Helia 2025, Vol. 48 (83), 94-102
https://doi.org/10.29329/helia.2025.1411.3

Original article

Embryo Culture Under Interspecific Hybridization in Genus *Helianthus*

Radostina Damyanova-Serbezova [a*], Miglena Drumeva [b], Nina Nenova [a], Daniela Valkova [a]

[a] Dobrudzha Agricultural Institute, 9520, General Toshevo; Agricultural Academy, Bulgaria
[b] Technical University, Varna, 9000, Bulgaria

Abstract

Interspecific hybridization was carried out between cultivated sunflower *Helianthus annuus* L. and accessions from perennial wild species of genus *Helianthus* with the aim to develop hybrid plants with enriched heritability and higher resistance to abiotic and biotic stress factors. In order to overcome the difficulties in the process of crossing, the *embryo rescue* method was applied. Two CMS lines developed at Dobrudzha Agricultural Institute – General Toshevo (DAI) were included as mother components in hybridization. Accessions from five perennial *Helianthus* species- *H. grosseserratus, H. maximiliani, H. microcephalus, H. decapetalus*and *H. strumosus* with different ploidy levels were used as male component in the crosses.These accessionswere maintained in the wild sunflower collection in DAI. Ten hybrid crosses were made. Thirty-eight embryos were obtained, which were cultivated on nutrition medium. Six F_1 interspecific hybrids were produced -five plants from cross 10517AxM-134 and one from the combination 10517AxM-152. Theywere distinguished from each other by their habit type. All hybrid plants were sterile but they could be grown vegetatively.

Keywords: Embryo Rescue, Interspecific hybridization, *Helianthus*, Wild Species

Received: 09 November 2025 * **Accepted:** 29 December 2025 * **DOI:** https://doi.org/10.29329/helia.2025.1411.3

*** Corresponding author:**

Radostina Damyanova-Serbezova, Dobrudzha Agricultural Institute, 9520, General Toshevo; Agricultural Academy, Bulgaria
Email: e-mail: damianova.r@abv.bg

INTRODUCTION

Sunflower has been grown by the indigenous people of North America since 4000 years and was introduced to Europe in the 16th century (Chernova, 2021). In Bulgaria, during and after WW2, it was a main oilseed crop with regard to food supply (Valkova, 2013). Sunflower oil is valuable because of the high content of unsaturated fatty acids, primarily oleic and linoleic, which have favorable effect on human health (Jingetal, 1997; Huetal, 2001). The protein in the sunflower seeds is of high quality, without toxic components and rich in vitamins of group B (Ivanov, 1991). Especially important essential amino acids for the living organisms such as, isoleucine, leucine, lysine, methionine, phenylalanine, histadine, tryptophan and valine, are contained in sunflower protein (Fernandez-Martinez et al., 2010).

Sunflower is a high-yielding crop but yield is often compromised under the effect of certain factors of biotic and abiotic nature. Some diseases as phomopsis, sclerotinia, downy mildew and alternaria, as well as the parasite *Orobanche*, considerably reduce the harvest from this crop. The recurrent droughts also affect negatively the yields. The control of these unfavorable factors is most successful when applying breeding methods. Developing resistant varieties and hybrids is an appropriate method for overcoming the negative influence of the environment. The potential of cultivated sunflower as a source of genes for resistance to biotic and abiotic stress is limited; therefore the use of interspecific hybridization is a useful solution to the problem. The wild sunflower species carry genes for resistance to diseases, parasites, drought and herbicides (Christov *et al*., 1996; Vega *et al*., 2012; Valkova and Encheva, 2021; Damyanova-Serbezova *et al.,* 2024).

Sometimes the hybridization between cultivated sunflower and a wild species is impossible, because there are so incompatible barriers and for these reasons is necessary to use the *embryo rescue* method.

The aim of the research is to investigate the possibility of applying the embryo rescue method to create interspecific hybrids possessing genes for resistance to biotic and abiotic stress factors.

MATERIAL AND METHODS

The experiment was carried out at DAI during 2023. Two CMS sunflower lines (830 A and 10517 A), sown later and grown under greenhouse conditions, were used as maternal plants. Hybridization was done between the cultivated sunflower and accessions from five perennial species with different level of ploidy (Table 1). Each sample of the collection has its own catalog number, official for the collection's register and listed in the FAO catalogue.

Table 1. Perennial wild sunflower species used in the study

Perenial wild species	Accession	Number of chromosomes (2n)
H. grosseserratus	GT-M-014	34
H. maximiliani	GT-M-017	34
H. microcephalus	GT-M-080	34
H. decapetalus	GT-M-134	68
H. strumosus	GT-M-152	68

Interspecific crosses according to the scheme cultivated sunflower x wild species were grown in greenhouse conditions.

CMS lines were pollinated with each of the wild species accessions included in the study. Ten crosses were made, from of which 38 embryos were obtained.

The technique embryo culturing using the methods of Azpiroz *et al.* (1988) was applied to overcome in company.

The embryos from the hybridization were obtained by applying classical selection methods and embryo cultivation (Nenova, 2002; Nenova and Drumeva, 2012)

All obtained embryos were cultivated on *in vitro* modified nutrition medium B5 (Gamborg et al., 1968).

Embryo preparation and sterilization

The inflorescences of the pollinated plants were cut off, the bracts were removed and the young formed seeds were detached from the head with pincers. Then they were sterilized in 70% solution of commercial bleach and washed five times with sterilized distilled water.

Cultivation and nutrition medium

For embryo cultivation, modified and enriched B5 nutrition media was used. The process wascarriedout in a laminar box under sterile condition. Using sterile forceps and a scalpel, the membranes are removed and the embryos are placed in Petri dishes with pre-distributed culture medium. The culture medium is previously sterilized in an autoclave.

The Petri dishes were cultivated at temperature 24 ± 2^0 C and illumination of 2500-3000 lux. The photoperiod was 16/8 day/night. The development of the plants was recorded (Figure 1).

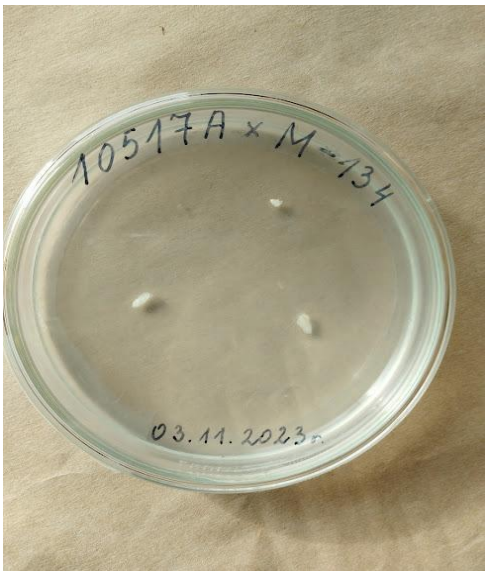

Figure 1. Embryos from cross 10517AxM-134.

Transfer of plants to soil

Depending on the condition and development of the plants, the time for their transfer to soil substrate composed of soil and sand at ratio 1:1 was estimated. This was done on the 19th day after cultivation when the root reached 3-4 cm in length (Figure 2). At the beginning of May, the plant materials were transferred to the collection of wild perennial sunflower species maintained at DAI.

Figure 2. Plants from cross 10517AxM-134 transferred to soil

RESULTS AND DISCUSSION

Ten crosses were made according to the scheme “cultivated sunflower × wild species”, five with line 10517 A and five with line 830 A. The hybridization was successful in three crosses: 10517AxGT-M-134, 10517AxGT-M-152 and 830AxGT-M-17, which constituted 30%of all crosses made.

The embryos were cultivated in modified B5 nutrition medium. On the third day, the emergence of a root was observed, and on the fifth – of leaves. The three-day old embryos from cross 830A x GT-M-17 perished in 10 days.

Nine plants developed from the cultivated 38 embryos, which constituted 23% of all cultivated embryos. The low percent of the produced embryos and of the subsequent plants, which developed from them, indicated difficult interspecific hybridization between the cultivated and wild perennial species of genus *Helianthus*. The resulting plants are from the crosses 10517A x GT-M-134 and 10517A x GT-M-152, in which the paternal forms are tetraploid. No plants were obtained from the crosses with perennial diploid species used in the study. This confirms the statement that hybridization between cultivated sunflower and perennial wild species occurs more easily when the perennial species is tetraploid and very difficult when the perennial species is diploid, regardless of the same ploidy level as the cultivated sunflower.

Table 2. Results from the use of embryo culture of immature embryos from the initial crosses

Crosses	Number of crosses	Obtained embryos	Obtained plants
830A × GT-M-014	1	0	0
830A x GT-M-017	1	13	0
830A x GT-M-080	1	0	0
830A x GT-M-134	1	0	0
830A x GT-M-153	1	0	0
10517A x GT-M-14	1	0	0
10517A x GT-M-17	1	0	0
10517A x GT-M-148	1	0	0
10517A x GT-M-134	1	15	6
10517A × GT-M-152	1	10	3
Total number	10	38	9

Vitrification was observed in three plants: two from cross 10517A x GT-M-134 and one from10517A x GT-M-152, which was 30 % from all plants (Figure 3).

Figure 3. Vitrification in cross10517A x GT- M-134

The remaining six plants were transferred to soil substrate and grown under greenhouse conditions. These were five plants from cross 10517A x M-134 and one from the combination 10517A x M-152. In two of the plants from the cross 10517A x M-134, a shortened stem was observed and the leaves formed a rosette (Figure 4). Anthocyanin coloration was not observed. In the other three plants from the same cross, a branched stem developed, with additional shoots at a later stage (Figure 5). In two of the plants, anthocyanin coloration on the stem and the petioles was found. Such coloration was also noticed in the plant from the combination 10517A x M-152. All obtained plants had perennial cycle of development. The presence of anthocyanin color, branched stem and perennial cycle of development implied gene transfer from the wild parent to the genome of the cultural sunflower (Christov, 2006).

Figure 4. Shortened stem of a plant from cross 10517A x GT- M-134

Figure 5. Difference in the habit type between the plants from cross 10517Ax GT- M-134

All plants derived from the above crosses were sterile (Figure 6). The explanation can be the widespread incompatibility and selectivity at pollination. The sterility under distant hybridization is a common phenomenon. It can be the result from incompatibility between the cytoplasm and the genotype (Nenova, 2002). The plants were grown under field conditions in a stationary collection of perennial wild sunflower species at DAI.

Figure 6. Sterility in the plants from cross 10517A x M-134

The phenological and morphological characterizations showed that the mean values of the parameters of the hybrid combinations were intermediate of the parental forms (Fig. 7 and 8)

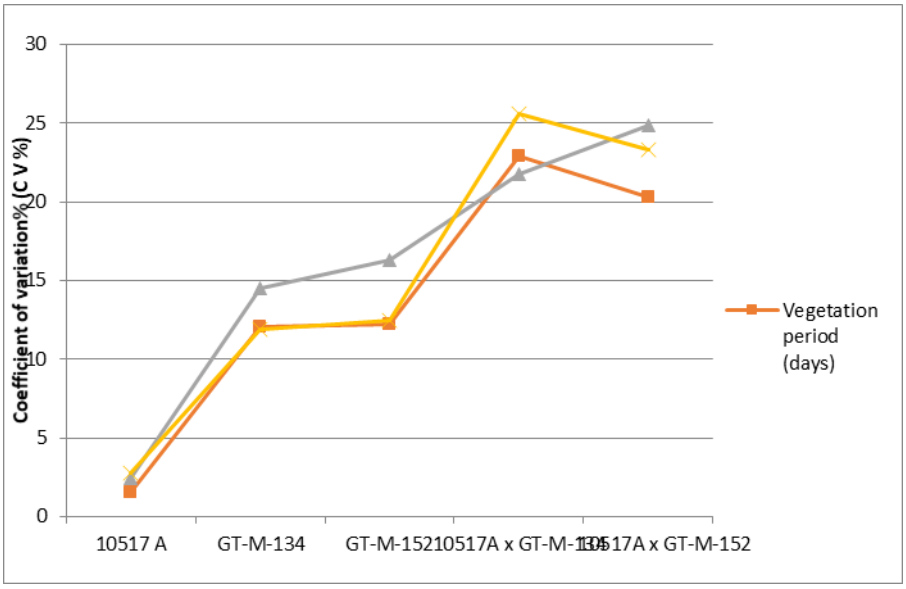

Figure 7. Comparative phenological characterization of the parental forms and their -hybrid combinations

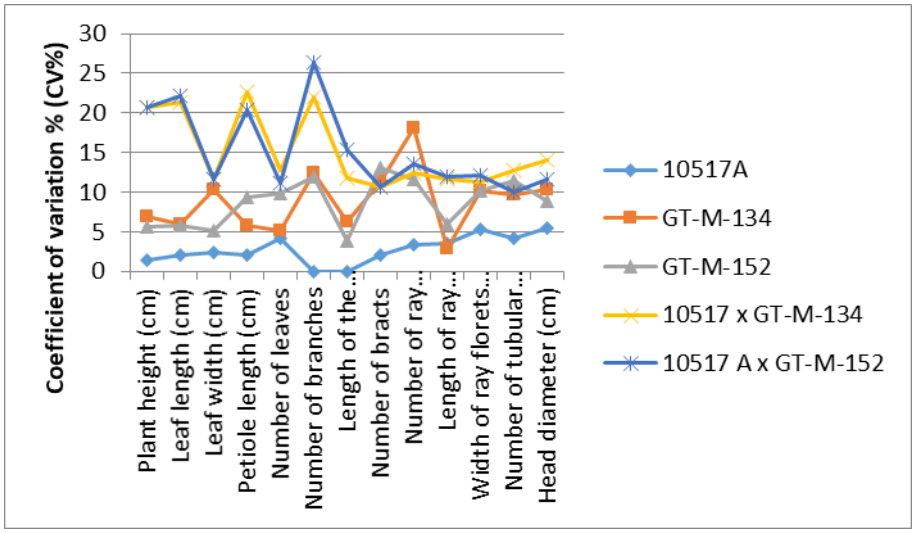

Figure 8. Comparative morphological characterization of the parental forms and their hybrid combination

Figures 7 and 8 show that the coefficients of variation (CV %) of the investigated phenological parameters can be grouped as follows: They were lowest in cultivated sunflower (10517 A), which is normal and can be explained by the uniformity of the line. In the wild speciesaccessions GT-M-134 and GT-M-152, the coefficients of variation of the studied parameters were higher in comparison to cultural sunflower.

Highest were the coefficients of variation of the obtained hybrid combinations. This implied plasticity of the traits in the hybrid forms with wild species, which can be used for modeling of the breeding process in sunflower. In the hybrid combinations, there was a wide genetic base coming from the parental forms, which can be applied to the development of new sunflower varieties and hybrids with specific traits and parameters.

In future studies, F1 plants will be subjected to colchiploidy in vitro or in vivo for amphidiploidization, as crosses with these plants can lead to fertile progeny.

CONCLUSION

Ten interspecific crosses between cultivated and perennial sunflower were made. By using the *embryo rescue* method, six plants were obtained, which were transferred to soil substrate and grown under field conditions.

All plants were sterile but are suitable for vegetative reproduction.

The coefficients of variation of the phenological characteristics and the morphological traits were high and provided a wide genetic basis for realization of the breeding process aimed at developing varieties and hybrids with desirable traits.

The perennial species from genus *Helianthus* maintained and stored in the DAI collection can be used as breeding material to enrich the genome of cultivated sunflower.

F_1 plants will be subjected to colchiploidy in vitro or in vivo for amphidiploidization, as crosses with these plants can lead to fertile progeny.

Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

Azpiroz, H.S., Vincourt P., Serieys H. Gallais A. 1988. In vitro immature embryo culture for accelerating the breeding cycle of sunflower lines and its morphovegetative effects. Sci. Bull. FAO Res. Network Romania (1987). Helia (10): 35-38

Christov, M. (2006). Contribution of interspecific hybridization to breeding of sunflower. Field Crops Studies. III-2

Christov, M., Shindrova, P. & Encheva, V. (1996). Transfer of new characters from wild

Helianthus species to cultivated sunflower. Genet. a Slecht., 32(4): 275-286.

Gamborg, O. L., Miller, R,, & K. Ojima, (1968). Nutrient requirements of suspension cultures of soybean root cells. Experimental cell research. 50 (1): 151

Nenova, N. (2002). Combining methods of interspecific hybridization with in vitro techniques for enriching the genome of cultivated sunflower (H. annuus L.).

Thesis for the awarding of the academic and scientific degree of Doctor. Sofia. p. 79.

Nenova, N. and Drumeva, M. (2012). Combined application of different methods for creating lines resistant to economically important diseases and parasites, fertility restorers for sunflower. Third international scientific. Congress "50 Years of Technical University Varna", pp. 40-42.

Vega, T., Breccia, G., Gil, M., Zorzoli, R., Picardi, L., Nestares G. (2012). Acetohydroxyacid synthase (AHAS) in vivo assay for screening imidazolinone-resistance in sunflower (*Helianthus annuus* L.). Plant Physiology and Biochemistry. 61: 103-107. Doi: 10.1016/j.plaphy.2012.09.013.

Valkova, D., & Encheva, V. (2021). Wild annual Helianthus species as sources for resistance to some foliar pathogens on sunflower. Bulgarian Journal of Agricultural Science. 27 (5): 942–94.