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СЪЗДАВАНЕ НА МУТАНТНИ В ЛИНИИ СЛЪНЧОГЛЕД (*HELIANTHUS ANNUUS* L.) ЧРЕЗ ИНДУЦИРАН МУТАГЕНЕЗ

ЮЛИЯ ЕНЧЕВА, ПЕТЪР ПЕТРОВ, ПЕПА ШИНДРОВА

DEVELOPING MUTANT B LINES IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) TROUGH INDUCED MUTAGENESIS

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Abstract

Immature zygotic embryos of sunflower line 197 B were treated with ultrasonic before plating to embryo culture medium. Some mutant plants were isolated and self-pollinated for several generations. New sunflower forms with inherited phytopatological, morphological and biochemical changes were obtained through selection and self-pollination. Reduction and increasing was observed for the character plant height. Positive and negative changes according to the control line were observed for the character 1000 seed weight. Significant increasing in the mean arithmetic value were establish in characters seed weight, seed thickness and stem diameter. Mutation for resistance to the local population of *Orobanche cumana* (race A-E) was obtained from the susceptible Bulgarian control line 197 B.

Key words: *Helianthus annuus*, immature zygotic embryos, ultrasound, mutagenesis, new breeding material, resistance, *Orobanche cumana*

Introduction

Spontaneous mutations occur in nature with extremely low frequency. In this connection induced mutagenesis provide tools for the rapid creation and increase in variability in crop species (in rice-[5]; in sunflower-[1].

Broomrape (*Orobanche cumana* Wallr.) is a parasite on the roots of sunflower plants and causes serious damages to sunflower production.

Furthermore, the parasite forms new more virulent races which overcome the resistance of the varieties and hybrids commonly used in production ([11]; [4]. This complicates the control of broomrape. This leads to considerable losses expressed, on the one hand, in yield decrease, and on the other - in worsened quality of the obtained produce ([12]. With a view of limiting the parasite's distribution and decreasing the losses it causes, it would be preferable to develop new lines resistant to the broomrape. Instead of wild species, induced mutagenesis

on immature zygotic embryos can be another source for producing plants resistant to the parasite *Orobanche*.

The aim of this study was: to developed variable B line from sunflower by induced mutagenesis; to accelerate the breeding process by applying the embryo culture method; to carry out biometric and biochemical investigations on the new lines B5 generation; to evaluated the new genetic materials for resistance to the parasite *Orobanche cumana*.

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. The morphological and biochemical traits of the new mutant lines and the control genotype were studied during 2006-2008.

A/ Developing of mutant lines

The Bulgarian self-pollinated line 197 B with normal cytoplasm, witch is highly homozygtic (over 30 generations), was used as donor material.

Plants were grown in the field and were hand-pollinated. The aseptically isolated immature zygotic embryos (11-13 days old) were treated with ultrasound at dose 25.5 W/cm^2 for 1, 3 and 5 min before plating on nutrition medium M for further growing: 1/2 MS [6] macro salts, MS micro salts, B5 vitamins [3], 20 g/l sucrose, pH-5.7. Sixty zygotic embryos were plated for each variant.

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 under greenhouse conditions.

B/ Field experiments

Biometric evaluation of control line 197 B and mutant lines 74 B, 78 B, 85 B and 88 B

As a result from long-term selfing and individual selection, new sunflower lines were produced in B5 generation. The main criterion for selection was resistance to *Orobanche cumana*. The lines were investigated with regard to some main characteristics concerning breeding in sunflower, also.

C/ Biochemical analysis

To determine the oil content of air-dry deeds from the materials included in the study, Nuclear-magnetic resonance [7] was used.

D/ Phytopatological evaluation

The phytopathological evaluation was performed with regard to the local *Orobanche* population (race A-E) at the Sunflower Phytopathology Laboratory during 2005-2007. Broomrape resistance was evaluated under greenhouse conditions according [9]. Broomrape resistance was calculated as percentage of non-infected plants. The reaction of 50 plants from each genotype was recorded using the following scale: 0 % = S (sensitive); 100 % = R (resistant).

D/ Statistical analysis

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

The following statistical analysis was performed: a) Cluster analysis by Euclidean linkage distances [2]. Analysis of the experimental data was by the statistical package BIOSTAST 6.0. [10].

Results and Discussion

Evaluation according to quantitative traits in mutant lines 74 B, 78 B, 85 B and 88 B

Immature embryos-11-13 days old were treated with ultrasound before planting on nutrition medium M for father growing. After selfing and individual selection of materials new B lines were obtained. Lines 74 B, 78 B, 85 B and 88 B (Figure 1) were preferred due to their significant differences with control line 197 B concerning resistance to the parasite *Orobanche* and some important morphological and biochemical characters.

Plant height is one of main biometric indices in sunflower which has been subjected to investigation (Table 1). The decrease was significant for the line 74 B and was with 5.6 cm. Statistical increasing of mean arithmetic value with 7.7 cm. were established in line 78 B.

Values higher than that of control line 197 B were registered in all of new lines for the character stem diameter (Table 1). Differences from 1.6 cm. to 2.1 cm. concerning lines 74 B, 78 B and 88 B were with high degree of significance. Positive and negative changes according to the control line were observed for the character 1000 seed weight. While lines 74 B and 88 B demonstrated exceeding of 4.3 to 6.7 g., lines 78 B and 85 B had lower values within the range of 4.3 to 5.4 g. Highly significant increasing in the mean arithmetic value were establish in characters seed weight (with 0.4 mm. and 0.5 mm.) and seed thickness (with 0.6 mm. and 0.7 mm.).

All investigated new lines posses lower value of index seed length. A significant decrease, by 0.5 mm. was noted only in the line 85 B (Figure 2).

In our study 1000 seed weight was the least stable of all investigated characters, and oil content and petiole length the most stable one. The highest number of changes indexes (6. from 12) was observed in line 74 B i.e. 50 % of the total number of characters.

Fig. 1: Mutant line 88 B

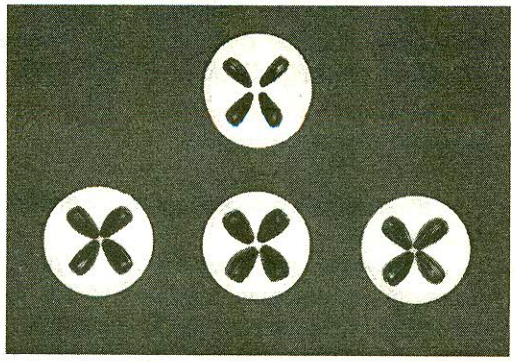

Fig. 2: Seeds of control line 197 B and mutant lines 74 B, 78 B and 88 B

Table 1. Effect of ultrasound treatment on some morphological and biochemical characteristics of mutant lines, produced through induced mutagenesis of immature zygotic embryos from genotype 197 B. Harvest years 2006-2008, average data.

traits	Control line 197 B	Line 74 B	Line 78 B	Line 85 B	Line 88 B	LSD
Plant height (cm)	97.17	91.60	**104.87+a**	93.10	**90.83-a**	Gd5%=5.61
Head diameter (cm)	15.43	**16.67+b**	17.20	15.48	16.27	Gd5%=1.27
Leaf width (cm)	14.73	15.53	14.48	**13.63-a**	14.68	Gd5%=0.98
Leaf length (cm)	15.47	15.60	14.60	**14.29-a**	14.77	Gd5%=1.06
Petiole length (cm)	7.50	7.73	7.67	7.37	7.68	Gd5%=0.36
Number of leaves (no)	26.0	26.0	26.0	26.0	**25.00-a**	Gd5%=0.72
Stem diameter (mm)	16.37	**18.05+b**	**17.95+a**	16.43	**18.42+b**	Gd5%=1.22
Oil content in seed (%)	38.29	40.11+b	40.39	39.29	39.98	Gd5%=2.11
1000 seed weight (g	60.29	**64.57+b**	**55.74-b**	**54.89-c**	**66.94+c**	Gd5%=2.76
Seed width (mm)	5.40	**5.87+b**	5.33	5.07	**5.80+a**	Gd5%=0.33
Seed length (mm)	11.33	11.13	11.07	**10.86-b**	11.13	Gd5%=0.30
Seed thickness (mm)	3.16	**3.80+c**	3.43	3.13	**3.86+c**	Gd5%=0.26

Cluster analysis for morphological and phytopatological traits

Investigation on the Euclidean distance between control line 197 B and mutant lines 74 B,
78 B, 85 B and 88 B

In order to characterize the investigated lines as fully as possible and to determine the relations between them and the similarity in their response to various conditions, cluster analysis was applied. The dendrogram of phytopatological, morphological and biochemical classification resulted in the differentiation of the control genotype and the new mutant lines into two main clusters (figure 3). The big distance of mutant lines and control line 197 B was due to the fact that they differ mainly with resistance to the parasite *Orobanche cumana.*

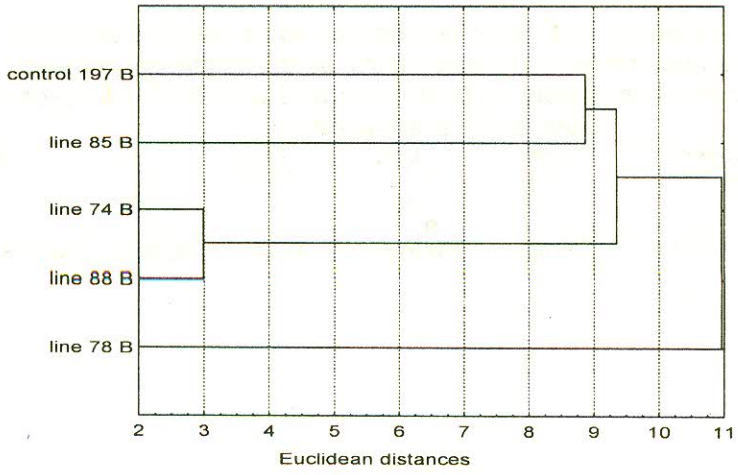

Figure. 3: Degree of similarity between control line 197 B and mutant lines 74 B, 78 B, 85 B and 88 B

The new lines have lower and thick steam, higher 1000 seed weight, larger seed width and seed thickness. Despite a big difference between the investigated lines and check line 197 B, the dendrogram shows a lower distance between line 74 B and 88 B. That may be explained by similar resistance to the parasite broomrape, morphological traits and reaction to the factors of environment.

Evaluation of the sunflower mutant lins for resistance to local broomrape population

Broomrape presents serious problem to sunflower production in Bulgaria. It is constantly expanding its distribution area, forming new more virulent races [11].

In our study except morphological and biochemical changes a mutation was observed in reaction of the new lines towards *Orobanche cumana* parasite. The control genotype 197 B was susceptible to broomrape. The mutant lines 74 B, 78 B, 85 B and 88 B showed 100 % resistance to the local broomrape population. These results were confirmed during three years of evaluation. On the base of this data the conclusion was drown that the resistance of the new lines was due to the mutagenic treatment with ultrasound. The results allow us to assume that the resistance of the mutant sunflower lines to *Orobanche* occurred as a results of a single dominant gene. According to [8] the gene Or6 provides resistance to all races from A to F. The same mutation resistance to broomrape was obtained in all variants involving the initial genotype 197 B. This allow us to assume that their are mutable locations in the cultural sunflower genome resulting from induced mutagenesis.

CONCLUSION

Induced mutagenesis of immature zygotic sunflower embryo allows to develop economically useful traits, including resistance to the parasite *Orobanche*. Combining induced mutagenesis in immature zygotic embryo with the embryo culture method, it can be assumed that the new variability obtained is due only 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.

Further evaluation is needed to achieve a more complete description of the new lines, produced in terms of general combining ability.

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