Journal of Medicinal Plants and By-products (2023) 3: 225-231
https://doi.org/10.22092/jmpb.2022.355966.1398

Original Article

The Effect of Ethyl Methylsulfonate on Germination and Morphological Traits of *Camelina* as a Medicinal Plant

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Article History

Received: 17 September 2021
Accepted: 21 January 2022

Keywords
Brassicaceae family
Fatty acid
Mutation breeding
Oilseed plant

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ABSTRACT

Camelina sativa (L.) Crantz is an annual flowering plant that belongs to the *Brassicaceae* family. *C. sativa* as a dicotyledonous plant, one of its characteristics is morphological formability to respond to large environmental changes. This study aimed to evaluate the effect of ethyl methylsulfonate on germination and morphological traits of *C. saiva*. Ethyl methanesulfonate (EMS) is one of the most potent mutagenic and carcinogenic compounds that cause point-substitution-mutation in the genome. To induce mutations in this experiment, ethyl methanesulfonate with concentrations of 0.1% and 0.5% was used in two periods of 8 and 16 hours on 200 seeds of *Camelina*. The results of variance analysis for plant heights, number of leaves per sub-branch, number of seeds per pod, number of main branch leaves, stem diameter, and pod length did not show the significant difference, but the only significant difference was for the trait of pod width. Also, results showed that there was a significant difference between the tested treatments in terms of germination percentage, longest root length, mean root length, mean shoot length, seedling fresh weight, and seedling dry weight. According to the results of germination experiments, EMS treatment widely affected all six studied traits, therefore, the amounts of the largest root length and shoot length decreased with increasing EMS concentration, but this relationship was not observed in other traits. Although EMS is classified as toxic, for germination percentage, it was observed that with increasing concentration and time of EMS treatment, germination percentage also increased. According to the correlation results, all traits had a positive and significant relationship with each other, which is probably due to the nature of the traits and their additive effect.

INTRODUCTION

Camelina sativa (L.) Crantz is an annual flowering plant that belongs to the Brassicaceae family. Today, it has been widely studied by researchers and farmers in the united states and European countries due to satisfactory seed performance, adaptation to environmental conditions, and the presence of significant amounts of oil, which has a high value in terms of food and industrial uses [1, 2].

Camelina is native to southwestern Asia and southeastern Europe, and its cultivation history is back to 4,000 years ago in these areas. Research has shown that *C. sativa* with seed oil (36-47%) can produce up to twice as much oil as soybean (18-22%) [3]. The amount of saturated fatty acids is less than 10%, which makes this plant very valuable [4]. The number of its chromosomes is 2n = 40 and the size of the genome is 750 Mbp [5]. The presence of the *C. sativa* reference genome (n = 20) with an estimated size of 70 Mbp has been confirmed by its allohexaploid nature, which is relatively conserved in the three sub-genomes [6]. Lack of interspecific and intraspecific diversity among crops can cause many problems such as reducing resistance to pests and diseases and non-biological stresses [7].

Ethyl methanesulfonate (EMS) is one of the most potent mutagenic and carcinogenic compounds that cause point-substitution-mutation mutations in the genome [8]. The mutagenicity of this substance depends on factors such as plant genus and species, dose and time of use, and physiological conditions for these reasons, it is very important to be aware of the effects of EMS on different plants [9-11]. For example, research on the amount of iron and manganese in chickpeas has shown that at low concentrations of EMS there was a positive and significant relationship in mutagenesis plant between iron and manganese with chickpea yield [12]. Research has also shown that the use of ethyl methyl sulfonate significantly reduces the composition of vernolic acid and significantly increases the composition of linoleic, oleic, palmitic, stearic, and arachid fatty acids in mutants of the plant *Vernonia centrapalus* var Pauciflorus (Willd) [13]. Research has shown that the treatment of okra seeds with concentrations of 0.5 to 1% causes changes in germination traits. The relationship between these changes has been such that increasing the concentration of EMS has reduced all the germination traits in this plant [14]. This study aimed to induce mutation in Camelina by treating plant seeds and investigating the genetic changes of mutants by studying germination traits and morphological characteristics.

MATERIALS AND METHODS

Induction of Chemical Mutation in Seeds

induce mutations in this experiment, ethyl methanesulfonate with concentrations of 0.1 and 0.5% was used in two periods of 8 and 16 hours on 200 seeds (for each treatment) in separate Petri dishes (50 seed per petri dish).

Germination test

To investigate the effect of EMS on germination, the mentioned treatments were used in a completely randomized design with three replications in the laboratory of Razi University. After applying the treatments, germination traits (germination percentage, the longest root length (mm), average root length (mm), average shoot length (cm), seedling dry weight (g), and fresh weight (g) were measured after 6 days., The method of Jander *et al*. 2003 was used to continue the steps.

Seed cultivation and morphological traits test

Seeds were treated with 0.1% and 0.5% EMS and they were planted by RCBD design in three replications with a distance of 20 cm and a distance of 5 cm on each row. The number of germinated seeds, the number of mature plants, the number of plants that did not enter the reproductive stage, the number of plants that were not affected by the mutation, and other survival traits (such as growth period, seed production rate, seed germination capacity, etc) were recorded for each treatment. The harvested seeds of the first generation (M1) of each plant were planted on a separate row the following year and their characteristics were recorded. The same procedure was followed for the M2 and M3 generations. Various conventional morphological traits of *Camelia* (such as plant height, number of seeds per pod, number of leaves in sub-branch, number of leaves in the main branch, stem diameter (mm), pod length (mm), and pod width (mm) were recorded in M3 generation.

Data analysis

Excel software 2010 was used to categorize and sort the data. Normality test, correlation and cluster analyses were done by SPSS software ver. 16.0. Analysis of variance was carried out by SAS software ver. 9.1. The comparison of mean test was performed by SPSS software ver. 16.0, using least significant difference (LSD). Cluster analysis was done for grouping cultivars/lines by the UPGM method.

RESULTS

Morphological Analysis

The results of variance analysis for morphological traits showed that there was no significant difference between plant heights, number of leaves per sub-branch, number of seeds per pod, number of main branch leaves, stem diameter, and pod length, and the only significant difference was for pod width (Table 1).

The results of comparing the mean by LSD method and the values of minimum and maximum width are given in Table 2. According to the comparison results, the mean of the lowest value for pod width attribute was for those seeds that were treated with 0.5% EMS for 16h (26mm) and the highest for seeds with 0.5% EMS for 8h (46mm). The results show that there is a significant difference between the treated seeds with 0.1% EMS (42mm) and 0.5% EMS (46mm) for 16h with the control group (30mm). Also, a significant difference was observed between treated seeds with 0.5% for 8h (26mm), treated seeds with 0.1% EMS (42mm), and treated seeds with 0.1% EMS for 16h (26mm)

Pearson correlation analysis was used to determine the presence or absence of relationships between variables. The matrix of correlation coefficients of morphological traits is presented in (Table 3). Based on the results, plant height and number of seeds per pod had a positive and significant correlation. Also, plant height had a negative and significant correlation with pod length and pod width. Based on the results, some traits had a significant relationship with each other. There was also a significant positive correlation between plant height and the number of seeds per pod. Therefore, it can be concluded that plant height may also be associated with yield, and breeding this trait may increase the yield.

Table 1 Results of morphological traits variance analysis of Camelina sativa (L.) Crantz (mutant)

		Mean squares						
SOV	d.f.	PH	NSP	NLSB	NLMB	SD	PL	PW
Treat	4	195.32 ns	1.65 ns	0.93 ns	4.57 ns	0.12 ns	0.01 ns	0.03 *
Error	16	115.52	4.45	0.95	4.25	0.15	0.01	0.01
CV		15.11	25/60	12.25	12.33	17.88	23.44	24

ns and * show nonsignificance and significance at the level of 5%, respectively. Where PH: plant height; NSP: number of seeds per pods; NLSB: number of leaves in the sub-branch; NLMB: number of leaves in the main branch; SD: stem diameter; PL: pod length; PW: pod width

Table 2 Results of comparing the mean of the width of the pod

Treat name	Treat	Trait
		pod width (mm)
C	Control	30 ± 0.1 c
T1	0.1% EMS for 8 h	34 ±5 abc
T2	0.1% EMS for 16 h	42 ± 3 ab
T3	0.5% EMS for 8 h	26 ± 4 c
T4	.5% EMS for 16 h	46 ± 2.4 a

The existence of a common letter indicates non-significant differences

Table 3 Correlation coefficients of morphological traits of Camelina sativa (L.) Crantz (mutant)

Traits	PH	NSP	NLSB	NLMB	SD	PL	PW
PH	1	-	-	-	-	-	-
NSP	0.45 *	1	-	-	-	-	-
NLSB	0.27 ns	0.08 ns	1	-	-	-	-
NLMB	0.1 ns	0.005 ns	-0.03 ns	1	-	-	-
SD	0.4 ns	0.15 ns	-0.3 ns	-0.21 ns	1	-	-
PL	-0.55 **	-0.34 ns	0.3 ns	-0.3 ns	0.21 ns	1	-
PW	-0.55 **	0.19 ns	-0.1 ns	-0.21 ns	0.28 ns	0.15 ns	1

ns and * show insignificance and significance at the level of 5%, respectively; Where PH: plant height; NSP: number of seeds per pods; NLSB: number of leaves in the sub-branch; NLMB: number of leaves in the main branch; SD: stem diameter; PL: pod length; PW: pod width

Table 4 Analysis variance of germination parameters of mutant camelina sativa (L.) Crantz seeds

		Mean squares					
S.O.V	df	Germination percentage	Longest root length	Mean root length	Mean shoot length	Seedling fresh weight	Seedling dry weight
Treat	4	705.83 **	597.6 **	299.73 **	390.10 **	0.0003 **	0.000002 **
Error	10	41/66	3.73	4	15.33	0.00002	0.0000002
CV	-	8.96	11.50	18.29	24.27	12.78	14.21

ns and ** They show non-significance and significance at the level of 1%, respectively

Germination Analysis

The results of the analysis of variance (ANOVA) showed that there was a significant difference between the tested treatments in terms of germination percentage, longest root length, mean root length, mean shoot length, seedling fresh weight, and seedling dry weight, which are presented in Table 4.

The mean comparison results for germination traits and the minimum and maximum values of the measured traits are given in Table 5 . Based on these results, the mean of the control group (86.66%), treated seeds with 0.5% EMS for 8h (78.33%), and treated seeds with 0.5% EMS for 16h (78.33%) showed the highest germination percentage. The lowest germination percentage belonged to treated seeds with 0.1% EMS for 16h (46.66%). Also, a significant difference was observed between treated seeds with 0.1% EMS for 16h (46.66%), treated seeds with 0.1% EMS for 8h (70%), and control (86.66%) treatments for germination percentage ($P < 0.05$).

The results showed that the highest root length was for the control group (41.33 mm), and the lowest values of the root length were for treated seeds with 0.1% EMS for 8h (14.33 mm), treated seeds with 0.5% EMS for 16h (12.33 mm), and treated seeds with 0.1% EMS for 16h (5.33 mm). Also, for this trait, there was a significant difference between treated seeds with 0.1% EMS for 8h (14.33 mm), treated seeds with 0.5% EMS for 16h (12.33 mm), treated seeds with 0.5% EMS for 8h (10.67 mm), and treated seeds with 0.1% EMS for 16h (5.33 mm) ($P < 0.05$).

About the trait of shoot length, The lowest mean was for treated seed with 0.1% EMS for 16 (3.33 mm), and the highest mean belonged to the control group (31.66 mm). Also, all measured treatments had significant differences from the control group (31.66 mm) ($P < 0.05$).

For the seedling fresh weight trait, a significant difference was observed between the control group (0.05g) and other treatment groups (P<0.05).

The highest and the lowest values of seedling dry weight were for the control treatment (0.004 g) and treated seeds with 0.1% EMS for 16h (0.002 g), respectively. Also for this trait, there was a significant difference between control groups and other treatment groups (P<0.05).

Pearson correlation analysis was used to determine the presence or absence of relationships between variables. The results of the correlation coefficient matrix for germination traits are presented in Table 6 Germination percentage had a positive and significant correlation with the traits of longest root length, mean root length, mean shoot length, seedling fresh weight, and seedling dry weight.

The length of the tallest root had a positive and significant correlation with the traits of mean root length, mean shoot length, seedling fresh weight, and seedling dry weight.

There was a positive and significant correlation between mean root length and seedling fresh weight and seedling dry weight. Seedling fresh weight trait had a positive and significant correlation with seedling dry weight trait.

DISCUSSION

According to the results of this study, the existence of insignificant differences between mutagenicity causes a low impact on morphological traits. Hasanzade *et al*. [15] reported that seed yield traits and the number of pods per lateral branch had a significant positive correlation with yield in Karaj region. Also, in Kashmar region, results showed that seed yield per square meter had a positive and significant correlation with the number of pods in the lateral branch, pod weight, and grain yield per plant.

Table 5 Mean comparison results for germination traits of mutant Camelina seeds

		Traits					
Treat name	Treat	Germination percentage	Longest root length (mm)	Mean root length (mm)	Mean shoot length (mm)	Seedling fresh weight (g)	Seedling dry weight (g)
C	Control	86/66 ± 1.66 a	41.33 ± 1.79 a	28.66 ± 2.18 a	31.66 ± 1.76 a	0.05 ± 0.003 a	0.004 ± 0.0002 a
T1	C:1 T: 8h	70 ± 2.88 b	14.33 ± 0.33 b	7.66 ± 0.66 b	7.66 ± 0.88 d	0.03 ± 0.001 a	0.003 ± 0.001 bc
T2	C:1 T: 16h	46.66 ± 4.40 c	5.33 ± 0.33 d	4.33 ± 0.88 b	3.33 ± 0.33 d	0.02 ± 0.001 b	0.002 ± 0.00001 c
T3	C:5 T: 8h	78.33 ± 1.66 ab	10.67 ± 1.20 c	7 ± 0.57 b	22.66 ± 3.84 b	0.03 ± 0.004 a	0.003 ± 0.0004bc
T4	C:5 T: 16h	78.33 ± 6.01 ab	12.33 ± 1.20 bc	7 ± 0.57 b	15.33 ± 2.60 c	0.03 ± 0.001 a	0.003 ± 0.001 bc

The existence of a common letter indicates no significant differences; P = 0.05

Table 6 Correlation coefficients between germination traits for mutant Camelia seeds

Trait	Longest root length (mm)	Mean root length (mm)	Mean shoot length (mm)	Seedling fresh weight (g)	Seedling dry weight (g)	Longest root length (mm)
germination percentage	1	-	-	-	-	-
longest root length (mm)	0.64 *	1	-	-	-	-
mean root length (mm)	0.55 *	0.95 **	1	-	-	-
mean shoot length (mm)	0.77 **	0.77 **	0.75 **	1	-	-
seedling fresh weight (gr)	0.63 *	0.88 **	0.93 **	0.88 **	1	-
seedling dry weight (gr)	0.65 **	0.78 **	0.79 **	0.82 **	0.92 **	1

* and ** They show significance at the level of 5% and 1%, respectively

Based on the results of the research by Meamari *et al*. [16], the simple correlation coefficient was negative and significant between seed yield and the height of the first lateral branch, the height of the first pod, pod length, and plant height. According to the research on sunflower plants [17], except for treated seeds with 0.6% EMS which 100% seeds were germinated, the percentage of germination and survival rate decreased with increasing EMS dose for all treatments compared to the control group. Also, with increasing EMS dose, a gradual decrease was observed for most of the studied traits such as leaf area, the number of inter-nodes, internode length, fresh and dry leaf weight, dry branch weight, and fresh and dry root weight compared to the control. However, a lower stimulatory effect of EMS was observed for shoot length in treated seeds with 0.2% EMS, root length in treated seeds with 0.4% EMS, and fresh shoot weight in treated seeds with 0.2% EMS in comparison with control. In addition, the results of the study by Wang *et al*. [18] on germination traits and mutation rate of rapeseed in different concentrations and times of EMS treatment have shown that different doses of EMS and different periods were significant for all treatments such as survival rate, germination, and physiological traits of seeds. Also, it found that increasing the dose and the period for each treatment leads to a significant reduction in the viability of the tested seeds. Based on the results of the research by Ram Kumar and Dhanavel, 2019, on the lethality of different concentrations of EMS on the seeds of *Panicum sumatrense*, it was found that growth is reduced to 50% under lethal dose of EMS. They also stated that the germination rate decreases with increasing EMS concentration.

A study on simple correlation coefficients of salinity-resistant cultivars seeds in rice showed that among the traits, the most positive and significant correlation was related to germination percentage (r = 0.89) [19]. Also, studies on rapeseed seeds under salinity stress showed that the correlation analysis between the evaluated traits on the germination test and callus growth indicates that there is no correlation between callus growth parameters and germination [20,21].

Based on the results of the current study, a positive and significant correlation was observed between all germination-related traits. Probably due to the nature of the treatments and the close relationship between the traits, significant correlations have been observed. For example, increasing the length of the longest root also increased the average length of the root. It is also expected that due to the toxicity of the experimental treatments for germination, only seeds with good germination power will germinate, which will increase the average of the traits. Also, according to the correlation results, all traits have a positive and significant relationship with each other, which is probably due to the nature of the traits and their additive effects. However, Camelina oilseed is a new plant for Iranian agriculture and more research is needed on it [22-24].

CONCLUSION

Examination of eight morphological traits showed that only the width of the pod was affected by EMS treatment. These results indicated that EMS treatment did not significantly affect morphological traits and could not cause significant changes in the phenotype of mutant seeds. Still, it did affect the appearance of pods and different phenotypes compared to the control group and different concentrations. It can be stated that this treatment probably has little effect on phenological pathways. Also, based on the results of the correlation between traits, it can be said that in the *Camelina* plant, plant height has a positive correlation with seed yield.

According to the results of germination experiments, EMS treatment widely affected all six studied traits, so that with increasing EMS concentration, the amount of largest root length and shoot length decreased, but this relationship was not observed in other traits. Although EMS is classified as a toxic substance, germination percentage trait increased with increasing concentration and time of EMS treatment. Also, according to the correlation results, all traits have a positive and significant relationship with each other, which is probably due to the nature of the traits and their additive effects.

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