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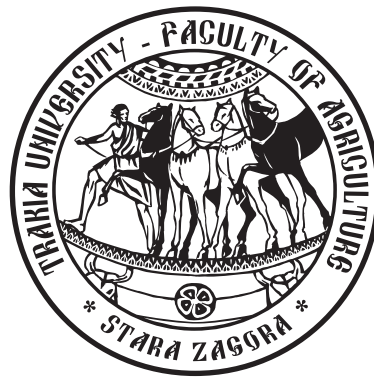
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Developing sunflower fertility restorer lines from commercial hybrids by using *in vitro* technique

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Abstract. The experimental research work encompassed the period 2007 – 2009 and was carried out at Dobrudzha Agricultural Institute, General Toshevo. Six highly productive commercial hybrids (X1, X2, X3, X4, X5 and X6) were used as initial material for production of sunflower fertility restorer lines by the method of gamma-induced parthenogenesis. Mixed pollen treated with dose 600 Gy was used for parthenogenetic induction. Thirty plants from each hybrid were pollinated with irradiated pollen. A total of 833 immature embryos were obtained from the six hybrids, which were further grown under *in vitro* conditions till formation of young plantlets. Normal growth and development were observed in 569 embryos. The obtained young plantlets were transferred to soil and grown till maturity in a greenhouse. Of these plants, 326 were fertile and were potential double haploid lines. After selfing under greenhouse conditions, a total of 6 016 P2 seeds were obtained from them. In 2008 the P2 progeny was reproduced to fertile plants under field conditions. One hundred ninety-eight double haploid R lines were selected. In 2009 the double haploid lines were investigated under field conditions, performing morphological, biometrical, phytopathological and phenological evaluation of the lines. The lines with origin from hybrids X3 and X1 possessed better developed vegetative mass, well formed and large heads on the side branches and powerful stems. One hundred fifty-nine lines were selected. Ninety-eight of them demonstrated full resistance to downy mildew race 731. The resistant lines originated from hybrids X1, X2, X3, X4 and X6. The lines obtained from hybrid X5 did not carry genes for resistance to downy mildew race 731. Phytopathological evaluation was done also with regard to the diseases phoma, phomopsis and alternaria under field conditions. Ninety-five percent of the lines showed full resistance to phoma (rank 0) and high resistance to moderate susceptibility to phomopsis (rank 1 – 3). In 16 % of the lines full resistance to alternaria (rank 0) was found.

Keywords: sunflower, fertility restorer lines, *in vitro* methods, parthenogenesis

Introduction

Sunflower (*Helianthus annuus* L.) has indisputable economic importance in Bulgaria and elsewhere in the world. Higher yields, seed quality and resistance to diseases and pest are major aims of sunflower breeding, the end purpose being the development of new hybrids which meet the modern requirements. A good approach for the timely realization of the breeding programs and their aims is to combine the conventional breeding methods with some biotechnology techniques. The possibility to significantly shorten the time necessary for developing of genetically stable lines is one of the achievements of plant biotechnology. Since sunflower is one of the crops with rather conservative response to the use of *in vitro* techniques such as the anther, pollen and ovular techniques, by combining conventional and biotechnology approaches, an efficient method was developed at Dobrudzha Agricultural Institute (DAI) for production of double haploid sunflower lines based on gamma-induced parthenogenesis. (Todorova et al., 1997; Todorova and Ivanov, 2000; Todorova, 2002). This method significantly reduces the time necessary for development of parental lines for heterosis breeding in sunflower. The properties of the double haploid lines obtained through this method are determined by the genetic potential of the initial material. This method allows producing genetically stable lines from sunflower within 2 years.

The gamma-induced parthenogenesis is applicable to other plant species as well. Good examples in this respect are the results obtained in tobacco (Musial and Przywara, 1999; Pandey and Phung, 1982), cabbage (Dore, 1989), apple (Zhang and Lespinasse, 1991), onion (Dore and Marie, 1993), rose (Meynet et

al., 1994; Meynet et al., 1996), cucumber (Faris et al., 1999; Caglar and Abak, 1999; Lotfi et al., 1999) and other crops.

The aim of this investigation was to develop fertility restorer lines in sunflower by using the gamma-induced parthenogenesis method.

Material and methods

The experimental work encompassed the period 2007 – 2009 and was carried out at DAI. The method of gamma-induced parthenogenesis (Todorova, 2002) was applied. Six commercial hybrids of high productivity were used as initial material for obtaining fertility restorer sunflower lines (X1, X2, X3, X4, X5 and X6). The high production potential of the hybrids and their resistance to some economically important diseases on sunflower made them valuable initial material for production of fertility restorer lines.

Ten fertility restorer lines developed at DAI were used as pollen sources. The pollen collected from these lines was mixed at equal parts and after gamma-irradiation it was used for pollination of the mother plants. The pollen was irradiated with dose 600 Gy, using Cs 137 as a source of ionizing radiation. To sterilize the male gametophyte, the initial plants from the hybrids were treated with water solution of gibberellic acid (GA3) at concentration 45 mg/l. The solution was applied directly on the forming bud (1 – 1.5 cm in diameter) and the top pair of leaves. Double treatment was applied at 2-day interval. To avoid foreign pollination, the plants were isolated immediately before beginning of flowering. Thirty plants from each hybrid were pollinated and it was found that the chemical

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castration was effective and there were no traces from the plant's own pollen.

The isolation and cultivation of the obtained embryos was performed according to the methodology of Azpiroz et al. (1988). The formed young parthenogenetic plants (P1) were transferred to soil and stabilized at temperature 20°C/15°C day/night, 70% relative air humidity and photoperiod 16/8 h day/night for 30 day, then the daily temperature was increased to 25°C±2°C, and the night temperature to 20°C±2°C. Under these conditions the plants were self-pollinated and grown till maturity. The progeny (P2) of the fertile diploid plants was tested for resistance to downy mildew (*Plasmopara helianthi*) under greenhouse conditions. The investigated plants with 100% resistance were evaluated for morphological traits under field conditions. Five plants from each number with uniformity in their P2 progeny were selected and self-pollinated. The P3 progeny was tested for resistance to downy mildew, phoma, phomopsis and alternaria.

The resistance of the obtained double haploid plants to economically important diseases such as downy mildew, phoma, phomopsis and alternaria was investigated at the Plant Pathology Laboratory and in the infection fields of DAI. The following methods for evaluation were used:

- The inoculation of the breeding materials with downy mildew (*Plasmopara helianthi* Novot.) was carried out according to a standard methodology (Vear and Tourvieille, 1987) adapted for work under the conditions of DAI. The evaluation for resistance was done according to a 0-100% scale.

- The evaluation for resistance to phoma (*Phoma macdonaldii* Boerema / *Phoma oleracea* var. *helianthi-tuberosi* Sacc) was carried out under infection field conditions. Inoculation was performed at stage budding – beginning of flowering by the method of Fayzalla and Maric (1981). The pathogen rate of attack was read in a scale from 1/3 to 3/3.

- The evaluation for resistance to phomopsis (*Phomopsis helianthi* Munt. – Cvet.) was done in infection field. The method of Encheva and Kiryakov (2000) was applied for inoculation of the materials. The attacking rate of the pathogen was read according to a 0-4 scale.

For evaluation of the parthenogenic response of the hybrids involved in this trial, one-factor dispersion analysis was used (Genchev et al., 1975) as well as t-criterion. All data were processed with the help of Biostat Version 5.1 (Penchev, 1998) and Statistica, Version 5.1 D, Stat Soft. Inc.

Results and discussion

From the six initial hybrids, a total of 833 immature embryos were obtained after applying the method of gamma-induced parthenogenesis, with different percent of the respective hybrids (Figure 1). Highest number of parthenogenetic embryos was obtained from hybrids X2, X3, X4, X1, and significantly lower from hybrids X5 and X6, which can be explained with the different responsiveness of these genotypes to parthenogenetic induction (Todorova et al., 2004). The embryos were grown *in vitro* till formation of young plantlets. Normal growth and development were observed in 569 embryos. The young plantlets obtained from them were further grown under greenhouse conditions till maturity. Out of these plants, 326 were fertile. After selfing in a greenhouse, a total of 6016 P2 seeds were obtained from them (Table 1).

Following the evaluation for resistance to downy mildew, 269

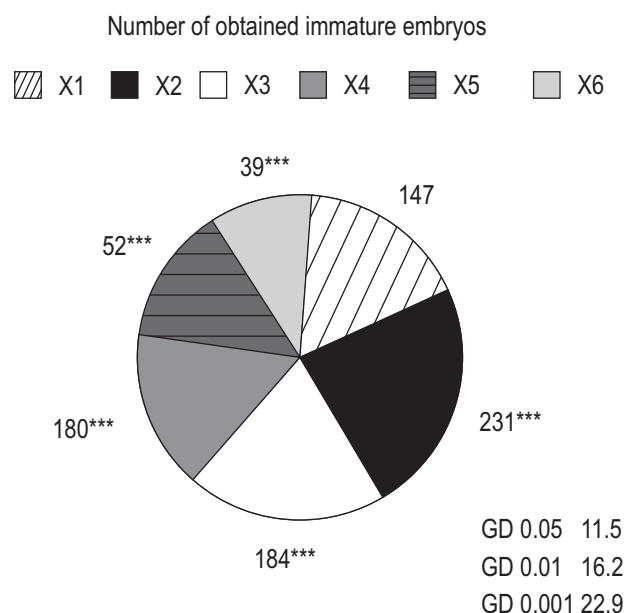


Figure 1. Responsiveness of *Helianthus annuus* L. hybrids to induction of parthenogenetic development by use of irradiated pollen

*, **, *** – significances of the differences in the parthenogenic responsiveness of the initial hybrids at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$

Table 1. Number of young plantlets and seeds obtained from parthenogenetic embryos

Initial hybrids	No of young plantlets	No of sterile plants	No of fertile plants	No of produced seeds
X1	76	34	42	741
X2	160	71	89	1841
X3	154	64	90	1680
X4	143	59	84	1363
X5	30	10	20	384
X6	6	3	1	7
Total	569	241	326	6016

ones with 100% resistance to downy mildew race 731 were chosen for reproduction and selection under field conditions. The progeny of the single fertile plant obtained from hybrid X6 showed complete susceptibility to downy mildew and was discarded from further investigations. After field evaluation, 196 double haploid R lines with good morphological uniformity and stem branches were selected. The lines with origin from hybrids X4 and X5 possessed better vegetative mass, with large and well-shaped inflorescences on the side branches and powerful stem. During the next reproduction and selection, 159 lines were chosen, 98 of which exhibited full resistance to downy mildew race 731. The resistant lines were with origin from hybrids X2, X3, X4 and X5 (Figure 2).

The lines obtained from hybrid X1 did not carry genes for resistance to downy mildew race 732. The phytopathological evaluation for resistance to phoma, phomopsis and alternaria showed that 95 % of the lines had full resistance to phoma (rank "0") and high resistance to moderate susceptibility to phomopsis (rank 1

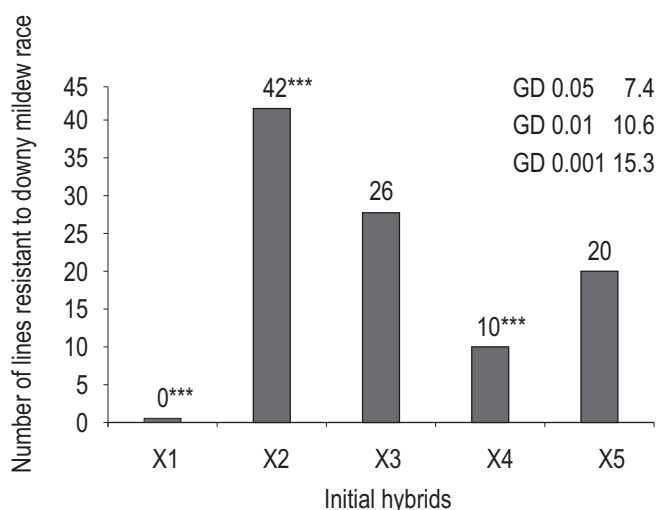


Figure 2. Lines resistant to downy mildew race 731 obtained from commercial sunflower hybrids through the method of gamma-induced parthenogenesis

*, **, *** – significance of the differences in the number of obtained lines with resistance to downy mildew at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$

– 3). In 16% of the lines full resistance to alternaria was found (rank "0").

The complex evaluation of the lines showed that 159 of them had good morphological uniformity and resistance to the economically important diseases on sunflower. The highest number of lines with good economic characteristics was obtained from hybrids X2 and X3 (Figure 3). The relative percentage of the hybrids from the total number of developed lines with valuable indices did not preserve the ratios determined when reading the number of produced parthenogenetic embryos. From hybrid X5, the relative percentage of which was significantly lower than the percentage of hybrids X1 and X4 with regard to the number of produced immature embryos as a result from induced parthenogenetic development, greater number of lines with economic importance were obtained in comparison to hybrid X1, the number being almost equal to hybrid X4 (Figure 3). This can be explained by the fact that the breeding value of the double haploid lines is determined by the genetic potential of the initial material with regard to the valuable breeding indices, while the number of the obtained parthenogenetic embryos is a function of the parthenogenetic responsiveness of the initial material.

The results from the experiment and their analysis show that the use of hybrid X6 for production of doubled haploid lines through the method of gamma-induced parthenogenesis is not efficient. In this case to produce a sufficient number of lines from this hybrid it is recommendable to apply forced self-pollination and selection for obtaining of genetically stable lines.

Conclusion

Hybrids X2, X3, X4 and X1 have well expressed parthenogenetic responsiveness and are suitable initial material for production of lines by the method of gamma-induced parthenogenesis. The use of hybrid X6 for production of double haploid lines by the method of gamma-induced parthenogenesis is

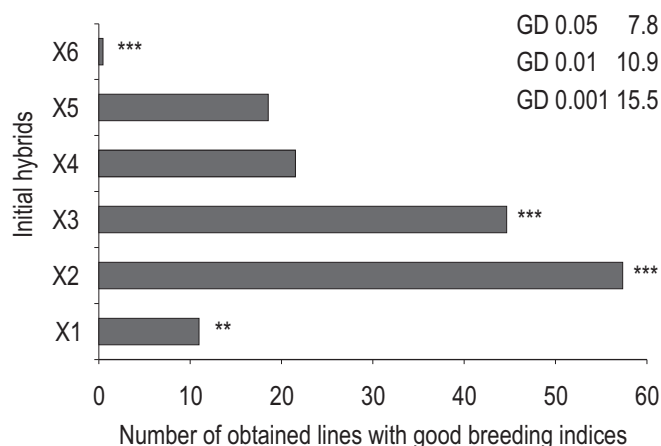


Figure 3. Economically valuable parthenogenetic lines obtained from *Helianthus annuus* L. hybrids

*, **, *** – significance of the differences between the hybrids with regard to the number of obtained lines with good economic properties at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$

not efficient. By the combined use of conventional and *in vitro* methods, 159 fertility restorer lines with good breeding characteristics were obtained.

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