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Original Article

Engineering erucic acid biosynthesis in camelina (*Camelina sativa*) via *FAE1* gene cloning and antisense technology

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Abstract

Oil seeds now make up the world's second-largest food source after cereals. In recent years, the medicinal- oil plant *Camelina sativa* has attracted much attention for its high levels of unsaturated fatty acids and low levels of saturated fatty acids as well as its resistance to abiotic stresses. Improvement of oil quality is considered an important trait in this plant. Erucic acid is one of the fatty acids affecting the quality of camelina oil. Altering the fatty acid composition in camelina oil through genetic manipulation requires the identification, isolation, and cloning of genes involved in fatty acid biosynthesis. The Fatty Acid Elongase 1 (*FAE1*) gene encodes the enzyme β-ketoacyl CoA synthase (KCS), a crucial enzyme in the biosynthesis of erucic acid. In this study, the isolation and cloning of the *FAE1* gene from *Camelina sativa* were conducted to construct an antisense structure. The molecular homology modeling of *DFAE1* proteins using the SWISS-MODEL server on ExPASy led to the generation of the 3D structures of *FAE1* and *DFAE1* proteins. The GMQE values of 0.44 for *FAE1* and 0.08 for *DFAE1* suggest high accuracy in the structural estimation of these genes. The fragments were isolated from the DNA source of the genomic Soheil cultivar with an erucic acid content of about 3% (in matured seeds) using PCR. After cloning the *FAE1* gene into the Bluescript II SK+ vector and sequencing, the resulting fragments were utilized to construct the antisense structure in the pBI121 plant expression vector. The approved antisense structure was introduced into the Camelina plant using the Agrobacterium-mediated method, with optimization of tissue culture and gene transfer conditions. This approach holds potential to advance our knowledge of fat biosynthesis, leading to potential improvements in oil quality in *Camelina sativa*.

Keywords: *DFAE1*, *FAE1*, Gene transfer, KCS enzyme, Transgenic plant

1. Introduction

Camelina (*Camelina sativa* [L.] Crantz) is an oilseed crop belonging to the *Brassicaceae* family, commonly known as false flax or gold-of-pleasure. It is adapted to temperate growing regions and has its origins in East Europe and West Asia. The crop has been utilized since the late Neolithic Era when it was domesticated in Southeast Europe [1, 2].

Since the 1980s, the interest in Camelina has been revitalized due to the growing demand for both food and biofuel oils. This resurgence has led to the establishment of pilot-scale productions in European countries and North America. Camelina is notable for its low-input requirements [3] and remarkable resistance to common *Brassicaceae* diseases and pests, such as blackleg disease and [4] flea beetles [5].

In most *Brassicaceae* oilseeds, such as non-canola-quality rapeseed cultivars or crambe, erucic acid (C22:1) is synthesized. The interest in these unusual fatty acids stems from their unique physicochemical properties and the broader range of oleochemical reactions they enable, making them suitable for various applications beyond those of common vegetable oils [6,7].

Camelina seed oil is distinctive in two key aspects: (i) the polyunsaturated alpha-linolenic acid (C18:3) is the primary fatty acid, and (ii) the concentration of erucic acid (C22:1) is relatively low compared to other Brassicaceae species, while eicosenoic acid (C20:1) is synthesized instead as a long-chain fatty acid [7-9].

Apart from acceptable yield and processing potential, fully developed tools are available for breeding and trait improvement through genetic engineering in Camelina. This is due to the high sequence identity of genes in Camelina, which has led to numerous successful transformation reports over the years. Apart from selection markers, genes for altering fatty acid composition, improved seed and oil yield, or drought resistance have been expressed in Camelina [10, 11]. For example, RNAi suppression of fatty acid desaturase (*FAD2*) and elongase (*FAE1*) genes resulted in reduced concentrations of linoleic, linolenic,

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and eicosaenoic acids, while oleic acid was accumulated at a level of 66% [12]. Compared to natural genetic or environmental variation in fatty acid composition, suppression lines have a significant impact on tailoring oil composition traits. Additionally, other strains of Camelina engineered with fatty acid desaturase and elongase gene cassettes have produced up to 31% eicosapentaenoic acid (EPA, C20:5-omega-3) or up to 14% docosahexaenoic acid (DHA, C22:6-omega-3) [13].

Camelina has a low-risk biosafety status due to its low outcrossing rate [14, 15], no intercrossing with common *Brassicaceae* species [16] and weak weed potential [17] compared to other *Brassicaceae* crops, thus making it particularly suitable as a platform crop.

Gene silencing, particularly through antisense technology, is a crucial strategy for modifying fatty acid compositions [18]. Antisense technology involves the inhibition of gene expression by a double-stranded RNA molecule that hinders the expression of a specific gene homologous to the RNA. This process leads to mRNA degradation, ultimately resulting in a reduction of the targeted gene product. In their study, researchers focused on tissue culture, transformation, and adaptation of the seed-specific *FAE*1 gene from Camelina sativa in both sense and antisense orientations. This genetic manipulation aimed to enhance the oil quality of Camelina plants by modifying the fatty acid composition.

2. Materials and Methods

2.1. Plant materials

The experiments were conducted using the *Camelina sativa* cultivar 'Soheil', developed by Biston Shafa Company in Iran. This cultivar, derived from a cross between Blaine Greek and Calena cultivars, was purified using the doubled haploid method. The 'Soheil' cultivar features approximately 3% erucic acid, 35% oil content, 90% unsaturated fatty acids, and around 35% omega-3 fatty acids in matured seeds. Genomic DNA for the experiments was extracted from young leaves using a kit from Vivantis.

2.2. Plasmids and bacterial strains

The Bluescriprt II SK+ plasmid and *Escherichia coli*-DH5-alpha were used for cloning and sequencing. The plasmid *pBI121* (Novagen) and *A. tumefaciens* LBA4404 were used in order to produce competent cells and plant transformation.

2.3. Three-dimensional structure of *FAE1* and *DFAE1*

SWISS-MODEL is a renowned server for automated comparative modeling of three-dimensional (3D) protein structures. It has been a pioneer in automated modeling since 1993 and remains one of the most widely- used free web-based tools for this purpose. The SWISS-MODEL server is continuously evolving to enhance the integration of expert knowledge into a user-friendly platform.

2.4. Amplification and cloning of the *FAE1* and *DFAE1* genes

In the project, primer pairs were designed and synthesized based on the *FAE1* sequences in GenBank (Accession no. GU929420.1) for the *FAE1* (sense sequence) and *DFAE1* (antisense sequence) (Table 1). The FAE1 primers were utilized to amplify the full-length *FAE1* gene. Two strategies were implemented to modify the erucic acid content: a) the creation of an antisense structure from the entire *FAE1* gene, referred to as *RFAE1*, with its specific promoter to reduce erucic acid content [19]; and b) generating an antisense structure from a portion of the *FAE1* gene (first domain), known as *DFAE1*, with its specific promoter to lower erucic acid levels. Subsequent PCR analysis of transgenic plants involved the detection of the integrated *TRFAE1* construct by amplification with TFAE1 F and TFAE1 R primers.

Genomic DNA was isolated from leaves of *C. sativa* with a Vivantis kit (Plant DNA extraction). The *FAE1* genes were amplified from the genomic DNA using the primers mentioned above (Vivantis kit). The purified *FAE1* genes were double digested with *Cfr9I* and *SacI* enzymes and sub-cloned into the PTG19-TPCR cloning vector (Vivantis kit) which were digested with the same enzymes.

Following the TA-cloning scheme, tailed PCR products were ligated into the PTG19-T Vector using a 1:3 molar ratio of vector to insert, based on the manufacturer's instructions. The ligation reaction was set up in a 30 µl volume, including 2 µl PTG19-T plasmid, 16 µl fresh PCR product, 1 µl T4 DNA ligase enzyme, 1 µl 10X buffer, and 10 µl nuclease-free distilled water. After gentle mixing and brief centrifugation, the ligation reaction mixture was incubated overnight at 10ºC. Recombinant vectors were stored at -20°C until transformation. Competent cells were prepared from Escherichia coli strain DH5-alpha using the calcium chloride method. The PCR product was cloned into the PTG19-T vector and transformed into *E. coli* (DH5-alpha strain). The presence of inserts in the transformed colonies was screened by selection on MacConkey agar medium containing 100 mg/L ampicillin and colony PCR with specific primers. Subsequently, the recombinant plasmids underwent further analysis through sequencing.

2.5. Screening of clones containing pBI121 recombinant plasmids

The plasmid was extracted from the clones that were cultured on LB medium supplemented with kanamycin and specific antibiotics for the Agrobacterium strain. Sub-

Table 1. The sequence of primers used for the *FAE1* gene and *TRFAE1* construct as a complete antisense of the *FAE1* gene. Where, F: Forward; R: Reverse.

Name	Primer	Sequence (5′→3′)
FAE1	F	CGC GAT AAT TTA TCC TAG TTT GC
	R	CTA CAA TGC GTT GGT GGA AG
DFAE1	F	**CCC GGG**[1] CGT AAT AGT GCT TGA TCT T
	R	**GAG CTC**[2] TTA ACG TGC CCC CAC GAA A
TFAE1	F	GTC GCC TAA GGT CAC TAT CAG CTA GC
	R	CTC TCA TCG TCT CCT TGT

sequently, the extracted plasmid was verified and confirmed through a control procedure (Jumping).

2.6. Sequencing of cloned *DFAE1* gene

To conclusively confirm the obtained fragments, clones harboring the desired fragments were sequenced. Following the measurement of purified plasmid concentration, 30 nanograms of each sample was submitted to Gene Technologies Company for sequencing. The clones sent for sequencing were identified using standard primers from both ends. Upon validating the accuracy of the amplified fragments through sequencing, the gene sequence under study was analyzed alongside other sequences in the BLAST and Alignment databases at both the nucleic acid and amino acid levels.

2.7. Antisense construction of *FAE1* gene

In the antisense construction, the *FAE1* gene was substituted for the β-glucuronidase (GUS) gene in the modified pBI121 vector. To achieve this, the modified pBI121 vector was cleaved with *Cfr9I* and *SacI* restriction enzymes to excise the GUS gene. Consequently, the PCR fragment utilized for the antisense construction had a *SacI* site at the beginning and a *Cfr9I* site at the end of the gene. The presence and orientation of the constructs in the recombinant pBI121 vector were assessed through PCR analysis and restriction enzyme digestion.

2.8. Plant tissue culture, transformation and regeneration

In the experiment, seeds of *Camelina sativa* were sterilized with 70% ethanol for 1 minute and 5% sodium hypochlorite by shaking vigorously for 10 minutes. Subsequently, the seeds were rinsed thrice with sterile distilled water and germinated under aseptic conditions on 1/2 MS medium [20] in glass bottles (30- 40 seeds per bottle) at 25°C under a 16-hour light/8-hour dark photoperiod. Plant transformation and regeneration were carried out according to a specified procedure [21]. Cotyledon segments from seedlings of different ages (5, 7, 9, and 14 days) were used as explants to assess shoot regeneration. The media composition varied in terms of macro salts, types of antibiotics such as Cefotaxime (50, 100, 200 and 400 mg/L) and Meropenem (50, 100, 200 and 400 mg/L), sucrose (30 and 20 g/L), and phytohormones such as BAP (0.5, 1.5, 2 and 2.5 mg/L) and kin (0.5, 1.5, 2 and 2.5 mg/L) for regenerating and NAA (0.2, 0.4, 0.5 and 1 mg/L) and IBA (0.1, 0.2, 0.4 and 0.8) for rooting.

Vitrification, a common issue in the tissue culture process of Camelina, can significantly impact shoot multiplication, culture vigor, and the successful acclimatization of micro-propagated plants to in vivo conditions [22]. In this study, ammonium-to-nitrate ratios (1/6, 1/3 and 1/2) and agar values (8.5, 8 and 7.5 gr) were investigated.

During the experiment, care was taken to remove the apical meristem that may sometimes adhere to the petioles. Colonies of *A. tumefaciens* strain LBA4404 containing the modified binary plasmid recombinant pBI121 (antisense constructs) were cultured overnight at 28°C in LB medium supplemented with 10 mg/L kanamycin. Explants were then inoculated with *A. tumefaciens* for 10 minutes, followed by cultivation on the same medium solidified with agar at 25°C in the dark. After 72 hours of co-cultivation, the explants were transferred to medium containing kanamycin for selection of transgenic plant cells, along with Cefotaxime and Meropenem to eliminate Agrobacteria. Subsequent subculturing was performed at 14-day intervals. Transgenic plants were identified based on kanamycin resistance, mature plants were regenerated, cultured in perlite, and then transferred to soil for growth to maturity. To prepare a cotyledon explant, germinated seeds aged 5 to 7 days will be used. After removing the terminal buds, the cotyledon leaves will be positioned on the culture medium with approximately 2 mm of the petiole immersed in the medium [23].

2.9. Statistical analysis

ANOVA was used for analysis of variance and Duncan's multiple range test was used to compare means. SPSS software was used to obtain the variance analysis and compare the average of the effect of explant age on the production of green seedlings, effect of hormone concentration on regeneration rate, effect of antibiotics on the rate of regeneration and effect of hormone concentration on rooting rate.

3. Results

3.1. Three-dimensional structure of *FAE1* and *DFAE1*

Molecular homology modeling of *DFAE1* proteins using the SWISS-MODEL server on ExPASy generated the 3D structure of *FAE1* and *DFAE1* proteins. The GMQE values of 0.44 for *FAE1* and 0.08 for *DFAE1* suggested a high level of accuracy in the structural prediction of these genes (Figure 1).

3.2. Isolation of *FAE1* gene from Camelina using PCR

PCR amplification of genomic DNA from the Sohil variety of *Camelina sativa* using specific primers (FAE1 F and FAE1 R) resulted in a PCR product with a size of 1518 bp (Figure 2) for the *FAE1* gene. Additionally, PCR amplification with specific primers (DFAE1 F and DFAE1 R) produced PCR products, with the *DFAE1* gene size being 723 bp (Figure 2).

The purified *DFAE1* gene was inserted into the PTG19-T vector and then introduced into *Escherichia coli* (DH5-

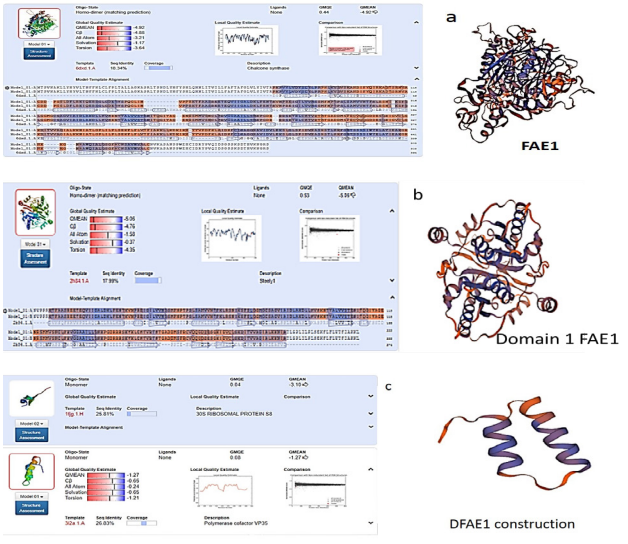

Fig. 1. a: Three-dimensional structure of *FAE1* protein, b: Three-dimensional structure of Domain 1, *FAE1* protein and c. Three-dimensional structure of *DFAE1* protein.

alpha strain) through transformation. The presence of the insert in the transformed colonies was verified by selecting them on MacConkey agar medium (as shown in Fig. 3a) and conducting colony PCR with specific primers (as illustrated in Figure 3b).

The *DFAE1* gene was isolated, subjected to restriction enzyme digestion, and cloned into the pSK+ plasmid. The clones were verified through PCR, restriction enzyme analysis, and sequencing. Subsequently, the validated PCR fragments were sub-cloned into a plant binary vector (recombinant pBI121), and the resulting clones and orientation of the construct were confirmed through PCR and restriction enzyme digestion, as shown in Figure 4 [23].

3.3. Enzymatic digestion of vector pBI1400

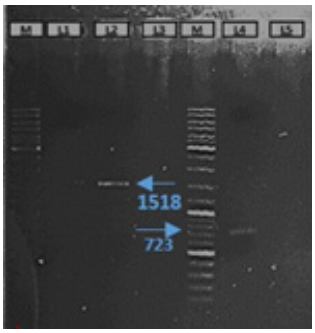

Fig. 2. The *FAE1* gene *of* the amplification by PCR. L2 Amplification of the *FAE1* gene with specific primers (1518 bp). The *DFAE1 of* the amplification products by PCR L4 with specific primers (723 bp). L1 and L5: negative control.

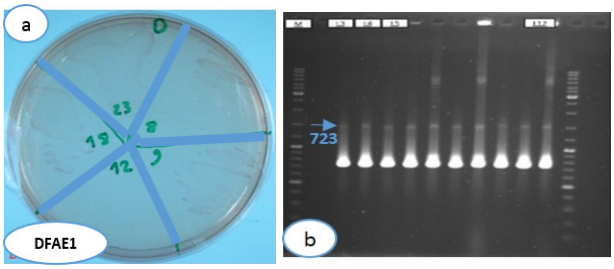

Fig. 3. Colonies were screened by selection on Mac Conkey agar medium and Confirmation by PCR of *DFAE1* construct. a) Colonies were screened for *DFAE1* construct. **b)** Confirmation by PCR; Lane M: 50 bp plus ladder. L1 and L2: Negative control (723 bp).

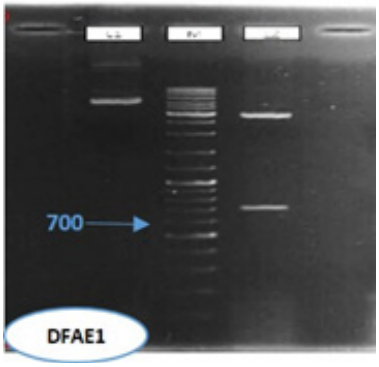

Fig. 4. Confirmation of *DFAE1* construct cloned into recombinant pBI121 via restriction enzyme analysis. M: 100 bp plus ladder. L2 digestion of the *DFAE1* construct with *Cfr91*, *Sac I*, and L1 pBI1400 plasmid.

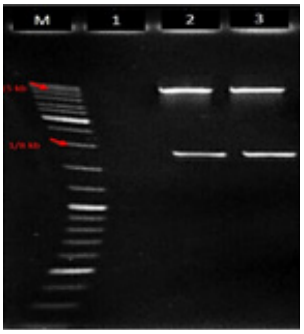

Fig. 5. Electrophoresis pattern of pBI1400 enzymatic digestion product containing gus fragment. L M: marker (Mix 100bp) DNA; L2 and 3: Gus gene removed from pBI1400 plasmid with *Cfr9I* and *SacI* enzymes.

To prepare the plant expression vector PBI1400, the vector's quality was verified, followed by enzymatic digestion with *Cfr9I* and *SacI* enzymes at 37 degrees Celsius for four hours on a 1% agarose gel. Subsequently, the desired fragments resulting from the enzymatic digestion were purified from the gel using an extraction kit. The outcome of this reaction is depicted in Figure 5 [23].

3.4. Sequencing of Colonies *DFAE1* gene

Following the verification of the amplified fragments through sequencing, the gene sequence under study was compared with other sequences in the BLAST and Alignment databases at both the nucleic acid and amino acid levels, as depicted in Figure 6.

3.5. Cloning of *DFAE1* fragments in pBI1400 plasmid vector

The *DFAE1* fragments were ligated with the cut pBI1400 vector, and the binding reaction was executed.

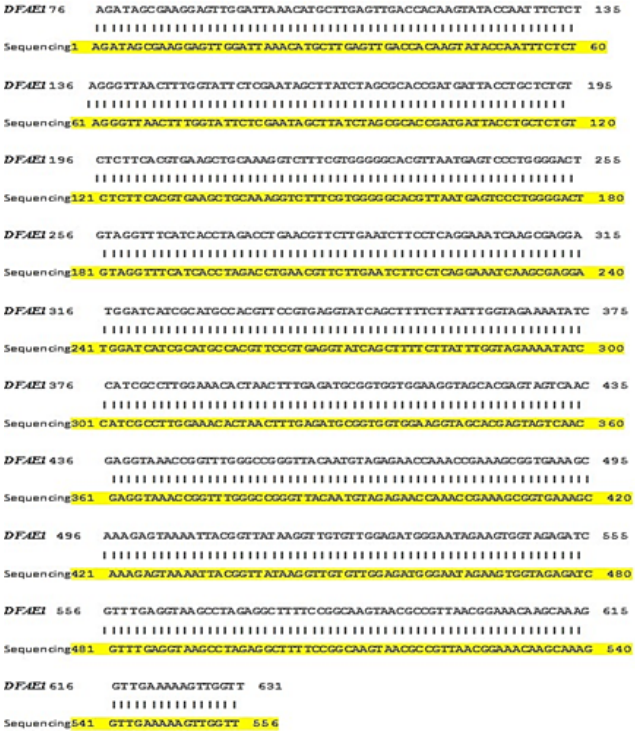

Fig. 6. Examining the similarity of the sequencing result with the main sequence of the *DFAE1* gene.

The recombinant plasmids were then introduced into susceptible *E. coli* DH5-alpha cells, and initial screening was conducted on a solid medium supplemented with kanamycin. To confirm successful cloning, plasmid extraction was performed from multiple colonies grown on the solid medium. The homology of the *DFAE1* fragments within the pBI1400 plasmid vector was validated using the PCR method, as shown in Figure 7.

The constructs were transferred to *A. tumefaciens* LBA4404 using the freeze-thaw standard method. Subsequently, the Agrobacterium strains were utilized to transform *Camelina sativa* via Agrobacterium-mediated petiole cotyledonary transformation. In investigating the age of cotyledon explants on the production of green seedlings, an experiment was used in a completely randomized design with three replications. The results of the analysis of variance and comparison of means by Duncan's method can be seen in Tables 2 and 3 that the five-day-old explants with the production rate of green seedlings were superior to the other two ages.

In order to evaluate the effect of hormone concentration on the regeneration rate, a factorial experiment was carried out in the form of a completely randomized design with two replications. The results of analysis of variance

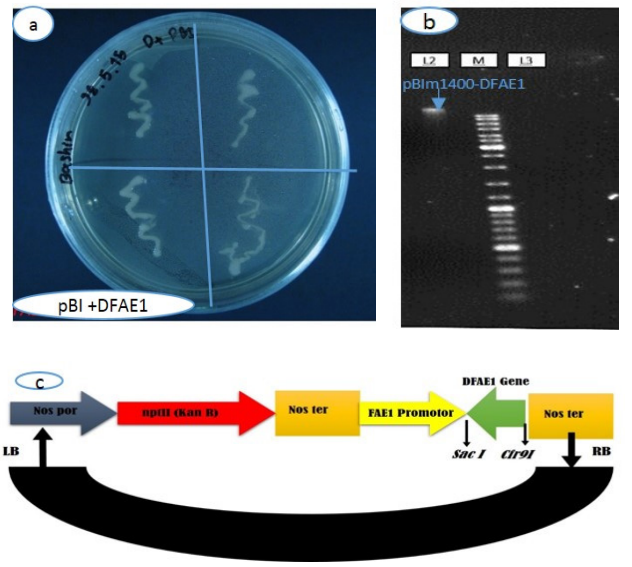

Fig. 7. a) *DFAE1* colonies in pBI1400 plasmid. **b)** Confirmation of construct was transferred to *A.tumefaciens* LBA4404 by PCR, L2 product of the recombinant pBI121 plasmid containing *DFAE1* construct. L3: negative control. **c)** The transformation vector used in the study.

Table 2. Variance analysis of the effect of explant age on the production of green seedlings.

Source	Df	MS
age	2	40.44**
Error	6	3.22
CV	13.5	

Table 3. Comparing the average effect of explant age on the production of green seedlings.

Age (day)	SE=3.222
5	17.667[a]
7	11.667[b]
10	11[b]

of this plan are in Table 4 and the comparison of averages was done by the LSD method, the results of which are presented in Table 5. The results show that there is a significant difference between different concentrations of BAP, Kin and their interactions. Explants at a concentration of 1.5 mg/L of BAP are superior to other concentrations.

In order to choose the best antibiotics and their concentrations, a factorial experiment was used in the form of a completely randomized design with two replications. The results of analysis of variance and comparison of means by LSD method are presented in Tables 6 and 7. The results show that there is a significant difference between different concentrations of antibiotics and their interactions. The explants in concentrations of 200 mg/L of cefotaxime and 100 mg of meropenem were superior to other concentra-

Table 4. Variance analysis of the effect of hormone concentration on regeneration rate.

Source	Df	MS
BAP	3	36.78**
Kin	3	9.94**
BAP*Kin	9	1.3*
Error	16	0.406
CV	17.33	

Table 5. Comparing the average of the effect of hormone concentration on regeneration rate.

Hormone	concentration	SE=0.4
BAP	0.5	6.62[a]
	1.5	3.87[b]
	2	2.75[c]
	2.5	1.62[d]
Kin	0.5	5.37[a]
	1.5	3.37[b]
	2	3.12[b]
	2.5	3.00[b]

Table 6. Variance analysis of the effect of antibiotics on the rate of regeneration.

Source	Df	MS
Cefotaxime	3	10.45**
Meropenem	3	9.28**
Cefotaxime * Meropenem	9	4.87*
Error	16	0.187
CV	22.34	

Table 7. Comparing the average of the effect of antibiotics on the rate of regeneration.

Hormone	concentration	SE=0.4
Cefotaxime	50	3.62[a]
	100	1.62[b]
	200	1.37[bc]
	400	1.12[c]
Meropenem	50	3.5[a]
	100	1.75[b]
	200	1.37[bc]
	400	1.12[c]

tions.

In order to investigate the best concentration of NAA and IBA hormones on the rate of rooting, a factorial experiment was carried out in the form of a completely randomized design with two replications. The results show that there was a significant difference between different concentrations of NAA. Seedlings had root production at a concentration of 1 mg/L of NAA (Tables 8 and 9).

The transgenic plants exhibited a regeneration frequency of approximately 30% in the medium containing 8 mg/L of kanamycin. Following acclimatization of the rooted plantlets to in vivo conditions, they were allowed to flower and produce seeds (Figure 8).

3.6. Molecular analysis of transgenic plants

Genomic DNA from putative transgenic and non-transgenic plants was examined for the presence of the *TRFAE1* gene using PCR with the TFAE1 F and TFAE1 R primers. PCR amplification yielded a 750 bp fragment in the transgenic plants, while no amplification was detected in the control plants, as illustrated in Figure 9.

4. Discussion

Camelina is an oilseed crop that has recently been developed for oil production and diversification of modern crop production systems [24-26]. Biotechnology provides an effective tool for rapid Camelina improvement and biological research [27, 28]. By employing RNA interference (RNAi) targeting crucial genes in fatty acid synthesis, seed fatty acids can be efficiently modified through a straightforward transfection approach. This approach opens avenues for enhancing Camelina for various agronomic traits, such as altering fatty acid composition in seed oil, thereby positioning this crop as a sustainable bioenergy source.

Improving the quality of Camelina oil and meal presents a valuable goal for enhancing this plant. Camelina oil can serve purposes in human nutrition or industrial applications, while the protein-rich meal can be utilized in livestock feed or as a fertilizer. The quality of Camelina oil is influenced by its fatty acid composition, as well as factors such as anti-nutritional components (e.g., glucosinolates) and the balance of protein and fiber content. The ratio of oil to protein in the seed, particularly the oil content due to its economic value, plays a significant role in determining the overall quality of Camelina [19].

Improving the quality of Camelina oil involves addressing the presence of erucic acid, an undesirable compound that impacts the oil's food quality when exceeding two percent. Genetic modification methods, such as gene transfer, offer a means to reduce erucic acid levels by targeting the biosynthesizing enzyme controlled by the *FAE1* gene. By identifying, isolating, and cloning the *FAE1* gene and introducing the appropriate structure into Camelina plants, the effects on oil quality can be assessed. Antisense structures of *RFAE1* and *DFAE1* were developed to inhibit the *FAE1* gene, with computer analysis aiding in primer design for cell isolation and amplification. Structural analysis revealed the potential efficacy of the antisense structure *DFAE1* in reducing erucic acid levels, supported by genomic similarities of the proteins [7,19, 29, 30].

Regeneration studies in Camelina have shown that various species of the cruciferous family and hypocotyl explants are efficient for initiating organogenesis in laboratory conditions. Experimentation by Yamets et al. (2013) involved utilizing *Camelina sativa* seedlings of different ages (five, seven, nine, and 14 days) on culture medium to generate explants. Previous research has highlighted that cytokinins play a crucial role in promoting stem formation by initiating cell division and branch differentiation in several cruciferous species, alongside different combina-

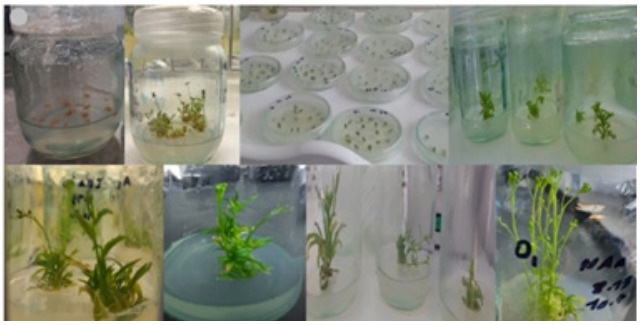

Fig. 8. Development and elongation of shoots after transformation.

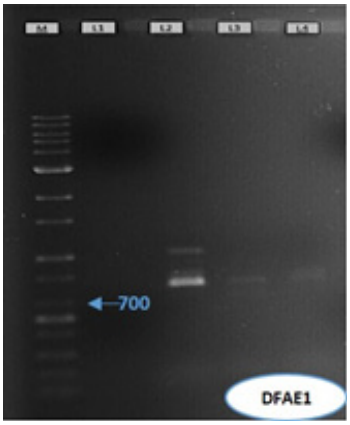

Fig. 9. PCR analysis of transgenic plants with integrated *TDFAE1* construct. L1: Negative control**.** L2, L3 and L4, PCR amplification produced a fragment of 750 bp in the transgenic plants with *TDFAE1* construct.

Table 8. Variance analysis of the effect of hormone concentration on rooting rate.

Source	Df	MS
NAA	3	109.36**
IBA	3	2.28ns
NAA * IBA	9	4.72*
Error	16	1.21
CV	27.81	

Table 9. Comparing the average of the effect of hormone concentration on the rooting rate.

Hormone	concentration	SE=1.21
NAA	0.2	4.62a
	0.4	4.12ab
	0.5	3.75ab
	1	3.37b
IBA	0.2	9.5a
	0.4	2.37b
	0.5	2.25b
	1	1.75b

tions of auxin and cytokinin. Efficient shoot regeneration in Camelina has been achieved in media containing cytokinins combined with auxin [31-35].

In a study by Zebarjedi et al. (2006), the *FAE* gene was transferred to canola using sense and antisense structures via the Agrobacterium method. This transfer was conducted in both low erucic acid cultivars (LEAR, e.g., PF) and high erucic acid cultivars (HEAR, e.g., Maplus). The transgenic plants were screened in a kanamycin-containing environment, followed by analysis using PCR and Southern blotting methods. The erucic acid content in the first generation of transgenic plants (T0) was assessed through chromatography, revealing significant alterations in fatty acid composition between transgenic plants harboring meaningful and non-functional *FAE* gene structures [23]. Additionally, in a study by Bashiri et al. (2023), plants containing the *RFAE1* construct displayed reduced erucic acid levels compared to control plants, as analyzed by gas chromatography [19].

In a separate study, a fluorescent protein (DsRed) served as a visible selective marker in the genetic modification of Camelina. This approach enabled the straightforward screening of mature transgenic seeds from a large pool of non-transgenic seeds, resulting in the successful acquisition of over one percent transgenic seeds. Genetic analysis revealed that the majority of transgenic plants carried a single transgenic version. The results demonstrated the efficacy of genetic engineering in enhancing the agronomic characteristics of Camelina, particularly in modifying the fatty acid profile of its seed oil [21].

In a study by Wu et al. (2008), the complete coding sequences of the *FAE* gene were extracted from eight rapeseed cultivars with varying levels of erucic acid content. It was observed that four base pair deletions occurring between T1366 and G1369 in the *FAE1* gene resulted in a frame-shift mutation in certain instances, leading to premature termination of translation. This mutation highlighted a genetic variation that could impact the synthesis of erucic acid in rapeseed [36].

The four-base pair deletion was primarily observed in the C-genome of *B. napus* and infrequently in the A-genome. In the yeast system, gene isoforms resulting from this deletion generated truncated proteins lacking enzymatic activity, unlike the intact *FAE1* gene that produced very long chain fatty acids. While the *FAE1* gene isoforms were transcribed by eliminating four base pairs during rape seed development, these isoforms failed to translate into functional proteins. Through this mutation-induced deletion of four base pairs, researchers achieved the production of low erucic acid in rapeseed [37].

In a study by Kang et al. (2011), three *FAD2* genes were identified in Camelina for the production of oils suitable for industrial applications using the antisense method. The aim was to enhance oleic acid (monounsaturated fatty acid, MUFA) content and reduce levels of unsaturated fatty acids (polyunsaturated fatty acids, PUFA) such as linoleic acid and linolenic acid in industrial oils. Despite the high sequence similarity among the three *CsFAD2* genes, they exhibited distinct expression patterns. *CsFAD2-1* was found to be expressed in various tissues, including developing seeds, flowers, leaves, roots, and stems, while *CsFAD2-2* and *CsFAD2-3* were primarily expressed in seeds [15].

Yemets et al. (2013) focused on optimizing tissue culture and regeneration processes in Camelina cultivars Peremozhets and Mirazh. The study successfully determined the most effective concentrations of disinfectants, treatment durations for plant material, phytohormone ratios, sucrose concentrations for branching formation, and NAA concentration for seedling rooting. Additionally, transgenic Camelina plants were generated through the Agrobacterium method utilizing the pGH217 vector carrying the β-glucuronidase (Gus) reporter gene [33].

Yan et al. (2015) conducted a study on *Brassica rapa* lines lacking erucic acid, revealing that the development of plants with reduced erucic acid content was not associated with changes in the *FAE1* coding sequence but rather linked to decreased *FAE1* expression. The *FAE1* promoter sequences in low erucic acid and high erucic acid materials exhibited a 95% similarity. Notably, deletions of twenty-eight bases, including a 24-base AT-rich region, were identified approximately 1300 bp upstream from the *FAE1* start codon in the low erucic acid accessions. These promoter variations could impact *FAE1* expression levels, suggesting novel regulatory mechanisms for erucic acid synthesis [37].

Camelina, an under-exploited crop species, holds significant economic potential. Research demonstrates that Camelina can be efficiently transformed using a simplified method that bypasses complex tissue culture procedures. Unique properties of Camelina, such as low agricultural requirements and resilience to pests and diseases, make it an attractive option for cultivation. Its cost-effective production in various climates positions Camelina as a favorable choice for biofuel production compared to other vegetable oils. However, the presence of erucic acid, a component of the *Brassicaceae* family of fatty acids, poses health risks, prompting efforts to develop erucic acid-free varieties. Modifying erucic acid levels in seeds is a key focus for enhancing the quality of Brassica oil [25].

Abbreviations

Enzyme β-ketoacyl CoA Synthase (KCS); Global Model Quality Estimation (GMQE); Basic Local Alignment Search Tool (BLAST); β-glucuronidase (GUS); Benzyl Amino Purine (BAP); Naphthalene Acetic Acid (NAA); Indole-Butyric Acid (IBA); Mono Unsaturated Fatty Acids (MUFA); Poly Unsaturated Fatty Acids (PUFA); Eicosapentaenoic Acid (EPA); Fatty Acid Desaturase 2 (*FAD2*); Fatty Acid Elongase 1(*FAE1*).

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References

1. Donmez EO, Belli O (2007) Urartian plant cultivation at Yoncatepe (Van), Eastern Turkey. Econ Bot 61: 290–298. doi.org/10.1663/0013.
2. Larsson M (2013) Cultivation and processing of *Linum usitatissimum* and *Camelina sativa* in southern Scandinavia during the Roman Iron Age. Veget Hist Archaeobot 22: 509–520. doi:10.1007/s00334-013-0413-3.
3. Putnam DH, Budin JT, Field LA, Breene WM (1993) Camelina: A promising low-input oilseed. In: Janick, J., Simon, J.E. (Eds),

New Crops, Exploration, Research and Commercialization, Proceedings of the Second National Symposium, Indianapolis, Oct. 6–9, 1991, John Wiley and Sons, Inc., New York pp. 314–322. http://www.hort.purdue.edu/newcrop/proceedings

4. Li H, Barbetti MJ, Sivasithamparam K (2005) Hazard from reliance on cruciferous hosts as sources of major gene-based resistance for managing blackleg (*Leptosphaeria maculans*) disease. Field Crops Res 91: 185–198.
5. Henderson AE, Hallett RH (2004) Prefeeding behavior of the crucifer flea beetle, *Phyllotreta cruciferae*, on host and nonhost crucifers. J Insect Behavior 17: 17–39. doi: 10.1023/B.
6. Vollmann J, Laimer M (2013) Novel and traditional oil crops and their biorefinery potential. In: Yang, S.-T, El Enshasy HA, Thongchul N. (Eds), Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers, John Wiley & Sons, Inc., Hoboken, New Jersey, USA. pp. 47–59. doi:10.1002/9781118642047.ch3
7. Gugel RK, Falk KC (2006) Agronomic and seed quality evaluation of *Camelina sativa* in western Canada. Can. J Plant Sci 86: 1047–1058. doi.org/10.4141/P04-081
8. Vollmann J, Moritz T, Kargl C, Baumgartner S, Wagentristl H (2007) Agronomic evaluation of camelina genotypes selected for seed quality characteristics. Ind Crops Prod 26: 270–277. doi.org/10.1016.
9. Zubr, J, Matth B (2002) Effects of growth conditions on fatty acids and tocopherols in *Camelina sativa* oil. Ind Crops Prod 15: 155–162. doi: 10.1016/S0926-6690(01)00106-6.
10. Lu C, Kang J (2008) Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by Agrobacterium-mediated transformation. Plant cell Reprod 27: 273–278. doi:10.1007/s00299-007-0454-0.
11. Liu X, Brost J, Hutcheon C, Guilfoil R (2012) Transformation of the oilseed crop *Camelina sativa* by *Agrobacterium*-mediated floral dip and simple large-scale screening of transformants. In Vitro Cell Dev Biol Plant 48: 462–468. doi: 10.1007/s11627-012-9459-7.
12. Horn PJ, Silva JE, Anderson D, Fuchs J (2013) Imaging heterogeneity of membrane and storage lipids in transgenic *Camelina sativa* seeds with altered fatty acid profiles. Plant J 76: 138–150. doi: 10.1111/tpj.12278.
13. Ruiz-Lopez N, Haslam RP, Napier JA, Sayanova O (2014) Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. Plant J 77: 198–208. doi: 10.1111/tpj.12378.
14. Walsh KD, Puttick DM, Hills MJ, Yang RC (2012) First report of outcrossing rates in camelina [*Camelina sativa* (L.) Crantz], a potential platform for bioindustrial oils. Can J Plant Sci 92, 681–685. doi/pdf/10.4141/cjps2011-182.
15. Groeneveld JH, Klein AM (2014) Pollination of two oil-producing plant species: Camelina (*Camelina sativa* L. Crantz) and pennycress (*Thlaspi arvense* L.) double-cropping in Germany. GCB Bioenergy 6: 242–251. doi.org/10.1111/gcbb.12122.
16. Julié-Galau S, Bellec Y, Faure JD, Tepfer M (2014) Evaluation of the potential for interspecific hybridization between *Camelina sativa* and related wild Brassicaceae in anticipation of field trials of GM camelina. Transgenic Res 23: 67–74. doi: 10.1007/s11248-013-9722-7.
17. Walsh KD, Raatz LL, Topkina KC, Hall LM (2013) Transient seed bank of camelina contributes to a low weedy propensity in Western Canadian cropping systems. Crop Sci *53*, 2176–2185. doi. 10.2135/cropsci2013.03.0142.
18. Shi J, Lang C, Wang F, Wu X, Liu R, Zheng T, Wu G (2017) Depressed expression of *FAE1* and *FAD2* genes modifies fatty acid profiles and storage compounds accumulation in *Brassica napus* seeds. Plant Sci 263: 177-182. doi: 10.1016/j.plantsci.2017.07.014.
19. Bashiri H, Kahrizi D, Salmanian A H, Rahnama H, Azadi P (2023) Control of erucic acid biosynthesis in Camelina (*Camelina sativa*) by antisense technology. Cell Mol Biol 69(7): 212-217. doi.org/10.14715/cmb/2023.69.7.34
20. Murashing T, Skoog F (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Plant Physiol 15: 473-497. doi.org/10.1111/j.1399-3054.
21. Moloney MM, Walker JM, Sharma KK (1989) High efficiency transformation of *Brassica napus* using Agrobacterium vectors. Plant Cell Reprod 8: 238-242. doi: 10.1007/BF00778542.
22. Hammerschlag F A (1986) Temperate fruits and nuts. In: "Tissue culture as a plant production system for horticultural crops". 221–236.
23. Zebarjadi A, Jalali Javaran M, Karimzadeh G, Moeini A, Mousavi A, Salmanian A. H (2006) Transformation of rapeseed (*Brassica napus* L.) plants with sense and antisense constructs of the fatty acid elongase gene. Iran J Biotechnol 4(2): 79-87.
24. Seyed Hassan Pour, SM, NejadSadeghi L, Kahrizi D, Shobbar ZS (2024) Bioinformatic and Phylogenetic Investigation of WRKY Genes Involved in Drought Stress in Camelina sativa Plant. Agrotech Ind Crops 4(2): 65-79. doi: 10.22126/atic.2023.8830.1084.
25. Berti M, Gesch R, Eynck C, Anderson J, Cermak S (2016) Camelina Uses, Genetics, Genomics, Production, and Management. Ind Crops Prod 94: 690–710. https://doi.org/10.1016.
26. Obour A K, Sintim H Y, Obeng E, Jeliazkov D V (2015) Oilseed Camelina (*Camelina sativa* L. Crantz): Production Systems, Prospects and Challenges in the USA Great Plains. Adv. Plants Agric Res 2: 68–76. doi.10.15406/apar.2015.02.00043.
27. Collins-Silva J E, Lu C, Cahoon E B (2011) Camelina: A designer biotech oilseed crop. INFORM. 22: 610–613.
28. Hines P J, Travis J (2016) The new harvest. Science 353: 1218. doi: 10.1126/science.353.6305.1218.
29. Bashiri H, Kahrizi D, Salmanian A H, Rahnama H, Azadi P (2023) Bioinformatic analysis of *FAE1-A* and *FAD2-A* genes in *Camelina sativa.* Iranian J of Gene and Plant Breeding. 12(2): 49-59. doi: 10.30479/IJGPB.2024.18866.1344.
30. Lang Ch, Wang F, Liu R, Zheng T, Hu Zh, Wu X, Wu G (2022) Genetic Regulation of Fatty Acid Biosynthesis in *Brassica napus* Seeds Based on FAE1 and FAD2 Genes. Mol Plant Breed 13 (3): 1-9. doi: 10.5376/mpb.2022.13.0003.
31. Tang G H, Zhou W Z, Li H Z, Mao B Z, He Z H, Yoneyama K (2003) Medium, explant and genotype factors influencing shoot regeneration in oil_ seed *Brassica* spp. Agr. Crop Sci 189: 351–358. doi: 10.1007/s002990050482.
32. Zhang Yu, Xu J, Han L, Wie W, Guan Z, Cong L, Chai T (2006) Efficient shoot regeneration and agrobacterium_mediated transformation of brassica juncea. Plant Mol Rep 24: 255.
33. Yemets AI, Boychuk YN, Shysha EN, Rakhmetov DB, Blume YB (2013) Establishment of in Vitro Culture, Plant Regeneration, and Genetic Transformation of *Camelina sativa*. Cytol Genet 47(3): 138–144. Doi: 10.3103/S0095452713030031.
34. Sparrow P A C, Townsend T M, Morgan C L, Dale P J, Arthur A E, Irwin J A (2004) Genetic analysis of in vitro shoot regeneration from cotyledonary petioles of *Brassica oleracea*. Theor Appl Genet 108: 1249–1255. doi: 10.1007/s00122-003-1539-y.
35. Tattersall A, Millam S (1999) Establishment and in vitro regeneration studies of the potential oil crop species *Camelina sativa*. Plant Cell Tiss. Organ Cult 55: 147–149.
36. Wu G, Wu Y, Xiao L, Li X, Lu Ch (2008) Zero erucic acid trait of rapeseed (*Brassica napus* L.) results from a deletion of four base pairs in the fatty acid elongase 1 gene. Theor Appl Genet 116: 491–499. doi: 10.1007/s00122-007-0685-z.

37. Yan G, Li D, Cai M, Gao G, Chen B, Xu K, Li J, Li F, Wang N, Qiao J, Li H, Zhang T, Wu X (2015) Characterization of *FAE1* in the zero erucic acid germplasm of *Brassica rapa* L. Breed Sci 65: 257–264. doi: 10.1270/jsbbs.65.257.