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MUTATION IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) B LINES CREATED THROUGH ULTRA SONIC

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Abstract

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Immature sunflower (*Helianthus annuus* L.) zygotic embryos of sunflower Bulgarian line maintainer of sterility 2607 B were treated with ultra sonic at dose of 25.5 W/cm^2 for 1, 3 and 5 min and at dose of 51.0 W/cm^2 for 7 min, 9 min and 11 min before plating in the embryo culture medium M. As a result some Chlorophyll mutations, Leaf mutations, Stem mutations, Inflorescence mutations, Seed mutations and Physiological mutations were observed.

Key words: *Helianthus annuus*, embryo cultural method, ultra sonic, mutant line

Резюме

Юлия Енчева, 2014. Мутация в слънчогледови (Helianthus annuus L.) В линии, създадена чрез ултра звук. FCS 9(2):243-248

Незрели слънчогледови (*Helianthus annuus* L.) зиготни зародиши на Българската линия закрепител на стерилността 2607 В са третирани с ултра звук в доза 25.5 W/cm^2 за 1, 3 и 5 min и в доза 51.0 W/cm^2 за 7 min, 9 min и 11 min преди платиране върху хранителна среда М. Като резултат са наблюдавани някои Хлорофилни мутации, Лестни мутации, Стъблени мутации, мутации по съцветието, мутации по семената и Физиологични мутации.

Ключови думи: *Helianthus annuus,* метод ембрио култура, ултра звук, мутантна линия

INTRODUCTION

In recent years, the creation of highly productive varieties and hybrids of sunflower is severely limited by the narrow genetic bases. This is the main reason of genetic uniformity of raised varieties and hybrids.

According Ahloowalia, 1994 spontaneous mutations occur with a low frequency in all species. Most mutations are recessive and deleterious. Genetic mutations and recombinations

were improve plasticity and enhancement of the genetic potential of sunflower in particular.

Mutagenesis, both physical and chemical, proved favorable for mutation induction in tissue cultures. It is a technique which allows widening 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. Mutation breeding has been used successfully to improve yield, oil content, disease resistance, change plant architecture, shorten the growing

season etc. Most often subjected to treatment with mutagens are sunflower mature seeds (Jambhulkar *et al*., 1999; Sagadeesan *et al*., 2008). Christov and Nikolova, 1996 obtained mutation as leaf, stem, inflorescence, seed and physiological mutations after treatment of mature seeds of variety Peredovik and inbred line 24 Mit, 130 Mit, 2969 R, 3004 R and Ha-89 with ultra sonic. Lyakh *et al.,* 2005 studied the frequency and spectrum of morphological mutations, raised in M2 after sunflower mature and immature seed treatment with ethylmethanesulphonate (EMS). They observed 18 type of mutation, which were classified as chlorophyll deficiency, leaf, stem and ray floret shape and color mutations. Thirty-three types of chemical mutation (EMS) at sunflower immature embryos were found by Soroka and Lyakh, 2009. They were described and classified into the following groups: Chlorophyll deficiency mutations, cotyledon, leaf, stem, inflorescence, seed and physiological mutations.

Initial experiments on sunflower induced mutations *in vitro* and their use in breeding program of Dobroudja agricultural institute were started at 2001. The main objective was to develop inbred lines with high yield, high oil content, and resistance to diseases, early flowering and good combining ability.

The aim of this study was: to increase genetic variability of sunflower by treatment of immature embryos with ultra sonic- 2.5 W/cm^2 for 1, 3 and 5 min and 51.0 W/cm^2 for 7, 9 и 11 min before plating to nutrition medium M.

MATERIAL AND METHODS

The experiments were carried out under laboratory conditions of Dobroudja Agricultural Institute-General Toshevo.

Developing of mutant lines

The Bulgarian line maintainer of sterility 2607 B, witch is highly homozygotic, was used as donor material. The control plants were grown under field conditions.

A main requirement to the initial plant material used according to the methods of embryo culture in combination with ultra sonic is to be genetically pure, i.e. homozygotic to the highest possible degree. Therefore the control line 2607 B (more 35 year selfing) with very good morphological uniformity was chosen as initial material for induced mutagenesis.

Plants were grown in the field and were hand-pollinated. The immature seeds (12-18 days old) were treated with ultra sonic at dose 25.5 W/cm^2 for 1, 3 and 5 min and 51.0 W/cm^2 for 7, 9 and 11 min. Immature seeds were sterilized under the following conditions: 1) 1 min in 95 % ethanol; 2) 15 min in bleaching solution (2.7 % Cl); 3), followed by several washings with sterile distilled water. Immature zygotic embryos were aseptically isolated and plating on nutrition medium M for further growing (Azpiroz *et al*.): 1/2 MS (Murashige, T. & F. Skoog, 1962) macro salts, MS micro salts, B5 vitamins (Gamborg *et al*., 1968), 20 g/l sucrose, pH-5.7. The conditions for cultivation were: 25^o C, 16/8 h photoperiod for one week. The plants which formed roots were transferred to soil and were further grown and self-pollinated in greenhouse.

RESULTS AND DISCUSION

Immature sunflower (*Helianthus annuus* L.) zygotic embryos of sunflower line maintainer of sterility 2607 B were treated with ultra sonic before plating to embryo culture medium. Embryo culture method allowed isolation of embryos before terminating their development and their plating in nutrition medium to grow *in vitro* seedlings. The plants obtained were self-pollinated under greenhouse conditions.

The data in table 1 indicate that the treatment with ultra sonic at dose of 25.5 w/cm^2 for 1 min had a stimulating effect on plant production (75 %). The dose of 51.0 W/cm^2 for

9 min and 11 min (Table 2) increased the number of plant produced (78.9 % and 83.3 %, respectively), also.

Stimulating effect on index seed produced (218 and 304 number, respectively) was registered for the dose of 25.5 W/cm^2 for 3 min and 5 min (Table 1). The data of table 2 showed considerably increasing of seeds produced for variant with 11 min (331 numbers).

According the index seed production the variant of treatment with ultra sonic step II (51.0 w/cm^2) showed better results-660 total number of seed produced in comparison to step I (25.5 w/cm^2) 633.

Table 1. Responsiveness to treatment with ultra sonic at dose 25.5 W/cm^2 (step I) of immature zygotic embryos and plant produced.

Таблица 1. Отговор на незрели зиготни зародиши към третиране с ултра звук в доза 25.5 W/cm^2 (I степен) и получени семена.

Variant of treatment with ultra sonic I step / Вариант на третиране с ултра звук I степен	Immature embryos Treated / Третирани незрели ембриони	Plants produced / Произведени растения	%	Seeds produced / Произведени семена	Mean number of seeds per plant / Среден брой семена от растение
1 min	20	15	75.0	111	7.4
3 min	30	15	50.0	218	14.5
5 min	26	15	57.7	304	20.3
Total	76	45	59.2	633	14.1

Table 2. Responsiveness to treatment with ultra sonic at dose 51.0 W/cm^2 (step II) of immature zygotic embryos and plant produced.

Таблица 2. Отговор на незрели зиготни зародиши към третиране с ултра звук в доза 51.0 W/cm^2 (II степен) и получени семена.

Variant of treatment with ultra sonic II step / Вариант на третиране с ултра звук II степен	Immature embryos Treated / Третирани незрели ембриони	Plants produced / Произведени растения	%	Seeds produced / Произведени семена	Mean number of seeds per plant produced / Среден брой семена от растение
7 min	27	15	55.6	168	11.2
9 min	19	15	78.9	161	10.7
11 min	18	15	83.3	331	22.1
Total	64	45	70.3	660	14.7

Treating embryos with ultra sonic caused a wide range of Chlorophyll deficiency mutations, Leaf mutations, Stem mutations, Inflorescence mutations, Seed mutations and Physiological mutations. Their brief description is given in Table 3. Twenty-five types of mutations were found in our study. Mutation process included chlorophyll mutations as *Aurea* and *Xanthovirescens* (Figure 1), *Albovirescens* (Figure 2), *Xanthovirescens* and *Albovirescens* (Figure 3), *Aurea* (Figure 4). Some of the *Xanthovirescens* and *Albovirescens* cotyledons were developed to green plants. Figure 5 presented chlorophyll mutation as *Viridomaculata*.

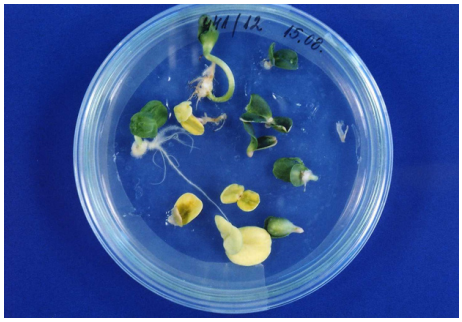

Figure 1. Chlorophyll mutation *aurea* and types *Xanthovirescens*
Фигура 1. Хлорофилна мутация *aurea* и *Xanthovirescens* тип

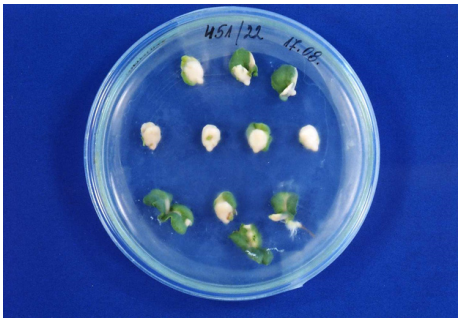

Figure 2. Numerous chlorophyll mutations-*Albovirescens*
Фигура 2. Множество хлорофилни мутации- *Albovirescens*

Figure 3. Sunflower mutant plants with chlorophyll types *Xanthovirescens* (in the middle) and *Albovirescens (*left and right)
Фигура 3. Слънчогледови мутантни растения с хлорофилен тип *Xanthovirescen* (в средата) и *Albovirescens (*ляво и дясно)

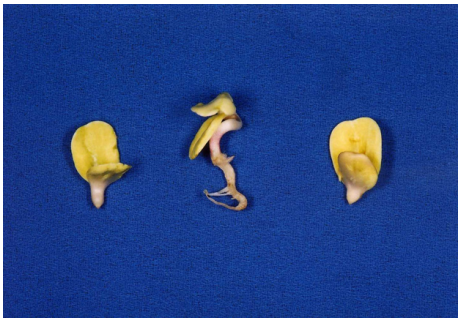

Figure 4. Chlorophyll mutation *aurea*
Фигура 4. Хлорофилна мутация *aurea*

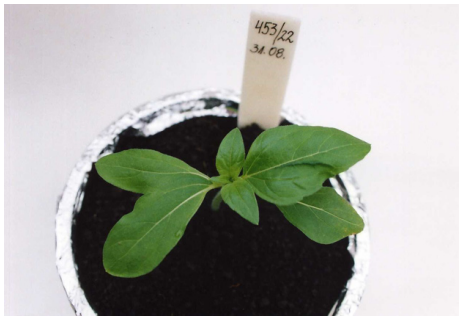

Figure 6. Leaf mutations (leaf with double top)
Фигура 6. Листни мутации (листа с двоен връх)

Figure 5. Sunflower mutant plants with chlorophyll types *Viridomaculata*
Фигура 5. Слънчогледови мутантни растения с хлорофилен тип *Viridomaculata*

Another type of observed mutation (Table 3) was leaves without top or with double tops, full sterile plants, dwarf plants, early flowering plants, plants with decrease number of ray flowers, malformed leaf, corrugated leaf, leaves with lanceolate shape, absence of ray florets, small and malformed capitulums, decreased number of ray florets, replacement of ray flowers with bract leaves, albino seeds and seed with gray stripes.

Some of these mutations were published by Soroka and Lyakh (2009) after treatment of immature zygotic embryo with EMS.

Table 3. Type of morphological mutations in M1 and their descriptions.
Таблица 3. Тип на морфологични мутации в М1 и тяхното описанание.

№	Type of mutation /Тип мутация	Characteristic / Характеристика
	I. Chlorophyll deficiency mutations	
1	Aurea	Yellow cotyledons
2	Xanthovirescens	Yellow-green cotyledons
3	Albovirescens	White-green cotyledons
4	Viridomaculata	Yellow-green leaves
5	Xantha	Yellow-green spots on the leaves
6	Xantha of necrotic type	Yellow-green spots on the leaves, transforming into necrotic segments
	II. Leaf mutations	
7	Malformed lea	Malformed, split leaf blades
8	Malformed leaf	Malformed, leaves with double top
9	Malformed leaf	Malformed, leaves without top
10	Dichotomous venation	Fan-shaped venation
11	Leaves with calceolate shape	
12	Corrugated leaf	
	III. Steam mutations	
13	Low-growing	Plant reduced in height
14	High-growing	Plant increased in height
15	Disturbed growing plant peak	Disturbed development of plant peak
	IV. Inflorescence mutations	
16	Malformed capitulum	Malformed
17	Absence of ray florets	Replacement of ray leaves with bract leaves
18	Few ray florets	Decrease number of ray florets
19	Small capitulum	Decreased size of capitulum
20	Malformed capitulum	Inflorescence consisting of many malformed heads
21	Full sterility	Anthers without pollen
	V. Seed mutation	
22	Albino	Seed with white color
23	Seed with gray stripes	
	VI. Physiological mutations	
24	Early flowering	
25	Late flowering	

CONCLUSION

As a result of treatment of immature zygotic embryos of sunflower genotype 2608 B with ultra sonic some Chlorophyll deficiency mutations, Leaf mutations, Stem mutations, Inflorescence mutations, Seed mutations and Physiological mutations chlorophyll,

mutations were observed.

Stimulation effect of ultra sonic at a dose 51.0 W/cm^2 (step II) was observed according indexes plants produced and seeds produced.

The available literature on sunflower does not provide data on treatment of immature zygotic embryos with ultra sonic. In this respect the approach is especially valuable due to the fact that immature sunflower zygotic embryos are treated at an early stage of development, i.e. this is functional tissue. This is expected to increase to a higher rate the frequency of mutations in comparison to the classical approach of treating air dry seeds.

Having combined induced mutagenesis with embryo culture method, it was concluded that the new variability was exclusively due to the effect of the mutagen. This assumption is confirmed by the fact that the embryo culture method alone does not generate variation due to the lack of mutagen factors in the nutrition medium and the short period of *in vitro* cultivation of the immature zygotic embryos. The advantage in this case is that this allows isolation of embryos before terminating their development and their plating onto nutrition medium to grow *in vitro* seedling.

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