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Hybridization between cultivated sunflower and wild annual species *Helianthus petiolaris* Nutt.

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Abstract. *Interspecific hybridization was carried out between sterile analogues of cultivated sunflower lines with normal cytoplasm and wild annual Helianthus petiolaris accessions from a collection of DAI-General Toshevo. The obtained F, progenies were characterized from morphological, phenological, biochemical and phytopathological point of view. Hybrid forms with resistance to leaves pathogens, downy mildew and broomrape were obtained. They were distinguished with diversity of seed oil content. The most variable phenological phases of hybrid plants from all crosses were duration of flowering period and germination. The hybrid plants from crosses 325 A x E-142 and 813 A x E-142 were characterized with higher seed oil content than the other studied crosses. All hybrid plants, originating from wild Helianthus petiolaris accession E-142 were resistant (100%) to downy mildew and to the parasite broomrape. They were also with immune type of reaction to the leaves pathogens and with the highest oil content in seeds. Among the included in the investigation accessions, E-142 was characterized with the highest crossability. The hybrid plants of the crosses included in this study were carriers of Rf genes and could be used for developing restorer lines.*

Keywords: interspecific hybridization, *Helianthus petiolaris,* crossability, resistance, oil content

Introduction

Wild species from the genus *Helianthus* possess not only considerable variability for most of the traits but also excellent survival environmental mechanisms. They have been included in the breeding process as sources of valuable characters for the cultivated sunflower, but their use was connected with some difficulties. Some of the problems in interspecific hybridization were very low or lack of crossability, sterility of hybrid progenies, long period of dormancy, abortion of hybrid embryo and obtaining seeds with immature endosperm. *H. petiolaris* Nutt. is a wild annual species, used in genetic improvement of cultivated sunflower as a donor for cytoplasmic male sterility. In 1969, Leclercq found out the first and widely used in heterosis breeding source of cytoplasmic male sterility PET 1 from the cross between wild species *H. petiolaris* and cultivated sunflower (variety Armavirskii 9345). Tsvetkova and Shopov (1976) obtained by interspecific hybridization with *H. petiolaris* Nutt. sources of fertility restorer genes (Rf genes). The wild species *H. petiolaris* is also a source of genetic resistance to diseases – *Sclerotinia sclerotiorum* (Cáceres et al., 2006), *Phomopsis helianthi* (Cáceres et al., 2007), *Plasmopara helianthi* (Christov, 2008), drought tolerance (Onemli and Gucer, 2010; Vassilevska-Ivanova et al., 2001), tolerance to certain herbicides (Miller and Seiler, 2005; Terzic and Atlagic, 2008). The accessions of this species were used in breeding programs for improving oil quality of cultivated sunflower seeds (Carrera and Poverene, 1995). *H. petiolaris* Nutt. hybridizes with cultivated sunflower *H. annuus* L., either artificially or spontaneously. Both species are diploids and in the same taxonomic section.

The aim of this investigation was to obtain hybrid plants, carriers of Rf genes, resistant to some diseases (*Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii*) and to the parasite broomrape, with varied seed oil and protein content, suitable for including in sunflower breeding programs for developing diverse restorer lines.

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Material and methods

The investigation was carried out in Dobrudzha Agricultural Institute, General Toshevo. Four accessions of wild annual species *H. petiolaris,* ssp. *petiolaris* were included in this study – E-020, E-021, E-024 and E-142. Previous investigations showed that these accessions possessed resistance to downy mildew, gray and black spots on sunflower, and to the parasite broomrape (Valkova, 2013). The cultivated sunflower was represented by three CMS lines – 325 A, 813 A and 846 A. The classical methods of interspecific hybridization were applied for obtaining hybrid seeds. Interspecific crosses *cultivated sunflower x wild species* were performed and the obtained hybrid plants were grown in field conditions. As paternal components in the realized crosses were the accessions of wild *Helianthus* species. The sterile analogues of fertile sunflower lines with normal cytoplasm were used as maternal parents.

Phytopathological evaluations of F_1 hybrid progenies were carried out in laboratory conditions and on an artificial infection plot. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berlese et de Toni) was carried out by 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 by the method of Encheva and Kiryakov (2002) in field conditions on an artificial infection plot. Evaluation for resistance to black spots on sunflower (*Phoma macdonaldii* Boerema / *Phoma oleracea* var. *helianthi-tuberosi* Sacc) was carried out by the method of Fayralla and Maric (1981) in field conditions on an artificial infection plot. The seed oil and protein content was determined by the method of Rushkovskii (1957). The weight of 1000 seeds was measured on three samples, where each sample consists of 25 or 50 seeds. Ten plants per accession, grown in field conditions, were used for this investigation in order to collect sufficient pollen quantity. The following phenological characters, conforming to UPOV characteristics, were determined: germination (days), beginning of

button formation (days from germination), beginning of flowering (days from germination), period of flowering (days), beginning of maturity of central inflorescence (days from germination), vegetation period (days). Germination was reviewed at cotyledons emergence of 75% of the sown seeds. Beginning of button formation was reviewed at inflorescence formation of the central stem, and beginning of flowering – beginning of flowering of central head for 25% of the plants. For the end of vegetation period was accepted the withering of stems and leaves of all plants. The seed set (%) was calculated as a correlation between number of inseminated disk florets to the total number of disk florets in one inflorescence. The analysis of experimental data was done by the statistical package BIOSTAT 6.0. For analyzing the obtained results, consistent to the aims of investigation, the following quantities were analyzed: arithmetic mean and the coefficient of variation (CV), which showed the relative uniformity or variability of the studied characters.

Results and discussion

Plants from four populations of annual species *H. petiolaris* were crossed with sterile analogues of cultivated sunflower lines. All obtained F_1 interspecific hybrid plants were fully branched with or without central head. Cultivated lines were not branched. The branching and anthocyanin coloration were typical characters for wild species. Anthocyanin coloration was observed on stems, leaves and rarely on the petioles of the obtained hybrid plants. The presence of anthocyanin pigmentation and branching on F_1 plants were the suitable markers for early establishment of the hybrid type of the obtained plants.

The obtained hybrid plants were 110 cm to 170 cm tall. Some differences in plant height, color of disk florets and leaves, length of branches were observed among plants from one and the same cross. This was due to the fact that the paternal parent was a population. Leaves were mostly alternate, ovate and slightly serrate on the margins, truncate at the base. The crossability between *H. petiolaris* accessions and cultivated sunflower varied from 55.5% to 100%. This was because both species were close from taxonomic point of view (Seiler, 1992). The lowest crossability was determined for accession E-020, and the highest – for accessions E-024 and E-142. Seeds were obtained from all pollinated inflorescences with pollen from accessions E-024 and E-142, which proved their best crossability. Similar results were obtained by Hristov (1990), Nikolova (1998), Valkova (2013). *H. petiolaris* is the wild species which stands out among the other annual species with higher crossability (Christov, 2008). The difficulties connected to the low crossability of these two species resulted in obtaining seeds with immature endosperm and sterility of the hybrid plants.

The results from hybridization showed that the seed set was lower and varied from 9.8% for the hybrid 813 A x E-020 to 26. 4% for the hybrid 325 A x E-142 (Table 1). Some differences in viability of hybrid seeds were established. The percentage of viable F_1 plants varied from 39.7% for the hybrid 813 A x E-020 to 78.5% for the hybrid 325 A x E-142, which was distinguished also with the biggest number of seeds per head. The accession E-142 was characterized with better crossability and the biggest number of obtained hybrid seeds originated from it.

The phenological observations were done and the main phenological phases were studied. The mean values of the studied phenological phases and their variation for the parents and the obtained hybrid F_1 progenies were presented in Table 2. Hybrid plants were distinguished with higher variation than their parents regarding all studied phenological phases. Differences were observed both between plants from the different crosses and plants from the same cross. This was because the accessions were maintained as populations. The vegetation period of hybrids was shorter than that of wild species *Helianthus petiolaris* and varied from 95 to 115 days for the earlier to 120 – 135 days for the other progenies. The duration of flowering period of hybrid plants was determined as the most variable phenological phase for all studied progenies, followed by variation in germination.

Table 1. Crossability between wild species *Helianthus petiolaris* and cultivated sunflower lines (*H. annuus*).

Hybrid combination	Pollinated inflorescences			Obtained seeds			Obtained hybrid plants	
	Total number	With seeds		Average per head	Total number	Seed set, %	Total number	Average per seeds, %
		number	%					
846 A x E-020	3	1		20	50	11.7	32	48.5
813 A x E-020	3	2		17	34	9.8	29	39.7
325 A x E-020	3	2		25	50	19.1	36	53.3
H.annuus x E-020	9	5	55.5	20.7	134	13.5	72	43.1
846A x E-021	3	3		30	111	15.2	40	51.38
813 A x E-021	3	2		24	102	11.6	31	41.4
325 A x E-021	3	2		38	104	20.1	43	64.5
H.annuus x E-021	9	7	77.8	30.7	317	15.6	233	78.46
846 A x E-024	3	3		37	103	17.2	48	59.3
813 A x E-024	3	3		34	100	14.5	36	45.4
325 A x E-024	3	3		44	115	22.3	58	70.2
H.annuus x E-024	9	9	100	38.3	318	18	233	78.46
846 A x E-142	3	3		47	121	19.2	57	62.3
813 A x E-142	3	3		44	105	18.3	41	48.4
325 A x E-142	3	3		52	126	26.4	65	78.5
H.annuus x E-142	9	9	100	47.7	352	21.3	233	78.4

Table 2. Mean values and variation coefficients of the studied phenological phases of the parents and their F1 hybrids.

Phenological phases	P1 cultivated sunflower (*H. annuus* L.)		P2 *Helianthus petiolaris*		F1 *H. annuus x H. petiolaris*	
	Mean	VC	Mean	VC	Mean	VC
Germination, days	10.71	3.32	14.66	9.32	12.5	12.5
Beginning of button formation, days from germination	43.95	3.23	57.67	3.9	46.5	46.5
Beginning of flowering, days from germination	55.91	7.28	72.33	2.96	58.16	58.16
Flowering, days	6.33	13.58	88.34	4.09	22.83	22.83
Beginning of maturity of central inflorescence, days from germination	92.95	6.16	104	3.94	94.5	94.5
Vegetation period, days	111.5	5.24	185.31	1.83	121.16	121.16

Variation was also observed in some biochemical characters connected to seed oil and protein content of parental forms and the obtained progenies. The average values of the studied biochemical characters were presented on Figure 1. Seeds of wild *H. petiolaris* were characterized with low 1000 seeds weight and seed oil content, and high content of hull. The seeds of sunflower hybrid plants were with higher content of oil in seeds and kernels. The oil content of hybrids in kernel varied from 64,5% to 71% and in seeds – from 35,8% to 49%. The highest seed oil content (49%) was determined for the crosses 325 A x E-142 and 813 A x E-142.

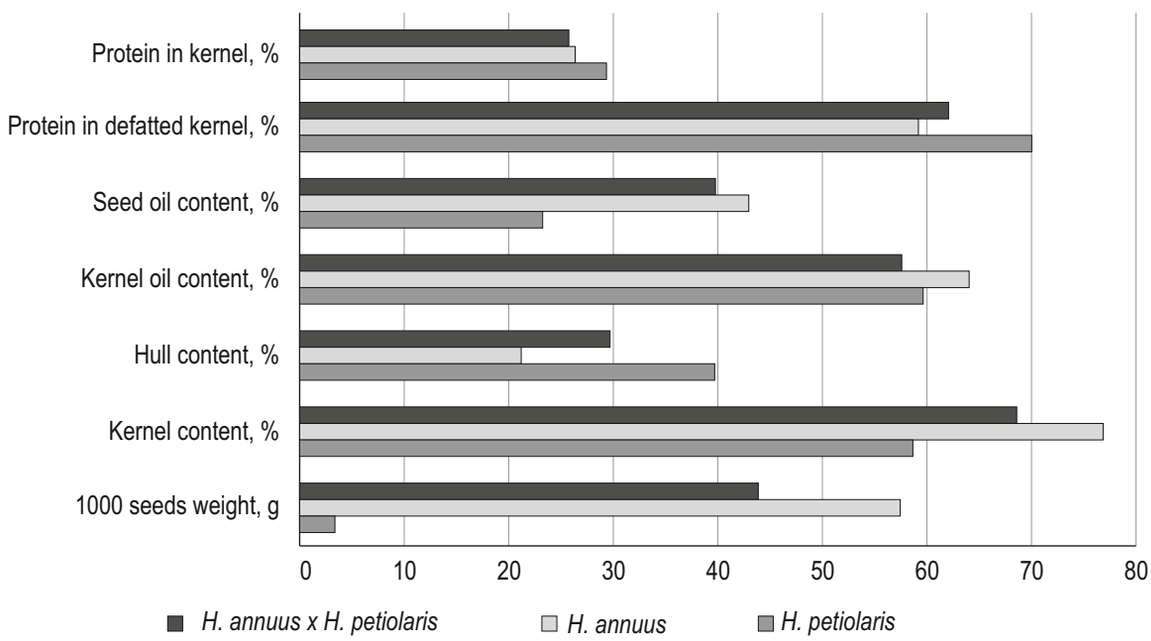

Figure 1. Average values of characters, connected to seed protein and oil content of hybrid plants and their parents.

The seeds of wild sunflower species have lower oil content compared to the cultivated sunflower, but it could be changed quickly to acceptable level by backcrossing with cultivated sunflower lines (Seiler and Rieseberg, 1997). The kernel content (%) was of importance for the total oil yield per hectare. Increasing of kernel content gave the opportunity for accumulation of bigger oil quantity in it and led to increasing the seed oil content, respectively, increasing the seed yield. Seed oil content was a character which reflected simultaneously on the kernel content and the oil content in it. Therefore, the sunflower seed oil content of hybrid plants was set up by the relative portion of kernel to the whole seeds, and the content of oil in it. The seed oil content of the new registered hybrids and cultivars was increased due to decreasing the hull content predominantly to increasing the other characters (Nikolova, 1987). According to this dependence, hybrid plants with the lowest hull content and the highest oil content were selected.

The reaction of hybrid materials to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* and the parasite broomrape (*Orobanche cumana*) was studied with the aim to establish the sources of resistance to these pathogens (Tables 3 and 4).

The hybrid combinations 325 A x E-142 and 813 A x E-142 were resistant (100%) to downy mildew and the parasite broomrape. They were characterized with immune type of reaction to the pathogens that caused grey and black spots on sunflower and with 49% oil content in seeds. Their vegetation period was 115 – 118 days. Certain resistance to these two pathogens was established for the other hybrids, obtained with participation of the accessions E-020 and E-021. They could be successfully included in the sunflower breeding programs for developing new resistant lines.

Table 3. Phytopathological evaluation of F1 hybrid progenies for resistance to *Pl. helianthi* and Orobanche cumana.

Resistance, %	Hybrid combination	
Resistance 100% to Pl. helianthi Novot. and 76 – 99% to *Orobanche cumana Wallr.*	325 A x E-142	813 A x E-142
Resistance 76 – 99% to Pl. helianthi Novot. and *Orobanche cumana Wallr.*	325 A x E-022	846 A x E-022
	325 A x E-020	846 A x E-020
	813 A x E-022	846 A x E-021

Table 4. Phytopathological evaluation of F1 hybrid progenies for resistance to *Phomopsis helianthi Munt.-Cvet.* et al. and *Phoma macdonaldii Boerema*.

Type of reaction	Hybrid combination	
Immune to Phomopsis helianthi and Phoma macdonaldii	325 A x E-142	813 A x E-142
	846 A x E-142	325 A x E-022
Resistant to *Phomopsis helianthi* and *Phoma macdonaldii*	325 A x E-024	846 A x E-022
	325 A x E-020	846 A x E-020
	813 A x E-022	846 A x E-021

Immune type of reaction to the pathogens causing grey and black spots on sunflower showed four hybrid forms and other six hybrids were resistant.

Conclusion

Wild *Helianthus* species have been included in sunflower breeding programs mainly as donors for resistance to diseases and to the parasite broomrape. Transfer of genes that control the resistance into cultivated sunflower lines gave the opportunity for diversification of cultivated sunflower. Resistance 100% to downy mildew and immune type of reaction to the pathogens *Phomopsis helianthi* and *Phoma macdonaldii* was established for all crosses with participation of E-142 accession.

Plants from the hybrid combinations 325 A x E-142, 813 A x E-142, 846 A x E-142 and 325 A x E-022 could be used as donors for resistance. They were also distinguished by 45 – 49% seed oil content. The obtained hybrid material was a useful initial material to be applied in sunflower breeding programs for producing restorer lines with high seed oil content and resistance to the main diseases with economic importance.

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