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Tissue Culture Optimization and Construction of a Suitable Vector for Engineering Very Long-Chain Fatty Acids in *Camelina sativa* L.

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ABSTRACT

Oil quality from oilseeds is determined by the fatty acid (FA) content. Fatty Acid Elongase 1 (FAE1) is an essential FA gene that has been genetically modified to change the constitution of fatty acids in oilseed plants. In this study, recombinant vector construction for genome editing of the *CsFAE1B*, *CsFAE1A*, and *CsFAE1C* genes using CRISPR/Cas$_9$ was used to improve the quality of *Camelina Sativa* oil (which contains about 50% oil and 4% erucic acid). Additionally, camelina tissue culture optimization for recombinant vector transfer was performed concurrently. Finally, gRNA was designed to target three copies of the *CsFAE1* genes, and after transfer to the pFGC-pcoCas9 vector, the recombinant vector was confirmed by PCR and enzymatic digestion. The tissue culture optimization results indicated that hormone ratios of 0.5 mg L^{-1} NAA and 3 mg L^{-1} BAP for cotyledon and hypocotyl in the Gamborg medium induced embryogenic calluses three weeks after cultivation. Additionally, hormone ratios of 0.5 NAA, 2 BAP, and 1 (mg L^{-1}) Kin led to direct regeneration in cotyledon explants. In future studies, tissue culture optimization and recombinant vector construction for genome editing of FAEs genes could improve oil quality with the genetic transformation of camelina.

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1. Introduction

Camelina sativa L., an oilseed crop in the Brassicaceae family, has superior agronomic traits, including low water and fertilizer requirements, high adaptability, and drought resilience (Ahmad *et al.*, 2023). Its short life cycle, ease of genetic modification, and available genome data enable rapid metabolic engineering to produce ecologically friendly and sustainable unusual fatty acids and modified oils (Ghidoli *et al.*, 2023). These oils are valuable for high-value chemicals, biofuels, culinary uses, and health-promoting foods and medications (Alberghini *et al.*, 2022). However, genetic engineering of camelina requires developing tissue culture techniques, and its hexaploid nature complicates trait modification due to complementary gene effects (Ghidoli *et al.*, 2023). Genome editing systems like zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-Cas$_9$ enable precise DNA editing (Saraswat *et al.*, 2023). However, ZFNs and TALENs often lack predictability and face challenges in selective gene targeting. CRISPR-Cas$_9$ surpasses these methods due to its unmatched efficiency, ease of use, and precise DNA targeting capabilities (Cromwell *et al.*, 2018). CRISPR-Cas$_9$-generated mutant plants are highly favored as they leave no foreign DNA traces, avoiding costly deregulation processes. The CRISPR-Cas$_9$ system is a versatile and powerful genome engineering tool, enabling precise, site-specific modifications across various biological systems, including plants (Wada *et al.*, 2020). CRISPRs are DNA fragments in prokaryotes encoded by CRISPR RNA (crRNA). The CRISPR/Cas$_9$ system targets DNA using an artificial single-guide RNA (sgRNA),

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particularly a 20-nucleotide guide sequence (gRNA) at the sgRNA's 5' end. Accurate DNA editing requires target-specific sgRNA molecules, influencing both specificity and cutting efficiency. However, the system faces challenges with off-target effects, where Cas_9 activity at unintended genomic sites may cause unexpected silencing (Nishimasu *et al.*, 2018). Recent experimental techniques have focused on reducing off-target effects by modifying Cas_9 activity, leading to the development of highly specific gRNA and improved CRISPR/Cas_9 targeting precision (Kim *et al.*, 2020). However, bioinformatics techniques can design sgRNA sequences to minimize off-target effects (Liu *et al.*, 2019). CRISPOR provides a visual online platform for target selection, primer creation, and cut site identification, enhancing efficiency and specificity using gene sequence data at a large scale. It offers versatile sgRNA design options with additional scoring and ranking criteria (Ebrahimi and Hashemi, 2024).

FAE1, encoding fatty acid elongase1, is essential for erucic acid biosynthesis, catalyzing the initial process step in very long-chain fatty acid production and *C. sativa* chromosomes have three functional FAE1 alleles. *CsFAE1* gene expression is closely linked to oleic acid levels in rapeseed. Studies show *FAE1* knockout increases oleic acid and reduces erucic acid content (Ozseyhan *et al.*, 2018). Plant tissue culture is a cornerstone of plant biology, enabling applications like plant preservation, large-scale production of plants, genetic modification, secondary metabolites synthesis, and somaclonal variation (Ghosh *et al.*, 2021). Tissue culture allows scientists to modify plant cells in controlled environments, offering opportunities to advance plant knowledge and harness their benefits. So optimizing these processes is key to improving in vitro propagation efficiency (Balamurugan *et al.*, 2024).

The primary objective of this study is to optimize tissue culture conditions, design gRNA, and construct a recombinant vector for the CRISPR/Cas9-mediated genome editing of the CsFAE1A, CsFAE1B, and CsFAE1C genes in *C. sativa*. Targeting these genes could alter the fatty acid composition of camelina oil in future studies, specifically reducing the levels of erucic acid and eicosenoic acid, which are associated with health risks, while enhancing the production of beneficial fatty acids. Additionally, this research seeks to establish an efficient in vitro regeneration system for *C. sativa* to facilitate the successful introduction of the recombinant vector via *Agrobacterium*-mediated transformation, ultimately producing camelina plants with improved oil quality suitable for human consumption and industrial applications. We assume that the CRISPR/Cas_9-mediated knockout of the *CsFAE1A*, *CsFAE1B*, and *CsFAE1C* genes in *C. sativa* will significantly reduce the accumulation of erucic acid and eicosenoic acid in the seeds, leading to an improved fatty acid profile in the oil. Furthermore, optimizing tissue culture conditions, particularly the hormone ratios in the growth medium, will enhance embryogenic callus formation and direct regeneration, thereby facilitating the successful genetic transformation of *C. sativa*. This approach will result in the development of camelina lines with oil compositions that are more suitable for nutritional and industrial uses.

2. Materials and methods

2.1. Bioinformatics studies

2.1.1. gRNA design and finding FAEs gene sequences in camelina

The selection criteria for gRNA include proximity to the 5' end of exon 2. Comprehensive coverage of all three *CsFAE1* genes, 3. A better efficiency rating, 4. no off-target locations, 5. Regions that are at least self-complementary, 6. High GC content, ideally between 40% and 70%. The sequences of the desired genes were obtained from https://www.ncbi.nlm.nih.gov/. Subsequently, the http://crispor.org became accessible, and the exon sequences of the *C. sativa* genes—*FAE1A*, *FAE1B*, and *FAE1C*—were entered independently into the program page. The appropriate genome and the type of CRISPR nuclease to be used are determined in the second and third steps. Genomes can be searched using the organism's scientific name. In the 'Select a Protospacer Adjacent Motif' section, the PAM sequence type is selected like Fig. 1. After a quick calculation, the website output shows a graphical depiction of each input sequence for sgRNA design (Fig. 2). The best gRNA that fits the above criteria and can target the three alleles of the FAE1 gene (*FAE1A*, *FAE1B*, *FAE1C*) was selected and then examined for off-target sites using the web-based Cas-OFFinder software (http://www.rgenome.net/cas-offinder).

2.1.2. Design of the gRNA cassette

In this project, an expression cassette (417 base pairs long) containing the AtU6-1 promoter (305 base pairs

long), the tracrRNA region (RNA scaffold) (76 base pairs long), and a poly-dT terminator sequence (8 base pairs long) was designed to insert any crRNA sequence into the sgRNA expression cassette. The AtU6-1 promoter, which is a component of the U6 snRNA protein in Arabidopsis, is expressed in all plant organs, including the stems, leaves, and roots (Li *et al.*, 2013; Li *et al.*, 2007). As a result, in our experiment, the sgRNA expression cassette was driven by the AtU6-1 promoter. This promoter has a single purine nucleotide (A or G) as the transcription start nucleotide sequence and an oligo-dT of 8-6 nucleotides as the terminator sequence. Diguanosine 5'-triphosphate (dGTP) was employed as the transcription start nucleotide at the 5' end of the crRNA in the specific sgRNA expression cassette, and eight nucleotides of dT were used as the terminator sequence at the 3' end of the scaffold (Fig. 3). The expression cassette has *EcoRI* and *XbaI* restriction sites at both ends, which are required for cloning into the pFGC-pcoCas9 expression vector. Vector NTI software was used to design the gRNA cassette's forward and reverse primers (Table 1).

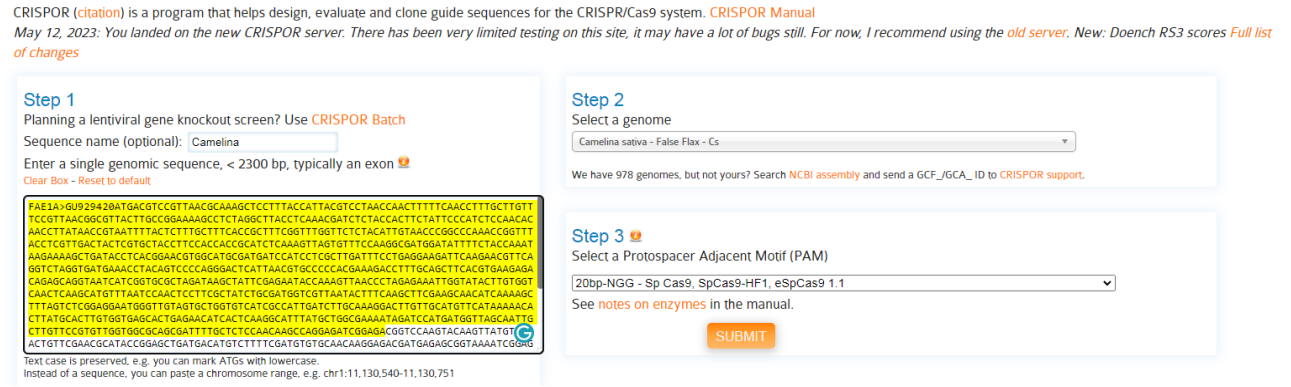

Figure 1. CRISPOR Online Software available on "http://crispor.org"

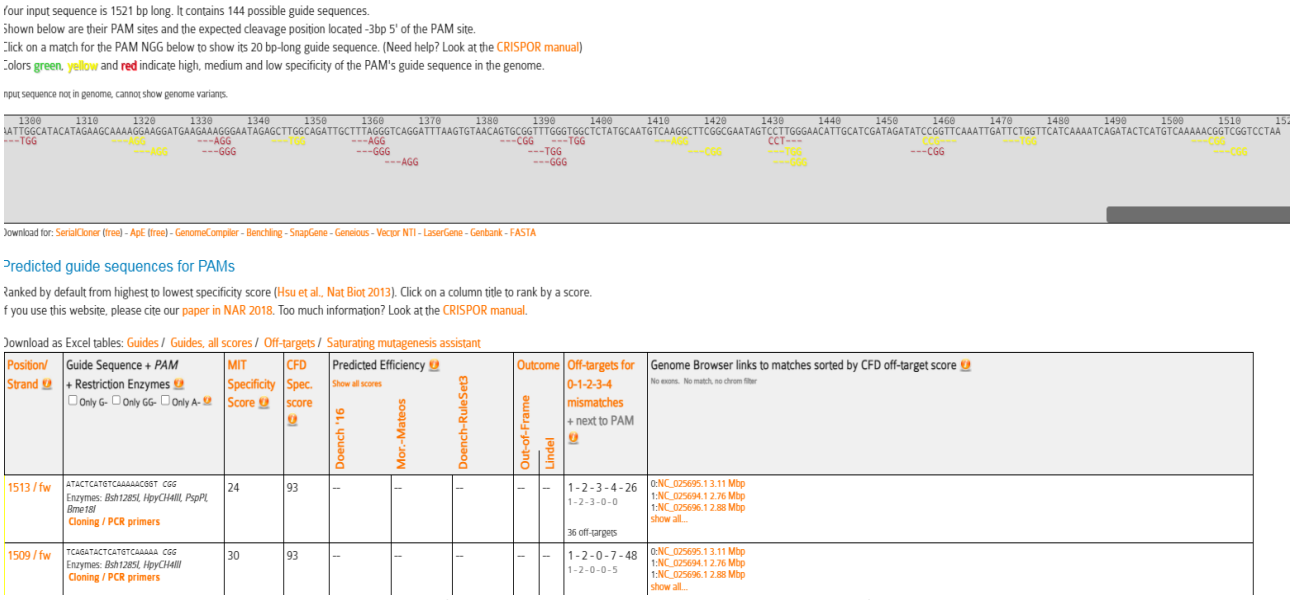

Figure 2. CRISPOR on web software output with a graphical representation for each FAE1 gene sequence

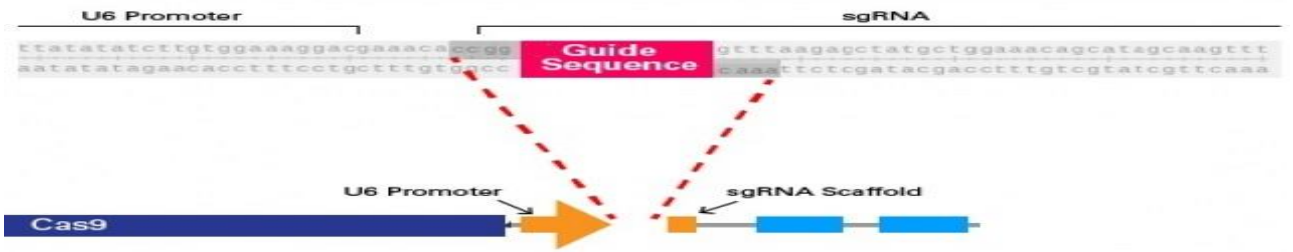

Figure 3. sgRNA cassette including the Arabidopsis U6 promoter, CRISPR RNA, Cas_9-binding scaffold RNA, terminator sequence, and restriction sites for bacterial cloning.

Table 1. Forward and reverse primers of the gRNA cassette

Primer name	Sequence	Tm	Product size
Forward	5' CGACTTGCCTTCCGCACAATACATC 3'	62.3	490 bp
Reverse	5' CGCCGAAGAACAGAGGAAGA 3'	60.8	

2.2. Laboratory studies

2.2.1. Digestion of pFGC-pcoCas9 vector and PUC19 vector including gRNA

Plasmid extraction from PUC19 containing gRNA was performed using the miniprep method (Birnboim and Doly, 1979), and plasmid extraction from pFGC-pcoCas$_9$ was carried out using the plasmid extraction kit (DNA FAVORGEN Biotech Co., FAPDE 004, Taiwan). The gRNA and pFGC-pcoCas$_9$ fragments were separately digested with the restriction enzymes *EcoRI* and *XbaI*. The digestion reaction had a total volume of 20µl, containing 2µl of DNA, 2µl of 10X buffer, 1µl (2 units) of each restriction enzyme, and 14µl of ddH_2O. The reactions were incubated at 37°C for two hours. Finally, the digested products were loaded onto a low-melting-point agarose gel (LMP1%).

2.2.2. Construction of the recombinant expression vector pFGC-pcoCas9-FAE1

The digested products of the pFGC-pcoCas$_9$ vector and gRNA after loading on a 1% LMP agarose gel, excised using a blade, and the gel slices purified with LMP buffer. The gRNA fragment was ligated with the pFGC-pcoCas$_9$ plasmid using the T_4 DNA ligase enzyme in a ligation reaction. The plasmid containing the gRNA cassette was named pFGC-pcoCas$_9$-FAE1. Finally, this recombinant vector was transferred into competent *E. coli* strain BL21 via the heat shock method.

2.2.3. Confirmation of the construction pFGC-pcoCas9-FAE1 recombinant vector

2.2.3.1. Polymerase chain reaction (PCR) on recombinant colonies

PCR reaction on colonies was performed to amplify FAE1 genes cassette with a pair of forward (CGACTTGCCTTCCGCACAATACATC3)' and reverse primers 5'(CGCCGAAGAACAGAGGAAGA 3') in final volume of 25 µl containing 50-100ng DNA of recombinant vector, 400 nM gene-specific primers, and 12.5 µl 2XPCR Master Mix, and 0.5µl Taq DNA Polymerase (Sinacolon company). The PCR reaction was performed according to the following temperature program: initial denaturation at 94°C for 5 min, 35 cycles including denaturation at 94°C for 1 min, primer annealing at 55°C for 45 s, extension at 72°C for 45 s, and final extension at 72°C for 5 min.

2.2.3.2. Digestion reaction on extracted recombinant plasmids

Enzyme digestion was used to confirm the presence of recombinant plasmids containing gRNA genes in colonies resulting from the ligation reaction. Plasmids that had the gene confirmed by polymerase chain reaction (PCR) using particular primers for the gRNA gene were digested and validated with the restriction enzymes *EcoRI* and *XbaI*, respectively.

2.3. Plant materials and growth conditions

The *C. sativa* seeds, obtained from a double haploid project introduced by Dr. Danial Kahrizi from Razi University, Kermanshah, Iran, were utilized in this experiment. Basic MS and Gamborg medium, together with growth regulators BAP, KIN, PIC, NAA, and 2,4-D, were used. The seeds were initially sterilized by immersing them in 96% alcohol for 60 seconds. After three washes with sterile distilled water, they were immersed in a 2% sodium hypochlorite solution for ten minutes. Subsequently, the seeds were washed three times with sterile distilled water. For germination, the seeds were sown in a jam jar containing 50ml of sterile MS medium (Murashige and Skoog, 1962) and Gamborg medium (Gamborg *et al.*, 1968), and maintained in a growth room under a photoperiod of 16 hours light and 8 hours darkness with a light intensity of 2400 lux and a temperature of 24°C for one week (Fig. 4). Hypocotyls and cotyledons from sterile seedlings were cut to appropriate sizes. The explants were then vertically grown in Petri dishes with 20ml of Gamborg media with varied doses of plant growth regulators. These growth regulators included various ratios of cytokinins (Kin and BAP) and auxins (NAA, 2,4-D, and Picloram). The percentage of callus induction and regeneration in each treatment combination was measured three times. The data were statistically analyzed using SPSS software version 23, and mean comparisons were made with Duncan's multiple range test at the 5% probability level.

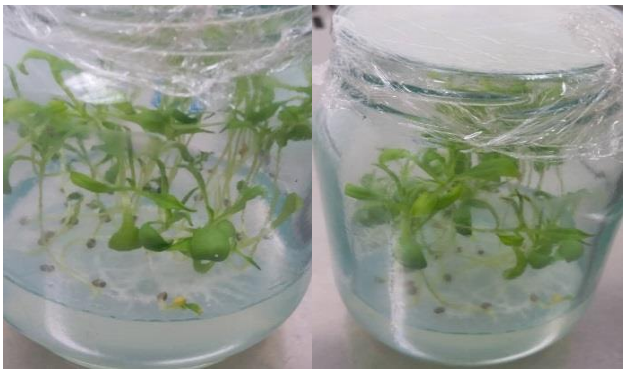

Figure 4. Seedlings for preparation of cotyledon and hypocotyl explants

3. Results and discussion

3.1. Validation of PUC19-gRNA and pFGC-pcoCas9 plasmid digestion

To clone the gRNA cassette into the pFCG-pcoCas9 vector, restriction digestion was used. To begin, plasmid extraction was performed using the PUC19-gRNA and the empty pFCG-pcoCas9 vector. Double digestion was performed on the gRNA plasmids using the restriction enzymes *XbaI* and *EcoRI*, resulting in a band of approximately 500kb. The pFCG-pcoCas9 plasmids were also digested, resulting in a band of about 13330 bp. Fig. 5A shows the electrophoresis image of gRNA digestion, while Fig. 5B shows the electrophoresis image of pFCG-pcoCas9 digestion. In a ligation reaction, the gRNA fragment was ligated to the pFGC-pcoCas9 plasmid using the T4 DNA ligase enzyme. The plasmid with the gRNA cassette was designated pFGC-PcoCas9-FAE1.

3.2. Confirmation of gRNA cassette cloning in pFGC-pcoCas9 vector

The recombinant pFGC-pcoCas9 vector was analyzed using PCR and enzyme digestion on the recombinant colonies. Ultimately, using the heat shock technique to introduce this recombinant vector into competent *E. coli* strain BL21, to verify the recombinant nature of the bacteria and the existence of pFGC-PcoCas9-FAE1, PCR was performed on the colonies and double digestion was carried out on the pFGC-PcoCas9-FAE1 plasmids using the restriction enzymes *XbaI* and *EcoRI*. The electrophoresis image of PCR on recombinant *E. coli* BL21 colonies containing the recombinant vector pFGC-PcoCas9-FAE1 is shown in Fig. 6A. Additionally, the electrophoresis image of the digested pFGC-PcoCas9-FAE1 plasmids extracted from the recombinant bacteria is presented in Fig. 6B. The circular schematic of the recombinant vector pFGC-pcoCas$_9$ –gRNA is shown in Fig. 7.

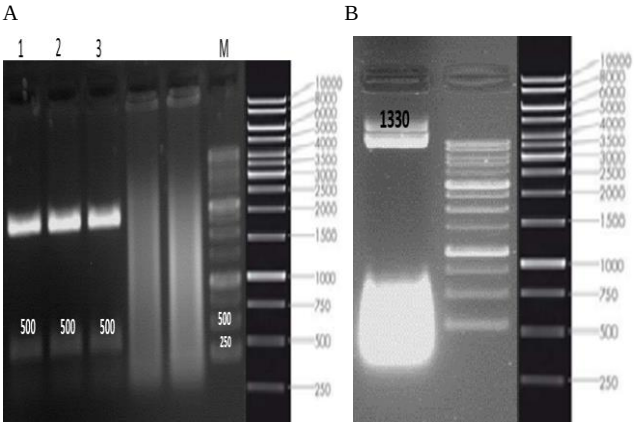

Figure 5. A: Electrophoresis image of the double digest of the PUC$_{19}$ cloning vector with the restriction enzymes *EcoRI* and *XbaI* on an agarose gel. The 500 bp band corresponds to the expected size of the gRNA sequence. 1, 2, and 3 are independent replicates (M = 1 Kb DNA Ladder marker). B: Electrophoresis image of the double digest of the pFGC-pcoCas$_9$ expression vector with the restriction enzymes *EcoRI* and *XbaI* on an agarose gel. The 13,330 bp band corresponds to the expected size of the pFGC-pcoCas9 vector. (M = 1 Kb DNA Ladder marker).

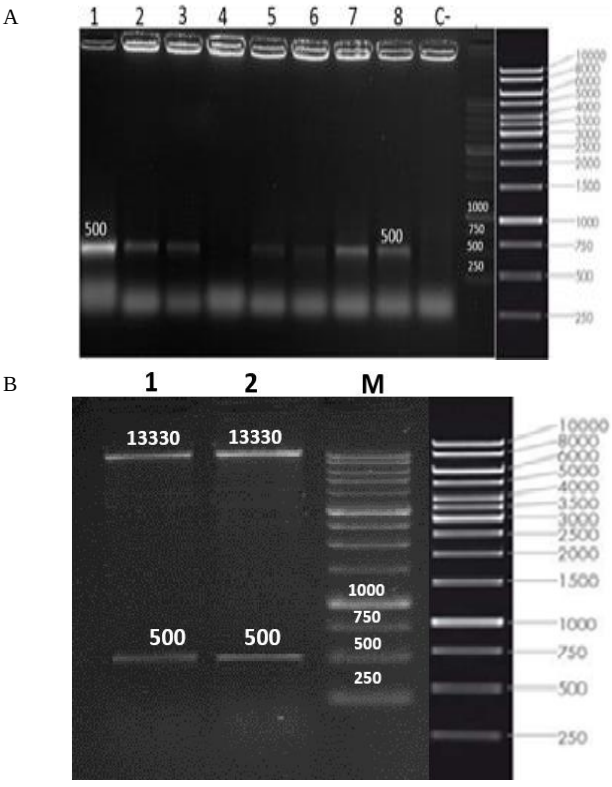

Figure 6. A: Electrophoresis image of the PCR product with gRNA-specific primers on recombinant *E. coli* BL21 colonies. The 500 bp band corresponds to the expected size of the gRNA sequence. 1, 2, 3, 5, 6, 7, and 8 are independent replicates. C- = negative control (M = 1 Kb DNA Ladder marker). B: Electrophoresis image of the double digest of the recombinant expression vector pFGC-pcoCas9 containing gRNA with the restriction enzymes *EcoRI* and *XbaI* on an agarose gel. The 500 bp band corresponds to the expected size of the gRNA gene (M = 1 Kb DNA Ladder marker).

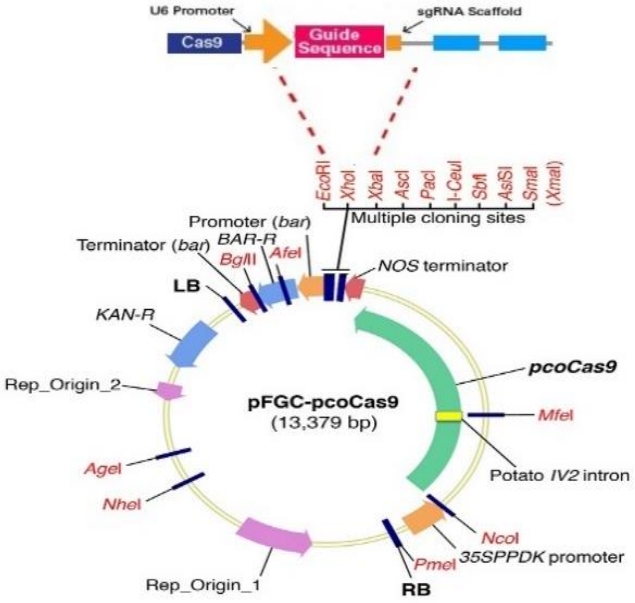

Figure 7. Circular schematic of the recombinant vector containing gRNA

3.3. Callus induction and regeneration of C. sativa

During the investigation of the effect of sterilization on *C. sativa* seeds, it was found that irrespective of the treatment duration, contamination of the seeds was observed on the fifth day when using 1% sodium hypochlorite. Analysis of the effect of sterilization conditions on seed germination indicated that the optimal treatment for *C. sativa* seeds involves using 2% sodium hypochlorite for 6 to 7 minutes, as no contamination was observed under these conditions, and the seed germination rate was 100% (Fig. 4).

In our study on shoot regeneration, a range of concentrations of BAP with NAA, Kin with NAA, and Kin with Pic combinations were tested. Fifteen different media, varying in growth regulator compositions, were evaluated. Explants in each treatment combination were subcultured to fresh media every three weeks. After three or four weeks of culture, yellow-green callus formation with distinct initiation points of new shoots was observed on various media types on the cotyledon and hypocotyl sections of camelina. For embryogenic calluses, the combination 0.5NAA+3BAP achieved the highest callus induction at around 70%, indicating the strongest effect of this treatment. In contrast, combinations such as 0.5 mg L^{-1} 2,4-D+2BAP and 0.1 mg L^{-1} 2,4D+1KIN resulted in much lesser callus induction (Fig. 8), and for direct regeneration, the maximum regeneration was found with the hormone combination 0.5NAA+2BAP+1KIN, denoted as 'a'. Combinations such as 0.3NAA+2BAP have lower regeneration percentages, denoted as 'c' (Fig. 9). In the remaining hormonal ratios, non-embryogenic and vitrification calluses were found. So camelina shoot regeneration was shown to be most efficient in medium containing both cytokinins and auxins.

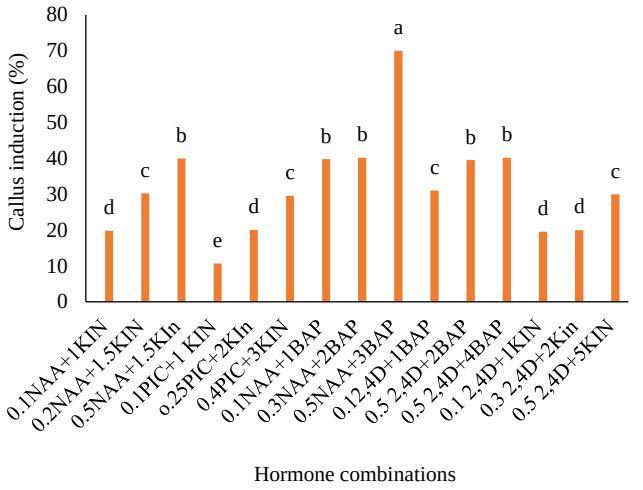

Figure 8. Mean comparison of hormone combinations on callus induction by Duncan's Multiple Range Test at alpha=0.05.

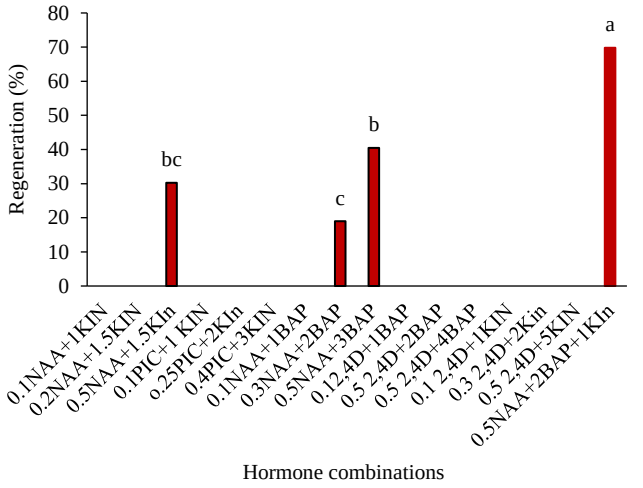

Figure 9. Mean comparison of hormone combinations on direct regeneration by Duncan's Multiple Range Test at alpha=0.05.

It should be highlighted that media containing both BAP and NAA had a better efficiency for somatic embryogenesis and direct regeneration than media containing only BAP. Furthermore, cotyledon explants were substantially more efficient in somatic embryogenesis than hypocotyls, and only cotyledon explants exhibited direct regeneration. Fig. 10A-10C, and Fig. 10F-10L show callus formation on cotyledon sections, whereas Fig. 10D and 10E show callus formation on hypocotyl sections. Fig. 11 shows the direct regeneration of cotyledon sections.

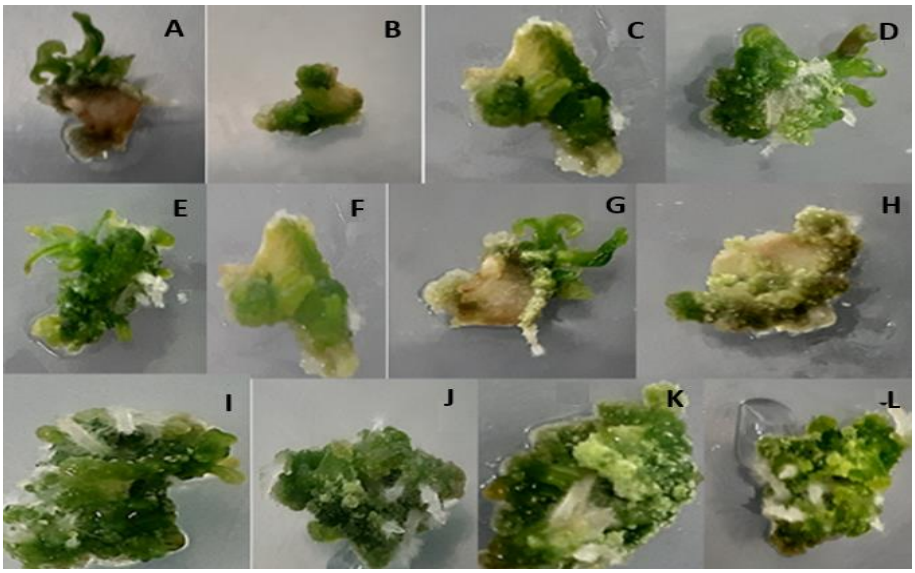

Figure 10. Embryogenic callus production in *C. sativa* cotyledon and hypocotyl explants three weeks after cultivation with BAP and NAA hormonal combinations.

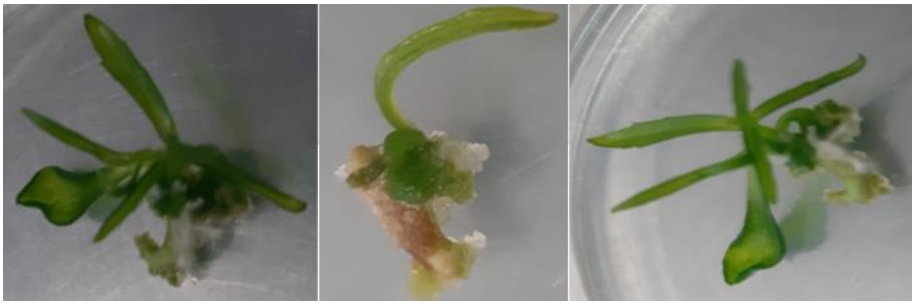

Figure 11. Direct regeneration in *C. sativa* cotyledon explants three weeks after cultivation in MS medium with hormonal combinations of KIN, BAP, and NAA

Camelina, an oilseed crop, has been developed to diversify modern agricultural production methods and produce oil (Bashiri *et al.*, 2023). According to camelina regeneration tests, a variety of cruciferous species and hypocotyl explants are successful at initiating organogenesis in laboratory conditions. To produce plantlets, Yamets et al. (2013) used camelina seedlings of varying ages (five and seven days) on culture media. Previous research has shown that, in addition to diverse auxin and cytokinin combinations, cytokinins are required for stimulating stem formation by initiating cell division and branch differentiation in a variety of cruciferous species. Camelina has shown effective shoot regeneration in medium containing auxin and cytokinins (Yemets *et al.*, 2013). The current study investigated the impact of two cotyledon and hypocotyl explants treated with various types and quantities of PGRs on somatic embryogenesis and regeneration in *C. sativa*. We provide cotyledon explants as candidates for Agrobacterium-mediated transformation in *C. sativa*. Using cotyledon as an explant source facilitated quicker growth and the production of a large volume of starting material for transformation in a short period of time. However, shoot apical meristem cells have been employed for genetic transformation in other plant species (Barlass and Skene, 1978; Sabbadini *et al.*, 2019). There has been no previous publication on cotyledon transformation using vector-based CRISPR technology on camelina. The hypocotyl had very little ability to generate embryogenic calli, cotyledons in hormone concentrations of 0.5 mg L^{-1} NAA and 3 mg L^{-1} BAP had the highest number of embryogenic calli (Fig. 10) and cotyledon in phytohormone concentrations of 0.5 mg L^{-1} NAA, 2 mg L^{-1} BAP, 1 mg L^{-1} Kin plus casein on B5 culture medium possessed the greatest number of direct regeneration (Fig. 11). The embryogenesis of the cotyledon explants was greater to those of the hypocotyl and direct regeneration was observed only in the cotyledons. Other researchers in a different study also emphasized appropriate reaction of cotyledon explants in camelina tissue culture (Rezaeva *et al.*, 2024).

The *FAE1* gene, which encodes ketoacyl-CoA synthase (KCS), plays a critical role in the biosynthesis of eicosenoic acid (20:1) and erucic acid (22:1). The gene was first identified in Arabidopsis, and following researches have shown the presence of an extensive *FAE1* gene family in Brassica species (Patra *et al.*, 2025). Camelina is an underutilized cultivated species but has a considerable potential for economic significance (Bashiri *et al.*, 2024). However, some undesired traits such as very long chain fatty acids (VLCFAs) and glucosinolate must be removed. Biotechnology provides an efficient instrument for expedited camelina improvement and biological research. In this study, we focused on the VLCFAs decrease by aiming FAE1 enzymes which extend the 18:1 substrate to 20:1 and 22:1. Newly synthesized 18:1 fatty acids can be either elongated in the acyl-CoA pool by FAE1 or desaturated on PC pool by FAD2 and FAD3 enzymes (James *et al.*, 1995). A study demonstrated that synthetic microRNA can be employed to target the *FAE1* gene in Arabidopsis, and 20:1 fatty acids decreased were from 15.4% to 1.9% (Belide *et al.*, 2012). More recently, RNA interference (RNAi) has been utilized to specifically inhibit BjFAE1 expression to reduce seed eicosanoid acid (EA) content (Sinha *et al.*, 2007; Wang *et al.*, 2022). CRISPR/Cas_9 provides potentially enhanced precision and efficacy in accomplishing targeted gene disruption in comparison to RNA interference (RNAi), as documented by recent investigations. CRISPR/Cas_9 facilitates direct modification of the target genomic DNA, potentially resulting in stable gene knockout. In contrast to RNAi, which has uneven levels of gene silence in transgenic lines, CRISPR/Cas_9-mediated gene deletion can generate plants with persistent inheritance of the late embryogenesis abundant (LEA) characteristic across generations. (Patra *et al.*, 2025). Allohexaploid structure of camelina complicates the entire knockout of *FAE1* genes since there are three active *FAE1* copies which participate in the order of *FAE1-B* >*FAE1*-C >>> *FAE1-A*. To achieve successful knockout of *FAE1* genes, all alleles should be targeted simultaneously because silencing a single copy of *FAE1* gene did not lead total knockout. For example, sequencing results of *FAE1* EMS mutant revealed that only *FAE1-B* was mutated, however, other two copies were still active, so that camelina continued to produce VLCFAs. In this research, we designed a new sgRNA that targeting all three *FAE1* genes using the CRISPR/Cas9 technology, we will obtain knock-out mutants that accumulate very small amounts of VLCFAs without causing deleterious effects on seed traits and plant growth. In summary, the oilseed crop camelina possesses considerable capacities in food and non-food. It is essential to enhance fatty acid composition in its oils to satisfy various needs.

4. Conclusion

C. sativa is an oilseed plant with a lot of potential for use in both food and non-food applications. To satisfy a variety of demands, its oils' fatty acid composition must be improved. In order to lower the amounts of eicosenoic acid and erucic acid, we designed gRNA for the *C. sativa* genes *FAE1A*, *FAE1B*, and *FAE1C* using the CRISPOR site and then inserted it into the chosen pFGC-pcoCas$_9$ vector. PCR and enzyme digestion were used to validate the recombinant vector pFGC-PcoCas9-FAE1. Furthermore, tissue culture optimization for this plant was examined, along with the best conditions for creating an in vitro culture, such as the ideal sterilizing agent concentration and seed treatment duration for *C. sativa*, the kind of explants chosen for somatic embryogenesis and regeneration (cotyledon and hypocotyl), and the concentration of different plant growth regulators (NAA, BAP, KIN, and PIC) were investigated. This made it possible to use Agrobacterium co-cultivation to introduce the recombinant vector into *C. sativa* plants, producing plants with a fatty acid composition that is appropriate for human consumption.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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