HELIA, **31**, Nr. 48, p.p. 61-72, (2008) UDC 633.854.78:631.523
DOI: 10.2298/HEL0848061E

DEVELOPING MUTANT SUNFLOWER LINES (*Helianthus annuus* L.) THROUGH INDUCED MUTAGENESIS

Encheva, J.*, Shindrova, P., and Penchev, E.

Dobroudja Agricultural Institute, General Toshevo 9520, Bulgaria

Received: February 25, 2007
Accepted: March 28, 2008

SUMMARY

Immature zygotic embryos of sunflower fertility restorer line 147 R were treated with ultrasound before plating to embryo culture medium. 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. Genetic changes that occurred during the regeneration procedure included fifteen morphological and biochemical characters. In this study, negative genetic changes were registered for most of the indices. Positive changes were registered for leaf petiole length and number of leaves. Plant height was the least stable of all characters under study. Mutation for resistance to the local population of *Orobanche cumana* (race A-E) was obtained from the susceptible Bulgarian control line 147 R. All five investigated mutant restorer lines possessed 100% resistance to *Orobanche* and stable inheritance in subsequent generations. Our results showed that mutagenesis in sunflower can be successfully used to develop new lines useful for heterosis breeding.

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

INTRODUCTION

The development of variable breeding material is a primary task of the genetic and breeding programs in sunflower. New approaches, such as tissue culture in combination with induced mutagenesis, provide an additional possibility to enrich the genetic variability in the sunflower.

Studies on genetic variation in sunflower regenerants are not numerous. Significant changes and molecular evidence for genetic variation in sunflower regenerants

* Corresponding author

obtained by the method of direct organogenesis were reported by Encheva *et al.* (2003).

Mutagenesis, both physical and chemical, proved favorable for mutation induction in tissue culture. Encheva *et al.* (1993, 2002, 2003) reported statistically significant changes in morphological and biochemical characters of plants regenerated from immature zygotic embryos of sunflower, independently and in combination with gamma irradiation. Positive results were obtained when induced mutagenesis and tissue culture were appropriately combined in tomato (Gavazi *et al.*, 1987), maize (Novak *et al.*, 1988) and wheat (Cheng *et al.*, 1990).

Although sunflower breeding has been very successful in the recent decades, a number of aims remained to be achieved, *e.g.*, resistance to various diseases and the parasite *Orobanche*. However, these efforts are obviously limited by the narrow genetic base of commercial sunflower which has to be enlarged by the utilization of wild species, mutagenesis or tissue culture. Development of new lines resistant to broomrape is an important objective in sunflower breeding.

Broomrape (*Orobanche cumana* Wallr.) is a parasite of the roots of sunflower plants and it causes serious damage to sunflower production (Škorić, 1994). The genus is spread on 16 million hectares in the Mediterranean region and south-west Asia. The most important species, *O. cumana*, has become a limiting factor for the crop production in Eastern Europe and Spain (Casteljon-Munoz *et al.*, 1991). The parasite has a high propagation coefficient allowing it to multiply rapidly, to expand its distribution area and to increase its attack rate. According to Kaya *et al.* (2004), about 80% of the sunflower areas in Turkey (Trakya region) are infested with seeds of the parasite. According to these authors, the broomrape occurs on an epiphytotic scale in this region every 20 years. Furthermore, the parasite keeps forming new, more virulent races which overcome the resistance of the currently used commercial varieties and hybrids (Kaya *et al.*, 2004; Pacureanu-Joita *et al.*, 1998; Alonso, 1996; Fernandez-Martinez *et al.*, 2000). This complicates the control of broomrape.

Broomrape also presents serious problems to sunflower production in Bulgaria. It keeps expanding its distribution area, and keeps forming new more virulent races (Shindrova, 1994). This leads to considerable losses expressed, in yield reductions on the one hand, and inferior quality of oil on the other (Shindrova *et al.*, 1998). 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. In addition to wild species, induced mutagenesis of immature zygotic embryos can be a source for producing plants resistant to *Orobanche*.

The aims of this study were: a) to develop variable R lines through *in vitro* induced mutagenesis in the initial sunflower genotype 147 R, b) to evaluated new genetic material for resistance to the local population of the parasite *Orobanche cumana* (races A-E), and c) to carry out biometric and biochemical investigations on the new lines (R_5M_5 generation).

This paper is the first report on mutation induction for resistance to *Orobanche* in immature zygotic embryos of sunflower.

MATERIAL AND METHODS

A part of the experiments was carried out under laboratory conditions, and another part at the experiment field of Dobroudja Agricultural Institute in General Toshevo.

Morphological and biochemical characters of the new mutant lines and the control genotype were studied during 2003-2005.

A/ Developing mutant lines

The Bulgarian fertility restorer line 147 R, which is highly homozygous, was used as donor material. A main requirement for the initial plant material used according to embryo culture methods in combination with ultrasound is to be genetically pure, *i.e.*, homozygous to the highest possible degree. Therefore, the control line 147 R, with very good morphological uniformity due to long selfing (over 30 generations), was chosen as initial material for induced mutagenesis.

Plants were grown in the field and were hand-pollinated. Immature zygotic embryos (11-13 days old) were aseptically isolated and sterilized under the following conditions: 1) 1 min. in 95 % ethanol; 2) 15 min. in bleaching solution (2.7% Cl); 3) several rinsings with sterile distilled water. Sixty zygotic embryos were plated for each variant.

The isolated immature zygotic embryos were treated with ultrasound dose of 25.5 W/cm^2 for 5, 7, 9, 11 and 13 min before plating on nutrition medium M for further growing (Azpiroz *et al.*, 1988): 1/2 MS (Murashige and 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 day/night photoperiod for one week. The embryo culture method allows isolation of embryos before terminating their development and their plating onto nutrition medium to grow *in vitro* seedlings. 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 the control line 147 R and the mutant lines 116 RM, 117 RM, 118 RM, 119 RM and 120 RM

As a result of long-term selfing and individual selection, new sunflower lines were produced in the R_5 generation. The main criterion for selection was resistance to *Orobanche cumana.* The lines were assessed with regard to some major characters important in sunflower breeding. Each generation of plants was examined for biometric parameters and biochemical characterization of seeds was carried out.

The biometric evaluation and biochemical analysis of the control genotype and the newly developed mutant lines were made on 10 plants each year, and it included 15 agronomic characters: oil content in the kernel, 1000-seed weight, plant height, leaf width, leaf length, number of leaves, petiole length, head diameter, number of branches, length of branches, diameter of lateral head, stem diameter, seed width, seed length and seed thickness. 1000-Seed weight (g) was determined using three samples each with 50 seeds per head. The control data were collected from plants of the original line 147 R which was grown in field together with the mutagenic plants.

Biochemical analysis

Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) was used to determine oil in the kernel from the developed mutant lines as compared with the control line.

C/ Phytopathological evaluation

The phytopathological evaluation of the control genotype 147 R and the obtained mutant lines was performed with regard to the local *Orobanche* population (race A-E) at the Sunflower Phytopathology Laboratory during 2003-2005. Broomrape seeds were collected from several regions in Bulgaria. Broomrape resistance was evaluated under greenhouse conditions according to the method of Panchenko (1975), slightly modified to fit the local conditions. A mixture of soil and sand (2:1) was prepared, and 0.2 mg of broomrape seeds were added to each kilogram of the mixture. Sunflower was grown in this substrate in the following order: 50 plants + 10 plants (standard-AD-66) in every container. They were placed in a greenhouse under controlled conditions with irrigation. Forty-five days after planting, the roots of all sunflower plants were cleaned and checked for the existence of the parasite. 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).

Statistical analysis

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

The following statistical analyses were performed: a) variance analysis using the following model: $Y_{ijk}=\mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+\varepsilon_{ijk}$ (Everitt, 1984), b) cluster analysis by Euclidean linkage distances (Elliott *et al.*, 1982). The analyses of the experimental data were performed with the statistical package BIOSTAST 6.0.

RESULTS AND DISCUSSION

I. Evaluation of quantitative characters in mutant lines

The study included morphological and biochemical characters of economic importance. Mutant lines (Figure 2) originating from the fertility restorer line 147 R (Figure 1) were selected on account of their statistically significant morphological and biochemical changes and resistance to *Orobanche cumana*.

Figure 1: Control genotype 147 R from Bulgaria

Figure 2: Mutant lines 116 RM, 117 RM and 118 RM developed by induced mutagenesis of immature zygotic embryos

Differences were established in the genetic potential for the following indices: plant height, leaf width, number of leaves and 1000-seed weight (Table 1).

Plant height is one of the most often investigated morphological characters in cultural sunflower; it is considered a quantitatively inherited character. Reduced plant height controlled by recessive genes in lines with a reduced number of leaves has been reported by Fick (1978), Vranceanu (1974) and Beretta de Berger and Miller (1985).

In our study, significant changes occurred in the lines 116 RM, 117 RM, 118 RM, 119 RM and 120 RM (Figure 2), which decreased their mean index value by 13.8 to 22.4 cm in relation to the control 147 R. Stem breaking due to adverse growing conditions can cause significant yield reductions in some years. Reduced plant height may lead to increased sunflower yield due to improved standability. Reduction in plant height has been achieved in somaclonal lines (Encheva *et al.*, 1993, 2002, 2003) and by using the direct organogenesis method in combination with gamma irradiation (Encheva *et al.*, 1993, 2002). Novak *et al.* (1988) reported plant height reduction after treatment of immature zygotic embryos of maize with a dose of 5 Gy. Reduction in plant height of sunflower plants was also observed by

Hristov (1996), after treatment of air-dry seeds with gamma ray doses of 150 Gy and 200 Gy. One of the few positive changes observed among the mutant lines was in the number of leaves. In the line 116 RM, the mean number was 29, against 23 in the check line 147 R. This difference was highly significant.

Significant decreases in leaf size were registered in all lines. Most significant were the differences between the line 118 RM (17.4 cm leaf width and 16.0 cm leaf length) and the check variant (20.8 cm and 18.2 cm, respectively). Reduction of head size from 2.7 to 3.9 cm had the highest degree of significance in all new lines developed. In the lines 117 RM and 118 RM, reductions in the length of branches ranged from 8.4 to 9.5 cm. These data too were highly significant. Also, significant decreases in the number of branches (from 3 to 5) were observed in the lines 116 RM, 117 RM and 118 RM. Significant decreases in the mean value of stem diameter, from 2.8 to 5.6 mm, were noted in all mutant lines. Negative and highly significant changes in the mean 1000-seed weight were registered in all investigated lines. The decreases ranged from 19.4 to 23.2 g. The oil content in kernel demonstrated considerable stability. A significant decrease, by 4.3%, was noted only in the line 118 RM. Considering the data for the three seed characters of the mutant lines, seed thickness, seed width and seed length, it can be said that changes in seed form and size did occur (Figure 3). Highly significant decreases in the mean arithmetic value were registered in all three characters, the most significant being for seed thickness (0.8-2.9 mm).

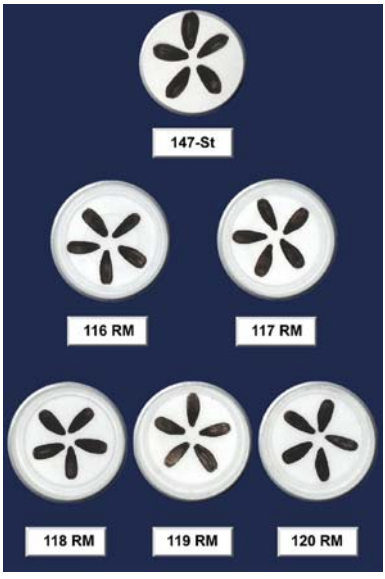
Table 1: Means of squares for the studied indices

Index	MSA	MSB	MSA × B	MSE
Plant height	179 **	442.1 ***	244.5 ***	12.65
Head diameter	2.35	18.38 **	7.17 *	1.1
Leaf length	2.83	29.55 *	2.45	3.49
Leaf width	44.2 *	27.4 *	28 *	4.16
Stem diameter	19.1	67.4 *	17.8	13.6
Length of branches	169.2	662.8 **	91.9	59.4
Number of leaves	74 *	141***	49 *	7
Diameter of lateral head	3.1	6.74	4.06	1.21
1000-seed weight	228.2 *	759.2 ***	144	31.1
Seed width	0.58	1.29	1.85 *	0.37
Seed thickness	0.7	0.8	1.88 *	0.39
Seed length	0.19	0.08	0.11	0.18
Oil in the kernel	0.99	26.3 *	7.57	5.9
Petiole length	10.99	34.2 *	3.84	5.4
Number of branches	45	149	34	19
df	5	2	10	34

A-genotype, B-environmental conditions, *-statistically significant at p=0.05,
-statistically significant at p=0.01, *-statistically significant at p=0.001

Significant changes in seed width, seed length and seed thickness were observed in all mutant lines, although they were most significant in line 118 RM where the decreases were 1.2, 0.52 and 1.1 mm, respectively. Mutations were also registered in quality characters such as seed color. The initial genotype 147 R has black seeds with gray stripes, both at the ends and at the sides. The mutant lines did not have stripes. The lines 116 RM, 118 RM and 120 RM have black seeds, the lines 117 RM and 119 RM brownish seeds (Figure 3).

As a result of our study, negative genetic changes were registered for oil content in the kernel, 1000-seed weight, plant height, leaf width, leaf length, head diameter, number of branches, length of branches, diameter of lateral head, stem diameter, seed width, seed length and seed thickness. This accounted for 86.7% of the changes of all studied characters. The positive changes, concerning the leaf petiole length and number of leaves, account for 13.3% of all characters studied. Plant height was the least stable of all investigated characters, and oil content in the kernel the most stable one. The line 118 RM had the highest percent of significant negative changes (93%) for all investigated characters.



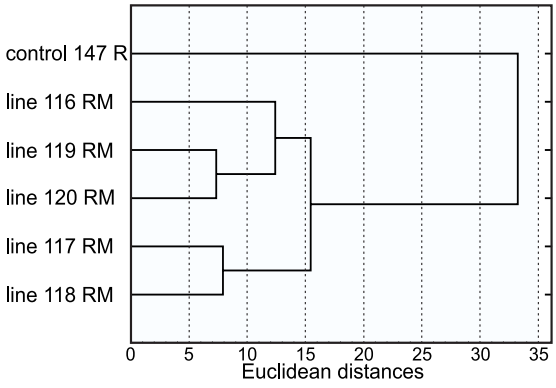


Figure 4: Degree of similarity between control line 147 R and mutant lines 116 RM, 17 RM, 118 RM, 119 RM and 120 RM

Figure 3: Seed shape and color of control line 147 R and mutant lines 116 RM, 117 RM, 118 RM, 119 RM and 120 RM

Factor B (environmental conditions) had a significant effect on a number of characters such as plant height, 1000-seed weight, number of leaves, head diameter, length of branches, leaf length, leaf width, stem diameter, oil content in the kernel, petiole length and the number of branches.

It was found that the diameter of branch head, seed width, seed thickness and seed length were stable and they were not affected by changes in the climatic conditions.

The interaction of the two investigated factors (A and B) was significant for plant height, head diameter, number of leaves, seed width and seed thickness.

Highest statistical significance among the investigated factors, including genotype × environment (G × E) interactions, was established for plant height.

Cluster analysis for agronomic and morphological characters

Euclidean distances between control line 147 R and mutant lines 116 RM, 117 RM, 118 RM, 119 RM and 120 RM

Cluster analysis was carried out to calculate the Euclidean distances between the investigated lines. The dendrogram of morphological and biochemical characters (Figure 4) differentiated the control and the new mutant lines into tree main clusters. The control line 147 R is in the first cluster, the lines 119 RM and 120 RM in the second and the lines 117 RM and 118 RM in the third cluster. The dendrogram shows a large Euclidean distance between the newly developed lines and the control line. Mutually closest and simultaneously standing at the greatest distance from 147 R were lines 119 RM and 120 RM and 117 RM and 118 RM, respectively.

Evaluation of sunflower mutant lines for resistance to the local *Orobanche* population

Broomrape is a major disease in parts of Europe, especially Spain, the Near East and China (Škorić, 1994). In most countries where sunflower is grown commercially, successful production is endangered by many fungal pathogens and parasites. Under extreme circumstances, losses may be severe, near 100% in parts or even entire fields. Because of the narrow germplasm of cultivated sunflower, mutagenesis is applied as an alternative method to conventional ones.

Except for the established morphological and biometrical changes, a mutation was observed in the reaction of the genotypes towards *Orobanche* parasite (Figure 5). The initial genotype 147 R was susceptible to Orobanche. The mutant lines produced from immature zygotic embryos by ultrasound treatment showed 100% resistance to the local broomrape population. These results were confirmed during the three years of evaluation. On the basis of these data, the conclusion was drawn that the resistance of the lines was due to the mutagenic treatment with ultrasound.

Figure 5: Evaluation of mutant line 116 RM, 117 RM and 118 R M (left) and control line 147 R (right) for resistance to the broomrape

The results allow us to assume that the resistance of the mutant sunflower lines to *Orobanche cumana* occurred as a result of mutation of a single dominant gene. Similar conclusion was made by Christov *et al.* (1996), analyzing the type of resistance to broomrape of mutant sunflower forms obtained through irradiation of air dry seeds with gamma rays. The authors found that the resistance was controlled by a single dominant gene. Resistant mutants with dominant genes were obtained after EMS treatment of pollen of tomato (Gavazi *et al.*, 1987). Many studies show that sunflower resistance to races A, B and C is monogenic (Pogorletskii and Geshele, 1976; Burlov and Kostiuk, 1976; Burlov and Arteminko, 1983; Tolmachev, 1984). According to Pacureanu-Joita *et al.* (1998), the gene *Or6* provides resistance to all races, from A to F.

In nature, polyploid perennial species are considered as sources of resistance. Among them, *H. tuberosus* has been most frequently used as a source of resistance to *Orobanche* (Pustovoit, 1978; Christov, 1996). Nikolova *et al.* (1998) observed a high degree of resistance to the local *Orobanche* population in the wild species *H. divaricatus* (M-015), *H. giganteus* (M-011), *H. glaucophyllus* (M-12), *H. grosseserratus* (M-014), *H. mollis* (M-082), *H. nuttallii* (M-088) and *H. smithii* (M-008). When hybridizing 100% resistant wild species with cultural sunflower, they observed variation in the resistance of the hybrid materials from 10 to 100%.

In our experiment we proved that 100% stable resistance of sunflower mutant lines to the local *Orobanche* population can also be obtained through induced mutagenesis, by treatment of immature zygotic embryos with ultrasound in particular. The same mutation, resistance to broomrape, was obtained in all variants involving the initial genotype 147 R. This allows us to assume that there are mutable locations in the cultural sunflower genome resulting from induced mutagenesis. Although induced mutagenesis is a random and unpredictable process, it is an irrefutable fact that the achieved mutation of resistance to broomrape is reliably inherited by the progenies of the fertility restorer lines.

Our results confirmed the conclusion of Skirvin (1978) who claimed that mutagenesis, physical or chemical, is effective for induction of mutations in tissue cultures. It was established that induced mutagenesis is more effective when using embryos at an early stage of development, as compared with air dry seeds (Atanassov, 1988).

CONCLUSION

Induced mutagenesis of immature zygotic sunflower embryo allows to develop economically useful characters, including resistance to *Orobanche*. Programs of sunflower breeding at DAI has produced morphological, biochemical and phytopathological variability by ultrasound treatment. Having combined induced mutagenesis in immature zygotic embryos with the embryo culture method, it was concluded that the new variability was exclusively due to the effect of the mutagen. This conclusion is confirmed by the fact that the embryo culture method alone does

not generate variation, due to a lack of mutagen factors in the nutrition medium and a short period of *in vitro* cultivation of immature zygotic embryos. Isolation of embryos before terminating their development allows their plating onto nutritive medium to grow *in vitro* seedlings.

The available literature on sunflower does not provide data on the treatment of immature zygotic embryos with ultrasound. In this respect our 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 already a functional tissue. This is expected to increase the frequency of mutations in comparison with the classical approach of treating air-dry seeds. Mutation techniques in combination with the embryo culture method allow plant breeders to produce a desired variation and obtain 5 generations within a single year.

Further evaluation is needed to obtain a more complete description of the new lines in terms of fertility restoration and general combining ability.

REFERENCES

Alonso, C., 1996. New highly virulent sunflower broomrape (*Orobanche cumana* Wallr.) phenotypes in Spain. Advances in parasitic plant research. 6th International Parasitic Weed Symposium. April 16-18, Cordoba, Spain.

Atanassov, A., 1988. Biotechnology in Agriculture, Zemizdat, Sofia: pp. 1-278.

Azpiroz, I.S., Vincourt, P., Serieys, H. and Gallais, A., 1988. La culture *in vitro* des embryous immatures dans l'acceleration du cycle de selection des lignees de tournesol et ses effects morphovegetatifs. Helia 10: 35-38.

Berretta de Berger, A.M., and Miller, J.F., 1985. Estudio genetico de seis fuentes de estatura reducida de planta en girasol. In: Proc. 11th Int. Sunflower Conf., Mar del Plata, Argentina, 10-13 March. Int. Sunflower Assoc. Toowoomba. Qld. Australia, pp. 651-657.

Burlov V.V. and Kostyuk, S.V., 1976. Inheritance of resistance to a local race of broomrape (*O. cumana* Wall.). Genetika 12: 151-155.

Burlov V.V. and Arteminko, Y.P., 1983. Resistance and virulence germplasm in the evolutionary associated pair of sunflower (*H. annuus* L.) and broomrape (*Orobanche cumana* Wall.). Genetika 19: 659-664.

Casteljon-Munoz, M., Romero-Munoz, Garcia-Torrez, 1991. *Orobance cernua* dispersion and its incidence in sunflower in Andalusia (Southern Spain). In: Wegmann, K., Musselman, L.J. (eds), Progress in *Orobanche* Research, Tübingen, Germany, Eberhard-Karls University, pp. 44-48.

Cheng, X.Y., Gao, M.W., Ling, Z.Q. and Lin, K.Z., 1990. Effect of mutagenic treatments on somaclonal variation in wheat (*Triticum aestivum* L.). Plant Breeding 105: 47-52.

Christov, M., 1996. Characterization of wild *Helianthus* species as sources of features for sunflower breeding. In: P.D.S. Calgari and D.J.N. Hind (eds). *Compositae*: Biology and Utilization. Proceeding of the International Composite Conference, Kew, 1994. (D.J.N. Hind, Editor-in-Chief), Royal Botanical Gardens, Kew, vol. 2. pp. 547-570.

Elliott, D.F., and Rao, K.R., 1982. Fast Transforms: Algorithms, Analyses, Applications. Academic Press, Inc., Orlando, FL, pp. 82-92.

Encheva, J., Ivanov, P., Tsvetkova, F. and Nikolova, V., 1993. Development of a new initial breeding material in sunflower (*Helianthus annuus* L.) using direct organogenesis and somatic embryogenesis. Euphytica 68: 181-185.

Encheva, J., Tsvetkova, F. and Ivanov, P., 2002. Creating genetic variability in sunflower through the direct organogenesis method, independently and in combination with gamma irradiation. Helia 25(37): 85-92.

Encheva, J., Kohler, H., Friedt, W., Tsvetkova, F., Ivanov, P., Encheva, V. and Shindrova, P., 2003. Field evaluation of somaclonal variation in sunflower (*Helianthus annuus* L.) and its application for crop improvement. Euphytica 130: 167-175.

Encheva, J., Tsvetkova, F. and Ivanov, P., 2003. Comparison between somaclonal variation and induced mutagenesis in sunflower (*Helianthus annuus* L.). Helia 26(38): 91-98.
Everitt, B.S., 1984. An introduction to latent variable models. New York, Chapman and Hall. pp. 125-132.
Fick, G.N., 1978. Sunflower breeding and genetics. In: J.F. Carter (ed.) Sunflower Science and Technology. Agron. Monograph. 19: 279-337.
Fernandez-Martinez, J., Malero-Vara, M.J., Minoz-Ruz, J., Ruso, J. and Domingez, J., 2000. Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance of the *Or-5* gene. Crop Science 40(2): 550-555.
Gamborg, O.L., Miller, R.A. and Ojima, K., 1968. Nutriment requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50: 151-158.
Gavazzi, G., Tonelli, C., Todesco, G., Arreghini, E., Raffaldi, F., Vecchio, F., Barbuzzi, G., Biasini, M., and Sala, F., 1987. Somaclonal variation versus chemically induced mutagenesis in tomato (*Licopersicum esculentum* L.). Theor. Appl. Genet. 74: 733-738.
Kaya, Y., Demerci, M. and Evci, G., 2004. Sunflower (*Helianthus annuus* L.) breeding in Turkey for broomrape (*Orobanche cernua* Loeffl.) and herbicide resistance. Helia 27(40): 199-210.
Murashige, T. and Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissues cultures. Plant Physiol. 15: 473-497.
Newport Instrument Ltd., 1972. Use of the Newport quantity analyzed as a replacement for solvent extraction for measuring the oil and fat content of oil seeds, chocolate, meat and other material. Newport Pagnell, England.
Novak, F.J., Daskalov, S., Brunner, H., Nestincky, M., Afza, R., Dolezelova, M., Lucretti, S., Herichova, A. and Hermelin, T., 1988. Somatic embryogenesis in maize and comparison of genetic variability induced by gamma radiation and tissue culture techniques. Plant Breeding 101: 66-79.
Nikolova, L., Christov, M. and Shindrova, P., 1998. New sunflower forms, resistant to *Orobanche cumana* Wallr., originating from interspecific hybridization. Proceedings of Fourth International *Orobanche* Workshop. September 23-26, Albena, Bulgaria.
Pacureanu-Joita, M., Vranceanu, A.V., Soare, G., Marinescu, A. and Sandu, I., 1998. The evaluation of the parasite-host interaction in the system *Helianthus annuus* L.-*Orobanche cumana* Wallr. in Romania. Proc. 2nd Balkan Symposium on Field Crops, June 16-20, Novi Sad, Yugoslavia, 1: 153-155.
Panchenko, A.N., 1975. An early diagnostic method for resistance to *Orobanche cumana* Wallr. Agricultural newspaper. N 2. 225-228 (in Russian).
Pogorletskii, P.K. and Geshele, E.E., 1976. Sunflower immunity to broomrape, downy mildew and rust. Proc. 7th Int. Sunflower Conf. Krasnodar, Russia, pp. 238-243.
Pustovoit, G.V. and Skuropat, Z.J., 1978. The inheritance in wild species of genus *Helianthus* (In Russian). Zbornik -VNIMK Pest and Diseases of Oil Crops. Krasnodar, Russia. pp. 44-48.
Shindrova, P., 1994. Distribution and race complex of broomrape (*Orobanche cumana* Wallr.) in Bulgaria. In: Pieterse A.H., Verkleij J.A.C., ter Borg S.J., (eds.) Proceedings of the 3rd International Workshop on *Orobanche* and related Striga research. Amsterdam, The Netherlands: Royal Tropical Institute. pp. 112–120.
Shindrova, P., Ivanov, P. and Nikolova, V., 1998. Effect of broomrape (*Orobanche cumana* Wallr.) intensity of attack on some morphological and biochemical indices of sunflower (*Helianthus annuus* L.). Helia 21(29): 55-62.
Škorić, D., 1994. Sunflower breeding for resistance to dominant diseases. *EUCARPIA Oil and Protein Crops Section*. Albena (Bulgaria), October 22-24, pp. 30-28.
Skirvin, R.M., 1978. Natural and induced variation in tissue culture. Euphytica 27: 241-266.
Tolmachev V.V., 1984. Geneticheskij kontrol priznaka zarazihoustoichivosti (*Orobanche cumana* Wallr.) u resistentnogo analoga linii podsolnechnika BK 373 B. Nauch. Techn. Bull. VNIMK. Krasnodar, Vir 84: 47-47.
Vranceanu, A.V., 1974. Sunflower. Academy of Romania Socialist Republic, pp. 1-332.

DESARROLLO DE LÍNEAS MUTANTES DE GIRASOL (*Helianthus annuus* L.) A TRAVÉS DE MUTAGÉNESIS INDUCIDA

RESUMEN

Se trataron embriones cigóticos inmaduros de la línea restauradora de girasol 147 R con ultrasonido antes de su traspaso a un medio de cultivo de embriones. Algunas plantas mutantes se aislaron y autofecundaron durante varias generaciones. Se obtuvieron nuevas variantes de girasol con cambios morfológicos y bioquímicos heredables a través de selección y autofecundación. Los cambios genéticos ocurridos durante la regeneración incluyeron quince caracteres morfológicos y bioquímicos. Como resultado de nuestro estudio, se registraron cambios genéticos negativos para la mayoría de los índices. Los cambios positivos opuestos incluyen los índices de largo de pecíolo y número de hojas. El índice altura de planta fue el menos estable de todos los caracteres estudiados. Se obtuvo una mutación para resistencia a la población local de *Orobanche cumana* (razas A-E) a partir de la línea búlgara susceptible 147 R. Las cinco líneas restauradoras mutantes investigadas poseyeron 100% de resistencia a *Orobanche*, cuya herencia fue estable en las generaciones siguientes. Nuestros resultados muestran que se puede utilizar exitosamente la mutagénesis en girasol para desarrollar nuevas líneas útiles para el mejoramiento basado en la heterosis.

DÉVELOPPEMENT DE LIGNÉES MUTANTES DE TOURNESOL (*Helianthus annuus* L.) PAR MUTAGENÈSE

RÉSUMÉ

Les embryons immatures de tournesol de la lignée restauratrice de fertilité 147 R ont été traité avec des ultrasons avant de les semer. Certaines plantes mutantes ont été isolées et autofécondées sur plusieurs générations. Les nouvelles formes de tournesol avec les changements morphologiques et biochimique dont elles ont hérité ont été obtenues au travers d'une sélection et d'une autofécondation. Les changements génétiques qui se sont produits durant la procédure de régénération ont inclus 15 caractères morphologiques et biochimiques. Notre étude montre des changements génétiques négatifs ont été enregistrés pour la plupart des indices. A l'opposé, les changements positifs concernent les indices de longueur du pétiole de la feuille ainsi que le nombre de feuilles. La hauteur de la plante a été le moins stable de touts les caractères étudiés. La mutation pour la résistance à la population locale de *Orobanche cumana* (race A-E) a été obtenue à partir de la lignée bulgare 147 R de témoin de sensibilité. La totalité des 5 lignées restauratrices mutantes étudiées possèdent 100% de résistance à l'*Orobanche* et ceci est fixé dans les générations suivantes. Nos résultats montrent que la mutagénèse du tournesol peut être utilisée avec succès pour développer de nouvelles lignées utiles à la sélection pour l'heterosis.