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MUTANT SUNFLOWER LINE 338 B CREATED BY *IN VITRO* MUTAGENESIS

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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 before plating in the embryo culture medium M. New sunflower forms with inherited morphological and biochemical changes were obtained through selection and self-pollination. Line 338 B subjected to investigation in this study was characterized with significant changes concerning most of the studied characters. Based on all 12 agronomic characters investigated, it can be determined that the increasing the value were registered for plant height, seed length and oil in the seeds. Decreasing the value of the indexes was observed for head diameter, seed thickness, seed width, 1000 seed weight, leaf width and leaf length. The indexes number of leaves and stem diameter were the most stable. Mutant line 338 B was characterized with differences in comparison to the control line 2607 B concerning some of the quality characters as color of seeds in particular. The increased oil content in seeds in line 338 B is desired mutation in the breeding programme of sunflower.

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

Резюме

Юлия Енчева, Галин Георгиев, Даниела Вълкова, 2014. Мутантна линия слънчоглед 338 В, създадена чрез ин витро мутагенез. FCS 9(2):233-241

Незрели слънчогледови (*Helianthus annuus* L.) зиготни ембриони на Българската линия закрепител на стерилността 2607 В са третирани с пултра звук преди платиране върху хранителна среда М. Чриз селекция и самоопрашване са получени нови слънчогледови форми с наследяеми морфологични и биохимични изменения. Линия 338 В обект на настоящото изследване се характеризира с доказани изменения, отнасящи се до голяма част от изследваните признаци.

Базирайки се на 12 изследвани агрономически характеристики, може да се определи повишаване на стойностите на височината на растенията, дължината на семената и маслеността в семената. Намаление в стойността на индексите е наблюдавано при диаметъра на питата, дебелината на семето, ширината на семето, масата на 1000 семена, ширината и дължината на листата. Най-стабилни са индексите брой листа и дебелина на стъблото. Мутантна линия 338 В се характеризира с различия в сравнение с контролната линия 2607 В, отнасящи се до качествени признаци като цвят на семената. Увеличеното съдържание на масло в семето при линия 338 В е желана мутация в селекционната програма при слънчогледа.

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

INTRODUCTION

The aim of the breeders of many crops is to establish and recombine the available genetic variability. When existing germplasm fails to provide the desired recombinant, it is necessary to resort to other sources of variation as interlinear hybridization, induced mutagenesis, polyploidy and tissue cultures.

Mutagenesis began to be an important research approach since 1928 when Stadler, Muller and Altenberg demonstrated that X ray and radium cause heritable changes in barley, maize, and Drosophila (Bird and Neuffer, 1986). Spontaneous mutations occur in nature with extremely low frequency. In this connection induced mutagenesis provides tools for the rapid creation and increase in variability in crop species and in sunflower in particular. According Anashchenko (1977) the most important effects of physical and chemical mutagenesis are the production of a large number of recessive genes and cytoplasmic mutations, both of which increase the variability in the cultivated sunflower. Under the influence of the mutagenic factors a large spectrum of heritable mutations were induced (Christov, 1990). The most interesting mutation obtained under the influence of ultra sonic at mature sunflower seeds was the CMS from cultivar Peredovik (Christov and Nikolova, 1996). Costov *et al.* (2007) increased the resistance of tomato *Orobanche ramosa* L after treatment of seeds with ethylmethanesulphonate (EMS). Encheva *et al*., 2008 and Encheva, 2009 created sunflower lines with statistically significant changes in morphological characters and resistant to parasite *Orobanche cumana* after treatment of immature zygotic embryos with ultra sonic. Soroka and Lyakh, 2011 described the inheritance of some mutant traits in cultivated sunflower which were found after treating immature embryos of ZL-95 line with a chemical mutagen ethylmethanesulphonate. It was shown that the traits of dichotomous leaf venation and tobacco-like plant are recessive and inherited independently as monogenic traits when cross-bred with the source line.

Sunflower is the main oleaginous crop for Bulgaria where the total cultivated sunflower areas are approximately 788 000 ha. Sunflower production increses each year and in 2014 Bulgaria is on the third place after France and Romania in Europe. This requires the creation of a wide variety of inbred lines components of future commaersial hybrids which posses high yield, high oil content, resistance to diseases, early flowering and good combining ability.

The aim of this study was: a) to develop variable B lines from sunflower by treatment of immature zygotic embryos 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 и 11 min before plating to nutrition medium and b) to verify mutant line 338 B and control line 2607 B for some main characteristics.

MATERIAL AND METHODS

A part of the experiments were carried out under laboratory conditions, and another – at the trial field 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. A main requirement to the initial plant material used according to the methods of embryo culture in combination with ultrasound is to be genetically pure, i.e. homozygotic to the highest possible degree. Therefore the control line 2607 B (more 30 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 isolated 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 и 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. and 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° 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.

Biometric evaluation of control line 2607 B and mutant line 338 B

The biometric evaluation and biochemical analysis of the control genotype and the new developed mutant line 338 B were made on 10 plants for three years and included 12 main agronomic traits as oil in the seed, 1000 seed weight, plant height, leaf width, leaf length, number of leaves, petiole length, head diameter, stem diameter, seed length, seed thickness and seed width. 1000 seed weight (g) was determined on three samples of 50 seeds per head each. The control data were collected from plants of the original line 2607 B which was grown in the field together with mutagenic plants of line 338 B.

Biochemical analysis

To determine the oil content of air-dry seeds from the materials included in the study, Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) was used.

Statistical analysis

The control line 2607 B and developed mutant line 338 B were analyzed statistically with regard to the agronomic traits such as oil in the seed, 1000 seed weight, plant height, leaf width, leaf length, number of leaves, petiole length, head diameter, stem diameter, seed length, seed thickness and seed width.

Analysis of the experimental data was made by the statistical package BIOSTAST 6.0.

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.

Evaluation according to quantitative traits in mutant line 338 B

In our study some mutant plants were isolated and self-pollinated for several generations. New sunflower forms with inherited morphological and biochemical changes were obtained through selection and self-pollination. Mutant lines 338 B (Figure 2) originating from the Bulgarian line maintainer of sterility 2607 B (Figure 1) was selected due to their statistically significant morphological and biochemical changes. Statistically significant differences were established in the genetic potential of the indices plant height, head diameter, seed length, seed thickness and seed width and oil in the seed of the studied line 338 B.

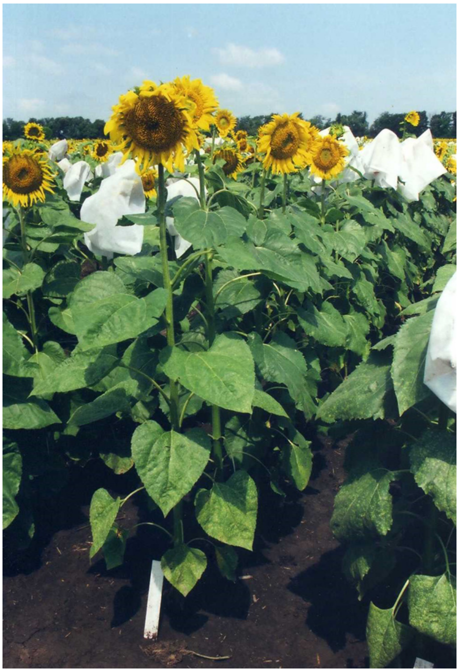

Figure 1. Control line 2607 B
Фигура 1. Контролна линия 2607 В

Figure 2. Mutant line 338 B
Фигура 2. Мутантна линия 338 В

Figure 3 presents data on the significant change of the mean value for the character plant height. The new mutant line 338 B possessed plant heights with 15.2 cm. more than the control 2607 B. Plant height is one of the morphological indices most often investigated in cultural sunflower. It is considered a quantitatively inherited character. Increased plant height is controlled by dominant genetic effects (Putt 1966), 57 % dominant effects and 30 % additive effects (Lay and Khan, 1985) and dominance to over dominance (Kovacik and Skaloud, 1990). Similar mutations with high stem and large leaves are obtained by Christov, M and V. Nikolova, 1996 after treatment of air dry seeds of sunflower lines L-2969 and L-3004 with ultra sonic and at line L-1607 and L-3004 after treatment with gamma rays.

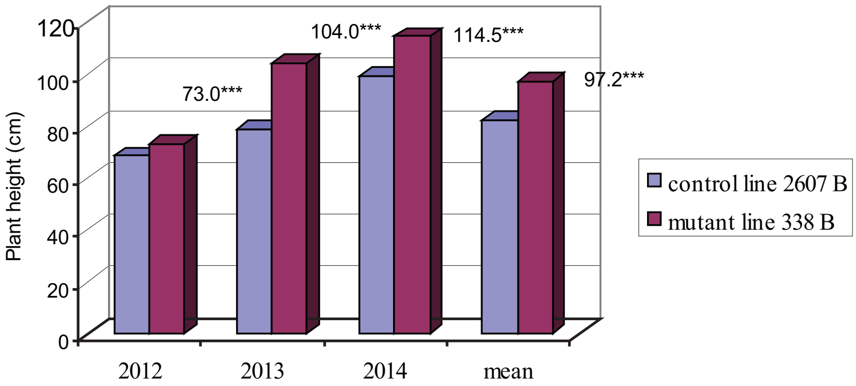

Figure 3. Plant height (cm) of control line 2607 B and mutant line 338 B (***-P=0.001%)
Фигура 3. Височина на растение (cm) на контролната линия 2607 В и мутантна линия 338 В (***-P=0.001%)

A significant decrease of the mean value of the index head diameter (Figure 4) with 2.4 cm was noted in mutant line. Differences were established in the shape of head. In control line 2607 B it is convex (Figure 5) and vice versa in mutant line 338 B it is flat (Figure 6).

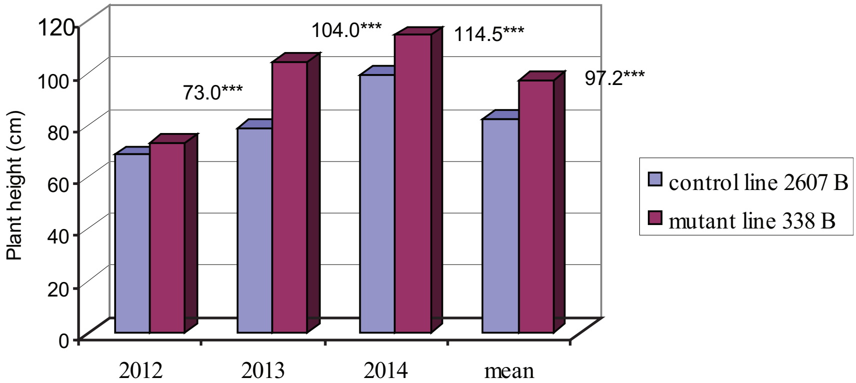

Figure 4. Head diameter (cm) of control line 2607 B and mutant line 338 B (***-P=0.001%) **Фигура 4.** Диаметър на пита (cm) на контролната линия 2607 B и мутантна линия 338 B (***-P=0.001%)

Figure 5. Head of control line 2607 B
Фигура 5. Пита на контролна линия 2607 B

Figure 6. Head of Mutant line 338 B
Фигура 6. Пита на мутантна линия 338 B

Summarizing the data on the three indices of the seeds of mutant line: seed thickness, seed width and seed length, it can be said that changes in the form and size of seeds occurred (Figure 7, 8, and 9). A decrease in the mean arithmetic value with a high degree of significance was registered in the two indices- seed thickness and seed width (with 0.06 mm and 0.18 mm, respectively).

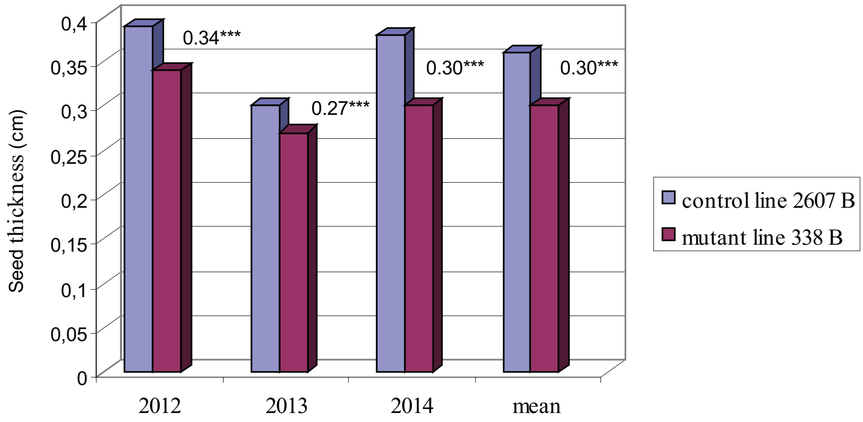

Figure 7. Seed thickness (mm) of control line 2607 B and mutant line 338 B (***-P=0.001 %)

Фигура 7. Дебелина на семе (mm) на контролната линия 2607 В и мутантна линия 338 В (***-P=0.001%)

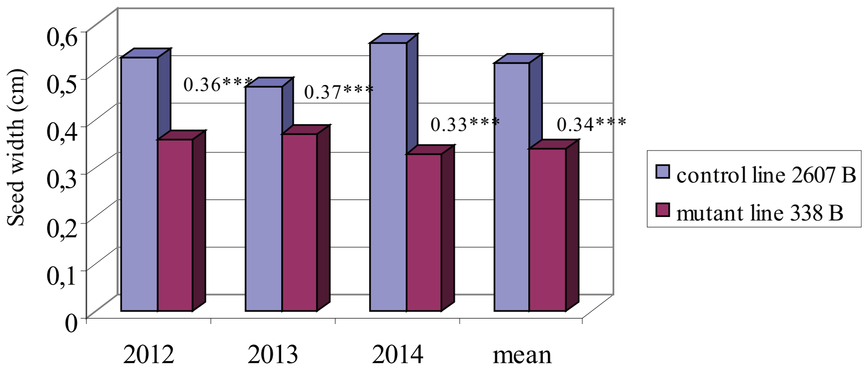

Figure 8. Seed width (mm) of control line 2607 B and mutant line 338 B (***-P=0.001 %)

Фигура 8. Ширина на семе (mm) на контролната линия 2607 В и мутантна линия 338 В (***-P=0.001%)

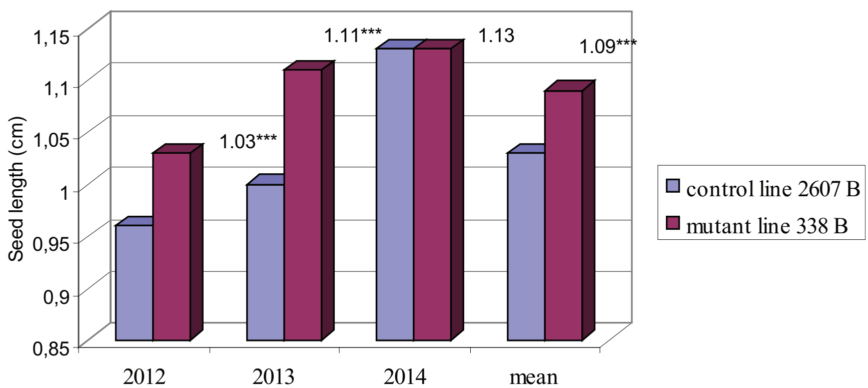

Figure 9. Seed length (mm) of control line 2607 B and mutant line 338 B (***-P=0.001 %)

Фигура 9. Дължина на семе (mm) на контролната линия 2607 В и мутантна линия 338 В (***-P=0.001%)

Significant increasing with 0.06 mm of the character seed length for the mutant line 338 B was observed.

Mutations were registered also in the quality indices such as seed color (Figure 10). The seeds color of control line 2607 B is black with lateral gray stripes, while the mutant line 338 B has white color.

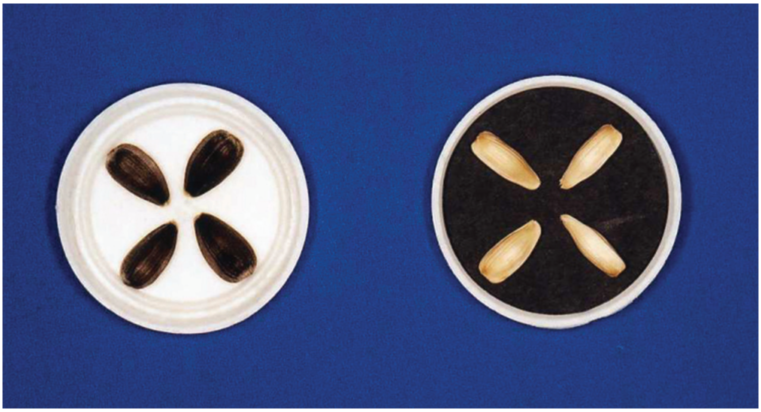

Figure 10. Seed shape and color of control line 2607 B (left) and mutant (albina) line 338 B (right)

Фигура 10. Форма и цвят на семе на контролната линия 2607 В (ляво) и мутантна (албинос) линия 338 В (дясно)

Oil content in seed is one of the most important agronomic indices (Figure 11). The results from dispersion analysis demonstrated a significant increase with 7.1 % at mutant line. Increasing of oil content was reported at lines CG-3663 and CG-3606 after mutagenic treatment of dry sunflower seeds with gamma ray (Vranceanu and Iuoras, 1990). Christov and Nikolova, 1996 published increasing oil content in sunflower lines MF P-13 and L-3004 after treatment of dry seeds with gamma rays.

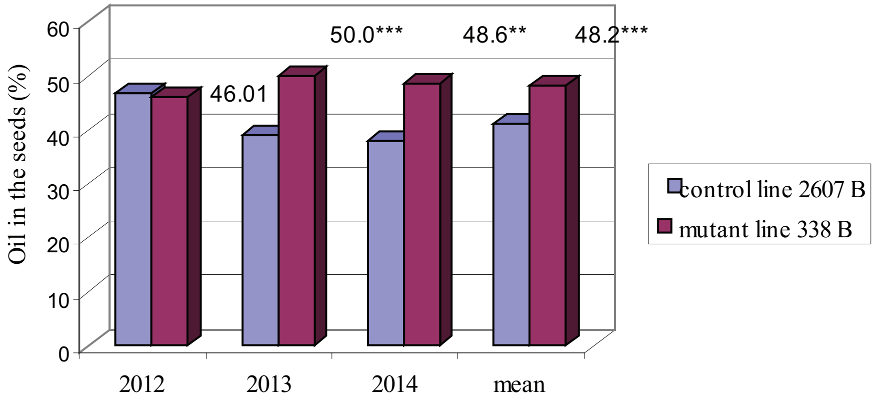

Figure 11. Oil in the seeds (%) of control line 2607 B and mutant line 338 B (**-P=0.01 %; ***-P=0.001 %)

Фигура 11. Масло в семето (%) на контролната линия 2607 В и мутантна линия 338 В (***-P=0.001%)

Based on all 12 agronomic characters investigated, it can be determined that the increasing the value of were registered f plant height, seed length and oil in the seeds.

Decreasing the value of the indexes was registered for head diameter, seed thickness, seed width, 1000 seed weight, leaf width and leaf length. The indexes number of leaves and stem diameter were the most stable.

Differences of characteristics of mutant line 338 B and control line 2607 B

Table 1 present differences of mutant line 338 B from control line 2607 B concerning 17 characteristics (Table 1). While head shape of grain side of control line is convex the new mutant line have flat one. Considerable clear is the index leaf-shape of cross section. It is convex at mutant line 338 B and weakly concave at a control line 2607 B. The most investigated index plant height is medium for line 2607 B, while at new line is high.

Table 1. Differences of some characteristics of sunflower mutant line 338 B from control line 2607 B.
Таблица 1. Различия при някои признаци на слънчогледова мутантна линия 338 B от контролна линия 2607 B

Characteristics Признаци	Control line 2607 B Контролна линия 2607 B	Mutant line 338 B Мутантна линия 338 B
plant height	medium	high
head shape of grain side	convex	flat
leaf- blistering	absent or very weak	medium
leaf-shape of the cross section	weakly concave	convex
leaf-angle of lateral veins	right angle or nearly right angle	obtuse
leaf-color	light green	medium green
leaf-блясък	strong	medium
time of flowering	Late	medium
ray flowers number	medium	Many
ray flowers shape	elongated	ovoid-elongated
ray flowers length	long	medium
ray flowers coloring	orange	orange yellow
seed-shape	ovoid wide	elongated
seed-color	black	white
seed-stripes	present	absent
seed position of stripes	lateral	absent
seed color of stripes	gray	absent

The leaf color of new line 338 B is medium green. Control line 2607 B posses light green leaves. While ray flower shapes of standard were elongated, the new mutant line 338 B have ovoid-elongated. Considerable difference was observed at index leaf blistering. While control line posses absent or very weak blistering, mutant line have medium one. The control line was characterized with orange color of ray flowers in contrast of mutant line which demonstrated orange yellow. Seed-shape also differs. Line 2607 B posses ovoid wide seeds, while mutant 338 B has elongated shape. Considerable clear is mutation concerning seed color. It is white at mutant line 338 B and black with lateral gray stripes at a control line 2607 B.

CONCLUSION

As a result of treatment of immature zygotic embryos of sunflower genotype 2607 B with ultra sonic some morphological and biochemical mutations were observed. The same mutation was registered among regenerants obtained with both variants of the treatment (25.5 W/cm^2 and 51.0 W/cm^2) of the control genotype 2607 B.

As a result of continuous self-pollination and selection line 338 B was obtained, which was distinguished with its morphological and biochemical differences. The statistically obtained changes in some of investigated morphological indexes leading to the appearance of line with altered architecture.

The changes in the mutant line 338 B are variations in the values of important agronomic performance, but the emergence of new indexes were not observed.

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. This allowed us to conclude that the new variability was exclusively due to the effect of the mutagen.

Induction of mutations is unpredictable process, but in our study were obtained morphological and biochemical mutations with stable inheritance in the descendants of the new mutant lines.

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