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Heterosis for Agronomically Important Traits in Sunflower Hybrid Rada, Developed with Mutant Restorer Line 12002 R

Abstract: Hybrid Rada was developed with simple cross of Bulgarian line cms2607 and mutant line 12002 R. Mutant line was developed after treatment of immature sunflower (*Helianthus annuus* L.) zygotic embryos of fertility restorer line R 2574 with gamma irradiation at dose of 8 Gy for 1 min. Hybrid Rada was tested for three years in testing plots of Dobroudja Agricultural Institute – General Toshevo and for two years at the seven locations of State Variety Testing Commission. The ANOVA procedure proves that the parents (2607 A and 12002 R) and the received hybrid Rada are with different genetic potential in the studied indices. In our study, all six characters recorded positive and significant heterosis in the direction of both relative to parental average (h1) and relative to better parent (h2). The correlations of the hybrid Rada with the mean values of the parental lines (h1) and with the mean value of the parent with higher indices (h2) were statistically significant. In h1, the variation was from 1.52% to 17.92%; in h2, the variation was within 1.49–12.81%. The highest positive heterotic effect (h1 = 17.92%) in the hybrid Rada was for trait seed yield per head relative to parental average h1. The model of inheritance of studied indices is additive dominant with partial or full dominance in relation to parent with the higher value. This was markedly expressed for indices seed yield per head.

Keywords: *Helianthus annuus*, gamma irradiation, mutant line, hybrid, heterosis, resistance, *Plasmopara helianthi*, *Phomopsis helianthi*, *Phoma macdonaldii*, *Orobanche cumana*

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Introduction

Intense competition existing in the domestic and global sunflower markets makes it necessary to constantly develop new hybrids with a higher genetic potential for seed and oil yields per unit area, a wider range of disease resistance, and greater adaptability than that can be found in the existing hybrids (Skorić *et al.*, 2004). Worldwide there are hybrids tolerant or resistant to the economically important diseases on sunflower and the parasite *Orobanche* (Skoric, 1985); they, however, quickly drop out of national production lists due to the occurrence of new more virulent races which impose the necessity of new resistant varieties and hybrids. Sunflower is a crop of high risk due to losses caused by diseases. Under conditions favorable for development of the fungal pathogens causing *Plasmopara helianthi*, *Phoma macdonaldii*, *Phomopsis helianthi*, and the parasite *Orobanche cumana*, the crop may be entirely compromised.

The changes in the varietal structure during the last years and in the agronomy practices of sunflower cultivation created favorable conditions for propagation and rapid distribution of parasite *Orobanche cumana* in Bulgaria. As a result it is expanding to new territories, and the percent and rate of attack have increased. Significant changes occurred in the broomrape population as well: it enriched with new races of greater virulence (Shindrova, 1994; Shindrova, 2006). Similar processes are observed in other countries as well – Turkey, Romania, Spain, and so forth (Pacurenu-Joita *et al.*, 1998; Kaya *et al.*, 2004; Fernandez-Martinez *et al.*, 2000). This situation requires searching for means of disease control and diminishing the losses they cause. The appearance of new races that overcome preceding resistances maintains a challenge between *Orobanche* and breeders. The *Orobanche cumana* resistance sources come from old cultivated sunflower populations, from wild *Helianthus*, or from mutagenesis (Bervillé and Seriey, 2001).

Several sunflower mutant lines were obtained from interspecific hybridization between *H. annuus* × *H. eggertii* (F13) line 7041, *H. annuus* × *H. tuberosus* (F13) line 7009, and *H. rigidus* × *H. annuus* (F13) line 7043. Some of the sunflower mutant lines and remote hybrids showed a complete resistance or an increased resistance to *Orobanche cumana* (Batchvarova, 2001). Christov *et al.* (2009) reported 13 perennial and 3 annual *Helianthus* species resistant to parasite broomrape, race E. Resistance to the race F broomrape populations was found in germplasm of both cultivated and wild sunflower (Akhtouch *et al.*, 2002; Fernandez-Martinez *et al.*, 2010; Seiler, 2010; Gulya *et al.*, 2010). The resistance to broomrape, race G established in annual *H. argophylus* was successfully transferred to cultivated sunflower (Valkova *et al.*, 2009).

Instead wild species positive results were obtained when induced mutagenesis and tissue cultivation were combined appropriately. Several sunflower mutant lines were obtained after treatment with gamma rays (Co^{60}) at doses of 150 and 200 Gy from cultivar VNIIMK 8931: No. MX199, No. MX655, No. 6116. Some of the sunflower mutant lines and remote hybrids showed a complete resistance or an increased resistance to *Orobanche cumana* (Batchvarova, 2001). Encheva *et al.* (2003, 2008a, 2008b) and Encheva (2009) reported the development of sunflower lines resistant to diseases and *Orobanche cumana* through the embryo culture method in combination with ultra sound.

Heterosis and hybrid seed production in sunflower has played a major role in improving crop productivity and quality in order to feed the ever-increasing human population. An important direction of research work on sunflower is heterosis breeding. It became possible after the discovery of the first CMS source in sunflower by Leclercq (1969) and the finding out of efficient fertility restorers (Enns *et al.*, 1970; Kinman, 1970; Leclercq, 1971; Vranceanu and Stoenescu, 1971).

The phenomenon of heterosis is not present in all hybrid combinations of the F1 generation; however, heterotic effects differ from trait to trait. The manifestation of heterosis for agronomically important traits is a prerequisite for obtaining productive hybrids (Skorić *et al.*, 2002).

The first Bulgarian hybrids based on CMS were tested in competitive varietal trials during 1973 (Stoyanova *et al.*, 1977; Ivanov *et al.*, 1988). In 1979 the first Bulgarian hybrid Super Start was released and distributed on the entire territory of Bulgaria (Gotsov *et al.*, 1981; Ivanov *et al.*, 1988). After triple testing, hybrid Albena was introduced in France, occupying at its prime about 40% from the total area sown with sunflower in this country. Due to its good adaptability and productivity, it gained the position of a world standard. It was successfully grown in Germany and Austria. In Bulgaria, over 20 sunflower hybrid varieties have been developed and released.

In the recent years, the producers in Bulgaria and abroad prefer higher yielding hybrids that possess the ability to overcome the constantly changing stress factors of the environment (diseases, parasites, pests on sunflower, changeable environmental and meteorological conditions) with the aim to limit excess expenditures for production and storage of sunflower seeds.

The aims of this study were:

(a) to estimate the degree of heterosis over a parental mean and better parent for some important agronomical traits in sunflower hybrid Rada (2607 A $\times$ 12002 R) and
(b) to evaluate hybrid Rada for resistance to *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii*, and parasite *Orobanche cumana*.

Materials and methods

A part of the experiments were carried out at the trial field of Dobroudja Agricultural Institute (DAI) – General Toshevo and State Variety Testing Commission, and another under laboratory conditions. The Bulgarian fertility restorer line R 2574, which 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 gamma irradiation is to be genetically pure, i.e. homozygotic to the highest possible degree. Therefore, the control line R 2574 with very good morphological uniformity due to long selfing (over 32 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) followed by several washings with sterile distilled water. Sixty zygotic embryos were plated for each variant.

The isolated immature zygotic embryos were treated with gamma irradiation (Cs137) at dose of 8 Gy 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 and 16/8 h 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 that formed roots were transferred to soil and were further grown and self-pollinated under greenhouse conditions.

Field experiments

As a result from long-term selfing and individual selection, new sunflower line 12002 R was produced in R9 generation. The main criteria for selection were resistance to *Orobanche cumana* and good combining ability.

Hybridization

To determine the combining ability of the new developed sunflower mutant line 12002 R, the sterile analogue of the Bulgarian selfed line 2607 was used. The standards for comparing the new hybrid Rada developed were the Bulgarian commercial hybrids Albena, San Luka, Perfect, and Mercury and French hybrid Diabolo PR.

The obtained hybrid was tested for three years 1997–1999 at the trial field of DAI – General Toshevo and for two years 2002 and 2004 at the seven locations of State Variety Testing Commission according to the block-design method, in three replications, the area of each replication being 25 m^2.

Biometric evaluation of parental lines and hybrid Rada

The biometric evaluation and biochemical analysis of parental lines and hybrid Rada and standards was made on ten plants for each individual year, and included main agronomic traits as plant height, head diameter, stem diameter, 1,000 seed weight, total seed number per head, and seed yield per head. 1,000 seed weight (g) was determined on three samples of 50 seeds per head each. Heterosis effect was calculated for plant height, head diameter, stem diameter, 1,000 seed weight, total seed number per head, and mean value of seed yield per head.

Phytopathological evaluation

The phytopathological evaluation of mutant line 12002 R, hybrid Rada, and standards was performed with regard to the parasite *Orobanche cumana* and the diseases *Plasmopara helianthi, Phomopsis helianthi*, and *Phoma macdonaldii* at the Sunflower Phytopathology Laboratory and infection fields of DAI – General Toshevo.

The evaluation for resistance to *Plasmopara helianthi* and *Orobanche cumana* were done according to standard methodologies (Panchenko, 1973; Gulya *et al*., 1991). The phytopathological evaluation of hybrid was performed with regard to *Plasmopara helianthi* (Farl.) Berlese & de Toni-race 300, 700, and 731 and to the *Orobanche cumana* races F and G at the Sunflower Phytopathology Laboratory.

With a view to characterizing the resistance to *Plasmopara helianthi* was used the method suggested by Gulya *et al*. (1991). The evaluation of 50 plants from hybrid was carried out using standard methodologies: 0% = S (sensitive); 100% = R (resistant). *Orobanche cumana* resistance was evaluated under greenhouse conditions according to Panchenko (1975), slightly modified to local conditions.

Orobanche cumana resistance was calculated as percentage of non-infected plants. The reaction of 50 plants from each: mutant line, hybrid Rada, and standards was recorded using the following scale:

0% = S (sensitive);
100% = R (resistant).

The evaluation for resistance to attacks of gray spots (*Phomopsis helianthi*) and black spots (*Phoma macdonaldii*) was performed in field and in artificial infection plots. The type and severity of the attack were read one week after mass flowering according to the following scales:

Type of gray spot attack:
0 – no symptoms;
1 – a necrotic lesion up to 5 cm in diameter;
2 – a necrotic lesion over 5 cm in diameter;
3 – several merged necrotic lesions on the stem;
4 – a stem broken at the place of infection.

Type of black spot attack:
0 – no symptoms;
1 – a necrotic lesion near the petiole;
2 – several necrotic lesions on the stem;
3 – the entire stem is covered with necrotic lesion or the stem is broken.

Attacking rate: what portion of the plant's stem is covered with lesions of the pathogen (1/3, 2/3, or 3/3) (Encheva and Kirykov, 2000).

Statistical analysis

The means of parent lines and hybrid Rada according to the investigated indices, such as plant height, head diameter, stem diameter, 1,000 seed weight, total seed number per head, and seed yield per head, mean errors, and reliability intervals were calculated.

The coefficients of heterosis effect h1 and h2 (Mather and Jinks, 1995; Lynch and Walsh, 1998), on which the evaluation of the cross relative to the parents by the above indices was based, were also calculated.

The experimental data are analyzed by one-way ANOVA model with replications (Falconer and Mackay, 1996). The data were analyzed using statistical packages BIOSTAT 6.0 and SPSS 17.0.

Results and discussion

The aim of this study was to estimate the degree of heterosis over a parental mean (h1) and better parent (h2) for some important agronomical traits in sunflower hybrid Rada (Figure 1) and to evaluate the hybrid for resistance to diseases *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii*, and parasite *Orobanche cumana*.

The farther line 12002 R was produced by treatment with gamma irradiation at dose of 8 Gy for 1 min of immature zygotic embryos and in combination with embryo culture method at initial genotype R 2574.

To determine the combining ability of the new developed mutant sunflower line 12002 R, the sterile analogue of the Bulgarian selfed line 2607 A was used. Sterile line was developed at the base of cytoplasm Pet 1.

Figure 1: Hybrid Rada developed by the cross 2607 A × 12002 R.

Heterosis for agronomically important traits

Heterosis effect at F1 plants has allowed sunflower to become one of the major oilseeds in many countries and Bulgaria in particular.

The ANOVA procedure (Table 1) proves with probability $p = 0.001$ of the alternative hypothesis that the parents and the received cross are with different genetic potential in the studied indices. Based on this result it is possible to make the conclusion for good effect of the heterosis.

Table 1: Dispersion analysis of the studied indices.

Traits	MS genotype	MS error	*F* exp
Plant height	12,760.8	22.3	571.8***
Head diameter	489.2	1.6	310.8***
Stem diameter	416.4	5.1	82.4***
Total seed number per head (no)	1,459,362.0	48,349.7	30.2***
1,000 seed weight	3,542.9	78.6	45.1***
Mean value of seed yield per head (g)	12,840.4	73.2	175.5***

Note: *** – statistically significant at $p = 0.001$.

Table 2 presents the mean values of the indices of parents and cross, as well as the mean value error. According to the data, the cross 2607 A × 12002 R exceeded both parents for all studied indices, especially by total seed number per head and seed yield per head. For the indices mentioned above, the values were several times higher than the respective values for the parental lines.

Table 2: Mean values of plant height, head diameter, stem diameter, 1,000 seed weight, total seed number per head, and mean value of seed yield per head of parents and hybrid Rada (2607 A × 12002 R).

Traits	2607 A	12002 R	2607 A × 12002 R
Plant height (cm)	121.5 ± 2.40	117.0 ± 2.20	181.0 ± 3.30
Head diameter (cm)	14.0 ± 0.32	9.6 ± 0.21	23.3 ± 0.42
Stem diameter (cm)	16.7 ± 0.34	13.6 ± 0.27	26.0 ± 0.48
Total seed number per head (no)	149.5 ± 2.90	38.1 ± 0.92	748.4 ± 9.70
1,000 seed weight (g)	31.4 ± 0.62	42.6 ± 0.83	68.2 ± 1.40
Mean value of seed yield per head (g)	5.1 ± 0.40	2.2 ± 0.09	65.7 ± 1.20

This fact was also confirmed when evaluating the coefficients of heterosis effect h1 and h2 (Table 3).

The correlations of the cross with the mean values of the parental lines (h1) and with the mean value of the parent with higher indices (h2) were statistically significant.

In our study, all six characters recorded positive and significant heterosis in the direction of both relative to parental average (h1) and relative to better parent (h2).

Table 3: Heterosis for plant height, head diameter, stem diameter, 1,000 seed weight, total seed number per head, and seed yield per head relative to parental mean (h1) and better parent (h2).

Traits	h1	h2
Plant height (cm)	1.52**	1.49**
Head diameter (cm)	1.97***	1.66**
Stem diameter (cm)	1.72**	1.57**
Total seed number per head (no)	7.98***	5.0***
1,000 seed weight (g)	1.84**	2.17***
Mean value of seed yield per head (g)	17.92***	12.81***

Notes: ** – statistically significant at $p = 0.01$, *** – statistical significant at $p = 0.001$.

According to Skoric *et al.* (2006), significant manifestation of heterosis for agronomically important traits is the main precondition for obtaining productive sunflower hybrids.

In hybrid combination 2607 A × 12002 R, heterosis values for plant height, head diameter, stem diameter, total seed number per head, 1,000 seed weight, and seed yield per head were highly significant and positive. The magnitude of heterosis when compared with parental average h1 ranging from 1.52% to 17.92%. Heterosis value of characters mentioned above was highly significant to better parent h2 (1.49–12.81%), also. Such positive heterosis for total seed number per head and seed yield per plant of sunflower was published by Hladni *et al.* (2007).

The highest positive heterotic effect (h1 = 17.92%) in the hybrid combination 2607 A × 12002 R was for trait seed yield per head relative to parental average h1. The restorer line 12002 R in this combination had lower value of seed yield per head in the experiment. High levels of heterosis were observed for the hybrids TS-17 × TR-120 and TS-18 × TR-120 for important agronomic characters such as seed weight per head and weight of filled seeds per head (Khan *et al.*, 2004). Hladni *et al.* (2005) reported a positive and highly significant heterosis values for seed yield per plant relative to both the parental mean and the better parent.

In our study, heterosis for plant height, head diameter, stem diameter, 1,000 seed weight, total seed number per head, and seed yield per head varied according to both, traits and genotypes.

An additive dominant model of inheritance was established with partial or full dominance of the all studied traits in the direction of parent with the higher value.

Evaluation of mutant line 12002 R and hybrid Rada for resistance to some economically important diseases and parasite on sunflower

Hence, there is a need for greater variability providing additional sources of resistance to diseases, and insects, and seed quality characteristics among modern cultivated sunflower (Seiler, 1992). At present cultivated hybrid sunflower utilizes an extremely narrow genetic base, mainly the male sterile cytoplasm derived from the wild species *H. petiolaris* (Leclercq, 1969).

The parasite *Orobanche cumana* grows on sunflower roots, resulting in weak and dwindled plants, with thin steam. The parasite enhances the transpiration of damaged plants, which in drought conditions are withering, even if attacked by a small number of parasitic plants (Iliescu *et al.*, 1998). Broomrape presents serious problems to sunflower production in Bulgaria, as well (Shindrova, 2006).

Jan and Fernandez-Martinez (2002) reported successful transferring of genes for resistance to race F from perennial wild species *H. angustifolius*, *H. cusickii, H. divaricatus*, *H. grosseseratus*, and *H. maximiliani*. Seiler and Fredrick (2011) found that species *H. grosseserratus, H. maximiliani*, and *H. divaricatus* possessed resistance to race F and *H. debilis ssp. tardiflorus* possessed resistance to race G. Christov (2012) reported new lines with full resistance to *Orobanche cumana* race G, developed from interspecific hybridization with *H. pauciflorus, H. tuberosus, H. divaricatus, H. hirsutus*, and *H. bolanderi*.

Instead wild species genetic variability, as resistance to parasite *Orobanche cumana* in particular, may be increased using induced mutagenesis (Batchvarova *et al.*, 2001; Encheva *et al.*, 2008a, 2008b). Resistant mutants to *Verticillium* with dominant genes were obtained after EMS treatment of pollen of tomato (Gavazzi *et al.*, 1987). Kostov *et al.* (2007) reported mutant tomato plants resistant to *Orobanche ramosa*, produced by EMS treatment of seeds.

In our study, a mutation was observed in the reaction of the line toward *Orobanche cumana* parasite. The initial genotype R 2574 was susceptible to broomrape. The mutant line 12002 R showed 100% resistance to *Orobanche cumana* race F. These results were confirmed during several years of evaluation. In 2011, the line showed 83.3% resistance to the new race G.

On the basis of these data, the conclusion was drawn that the resistance of the new line 12002 R was due to the mutagenic treatment with gamma irradiation. The same mutation, resistance to the parasite broomrape, was obtained in all variants of treatment with gamma rays of the initial genotype 2574 R. Mutation for resistant to *Orobanche cumana* was produced after treatment of immature zygotic embryos of line 2574 R with ultra sonic, also (Encheva *et al.*, 2012). This allows us to assume that there are mutable locations in the cultural sunflower genome resulting from induced mutagenesis.

Combining induced mutagenesis in immature sunflower 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.

The result allows us to assume that the resistance of the new line 12002 R occurred as a result of mutation of a single dominant gene. Similar conclusion was made by Christov (1996) analyzing the type of resistance to *Orobanche cumana* of mutant sunflower forms obtained through irradiation of air dry seeds with gamma rays. Monogenic and dominant inheritance to sunflower *Orobanche cumana* was confirmed by genetic studies of other authors (Tolmachev, 1984; Sukno *et al.*, 1999), although two dominant genes (Dominguez, 1996b). According to Pacureanu-Joita *et al.* (1998), the gene *Or6* provides resistance to all races – from A to F. The race F resistant population BR4, derived from wild species *H. divaricatus* and *H. grosseserratus*, was found to be under the control of a single dominant gene designated *Or6* (Perez-Vich *et al.*, 2002). Pacureanu *et al.* (2004) reported also a single dominant gene controlling the resistance to race F in Romania.

Instead of line 12002 R a study was carried on the hybrid Rada for resistance to the diseases *Phomopsis helianthi*, *Phoma macdonaldii*, *Plasmopara helianthi*, and the parasite *Orobanche cumana* (Table 4). Our later study showed 83% resistance of line to *Orobanche cumana* race G.

Table 4: Two-years evaluation of standard-commercial hybrids Albena, Perfect, Diabolo PR, and new hybrid Rada for resistance to *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii*, and parasite *Orobanche cumana, %.*

Hybrid	*Phomopsis helianthi*		*Phoma macdonaldii*		*Plasmopara helianthi*		*Orobanche cumana*
	Degree of infection	Type	Degree of infection	Type	Race 300	Race 700	Race F
					Resistance %	Resistance %	Resistance %
Standard Albena	2	2	1/3	1	100.0	95.0	0
Standard Diabolo	2	2	1/3	1	100.0	95.0	100.0
Standard Perfect	1	1	1/3	1	90.5	84.5	100.0
Hybrid Rada	1	1	2/3	2	100.0	100.0	70.0

The hybrid Rada was characterized with 70% resistance to the parasite *Orobanche cumana* race F. That was inherited from the mutant restorer line 12002 R because mother line in the cross 2607 A was susceptible.

The new hybrid has full resistance to the *Plasmopara helianthi* race 300 and 700. Hybrid Rada carries resistance to *Plasmopara helianthi*, inherited from the Bulgarian mother line of the hybrid (2607 A).

The response of the hybrids to gray and black spot attack is given in Table 4, also. Hybrid Rada demonstrated resistance to the attack of *Phomopsis helianthi*. At the same time, the standard Albena and Diabolo PR showed lesions typical for the pathogen covering 2/3 of the stems (gray spots).

Our result demonstrated that induced mutagenesis *in vitro* leads to genetically heritable variation of sunflower that is suitable for use in a program for the development of initial breeding material.

The new hybrid resistant to diseases and parasite *Orobanche cumana* will be useful for practice and will guarantee restricted propagation of the pathogens and cleaning of the infected fields.

Conclusions

We succeed to create mutant sunflower line 12002 R with positive changes as resistance to parasite *Orobanche cumana* race G. The line was created at initial susceptible genotype R 2574. Mutation was induced after treatment of immature sunflower (*Helianthus annuus* L.) zygotic embryos of fertility restorer line R 2574 with gamma irradiation at dose of 8 Gy for 1 min. 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 functional tissue.

The new line 12002 R possesses very good combining ability. Hybrid Rada was developed with simple cross of Bulgarian line cms2607 and mutant line 12002 R.

Significant differences existed among the genotypes (line 2607 A, line 12002 R, and hybrid Rada) in their mean values of all studied indices.

The heterosis values for the all six characters studied were positive and highly significant in hybrid Rada both relative to parental mean and relative to better parent.

Seed yield per head had a much more pronounced heterosis effect (17.92% and 12.81%) relative to parental average (h1) and relative to better parent (h2) than other traits.

The hybrid simultaneously possessing resistance to the parasite *Orobanche Cumana* race F and full resistance to *Plasmopara helianthi* races 300 and 700.

Higher yield of hybrid Rada and resistance to some economically important diseases and parasite *Orobanche cumana* are a guarantee that it is suitable for mass production.

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References

Akhtouch, B., Muñoz-Ruz, J., Melero-Vara, J.J., Fernández-Martínez, J.M., Domínguez. Plant Breeding 121: 266–268.

Azpiroz, I.S.,Vincourt, P., Serieys, H., 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.

Bervillé, A., Serieys, H., 2001. Perennial *Helianthus* as a source for *Orobanche* resistance. *In*: Parasitic Plant Management in sustainable Agriculture Workshop "State of the art in Orobanche control". 18–20 October, 2001, Bari, Italy, p. 34.

Batchvarova, R., Hristov, M., Slavov, S., Valkov, V., 2001. Development of sunflower lines resistant to *Orobanche cumana* Wallr. through mutagenesis and interspecific hybridization. *In*: 7th International Parasitic Weed Symposium. 5–8 June, Nantes, France.

Batchvarova, R., 2001. Development of sunflower lines resistant to *Orobanche*. *In*: Parasitic Plant Management in sustainable Agriculture Workshop "State of the art in *Orobanch*e control". 18–20 October, 2001, Bari, Italy.

Dominguez, J., 1996b. R-41, a sunflower restorer inbred line, carrying two genes for resistance against a highly virulent Spanish population of *Orobanche cernua*. Plant Breeding 115: 203–204.

Gotsov, K., Karaivanov, A., Tsvetkova, F., Tsvetkov, S.,Velkov, V., Radkov, P., 1981. Achievements and problems of plant breeding at IWS-General Toshevo. Section "Sunflower". *In*: Scientific-theoretical conference at the field of plant breeding. NAPS, Sofia, pp. 32–36.

Khan, M.S., Khalil, I.H., Swati, M.S., 2004. Heterosis for yield components in sunflower (*Helianthus annuus* L.). Asian Journal of Plant Sciences 3: 207–210.

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

Christov, M., Piskov, Al., Encheva, J., Valkova, D., Drumeva, M., Nenova, N., Nikolova, V., Encheva, V., Shindrova, P., Petrov, P., Georgiev, G., 2009. Developing sunflower hybrid cultivars with increased productivity, resistant to disease and broomrape using classical and biotechnological methods. Science-technical bulletin. Institute for oilseed crops. UOSC, N° 14: 74–87.

Christov, M., 2012.Contribution of interspecific hybridization to sunflower breeding. Helia 35(57): 37–46.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, 4th ed., Addison Wesley Longman, Harlow, Essex.

Fernandez-Martinez, J.M., Malero-Vara, J., Minoz-Ruz, J., Ruso, J., 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.

Fernandez-Martinez, J.M., Perez-Vich, B., Velasco, L., 2010. Oil Crop Handbook of Plant Breeding -Sunflower, Vol. 4, pp. 155–232.

Enns H., Dorrell, D.G., Hoes, J.A., Chubb, W.O., 1970. Sunflower research, a progress report. *In*: Proc 4th Int Sunflower Conf, Memphis, Tennessee, pp. 162–167.

Encheva, V., Kirykov, I., 2000. A method for evaluation of sunflower resistance to Diaporte/ *Phomopsis helianthi* Munt.- Cvet at al. Bulgarian Journal of Agricultural Science 8: 219–222.

Encheva, J., Christov, M., Nenov, N., Tsvetkova, F., Ivanov, P., Shindrova P., Encheva, V., 2003. Developing genetic variability in sunflower (*Helianthus annuus* L.) by combined use of hybridization with gamma radiation or ultrasound. Helia 26(38): 99–108.

Encheva, J., Shindrova P., Penchev. E., 2008a. Developing mutant sunflower lines (*Helianthus annuus* L.) through induced mutagenesis. Helia 31(48): 61–72.

Encheva, J., Christov, M., Shindrova, P., 2008b. Developing mutant sunflower lines (*Helianthus annuus* L.) by combined used of classical method with induced mutagenesis and embryo culture method. Bulgarian Journal of Agricultural Science 14(4): 397–404.

Encheva, J., 2009. Sunflower (*Helianthus annuus* L.) mutant line, developed using induced mutagenesis. In: Breeding and technical crops. Field Crop Studies V(1): 109–117.

Encheva, J., Shindrova, P., Encheva, V., Penchev, E., 2012. Sunflower commercial hybrid Yana, developed with mutant restorer line R 12003. Helia 35(56): 47–60.

Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutriment requirements of suspension cultures of soybean root cells. Experimental Cell Research 50: 151–158.

Gavazzi, G., Tonelli, C., Todesco, G., Arreghini, E., Raffaldi, F., Vecchio, F., Barbuzzi, G., Biasini, M., Sala, F. 1987. Somaclonal variation versus chemically induced mutagenesis in tomato (*Licopersicum esculentum* L.). Theoretical and Applied Genetics 74: 733–738.

Gulya, T. J., Miler, J. F., Firanyi, F., Sackston, W.E., 1991. Proposed internationally standardized method for race identification of *Plasmopara halstedii*. Helia 14: 11–20.

Gulya, T.J., Marek F.L., Gavrilova, V., 2010. Disease resistance in cultivated sunflower derived from public germplasm collections. *In*: Proc Int Symp "Sunflower breeding on resistance to disease". June 23–24, Krasnodar, Russia, pp. 7–19.

Hladni N., Škorić, D., Kraljević-Balalić, M., 2005. Heterosis for seed yield and yield components in sunflower. Genetika 37(3): 253–260.

Hladni, N., Skoric, D., Kraljevic-Balalic, M., Sakac, Z., Miklic, V., 2007. Heterosis for agronomically important traits in sunflower (*H. annuus* L.). Helia 30(47): 191–198.

Ivanov, P., Velkov, V., Petrov, P., Georjiev, I., Shindrova, P., Tsvetkova, F., 1988. Direction of advanced plant breeding work in sunflower. Agricultural Science XXVI(1): 40–50.

Jan, C.C., Fernandez-Martinez, J.M., 2002. Interspecific hybridization, gene transfer and the development of resistance to the broomrape race F in Spain. Helia 25(36): 123–136.

Kaya, Y., Demerci, M., Evci, G., 2004. Sunflower (*Helianthus annuus* L.) breeding in Turkey for broomrape (*Orobanche cernua* Loeffl.) and herbicide resistance. Helia 27(40): 199–210.

Kinman, M.L., 1970. New development in the USDA and state experiment station sunflower breeding programme. *In*: Proc of the 4th International Sunflower Conference. Memphis, USA, pp. 181–183.

Kostov, K., Batchvarova, R., Slavov, S., 2007. Application of chemical mutagenesis to increase the resistance of tomato to *Orobanche ramosa* L. Bulgarian Journal of Agricultural Science 13: 505–513.

Leclercq, P., 1969. Une sterilite male chez le tournesol (A male sterility in sunflower). Annales d Amelioration des Plantes 19: 99–106.

Leclercq, P., 1971. La sterilite male cytoplasmique du tournesol. I. Premieres etudes sur la restauration de la fertilite. Annales de l'Amelioration des Plantes 21: 45–54.

Lynch, M., Walsh, B., 1998. Genetics and Analysis of Quantitative Traits, Sinauer, Sunderland, MA.

Mather, K., Jinks, J., 1995. Biometrical genetics, Birmingham. Biometrical Journal 5: 72–80.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissues cultures. Plant Physiology 15: 473–497.

Pancenko, A.N., 1973. Rannija diagnostika zarazihoustoicivosti pri selekcii podsolnecnika. Zbirnik VNIIMK, pp. 107–115.

Panchenko, A.N., 1975. An early diagnostic method for resistance to *Orobanche cumana* Wallr. Agricultural Newspaper. N 2: 225–228 (in Russian).

Pacureanu-Joita, M., Vranceanu, A.V., Soare, G., Marinscu, A., Sandu, I., 1998. The evaluation of the parasite-host interaction system (*Helianthus annuus* L.)-(*Orobanche cumana* Wallr.) in Romania. *In*: Proceedings of 2nd Balk Symposium on Field Crops. 16–20 June, 1998. Novi Sad, Yugosllavia: pp. 153–155.

Pacureanu, M., Veronesi, C., Raranciuc, S., Stanciu, D., 2004. Parasite-Host plant interaction of *Orobanche cumana* Wallr. (*Orobanche cernua* Looefl.) with *Helianthus annuus*. *In*: Seiler, G.J. (ed). *In*: Proc 16th Int Sonf. Conf., Fargo, ND, 28 August–2 September, 2004, Int. Sunflower Assoc., Paris, pp. 171–177.

Perez-Vich, B., Akhtouch, B., Munoz-Ruz, J., Fernandez-Martinez, J.M., Jan, C.C., 2002. Inheritance of resistance to a highly virulent race "F" of *Orobanche cumana* Wallr. in sunflower line derived from interspecific amphiploids. Helia 25: 137–144.

Iliescu, H.C., Iordache, E., Jinga, V., Ionita, A., 1998. Response of some sunflower hybrids to attack of the parasitic phanerogame *Orobanche cumana* Wallr. *In*: Wegmann, K., Musselman, L.J., Joel, D.M. (eds) Current problems of *Orobanche* Researches, Proceedings of the Fourth International Workshop on *Orobanche*. Albena, 23–26 September, Bulgaria, pp. 291–294.

Seiler, G.J., 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crop Research 30, 195–230.

Seiler, G.J., 2010. Utilization of wild *Helianthus* species in breeding for disease resistance. *In*: Proc Int Symp "Sunflower breeding on resistance to disease". June 23–24, Krasnodar, Russia, pp. 36–51.

Seiler, G.J., Fredrick, M.L., 2011. The USD-ARS sunflower collection as a source of genetic diversity for cultivated sunflower. http://www.sunflowernsa.com/uploads/resources/607/sunflower-collection-as-a-source-of-genetic-diversity-for-sunflower-seiler.pdf

Shindrova, P., 1994. Distribution and race complex of Broomrape (*Orobanche cumana* Wallr.) in Bulgaria. *In*: Proceedings of the Third International Workshop on *Orobanche* and related Striga research. Amsterdam, pp. 142–145.

Shindrova, P., 2006. Broomrape (*Orobanche cumana* Wallr.) in Bulgaria-Distribution and race composition. Helia 29(44): 111–120.

Skoric, D., 1985. Sunflower breeding for resistance to Diaporthe (*Phomopsis helianthi* Munt.-Cvet). Helia FAO 8: 21–24.

Skoric, D., Marinkovic, R., Jocic, S., Jovanovic, D., Hladni, H., 2002. Dostignuca I dalji pravci u oplemenjivanju suncokreta I izbor hibrida za setvu u 2002. Zbornik radova naucnog institute za ratarstvo I povratarstvo. Novi Sad 36: 147–159.
Skoric, D., Jocic, S., Malidza, I.G., 2004. Novi hibridi suncokreta Pobednik i RIMI. Zbornik radova sa 45. Savetovanja industrije ulja. Petrovac na Moru, 21–31.
Skoric, D., Jocic, S., Jovanovic, D., Hladni, N., Marinkovic, R., Atlagic, J., Pankovic, D., Vasic, D., Miladinovic, F., Gvozdenovic, S., Terzic, S., Sakac, Z., 2006. Achievements of sunflower breeding (in Serbian). Periodical of Institute of Field and Vegetable Crops, Novi Sad. 42: 131–173.
Stoyanova, J., Simeonov, B., Sabev, G., Petrov, D., Georgiev, I., Dimitrov, I., Georgieva-Todorova, J., Rangelov, L., Petrova, M., Ivanov, P., Palazov, P., Kontev, C., 1977. Sunflower in Bulgaria, BAS, Sofia.
Sukno, S., Melero-Vara, J.M., Fernandez-Martinez, J.M, 1999. Inheritance of resistance to *Orobanche cumana* Loefl. in sex sunflower lines. Crop Science 39: 674–678.
Tolmachev, V.V., 1984. Geneticheskij control priznaka zarazihoustoichivosti i resistentnovo (*Orobanche cumana* Wallr.) analoga linii podsolnechnika BK 373 B. Nauchn Technolgy Bulletin. VNIMK. Krasnodar, Vip 84: 47–47.
Valkova, D., Christova-Cherbadzi, M., Christov, M., 2009. Exploration of *Helianthus argophyllus* accessions. *In*: Proc Int Sci Conf "technical crops in modern agriculture". Balti, Moldova, 7–8 August, 2009, pp. 106–113.
Vranceanu, A.V., Stoenescu, F., 1971. Pollen restorer gene from cultivated sunflower (*Helianthus annuus* L.). Euphytica 20(4): 536–541.