

STUDY OF HYBRID MATERIAL ORIGINATED FROM INTERSPECIFIC CROSSES WITH WILD *Helianthus annuus* L. FOR RESISTANCE TO DISEASES AND PARASITE BROOMRAPE

DANIELA VALKOVA*, JULIA ENCHEVA, VALENTINA ENCHEVA, PEPA SHINDROVA, MICHAIL CHRISTOV
Dobroudja Agricultural Institute, General Toshevo, Bulgaria

*E-mail: valkova_d@abv.bg

Abstract

Hybrid plants were obtained by crossing between five male sterile sunflower lines and 20 accessions of wild annual species *Helianthus annuus* L. from the collection of wild sunflower species in DAI, General Toshevo. Resistance of F₁ and F₂ hybrid plants to the cause agents of gray spots (*Phomopsis helianthi* Munt.-Cvet. et al.) and black spots on sunflower (*Phoma macdonaldii*), *Alternaria* leaf spot, downy mildew (*Plasmopara helianthi* Novot.) and the parasite broomrape (*Orobanche cumana* Wallroth) was studied. Some morphological, phenological and biochemical characters were established. Data obtained from the quarantine plots and in laboratory conditions showed that plants from some of the crosses were with resistant type of reaction to one or more diseases. Differences in morphological characters were observed between plants from different crosses and among plants from the same cross. Transfer of fertility restorer genes for the CMS PET1 was established.

Key words: hybridization, resistance, *Helianthus annuus*

INTRODUCTION

The genus *Helianthus*, continues to contribute specific characteristics for cultivated sunflower improvement. The wild sunflower species are adapted to a wide range of habitats and possess considerable variability for their reaction to insects and disease pathogens, and for most agronomic characters.

When screening wild sunflower species as potential sources of genes, it should be realized that plants within a single population of a species may exhibit different levels of resistance to a given pathogen due to segregation, since the native populations are open-pollinated and segregating for many traits. It is also important to consider more than one population of a species when characterizing resistance genes from a single species. There is a continued need to collect, maintain, evaluate and enhance wild *Helianthus* germplasm for future improvement of cultivated sunflower.

The wild *Helianthus annuus* L. variability is high and represented some specific adaptations, which play an important role in the study of its genetic potential.

The aim of our study was to investigate the

potential opportunities of 20 accessions of wild species *Helianthus annuus* L. to transfer in their hybrid progenies the resistance to economically important pathogens downy mildew, *Alternaria* leaf spot, *Phomopsis* stem canker, *Sclerotinia* wilt/rot, and parasitic weed broomrape. Obtaining of hybrid plants, carriers of genes for fertility restoration of CMS PET-1 were also of interest for the present investigation.

MATERIAL AND METHODS

The investigation was carried out in Dobroudja Agricultural Institute, General Toshevo (DAI). Twenty accessions of wild species *Helianthus annuus* included in the collection of wild species in DAI were successfully crossed with sunflower inbred lines AK-19A, AK-42A, AK-109A, AK-126A, AK-383A, developed in DAI and their F₂ generations were included in the study.

The evaluation of hybrid material for downy mildew resistance (*Plasmopara helianthi* Novot.) was made according to the standard method (Tourvieille et al., 2000).

The evaluation of hybrid material for broomrape resistance (*Orobanche cumana* Wallroth) was carried out in greenhouse conditions accord-

ing to the standard method (Panchenko, 1975), slightly modified to the local conditions. Resistance was calculated as percentage of non-infected plants on the scale: 0% = S (sensitive) – 100% = R (resistant).

The evaluation of hybrid material for resistance to grey spots on sunflower (*Phomopsis helianthi* Munt.-Cvet. et al.), brown spots (*Alternaria* sp.) and black spots (*Phoma macdonaldii*) was carried out according to the method of Encheva and Kiryakov (2002) under field conditions in artificial infection plot.

The evaluation of hybrid material for resistance to sclerotinia (*Sclerotinia sclerotiorum* (Lib) de Bary) was carried out according to methods of Encheva and Kiryakov (2002) and Christov et al. (2004) under field conditions.

RESULTS AND DISCUSSION

All of the obtained hybrid combinations were similar in their morphological characters. Hybrid plants were with erect and branched, haired, dark green and ribbed stems. Plants height varied from less than 1 m to about 2 m.

Different types of branching were observed: mainly at the base, at the tip and whole plant. Differences were observed in the position of the nearest lateral head to the central. At the end of its growth the lateral heads reached the central head height or were located below or above it. Leaves were alternatively situated and covered with hairs in both sides. They were with intermediate type of inheritance and size varied from smallest (10 cm/15 cm) for crosses AK-19A × GT-E-066 и AK-19A × GT-

E-045, medium (18 cm/21 cm) – for the cross AK-383A × GT-E-043, to largest (28 cm/35 cm) for the crosses AK-109A × GT-E-077 и AK-109A × GT-E-081. Leaves color varied from light green to dark green with slight anthocyanin coloration. For some accessions the leaf blistering was very strong and for others it was absent. Variations were observed also for leaf serration – from absent or very weak to strong. Different leaves shapes were examined – elongated, triangular and ovate. Leaf petioles were with different length, haired and colored with anthocyanin in different degree. Bract leaves were with different size and length of the tip. Different diameter and intermediate type of inheritance were examined for the inflorescences. Their ray florets were with different color – lemon, yellow, orange or dark red. Their number and size differed for all cross combinations.

Seeds size, shape and color varied for all crosses. Number of days to flowering was different. The longest vegetation period (130 days) was established for crosses AK-42A × GT-E-165, AK-126A × GT-E-167, as well for separate plants from the cross AK-42A × GT-E-167, and the shortest (98 days) – AK-383A × GT-E-123.

Greater part of the obtained hybrid material was fertile and the presence of Rf genes for CMS PET1 was proved. It could be included in the breeding program for developing new sunflower forms and hybrids.

All 100 hybrid combinations were tested for resistance to downy mildew and to the parasite broomrape at laboratory conditions (Table 1).

Full resistance to downy mildew showed 20 hybrid combinations, but only 9 of them were

Table 1. Results of testing for resistance to downy mildew and to the parasite broomrape

Hybrid forms	Resistance		Seed oil content, %	Vegetation period
	downy mildew, %	broomrape, %		
AK-19A × GT-E-088	100	100	43.44	120
AK-42A × GT-E-128	100	100	42.30	125
AK-42A × GT-E-088	100	100	42.10	125
AK-109A × GT-E-088	100	100	42.60	110
AK-109A × GT-E-128	100	100	41.50	125
AK-126A × GT-E-088	100	100	41.30	115
AK-126A × GT-E-128	100	100	42.50	120
AK-383A × GT-E-088	100	100	42.25	115
AK-383A × GT-E-128	100	100	43.50	117

Table 2. Results from the testing of hybrid materials of F₂ generation for resistance to diseases and the parasite broomrape

Hybrid forms	Resistance					
	downy mildew, %	broomrape, %	<i>Phomopsis</i> *	<i>Phoma</i> *	<i>Alternaria</i> *	<i>Sclerotinia</i> **
AK-19A × GT-E-088	100	100	R	R	R	0-1
AK-42A × GT-E-092	100	80	R	R	R	0-1
AK-42A × GT-E-128	100	100	R	R	R	0-1
AK-383A × GT-E-133	100	75	R	R	R	0-1
AK-109A × GT-E-166	100	80	R	R	R	0-1
AK-383A × GT-E-169	100	75	R	R	R	0-1
AK-109A × GT-E-171	100	80	R	R	R	0-1
AK-126A × GT-E-174	100	70	R	R	R	0-1
AK-109A × GT-E-045	75	0	I	R	R	0-1
AK-126A × GT-E-058	75	0	I	R	R	0-1
AK-383A × GT-E-155	80	0	I	R	R	0-1
AK-42A × GT-E-088	100	100	I	R	R	0-1
AK-42A × GT-E-155	80	0	I	R	R	0-1
AK-126A × GT-E-133	100	75	I	R	R	0-1
AK-126A × GT-E-171	100	80	I	R	R	0-1
AK-109A × GT-E-092	100	80	I	R	R	0-1
AK-109A × GT-E-088	100	100	I	R	R	0-1
AK-109A × GT-E-128	100	100	I	R	R	0-1
AK-126A × GT-E-092	100	80	I	R	R	0-1
AK-126A × GT-E-088	100	100	I	R	R	0-1
AK-126A × GT-E-128	100	100	I	R	R	0-1
AK-383A × GT-E-092	100	80	I	R	R	0-1
AK-383A × GT-E-088	100	100	I	R	R	0-1
AK-383A × GT-E-128	100	100	I	R	R	0-1

*Category: I – immune; R – resistant; **Type of infection: 0-1 – resistant.

resistant to broomrape. These crosses were obtained with participation of the accessions GT-E-088 and GT-E-128.

For resistance to *Phomopsis* were tested all hybrid combinations in artificial infection plot under field conditions. Immune type of reaction showed 34 combinations. These are all hybrid combinations obtained with the participation of the accessions GT-E-088, GT-E-092, GT-E-128, GT-E-133, GT-E-166, GT-E-169, GT-E-171 и GT-E-174. Another 49 combinations reacted with type of infection – 1, which referred them to the group of resistant materials. Moderately resistant

hybrid materials were 77, moderately susceptible were 9.7% and susceptible – 2%.

For resistance to *Phoma* were tested all hybrid combinations. Immune type of reaction showed 54 combinations. These are all hybrid combinations obtained with the participation of the accessions GT-E-045, GT-E-058, GT-E-088, GT-E-092, GT-E-128, GT-E-133 and GT-E-155.

For resistance to *Alternaria* were tested all hybrid combinations. Immune type of reaction showed 58 combinations. Another 32 combinations reacted with type of infection – 1, which referred them to the group of resistant materials.

The evaluation of the hybrid material for resistance to *Sclerotinia* was made under field conditions after artificial inoculation. Different type of plants reaction in the same accession was observed for some hybrid forms. Resistant type of attacks showed 37 accessions from all infected plants. Resistant and susceptible plants were found among another 33 accessions. All other accessions were susceptible.

In Table 2 are presented the general results of testing of the hybrid combinations, which showed best results.

CONCLUSIONS

Results of the testing showed that greater parts of the new fertile forms are carriers of Rf genes.

The hybrid forms possessed resistance to one, two, or more diseases and parasite broomrape. Hybrid combinations obtained with participation of accessions GT-E-088 and GT-E-128 were resistant to all studied diseases. The resistant material could be included in the breeding programs for improving cultivated sunflower.

REFERENCES

- Bohorova, H.** 1983. Tzitogenetichno prouchvane na mejduvidovi hibridi *H. scaberimus* ($2n = 68$) x *H. annuus* ($2n = 34$). Otdalechena hibridizatsia pri slanchogleda. BAS, Sofia, p. 219-228
- Christov, M.** 1996a. Characterization of wild *Helianthus* species as sources of new features for sunflower breeding. In P. D. S. Caligari & D. J. N. Hind (Eds). *Compositae: Biology & Utilization*. Proceedings of the International Compositae Conference, Kew, 1994. (D. J. N. Hind, Editor-in-Chief). vol. 2, p. 547-570. Royal Botanic Gardens, Kew.
- Christov, M.** 1996b. Hybridization of cultivated sunflower and wild *Helianthus* species. In P. D. S. Caligari & D. J. N. Hind (Eds). *Compositae: Biology & Utilization*. Proceedings of the International Compositae Conference, Kew, 1994. (D. J. N. Hind, Editor-in-Chief), vol. 2, p. 603-615. Royal Botanic Gardens, Kew.
- Christov, M., P. Shindrova, V. Entcheva.** 1996. Transfer of new characters from wild *Helianthus* species to cultivated sunflower. *Genet. a Slecht.*, 32, (4): 275- 286
- Georgieva-Todorova, I.** 1964. Mejduvidova hibridizatsia v rod *Helianthus*. BAS, Sofia.
- Georgieva-Todorova, I.** 1976. Mejduvidovi otnoshenia v roda *Helianthus*. BAS, Sofia.
- Christov, M.** 1988. Hybridization between *H. argophyllus* T. & G. ($2n = 34$) and *H. annuus* L. ($2n = 34$). *Genetics and Breeding*, vol. 21(6).
- Encheva, V. and I. Kiryakov.** 2002. A method for evaluation of sunflower resistance to Diaporthe/Phomopsis helianthi Munt.-Cvet. et. al. *Bulgarian Journal of Agricultural Science*, 8: 219-222
- Laferriere, J. E.** 1986. Interspecific hybridization in Sunflowers: An illustration of the importance of wild genetics resources in plant breeding. *Outlook Agriculture*, 15 (3), p. 104-109
- Pustovoit, G. V.** 1975. Selektzia podsolnechnika na gruppovoi immunitet metodom mejvidovoi gibridizatsii. In *Podsolnechnik. Kolos*, Moscow, p. 164-209
- Satziperov, F. A.** 1916. Opit skreshivania dvuh form podsolnechnika. *H. annuus* x *H. argophyllus*. Tr. Prikl. Bot. *Genet. i Sel.*, 9.
- Skoric, D.** 1992. Results obtained and future directions of wild species use in sunflower breeding. Proc. of the 13th Inter. Sunfl. Conf., Pisa, Italy, p. 1317-1348
- Seiler, G. J.** 1988. The genus *Helianthus* as a source of genetic variability for cultivated sunflower. Proc. of the 12th Int. Sunflower Conference, Novi Sad, p. 17-58
- Seiler, G. J.** 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. *Field Crops Research*, 30, p. 195-230
- Skoric, D.** 1988. Sunflower breeding. *Ujarstvo*, No.1, Beograd.
- Thompson, T. E., D. C. Zimmerman & C. E. Roger.** 1981. Wild *Helianthus* as a genetic resource. *Field Crops Res.*, 4, p. 333-343