Valkova, D., Penchev, E. & Encheva, V. (2017). Hybridization between cultivated sunflower and wild species Helianthus praecox Engelm.& Gray. *Rastenievadni nauki/Bulgarian Journal of Crop Science, 54*(4), 9-13

Hybridization between cultivated sunflower and wild species *Helianthus praecox* Engelm. & Gray

Daniela Valkova*, Emil Penchev, Valentina Encheva
Dobrudzha Agricultural Institute, 9520 General Toshevo, Bulgaria
*e-mail: *valkova_d@abv.bg*

Abstract

Interspecific hybridization was carried out between sterile analogues of cultivated sunflower lines with normal cytoplasm and wild annual *Helianthus praecox,* accession GT-E-144, from collection of DAI-General Toshevo. Hybrid plants were produced using classical breeding methods. The degree of crossability and inheritance of some morphological traits were determined. The obtained F_1 progenies were characterized from morphological, phenological and phytopathological point of view. The hybrid plants, carriers of Rf genes for CMS Pet 1, could be used in sunflower breeding programs for developing restorer lines. They were distinguished with resistance to phomopsis stem canker, phoma and sunflower downy mildew.

Keywords: sunflower; hybridization; Helianthus praecox; inheritance; resistance to diseases

Резюме

Осъществена е междувидова хибридизация между стерилните аналози на линии културен слънчоглед с нормална цитоплазма и дивия едногодишен вид *Helianthus praecox,* образец GT-E-144, от колекцията в ДЗИ – Генерал Тошево. Хибридни растения са получени чрез прилагане на класически селекционни методи. Определени са степента на кръстосваемост и наследяването на някои морфологични признаци. Получените F_1 потомства са характеризирани от морфологична, фенологична и фитопатологична гледна точка. Хибридните растения, носители на Rf гени за CMS Pet 1, могат да се използват в селекционните програми при слънчогледа за създаване на линии-възстановители. Те се отличават с устойчивост към фомопсис, фома и мана.

Ключови думи: слънчоглед; хибридизация; *Helianthus praecox*; наследяване; устойчивост към болести

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 sunflower production in some years. Genetic variability of 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 (Seiler, 1992; Skoric, 1992; Hladni and Miklič, 2012). That is why they were widely used in sunflower breeding programs. The wild annual species *Helianthus praecox* Engelm. & Gray (2n=34) can be a source of resistance to downy mildew and phomopsis stem

canker (Korell et al., 1996), high oleic acid content (Seiler, 1988) and new CMS types (Christov, 1993). 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 F_1 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 F_1 hybrids was very close to the female parent.

The aim of this study was to obtain interspecific hybrid progenies with participation of wild species *H. praecox*, to 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 2014-2016. The cultivated sunflower was represented by seven CMS lines – 10 A, 325 A, 217 A, 774 A, 762 A, 753 A, 736 A. The wild species *Helianthus praecox*, accession GT-E-144 (received from the USA, Texas) 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. praecox*, previously excised. Seeds from interspecific crosses were obtained, applying the methods of classical breeding (Christov and Petrov, 1988). 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 on artificial infection plot. Evaluation for resistance to sunflower 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 by 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 by 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 used the accession of wild *H. praecox*. 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 14.3% to 57.1% and the average percentage for all crosses was 30.6%. The highest crossability rate was establish for the cross 736 A x GT-E-144, followed by the cross 10 A x GT-E-144. The seed set of one head (the percentage of insemination) was very low and varied from 5.2% for the cross 325 A x GT-E-144 to 12.1% for the cross 753 A x GT-E-144.

Some differences were established in the viability of hybrid seeds. The percentage of obtained F_1 plants varied from 33.3% to 61.7%. At the average, hybrid plants were obtained from 49% of all obtained seeds. The highest number of hybrid plants was obtained from crosses 774 A x GT-E-144, 736 A x GT-E-144 and 325 A x GT-E-144, from 61.7% to 53.8% compared to seeds, respectively.

Hybrid plants from all crosses were characterized morphologically. They had erect and branched stem with weak or heavy expressed anthocyanin coloration. Weak bract pigmentation was also observed. The phyllaries were tapered gradually to a point. Thin grayish-white hairs covered stems, leaves, bracts and petioles. The leaves were mostly alternate, blades were ovate to triangular. They were light green and serration was different and well expressed. Ray florets were yellow and orange-yellow. For some inflorescences disk florets were purplish-brown. These traits were not observed in cultivated sunflower, but they were typical for wild species. Their presence was suitable morphological marker for early determination of hybrid type of obtained F_1 plants. The central stem was longer than the branches. Plants had central inflorescence and many small heads, formed on branches. The number of branches varied from 4 to 10.

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. 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

Table 1. Crossability of wild species *H. praecox* (GT-E-144) 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	%					
0 A x GT-E-144	7	3		12	36	7.5	12	33.3
325 A x GT-E-144	7	1		13	13	5.2	7	53.8
217 A x GT-E-144	7	1		14	14	6.1	6	42.8
774 A x GT-E-144	7	2		17	34	10.7	21	61.7
762 A x GT-E-144	7	2		18	36	8.9	18	50.0
753 A x GT-E-144	7	2		18	36	12.1	17	47.2
736 A x GT-E-144	7	4		17	68	11.5	37	54.4
***H. annuus* x GT-E-144**	**49**	**15**	**30.6**	**15.6**	**237**	**8.8**	**118**	**49.0**

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 variation of main phenological phases was studied. The variation coefficients of all characters of hybrids were higher, than of their parents. The duration of flowering, vegetation period and germi-

Table 2. Variation in characters and type of inheritance for parents and F_1 progeny

Traits	*H. annuus*		*H. praecox*		*H. annuus x H. praecox*			
	$\bar{x}$	VC	$\bar{x}$	VC	$\bar{x}$	VC	d/a*	H^2
Plant height, cm	145	11.5	160.5	4.7	159.7	25.1	0.09 i	0.85
Stem diameter, cm	3.6	8.3	0.7	5.2	1.4	11.7	-0,49 i	0.94
Number of branches	-	-	21	14.5	11.7	11.2	0.38 i	0.89
Length of the longest branch, cm	-	-	99.5	14.6	113.5	13.3	3.24 h	0.96
Leaf length, cm	33	7.7	14	7.8	17	10.5	-0.23 i	0.91
Leaf width, cm	36	6.3	11	5.1	15.6	15.3	-0.57 pd	0.92
Leaf petiole length, cm	16.4	9.7	8.7	14.2	9.7	10.2	-0.59 pd	0.92
Head diameter, cm	19.7	11.2	2.5	6.4	7.3	21.8	-0.24 i	0.89
Number of bracts	69	10.4	30	7.2	77.6	17.2	1.92 h	0.94
Number of ray flowers	39	8.8	16	9.4	33.9	9.1	0.23 i	0.95
Ray flowers length, cm	6.8	8.2	2.6	6.7	3.8	23.5	-0.03 i	0.9
Ray flowers width, cm	2.9	6.1	0.6	5.6	1.5	15.4	0.68 pd	0.93
Number of disk florets	1510.2	17.1	198	11.2	523.3	18	-0.69 pd	0.87
Number of inseminated disk florets	1110.6	12.5	33.5	21.3	49.9	31.2	-0.66 pd	0.86
1000 seeds weight, g	79.5	7.1	4.9	4.1	49.9	24.3	0.48 i	0.79

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

Table 3. Variation of main phenological phases of studied F_1 hybrids

Characters	P_1		P_2		F_1	
	H. annuus		*H. praecox*		*H. annuus x H. praecox*	
	$\bar{x}$	VC	$\bar{x}$	VC	$\bar{x}$	VC
Germination, days	7.8	3.5	9.5	6.2	14.7	16.1
Beginning of button formation, days from emergence	41.9	3.2	51.3	3.1	41.5	10.8
Beginning of flowering, days from emergence	56.1	7.5	75.5	3.8	66.1	9.5
Duration of flowering, days	6.5	14.5	46.5	4.3	32.1	17.4
Beginning of main head maturity, days from emergence	92.5	6.1	89.8	2.7	70.2	10.1
Vegetation period, days	111.6	5.17	148.5	1.8	127.4	16.9

nation differed between progenies. The vegetation period of obtained hybrid plants was shorter than that of wild species and varied from 95-106 days for early progenies to 115-125 days for the late ones (Table 3). Plants from crosses 736 A x GT-E-144, 753 A x GT-E-144 and 10 A x GT-E-144 were distinguished with earliness (95-100 days).

The presence of fertility was established for all crosses. It varied from 17% to 56%. All fertile plants from the studied crosses were isolated and tested for resistance to diseases.

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 774 A x GT-E-144 and 753 A x GT-E-144 were resistant (100%) to downy mildew. The hybrid combinations 762 A x GT-E-144, 10 A x GT-E-144 and 736 A x GT-E-144 were resistant (76%-100%) to *Phomopsis helianthi* and *Phoma macdonaldii*. 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 resistance, into cultivated sunflower lines, gave the opportunity for diversification of cultivated sunflower and broadening its gene pool. Appling interspecific hybridization, some new forms could be obtained. Wild *Helianthus praecox* could be successfully used in breeding of sunflower. Plants from hybrid combinations, carriers of Rf genes for CMS Pet 1, could be used for obtaining new resistant restorer lines and included as initial material in sunflower breeding programs.

REFERENCES

Christov, M. (1993). Sources of cytoplasmic male sterility produced at IWS "Dobroudja". *Biotechnology & Biotechnological Equipment*, *7*(4), 132-135.

Christov, M. (1996). Hybridization of cultivated sunflower and wild *Helianthus* species. In: *P.D.S. Caligari& D.J.N. Hind (eds). Compositae: Biology & Utilization. Proc. Int. Compositae Conference,* Kew, 1994, vol. 2, pp. 603-615, Royal Botanic Gardens, Kew.

Christov, M. & Petrov, P. (1988). New sources of Rf genes for CMS based on Helianthus petiolaris. *Genetika i Selekcia*, *21*(5), 23-27.

Hladni, N., & Miklič, V. (2012). Old and New Trends of Using Genetic Resources in Sunflower Plant Breeding With The Aim of Preserving Biodiversity. In *Book Proceed., The Forth Joint UNS–PSU, Int. Conf. BioS. Biotechnol,* Novi Sad, Serbia, pp. 109-120.

IBPGR, 1985. Descriptors for cultivated and wild sunflower. AGPG. IBPGR/85/54, Roma, Italy.

Korell, M., Brahm, L., Fried, W. & Horn, R. (1996). Interspecific and intergeneric hybridization in sunflower breeding. *Plant Breed. Abstr.*, 66(8), 1081-1091.

Mather, K., Jinks, J. (1982). *Biometrical genetics.* Chapman & Hall, 430 p.

Rieseberg, L. H., Baird, S. J., & Desrochers, A. M. (1998). Patterns of mating in wild sunflower hybrid zones. *Evolution*, *52*(3), 713-726.

Seiler, G. J. (1988). The genus Helianthus as a source of genetic variability for cultivated sunflower. In: *Proc. 12 Int. Sunfl. Confer., Novi Sad, Yugoslavia*, vol.1, pp. 17-58.

Seiler, G. J. (1992). Utilization of wild sunflower species for the improvement of cultivated sunflower. *Field Crops Research*, *30*(3-4), 195-230.

Skoric, D. (1992, September). Results obtained and future directions of wild species use in sunflower breeding. In *Proceedings of the 13th International Sunflower Conference*, *Pisa, Italy*, vol. 2, pp. 1317-1348.

Thompson, T. E., Zimmerman, D. C., & Rogers, C. E. (1981). Wild Helianthus as a genetic resource. *Field Crops Research*, *4*, 333-343.

Whelan, E. D. P. (1978). Chapter 10. Cytology and interspecific hybridization. In: *Sunflower Science and Technology. The American Society of Agronomy. Monograph* (Carter J. F., ed.), pp. 339-369.