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HYBRIDIZATION BETWEEN CULTIVATED SUNFLOWER AND WILD SPECIES *HELIANTHUS BOLANDERI* A. GRAY

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Abstract

Interspecific hybridization was carried out between sterile analogues of cultivated sunflower lines with normal cytoplasm and wild annual *Helianthus bolanderi* accession E-009 from collection of DAI-General Toshevo. Hybrid plants were produced using classical breeding methods and the biotechnological method *embryo rescue*. The degree of crossability and the inheritance of some morphological traits were determined. The obtained F1 progenies were characterized from morphological and phytopathological point of view. Hybrid forms distinguished with resistance to stem canker, phoma and downy mildew were obtained. The hybrid plants, carriers of Rf genes for CMS Pet 1, could be used in sunflower breeding programs for developing restorer lines.

Keywords: interspecific hybridization, sunflower, *Helianthus bolanderi*, embryo rescue, resistance.

Introduction

Sunflower is the main oil crop in Bulgaria. The planting areas have increased in recent years because of higher profitability, low input requirements and better exporting possibilities, but higher rates of disease and pests have severely limited the sunflower production in some years. Genetic variability of the cultivated sunflower may be increased by interspecific hybridization with wild sunflower species. Wild species from genus *Helianthus* possess not only considerable variability for most of the traits but also excellent survival environmental mechanisms (Thompson et al., 1981). They possess genes for resistance to diseases (biotic stress), tolerance to abiotic stresses (drought, cold, soil salinity, certain herbicides) and high quality of proteins and oil (Hladni N. and Miklič V., 2012; Seiler, 1992; Skoric, 1992). That is why they were widely used in sunflower breeding programs. Whelan (1978) and Christov (1996) established that interspecific hybrids could be obtained, more or less easily, in crossings between annual wild species of the section *Helianthus* and cultivated sunflower, with or without embryo rescue techniques. In such interspecific hybrids, semi-sterility is a common trait due to strong genetic barriers: chromosomal translocations, inversions, etc. Sterility in F1 sunflower interspecific hybrids limits utilization of wild *Helianthus* species for the improvement of cultivated sunflower. According to Rieseberg *et al*., (1998) viable hybrids and fertile interspecific progenies could be produced and phenotype of obtained F1 hybrids was very close to the female parent. Jan C.C. and Chandler J.M. (1989) improved backcross seed set and the effectiveness of the colchicine chromosome doubling technique, analyzing the resultant tetraploids of H. annuus x H. bolanderi Gray. According to Sukno S. *et al.,* (1999) interspecific crosses *cultivated × wild sunflower*, showed higher proportion of fully developed embryos. Embryo culture proved to be a useful tool to overcome post-zygotic hybrid incompatibility (Nenova N., 2002). The aim of this study was to obtain interspecific hybrid progenies with participation of wild species *H. bolanderi*, determine the rate of crossability and inheritance mode of some traits as well as to find a resistant initial material for breeding purposes.

Material and methods

The investigation was carried out at Dobrudzha agricultural institute during 2011-2015. The cultivated sunflower was represented by seven CMS lines– HA 382, 325A, 217A, 704A, 349A, 353A,

383A. The wild species *Helianthus bolanderi*, accession E-009 was included in the investigation. The interspecific hybridization on the scheme *cultivated sunflower x wild species* was successfully applied in field conditions. The isolated sterile inflorescences of cultivated lines were pollinated by pollen from the inflorescences of *H. bolanderi*, previously excised. Seeds from interspecific crosses were obtained applying the methods of classical breeding and embryo rescue (Azpiroz *et al.*, 1988; Nenova N., 2002). Morphological and phenological characters were conformable with descriptors of IBPGR. The inheritableness *d/a* was calculated for F_1 progeny, using the coefficient of Mather and Jinks (1982). Phytopathological evaluations of F_1 hybrid progenies were carried out in laboratory conditions and in artificial infection plot. Evaluation for resistance to downy mildew (*Plasmopara halstedii* Farl. Berlese et 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 Fayralla and Maric (1981) in field conditions on artificial infection plot.

Results and discussion

Interspecific crosses *cultivated sunflower x wild species* were performed and the obtained hybrid plants were grown in field conditions. As paternal component in the realized crosses was the accession of wild *H. bolanderi*. The sterile analogues of fertile sunflower lines with normal cytoplasm were used as maternal parents. The data connected to crossability rate and seed set were presented on table 1. The results of hybridization showed that the crossability varied from 33% to 66% and the average percentage for all crosses was 42,8%. The seed set of one head (the percentage of insemination) wase very low and varied from 4,55% for the cross HA 382A x E-009 to 12,78% for the cross 353 A x E-009.

Table 1. Crossability of wild species H. bolanderi (E-009) and cultivated sunflower lines

Hybrid combination	Pollinated inflorescences			Obtained seeds			Hybrid plants obtained	
	Total number	with seeds		Average per head	Total number	Seed set, %	Total number	compared to seeds,%
		Number	%					
HA 382A x E-009	3	1		12	12	4,55	7	58,3
325 A x E-009	3	1		13	13	5,82	10	76,9
217 A x E-009	3	1		14	14	6,33	11	78,5
704 A x E-009	3	2		17	34	11,75	29	85,3
349 A x E-009	3	1		18	18	7,94	10	55,5
353 A x E-009	3	2		18	36	12,78	17	47,2
383 A x E-009	3	1		17	17	7,58	9	52,9
H.annuus x E-009	21	9	42,8	16	144	8,1	93	64,9

Some differences were established in the viability of hybrid seeds. The percentage of obtained F_1 plants varied from 47,2% for the cross 353 A x E-009 to 85,3% for the cross 704 A x E-009. At the average, hybrid plants were obtained from 64,9% of all obtained seeds. For overcoming the difficulties in applying of classical methods of breeding, connected to incompatibility of cultivated sunflower and with aim to obtain maximum number of hybrid plants, the method of *embryo rescue* was applied. The initial crosses were done in field conditions. The obtained 211 embryos from hybrid combinations were cultivated on firm plant tissue culture. The most suitable period for detachment of embryos were 10-12 days after pollination. F_1 hybrid plants were obtained from all crosses. The percentage of received hybrid plants varied from 65% to 100 %. This showed, that *embryo rescue*

could be successfully applied for obtaining of more than one generation per year. Hybrid plants from all crosses were characterized morphologically. They had erect and branched stem with weak or heavy expressed anthocyanin coloration. Thin greyish-white hairs covered stems, leaves, bracts and petioles. These traits were not observed in cultivated sunflower, but they were typical for wild species. Their presence were suitable morphological markers for early determining of hybrid type of obtained F_1 plants. The central stem was longer than the branches. Plants had larger central inflorescence and many smaller heads, formed on branches. The number of branched varied from 7 to 14. Leaves were green. Their shape was similar to that of cultivated sunflower, but their size was smaller and the serration was different and well expressed. The inheritance of some morphological traits was presented on table 2. The lowest were the indices of variation coefficient for traits, characterized the cultivated sunflower, which was presented by morphologically uniform lines. The paternal form and its F_1 combinations were characterized by higher indices of VC for the character number of inseminated disk florets, and that of the progeny was the highest.

Table2. Variation in characters and type of inheritance for parents and F_1 progeny

Traits	cultivated sunflower (H. annuus L.)		Helianthus bolanderi		H. annuus x H. bolanderi			
	$\bar{x}$	VC	$\bar{x}$	VC	$\bar{x}$	VC	d/a*	H2
Plant height, cm	145	11,5	160,5	4,7	159,7	25,1	0,09i	0.85
Stem-diameter, cm	3,6	8,3	0,7	5,2	1,4	11,7	-0,49i	0.94
Number of branches	-	-	21	14,5	11,7	11,2	0,38i	0.89
Length of the longest branch, cm	-	-	99,5	14,6	113,5	13,3	3,24h	0.96
Leaf-length, cm	33	7,7	14	7,8	17	10,5	-0,23i	0.91
Leaf-width, cm	36	6,3	11	5,1	15,6	15,3	-0,57pd	0.92
Leaf petiole-length, cm	16,4	9,7	8,7	14,2	9,7	10,2	-0,59pd	0.92
Head-diameter, cm	19,7	11,2	2,5	6,4	7,3	21,8	-0,24i	0.89
Number of bracts	69	10,4	30	7,2	77,6	17,2	1,92h	0.94
Number of ray flowers	39	8,8	16	9,4	33,9	9,1	0,23i	0.95
Ray flowers-length, cm	6,8	8,2	2,6	6,7	3,8	23,5	-0,03i	0.9
Ray flowers-width, cm	2,9	6,1	0,6	5,6	1,5	15,4	0,68pd	0.93
Number of disk florets	1510,2	17,1	198	11,2	523,3	18	-0,69pd	0.87
Number of inseminated disk florets	1110,6	12,5	33,5	21,3	49,9	31,2	-0,66pd	0.86
1000 seeds weight, g	79,5	7,1	4,9	4,1	49,9	24,3	0,48i	0.79

*i- intermediate; pd- partial dominance; d- dominance; h- heterosis.

Significant variation in plant height was not observed for the wild species, contrary to the variation for hybrid plants height. They were characterized by well displayed heterosis effect for the characters length of the longest branch and number of bracts. Partial dominance to maternal parent was established for the characters width of ray flowers. Regarding leaf width, length of petiole, number of disk florets and number of inseminated disk florets, the partial dominance to paternal parent was established. The indices of coefficient H^2 were high. The vegetation period of hybrids was shorter than that of wild species and varied from 94-110 days for early progenies to 115-135 days for the late ones.

The reaction of hybrid materials to the pathogens *Plasmopara helianthi, Phomopsis helianthi, Phoma macdonaldii* was studied with aim to establish the sources for resistance to these pathogens. The hybrid combinations 704 A x E-009 and 383 A x E-009 were resistant (100%) to downy mildew. The hybrid combinations 704 A x E-009, 349 A x E-009 and 353 A x E-009 were resistant (76%-100%) to *Phomopsis helianthi* and *Phoma macdonaldii*. Their vegetation period was 115-118 days. They could be successfully included in the sunflower breeding programs for developing new resistant restorer lines.

Conclusions

Wild *Helianthus* species have been included in sunflower breeding programs mainly as donors for resistance to diseases. Transfer of genes, controlling the resistance, into cultivated sunflower lines, gave the opportunity for diversification of cultivated sunflower and broadening its gene pool. E*mbryo rescue* could be successfully applied for overcoming the difficulties of classical breeding methods, connected to incompatibility of cultivated sunflower. Plants from hybrid combinations 704 A x E-009, 383 A x E-009, 704 A x E-009, 349 A x E-009 and 353 A x E-009, carriers of Rf genes for CMS Pet 1, could be used for obtaining of new resistant restorer lines and included as initial material in sunflower breeding programs.

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