СЪЗДАВАНЕ НА ЛИНИИ, ВЪЗСТАНОВИТЕЛИ НА ФЕРТИЛНОСТТА, ПРОИЗХОЖДАЩИ ОТ МЕЖДУВИДОВИ ХИБРИДИ
CREATION OF LINES RESTORERS OF FERTILITY ORIGINATING FROM INTERSPECIFIC HYBRIDS

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Abstract

Hybrid material, obtained by applying interspecific hybridization between cultivated sunflower and accessions of wild annual species *Helianthus annuus*, *H. petiolaris*, *H. debilis* and *H. neglectus*, maintained and preserved in the collection of *DAI-General Toshevo*, was used for the production of new sunflower fertility restorer lines. Sib-pollination, self-pollination and backcrossing were successfully applied. New R-lines were developed from preliminary tested hybrid material, obtained by repeated individual selection. They were characterized by resistance to leaf pathogens (*Phomopsis helianthi/ Diaporthe helianthi* Munt.-Cvet. et al., *Phoma macdonaldii* Boerema), downy mildew (*Plasmopara halstedii* (Farl.) Berl. anddeToni/*Pl. helianthi* Novot.) and to the broomrape parasite (*Orobanche cumana* Wallr.). The new restorer lines combined useful agronomic traits – suitable vegetation period, prolonged period of flowering, 1,000 seeds weight, high seed set, high seed oil content, as well as avery good general combining ability. As a result of the study, new hybrids with the participation of those *R-lines* were produced.

Key words: hybridization, *H. annuus*, *H. petiolaris*, *H. debilis*, *H. neglectus.*

INTRODUCTION

The inclusion of wild species from the genus *Helianthus* in the improvement breeding work on sunflower was of great importance for the increase of sunflower production. They possessed not only considerable variability for most of the traits, but also excellent survival environmental mechanisms. They possessed genes for resistance to diseases (biotic stress), tolerance to abiotic stresses (drought, cold, soil salinity, certain herbicides) and varied protein and oil content in seeds (Cáceres et al., 2006; Cáceres et al., 2007; Miller, J.F., and G.J. Seiler., 2005; Onemli, F. and Gucer, T., 2010; Terzic S. And Atlagic J., 2008; Vassilevska-Ivanova et al., 2001). During the last several decades, the diversity of sunflower was enriched and the system CMS-fertility restoration was successfully established. In 1969 Leclercq found the firsts table source of cytoplasmic malesterility CMSP et 1 in the cross between wild *H. Petiolaris* species and the cultivated sunflower (variety Armavirskii 9345), which was wide spread. Kinman identified the gene for restoration of fertility of the first CM Sin 1970 as *Rf1* (Vranceanu & Stoenescu, 1971). Sources for fertility restorer genes (Rf genes) were discovered by Skoric (1988), Christov (1996), Tavoljansky and Anaschenko (2001) and etc. They were transferred to lines with high combining ability (Skoric, 1992). On this base, the heterosis breeding on sunflower was successfully applied. The present worldwide interest in growing sunflowers as a crop is due to the increased yield of the new commercially available oilseed hybrids. This was obtained by increasing the portion of kernel to the whole achene. The decreasing of hull thickness led to facilitating the sunflower moth attacks and damages in process of harvest and post-harvest activities (Ivanov and Tsvetkova, 1984).

Sunflower was used as a traditional source of proteins. According to Christov (1990) if sunflower breeding was conducted in that direction, it needed a wide diversity of initial materials. It means that some forms of the genus *Helianthus* can be used as initial genetic source. According to Christov (1993) in the annual species, differentiation of a genotype is possible as a result of self-pollination of a single plant in natural or artificial conditions. In that way, a different number of stabilized genotypes, which, in total, expressed the diversity of the given species, represented each species. It was known that within each *Helianthus* species, there is a great diversity in form and the value of individual traits.

The farmers aspired to use hybrids, distinguished with high seed yield, ability to overcome different stress factors such ecological changes in the environment and diseases and pests attacks (Georgiev et al., 2012a; Georgiev et al., 2012b; Encheva and Georgiev, 2009).

The aim of this investigation was to present the results of the breeding work on development of fertility restorer lines (R-lines) obtained by applying of interspecific hybridization in the genus *Helianthus*.

MATERIALS AND METHODS

The hybrid material was created using some accessions from the collection of wild Helianthus species at Dobrudzha Agricultural Institute, General Toshevo. The following accessions of annual wild species were used for the purposes of hybridization: H. annuus E-004, E-035, E-045, E-121, E-123; H. argophyllus E-007 E-008; H. debilis E-010, E-011; H. petiolaris E-023, E-024; H. praecox E-027, E-145. Interspecific crosses *cultivated sunflower x wild species* were performed and the obtained hybrid plants were grown in field conditions. The cultivated sunflower was represented by four CMS lines – 325A, CAB-1, 813 A and846 A. R-lines were developed on the method of Christov and Petrov (1988).

The only fertile F_1 plants were self-pollinated individually under isolation bags. Backcrosses were carried out with the same sterile forms pollinated by pollen of F1 hybrid plants. Self-pollination and repeated individual selection were maintained until plant characteristics were stabilized and plants uniformed. This process needed several years. All advanced R-lines were tested for resistance to diseases and to broomrape. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berleseet de Toni) was carried out on the method of Vear and Tourvieille (1987). Evaluation for resistance to grey spots on sunflower (*Phomopsis/Diaporthe helianthi* Munt.-Cvet. et al.) was carried out on the method of Encheva and Kiryakov (2002) in field conditions on artificial infection plot. Evaluation for resistance to black spots on sunflower (*Phoma macdonaldii* Boerema/*Phoma oleracea* var. *helianthi-tuberosi* Sacc) was carried out on the method of Fayrallai Maric (1981) in field conditions on artificial infection plot. The seed oil content was measured on the method of Rushkovskii (1957). The sterile analogues of lines with normal cytoplasm 813 A, 217 A, 325 A and CAB-1 A were used as testers for evaluation of general combining ability (GCA). Only lines with very good general combining ability were included in hybridization with other lines 10A, 1017A, 325 A, 197 A and T6 A. The hybrid combinations were tested in variety testing trials at DAI.

RESULTS AND DISCUSSION

After prolonged self-pollination were obtained 135 new forms suitable for developing of new restorer lines and 23 new uniform R-lines. The breeding of new forms and the new R-lines was carried out by crossing of F_1, F_2 or BC_1 plants with male sterile lines (CMS Pet 1). The characterization of new R-lines showed that there existed a great variability among them. Short plants, fully branched were obtained using the accessions of *H. debilis* and *H. praecox*species. High plants, branched at the upper part of the plants were obtained using the accessions of wild *H. annuus* and *H. petiolaris*. High and unbranched or branched at the lower part of the plants were those obtained using the accessions of wild *H. annuus* and *H. argophyllus*. Most of forms obtained were with well-formed central head. For some others, originated from wild annual *H. debilis*, *H. praecox*and *H. petiolaris,* the central and lateral headswere almost equal in their size. Many of the branched R-lines had a prolonged period of flowering. For some of them it lasted more than 20 days. The vegetation period of the most new lines was medium. Medium maturity was established for lines originated from *H. petiolaris* and wild *H. annuus*. Early maturity was established for lines originated from wild species *H. praecox* and wild *H. annuus*. The head diameter varied from 9 to 23 cm. Number of branches was also varied from 7 to 17 per plant. Great variability was established for the characters – length, width and color of ray flowers. Seeds were distinguished by their size, weight and color. Most of them were black with grey or dark brown stripes. Seed size and weight for most of branched plants were small to medium. Unbranched plants had medium to large seed weight predominantly. Some biochemical characters connected to seed oil content were established. The kernel content varied from 72,2% to 85,7% (fig. 1). The oil content in the kernel varied from 58,2% to 69,7%, but the seed oil content varied from 43,2% to 52,7%. Protein content of seeds was significantly less and varied from 17,6% to 28%. The most variable character was 1000 seeds weight, which varied from 28 g to 72 g.

There action of advanced R-lines, characterized with high seed oil content, to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with aim to establish the resistant ones. Resistance 100% to *Pl. helianthi* Novot. and 76-99% to *Orobanche Cumana* Wallr. was reported for restorer lines originated from wild species *H. Annuus* (accessions E-045, E-123), *H. Argophyllus* (accession E-008), *H. Debilis* (accession E-010, E-011) and *H. Petiolaris* (accession E-023). Some restorer lines

originated from *H. Agrophyllus* (accession E-007) and *H. praecox* (accession E-027) demonstrated full resistance to broomrape. Most of the studied restorer lines were tolerant to *Phomopsis helianthi* and some of them, originated from H. agrophyllus (accessions E-007, E-008) were with immune type of reaction to this pathogen. Greater part of the studied lines were tolerant to the caused agent of black spots on sunflower too. Resistance 76-99% to *Pl. helianthi Novot*. And *Orobanche Cumana* Wallr. showed the studied restorers of fertility, originated from *H. Annuus* (accessions E-004, E-035, E-121), *H. Petiolaris* (accession E-024) and *H. praecox* (accession E-145).

Some morphological characters of the obtained new restorer lines were presented on table 1. The plant height varied from 100 cm to 170 cm. The new restorers were with higher seed oil content than the check. Longer vegetation period was established for restorer lines AM-21, AM-27, AM-31 and AM-42 originated from *H. argophyllus* accessions. The shortest period of vegetation was determined for lines JM-11, JM-14, JM-17 and JM-19, originated from wild *H. praecox*.

Some special morphological characteristics were also observed. The ray flowers of lines, originated from wild *H. argophyllus* were long with orangenuance. These lines were also distinguished with intensity of hairiness at the top of the stem. Fine to medium leaf serration was observed at lines, originated from the wild species *H. debilis*. Longest were the tips of bracts of lines, originated from wild *H. petiolaris* accessions.

Very good general and specific combining ability was established for lines KM-186, KM-187, KM-226, KM-231, KM-239, AM-31, PM-211, PM-234, JM-17. These lines were distinguished with highest seed set after self-pollination.

All hybrids obtained with participation of paternal lines AM-21,AM-27, AM-31 and AM-42 possessed resistance to broomrape. Resistance to downy mildew was established for hybridsobtained with participation of paternal lines DM-17, DM-22, PM-219,JM-19.

Some of obtained hybrids with durable resistance to grey spots on sunflower, downy mildew and broomrape were presented on table 2.

Fig. 1. *Biochemical characters connected to seed oil content of advanced R-lines*

Table 1. Some morphological charactersof the obtained new restorer lines

Line	Plant height, cm	Head diameter, cm	Days to maturity	Seed oil content, %	Origin of Rf genes
KM-114	145	19	100	46,6	*H. annuus* E-004
KM-163	100	19	101	48	*H. annuus* E-035
KM-186	150	21	98	47,5	*H. annuus* E-045
KM-187	150	20	98	48	*H. annuus*E-045
KM-226	145	20	100	49,2	*H. annuus* E-121
KM-231	160	19	102	50	*H. annuus* E-123
KM-239	155	18	102	51,2	*H. annuus* E-123
AM-21	164	14	105	46,8	*H. argophyllus* E-007
AM-27	168	14	104	47,2	*H. argophyllus*E-007
AM-31	164	15	105	48	*H. argophyllus* E-007
AM-42	170	14	108	47,6	*H. argophyllus*E-008
DM-17	120	13	102	48	*H. debilis* E-010
DM-22	110	14	100	46,9	*H. debilis* E-011
PM-211	160	13	103	52,7	*H. petiolaris* E-023
PM-219	155	13	103	49,5	*H. petiolaris* E-023
PM-234	160	13	103	49	*H. petiolaris* E-023
PM-248	140	11	101	50	*H. petiolaris* E-024
JM-11	110	14	86	48,9	*H. praecox* E-027
JM-14	120	15	86	47,5	*H. praecox* E-027
JM-17	130	17	82	46,9	*H. praecox* E-028
JM-19	135	17	84	47,2	*H. praecox* E-028
check	80	13	92	45,2	7009 R

Table 2. Characteristics of some hybrids developed by crossing of lines KM-186, KM-187, KM-226, AM-31, PM-211

Hybrid	Seed yield		Seed oil content,%	Oil yield		Plant height, cm	Vegetation period, days
	kg/da	% of average standard		kg/da	% of average standard		
GT-15 DV	351	102,3	47,8	215	103,1	140	110
GT-19 DV	394	110	50,3	234	107,9	160	118
GT-22 DV	375	105,4	49	228	105,8	150	115
GT-25 DV	405	112,8	51,1	241	111	170	118
GT-27 DV	364	103,4	48,2	219	104,2	140	110
GT-31 DV	381	106,7	49,5	229	106,2	150	115
GT-32 DV	390	108,2	49,5	232	107,3	155	118
Standard – average means of hybrids San Luka, Klarisa, LE 19							

CONCLUSIONS

1. Because of successfully applied interspecific hybridization, self-pollination, backcrossing and prolonged selection some new sunflower forms, lines and hybrids were developed.

2. The new genetic material was of importance for transferring of new genes, controlling important economic characters from wild *Helianthus* species to cultivated sunflower.

3. The obtained new hybrids could be successfully produced.

4. The obtained new and resistant to diseases restorer lines, which were distinguished by very good combining ability and high seed oil content, could be used in the breeding program on sunflower at Dobrudzha agricultural institute, General Toshevo.

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