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SUNFLOWER HYBRID RADA, DEVELOPED WITH MUTANT RESTORER LINE 12002 R

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Abstract

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Hybrid Rada was developed by 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 ^{137}Cs at doze of 8 Gy for 1 min. Hybrid Rada was tested for three years in testing plots of Dobroudja Agricultural Institute - General Toshevo and for 2 years in 7 locations of State Variety Testing Commission.

A dispersion analysis of hybrid Rada was carried out with regard to the main indices seed and oil yield at trial field of DAI-General Toshevo. The hybrid considerably exceeded standard commercial hybrid Albena by seed yield with 50.4 kg/dka, or 16.1% and standard commercial hybrid Super Start with 54.6 kg/dka or 17.7%. The oil yield was significantly higher with 39.3 kg/dka, or 28.3% in comparison to standard Super Start.

The hybrid Rada considerably exceeded the mean standard (the Bulgarian commercial hybrid Albena, Mercury and Perfect and French commercial hybrid Diabolo PR) by seed yield with 31.8 kg/dka, or 8.2% at the 7 locations of State Variety Testing Commission.

Instead higher yield hybrid Rada possessing resistance to the *Orobanche cumana* race F and full resistance to *Plasmopara helianthi* – races 300 and 700.

Key-words: *Helianthus annuus*, gamma irradiation, mutant line, combining ability, hybrid, seed yield, oil yield

Introduction

Sunflower (*Heliathus annuus* L.) oil is major edible oil worldwide. It is an important and valuable field crop to supply food for both animals and humans.

Sunflower breeders use various methods in order to get new genetic variability or new elite lines which are used for making new productiv hybrids after examining the combaining abilities showed by newly created lines (Skoric et al., 2012).

Improving plasticity and enriching genetic potential of sunflower can be realized through gene mutations and recombination. Among the few techniques used in modern breeding, the method of mutagenesis is considered an effected one. Mutagenesis, both physical and chemical, proved favorable for mutation induction in tissue cultures. Anashchenko (1977) concluded that the most important effect of physical and chemical mutagenesis is that they cost the development of a large number of recessive genes and cytoplasmic mutations, which increase the variability of sunflower. Some of them can be of interest as agronomically important characters; others can be used as marker traits.

Increase of genetic variability by mutagenesis at mature sunflower seeds (gamma rays-^{137} Cs, ultra sonic and ethyl methansulfonate) was studied by Christov (Christov, 1990; 1993, 1995; Christov et al., 1996, 2002). The mutant line M95-674, with altered petiole, leaf shape, shape of the inflorescence was developed from cultivar VINK8931 using 150 Gy ^{60}Co gamma rays (Christov, 1996); it also carries a recessive mutation that renders resistance to *Orobanche cumana*.

Berville et al., 1992 treated mature seeds o F1 hybrids with gamma rays ^{60}Co (100 Gy, 200 Gy, 300 Gy and 400 Gy) and 0.2% ethyl metansulfonate and obtained mutants resistance to bifenox and glyphosate.

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The mature seeds were subjected to mutagenic treatment more often (Jambhulkar et al., 1999; Sagadeesan et al., 2008). According to Jambhulkar et al., 1999 the dose of 200 Gy produced the largest number of mutations.

Lyakh et al., 2005 studied the frequency and spectrum of morphological mutations, raised in M2 after sunflower mature and immature seed treatment with ethylmethanesulphonate (EMS).

Regretfully use of induced mutagenesis did not bring the expected results concerning the resistance to diseases (Skoric et al., 2012). Positive results have been obtained only for resistance to *Alternaria* and parasite *Orobanche cumana*. Treating two sunflower genotype with gamma rays (150 Gy and 165 Gy) and ethyl methansulfonate (0.05 mol/dm^{-3}) De Oliveira et al., 2004 obtained mutants, which possessed sufficient tolerance to *Alternaria helianthi*.

Sunflower lines resistant to *Orobanche cumana* were created after treatment of immature zygotic embryos with gamma rays ^{137}Cs and ultra sonic by Encheva et al., 1993; 2002; 2003a; 2003 b; 2003c; 2004a; 2004b).

Instead creating of new mutant sunflower lines, new hybrids developed with mutant sunflower lines were not published. Mutant restorer line 12003 R was used as the male parent of the hybrid Yana (Encheva et al., 2012). Hybrid Yana was officially registered in 2009.

In Bulgaria, the total cultivated sunflower areas are approximately 73 000 acres. The larger part of this territory is situated in North East part of the country- Dobroudja region. Sunflower production increses each year and in 2013 Bulgaria is on the second place after Romania in Europe. This enforces developing of hegh productive hybrids resistant to biotic and abiotic stress factors. The induced *in vitro* mutagenesis is one of the suitable methods for creating lines intended for heterosis breeding.

The aim of this study was: (a) investigation on some agronomical traits of hybrid Rada in testing plots of DAI- General Toshevo and (b) testing of hybrid Rada at 7 locations of State Variety Testing Commission

Materials and Methods

A part of the experiments were carried out at the trial field of Dobroudja Agricultural Institute- General Toshevo and State Variety Testing Commission, and another under laboratory conditions.

Developing of mutant line 12002 R

The Bulgarian fertility restorer line R 2574, witch is highly homozygtic, 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 min in 95% ethanol;
- 15 min in bleaching solution (2.7% Cl);
- 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 radiation at dose of 8 Gy for 1 min. before plating on nutrition medium M for further growing (Azpiroz et al.,1988):

1/2 MS (Murashige and Skoog, 1962) macro salts, MS micro salts, B5 vitamins (Gamborg et al., 1968), 20 g/l sucrose, pH-5.7.

The conditions for cultivation were: 25^{o}C, 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 which 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 criterion for selection was mutation for resistance to *Orobanche cumana.*

Hybridization

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

The obtained hybrid was tested for three years 1997, 1998 and 1999 at trial field of Dobroudja Agricultural Institute-General Toshevo and for 2 years 2002 and 2004 at the 7 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 hybrid Rada

The biometric evaluation and biochemical analysis of hybrid Rada and standards was made on 10 plants for each in-

dividual year, and included main agronomic traits as seed yield, oil yield, 1000 seed weight, oil in the kernel (%), plant height, head diameter, stem diameter, vegetation period, total seed number per head and mean value of seed yield per head.

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

Biochemical analysis

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

Statistical analysis

The standards and a developed new hybrid Rada were analyzed statistically with regard to the agronomic traits such as seed yield, oil yield, oil in the kernel, plant height, head diameter and vegetation period .

Analysis of the experimental data from DAI-General Toshevo was made by the statistical package BIOSTAST 6.0.

Results and Discussion

Study on the production potential of hybrid Rada

The aim of this study was to investigate some important agronomic traits of hybrid Rada (Figure 3). The farther line 12002 R (Figure 2) 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 sunflower line 12002 R, the sterile analogue of the Bulgarian selfed line 2607 A (Figure 1) was used. Sterile line was developed at the base of cytoplasm Pet 1.

The 3 years testing of line 12002 R in the trial field of Dobroudja Agricultural institute showed 100% restoration ability and very good combining ability.

To determine the combining ability of the new developed sunflower line R 12003, the sterile analogue of the Bulgarian selfed line 2607 (Figure 1) was used. Sterile line was developed at the base of cytoplasm Pet 1.

A dispersion analysis of hybrid Rada (Table 1) was carried out with regard to the main indices seed yield. The hybrid considerably exceeded standard commercial hybrid Albena with 50.4 kg/dka, or 16.1% and commercial hybrid Super Start with 54.6 kg/dka or 17.7%.

The oil yield of hybrid Rada was significantly higher with 39.3 kg/dka, or 28.3% in comparison to standard Super Start. Plant height is another trait with significant difference in comparison to standard Albena and Super Start. The

Fig. 1. Male sterile line 2607 A

Fig. 2. Mutant fertility restorer line 12002 R

Fig. 3. Hybrid Rada developed by the cross 2607 A x R 12002 R

Table 1
Characteristics of hybrid Rada and standards-commercial hybrids Albena and Super Start. Location DAI-General Toshevo, average data

Traits	Standard hybrid Albena	Standard hybrid Super Start	hybrid Rada	LSD
Seed yield, kg	312.36a	308.13c	362.77	Gd 5% = 35.75
Oil yield, kg	145.46	138.83c	178.17	Gd 5% = 2.40
Plant height, cm	178.30b	177.8c	172.80	Gd 5% = 3.75
Diameter of head, cm	22.10d	22.73b	21.80d	Gd 5% = 0.44
Oil in the kernel, %	45.95d	45.47d	45.95d	Gd 5 % = 3.89
Vegetation period	106.75d	107.75d	113.25d	Gd 5% = 7.77

Values with the same letter is not significant at the level of P=5 %.

Table 2
Characteristics of hybrid Rada

Characteristics	Expression
Hypocotyl-antocyanin color /phase cotyledon/	absent or very weak
Leaf size	average
Leaf shape	cordate
Leaf color	medium green
Leaf-antocyanin color /at the edge of the leaves before flowering	absent
Leaf shining	present
Leaf blistering	absent or very week
Leaf - fineness of serration	medium
Leaf - shape of cross section	concave
Leaf - wings	absent
Leaf angle of lateral veins	acute
Leaf height of the edge of the leaf surface regarding the petiole –2/3 of the stem above the soil	average
Leaf angle between the lower part of the leaf petiole and the stem	right angle or nearly right angle
Stem-intensity of hairiness at the top	medium
Stem number of leaves on the main stem	average
Plant height	medium
Time of flowering	averagely early
Ray flowers number	average
Ray flowers shape	elongated
Ray flowers coloring	yellow
Disk flowers color	yellow
Disk flowers-antocyanin coloration of stigma	absent or very light
Head-number of the leaflets at the base of the raceme /bract leaves/	average
Bract -shape	elongated
Bract color of the external part	medium green
Bract- antocyanin color	absent
Head –position at maturity	turned down
Head- size at maturity	average
Head- shape at the seed side /at maturity/	convex
Plant-natural height /at maturity/	average
Plant-branching at maturity	absent
Seed-size	average

Table 2 continued

Seed-shape	ovoid-elongated
Seed-thickness of the husk /in a cross cut/	thin
Seed-main color /in a cross section/	black
Seed stripes	present
Seed-color of the stripes	grey
Seed position of the stripes	marginal
The hybrid Rada is not branched and does not form additional branches in maturity.	
Resistance to *Plasmopara helianthi*	full resistance to race 300, 700
Resistance to *Orobanche cumana*	resistant to race F
Resistance to *Phomopsis helianthi*	Type 1
Resistance to *Phoma macdonaldii*	Type 2
Utilization	for oil production

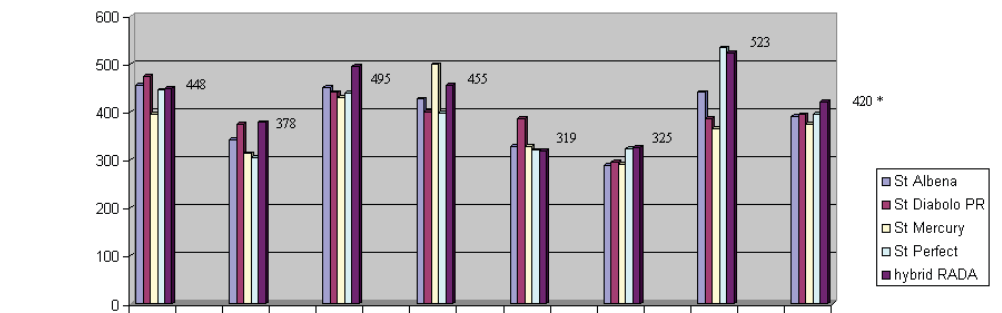
*: Type 1: a necrotic lesion up to 5 cm in diameter; Type 2: several necrotic lesions on the stem

observed difference at plant height was in direction towards decrease.

Hybrid Rada was included in testing at State Variety Testing Commission (in 7 different regions). A dispersion analysis of hybrid Rada-was carried out with regard to the main indices seed yield (Figure 4). The hybrid considerably exceeded the mean standard (the Bulgarian commercial hybrid Albena, Mercury and Perfect and French commercial hybrid Diabolo PR). The seed yield of hybrid Rada was significantly higher with 31.8 kg/dka, or 8.2%. The hybrid Rada exceeded the mean standard with regard of indices oil yield but this difference was statistically not significant (Figure 5).

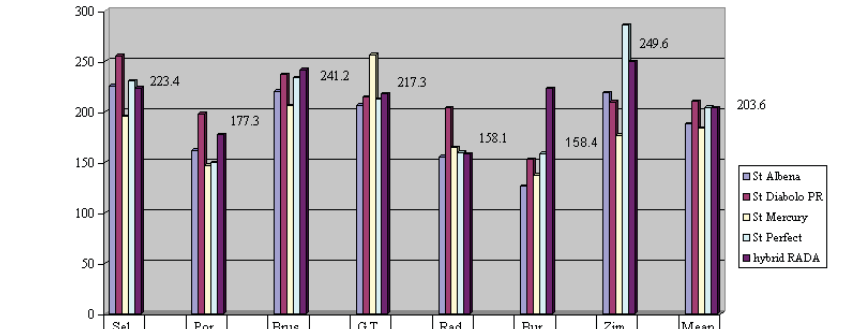
Biological characteristics of hybrid Rada

- Plant height of a hybrid Rada is from 131.3 to 172.0 cm
- Lodging or breaking of the head at maturity is not observed
- Average number of leaves on the main stem
- Lateral branches are not formed at maturity
- Leaves are average size, cordate and medium green
- Leaf blistering is week. Fineness of serration is medium
- The angle of lateral veins is acute.
- Concave leaf - shape of cross section
- Leaf wings absent
- Ray flowers elongated and yellow



	Sel.	Por.	Brus.	G.T.	Rad.	Bur.	Zim.	Mean
St Albena	456	342	450	426	328	288	440	390
St Diabolo PR	474	374	440	400	385	296	385	394
St Mercury	395	313	430	498	328	290	365	374
St Perfect	445	305	439	398	320	323	533	395
hybrid RADA	448	378	495	455	319	325	523	420

Fig. 4. Seed yield of hybrid Rada in comparison to mean standard of Bulgarian commercial hybrids Albena, Mercury and Perfect and French commercial hybrid Diabolo PR, at 7 locations of State Variety Testing Commission (*-P= 5 %)



	Sel.	Por.	Brus.	G.T.	Rad.	Bur.	Zim.	Mean
St Albena	225,7	161,5	220,2	206,4	155,4	126,2	218,7	187,7
St Diabolo PR	255	197,6	236,7	214,2	203,9	152,6	209,3	210
St Mercury	195,2	147	205,9	256,5	164,3	137	176,6	183,3
St Perfect	230,1	149,6	233,5	212,3	159,3	158,7	285,8	204,2
hybrid RADA	223,4	177,3	241,2	217,3	158,1	222,6	249,6	203,6

Fig. 5. Oil yield of hybrid Rada in comparison to and mean standard of Bulgarian commercial hybrids Albena, Mercury and Perfect and French commercial hybrid Diabolo PR, at 7 locations of State Variety Testing Commission

- Yellow disk flowers
- Anthocyanin coloration of disk flowers is absent
- Presence of pollen
- Bracts are medium green and elongated
- Turned position of a head at maturity
- Convex head-shape at the seed side/at maturity
- Seed-shape is ovoid-elongated with average size
- Seed is black with gray stripes
- The position of the stripes is marginal

Differences of hybrid Rada from standard Perfect are concerning 12 characteristics (Table 3). Considerable clear is the index leaf shining. It is absent at standard Perfect and present at a hybrid Rada. While ray flower shape of standard Perfect was oval, the new hybrid Rada has elongated one.

The hybrid Rada was characterized with turned down head position in contrast of standard Perfect which demonstrated half-turned down. While the seed shape of the standard was elongated at a hybrid Rada was avoid-elongated.

Table 3
Differences of some characteristics of hybrid Rada and standard Perfect

Characteristics	Standard hybrid Perfect	Hybrid Rada
Leaf size	large	average
Leaf shining	absent	present
Leaf- blistering	medium	absent or very week
Leaf- steam-number of leaves on the main stem	small	medium
Leaf- fineness of serration	fine	medium
Leaf-angle between the lower part of the leaf petiole and the stem	acute	right angle or nearly right angle
Stem-intensity of hairiness at the top	strong	medium
Ray flowers shape	oval	elongated
Date of flowering	medium-early	early
Head-position at maturity	half-turned down	turned down
Seed-shape	elongated	ovoid elongated
Seed position of the stripes	both marginal and lateral	marginal

Seed-position of the stripes was differing, also. Standard Perfect posses both marginal and lateral position, while hybrid Rada have marginal.

Conclusion

We succeed to create mutant sunflower line 12002 R with positive changes as resistance to parasite *Orobanche cumana*. The line was created at initial susceptible genotype 2574 R. Mutation was induced after treatment of immature sunflower (*Helianthus annuus* L.) zygotic embryos of fertility restorer line R 2574 with gamma irradiation ^{137}Cs at doze of 8 Gy for 1 min.

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

The tree years study in tasting plots of Dobroudja Agricultural Institute-General Toshevo and 2 years tasting at State Variety Testing Commission hybrid Rada considerably exceeded the standards (the Bulgarian commercial hybrid Albena, Super Start, Perfect and Mercury and French commercial hybrid Diabolo PR) by seed yield and oil yield.

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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