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NEW SUNFLOWER RESTORER LINES DEVELOPED BY DIRECT ORGANOGENESIS METHOD FROM INTERSPECIFIC CROSS *Helianthus annuus* L. (cv. ALBENA) × *Helianthus salicifolius* L.-DISEASE RESISTANCE, COMBINING ABILITY

Encheva, J.*, Christov, M., Shindrova, P., Drumeva, M. and Encheva, V.

Dobroudja Agricultural Institute-General Toshevo, 9520

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SUMMARY

The direct organogenesis method in immature F_1 embryos from sunflower was successfully applied for production of new forms from the interspecific cross *Helianthus annuus* (cv. Albena) × *Helianthus salicifolius*. After repeated selfing and continuous selection, a great diversity of new sunflower lines were developed. Some of the new lines possessed resistance to *Phomopsis*, *Phoma*, *Alternaria* and the parasitic angiosperm broomrape as well as very good combining ability. The hybrids developed from the new lines showed increases of the indices for seed and oil yields of 114.0% and 117.8%, respectively, compared with the mean standard (commercial hybrids Albena and Super Start), short vegetation period and reduced height. Combinations of these favorable changes are desirable in breeding work on sunflower.

Key words: sunflower, *Helianthus salicifolius*, direct organogenesis, disease resistance, combining ability

INTRODUCTION

An investigation on the productivity of cultivated sunflower showed that diseases are the most important limiting factor in the majority of countries producing sunflower. The widely spread diseases such as *Phomopsis helianthi*, *Phoma macdonaldii*, *Alternaria helianthi* and *Plasmopara helianthi* and the parasite *Orobanche cumana* drastically decrease sunflower yield especially in Central and Southern Europe. Wild *Helianthus* species are a potential source of genes for resistance to diseases (Škorić, 1987, Christov, 1990, Sackston, 1992; Seiler, 1992; Škorić and Rajčan, 1992; Škorić, 1995; Georgieva-Todorova, 1993, 1997).

* Corresponding author: e-mail: july_262002@yahoo.com

Application of new biotechnology methods can help in exceeding the present problems in interspecific hybridization of sunflower. By using the embryo culture method, hybrid plants resistant to *Phomopsis helianthi* (Dozet *et al.*, 1996) and partially resistant to *Sclerotinia* (Köhler *at al.*, 1999) were produced as a result of crossing cultivated sunflower with the wild species *Helianthus tuberosus*. With the help of the direct organogenesis method, hybrid plants resistant to *Orobanche cumana* were developed from crossing with the wild species *Helianthus tuberosus* (Encheva *et al.*, 2004).

The embryo rescue technique is most commonly used for overcoming the incompatibility between *H. annuus* L. and different alien species (Chandler and Beard, 1983; Kräuter *et al.*, 1991; Bohorova *et al.*, 1991; Friedt, 1992; Korell *et al.*, 1996a). However, it does not always contribute to the production of hybrid plants. This moved us to investigate possibilities of the direct organogenesis method, which has not been applied before, as an approach for overcoming the interspecific and intergeneric incompatibilities in sunflower hybridization (Encheva *et al.*, 1992). In comparison with the authors mentioned above, this study presents data on hybrid progenies in an advanced generation, as well as data on their combining ability.

The aim of this study was: a) to follow the reaction to the diseases *Phomopsis helianthi*, *Phoma macdonaldii*, *Alternaria helianthi* and *Plasmopara helianthi* and a local population of the parasite *Orobanche cumana* of the F_{10} hybrid progenies of the cross *H. annuus* (cv. Albena) × *Helianthus salicifolius* produced through the direct organogenesis method, and b) to investigate the combining ability of the new fertility restorer lines (R) against *H. petiolaris* cytoplasm.

MATERIALS AND METHODS

Cultivated sunflower (hybrid Albena-2n=34) and the wild species *Helianthus salicifolius*, accession M-078 (2n=34), were grown under field conditions at DAI-General Toshevo. Hybrid embryos were obtained by sterilizing the pollen of the female parent (hybrid Albena) with gibberellic acid and by hand pollination of the female plants with pollen from the male parental form, *Helianthus salicifolius.*

Direct somatic buds and plants were induced on nutrition media I, II and III (Encheva *et al.*, 1992). Electrophoretic studies confirmed the hybrid character of the obtained plants (Encheva *et al.*, 1992). As a result from repeated selfing and continuous individual selection in the hybrid materials, fertility restorer lines were produced in the F_{10} generation. All hybrid materials possessed a *cms* source of *H. petiolaris* from Leclerq (1969). In the crosses made, the female form had sterile cytoplasm of *H. petiolaris* and, therefore, only fertile forms were considered in this research, *i.e.*, the ones possessing a gene for restoration. The origin of this gene has not been proved by us. It may originate from both the female form - hybrid Albena,

and the wild male parent; there was evidence for the latter that it carries genes for restoration of this cytoplasm (Christov, 1996; Christov *et al.*, 1996).

The phytopathological evaluation

The phytopathological evaluation of the parental forms and the obtained hybrid progenies was performed with regard to the local broomrape (*Orobanche cumana* Wallr.) population and Phomopsis (*Phomopsis helianthi*, Munt.-Cvet. *et al.*), phoma *(Phoma macdonaldii*, Boerema/*Phoma oleracea* var. *helianthi-tuberosi* Sacc), Alternaria (*Alternaria helianthi)* and Downy mildew (*Plasmopara halstedii* (Farl.) Berl. & de Toni) at the Sunflower Phytopatology Laboratory and infection fields of DAI - General Toshevo. The evaluation was carried out in the period 1997-1999, following the standard methodologies. The estimation of resistance to Phomopsis was performed 20 days after inoculation of plants at budding stage (Tourvieille *et al.*, 1988). A six-degree scale was used:

- 0 = healthy plant;
- 1 = spots on the stem located around the leaf petiole;
- 2 = spots reaching up to 5 cm;
- 3 = spots girdling the stem;
- 4 = full necrosis of the pith and
- 5 = broken stems.

Intensity of infection with Alternaria and Phoma was estimated on the scale 0-4, in the infection field (Iliescu, 1992). The reaction of sunflower genotypes to broomrape was examined in the greenhouse using a technique described by Panchenko (1973). The reaction of 20 plants from each genotype was recorded using the following scale: 0%=S (sensitive); 100%=R (resistant). The method for determining the reaction of sunflower genotypes to downy mildew was described by Viranyi and Gulya (1995). Reaction of 20 plants from each genotype was recorded using the following scale: 0%=S (sensitive); 100%=R (resistant).

Biometric evaluation and biochemical analysis of hybrids 56, 81 and 88 developed from the new lines R107, R114 and R120

The biometric evaluation and biochemical analysis of the main agronomic characters of the new developed hybrids, seed yield and oil yield, were made on 30 plants in three replications for each individual year. Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) was used to determine oil content in air-dry seeds of the developed hybrids.

Hybridization

To determine the combining ability of the newly developed sunflower restorer lines, a sterile analogue of the Bulgarian selfed line ms2607 was used. The standards for comparing the new hybrids 56, 81 and 88 were the Bulgarian commercial hybrids Albena and Super Start. The obtained hybrid combinations were tested for

two years (1997 and 1999) at the breeding fields of DAI according to the block-design method, in three replications, the area of each replication being 10 m^2 (Barov and Shanin, 1965).

RESULTS AND DISCUSSION

A) Evaluation of the materials for some economically important diseases and parasites on sunflower

In most countries where sunflower is grown commercially, successful production is endangered by many fungal pathogens and parasites. Losses may be severe, near 100%, or even entire fields may perish under extreme circumstances.

Lines R107, R114 and R120 were considerably important as initial breeding material for sunflower selection and for hybrid seed production because in addition to resistance to some economically important diseases and parasite broomrape, they possessed very good combining ability.

The phytopathological evaluation of the parental form Albena and the obtained hybrid progenies was performed with regard to the local broomrape population and the diseases Phomopsis, Phoma, Alternaria and Downy mildew. The results from the three-year testing of promising R lines are given in Table 1. The 1997-1999 data show complete resistance to Phomopsis and 100% resistance to Downy mildew, as well as tolerance to Phoma of line R 114. Resistance to Altenaria and tolerance to Phoma were observed in line R120. The resistance of lines R114 and R120 to some of the economically important diseases probably comes from the wild species *H. salicifolius* which, according to the investigations of Christov, 1990, 1996 and Christov *et al*., 1996, has shown complete resistance to Downy mildew, Phomopsis and Phoma. Among the sources of resistance to phomopsis and alternaria, Škorić, 1987, pointed out *H. salicifolius*. The female form (hybrid Albena) is, on its part, susceptible to the diseases mentioned above, with the exception of downy mildew.

Table 1: Evaluation of advanced R lines from the interspecific hybrids *H. annuus* × *Helianthus salicifolius* and parental forms for resistance to Phomopsis, Phoma, Alternaria, Downy mildew race 300 (1997-1999) and Broomrape (1999)

Variant	Year												
	1997				1998				1999				
	a	b	c	d	a	b	c	d	a	b	c	d	e
hybrid Albena	2	2/3	2	100	2	2/3	2	100	2	2/3	2	100	0
H. salicifolius M-087	0	0	0	100	0	0	0	100	0	0	0	100	100%
R107	2	2/3	2	100	2	2/3	2	100	2	2/3	2	100	100%
R114	0	1/3	2	100	0	1/3	2	100	0	1/3	2	100	0
R120	2	1/3	0	100	2	1/3	0	100	1	1/3	0	100	0

Key: a) Phomopsis (scale 0-4); b) Phoma (scale 1/3-3/3);
c) Alternaria (scale 0-4); d) Downy mildew (scale 0-100%);
e) Broomrape (scale 0-100%)

Figure 1: Line R107 from the cross H. annuus (cv. Albena) × H. salicifolius

Figure 2: Hybrid 81 from the cross ms2607 × line R107

Figure 3: Hybrid 88 from the cross ms2607 × line R114

Broomrape is a major parasite in various parts of Europe, and especially in Spain, the Near East and China (Škorić, 1994). Therefore, development of new lines resistant to this parasite is essential for sunflower breeding. As a result of interspecific hybridization, line R107 was developed, which showed 100% resistance to broomrape against artificial infection background. The resistance of line R107 comes from the wild species *H. salicifolius*, which, according to Christov *et al*. (1996), has complete resistance to the parasite under both field and laboratory conditions.

B) Investigation on the productive potential of test hybrids developed from self pollinated sunflower lines from the cross *H. annuus* × *Helianthus salicifolius*

The results from the two-year testing of the test hybrids of the lines developed by the direct organogenesis method from the interspecific hybrid *H. annuus* (hybrid Albena) × *H. salicifolius* are presented in Figures 4-5 and Tables 2-3. The sterile analogue of the Bulgarian line 2607 was used as a tester.

Lines R107 (Figure 1) and R114 participated in the development of hybrids 81 (Figure 2) and 88 (Figure 3). The two-year testing revealed the very good production potential of the two hybrids.

Averaged for the two years, hybrid 81 gave a higher seed yield, by 37.0 kg/dka or 12.4%, than the mean standard (Figure 4). Hybrid 88 gave a higher seed yield than the standard, by 32.5 kg/dka or 110%. These differences were highly significant.

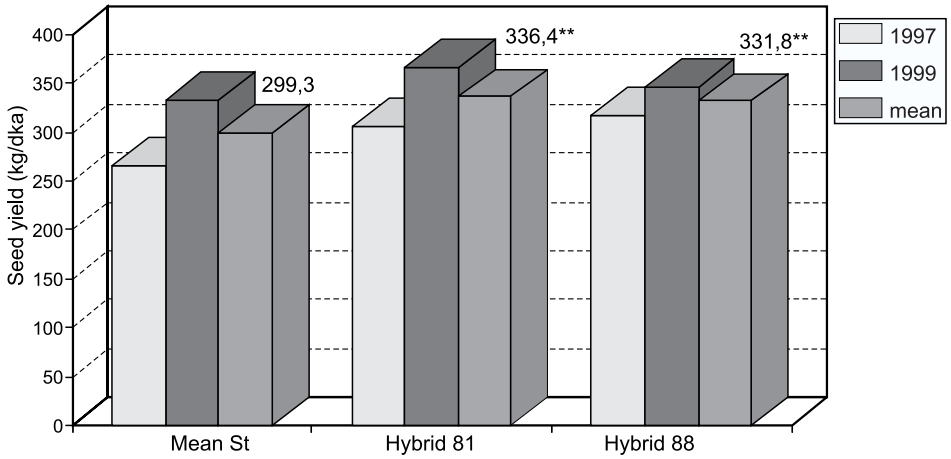

*Figure 4: Comparison of seed yields of hybrids 81 and 88 and the mean standard (commercial hybrids Albena and Super Start) during 1997 and 1999 (**P=0.1%)*

The data for oil yield (Figure 5) show that hybrid 81 exceeded the mean standard by 20 kg/dka or 15.2%. The oil yield of hybrid 88 was higher by 22.4 kg/dka or 14.0%. The results for hybrid 81 were highly significant.

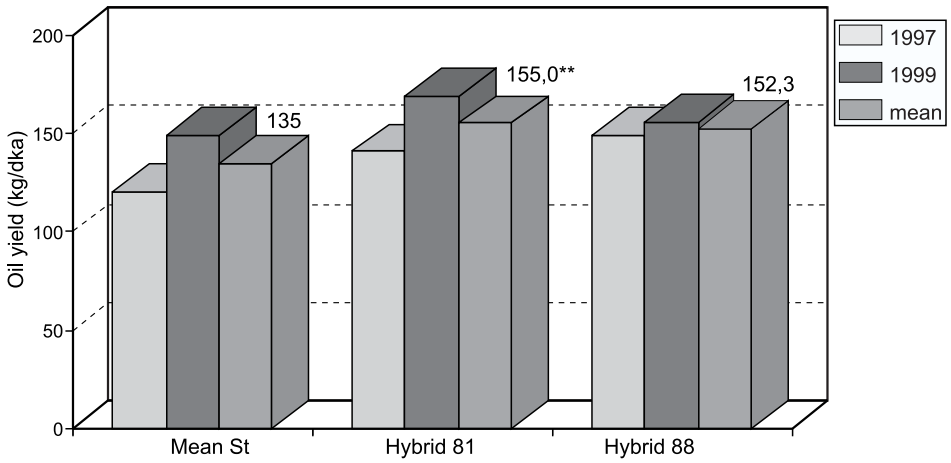

*Figure 5: Comparison of oil yields of hybrids 81 and 88 and the mean standard (commercial hybrids Albena and Super Start) during 1997 and 1999 (**P=0.1%)*

Table 2 provides data on the vegetation period and plant height for the two test hybrids. A statistically significant decrease of the vegetation period, by 4 days on average for the two years, was observed in hybrid 88. This hybrid showed the most significant decrease of plant height, by 19.5 cm, compared with the standard (Albena and Super Start) (Table 2). A significant difference of 4.5 cm was also observed in hybrid 81 in 1999.

Table 2: Phenological observation and morphological characterization of test hybrids 81 and 88 developed from lines R107 and R114, respectively; averaged data

Hybrid, year		Cross	Vegetation period difference against the mean standard, days			Plant height difference against the mean standard, cm		
1997	1999		1997	1999	mean	1997	1999	mean
No 47	No 81	ms2607 × R107	- 3	+ 2	- 1	-3.3	-4.5*	-4
No 52	No 88	ms2607 × R114	-5	-3	-4*	-19.8	-19.2	-19.5***

The indices for oil content in seed (%) and head diameter are given in Table 3. A high oil percentage was registered in hybrid 81, 3.4% above the standard. A considerable decrease, 1.4 cm, was determined in hybrid 81 with regard to the index for head diameter. The differences in both characters were not significant.

Table 3: Biochemical and morphological characterization of test hybrids 81 and 88 developed from lines R107 and R114, respectively; averaged data

Hybrid, year		Cross	Seed oil content difference against the mean standard,%			Head diameter difference against the mean standard, cm		
1997	1999		1997	1999	mean	1997	1999	mean
No 47	No 81	ms2607 × R107	104.0	102.7	103.4	-2.3	-0.5	-1.4
No 52	No 88	ms2607 × R114	106.0	100.0	102.2	-0.8	-0.4	-0.6

Beside the above advantages over the mean standard for seed and oil yield, hybrid 81 also showed 100% resistance to the parasite *Orobanche* in a test against an artificial infection background conducted in 1999. The resistance of hybrid 81 to *Orobanche* probably comes from line R107, which is 100% resistant to the parasite. Hybrid 81 resulted from the cross ms2607 × R107 and the resistance to broomrape was probably determined by the dominant gene in the male line, since the female line ms2607 is not resistant to the parasite.

The one-year results for test hybrid 56 developed from line R120 (Table 4) showed higher values than the mean standard for seed yield and oil yield, by 14.0% and 17.4%, respectively. These differences were not significant. Highly significant decreases of plant height (by 29.5 cm) and vegetation period (7 days) were observed.

Table 4: Biochemical and morphological characterization and phenological observations of test hybrid 56 developed from line R120 (1997); averaged data, difference against the mean standard

Hybrid	Cross	Seed yield		Oil yield		Plant height	Head diameter	Vegetation period
		kg/dka	%	kg/dka	%	(cm)	(cm)	(days)
No 56	ms2607× R120	303.8	114.0	141.7	117.4	-29.5***	-1.1	-7**

a, b and c = significance of differences at the levels of 0.05, 0.01 and 0.001, respectively

Beside the higher seed and oil yields, hybrids 81, 88 and 56 demonstrated a decreased plant height and a shorter growing season. These positive changes are important for the sunflower breeding program because they allow easier harvesting and earlier clearing of the fields for subsequent agrotechnological activities.

CONCLUSION

Using the direct organogenesis method in interspecific hybridization, fertility restorer lines were developed which possess very good combining ability in hybridization and resistance to some economically important diseases and parasites on sunflower. An early flowering line, R107, was developed which possesses 100% resistance to *Orobanche*. Hybrid 81, produced from line R107, exceeded the mean standard (hybrids Albena and Super Start) in seed and oil yields and it was 100% resistant to broomrape.

Line R114 demonstrated complete resistance to Phomopsis, tolerance to Phoma and 100% resistance to Downy mildew. This line had earlier dates with regard to the indices for beginning of flowering and mass flowering and a shorter vegetation period in comparison with the cultivated parent (hybrid Albena). Hybrid 88, developed line R114, exceeded the mean standard in seed and oil yields. It also had shorter plant height and growing season thanthe standard.

Line R120 developed from the cross with *H. salicifolius*, possessed an earlier date for beginning of flowering and a shorter vegetation period than the female par-

ent (hybrid Albena). The line demonstrated resistance to Alternaria, tolerance to Phoma and 100% resistance to Downy mildew. Hybrid 56 developed from line R120 showed higher seed and oil yields in comparison with the mean standard as well as shorter plant height and growing season.

The lines developed through interspecific hybridization are valuable initial material for sunflower breeding and hybrid seed production.

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NUEVOS RESTAURADORES DE GIRASOL FORMADOS POR EL MÉTODO DE ÓRGANOGENESIS DIRECTA DEL CRUZAMIENTO DE INTERESPECIES DE *Helianthus annuus* L. (cv. ALBENA) × *Helianthus salicifolius* – RESISTENCIA A ENFERMEDADES, HABILIDAD DE COMBINACIÓN

RESUMEN

El método de organogénesis directa en los embriones de girasol F_1 no madurados, fue aplicado con éxito para la formación de nuevas formas de cruzamientos de interespecies de *Helianthus annuus* (cv. Albena) × *Helianthus salicifolius*. Tras repetida autofecundación y selección continua, fue creado un elevado número de líneas que se diversificaban entre si significativamente. Algunas de ellas poseían resistencia a *Phomopsis*, *Phoma*, *Alternaria* y angiosperma parásita, el Hopo del girasol, tanto como buena habilidad de combinación. Los híbridos engendrados de las nuevas líneas, mostraron el incremento de índice para el rendimiento de semilla y aceite, de 114.0% y 117.8%, en relación con el valor medio del estándar (híbridos comerciales Albena y Super Start), corta vegetación y altura reducida. Las combinaciones de estos cambios positivos son deseables en el trabajo de selección en girasol.

NOUVELLES LIGNÉES RESTAURATRICES DE TOURNESOL DÉVELOPPÉES PAR ORGANOGÉNÈSE DIRECTE DU CROISEMENT INTERSPÉCIFIQUE *Helianthus annuus* L. (*cv*. ALBENA) x *Helianthus salicifolius* – RÉSISTANCE À LA MALADIE, APTITUDE COMBINATOIRE

RÉSUMÉ

La méthode d'organogénèse directe chez les embryons de tournesol F_1 immatures est utilisée avec succès dans la réalisation de nouvelles formes depuis le croisement interspécifique *Helianthus annuus* (*cv*. Albena) × *Helianthus salicifolius*. Après autofécondation répétée et sélection continue, un grand nombre de lignées significativement différentes ont été réalisées. Certaines des nouvelles lignées possédaient une résistance envers les *Phomopsis*, *Phoma*, *Alternaria* et l'angiosperme parasite de l'orobanche ainsi qu'une bonne aptitude combinatoire. Les hybrides créés à partir des nouvelles lignées ont montré une augmentation de l'indice de rendement en graines et en huile de 114,0% et de 117,8% relativement à la valeur moyenne standard (hybrides commerciaux Albena et Super Start), une végétation brève et une diminution de la hauteur. Des combinaisons de ces changements positifs sont souhaitables dans le travail de sélection sur les tournesols.