is consumed for the establishment of their organs. Yan et al. [33] reached a similar conclusion, but this is contradictory to the results of Scofield et al. [35]. Therefore, its mechanism needs to be further studied.

During the development of oil crop seeds, sugar metabolism not only provides nutrients and energy for seed growth and development but also is closely related to oil and fatty accumulation [36]. Some researchers have suggested that starch is not an important carbon source for oil and fatty acid synthesis during seed maturation [37,38]. Zhao et al. [39] also reported that the content of soluble sugar in Xanthoceras sorbifolia (*Xanthoceras sorbifolia* Bunge) seeds decreased almost linearly with the rapid accumulation of oil and fatty acids, while the starch that accumulated instantaneously was slightly degraded before the rapid accumulation of oil, but this did not meet the requirements of oil synthesis. Therefore, the most important carbon source of oil synthesis is soluble sugar rather than starch. Soluble sugar components undergo a significant changing period in the process of oil crop seed development, which will directly or indirectly affect the oil accumulation of late-stage seeds [40]. The expression of the sucrose synthase gene is higher in the middle and later stages of seed development, which may indicate the transformation of sugars to oil [41]. According to the correlation analysis results of the current study, the oil content of grain was negatively correlated with SS and SUC but positively correlated with ST, indicating that the decrease in SS and SUC contents is likely because they are the main carbon source of oil synthesis. However, starch had no significant correlation with oil synthesis, which is consistent with previous studies.

Alzamel et al. [42] reported that when organic fertilizer was used as a nitrogen source, the oil content of sunflower (*Helianthus annuus* L.) seeds was significantly increased. A change in the nitrogen source during seed ripening will affect the synthesis of FA, thus affecting the final proportion of fatty acids in mature seed oils. It has been reported that with the increase in nitrogen application amount, the absorption of N by crops increases, whereas the oil content of seeds decreases [14,43]. The high absorption rate of nitrogen leads to a decrease in the utilization rate of carbohydrates in oil synthesis but increases protein synthesis [44]. The reason for this correlation is related to the competition for carbon skeletons in the process of carbohydrate metabolism [45]. The synthesis of amino and fatty acids requires carbon compounds from the decomposition of carbohydrates. Since the carbohydrate content of proteins is lower than that of oil, increased N supply intensifies the synthesis of proteins at the expense of fatty acid synthesis and thus reduces the oil content of the seed [46]. We came to a similar conclusion, as organic manure increased the oil content of oilseed flax grains compared with chemical fertilizer and the control, and the application of sheep manure increased the oil content significantly. A comparison of different fertilization treatments showed that the grain oil content with a low-fertilizer application (N 112.5 kg ha^{-1}) was significantly higher than that with a high-nitrogen fertilizer application (N 225 kg ha^{-1}). Saraiva et al. evaluated the planting system with different fertilizer sources and found that the nitrogen release rate of sheep manure was significantly lower than that of chicken manure. Therefore, the high nitrogen efficiency of chicken manure enhanced protein synthesis and decreased oil synthesis, which may be the reason for the high oil content when using the sheep manure treatment [47]. High-fertilizer application amount reduced the grain oil content; however, because it increased the grain yield per unit area, the grain oil yield per unit area also increased significantly. The grain oil yield of the different varieties significantly increased with the 25 t ha^{-1} sheep manure treatment, on average by 8.00–43.63%, compared with the other treatments. In summary, the application of sheep manure is beneficial to increasing the oil content of oilseed flax under this experimental condition.

The concentration of unsaturated fatty acids in oilseed flax grain is very important for its final use and market price. There are significant differences in fatty acid contents among different flax varieties; it is reported that the LIN of bright-colored oilseed flax grains is higher than that of dark-colored oilseed flax varieties, while the contents of STE and OLE are relatively lower [48]. In the current study, there was no significant difference in PAL among the different varieties. The contents of STE, OLE, and LIN were the highest in the Dingya 26 variety, and while the Zhangya 2 variety is one of the few white-grain varieties in China, the LIN content of its grains was significantly higher than that of other dark varieties; this is consistent with previous reports. The correlation analysis showed that there was a significant negative correlation between OLE and LIN, which is because LIN results from the continuous desaturation of OLE and LIO [49]. Fertilization has a significant effect on the contents of different FA components in oilseed flax. When organic manure was used as a nitrogen source, the contents of PAL, STE, and LIO decreased, while the content of OLE increased significantly [50]. After the application of sheep manure, the contents of STE and LIO in tomato (*Solanum lycopersicum* L.) were lower than those treated with chemical fertilizer alone [51]. Our results are not exactly the same; after applying organic manure, the contents of PAL and STE in oilseed flax were lower than those subjected to chemical fertilizer treatment, while the contents of LIO in the different varieties were different with different fertilizer treatments. Considering the whole analysis after anthesis, organic manure increased the LIO content. Mohammadi [16] reached a similar conclusion. Compared with chemical fertilizer treatment, the application of organic manure significantly increased the contents of OLE and LIN, which may be due to the fact that, as a nitrogen source, organic manure can promote the hydrolysis rate of fatty acid complexes or their transport from proplastids to cytoplasmic compartments [50]. OLE is a monounsaturated fatty acid, whereas LIN and LIO are considered to be polyunsaturated fatty acids, so the application of organic manure increases the content of total unsaturated fatty acids. Unsaturated fatty acids are used as an important index to evaluate crop seed oil; the higher their content, the better the crop quality [52]. It can be seen that the application of organic manure can improve the quality of oilseed flax. The study of Zheljazkov et al. [53] on mustard (*Brassica juncea* (L.) Czern) suggested that the yields of LIO and LIN increased when using a high-nitrogen fertilizer. The high nitrogen level increased the content of unsaturated fatty acids, which was also confirmed by Xie et al. [54] for oilseed flax. We also concluded that with the increase in fertilizer application amount, the contents of PAL, STE, and OLE decreased, while the contents of LIO and LIN significantly increased with the increase in fertilizer application amount. The average LIN content of different varieties was the highest with the sheep manure 25 t ha^{-1} treatment, namely 0.64–3.90% higher than the other treatments. The nutritional value of the grain oil of oilseed flax is mainly due to its richness in α-linolenic acid and other unsaturated fatty acids, which are necessary for the human body. There is no doubt that organic manure has great application potential regarding oilseed flax.

FA metabolism is an important biological function of organisms, and plant oil metabolism engineering has become a prominent research goal. Currently, there are no studies or data concerning the effects of fertilizers on FA biosynthetic gene expression patterns in oilseed flax capsules with different LIN content genotypes. In the current study, qRT-PCR was used to detect the relative expression of *fad2a*, *fad3a*, and *fad3b* genes during grain development for three oilseed flax varieties with different LIN content genotypes using different fertilizer treatments. The *fad2a* gene plays a major role in the synthesis of LIO, while the *fad3a* and *fad3b* genes are primarily involved in the process of LIO desaturation to LIN. Gutierrez et al. [55] studied some commonly used housekeeping genes and found that the *apt1* gene was the most stably expressed; this was used for the relative quantification of the *fad* gene in the current study. Our results show that the expression of *fad2a*, *fad3a*, and *fad3b* genes in different varieties of oilseed flax capsules increased at first and then decreased with the change in days after anthesis, reaching a peak at about 21 days, indicating that they all contributed to the accumulation of LIN. In the process of grain development, the overall expression patterns of *fad3a* and *fad3b* were the same. Previous research highlighted the co-expression of *fad3a* and *fad3b* and their additive effects in LIN synthesis [56]. However, the expression levels observed in the current study were quite different from those of previous studies, which may be related to the state of the samples and environmental factors during sampling. Nevertheless, these data clearly support the role of *fad3a* and *fad3b* in the production of LIN in oilseed flax grains. The expression of FA desaturase genes can be induced by hormones, as well as biotic and abiotic (temperature, light, etc.) exposure factors [57]. Dvorianinova et al. [58] only analyzed the important roles of *FAD*2 and *FAD*3 genes in fatty acid synthesis and the differential expression of individual genes. We confirmed that it is possible to regulate the expression of these genes through fertilization, which can be used in agricultural practice to produce oilseed flax varieties with high OLE or high LIN. The average expression of the *fad2a* gene was significantly increased by applying 5.8 t ha^{-1} chicken manure, while treatment with 25 t ha^{-1} sheep manure was beneficial to the expression of the *fad3a* and *fad3b* genes and led to an increase in the LIN content. In terms of the effects of different fertilization treatments, the expression pattern of the target gene was basically consistent with the changing trend of fatty acids. These results indicated that organic manure increased the different components of FA by upregulating the expression of different *FAD* genes, but its underlying mechanism cannot be well explained and needs to be further explored. Rajwade et al. [59] revealed that in the low-LIN variety group, due to protein truncation, there was a loss of *fad3a* and *fad3b* gene activity; although the initial conversion to OLE was effective, later conversion to LIN seemed to be inefficient, resulting in the higher accumulation of OLE and LIO compared with LIN. This phenomenon was also found in our study, i.e., the relative expression of *fad2a* in low-LIN varieties (V2) was very high during the entire capsule development process, resulting in a significantly higher

LIO content in the V2 variety than in other varieties as well as the opposite expression levels of *fad3a* and *fad3b*. Therefore, there may be a stop codon mutation in *fad3a* in low-LIN varieties, which hinders its expression. Rajwade et al. [60] found that although there were some changes in nucleotide and AA sequences and intron/exon haplotypes in other flax varieties, except for a few varieties, these mutations did not change the structure of any desaturase protein. This may be irrelevant to the accumulation of LIN, so we speculate that there are significant differences in the accumulation modes of LIN among different varieties. Thambugala et al. [61] analyzed the correlations between the genotype and FA data of 120 flaxseed materials and found that only some *SAD* and *FAD* subtypes had a significant effect on FA composition. Therefore, the composition of FA may be determined not only by desaturase genes but also by other enzyme genes in the TAG synthesis pathway, although this hypothesis needs to be verified using more germplasm lines.

The *fad2b* and *fad3c* genes can be detected using qRT-PCR, which is a type of PCR testing, but the expression of the *fad2b* and *fad3c* genes is unstable, and their peak values are very low; therefore, the expression patterns of these two genes still need to be explored, as we did not analyze them in the current study.

5. Conclusions

In the current study, the effects of organic manure on the synthesis and accumulation of oil and fatty acid components in different oilseed flax varieties were explored, and the expression patterns of *fad2a*, *fad3a*, and *fad3b* genes, which play a key role in the process of fatty acid desaturation, were analyzed using different fertilizer treatments. It was found that organic manure can greatly increase grain oil yield and improve the quality of oilseed flax. The application of sheep manure significantly increased the content of NSCs in capsules, reduced the content of saturated fatty acids, and increased the content of unsaturated fatty acids in grains. Because *fad3* is involved in catalysis, which is the most important step of LIN formation in grains, and the application of 25 t ha^{-1} sheep manure significantly increased the expression of *fad3a* and *fad3b* genes, the proportion of LIN in oilseed flax grains may be further increased by optimizing fertilization measures. LIN is the most important quality indicator of oilseed flax, and its biosynthesis is regulated by many factors. It is necessary to further study the cloning of the *fad3* gene and the expression of the *SAD* and *fad2* genes. Future studies should also explore the underlying causes of *fad3* gene silencing, in order to further clarify their role in oilseed flax FA composition. Under the conditions of this experiment, 25 t ha^{-1} sheep manure treatment significantly increased the grain oil yield and improved the grain oil quality of different oilseed flax varieties, and the Dingya 26 variety exhibited the best grain oil yield. Thus, it can be used as a cultivation model for the high yield and high quality of oilseed flax in the dry areas of the Loess Plateau of China.

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