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INFLUENCE OF MATURE AND IMMATURE SUNFLOWER SEED TREATMENT WITH ETHYLMETHANESULPHONATE ON MUTATION SPECTRUM AND FREQUENCY

V. Lyakh[1*], A. Soroka[2], V. Vasin[3]

[1]*Zaporozhye State University, Zhukovskogo Str. 66, Zaporozhye, 69063, Ukraine*
[2]*Institute of Oilseed Crops, UAAS, Veseniaya Str. 1, Solnechny, Zaporozhye, 70417, Ukraine*
[3]*Melitopol State Pedagogical University, Lenin Str. 20, Melitopol, 72312, Ukraine*

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SUMMARY

The frequency and spectrum of morphological mutations, raised in M_2 after sunflower mature and immature seed treatment with ethylmethanesulphonate (EMS), were studied. Mature seeds of 3 self-pollinated lines (ZL9, ZL102, ZL169) and immature seeds of 2 lines (ZL102 and ZL169) were treated with EMS at the concentrations of 0.01%, 0.1% and 0.5% for 6 and 12 h and 0.01% and 0.1% for 6 and 12 h, respectively. We isolated and described 18 types of mutation, which were classified into the following groups: chlorophyll deficiency (6), leaf (5), stem and (4) ray floret shape and color (3). Genotypic differences were revealed for the spectrum of mutations occurring after mutagenic treatment of mature and immature seeds. Differences in mutation spectrum existed in the studied lines after treatment of mature seeds. Mutation frequency after immature seed treatment did not exceed the amount of mutations after mature seed treatment where the maximum frequency amounted to 13.2%. Chlorophyll deficiency mutations averaged a half and more of the visible morphological mutations.

Key words: sunflower, ethylmethanesulphonate, mature and immature seeds, morphological mutation, mutation spectrum and frequency

INTRODUCTION

Sunflower is the main oilseed crop in Ukraine. However, a narrow genetic diversity in cultivated sunflower limits essentially the possibilities to develop more productive and tolerant varieties and hybrids.

* Corresponding author, Phone: 380 61 289 12 04,
e-mail: Viktor5@hotpop.com

Mutagenesis is a technique which allows to widen a heritable variability by inducing new traits. Some of them can be of interest as agronomically important characters; others can be used as marker traits.

Mature seeds are typically treated with mutagens. New data have appeared recently on the effectiveness of mutagenic treatment of plant during vegetative period, of tissue culture *in vitro* or embryos of different age (Checheneva and Larchenko, 1997; Morgun *et al.*, 1993). Here we discuss on the effect of ethylmethanesulphonate treatment of mature and immature seeds of some self-pollinated sunflower lines on the frequency and spectrum of morphological mutations in the M_2 generation.

MATERIAL AND METHODS

Self-pollinated sunflower lines Zl 9, Zl 102 and Zl 169 used in this study have been developed at the Institute of Oilseed Crops and used as parent components of some commercial hybrids. Mature seeds and seeds at the age of 20 days were treated with ethylmethanesulphonate (EMS). Dry mature seeds of the lines Zl 9, Zl 102 and Zl 169 were soaked in aqueous solutions of the mutagen at of 0.01%, 0.1% and 0.5% concentrations for 6 and 12 h. After rinsing for 30 min., the seeds were directly sown into the soil. Twenty-day-old seeds of Zl 102 and Zl 169 lines were treated with EMS at 0.01% and 0.1% for 6 and 12 h. After rinsing, embryos were extracted and placed on a modified MS medium with a reduced quantity of phytohormones. After 5-7 days, seedlings with fully developed root system were planted in the plastic vessels. All the plants of the M_1 generation were selfed at the time of flowering. M_2 seeds of each M_1 plant were sown separately as individual plant-to-row progenies. Each M_2 family was the progeny of a single M_1 plant. In the M_2 generation the majority of visually selected mutants was selfed and advanced from M_2 to M_3. Each mutant line was raised along with its parents. Mutation frequency was calculated as percent of families with mutant plants in M_2.

RESULTS AND DISCUSSION

Mutation spectrum

Eighteen types of morphological mutations were found in the M_2 generation. These mutations were divided into the following groups: chlorophyll deficiency - 6, leaf - 5, stem - 4 and ray floret shape and color - 3. Their brief descriptions are given in Table 1.

Chlorophyll deficiency mutations

Six types of chlorophyll mutation were isolated in the M_2 generation, albina, chimera, virescent, viridis (Figure 1), xantha and "whitish". The first four types are

Table 1: Types of morphological mutations and their brief description

No.	Type of mutation	Characteristic
		I Chlorophyll deficiency mutations
1	Albina	Completely white or light yellow seedling, survived for 7-10 days
2	Chimera	White or yellow sectors on the leaves
3	Virescent	Completely yellow seedling which gradually turns green
4	Viridis	Light green seedling and plant
5	Xantha	Yellow green 4-6 upper leaves during capitulum formation, light, almost white, bottom of the capitulum
6	"Whitish"	White spots on the leaves remaining during the whole vegetation period
		II Leaf mutations
7	Dichotomous venation	Fan-shaped venation, smaller leaves
8	Sickle-shaped leaf	Arched central vein, one half of leaf plate less then another
9	Egg-shaped leaf	Egg-shaped leaf plate, small petiole – stem angle
10	Fringed leaf	Wavy leaf edge
11	Bubbled leaf	Large number of pimples on the leaves, small size of leaves
		III Stem mutations
12	Fasciation	Shortened internodes, flattened stem, large number of small leaves
13	Distorted growing point	Disturbed development growing point
14	Dwarfness	Shortened internodes, heavily hairy stem
15	Low-growing	Plant reduced in height
		IV Ray floret shape and color mutations
16	Small ray florets	Reduced length and width of ray florets
17	Lemony ray florets	Lemony colour of ray florets
18	Light yellow ray florets	Light yellow colour of ray florets

Figure 1: A light green mutant of viridis type (left) and a normal green plant (right)

Figure 2: A mutant with white spots that remain on the leaves during the entire vegetation period

often found in mutation studies of sunflower and other crops. Besides these mutation types, "whitish" plants were found (Figure 2). Such mutants have white spots which remain on the leaves for the whole vegetation period. This mutation essentially reduces the height of plant and decreases plant productivity. The genetic analysis revealed that it is governed by a single recessive gene (unpublished data). Chlorophyll mutation of xantha type was also uncommon – only upper leaves surrounding the capitulum were yellow-green (Figure 3).

Figure 3: A mutant showing yellow-green leaves surrounding the capitulum

Figure 4a: A mutant with bumps on the leaves

Figure 4b: A corrugated leaf mutant (view from above)

Leaf mutations

In the present study, 5 distinct types of morphological mutations involving shape of leaf lamina and venation were isolated. Sickle-shaped, egg-shaped, fringed and corrugated leaf mutations (Figure 4a, b) and the mutation of leaf dichotomous venation (Figure 5a, b) were found. The sickle-shaped leaf mutant, because of arched central vein, has one half of the leaf lamina shorter then another.

Stem mutations

Four mutations fall into the group of stem mutations - fasciation, dwarfness, low-growing and distorted growing point. The fasciated mutant, with a flattened stem, shortened internodes and a large number of small leaves, was a typical example of fasciation mutation. Low-growing mutants included plants whose reduced height was due to a smaller number of internodes. Dwarf plants (Figure 6a, b) had very short internodes. The stem of these mutants was covered with a large number of whitish hairs. The distorted growing point mutation was isolated from all three lines under study. The growing point in these mutants did not development normally and they did not form seeds.

Table 2: Mutation spectrum after EMS treatment of mature and unmature seeds of various sunflower lines

No.	Type of mutation	Line				
		ZI 9 (MS)	ZI 102 (MS)	ZI 169 (MS)	ZI 102 (US)	ZI 169 (US)
	I Chlorophyll deficiency mutations					
1	Albina	+	+	+		
2	Chimera	+	+	+		
3	Virescent	+	+			
4	Viridis		+			
5	Xantha			+		+
6	“Whitish”			+		+
	II Leaf mutations					
7	Dichotomous venation	+				
8	Sickle-shaped leaf			+		+
9	Egg-shaped leaf	+				
10	Fringed leaf					+
11	Bubbled leaf			+		
	III Stem mutations					
12	Fasciation	+	+			
13	Distorted growing point	+	+	+		
14	Dwarfness					+
15	Low-growing	+	+	+		
	IV Ray floret shape and color mutations					
16	Small ray florets	+				
17	Lemony ray florets		+			
18	Light yellow ray florets		+			
Total		10	10	9	0	5

MS – mature seeds; US – unmature seeds.

Figure 5a: Dichotomous venation on the backside of leaf lamina (right) and normal venation (left)

Figure 5b: A mutant with dichotomous venation of the leaf

Figure 6b: A dwarf mutant (view from the above)

Figure 6a: A dwarf plant with short internodes, very hairy stem and serrate leaf margin

Table 3: Mutation frequency after EMS treatment of mature seeds of different sunflower lines

Type of mutation	Line, treatment																	
	Zl102						Zl9						Zl169					
	6 h			12 h			6 h			12 h			6 h			12 h		
	0.01%	0.1%	0.5%	0.01%	0.1%	0.5	0.01%	0.1%	0.5%	0.01%	0.1%	0.5%	0.01%	0.1%	0.5%	0.01%	0.1%	0.5%
1	0	0	1.12	0	0	2.20	0	0	1.18	0	0	2.35	0	1.22	1.89	1.10	0	3.23
2	0	0	0	0	0	1.10	0	0	0	0	0	1.18	0	0	0	0	0	0
3	1.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	1.22	0	0	0	0
5	0	0	1.12	0	1.18	0	1.14	0	0	1.05	0	3.53	3.66	4.94	1.89	4.40	2.0	2.15
6	0	0	0	0	0	0	0	0	0	0	0	0	*2.44*	*2.47*	*5.66*	*5.50*	*2.0*	*1.08*
7	0	0	0	0	0	0	0	0	*1.18*	0	0	0	0	0	0	0	0	0
8	0	0	1.12	0	0	1.10	0	0	0	0	0	0	0	0	0	0	0	0
9	1.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	1.07	0	1.12	2.41	0	0	0	1.02	0	1.05	0	1.18	0	0	0	0	1.0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	1.18	0	1.14	0	1.18	1.05	1.02	0	0	0	0	0	0	0
13	0	0	0	0	2.35	1.10	0	0	1.18	0	0	0	1.22	1.23	0	1.0	0	0
14	0	0	0	0	0	0	0	0	*1.18*	1.05	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	*2.44*	*2.47*	*5.66*	*5.50*	*2.0*	*1.08*
16	0	0	0	0	0	0	1.14	1.02	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.10	0	0
Total, types	3	0	4	1	3	4	3	2	5	4	1	4	4	6	4	6	4	4
Total, frequency	3.2 ±1.82	0	4.4 ±2.18	2.4 ±2.04	4.7 ±2.29	5.5 ±2.60	3.4 ±2.22	2.0 ±1.43	4.7 ±2.30	4.2 ±2.06	1.0 ±1.02	7.1 ±2.78	7.3 ±2.88	11.1 ±3.49	9.4 ±2.84	13.2 ±3.42	5.0 ±2.18	6.4 ±2.55

Ray floret shape and color mutations

One ray floret shape mutation and two ray floret color mutations were isolated in the present study. The first mutation was characterized by narrow and short ray florets (Figure 7). All the analyzed lines in the control possessed ray florets of yellow color. After EMS treatment, ray florets of lemon-yellow (Figure 8) and light-yellow (Figure 9) color were found.

Figure 7: Ray florets reduced in length and width (right) and normal ray florets (left)

Mutation spectra of the lines ZL 9, ZL 102 and ZL 169 differed after mutagenic treatment of both mature and immature seeds (Table 2). The largest number of mutation types was isolated in ZL 9 and ZL 102 lines after mature seeds treatment. In spite of certain similarities, the mutation spectra for each genotype and each type of seeds were unique, especially for ZL169 after treatment of immature seeds.

Mutation frequency

On the whole, chlorophyll mutations accounted for more than a half of the isolated heritable changes (Tables 3 and 4). In the group of chlorophyll deficient mutations, "whitish" mutation was isolated with highest frequencies after mutagenic treatment of mature and immature seeds of the line Zl 169. Lethal mutations *albina* and *virescent*, which negatively influenced growth and development of plants, were found, as a rule, when maximum concentration of mutagene and maximum exposition were used for mature seeds treatment of different sunflower lines. More viable chlorophyll mutations, as viridis and xantha, were isolated after treatment with EMS 0.01-0.1% and often after 6 h exposition.

Among the morphological mutations of the leaf, the sickle-shaped leaf lamina mutation was present with the highest frequency. It was found in treatments with different concentrations of EMS and different expositions after treatment of mature and immature seeds of Zl 169 line. The other mutations, involving shape of leaf plate and leaf venation, had lower frequencies. They were mainly isolated after mutagenic treatment of mature seeds of Zl 9.

All stem mutations, except "dwarf" mutants, were isolated after mature seed treatment. These mutations were more frequent in the lines Zl 9 and Zl 102 than in Zl 169.

Mutations of ray floret shape and color were rarely noted. Color mutations were characteristic for the line Zl 102, shape mutations for Zl 9. Light-yellow color of ray florets was noted for high EMS concentrations, lemon-yellow color for low concentrations.

Figure 8: A mutant showing lemon-yellow color of ray florets (right) and a normal plant (left)

Figure 9: A mutant showing light yellow color of ray florets (left) and a normal plant (right)

Many of the isolated mutants had more than one morphological marker. Some mutants had mutations belonging to different groups. For example, a mutant of the line Zl 9 had fan-shaped venation of leaves (leaf mutations) and small ray florets (ray floret shape and color mutations). Such cases are italicized in Tables 3 and 4. When making calculation of the total mutation frequency for each line, frequency of only one mutation was taken into account.

Inheritance studies of mutations types similar to those isolated in the present study were carried out by Luczkiewicz (1975), Kovačik and Škaloud (1990), Vranceanu *et al.* (1993), Jambhulkar and Joshua (1999). However, some of the isolated mutations have not been reported before for sunflower and inheritance study of these mutations is in progress now.

A majority of the isolated mutants had reduced plant height and decreased plant productivity. Only some of the mutations, like viridis, xantha and the mutations of ray floret color, did not influence negatively the yield characteristics of plants and, perhaps, their combining ability. Taking into account that the self-pollinated lines Zl 9, Zl 102 and Zl 169 are parent components of many commercial hybrids, the above-mentioned mutants possessing conspicuous marker traits can be recommended for improved seed production.

Table 4: Mutation frequency after EMS treatment of immature seeds of sunflower lines

Type of mutation	Line, treatment							
	Zl 102				Zl 169			
	6 h		12 h		6 h		12 h	
	0.01%	0.1%	0.01%	0.1%	0.01%	0.1%	0.01%	0.1%
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	2.2	2.0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	6.5	*4.0*
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	*2.0*
12	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	*4.0*
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	*2.0*
18	0	0	0	0	0	0	0	0
Total, types	0	0	0	0	0	0	2	5
Total, frequency	0	0	0	0	0	0	8.7 ±4.16	8.0 ±3.84

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ENFLUENCIA DEL TRATAMIENTO DE LAS SEMILLAS MADURAS Y NO MADURAS DE GIRASOL CON ETILOMETANOSULFONATO SOBRE LA FRECUENCIA Y EL SPECTRO DE MUTACIONES

RESUMEN

La frecuencia y el spectro de mutaciones morfologicas separadas en M_2 despues del tratamiento de las semillas maduras y no maduras de girasol con etilometanosulfonato (EMS) fueron estidiados. Semillas maduras de tres lineas Zl9, Zl102, Zl169 y semillas no maduras de dos lineas Zl102, Zl169 fueran tratadas con EMS en las concentraciones de 0.01%, 0.1%, 0.5% durante 6 y 12 horas y 0.01%, 0.1% durante 6 y 12 horas, respectivamente. Fueron obtenidos y descritos 18 tipos de mutaciones que fueron combinados en los grupos siguientes: insuficiencia clorofilica (6), mutaciones de hoja (5), mutaciones de tallo (4), mutaciones de forma y color de flores de rayo (3). Diferencias genotipicas fueran reveladas para el spectro de mutaciones mostrado despues del tratamiento mutagenico de las semillas maduras y no maduras asi como las diferencias para el spectro mutacionico de las lineas estudiadas despues del tratamiento de las semillas maduras. La frecuencia mutacionica despues del tratamiento de las semillas no maduras no superada la cantidad de mutaciones despues del tratamiento de las semillas maduras donde la frecuencia maximala fue 13.2%. Las mutaciones de insuficiencia clorofilica han formado la mintad y mas de mutaciones morfologicas visibles.

EFFET DU TRAITEMENT DE LA SEMENCE MATURE ET IMMATURE À L'ÉTHYLE MÉTHANE SULPHONATE SUR LE SPECTRE ET LA FRÉQUENCE DE MUTATION

RÉSUMÉ

La fréquence et le spectre des mutations morphologiques dans la génération M_2 après le traitement de la semence mature et immature à l'éthyle méthane sulphonate (EMS) ont été analysés. Les semences matures de trois lignées autofécondées (ZL9, ZL 102, ZL169) ont été traitées au EMS en concentrations de 0,01%, 0,1%, 0,5% aux 6 et 12 heures et les semences immatures des deux lignées (ZL102 et ZL169) en concentrations de 0,01%, 0,1% aussi aux 6 et 12 heures. Nous avons isolé et décrit 18 types de mutations qui

ont été placées dans les groupes suivants : manque de chlorophylle (6), feuille (5), tige (4) et forme et couleur des fleurons ligulés (3). Après le traitement mutagène des semences matures et immatures, des différences génotypiques sont apparues dans le spectre de la mutation. Dans le cas des semences immatures, la fréquence de mutation n'a pas dépassé le nombre de mutations de la semence mature où la fréquence maximale était de 13,1%. Les mutations de type déficience en chlorophylle comptaient pour la moitié et plus des mutations morphologiques visibles.