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HETEROSIS EFFECTS FOR AGRONOMICALLY IMPORTANT TRAITS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Abstract

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Hybrid combination concluded Bulgarian male sterile line cms807 and mutant fertility restorer line 278 R. Father line was developed by treatment of immature zygotic embryo of restorer line 381 R with ultra sonic. Hybrid developed with the participation of line 278 R considerably exceeded the mean standard (the Bulgarian commercial hybrid San Luka and French commercial hybrid LG-5635) by seed and oil yield. Seed yield of hybrid combination was significantly higher in comparison to mean standard San Luka and LG-5635 with 71 kg/da, or 19.1%, and oil yield with 59 kg/da, or 141.8%. In our study 4 of 8 characters recorded positive and significant heterosis in the direction of both relative to parental average (h1) and relative to better parent (h2). In h1 the variation was from 120. 2% to 212.7%; in h2 the variation was within 111.9% to 132.9%. Highest magnitude of heterosis was observed at seed yield per plant in comparison to parental mean, followed by head diameter in comparison to better parent. Lack of heterotic effect was established for traits 1000 seed-weight, seed length, seed width and seed thickness in relation of parental mean and to better parent. At the base of calculated coefficients it can be concluded a dominant model of inheritance of the studded traits such as plan height, diameter of head, total seed number per head and seed yield per plant, in the direction of parental mean and to better parent. Instead higher seed and oil yield new hybrid combination simultaneously possessing high resistance to the *Orobanche* population of races-A-G distributed in Bulgaria and immunity to *Plasmopara helianthi*-races 300, 700 and 731.

Key words: Helianthus annuus, ultra sonic, mutant line, combining ability, hybrid, heterosis, resistance, *Plasmopara helianthi*, *Orobanche cumana*

Introduction

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 Leclerq (1969) and the finding out of efficient fertility restorers (Enns et al., 1970; Kinman, 1970; Leclerq, 1971; Vranceanu and Stoenescu, 1971).

Utilization of heterosis has allowed sunflower to become one of the major oil seed in many countries of Eastern and Western Europe, Russia and South America and is important crop in the USA, Australia, South Africa, China, India and Turkey. One of approximately 16.5 million hectares of sunflower grown in the major producing countries, 11.5 million hectares are planted to hybrids (Miller, 1998).

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 Start was released and distributed on the entire territory of Bulgaria (Gotsov et al.; 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

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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 aim of this study was: a) to estimate the degree of heterosis over a parental mean and better parent for some important agronomical traits in sunflower hybrid combination 807 A × 278 R and b) to study seed and oil yield of hybrid combination in comparison to mean hybrids (commercial Bulgarian hybrid San Luka and commercial French hybrid LG-5635) and c) to evaluate hybrid for resistance to *downy mildew* and parasite *broomrape.*

Material and Methods

A part of the experiments was carried out under laboratory conditions, and another one at the trial field of Dobroudja Agricultural Institute-General Toshevo. The morphological and biochemical traits of the new mutant line, the mother line and the hybrids were studied during 2012.

Developing of mutant line 278 R

The Bulgarian fertility restorer line 381 R, witch 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 ultrasonic is to be genetically pure, i.e. homozygotic to the highest possible degree. Therefore the control line 381 R with very good morphological uniformity was chosen as initial material for induced mutagenesis.

Plants were grown in the field and were hand-pollinated. The isolated immature zygotic embryos (11–13 days old) were treated with ultrasonic (during treatment the seeds were flooded in water, the whole surface well moistened) at dose 25.5 W/cm^2 for 1 min. before plating on nutrition medium M for further growing: 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. Immature embryos 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 conditions for cultivation were: 25^{o}C, 16/8 h photoperiod for one week. The rooted plants were transferred to soil and were further grown and self-pollinated under greenhouse conditions. After selfing and continuing selection restorer line was developed at R12M12 generation.

Developing of mother line 807 A

The sterile analog of Bulgarian line 807 B (15 generations) was used as mother component in the cross. The line has very good GCA and SCA, resistance to *Plasmopara helianthi* and to parasite *Orobanche cumanna*-race A-F.

Biometric evaluation of line 807 A, 278 R and hybrid 807 A × 278 R

The biometric evaluation and biochemical analysis of line 807 A, 278 R and hybrid 807 A × 278 R and standards San Luka and LG-5635 was made on 10 plants and included main agronomic traits as of plan height, diameter of head, total seed number per head, 1000 seed weight, seed yield per plant, seed length, seed width and seed thickness.

1000 seed weight (g) was determined on three samples of 50 seeds per head each.

Heterosis effect was calculated for plan height, diameter of head, total seed number per head, 1000 seed weight, seed yield per plant, seed length, seed width and seed thickness.

Biochemical analysis

To determine the oil content of air-dry seeds from the materials included in the study, Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) was used.

Hybridization

To determine the combining ability of the new developed sunflower line 278 R, the sterile analogue of the Bulgarian selfed line 807 was used. The standards for comparing the new hybrid 807 A × 278 R developed were the Bulgarian commercial hybrids San Luka и and the French commercial hybrid LG-5635.

The obtained of Dobroudja agricultural institute hybrid 807 A × 278 R was tested in 2012 at the breeding field according to the block-design method, in three replications, the area of each replication being 25 m^2**.**

Phytopatological evaluation

The phytopathological evaluation of parent lines, hybrid 807 A × 278 R and standards was performed with regard to *Orobanche cumana* – race G and the disease *downy mildew* at the Sunflower Phytopathology Laboratory and infection fields of DAI – General Toshevo in 2011.

The evaluation for resistance to downy mildew and broomrape were done according to standard methodologies (Panchenko, 1973; Gulya et al., 1991). The phytopathological evaluation of hybrid was performed with regard to downy mildew *Plasmopara helianthi* (Farl.) Berlese & de Toni-race

731 and to the local *Orobanche cumana* – race G at the Sunflower Phytopathology Laboratory during 2011.

With a view to characterizing the resistance to downy mildew were used the method suggesting by Gulya at al. (1991). The evaluation of 50 plants from hybrid was carried out using standard methodologies: 0% = S (sensitive); 100% = R (resistant).

Broomrape resistance was evaluated under greenhouse conditions according to Panchenko (1975), slightly modified to local conditions. Broomrape resistance was calculated as percentage of non-infected plants. The reaction of 50 plants from each genotype was recorded using the following scale: 0–100%.

Statistical analysis

The parent lines and a developed new hybrid 807 A × 278 R were analyzed statistically with regard to the agronomic traits such as plant height, diameter of head, total seed number per head, 1000 seed weight, seed yield per plant, seed length, seed width and seed thickness. Analysis of the experimental data was by the statistical package BIOSTAST 6.0. The means of parents and cross according to the investigated indices, such as plant height, diameter of head, total seed number per head, 1000 seed weight, seed yield per plant, seed length, seed width and seed thickness, mean errors and reliability intervals were calculated.

The coefficients of heterotic effect h1 and h2, on which the evaluation of the cross relative to the parents by the above indices was based, were also calculated. The data were analyzed using the statistical package SPSS Version 17.0.

Results and Discussion

Heterosis for agronomical important traits

Development of sterile *cms* analogues of lines used in sunflower breeding programs for commercial hybrid development is one of the practical applications of *cms* investigations. CMS PET-1 is a *cms* source which is widely used of in sunflower hybrid development.

The ANOVA procedure (Table 1) prove 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's possible to make the conclusion for good effect of the heterosis.

Table 1
Dispersion analysis of studied indices. Harvest year 2012

Traits	MS genotype	MSerror
Plant height, cm	1856,6 ***	29,6
Diameter of head, cm	449,4 ***	3,8
Total seed number per head, no	1431288 ***	7628,7
1000 seed weight, g	1891.7 ***	14.2
Seed yield per plant, g	11504.8 ***	115.6
Oil content in seed, %	37.3 ***	0.9
Seed length, mm	88.4 ***	0.27
Seed width, mm	36.05 ***	0.12
Seed thickness, mm	18.5 ***	0.11
df	2	29

Table 2
Mean values of plan height, diameter of head, total seed number per head, 1000 seed weight, seed yield per plant, oil content in seed, seed length, seed width and seed thickness of parents and the cross (807 A × 278 R). Harvest year 2012

Traits	807 A	278 R	807 A x 278 R
Plant height, cm	111.7 -,+ 0.5	117.0-,+ 12.0	137.5 -,+ 1.3
Diameter of head, cm	19.1 -,+ 0.5	12.0-,+ 2.6	25.4 -,+ 1.3
Total seed number per head, no	697.4 -,+8.2	87.4 -,+ 4.6	780.1-,+ 46.9
1000 seed weight, g	56.3-,+ 1.1	28.96 -,+ 1.2	39.9-,+ 1.3
Seed yield per plant, g	58.04 -,+ 0.8	3.5 -,+ 0.4	65.4 -,+ 5.8
Oil content in seed, %	45.6-,+ 0.3	46.2-,+ 0.3	49.2-,+ 0.3
Seed length, mm	13.1 -,+2.3	7.35-,+0,14	8.91-,+0.08
Seed width, mm	5.6-,+0.16	2.06-,+0.07	2.64-,+0.07
Seed thickness, mm	3.4-,+0.16	1.02-,+0.05	1.07-,+0.06

Fig. 1. Hybrid combination 807 A × 278 R

Table 2 presents the mean values of the indices of parents and cross, as well as the mean value error. According to all indices, the cross 807 A × 278 R (Figure 1) exceeded both parents, especially by plan height, diameter of head, total seed number per head, seed yield per plant and oil content in seed: for these indices the values were several times higher than the respective values for the parental lines.

According to 1000 seed weight, seed length, seed width and seed thickness new hybrid combination showed middle value. There were significant differences between the A-line, R line and their F1 hybrid in the mean value of studied characters.

Significant manifestation of heterosis for agronomical important traits is the main precondition for obtaining productive sunflower hybrids as reported by Skoric et al. (2006).

In our study 4 of 8 characters recorded positive and significant heterosis in the direction of both relative to parental average and relative to better parent (Table 3).

In hybrid combination 807 A × 278 R, heterosis value for plant height, diameter of head, total seed number per head and seed yield per plant were highly significant and positive, ranging from 120.24 to 212.68% relative to parental average h1.

Heterosis value of characters mention above was highly significant to better parent h2 (111.86–132.98%), 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 = 212.68%) in the hybrid combination 807 A × 278 R was for trait total seed number per head relative to parental average h1. The restorer line 278 R in this combination had lowers value of total seed number per head in the experiment.

Heterosis for plan height, diameter of head, total seed number per head and seed yield per plant varied according to both, traits and genotypes.

Lack of heterotic effect (h1 = 48.42–107.19%, h2 = 31.47–106.49%) was established for traits 1000 seed-weight, seed length, seed width and seed thickness.

At the base of calculated coefficients it can be concluded a dominant model of inheritance of the studded traits such as plan height, diameter of head, total seed number per head

Table 3

Heterosis for plan height, diameter of head, total seed number per head, 1000 seed weight, seed yield per plant, seed length, seed width and seed thickness relative to parental mean (h1) and better parent (h2). Harvest year 2012

Traits	h1	h2
Plant height, cm	120,24 **	117,52 *
Diameter of head, cm	163,34 ***	132,98 ***
Total seed number per head, no	198,80 ***	111,86 *
1000 seed weight, g	93,55	70,87
Seed yield per plant, g	212,68***	112,76 *
Oil content in seed, %	107,19	106,49
Seed length, mm	87.14	68.02
Seed width, mm	68,93	47,14
Seed thickness, mm	48.42	31.47

* – statisticaly significant p = 0.05; ** – statisticaly significant p = 0.01; *** – statisticaly significant p = 0.001

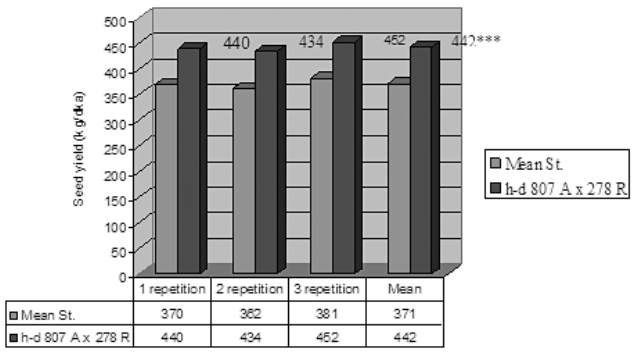

Fig. 2. Seed yield of hybrid 807A × 278 R and mean standard – San Luka and LG-5635 (* – p = 0.001%)**

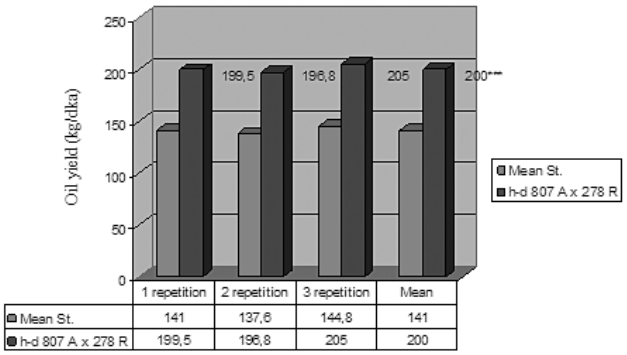

Fig. 3. Oil yield of hybrid 807 A × 278 R and mean standard – San Luka and LG-5635 (* – p = 0.001%)**

and seed yield per plant, in the direction of parental mean and to better parent. At indices 1000 seed weight, seed length, seed width and seed thickness this model is recessive. At trait 1000 seed weight the type of inheritance was additive.

Study on the production potential of hybrid 807A × 278 R

The aim of this study was to investigate some agronomic traits of sunflower hybrid 807A × 278 R (Figure 1), developed with mutant restorer line R 278.

To determine the combining ability of the new developed sunflower line R 278, the sterile analogue of the Bulgarian selfed line 807 was used. Sterile line was developed at the base of cytoplasm Pet 1.

The testing of line 278 R showed 100% restoration ability and very good combining ability. A dispersion analysis (Figure 2) of hybrid 807 A × 278 R was carried out with regard to the main indices seed yield (kg/da).

In 2012 hybrid 807 A × 278 R was testing at trial field of DAI-General Toshevo. The hybrid considerably exceeded the mean standard (the Bulgarian commercial hybrid San Luka and French commercial hybrid LG-5635) by seed yield. The seed yield of hybrid 807 A × 278 R was significantly higher with 71 kg/da, or 19.1%.

The results from the dispersion analysis of oil yield showed exceeding of the mean value of this index in hybrid 807A × 278 R with 59 kg/da, or 41.8% (Figure 3).

Evaluation of parent lines and hybrid 807 A × 278 R for resistance to Plasmopara helianthi and parasite Orobanche cumana

The main current problems of sunflower hybrid breeding are the further improvement of productivity by increasing seed and oil yields and the reduction of Genetic vulnerability to diseases and heat or water stress encountered in various ecological zones (Vranceanu, 1998).

A study was carried on the hybrid 807 A × 278 R for resistance to the diseases *Plasmopara helianthi* and the parasite *Orobanche cumana* (Table 4).

The new hybrid was immune to the *Plasmopara helianthi*-race 300, 700 and 731. While the standard San Luka and LG-5635 were susceptible to *Orobanche cumana* – race G, new hybrid combination showed resistance 60%. Mother line 807 A posses' resistance to *Orobanche cumana* – race F. Line 278 R showed 100% resistance to race G.

Resistance of hybrid 807 A × 278 R to downy mildew and broomrape and high seed and oil yield were very good combination in sunflower breeding program.

Table 4

Evaluation of parent lines, standards-commercial hybrid San Luka and LG-5635 and new hybrid combination 807 A × 278 R for resistance to *downy mildew* and parasite *broomrape,* 2011, %

Lines and hybrids	*Plasmopara helianthi*			*Orobanche cumana*
	race 300 resistance, %	race 700 resistance, %	race 731 resistance, %	race G resistance, %
Line 807 A	100.0	100.0	100.0	0
Line 278 R	100.0	100.0	100.0	100 %
Standard San Luka	100.0	95.0	100.0	0
Standard LG-5635	100.0	100.0	100.0	0
Hybrid 807 A × 278 R	100.0	100.0	100.0	60.0

Conclusion

The following can be concluded based on the study results.

– There were significant differences among the studied genotypes (inbred lines and hybrid) in the mean value of plan height, diameter of head, total seed number per head, 1000 seed weight, seed yield per plant, seed length, seed width and seed thickness.

– Heterosis effect for the agronomic characters under investigation was highly significant both relative to parental average and relative to better parent at the indices plan height, diameter of head, total seed number per head and seed yield per plant.

– The higher positive heterotic effect (h1 = 212.7%) in the hybrid combination 807 A × 278 R was for trait seed yield per plant relative to parental average h1, followed by diameter of head (h2 = 132.98%) in comparison to better parent.

– Negative heterotic effect was established for trait 1000 seed-weight, seed length, seed width and seed thickness.

– The development of hybrids with a high genetic potential for seed yield requires information on the manifestation of heterosis for agronomically important traits in the F1 generation.

– As a results of our study it can be concluded that the effect of heterosis is not the same for all investigated characters.

– High heterosis value can be expected in crosses of extremely divergent lines.

– Hybrid presented at this study was developed with simple cross of Bulgarian line 807 A and mutant line 278 R. Hybrid was tasted at DAI – General Toshevo and considerably exceeded the mean standard (the Bulgarian commercial hybrid San Luka and French commercial hybrid LG-5635) by seed and oil yield.

– The hybrid simultaneously possessing resistance to the *Orobanche cumana* – race G and immunity to *Plasmopara helianhi*-races 300, 700 and 731. Combination of high seed and oil yield and resistance to diseases and parasites is desire in sunflower breeding program.

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