Helia 2025, Vol. 48 (83), 74-83
https://doi.org/10.29329/helia.2025.1411.1

Original article

Interspecific Crosses Between Cultivated Sunflower (*Helianthus Annuus* L.) and the Perennial Species *Helianthus Mollis* (Gt M-020) Applying Embryo Culture

Nina Nenova [a], Daniela Valkova [a*]

[a] Dobrudzha Agricultural Institute, General Toshevo, Agricultural Academy, Sofia, Bulgaria

Abstract

The objective of this research was to carry out a comparative study of classical breeding methods and embryo-rescue technique for the production of interspecific F1 hybrids between wild perennial Helianthus mollis, accession GT M-020, and cultivated sunflower, as well as to analyze the contribution of applying embryo-rescue techniques for overcoming the environmental changes. Interspecific hybridization was done between cultivated sunflower Helianthus annuus L. and the perennial species Helianthus mollis, accession GT M-020. The embryo cultivation method was used for the successful performance of the crosses. Crosses were realized between this species and the male sterile line HA 300. Morphological, phytopathological and the biochemical studies were carried out. As a result of self-pollination and selection, hybrid materials in both F1 and F2 were obtained, and in advanced generations as well. Some of the progenies possessed higher seed oil content. Their resistance to economically important diseases (downy mildew, phoma and phomopsis) and parasite broomrape was presented. The conclusion of the research indicates that the selected hybrid materials differed significantly. Some of hybrid progenies demonstrated resistance to downy mildew, phoma and phomopsis. Applying of this method conducted to enrichment the gene pool of cultivated sunflower with diverse and useful traits, originated from wild sunflower species.

Keywords: sunflower, *Helianthus annuus*, *Helianthus mollis*, disease resistance, embryo culture, interspecific hybridization

Received: 30 September 2025 * **Accepted:** 23 December 2025 * **DOI:** https://doi.org/10.29329/helia.2025.1411.1

*** Corresponding author:**

Daniela Valkova, Dobrudzha Agricultural Institute, General Toshevo, Agricultural Academy, Sofia, Bulgaria
Email: nina_n13@abv.bg

INTRODUCTION

Biotechnological methods were applied to create or modify products, processes, or services, play a central role in different areas of knowledge and especially in wild hybridization (Skoric, 1992;Nenova *et al.,* 2014; Onisan *et al.,* 2025). The use of various wild species in breeding programs has shown that in most cases crossing of cultivated sunflower with wild species from the *Helianth*us genus is difficult (Christov, 1996; Dagustu, 2018; Lutzer *et al*., 2024; Nenova *et al.,* 2016; Nowakowska *et al*., 2024). This is why some wild species mostly the perennial diploid ones were rarely used as an initial gene plasm for breeding purposes. The successful resolution of this problem is to use embryo cultures, to isolate the embryo before abortion and to plant it in nutrient medium in order to grow an *in vitro* plant which can develop on its own (Azpiroz *et al.,* 1987; Lutzer *et al.,* 2024; Onisan *et al.,* 2025). Immature embryo rescue is one of the methods used for obtaining inbred lines in a shorter period, offering the advantage of seed harvesting at physiological maturity and the continuation of the line development process (Chandler *et al.,* 1983; Georgieva-Todorova, 1984; Hladni and Miklič, 2012). The genetic variability of cultivated sunflower could be increased by interspecific hybridization with wild sunflower species (Valkova *et al.,* 2015). Wild species from genus *Helianthus* possess not only considerable variability for most of the traits but also excellent survival environmental mechanisms (Karaaslan *et al.,* 2010; Sujatha and Prabakaran, 2001; Thompson *et al.,* 1981; Vear, 2016). They possess genes for resistance to diseases (biotic stress), tolerance to abiotic stresses (drought, cold, soil salinity, and certain herbicides) and high quality of proteins and oil (Nowakowska *et al.,* 2020; Seiler, 1992; Sukno *et al.,* 1999). 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.

This paper aims at a comparative study of classical breeding methods and embryo-rescue technique for production of interspecific F_1 hybrids between *Helianthus mollis*, accession GT M-020, maintained at the collection of wild *Helianthus* species in Dobrudzha Agricultural Institute and cultivated sunflower *Helianthus annuus* L

MATERIAL AND METHODS

Wild diploid perennial species *Helianthus mollis*, accession GT M-020, was included in the study. This species was maintained and reproduced in a stationary collection at Dobrudzha Agricultural Institute, General Toshevo. For the purposes of interspecific hybridization, the line HA 300 was also included as a mother line in crosses *H. annuus x H. mollis*. The seeds from A-line were used. Plants inflorescences from the wild species were hand emasculated. In the reciprocal *H.mollis x H.annuus* as a pollinator were used a mixed pollen from different lines – fertility restorers. Some of the inflorescences of the second type of crosses were left mature completely and the other part were

subjected to the embryo-recue method (Azpiroz *et al*., 1987; Nenova, N., *et al*, 2016; Dagustu, 2018). The normally shaped plants with well-developed roots were planted in soil under the greenhouse conditions (Rieseberg *et al.*, 1998; Terzić *et al.*, 2020; Valkova *et al.*, 2014). The plants from the two cross types were grown until flowering and were isolated for self-pollination. Some morphological and phenological studies of hybrid material during vegetation were carried out.

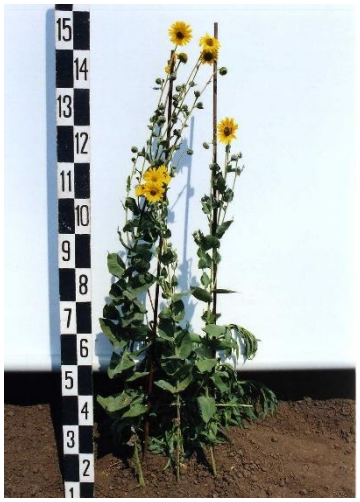
The phytopathological evaluation of the parental forms and the obtained hybrid progenies was performed with regard to the local broomrape (*Orobanche cumana* Wallr.) and downy mildew (*Plasmopara halstedii* Berl. & de Tori). The method for determining the reaction of sunflower genotype to downy mildew is described by Viranyi and Gulya (1995) by scale: 0%= S(sensitive); 100%=R(resistant). Broomrape resistance was calculated and checked as percentage of non-infected plants by scale: 0%=S (sensitive); 100%=R (resistant).

The obtained results were analyzed using various mathematical methods, applied depending on the purpose of the study. The average level of the trait was determined by the arithmetic mean value; the variation of the traits was determined by the coefficient of variation. Cluster analysis was applied to determine the distance between the parental forms and the newly obtained lines (Statistika, version 5.1, Stat. Soft.Inc).

RESULTS AND DISCUSSION

The use of various wild species in breeding programs has shown that in most cases crossing of cultivated sunflower with other species from the *Helianthus* genus is difficult (Christov, 1996). This is why some wild species mostly the perennial diploid ones were left unused as a gene-plasm for breeding purpose. The successful decision of this problem is to use embryo cultures, to isolate the embryo before abortion and plant it in nutrient medium in order to grow in vitro plant, which can develop on its own (Skoric,1992; Sujatha & Prabakaran, 2001; Silva Neto et al., 2025).

This paper aims to present a comparative study of classical breeding methods and *embryo-rescue* technique for production of interspecific F1 hybrids between *Helianthus mollis*, accession GT M-020 (Fig.1 and 2) and cultivated sunflower *H. annuus* L. (line HA 300).



A

B

Figure1 (A and B). *Helianthus mollis* (GT M-020)

Figure 2. Inflorescences from *Helianthus m*ollis (GT M-020)

The species *H. mollis*, accession GT M-020 is a perennial diploid species that reproduces by rhizomes, but under our conditions it can also be propagated by seeds. It is one of the most common species of the section *Divaricati*. In natural conditions it occurs in populations that are very different from each other - from single-stemmed to typically bushy. The stem height varied from 0,6 m to 1,5 m. The leaves are slightly rounded, with short petioles, almost sessile (Fig.1). The inflorescences are small, many on one stem (Fig.2). It blooms throughout August until mid-September. We selected the species *Helianthus mollis*, accession GT M-020, for our study because of the possibility of its being included in hybridization with the cultivated sunflower to improve its genetic potential.

The species *H. mollis*, accession GT M-020 is resistant to *Plasmopara helianthi, Phomopsis helianthi* (Georgieva-Todorova, 1984; Nenova *et al.,* 2014) and some authors have also identified populations of the species that carry genes for resistance to the important sunflower disease *Sclerotinia sclerotiorum* (Christov, 1996). The species could be used as a source for increasing protein content. From all positive characteristics of the species, it is clear that it can be used as a carrier of genes determining valuable economic qualities and can be used as a source of genetic material, which can be transferred into the genome of the cultivated sunflower applying hybridization.The incompatibility barriers between the species *Helianthus mollis* and the cultivated sunflower are difficult or almost insurmountable with conventional methods, and therefore we have included the *embryo rescue* method in the study to obtain successful F1 crosses (Nenova *et al.,* 2014; Valkova et al., 2015; Nenova *et al.,* 2016; Valkova *et al.,* 2016).

The study of self-compatibility of wild sunflower species is important in the application of interspecific hybridization. The difference among the number of fertilized plants in open and self-pollination show the nature of this species. The results from self-compatibility of wild *H. mollis* are

presented in Table 1. The analysis of these results reveals that pants of the accession produce seeds in self-pollination, although to a low degree from 0.93% to 1.86 %. The results of interspecific hybridization between the cultivated sunflower (line HA 300) and the wild perennial species *H. mollis* are given in Tables 2 and 3.

Table 1. Self-compatibility in wild species *H.mollis* GT M-020

Species/ Accession	**Open pollination**			**Self-pollination**		
	Number studied inflorescences	Fertilized inflorescences, (%)	Fertilization per plant, (%)	Number studied inflorescences	Fertilized inflorescences, (%)	Fertilization per plant, (%)
H.mollis (M-020)	10	100	48-72	10	30	0.93-1.86

Table 2. Hybridization between cultivated sunflower (line HA 300) and wild species *H. mollis.*

Crosses	Pollinated inflorescences		Number of seeds obtained	Number of hybrid plants obtained
	Total number of plants	Number of fertilized inflorescences		
M-020 x *H.annuus*	35	12	0	0
H.annuus x M-020	41	29	>100	19

Table 3. Production of interspecific hybrids by the embryo-rescue method.

Crosses	Number of isolated embryos	Number of plants obtained	Number of plants sown in soil	Number of dead plants	Number of seeds obtained	Green plants
M-020 x H. annuus	56	12	6	4	11	3
H.annuus x M-020	88	59	34	4	108	10

When *Helianthus mollis* used as a pollinator, a considerable number of seeds and hybrid plants are obtained. One of the difficulties in growing hybrid plants is that the greater percentage of the seeds obtained do not germinate and therefore the number of the hybrid plants is much smaller that the number of hybrid seeds. In some cases, self-pollinated plants from the female parent were obtained, too. Such cases contribute to the reduction of the hybrid plants number. Reciprocal crosses were made according to the classical breeding method (Table 2) and using embryo rescue culture (Table 3) and (Fig.3). It is evident that both exemplars produce a certain quantity of seeds, but no hybrid plants are obtained. Genetic distance between *H. annuus* and perennial *H. mollis* also manifests itself in the development of hybrids. A great part of the seeds do not germinate at all and the rest ones die in the early stage of their development.

A B C

Figure 3. Embryos young isolated (A), germinated (B) in vitro conditions and sown plant in greenhouses condition (C) from crosses M-020x HA 300.

Inflorescences from M-020 were covered with paper bags and emasculated. Some of them were pollinated with pollen from the line HA 300. These crosses were used to obtain interspecific hybrids by embryo rescue culture method (Fig. 3). Fifty-six embryos were isolated from the crosses using line HA 300 as a pollinator. They were produced 12 plants and 6 of them grown in greenhouse conditions. Another six died, when were transferred to the soil, just three of them were further grown in a greenhouse. All these observations indicate a remoteness of the species from the cultivated sunflower and a manifestation of incompatibility between them, expressed in disorders in reduction division during meiosis. Such conclusions were reported by Georgieva-Todorova (1984) and Christov (1996). Georgieva-Todorova (1984) assumes that in cases where complete or partial incompatibility is observed between perennial species of the genus *Helianthus* and the cultivated sunflower, postzygotic incompatibility occurs between the embryo and the endosperm, which prevents the development of hybrids.

Consequently, the hybridization between *Helianthus mollis* and *Helianthus annuus* in both directions is possible and the embryo rescue method is reliable for production of F_1 hybrid plants.

Characterization of hybrid material from HA 300 x M-020

In F_1 generation the plant height varied from 170-205 cm and the head diameter from 4.1-4.3 cm. The plants in F_1 generation proved to have 55.6% to 85% resistance to *Plasmopara helianthii*. In the second hybrid generation, a big segregation was observed and a great diversity of forms was obtained. Plant height varied from 175 to 320 cm and the head diameter - from 2.4 to 3.9 cm. In F_3 generation the segregation process continued. The plant height reached from 200 cm to 220 cm and the head diameter increases (Fig. 4). Some plants from this generation showed 100% resistance to broomrape and downy mildew. Plants, originated from the progenies F_2, BC_1, F_3, BC_1F_1 were also obtained and grown. Some of them reveal various degrees of resistance to mildew, broomrape and phoma (Table 4).

A B C

Figure 4. Hybrid material from cross HA 300 x M-020 (A-plant from F_1; B- plant from F_2; C-plant from F_3).

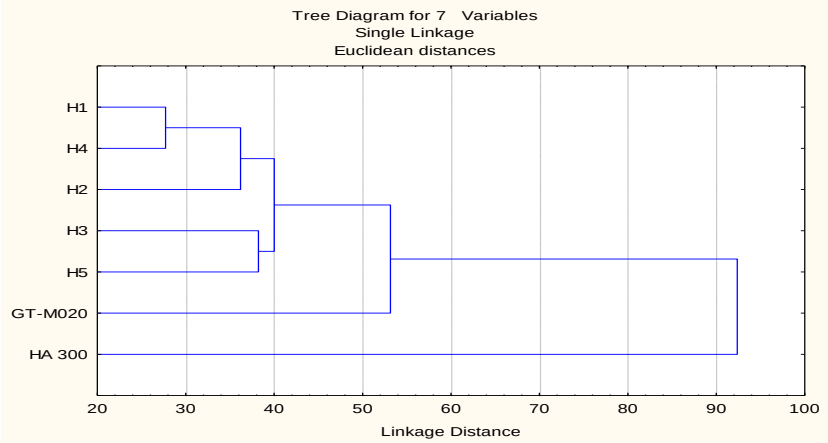


Figure 5. Clustering pattern between parental forms and their interspecific hybrid forms from the cross *HA-300 x Helianthus mollis- M-020*

The cluster analysis (Fig. 5) showed that the newly created hybrid combinations are separated into a cluster, where the Euclidean distance is closer to the wild parent *H. mollis*. This shows that in the initial generations, the hybrid crosses have a higher degree of similarity to that of the wild parent. Within the cluster itself, the new crosses are grouped again by degree of similarity and thus the differences between all newly created crosses can be taken into account.

Table 4. Date for the diversity of hybrid planst, obtained from the cross between *Helianthus annuus* L. x *Helianthus mollis* (GT M-020).

Accession/ № of plant	Plant height (cm)	Leaf length (cm)	Petiole length (im)	Length of the longest branch (cm)	Head diameter (cm)	Oil content in kernels (%)	Resistance to downy mildew (%)	Resistance to broomrape (%)
H 1	169	23.8	14.3	78	3.0	39	85	10
H 2	188	36	19	53	4.2	42	45	15
H 3	147	28	13	110	3.3	47	53	45
H 4	185	35	18	90	4.5	41	80	20
H 5	135	42	21	83	5.1	45	100	50
GT-M020	158	17	3	81	3.0	31	75	100
HA 300	114	21	12	0	25	47	100	100

Table 4 shows the characteristics of the diversity of several plants, that was obtained from crosses of the accession M-020. F_1 hybrid plants usually flowering very early, and this trait is inherited from the wild parent. The height of the plants is inherited intermediate. The diameter of the head is inherited by overdominance of the parent with a larger diameter. The number of tubular flowers was inherited intermediate to partial dominance of the parent with a higher number of florets. The seed set was inherited by overdominance of the wild parent with a weaker seed set. It was seen that a great diversity of plant forms was obtained. This enriches the gene pool of cultivated sunflower with diverse and useful traits.

The results presented in Tables 4 and 5 showed that the different indicators in the surviving and grown F_1 plants vary within quite wide limits, which indicates that it is necessary to perform more backcrosses to obtain balanced lines. The standard deviation for the character Plant height is 34.1cm, and the Coefficient of variation is 20.75%. The coefficient of variation (CV) is the lowest compared to the other four traits, but still within wide limits.

For the stabilized lines, CV should be up to 10%. For the remaining characters, CV is much higher from 31.19% to 50.13%. In sunflower interspecific hybridization, the variation of traits in the initial hybrid crosses is always observed. Therefore, even lines through interspecific hybridization are obtained slowly with many crosses, but at the expense of slower selection, more useful genes for important agronomic characters are recombined in newly created materials.

Table 5. Mean values, standard deviation and coefficient of variation of the resulting F_1 plants with origin *Helianthus annuus L. x H. mollis*, accession GT M-020.

Character	Average value	Standard Deviation	CV (%)
Plant height, (cm)	164.29	34.10	20.75%
Leaf length, (cm)	28.97	9.04	31.19%
Petiole length, (cm)	14.33	5.99	41.81%
Length of the longest branch, (cm)	70.71	35.45	50.13%
Head diameter, (cm)	3.31	15.92	48.68%

CONCLUSION

Hybridization between *H. mollis* (accession GT M-020) and *H. annuus* was made in both direct and reciprocal directions. Using the classical methods of hybridization, hybrid material was obtained but only in case when the cultivated sunflower was used as female parent. The low viability of the resulting hybrid seeds is probably due to various reasons, mainly related to the incorrect course of the development processes of the resulting embryos. These disorders are associated with the lack of seed germination, the death of the embryos in the early phases of their development.

Hybrid material was produced by crossing wild species x cultivated sunflower by applying the embryo-rescue method. These results suggest that the species *Helianthus mollis* (GT M-020) could be

crossed, yet to obtain hybrid material in both crossing directions it is necessary to use the embryo rescue method.

The great diversity of plant forms was obtained. This enriches the gene pool of sunflower with varied and useful traits.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

Azpiroz, H. S., Vincourt, P., Serieys, H., Gallais, A. (1987). La culture in vitro des embryons immatures dans l`acceleration du cycle de sélection des lignées de tournesol et ses effets morpho-vegetatifs. Helia, 10: 35-38.

Chandler, J.M., Beard, B.H. (1983). Embryo Culture of *Helianthus* Hybrids. Crop Science, 23: 1004-1007.

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.

Georgieva-Todorova, J. (1984). Interspecific Hybridization in the Genus *Helianthus* L. Z. Pflanzenzuchtung, 93:265-279.

Dagustu, N. (2018). In Vitro Tissue Culture Studies in Sunflower (*Helianthus* spp.). *Ekin J. Crop Breed. Genet. , 4*, 1.

Hladni, N**.,** Miklič, V. (2012). Old and New Trends of Using Genetic Resources in Sunflower Plant Breeding, With The Aim of

Preserving Biodiversity. Proc. Int. Conf. on BioScience: Biotechnology and Biodiversity“, Novi Sad, Serbia.

Karaaslan, D., Hatipoğlu, A., Türk, Z., Kaya, Y. (2010). Determination of Potential Sunflower (*Helianthus annuus* L.) Cultivars under Irrigated Conditions in Diyarbakir. Helia. 33 (52): 145-152.

Lutzer, A. V. B., Mueller, A. A., Oliveira, T. D. (2024). Environment in Industry 4.0: Applicability of Biotechnology as a Tool for Sustainability and Innovation. Revista de Gestão Social e Ambiental, *18*(11), e09860. https://doi.org/10.24857/rgsa.v18n11-150

Nenova, N, Valkova, D., Encheva, J., Taxin, N. (2014). Promising lines as a result of interspecific hybridization between cultivated sunflower (*H. annuus* L.) and the perennial. Turkish Journal of Agricultural and Natural Sciences. 2: 1654-1659.

Nenova, N., Valkova, D., Encheva, V., Georgiev, G. (2016). Comparative investigation of immature embryos growing interspecific hybrids. 19th International Sunflower Conference, Congress book – Association of Thrace Universities, Edirne, Turkey, pp. 460–464.

Nowakowska, M.; Pavlović, Ž.; Nowicki, M.; Boggess, S.L.; Trigiano, R.N. (2020). In Vitro Propagation of an Endangered *Helianthus verticillatus* by Axillary Bud Proliferation. *Plants, 9*, 712.

Nowakowska, M.; Pavlovic, Z.; Nowicki, M.; Boggess, S.L.; Trigiano, R.N. (2024). In Vitro Regeneration from Leaf Explants of *Helianthus verticillatus*, a Critically Endangered Sunflower. *Plants*, *13*, 285.

Onisan E, Petolescu C, Tenche-Constantinescu A, Petrescu I, Horablaga MN, Simina A, Gorinoiu G, Camen D, Popescu S, Mate C, (2025). Application of Biotechnology for Sunflower Inbred Line

Development: Immature Embryo Rescue. Applied Sciences. 15(3):1576. https://doi.org/10.3390/app15031576

Rieseberg, L.H., Baird, S., Desrocher, S.A. (1998). Patterns of mating in wild sunflower hybrid zones. Evolution, 52: 713-726.

Seiler, G. J. (1992). Utilization of wild Sunflower Species for the Improvement of Cultivated Sunflower. Field Crop Research, 30: 195-230.

Semerci, A., Y. Kaya, S. Durak. (2007). Economic Analysis of Sunflower Production in Turkey. Helia. 30: 47. 105-113.

Silva Neto, R. C. da, Pinheiro, L. A. L., Souza, J. P. da C., Nascimento, A. V. S. do, Nacif Junior, L. C., Almeida , S. do S. M. de A. de, Diniz, M. H. dos S. (2025). Environment and Biotechnology in the Context of Era 4.0. Revista De Gestão Social E Ambiental, *19*(1), e010919. https://doi.org/10.24857/rgsa.v19n1-108

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.

Sukno, S.; Ruso, J.; Jan, C.C.; Melero-Vara, J.M.; Fernández-martínez, J.M. (1999). Interspecific hybridization between sunflower and wild perennial *Helianthus* species via embryo rescue. Euphytica, *106*, 69–78.

Sujatha, M.; Prabakaran, A.J., (2001). High frequency embryogenesis in immature zygotic embryos of sunflower. Plant Cell Tissue Organ Culture, *65*, 23–29

Terzić S., Boniface M-C., Marek L., Alvarez D., Baumann K., Gavrilova V., Joita-Pacureanu M., Sujatha M., Valkova D., Velasco L., Hulke B.S., Jocić S., Langlade N., Muños S., Rieseberg L., Seiler G., Vear F., (2020). Gene banks for wild and cultivated Sunflower Genetic Resources. Oilseeds and fats, Crops and Lipids (OCL) 27: 9.

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

Valkova, D., Nenova, N., Georgiev, G., Encheva, V., Penchev, E., Encheva, J. (2014). Seed component diversity of hybrid forms, originated from wild *Helianthus* species. Turkish Journal of Agricultural and Natural Sciences. Sp. issue: 2: 1590-1595.

Valkova, D., Nenova, N., Encheva, V., Encheva, J. (2015). Creation of lines restorers of fertility originated from interspecific hybrids. Agricultural Sciences, VII (18): 75-79 (Bg).

Valkova, D., Nenova, N., Encheva, V., Encheva, J. (2016). Hybridization between cultivated sunflower and wild annual species *elianthus neglectus* Heiser. Proceedings 19th Intern. Sunflower Conference, 29 May-3 June, Edirne, Turkey, 2016, p. 454-459.

Vear, F. (2016). Changes in sunflower breeding over the last fifty years. Oilseeds and fats, Crops and Lipids (OCL), *23*, 2.

Viranyi, F. and T. Gulya. (1995). Proposed methodologies for inoculation of sunflower with *Plasmopara halstedii* and for disease assessiment. Bucharest, Romania, 25-28 July, 1995.

Whelan**,** E.D.P. (1978). Cytology and interspecific hybridization. pp. 371-386. In: Carter JF. Editor. Sunflower Science and Technology. The American Society of Agronomy. Monograph, 505 pages.