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DEVELOPING GENETIC VARIABILITY IN SUNFLOWER (*Helianthus annuus* L.) BY COMBINED USE OF HYBRIDIZATION WITH GAMMA RADIATION OR ULTRASOUND

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SUMMARY

As a result of hybridization between the Bulgarian fertility restorer line R 2574 and mixed pollen from a polycross population of known genetic composition, and treatment of immature embryos with gamma radiation or ultrasound, a large number of new fertility restorer lines were developed. Lines R 114, R 115, and R 116, subjected to investigation in this study, are characterized with significant changes concerning most of the studied characters. The increased number of seeds per head in lines R 115 and R 116, the increased 1000 seed weight in line R 114, the 100% resistance to the parasite *Orobanche* in lines R 114 and R 116, and the very good combining ability in hybridization are the desired combination in the breeding program of sunflower. The hybrids Rada and 80, developed with the participation of lines R 114 and R 116, considerably exceeded the mean standard (the Bulgarian commercial hybrids Albena and Super Start) in seed and oil yields, simultaneously possessing high resistance to the *Orobanche* population of races distributed in Bulgaria. In 2002, the hybrid Rada was registered for testing by State Variety Testing Commission and it exceeded the mean standard of the country. The combined use of interlinear hybridization with physical mutagenesis or ultrasound and the embryo culture method leads to increased genetic variability in sunflower and to a considerable shortening of the breeding process, producing 5 generations within a single year.

Key words: ***Helianthus annuus*, hybridization, embryo culture, gamma rays, ultrasound, new breeding material, resistance, *Orobanche cumana*, combining ability**

INTRODUCTION

Spontaneous mutations occur in nature with very low frequency. In this connection, interlinear hybridization and induced mutagenesis are some of the main means for fast development of genetic variability in plant species. Induced muta-

tions largely contribute to plant improvement worldwide and in some cases have a significant effect on the productivity of certain crops (Ahloowalia, 1990; Ashri, 1993; Maluszynski *et al.*, 1994; Micke, 1990, 1991; Novak, 1990, 1991).

Recently, the problem for acceleration of the breeding process has become especially important in many crops, particularly in sunflower. Necessity arises to use new methods and approaches for solving this problem. The embryo culture method has a special place among them. Using this method, up to 6 generations can be developed within one calendar year, which is not possible in conventional breeding. The embryo culture method allows isolation of embryos before terminating their development and their plating onto nutrition medium to grow *in vitro* seedlings. The embryo culture method has been used in 10-day old zygotic embryos for accelerated development of CMS-analogues in sunflower (Plotnicov, 1983). The authors developed 6 generations within one calendar year.

There are no publications among the available literary sources on the development of new breeding material in sunflower by the combined use of interlinear hybridization and gamma radiation or ultrasound, and acceleration of the breeding process through the embryo culture method.

The aim of this study was:

a to develop variable initial breeding material from sunflower by hybridization of line R 2574 to mixed pollen from a polycross population of known genetic composition (independently and after treatment of immature hybrid embryos with gamma rays at the dose of 8 Gy or ultrasound at the dose of 1.9 w/cm^2 for 1 min. before plating), and

b to accelerate the breeding process of the obtained hybrid materials by applying the embryo culture method.

MATERIAL AND METHODS

The Bulgarian self-pollinated branched fertility restorer line R 2574 (DAI-General Toshevo-resistant to *Plasmopara helianthi*) was used as a female component in interlinear hybridization. The male included mixed pollen from the following branched fertility restorer lines: R 2575 (resistant to *Plasmopara helianthi*), R 2572 (resistant to *Plasmopara helianthi*), R 2576 (resistant to *Plasmopara helianthi* and *Orobanche cumana*), R 1041 (resistant to *Plasmopara helianthi*), R 833 (resistant to *Plasmopara helianthi*), R 147 and R 7009 (both resistant to *Orobanche cumana*). The fertility restorer lines included in the polycross were of known quality composition, had good agronomic indices and good combining ability. The cross was realized under field conditions at DAI-General Toshevo. Hybrid embryos were obtained by sterilizing pollen from the female form with gibberellic acid (GA_3 - 0.045 g/l) and hand-pollination with mixed pollen from the polycross population.

***In vitro* cultivation of immature zygotic sunflower embryos**

The isolated immature zygotic embryos (13-16 days old) were plated on medium M for further growing 1/2 MS (Murashige & 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 and 16/8 h photoperiod for one week. A part of the embryos were treated with gamma radiation at the dose of 8 Gy, and another with ultrasound at the dose of 1.9 w/cm^2 for 1 min before plating on nutrition medium M. The plants which formed roots were transferred to soil and were further grown and self-pollinated under greenhouse conditions.

Biometric evaluation and biochemical analysis of mother line R 2574 and lines R 114, R 115 and R 116

The biometric evaluation and biochemical analysis of the female line and the newly developed ones were made on 10 plants each year, and they included 12 main agronomic characters. The weight of 1000 seed (g) was determined in three samples, each consisting of 50 seeds per head. Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) was used to determine oil content of air-dry seeds from the developed R lines (11 generation), as well as that of the female line. The statistical analysis of the results was performed according to Snedecor and Cochran (1957).

Hybridization

To determine the combining ability of the newly developed sunflower lines R 114, R 115 and R 116, the sterile analogue of the Bulgarian selfed line 2607 was used. The standards for comparing the new hybrids Rada, 80 and 79 were the Bulgarian commercial hybrids Albena and Super Start. The obtained hybrid combinations were tested for three years, 1997, 1998 and 2000, in the breeding fields of DAI according to the block-design method, in three replicates, the area of each replication being 10 m^2 (Barov and Shanin, 1965).

The phytopathological evaluation of the female genotype, the developed lines and hybrids was performed with regard to the local *Orobanche* population and the diseases *Phomopsis* and *Phoma* at the Sunflower Phytopathology Laboratory and infection fields of DAI - General Toshevo. The evaluation was carried out according to standard methodologies during the period 1997-2001 using 0-4 scale for *Phomopsis helianthi*, 1/3-3/3 scale for *Phoma macdonaldii* and 0 to 100% for *Orobanche cumana*.

RESULTS AND DISCUSSION

A large number of new fertility restorer lines were obtained as a result of selfing and individual selection of materials developed from the cross of line R 2574 and mixed pollen from the polycross population of known genetic composition. Lines R 114, R 115 and R 116 (Figures 1 and 2) were selected due to their good combining

ability and significant differences from the female line R 2574 concerning some important morphological and biochemical characters. Table 1 shows data on the significant changes of the mean values for six morphological characters. Regarding plant height, the observed difference was only in direction towards increase. The difference from the female form was considerable, from 14.14 cm to 26.24 cm.

Highly significant positive changes were registered also for the number of leaves (lines R 115 and R 116), leaf width (all lines), leaf length (all lines), petiole length (all lines), internode length (all lines), number of branches (all lines) and head diameter (all lines) (Tables 1 and 2). Only the observed values of the indices length of branches and head diameter of lines R 115 and R 116 (Table 2) and number of leaves of line R 114 (Table 1) were close to the corresponding values of the female parent. Significant morphological and biochemical changes in the indices of R lines (11 generation) developed from the cross of line R 2574 and mixed pollen from a polycross population for the period 1997-1999 (averaged data).

Table 1: Significant morphological changes in the indices of R lines (11 generation) developed from a cross of line R 2574 and mixed pollen from polycross population for the period 1997-1999 (averaged data)

Genotype	Plant height	Number of leaves	Leaf width	Leaf length	Petiole length	Internode length
	Mean (cm)	Mean (no)	Mean (cm)	Mean (cm)	Mean (cm)	Mean (cm)
Control 2574 R	90.63	21.00	15.20	16.23	10.73	4.83
Line R 114 - 8 Gy	104.77 +c	20.00	18.00 +c	18.43 +c	11.43 +c	6.03 +c
Line R 115 - us	107.30 +c	24.00 +c	19.93 +c	21.13 +c	12.63 +c	5.70 +b
Line R 116 - us	116.87 +c	24.00 +c	19.50 +c	18.77 +c	12.13 +b	6.03 +c

*,** and *** = significant differences at the levels of 0.05, 0.01 and 0. 001, respectively

Table 2: Significant morphological and biochemical changes in the indices of R lines (11 generation) developed from the cross of line R 2574 and mixed pollen from a polycross population for the period 1997-1999 (averaged data)

Genotype 2574 R	Number of branches	Length of branches	Stem diameter	Head diameter	Number of seeds per head	Oil percent	1000-seed weight
	Mean (no)	Mean (cm)	Mean (mm)	Mean (cm)	Mean (no)	Mean (%)	Mean (g)
Control	8.00	30.53	20.73	11.70	153.00	44.20	41.90
line R 114-8 Gy	17.00 +c	38.20 +c	25.87 +c	13.93 +c	201.00	41.50 -c	45.52 +a
line R 115- us	18.00 +c	30.43	26.43 +c	13.00 +b	385.00 +c	42.30 -b	36.70 -a
line R 116- us	23.00 +c	30.50	22.53 +a	13.03 +c	343.00 +b	46.20 +b	43.57

The changes in the number of seeds per head included positive changes in relation to the control within the range from 48 to 232. The data for lines R 115 and R 116 (Table 2) were significant. The data for the index 1000-seed weight included both positive and negative changes (Table 2). A significant increase of 3.6 g was observed only in line R 114. Data on the change of the mean values for oil content in seed, one of the most important agronomic indices are given in Table 2. A significant increase of 2% was observed in line R 116.

Figure 1: Line R 114

Figure 2: Line R 116

Figure 3: Hybrid Rada with participation of line R 114

Figure 4: Hybrid 80 with participation of line R 116

Evaluation of lines and hybrids for resistance to some economically important diseases on sunflower

A study was carried out on the promising lines R 114, R 115 and R 116, as well as on the hybrids Rada, 79 and 80, for resistance to the diseases *Phomopsis helianti*, *Phoma macdonaldii* and the parasite *Orobanche cumana*.

A high resistance of the lines to the population of the broomrape races distributed in Bulgaria was established: 100% for R 114, 51% for R 115 and 100% for R 116, as well as tolerance to *Phomopsis* and *Phoma*. The results from the test of the hybrids showed 76 to 81% resistance to broomrape. The hybrids Rada and 80 carry resistance to *Plasmopara helianthi*, inherited from the Bulgarian female line 2607.

Study of the production potential and biological characteristics of the hybrids Rada and 80 developed with participation of lines R 114 and R 116

A 3-year test of lines R 114 and R 116 showed 100% restoration ability and very good combining ability. The sterile analogue of the Bulgarian selfed line 2607 was used as the tester. A two-factor dispersion analysis of the hybrid Rada (Figure 3) and the hybrid 80 (Figure 4) was carried out for the indices of seed yield and oil yield per dekar and duration of the vegetation period (Figures 5, 6 and 7).

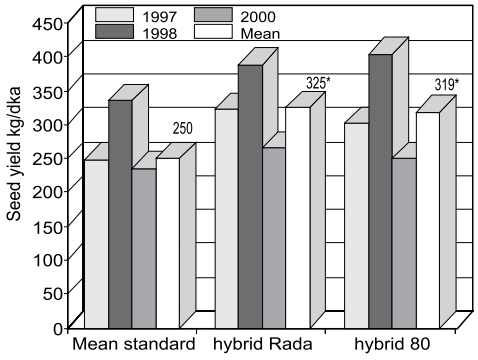

Figure 5: Seed yields of the hybrids Rada and 80 and mean standard (commercial hybrids Albena and Super Start) during 1997-2000 (-P = 5%)*

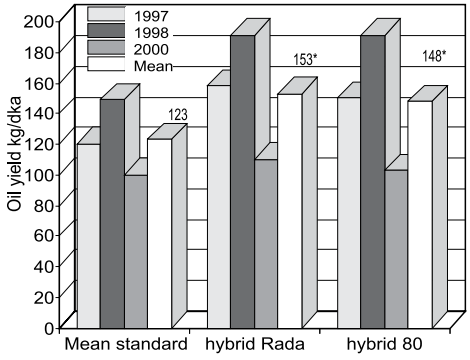

Figure 6: Oil yields of the hybrids Rada and 80, and the mean standard (commercial hybrids Albena and Super Start) during 1997-2000 (-P = 5%).*

The seed yield of hybrid Rada, averaged for the 3-year period of testing of line R 114, exceeded the mean of standards (the hybrids Albena and Super Start) by 75 kg/dka or 30%, which is a significant difference. The seed yield of the hybrid 80, developed with the participation of line R 116, was significantly higher by 69 kg/dka or 27.6%.

There was a significant difference for the index of seed yield also in factor B (year), which was probably determined by the different climatic conditions during each of the study years. In 1998, the seed yield was higher by 85.0 kg/dka or 29% in comparison with 1997 (total for the mean standard and the hybrids Rada and 80),

while in 2000 a reduction by 41.0 kg/dka or 14% was observed in the value of this index. The low seed yield in 1997 can be explained by the high precipitation during July and August (121.5 and 129.5 mm, respectively, the mean precipitation sums being 51.1 and 41.3 mm, respectively), i.e., during the stages of flowering, seed formation and filling. The low seed yield in 2000 was due to the low precipitation during July and August of 2.4 and 2.7 mm, respectively.

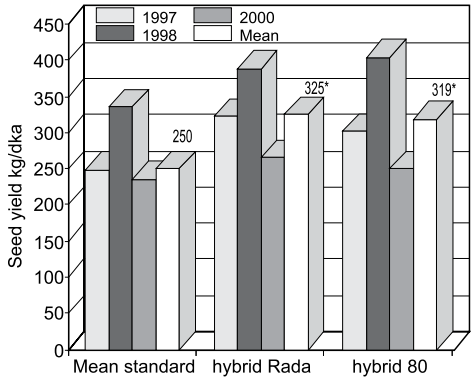

Figure 7: Vegetation periods of the hybrids Rada and 80, and the mean standard (the commercial hybrids Albena and Super Start) during 1997-2000 (-P = 5%).*

The results from the dispersion analysis of oil yield showed that the hybrids Rada and 80 exceeded the mean value of this index by 30 kg/dka and 25 kg/dka, respectively, or by 24.0% and 20.0%, respectively. These differences were statistically significant. There were also statistical differences with regard to factor B (year). The 1998 total for the mean standard and the two investigated hybrids exceeded the 1997 total by 34 kg/dka or 23.8%. In 2000, a decrease of the mean value in relation to 1997 was 39.0 kg/dka or 27%.

Besides their higher seed and oil yields, the hybrids Rada and 80 were characterized by a longer vegetation period (5 and 4 days, respectively). In contrast to the mean standard, the two hybrids showed a considerable variation in the vegetation period duration in all three years of study. There were also significant differences with regard to factor B (year). The vegetation period in 1997 was longer than in 1998 and 2000 by 15 and 12 days, respectively, probably due to the considerable rainfalls during the vegetation periods.

The two hybrids demonstrated a decreased plant height in relation to the mean standard by 4 and 5 cm, respectively. These differences were not statistically significant.

The hybrid 79 developed with the participation of line R 115 exceeded the mean standard in seed and oil yields by 41 kg/dka and 24 kg/dka, respectively, or by 14% and 18%, respectively. Due to the lack of 3-year data, this hybrid was not included in the figures.

CONCLUSION

The newly developed lines were characterized by increased oil content in seed, increased number of seeds per head, increased 1000-seed weight, resistance to the population of *Orobanche* races distributed in Bulgaria and very good combining ability, which is a desirable combination in the sunflower breeding programs.

The hybrid Rada, derived from line R 114, was registered for testing at State Variety Testing Commission in 2002 and it exceeded the mean standard during the tests.

It can be concluded on the basis of this study that the combined use of hybridization and physical mutagenesis or ultrasound and the embryo culture method leads to the development of variable initial material in sunflower and considerably shortens the breeding process by producing 5 generations within a single year.

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CREACIÓN DE VARIABILIDAD GENÉTICA EN GIRASOL (*Helianthus annuus* L.) COMBINANDO LA HIBRIDIZACIÓN CON LA RADIACIÓN GAMA O CON EL ULTRASONIDO

RESUMEN

Como resultado de hibridización entre el restaurador de fertilidad búlgaro R 2574 y el polen mezclado de una población policruzada del contenido genético conocido, en combinación con el tratamiento de embriones inmaduros con la radiación gama o con el ultrasonido, se ha creado un gran número de nuevas líneas restauradoras. Las líneas R 114, R 115 y R 116, que han sido objeto de esta investigación, se caracterizan por los significantes cambios, respecto a la mayoría de las propiedades investigadas. El número de semilla aumentado, por cabeza en las líneas R 115 y R 116, la masa aumentada de 1000 semillas en la línea R 114, una resistencia de 100% a parásitos *Orobanche cumana* en las líneas R 114 y R 116, y una habilidad muy buena de combinación en la hibridización, son la combinación más deseable para los programas de la mejora genética de girasol. El híbrido Rada y el híbrido 80, se han creado con la participación de las líneas R 114 y R 116, han superado significadamente el promedio de los híbridos estándar (híbridos comerciales búlgaros Albena y Super Start) respecto al rendimiento de semilla y aceite, y además tienen alta resistencia a las populaciones de *Orobanche cumana*, divulgado en Bulgaria. El híbrido Rada en el año 2002 fue comunicado para los *tests* en la Comisión de Variedades de Bulgaria, superando el promedio del estándar para ese país. La utilización combinada de la hibridización interlineal con la mutagénesis física o con el ultrasonido y del método de cultivo de embrión, lleva hasta el aumento de la variabilidad genética en girasol y significativamente acorta el proceso de la mejora genética produciendo cinco generaciones en un año.

CRÉATION DE VARIABILITÉ GÉNÉTIQUE DANS LE TOURNESOL (*Helianthus annuus* L.) PAR LA COMBINAISON D'HYBRIDATION ET DE RAYONS GAMMA OU D'ULTRASON

RÉSUMÉ

Un grand nombre de nouvelles lignes de restaurateurs a été créé par hybridation entre le restaurateur de fertilité bulgare R 2574 et le pollen mêlé d'une population à croisements multiples de structure génétique connue en combinaison avec le traitement d'embryons immatures par irradiation gamma ou ultrason. Les lignes R 114, R 115 et R 116 qui faisaient l'objet de cette étude, se caractérisent par des changements importants pour ce qui concerne la plupart des propriétés étudiées. Une augmentation du nombre de graines par tête dans les lignes R 115 et R 116, une augmentation de la masse de 1000 graines dans la ligne R 114, une résistance de 100% au parasite *Orobanche cumana* dans les lignes R 114 et E 116 et une très bonne aptitude de combinaison lors de l'hybridation sont les combinaisons les plus souhaitables pour les programmes de culture du tournesol. L'hybride Rada et l'hybride 80, créés avec la participation des lignes R 114 et R 116 ont dépassé de manière importante la moyenne des hybrides standard (hybrides commerciaux bulgares Albena et Super Start) pour ce qui concerne le rendement en graines et en huile et de plus, ils ont montré une grande résistance à la population

d'orobanche répandue en Bulgarie. En 2002, l'hybride Rada a été inscrit à des tests devant la commission des espèces de Bulgarie et a été supérieure à la moyenne du pays. L'usage combiné de l'hybridation entre lignes avec mutagénèse physique ou ultrason et les méthodes de culture des embryons conduit à une augmentation de la variabilité génétique dans le tournesol et abrège de manière importante le processus de culture en produisant cinq générations en une seule année.