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Research Article

Molecular and Biochemical Evaluation of Ethyl Methanesulfonate-Induced Mutant Lines in *Camelina sativa* L.

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Background: *Camelina sativa* is one of the most important oilseeds that has a proportionate profile of essential unsaturated fatty acids that are suitable for human nutrition. In this regard, we can mention a high percentage and a reasonable ratio of omega 3 and omega 6.
Objectives: In the current study, the created variation of second-generation mutant (M2) camelina lines in terms of fatty acid profiles and ISSR molecular markers in *C. sativa* was evaluated.
Materials and Methods: For this purpose, while producing the first-generation of mutant plants (M1), 200 M2 seeds with 0.1% and 0.5% ethyl methanesulfonate (EMS) mutations were treated in two replications for 8 and 16 hours based on a completely randomized design.
Results: The results of mean comparisons showed that there was no significant difference between treatments in terms of fatty acids of palmitic acid, stearic acid, linoleic acid, eicosadienoic acid, oleic acid and erucic acid. The cluster analysis revealed that all the treatments used with five replications were divided into eight groups. It was found that all replications of the treatment with a concentration of 0.1% and a time of 16 hours (C1T2) were in the second group with the lowest palmitic acid was present among other treatments. Therefore, C1T2 treatment is recommended as the best treatment to reduce palmitic acid. Examination of the information content of ISSR molecular markers also showed that markers 2, 5, and 6 were the best informative markers in the detection of camelina fatty acid profiles.
Conclusion: A significant variation has been created in the fatty acids profile and it can be applied in future breeding programs depending on the intended purpose.

Keywords: Camelina, Fatty acid profile, Genetic diversity, ISSR marker, Mutation, Palmitic acid

Abbreviations: Ethyl Methanesulfonate (EMS), Concentration (C), Time (T), first-generation of mutant plants (M1), second-generation mutant (M2), Gas Chromatography (GC), Optical density (OD) and inter-simple sequence repeat (ISSR).

1. Background

Camelina sativa is an annual flowering plant that belongs to the Brassicaceae family (1). It is a dicotyledonous plant whose morphological ductility is high in response to environmental changes and is a prominent feature of this plant. The height of this plant is usually between 65-115 cm (1). The seeds of this plant are very small (0.7 to 1.5 mm in terms of length), its one thousand seed weight range 0.8-1.8 g depending on the cultivar and their growth conditions during seed development (2, 3). The amount of seed oil has been reported from 30 to 40 $g.kg^{-1}$ (4-7). However, there is no high diversity for oil percentage and fatty acid content in this plant among its different accessions. Nevertheless, high diversity is

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required to carry out selection and breeding projects. Lack of interspecies and intraspecific diversity among crops can cause many problems (8-10).

One way to create diversity in low-diversity plant populations is to induce mutations (11, 12). Induction mutations create new alleles that can break the functional limitations of plant function (13). One of the methods of mutagenesis in plant seeds is the use of ethyl methanesulfonate (EMS), which is a multifunctional ethylation agent and in various types of genetic testing systems, its mutagenicity has been proven to break chromosomes (14). This mutagenic compound helps breeders to make the selection more easily by diversifying plants. (15). Research has also shown that the use of ethyl EMS significantly reduces the composition of vernolic acid and significantly increases the composition of linoleic, oleic, palmitic, stearic, and arachnid fatty acids in mutants of the plant Vern onia (Centrapalus pauciflorus (Willd) H.Rob) (16).

Advances in biotechnology in recent years have helped to identify genetic and phylogenetic relationships as well as plant breeding. In the last two decades, DNA-based molecular markers have been widely used for various purposes in plants and animals (17).

2. Objectives

This study aimed to induce mutation via EMS treatment of seeds in camelina and investigate the genetic changes of mutant lines by ISSR (Inter Simple Sequence Repeat) molecular marker and biochemical changes in fatty acid profiles.

3. Materials and Method

3.1. Select Plant Material and Induction of Mutation

The seeds of camelina (Soheil cultivar) were prepared from Biston Shafa Company, Iran. In order to induce mutations in this experiment, ethyl methanesulfonate (EMS) was used with concentrations of 0.1 and 0.5% in two periods of 8 and 16 hours on 200 seeds (for each treatment) in separate petri dishes (50 seeds per petri dish). Other steps were performed based on the method of Jander *et al*. (2003) (18). EMS-treated seeds were planted in a completely randomized design with 5 replications in separate pots inside the greenhouse as the firstgeneration mutant (M1). Then the seeds of each treatment were harvested separately and planted for the next generation (M2) via selfing.

3.2. Extraction of Oil from Seeds and Measurement of Fatty Acid Profiles

M2 generation was used to extract the oil and three replications were considered for each sample. In this way, the seeds of camelina mutant lines were powdered by mortar and pestle separately after cleaning from external contaminants. Then, oil was extracted from 500 mg of powdered seed samples using a Soxhlet apparatus and normal hexane as a solvent for eight hours. The free fatty acids in the oil were soaped by the method of Lopez Martinez *et al*. After removal of the solvent under vacuum conditions (19), the obtained fatty acids were then methylated using Lepage and Roy method (1984) (20). The methyl derivative of fatty acids was separated for the Gas Chromatography (GC) method after cooling at room temperature and removal of the solvent. Identification of fatty acids of gas chromatography device (Varian CP 3800) connected to FID detector and equipped with polar silica column (column length: 60 m, inner diameter: 0.25 mm, film thickness: 0.2 μm) was carried out. The GC was used to measure the profile of fatty acids and this analysis was carried out in Kermanshah's Mahidasht Agricultural Industrial Complex (Nazgol), Iran.

To separate and identify fatty acids, gas chromatograph (Varian CP3800) was used with connected to FID detector and equipped with polar silica column (TR-CN100 poly (bicyanopropyl) siloxane capillary) (column length: 60 m, inner diameter: 0.25 mm), Film thickness: 0.2 μm) (Teknokroma Co, Barcelona, Spain). The oil content of seed samples was calculated and reported based on their dry weight percentage and the amount of fatty acids based on the percentage of total oil and by comparing their subspecies level with standard samples including Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2), Alpha-Linolenic acid (C18:3), Arachidic acid (C20:0), Eicosenoic acid (C20:1), Eicosadienoic acid (C20:2), Eicosatrienoic acid (C20:3), Behenic acid (C22:0), Erucic acid (C22:1) and Nervonic acid (C24:1) (C: 12-C: 24, Sigma Company).

3.3. Extraction, Determination of Quality and Quantity of DNA

To extract DNA, the first five seed samples were prepared from each of the treatments. Then DNA samples were extracted from leaf samples separately by Doyle & Doyle method (1990) (21). The samples

were stored in the freezer at -20 °C for the next stages of the experiment. A specific concentration of DNA is required to perform the ISSR amplification reaction. Therefore, the quality and quantity of the extracted DNA were evaluated before the reactions.

Horizontal electrophoresis on 1% agarose gel in the presence of TBE buffer was used to determine the quality of the extracted DNA samples and to ensure that they were not broken. The presence of unclear and weak bands indicates fracture and poor DNA quality, and the presence of light polynucleotide bands at the end of the electrophoresis gel indicates RNA contamination. Samples with clear and sharp bands were considered as high-quality DNA. To quantify DNA samples, spectrophotometry was performed by optical absorption of DNA and proteins at 260 and 280 nm and by UV/VIS spectrophotometer.

So that if the OD260/OD280 ratio was in the range of 1.8-2, DNA would be of good quality. To calculate the appropriate DNA concentration, the absorbance at 260 nm was used according to the following formula.

DNA ng.μL^{-1}) = OD260 × Inverse dilution coefficient × 50

3.4. Molecular Analysis of DNA with ISSR Markers

To investigate genetic diversity, at the molecular level of DNA, 10 ISSR primers were used, the characteristics of which are listed in **Table 1**. According to the most polymorphic primers reported in camelina and rapeseed in different previous reports, primers were selected. Thus, for each treatment, 5 samples were considered for PCR reaction. In this experiment, the method of Williams *et al*. was used for polymerase chain reaction (22). The volume of the solution used was 25 µL and 100 samples of the master mix were prepared from Pishgam Company, Iran. The components of the reaction were: 0.5 µL DNA template (10ng), 2.5 µL primer (10mM), 4 µL Master Mix and 18 µL DDW. The reaction mixture corresponding to each sample was poured into separate microtubes and placed in a thermocycler. For the ISSR initiator, the reaction time program was executed as 1 cycle first denaturation (94 °C, 5 min), 30 cycles amplification [(denaturation 94 °C, 30 sec), (annealing in specific temperature, 40 sec) and (extension 72 °C, 80 sec)] and 1 cycle final extension (72 °C, 5 min).

3.5. Agarose Gel Electrophoresis and Staining

In order to separate the amplified DNA bands, 2% agarose gel was used in the TBE buffer. At this stage, 8 µL of DNA was gently mixed with 3 µL of loading buffer and each sample was loaded in a separate well. Gel electrophoresis was performed with a voltage of 100 for one hour and 30 minutes, then the strips were observed in the presence of UV rays with the Gel Doc device.

3.6. Analysis of Fatty Acid Profile Data

Data analysis including analysis of variance and mean comparison were performed by the LSD method using SAS software. Cluster analysis, detection function, correlation analysis and factor analysis were performed using SPSS ver: 21 software.

3.7. Molecular Data Analysis

After performing the polymerase chain reaction with ISSR primers, the presence and absence of each strip

Table 1. ISSR primers used for investigating the diversity of camelina mutant lines

No	Name	Sequence (5'-3')	Annealing (C°)
1	mm14	5'-CTC TCT CTC TCT CTC TRA-3'	49
2	mm15	5'-ACA CAC ACA CAC ACA CYT-3'	53
3	mm16	5'-ACA CAC ACA CAC ACA CYA-3'	49
4	p1	5'-GAG CAA CAA CAA CAA CAA-3'	49
5	p4	5'-CTG GTG TGT GTG TGT GTG T-3'	59
6	p6	5'-AGA GAG AGA GAG AGA GGC C-3'	60
7	p7	5'-AGA GAG AGA GAG AGA GGT G-3'	59
8	p8	5'-GAG AGA GAG AGA GAG AAC C-3'	59
9	p9	5'-GAG AGA GAG AGA GAG AAT C-3'	52
10	p10	5'-ATG ATG ATG ATG ATG-3'	37

Single-letter abbreviations for mixed-base positions: (Y= T or C, R= G or A)

were determined with numbers one and zero for all treatments.

After forming the raw data matrix, the marker parameters were calculated using Excel.2010. To calculate genetic similarity, the Jaccard similarity coefficient and principal component analysis were used by NTSYSpc 2.02e software. Treecon software was used to obtain dendrograms and bootstrap calculation, GenAlex software was used to analyze molecular variance XLSTAT ver: 2015 software was used for correlation between matrices obtained from ISSR molecular marker similarity coefficients and fatty acid profile data.

4. Results

4.1. Analysis of Variance and Mean Comparison

Examination of the results of the analysis of variance of fatty acid profile showed that a significant difference was observed for the treatment used in all fatty acids except behenic acid and omega-9-neuronic acid. The results of the mean comparison are presented in **Table 2**. Based on the comparison means results, the mutation induction by C2T16 treatment (7.67%) caused a significant increase in palmitic acid compared to the control (6.73%). Also, the use of C2T16 (7.67%) treatment increased the amount of palmitic acid compared to C1T16 (6.50%) and C2T8 (6.59%) treatments. However, the application of C1T16 (6.50%) and C2T8 (6.59%) treatments significantly reduced the amount of palmitic acid compared to the control. The highest and lowest levels of palmitic acid were observed for C2T16 (7.67%) and C1T16 (6.50%) treatments, respectively. For stearic acid, treatments C1T8 (3.52%), C1T16 (3.24%), and C2T16 (3.68%) significantly increased the amount of stearic acid compared to the control (2.53%). There was a significant difference in the amount of this fatty acid between C2T8 (3.29%) and C1T8 (3.52%), C1T16 (3.24%), and C2T16 (3.68%) treatments. The highest and lowest levels of stearic acid were (3.68%) C2T16 and (3.24%) C1T16, respectively. Comparison of the mean profile of fatty acids showed that the amount of omega-9-oleic acid in all treatments C1T8 (14.37%), C2T8 (14.37%), and C2T16 (14.53%) caused a significant decrease in this fatty acid compared to the control (17.20). The highest and lowest values of this fatty acid were observed for C1T16 (15.1%) and C1T8 (14.37%) treatments, respectively. The amount of omega-6-linoleic acid showed a significant increase in all C1T8 (24.07%), C1T16 (23.97%), C2T8 (23.6%), and C2T16 (24.47%) treatments compared to the control (20.15%). There was no significant difference between

Table 2. Mean comparison of the effect of different concentration and time treatments of EMS on fatty acid profiles of camelina mutants

Treat	Means										
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	linolenic acid	Arachidic acid	Eicosenoic acid	Eicosadienoic acid	Eicosatrienoic acid	Erucic acid	Others
C:1 T:8h	7.25 ab	3.52 ab	14.37 b	24.07 a	24.65 b	2.98 a	14.65 b	1.86 a	0.53 a	4.14 a	0.69 a
C:1 T:16h	6.50 c	3.24 b	15.1 b	23.97 a	25.32 b	3.09 a	14.47 b	1.65 b	0.49 a	3.53 bc	0.50 ab
C:2 T:8h	6.59 c	3.29 c	14.37 b	23.6 a	25.76 b	3.07 a	14.74 b	1.75 ab	0.57 a	3.93 ab	0.64 a
C:2 T:16h	7.67 a	3.68 ab	14.53 b	24.47 a	24.51 b	2.72 a	15.34 ab	1.61 b	0.57 a	3.51 bc	0.23 b
control	6.73 bc	2.53 c	17.20 a	20.15 b	28.74 a	1.86 b	16.26 a	1.64 b	0.4b	3.41 c	0.26 b

The means with a common letter indicates insignificance

the treatments for this fatty acid. The highest and lowest values of this fatty acid were for C2T16 (24.47%) and C2T8 (23.6%) treatments, respectively.

There was a significant decrease in the amount of omega-3-linoleic acid between C1T8 (24.65%), C1T16 (25.32%), C2T8 (25.76%), and C2T16 (24.51%) treatments compared to the control (28.74%) but we were found no significant difference between the treatments. The highest and lowest values of this fatty acid were in C2T8 (25.76%) and C2T16 (24.51%) treatments, respectively. There was a significant increase in the amount of arachidic acid in all treatments as C1T8 (2.98%), C1T16 (3.09%), C2T8 (3.07%), and C2T16 (2.72%) compared to the control (1.86%) but no significant difference was found between the treatments. The lowest and highest levels of this fatty acid were C2T16 (2.72%) and C1T16 (3.09%), respectively. Applications of C1T8 (14.65%), C1T16 (14.47%), and C2T8 (14.74%) treatments caused a significant reduction in the amount of omega-9-Eicosenoic acid (DK1) acid fatty acid compared to the control (16.26%). There was no significant difference between the treatments for this fatty acid and the highest and lowest values of this fatty acid were for C2T8 (14.74%) and C1T16 (14.47%) treatments, respectively.

There was a significant increase in the amount of omega-6-Eicosadienoic fatty acid for C1T8 treatment (1.86%) compared to the control (1.64%). Also, C1T8 (1.86%) treatment showed a significant increase compared to C1T16 (1.65%) and C2T16 (1.61%) treatments. The highest and lowest levels of this fatty acid were C1T8 (1.86%) and C2T16 (1.61%), respectively. For omega-3-eicosadienoic acid, C1T8 (0.53%), C1T16 (0.49%), C2T1 (0.57%), and C2T16 (0.57%) treatments significantly increased the amount of this fatty acid compared to the control (0.40%) but no significant difference was observed between treatments. The highest and lowest values of this fatty acid were for C2T16 (0.57%) and C1T16 (0.49%) treatments, respectively. Treatments C1T8 (4.14%) and C2T8 (3.93%) significantly increased the amount of omega-9-uric acid compared to the control (3.41%). Also, C1T8 (4.14%) treatment compared to C1T16 (3.53%) and C2T16 (3.51%) treatments showed a significant increase in the amount of this fatty acid. The highest and lowest levels of omega-9-erucic acid were C1T8 (4.14%) and C2T16 (3.51%), respectively. The amount of other fatty acids measured generally showed a significant increase compared to the control (3.41%) under the influence of C1T8 (0.69%) and C2T8 (0.64%) treatments. Also, C1T8 (0.69%) and C2T8 (0.64%) treatments significantly increased the amount of these fatty acids compared to C2T16 (0.23%) (**Table 2**). Therefore, according to the comparison of the obtained average, there is a great variety in terms of the amount of fatty acids among the treatments and this allows for a better breed of camelina. Since linolenic acid (omega 3), linoleic acid (omega 6), and oleic acid (omega 9) are essential components of fatty acid profiles and the human body is not able to produce them due to the lack of proper enzymes (23, 24). It was found that the use of mutagenic treatments increased the content of fatty acids in camelina compared to the control. The use of these mutated seeds is highly recommended to enhance the content of this essential fatty acid.

4.2. Correlations Between Fatty Acid Profiles

The matrix of correlation coefficients by the Pearson method based on fatty acid profiles is presented in **Table 3**. Palmitic acid had a positive and significant correlation with stearic acid. Stearic acid had a negative and significant correlation with omega-9-oleic acid, omega-3-linoleic acid, and omega-9-Eicosenoic acid and had a positive and significant correlation with omega-6 linoleic acid, arachidic acid and omega-6-Eicosadienoic acid. The correlation between omega-9-oleic acid with omega-3-linoleic acid and omega-9-Eicosenoic acid was positive and significant, also had a negative and significant correlation with omega-6-linoleic acid, arachidic acid, omega-6- eicosadienoic acid, and other fatty acids. Omega-6-linoleic acid had a positive and significant correlation with arachidonic acid, omega-9-Eicosenoic acid, and omega-6- eicosadienoic acid and had a negative and significant correlation with omega-3-linoleic acid. The correlation between omega-3 linoleic acid with omega-9-Eicosenoic acid and omega-9-neuronic acid was positive and significant, also had a negative and significant correlation with arachidonic acid and omega-6- eicosadienoic acid. There was a positive and significant correlation between arachidonic acid and omega-6- eicosadienoic acid and this fatty acid had a negative and significant correlation with omega-9-Eicosenoic acid and omega-9-neuronic acid.

The correlation between omega-9-Eicosenoic acid and omega-9-neuronic acid was positive and significant, also had a negative and significant correlation with

Table 3. Correlation coefficients based on fatty acid profiles in camelina mutant lines

Name (acid)	Palmitic	Stearic	(ω-9) Oleic	(ω-6) Linoleic	(ω-3) linolenic	Arachidic	Eicosenoic	Behenic	Eicosadienoic	Nervonic
Stearic	0.57*									
(ω-9) Oleic	-0.25	0.79**								
(ω-6) Linoleic	0.21	0.73**	-0.81**							
(ω-3) linolenic	-0.32	0.81**	0.81**	-0.74**						
Arachidic acid	0.05	0.66**	0.69**	0.63*	-0.68**					
Eicosenoic	0.02	-0.51*	0.60*	0.58*	0.53*	-0.83**				
Behenic acid	-0.01	0.15	-0.46**	0.33	-0.13	0.14	-0.34			
Eicosadienoic	0.4	0.85**	0.78**	0.62*	-0.66**	0.61*	-0.57*	0.23		
Nervonic	-0.18	-0.57*	0.45	-0.43	0.51*	-0.55*	0.67*	-0.06	-0.73**	
Others	-0.08	0.33	-0.58*	0.37	-0.39	0.45	-0.23	0.64**	0.21	0.15

omega-6- eicosadienoic acid. Behenic acid had a positive and significant correlation with other fatty acids. A significant negative correlation was observed between omega-6- eicosadienoic acid and omega-9-neuronic acid. Mostafavi *et al*.(2011) in evaluating yield, fatty acid composition, and amount of seed micronutrients in high-yielding rapeseed cultivars under the influence of different amounts of sulfur showed that oleic acid had a significant correlation with linoleic and linolenic palmitic fatty acids (25).

4.3. Factor Analysis Based on Fatty Acid Profiles

Factor analysis was performed by the principal component's method and varimax rotation on data. Eigenvalues of the amount of changes justified by each factor and the cumulative percentage of changes justified are presented in **Table 4**. The first three factors explained a total of 96.99% of the total variance. The first factor explained 62% of the total diversity. This factor was mainly affected by stearic acid, omega-6-linoleic acid, arachidic acid, and omega-9-neuronic acid with a positive factor charge and omega-9-oleic acid, omega-3-linoleic acid, and omega-9with negative factor charge.

The second factor explained 22.05% of the total diversity. This factor was mainly affected by behenic acid, other fatty acids, and palmitic acid with a positive factor charge. The third factor explained 12.93% of the total changes. This factor was mainly affected by palmitic acid with a positive factor charge. The use of factor analysis by the modifier can increase the ability to understand the relationship between variables and create the best choice in breeding programs. The agents were shown that for the combination of oil fatty acids, meal glucosinolates, and grain yield, four factors could explain 66.2% of the changes (26).

4.4. Cluster Analysis of ISSR Marker Results

Dendrogram obtained from cluster analysis based on ISSR marker using the Ward method in camelina mutant lines. The result of cluster analysis has been demonstrated in **Figure 1**. The numbers in parentheses indicate the type of treatment reported, which includes the type of concentration and time, and the numbers after the parentheses indicate the replication of that treatment. The clusters members were included as the first group: control samples, the second group: (C1T1) 1, (C1T1) 2, (C1T2) 2, (C1T2) 3, (C2T1) 3, (C1T2) 1, (C1T1) 3, (C1T1) 5, (C2T2 2, (C2T1) 2, (C1T2) 4, (C1T2) 5, third group: (C2T1) 4, (C2T1) 5, (C2T2) 1, fourth group: (C2T2) 3, fifth group: (C2T1) 1, sixth group: (C2T2) 5, seventh group: (C1T1) 4 and the eighth group (C2T2) 4. According to the mean comparison results, except for (C2T2) 2 from the second group and (C2T2) 1 from the third group, the other replications of the (C2T2) were grouped separately in the fourth, sixth, and eighth groups, and all of them had the highest levels of palmitic acid, stearic acid, omega-6-linoleic acid, arachidic acid, omega-9-Eicosenoic acid and omega-3-eicosatrienoic acid among the fatty acid profiles. In the third group, except for (C2T2)1, the two other cases, both of which were (C2T1) treatments, and had the highest significant levels of omega-6-linoleic acid, arachidic acid, omega-6- eicosadienoic acid, omega-3-eicosatrienoic

acid, omega-9-erucic acid. Except for (C1T1) 4, which is grouped separately in the seventh group, the other replications of the (C1T1) were all in the second group and had the lowest significant levels of omega-9-oleic acid, omega-3-linoleic acid, and omega-9-iconic acid. It was important to note that all treatment replications of (C1T2) were in the second group and had the lowest palmitic acid content among the other treatments. Since palmitic acid is one of the most saturated and harmful fatty acids for the human and increases the risk of heart attacks and increases LDL cholesterol (26), use a treatment with a concentration of 0.1% EMS with a time of 16 hours for it is recommended to reduce it.

In 2017, Yaman *et al*. identified two different clusters in the genetic region by examining 11 ISSR markers for camelina genetic diversity (27). Khatamian *et al*. 1390 to evaluate the yield, yield components, and some morphological characteristics of 16 rapeseed cultivars in the Arak region used cluster analysis to group the cultivars. They were divided into three separate groups

Table 4. Factor analysis of fatty acids in camelina mutant lines

Name	First factor	Second factor	Third factor
Palmitic acid	0.22	0.01	0.97
Stearic acid	0.88	0.19	0.41
ω-9 Oleic acid	-0.9	-0.36	-0.13
ω-6 Linoleic acid	0.96	0.18	0.16
ω-3 linoleic acid	-0.91	-0.24	-0.28
Arachidic acid	0.90	0.35	-0.23
ω-9 Eicosenoic acid	-0.80	-0.44	0.32
Behenic acid	0.11	0.99	-0.01
ω-6 Eicosadienoic acid	0.87	0.15	0.22
ω-9 Nervonic acid	0.95	0.25	0.14
Others	0.32	0.92	0.01
Palmitic acid	0.01	0.88	0.36
Stearic acid	0.35	0.84	-0.39
eigenvalue	8.06	2.86	1.68
relative variance	62	22.05	12.93
Cumulative variance	62	84.05	96.99

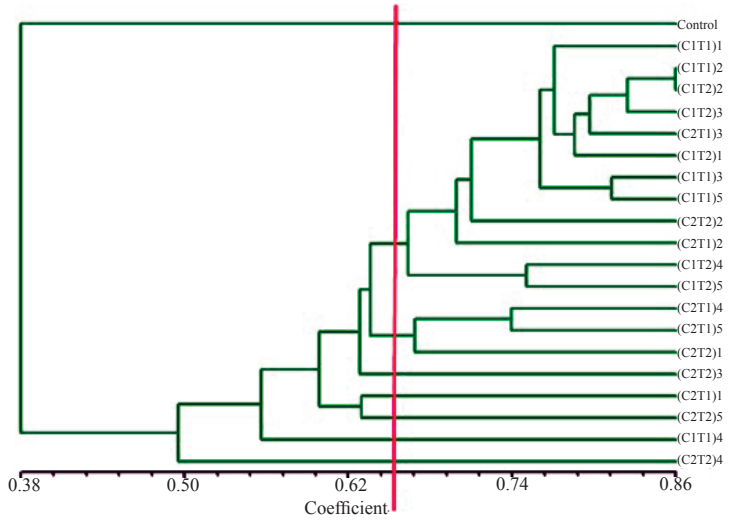

Figure 1. Dendrogram obtained from cluster analysis based on ISSR marker and using the Ward method.

and in each category were cultivars with similar traits (28). Talebi *et al*. In 2016 in the study of safflower genetic diversity through ISSR marking parameters showed that the samples were divided into three distinct groups (29). Li *et al*. In 2009 by studying the genetic diversity of *Vernicia fordii*, which is an oily plant, identified ISSR markers for three distinct groups (30).

4.5. ISSR Marker

In this study, 10 markers were used and the results of each marker are presented in **Table 5** separately. A total of 136 bands were produced by these markers, of which 113 were polymorphic bands (**Fig. 2**). The results showed that the average percentage of polymorphisms was 81.22%. The lowest polymorphic percentage was for marker 10 (46.66%) and the highest was for markers 2 and 5. Also, the highest content of polymorphic information was for marker 6 (1) and the lowest was for marker 9 (0.22). The highest resolution was 0.57 for marker 5 and the lowest was for marker 10 at 0.18. The highest marker index was related to marker 6 (14.06) and the lowest was related to marker 10 (0.84). The results of this Table showed that markers 2, 5, and 6 are the best informative markers in the recognition of camelina lines. Najaphy *et al*. (2011) also examined the ISSR marker parameters in the study of wheat genetic diversity and introduced the resolution and marker index as the best parameters for informative markers (31).

5. Discussion

Generally, the analysis of variance results of the treatments used to induce mutations showed that there were significant differences between the treatments in terms of the amount of fatty acids except for behenic acid and omega-9-neuronic acid. Comparison of means also showed a significant difference at the level of 5% for palmitic acid, stearic acid, omega-6-eicosadienoic acid, and omega-9-uric acid between the treatments. The results of cluster analysis showed that all treatments used with five replications were divided into eight different groups and it was found that all replications of treatment (C1T2) were in the second group and this regard had the lowest amount of palmitic acid among There were other treatments. Since palmitic acid is one of the saturated fatty acids and increases bad cholesterol, C1T2 treatment is recommended as the best treatment to reduce this harmful fatty acid. Simple correlation analysis between fatty acid profiles showed a significant relationship between most fatty acids. Principal factor analysis of Fatty acid profiles identified three factors as the main factors.

Examination of the information content of ISSR molecular markers also showed that markers 2, 5, and 6 are the best informative markers in the detection of camelina fatty acid profile. The prepared mutant lines and the marker information obtained from this research can be used for further research. For example, for drought resistance, there is little

Table 5. Investigation of ISSR marker parameters used in camelina mutant lines

Marker No.	Polymorphic ratio	Total number of amplified fragment	Number of polymorphic fragment	Content of polymorphic information	Polymorphism (%)	Marker index	Resolution
	6.23	13	9	0.61	63.29	3.81	0.29
	14.00	14	14	0.49	100	6.92	0.42
	12.07	14	13	0.56	92.85	6.83	0.41
	11.26	15	13	0.44	86.66	4.99	0.43
	16.00	16	16	0.71	100	11.49	0.57
	14.06	15	15	1.00	93.75	14.06	0.42
	9.09	11	10	0.65	90.90	9.1	0.56
	7.14	14	10	0.36	71.42	2.59	0.33
	4.00	9	6	0.22	66.67	0.91	0.25
	3.26	15	7	0.25	46.66	0.84	0.18
Total	97.67	136	113	5.29	812.2	61.54	3.86
Mean	9.767	13.6	11.3	0.529	81.22	6.154	0.386

information in camelina (4, 5); this is while the trait of drought resistance is very important (32). Also, it may be to study the feasibility of their cultivation; as it has been done in previous reports about camelina (7). Plant growth is of great agronomic and economic importance (32-34). The mutant lines obtained may also be used to study growth diversity. The presence of erucic acid may cause deposition in the heart and arteries and lead to cardiovascular disease (35). None of the treatments used were able to reduce erucic acid. Other molecular markers may be used to examine the more accurate diversity of mutant lines. Nanoparticles may also be used in the relationship (36). To further investigate the cause of fatty acid changes, additional analyses such as gene expression and proteomics are required. In recent years, new methods have been developed to study expression, gene sequencing, protein and mechanisms (37, 38). These methods can be used to evaluate the results more accurately.

If there are no changes in some traits after camelina mutation, it could be for several reasons. Mutations in the gene encoding that trait may not have been targeted (39). Another reason could be due to the genetics of camelina that is an allohexaploid plant and there are three copies of most genes (40). If the mutation manages to affect one version, there are still two untouched versions. This mutation may not affect the desired phenotype. Therefore, before modifying the camelina mutation, it is recommended that the purpose of the change be determined. If the trait is encoded by genes, each of which has three copies, we do not recommend the mutation method. For these, we recommend genomic editing methods such as CRISPR (41). However, there are reports (that the mutation has altered the expression of three-copy genes such as FAE1 in camelina. This gene is important in fatty acid biosynthesis (42). Due to the strategic importance of oilseed plants, it is necessary to conduct further studies of these plants (43-45).

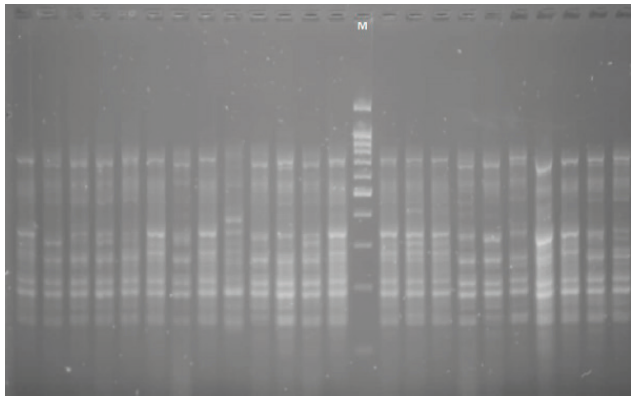

Figure 2. A sample of agarose electrophoresis gel of ISSR markers of camelina mutant lines. Lanes: M (size marker) and other lanes: ISSR marker of mutant lines.

6. Conclusion

According to these results, it can be said that there is variation in the profile of fatty acids and the plant breeder can use this variation in breeding programs depending on the intended purpose.

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References

1. Berti M, Wilckens R, Fischer S, Solis A, Johnson B. Seeding date influence on camelina seed yield, yield components, and oil content in Chile. *Ind Crops Prod*. 2011;**34**(2):1358-1365. doi:10.1016/j.indcrop.2010.12.008
2. Soorni J, Kazemitabar SK, Kahrizi D. *et al.* Genetic analysis of freezing tolerance in camelina [*Camelina sativa* (L.) Crantz] by diallel cross of winter and spring biotypes. *Planta*. 2021;**253**:9. doi: 10.1007/s00425-020-03521-z.
3. Rahimi T, Kahrizi D, Feyzi M, Ahmadvandi HR, Mostafaei M. Catalytic performance of MgO/Fe_2O_3-SiO_2 core-shell magnetic nanocatalyst for biodiesel production of Camelina sativa seed oil: Optimization by RSM-CCD method. *Ind Crops Prod*. 2021;**159**:113065. doi: 10.1016/j.indcrop.2020.113065.
4. Ghorbani, M., Kahrizi, D., Chaghakaboodi, Z. Evaluation of Camelina sativa Doubled Haploid Lines for the Response to Water-deficit Stress. *J Med Plants By-prod*. 2020;**9**(2):193-199. doi: 10.22092/jmpb.2020.351330.1240.
5. Borzoo S, Mohsenzadeh S, Moradshahi A, Kahrizi D, Zamani H, Zarei M. Characterization of physiological responses and fatty acid compositions of Camelina sativa genotypes under water deficit stress and symbiosis with Micrococcus yunnanensis. *Symbiosis*. 2020. doi:10.1007/s13199-020-00733-5.
6. Soltani MF, Zebarjadi AR, Abdoli-Nasab M, Jalali Javaran M, Kahrizi D. Isolation and Characterization of Delta 15 Desaturase (FAD3) Gene from Camelina sativa L. *J Appl Biotechnol Rep*. 2020;**7**(1):48-52. doi: 10.30491/jabr.2020.105920.
7. Kahrizi D., Rostami-Ahmadvandi, H., Akbarabadi, A. (2015). Feasibility Cultivation of Camelina (*Camelina sativa*) as Medicinal-Oil Plant in Rainfed Conditions in Kermanshah-Iran's First Report. *J Med Plants By-Prod*. 2015;**2**:215-218. doi: 10.22092/jmpb.2015.108911.
8. Ghamarnia H, Kahrizi D, Rostami Ahmadvandi H. Camelina, a Low Input and Adaptable Plant. 2020; Razi University Press. P118.

9. Raziei Z, Kahrizi D, Rostami-Ahmadvandi H. Effects of climate on fatty acid profile in Camelina sativa. *Cell Mol Biol*. 2018;**64**(5):91-96. doi: 10.14715/cmb/2018.64.5.15.
10. Fallah F, Kahrizi D, Rezaeizad A, Zebarzadi A, Zarei L. Evaluation of Genetic Variation and Parameters of Fatty Acid Profile in Doubled Haploid Lines of Camelina sativa L. *J Plant Genetic Res*. 2020;**6**(2):79-96. doiL 10.29252/pgr.6.2.79
11. Wei L, Zou Q. A review of DNA-binding proteins prediction methods. *Curr Bioinforma*. 2019;**14**(3):246-254. doi: 10.2174/1574893614666181212102030.
12. Xu L, Jiang S, Zou Q. An in silico approach to identification, categorization and prediction of nucleic acid binding proteins. *bioRxiv*. 2020. doi: 10.1093/bib/bbaa171.
13. Rostami-Ahmadvandi H, Kahrizi D, Cheghamirza K, Nosrati I, Zargooshi J. Molecular and agro- morphological genetic diversity assessment of Chickpea mutants induced via Ethyl Methane Sulfonate. *Cell Mol Biol.* 2016;62(11):8-12. doi: 10.14715/cmb/ 2016.**62**.11.2.
14. Altindal N. Molecular characterization of *Helianthus tuberosus* L. treated with ethyl methanesulfonate based on inter-simple sequence repeat markers. *Int J Environ Sci Technol*. 2019;**16**(9):5311-5318. doi: 10.1007/s13762-019-02486-1.
15. Kozgar M, Khan S, Wani M. Variability and correlations studies for total iron and manganese contents of chickpea (*Cicer arietinum* L.) high yielding mutants. *Am J Food Technol*. 2012;**7**(7):437-444. doi: 10.3923/ajft.2012.437.444.
16. Hadebe S, Modi AT, Hugo A, Shimelis HA. Seed oil content and fatty acid composition response to ethyl methanesulphonate mutagenesis in vernonia. *South Afr J Plant Soil*. 2019;**36**(5):375-380. doi: 10.1080/02571862.2019.1631399.
17. Martínez L, Cavagnaro P, Masuelli R, Rodríguez J. Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers. *Electron J Biotechnol*. 2003;**6**(3):244-253. doi: 10.2225/vol6-issue3-fulltext-11
18. Jander G, Baerson SR, Hudak JA, Gonzalez KA, Gruys KJ, Last RL. Ethylmethanesulfonate saturation mutagenesis in Arabidopsis to determine frequency of herbicide resistance. *Plant Physiol*. 2003;**131**(1):139-146. doi:10.1104/pp.102.010397.
19. López-Martínez JC, Campra-Madrid P, Rincón-Cervera MÁ, Guil-Guerrero JL. Ecological and simultaneous seed oil extraction/saponification/γ-linolenic acid concentration. *Eur J Lipid Sci Technol*. 2005;**107**(3):180-186. doi: 10.1002/ejlt.200401099.
20. Lepage G, Roy CC. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J Lipid Res*. 1984;**25**(12):1391-1396. doi: 10.1016/S0022-2275(20)34457-6.
21. Doyle JJ , Doyle JL. solation of plant DNA from fresh tissue. Focus. 1990;12:13-
22. Williams BL, Hornig M, Parekh T, Lipkin WI. Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *ISM*. 2012;**3**(1). doi: 10.1128/mBio.00261-11.
23. Gishini MFS, Zebarjadi A, Abdoli-Nasab M, Javaran MJ, Kahrizi D, Hildebrand D. Endoplasmic reticulum retention signaling and transmembrane channel proteins predicted for oilseed ω3 fatty acid desaturase 3 (FAD3) genes. *Funct Integr Genomics*. 2020;**20**(3):433-458. doi: 10.1007/s10142-019-00718-8
24. Napier JA, Graham IA. Tailoring plant lipid composition: designer oilseeds come of age. *Curr Opin Plant Biol*. 2010;**13**(3):329-336. doi: 10.1016/j.pbi.2010.01.008.
25. Mostafavi RM, Tahmasebi SZ, Mohammad MSS, Ghalavand A. Evaluation of yield, fatty acids combination and content of micro nutrients in seeds of high yielding rapeseed varieties as affected by different sulphur rates. *Electron J Crop Prod*. 2011;**4**(1):43-60.
26. Mokari_yamchi A, Sadeghian-Sharif S, Nattagh-Eshtivani E, Salehi-Sahlabadi A, Ghavami A, Barati M *et al* . Socioeconomic Inequality in Fruit and Vegetable Consumptions in Elderly People: A Cross Sectional Study in North West of Iran. *Nutr Food Sci Res*. 2019;**B**(4):17-22. doi: 10.29252/nfsr.6.4.17
27. Yaman H, Ulum KN, Hacioglu BT, Arslan Y, Subasi I. Molecular Characterization of Materials Selected from Some Camelina [*Camelina sativa* (L.) Crantz] Populations. *ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi*. 2017;**27**(2):123-126.
28. Khatamain O, Modares SS, Ghanati F, Mostavafi M. Evaluation of yield, its components and some morphological traits of sixteen rapeseed oil cultivars in Arak region. *J Agri Sci*. 2011;**21**(3):147-161.
29. Talebi R, Abhari SA. Evaluation of genetic diversity in safflower (Carthamus tinctorius L.) using agro-morphological, fatty acid composition and ISSR molecular markers. *Res J Biotech*. 2016;**11**(7):19-27.
30. Li P, Wang Y, Chen Y, Zhang S. Genetic diversity and association of ISSR markers with the eleostearic content in tung tree (*Vernicia fordii*). *Afr J Biotech*. 2009;**8**(19):4782-4788.
31. Najaphy A, Parchin RA, Farshadfar E. Evaluation of genetic diversity in wheat cultivars and breeding lines using inter simple sequence repeat markers. *Biotechnol Biotechnol Equip*. 2011;**25**(4):2634-2638. doi: 10.5504/bbeq.2011.0093.
32. Liu L, Li D, Ma Y, Shen H, Zhao S, Wang Y. Combined Application of Arbuscular Mycorrhizal Fungi and Exogenous Melatonin Alleviates Drought Stress and Improves Plant Growth in Tobacco Seedlings. *J Plant Growth Reg*. 2020;**13**:1-4. doi: 10.1007/s00344-020-10165-6.
33. Liu L, Li J, Yue F, Yan X, Wang F, Bloszies S, Wang Y. Effects of arbuscular mycorrhizal inoculation and biochar amendment on maize growth, cadmium uptake and soil cadmium speciation in Cd-contaminated soil. *Chemosphere*. 2018;**194**:495-503. doi: 10.1016/j.chemosphere.2017.12.025.
34. Wang L, Yang T, Wang B, Lin Q, Zhu S, Li C, Ma Y, Tang J, Xing J, Li X, Liao H. RALF1-FERONIA complex affects splicing dynamics to modulate stress responses and growth in plants. *Sci Adv*. 2020;**6**(21):eaaz1622. doi: 10.1126/sciadv.aaz1622.
35. Su Q, Liu Y, Lv XW, Dai RX, Yang XH, Kong BH. LncRNA TUG1 mediates ischemic myocardial injury by targeting miR-132-3p/HDAC3 axis. *Am J Physiol Heart Circ Physiol*. 2020;**318**(2):H332-H344. doi: 10.1152/ajpheart.00444.2019.
36. Guo H, Qian K, Cai A, Tang J, Liu J. Ordered gold nanoparticle arrays on the tip of silver wrinkled structures for single molecule detection. *Sens Actuators B Chem*. 2019;**300**:126846. doi: 10.1016/j.snb.2019.126846.

37. Zhang J, Liu B. A review on the recent developments of sequence-based protein feature extraction methods. *Curr Bioinforma*. 2019;**14**(3):190-199. doi: 10.2174/1574893614666181212102749.
38. Zhang W, Hu Y, Liu J, Wang H, Wei J, Sun P, Wu L, Zheng H. Progress of ethylene action mechanism and its application on plant type formation in crops. *Saudi J Biol Sci*. 2020;**27**(6):1667-1673. doi: 10.1016/j.sjbs.2019.12.038.
39. Laskar RM, Khan S. Mutagenic effectiveness and efficiency of gamma rays and HZ with phenotyping of induced mutations in lentil cultivars. *Int Lett Nat Sci*. 2017;**64**:17-31. doi: 10.18052/www.scipress.com/ILNS.64.17
40. Hutcheon C, Ditt RF, Beilstein M, Comai L, Schroeder J, Goldstein E, Shewmaker CK, Nguyen T, De Rocher J, Kiser J. Polyploid genome of Camelina sativa revealed by isolation of fatty acid synthesis genes. *BMC Plant Biol*. 2010;**10**(1):1-5. doi: 10.1186/1471-2229-10-233.
41. Jiang WZ, Henry IM, Lynagh PG, Comai L, Cahoon EB, Weeks DP. Significant enhancement of fatty acid composition in seeds of the allohexaploid, Camelina sativa, using CRISPR/Cas9 gene editing. *Plant Biotechnol J*. 2017;**15**(5):648-657. doi: 10.1111/pbi.12663
42. Ozseyhan ME, Kang J, Mu X, Lu C. Mutagenesis of the FAE1 genes significantly changes fatty acid composition in seeds of Camelina sativa. *Plant Physiol Biochem*. 2018;**123**:1-7. doi.10.1016/j.plaphy.2017.11.021.
43. Chaghakaboodi Z, Kakaei M, Zebarjadi A. Study of relationship between some agro-physiological traits with drought tolerance in rapeseed (Brassica napus L.) genotypes. *Cent Asian J Plant Sci Innov*. 2021;**1**(1):1-9. doi: 10.22034/CAJPSI.2021.01.01.
44. Farokhian S, Tohidi-Nejad E, Mohammadi-Nejad G. Studying the effect of bio-fertilizers on the yield components of Sesame (Sesamum indicum) genotypes under drought stress condition. *Cent Asian J Plant Sci Innov*. 2021;**1**(1):32-38. doi:10.22034/CAJPSI.2021.01.04.
45. Bakhshi B, Ahmadvandi HR, Fanaei HR. Camelina, an adaptable oilseed crop for the warm and dried regions of Iran. *Cent Asian J Plant Sci Innov*. 2021;**1**(1):39-45. doi: 10.22034/CAJPSI.2021.01.05.