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Promising Lines as a Result from Interspecific Hybridization between Cultivated Sunflower (*Helianthus annuus* L.) and the Perennial Species *Helianthus ciliaris* (M-092) via Embryo culture

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Abstract

Interspecific hybridization was done between cultivated sunflower H.annuus L. and the perennial species H.ciliaris, accession M-092. The embryo cultivation method was used for successful performance of the crosses. Crosses were realized between this species and sterile lines 2607 and HA-300. Different morphological characters were investigated. As a result of self-pollination and selection, hybrid materials in both F1 and F2 were obtained, and in advanced generations as well. Morphological, phenological and biochemical studies were carried out. Some of the progenies possessed higher seed oil content. Four lines among the selected, combined complete resistance to the pathogens of downy mildew and broomrape. These were the lines 1131/н, 1135/н, 1145/н, 1171/p, 1161/p and 1151/p.

Keywords: sunflower, *Helianthus annuus*, *Helianthus ciliaris*, disease resistance, embryo culture

Introduction

Many of the species from genus Helianthus have been found to be useful as carriers of genes controlling cytoplasmic male sterility, restorer genes, genes for high content of separate fatty acids, resistance to diseases and other stress factors. Therefore their involving in interspecific hybridization with cultivated sunflower is a promising method now more frequently applied in the breeding of this crop. To overcome non-crossability, the method of embryo cultivation has been successfully used (Nenova et al., 1990). The wild perennial species *Helianthus ciliaris* (Fig.1) belongs to section Divaricati. According to Christov (1996a, 1996b), this species possesses resistance to downy mildew and broomrape. The object of this investigation were the morphological, phenological and biometric traits of the interspecific hybrids developed by using *Helianthus ciliaris* accession M-092, and the possibility to apply embryo cultivation for overcoming the non-crossability with the wild species.

Materials and Methods

The investigations were carried out at Dobrudzha Agricultural Institute – General Toshevo. They involved accession M-092 of the perennial species Helianthus ciliaris, which is being maintained and propagated in our collection, as well as line 2607.

Morphological and phenological characterization was done of all cultivated sunflower lines, the obtained hybrid progenies and the species included in the investigation. The observations and measurements were performed on ten plants. The initial crosses were made under field conditions, and F1 was produced through embryo cultivation (Azpiroz et al., 1987) under laboratory conditions (Fig.1, 2, 3 and 4). The next generations were grown and observed in the field (Fig.6). The mathematical processing was carried out with the software BIOSTAT, version 1 (Penchev, 1998).

Results

Helianthus ciliaris is a perennial hexaploid species. It is resistant to economically important diseases such as downy mildew, phomopsis, alternaria, rust and the parasite broomrape. It shows tolerance to sclerotinia (Christov, 1996). The species multiplies through rhizomes, but under the conditions of Bulgaria propagation is comparatively successful through seeds as well. Flowering occurs throughout the entire month of August till mid-

September. Seeds are small and in most cases multicolored. The species is a carrier of fertility restorer genes for CMS based on Helianthus petiolaris. Due to all above-mentioned properties and traits, this species was included in hybridization for our investigations.

The initial crosses were made under field conditions. The obtained embryos were cultivated on solid nutrition medium (Azpiroz et al., 1987). The most suitable time for isolation of the embryos from this cross was on the 16th day after pollination. The F1 plants in the investigated combinations had anthocyanin coloration of the stem, and to a lesser degree – on the leaves, imitating the wild parent. All these traits were observed under greenhouse conditions. A total of 235 seeds were obtained from cross 2607 x M-092. On the next year, these seeds were planted in the field. In generation F2 a wide formative process was observed – a rich variety of forms by morphological, phenological and other traits. As a result from segregation, branched and non-branched plants were observed with different types of branching, shapes of leaves and shape and color of ray flowers. In some F4 progenies a tendency occurred toward uniformity of the plants by the following morphological traits: height, head diameter and general habit type. The branched phenotypes were predominant. Selection was carried out from generation F2 to generation F5 according to morphological traits and tests for resistance to downy mildew, broomrape and oil content in seed.

One of the tasks of contemporary breeding is finding out and combining the available genetic variability obtained from the wild gene plasm, and developing of suitable genotypes for specific environments.

In this direction, some important agronomy traits of the new lines obtained by using embryo cultivation were followed. Due to the polygenic nature of their inheritance, this was done with the help of biometric methods. The registered indices were among those most frequently investigated in the process of breeding of new sunflower lines. After precise study on the new materials, six lines were selected which possessed the necessary properties and could be included in the next stages of breeding. The followed indices were: plant height, head diameter, 1000 kernel weight, oil content in seed, resistance to diseases and parasites. Table 1 shows the mean values of these indices, the measurements being done in generations F6 - F7, when the lines are supposed to be uniform as a result from selection and imposed selfing. The statistically significant variations with regard to the more important indices included both positive and negative ones according to the parental form (line 2607).

Table 1. Mean values of main indices in lines derived from the hybrid combinations *Helianthus annuus x Helianthus ciliaris (M-092)*

LINES	Plant height, cm	Head diameter, cm	1000 kernel weight, g	Oil content, %
H.ciliaris	60	1.5	2.0	13.7
2607	139	28	48	42.1
1131/н	134	30	40	47.2
1135/н	136	32	47	48.5
1145/н	117*	29	42	45.2
1151/p	118*	23*	39*	48.0
1161/p	122	24*	38*	50.2
1171/p	112*	22**	45	49.5

- *5%; **- 1 %; ***- 0.1%

The plant height of the new lines was reduced in comparison to line 2607. The shortest plants were obtained from line 1171/н – 112 cm, the differences being significant at level 5%. Line 1135/н was almost as tall as the parental line. The height of the other four lines was reduced with 5 to 22 cm. Their variation coefficient was within the range 4.4 % - 9.5 %, showing good uniformity of the lines by this index. The shortest line 1171/н was also with the smallest head diameter (22 cm) at level of significance 1 %. The variation coefficient of this index was within the range 5.5% - 9.5%, revealing good uniformity of the lines according to this index. In the lines with origin from the cross 2607 x M-092 the plant height was reduced with 5 to 22 cm. This is desirable for the breeding lines because the plants are more resistant to lodging.

Another important index related to the production potential of the lines is 1000 kernel weight. The seeds of the species Helianthus ciliaris are with very small size and weight – 2 g. Therefore the new developed lines were compared only to the cultivated parent by this index (line 2607). Lowest 1000 kernel weight was registered in line 1161/p – 38 g, and highest – in line1135/н – 47g.

The wild forms have low oil content in seed. In the hybrid combinations, however, new materials are produced which possess high oil content (Christov, 1996). This was confirmed by the data we obtained, too. Accession M-092 had low percent of oil – 13.7%. The branched lines 1161/p and 1171/p were with the highest oil content of seeds: 50.2% and 49.5%, respectively. In the non-branched lines the oil percent of seeds was lower, within 46.2 - 49.2 %. The exceeding by this index according to line 2607 was with 4.6% - 5.9% for the branched lines and with 1.9% - 4.2% for the non-branched lines with origin from cross 2607 x M-092. These data indicated possible transgressions in the hybrid material with regard to oil content. It is evident that this is an index determined by multiple genes with additive effect and with complementary interaction.

The biological value of the fats depends on the content of unsaturated fatty acids. The demand of them is related to growth, metabolism, propagation, and also to the regulation of cholesterol and the level of lipids in the plasm of human liver. From this point of view, there is a need to carry out breeding for improved fatty acid composition in the oil of the sunflower seeds. The fatty acid composition of the new lines is given in Table 2.

All new lines had high content of linoleic acid in the oil determined by the inherited chromosome material of the species Helianthus ciliaris. Line 1171/p had the highest content of linoleic acid in the oil - 63.2%. It exceeded both parents: line 2607 with 6.6% and the wild parent with 3.7%. However, this line was with low oleic acid percent. The same tendency was observed in lines 1161/p and 1131/н. Line 1131/н had values similar to that of the parental lines by the two important unsaturated fatty acids – linoleic and oleic. The content of stearic and palmitic acids in all new lines was below that of line 2607 and close to the values of the wild parentM-092.

Table 2. Fatty acid composition of the lines derived from the interspecific cross *Helianthus annuus x Helianthus ciliaris*, accession M-092.

Parental forms and new lines	Linoleic acid, C18:2 %	Oleic acid, C18:1 %	Stearic acid, C18:0 %	Palmitic acid, C16:0 %
H.ciliaris M-092	59.5	29.9	3.8	6.8
2607	56.9	30.8	4.2	8.1
1131/н	59.2	31.0	2.6	7.2
1135/н	51.5	39.3	2.2	7.0
1145/н	54.6	36.4	2.0	7.0
1151/p	54.4	36.8	2.6	6.2
1161/p	50.2	40.4	1.9	5.6
1171/p	63.2	25.5	1.8	7.3

One of the main tasks of interspecific hybridization is directed toward transfer of genetic material from the wild species to the genome of the cultivated species for developing of forms resistant to diseases, parasites and pests. The lines were investigated for resistance to downy mildew

(Plasmopara helianthi Novot.) - race 700, phoma, phomopsis and broomrape (Orobanche cumana). The six lines derived from the cross Helianthus annuus x H. ciliaris were moderately resistant to the diseases phoma and phomopsis. All lines were resistant to downy mildew race 700. Transfer of resistance from the wild form was realized because line 2607 is susceptible to this pathogen.

In the breeding of sunflower hybrids with high production potential and valuable economic properties it is necessary to develop lines – parental components of the hybrids which possess good bio morphological traits and properties. These lines have to be evaluated to determine their breeding value. This is done by investigations on their combining ability, i.e. their ability to produce after crossing hybrid generation with high heterosis effect. The investigations on the combining ability of the new lines began in generations

In spite of the results up to now, to confirm the good combining ability, additional investigations are necessary with a part of the lines. In Tables 3 and 4 data are presented on the production potential of the lines with origin from hybrid combination *Helianthus annuus x H. ciliaris*. The sterile analog of line 2607 was used as a tester. Hybrids San Luka, Maritsa and Brio were involved as standards.

All hybrid combinations demonstrated good combining ability. During the three years of the investigation they exceeded the mean standard by this index. In the hybrid combinations involving non-branched lines the exceeding was with 14.8 %, and in the hybrids involving branched lines it was 6.6 %. The hybrid combinations involving branched lines (p) showed 100 % restoration ability, while those involving non-branched lines (н) – 100 % sterility. This indicates that the species Helianthus ciliaris has well expressed correlation between branching and restoration ability.

Table 3. Seed yield from hybrid combinations with father lines derived from the cross *Helianthus annuus x H. ciliaris,* in % from the mean standard.

Hybrid combination	Seed yield, %			
	2010	2011	2012	Mean
ms 2607 x 1131/н	119.7	118.4	107.1	115.1**
ms 2607 x 1135/н	107.3	116.5	111.9	111.9*
ms 2607 x 1134/н	117.8	102.9	124.5	115.1**
ms 2607 x 1151/p	108.0	105.3	105.2	106.2
ms 2607 x 1161/p	105.0	107.5	107.9	106.8
ms 2607 x 1171/p	106.2	103.2	109.1	106.2

By the index oil yield, hybrid 2607 x 1145/н was with the highest percent above the standard, averaged for the three years of testing – 18.3 %. The lines with origin from the cross Helianthus annuus x Helianthus ciliaris M-092 demonstrated stable exceeding of the mean standard throughout all years of testing by the index oil yield, while the same could not be said for the greater part of the lines we have tested with origin from interspecific crosses of cultivated sunflower with some other species of genus Helianthus.

Conclusion

Based on all results obtained from the interspecific hybridization of the species Helianthus ciliaris accession M-092 with the cultivated species Helianthus annuus L with the help of the embryo rescue method, the following conclusions can be made:

• The isolation of the embryos from this cross should be done on the 16th day after pollination;

• After individual selection, uniform branched and non-branched lines were obtained;

• The height of the new lines developed was reduced in comparison to parental line 2607;

• The new lines have higher content of oil in seeds in comparison to the parental forms;

• A part of the breeding lines have higher content of oleic acid in oil, and line 1171/p is with higher linoleic acid content;

• The hybrid combinations of the new lines demonstrated stable values above the mean standard by the indices seed yield and oil yield during the three-year testing.

• The hybrids involving branched lines showed 100 % restoration of fertility, and the hybrids involving non-branched lines were 100 % sterile. They are reliable initial material as B and R lines for developing of new hybrids after additional observations and analyses. This indicates that the species Helianthus ciliaris has well expressed correlation between branching and restoration ability. This correlation can certainly be used as a marker for the ability of fertility restoration in materials derived from this species

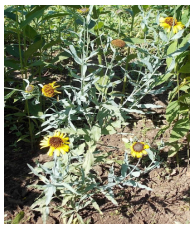

Figure 1. *Helianthus ciliaris* M-092

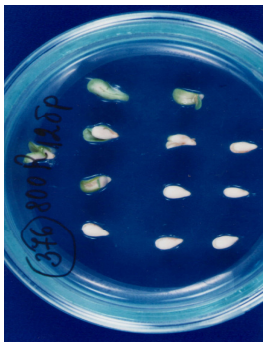

Figure 2. Embryos from cross *H. annuus x H. ciliaris*

Figure 3. *In vitro* plant from cross *H. annuus x H. ciliaris*

Figure 4. Young F1 plant from cross *H. annuus x H. ciliaris*

Figure 5. Isolated F1 plant from cross *H.annuus x H.ciliaris*

Figure 6. Line 1171/p

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