

Characteristics of some hybrid progenies produced by crossing of *Helianthus annuus* L. with *H. maximiliani* SCHRADER

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ABSTRACT

- *H. maximiliani* is very interesting species from the agronomic point of view. Some accessions are sources of new CMS, *Rf* genes for CMS Pet-1 and resistant to *Sclerotinia sclerotiorum*. The aim of our investigation is: 1) to receive hybrids from crossing between cultivated sunflower *Helianthus annuus* and the wild perennial diploid species *Helianthus maximiliani*; 2) to characterize some hybrid progenies; 3) to obtain new inbred lines with useful for the sunflower breeding characters as: good general (GCA) and specific combining ability (SCA), resistance to downy mildew, phomopsis and broomrape and etc.
- Three accessions (M 175, M 176 and ND 19) from the perennial diploid species *Helianthus maximiliani* ($2n = 34$) were included in hybridization with annual diploid cultivated sunflower *Helianthus annuus* L, represented by a cultivar (Peredovik) and 4 lines (sterile analogues: 2607A, 6116A, 6234A and HA89A in CMS Pet-1 and fertile lines: 2607B and HA 89B). Self-pollination of first generation and back-crossing (once) of F_1 with cultivated sunflower (cross HA 89A x F_1) were made with aim to obtain F_2 and BC_1 . All next generations were obtained by self-pollination. The investigation was carried out on standard methods. Combining ability was tested applying diallel crosses with multivariate analyses.
- The investigation was carried out during the period 2000-2010. Direct and reciprocal crosses were made. The crossability rate was low. Seeds were obtained at both directions of crossing and hybrid plants - from the direct crosses. F_{10} and F_8BC_1 generations were produced. All F_1 plants were with annual cycle of growth. The F_1 plants had an intermediate type of heritability, but they strongly resembled the wild parent by the most important biomorphological characters. Reduction division of PMC in the F_1 plants occurred with deviations. All plants from second and third generation were branched. Some unbranched plants appeared in the next generations. Second hybrid generation showed morphological difference from the parents that steadily was inherited and it was suitable for markers - light lemon-yellow ray flowers. It was established that the subspecies carried *Rf* genes for CMS Pet-1. As a result of selection seven new inbred lines with high seed oil content, resistance to diseases downy mildew, phomopsis and the parasite broomrape and good combining ability were obtained.
- These results showed that species *Helianthus maximiliani* possesses some important characters which were already transferred or could be transferred to sunflower in the process of hybridization between these two diploid species. The obtained on this base, new inbred lines, were parts of a whole work on widening of genetic diversity in sunflower.
- Three from the seven obtained inbred lines were included in a sunflower breeding program for developing hybrids based on heterosis.

Key words: backcross - *Helianthus annuus* - *Helianthus maximiliani* - hybridization - lines - sunflower

INTRODUCTION

Interspecific hybridization is an important source of variability in sunflower (Whelan, 1978a). Cytoplasmic male-sterility, fertility restoration genes, and resistance to several diseases have been obtained by this technique (Whelan and Dorrell, 1980). *H. maximiliani* Schrader is perennial diploid ($2n = 34$) species and belongs to the section *Divaricati*, series *Corona-solis* of the genus *Helianthus* (Schilling and Heiser, 1981). This species is very interesting from the agronomic aspect, because it was found that some accessions are sources of new CMS (with FAO code MAX1 - Whelan, 1980 and MAX2 - Jan, 1994; reported by Serieys and Christov, 2005) and some of their populations (Škorić and Rajčan, 1992) and clones (Henn et al., 1997) are resistant to *Sclerotinia sclerotiorum*. Although a gene for resistance to this disease has not been found (Aćimović, 1992) Hristova-Cherbadzi and Christov (2008) reported for lines with resistance to *Sclerotinia*. Whelan (1978b) reported the first successful hybridization between perennial species *H. maximiliani* and the annual wild *H. annuus*. No hybrids were obtained when cultivated sunflower was used as the pollen parent (Whelan and Dorrell, 1980). Scoric et al. (1987) reported for F_1 hybrids from reciprocal crosses of cultivated sunflower with 27 wild species - 11 annual and 16 perennial. F_1 hybrids received with the participation of *H. mollis*, *H. nuttallii* and *H. maximiliani* were sterile and with abortive seeds. Atlagic et al. (1995) investigated the meiosis and pollen grain viability in *Helianthus mollis*, *Helianthus salicifolius*, *Helianthus maximiliani* and their F_1 hybrids with the cultivated sunflower. The results showed that this diploid perennial species could be crossed with cultivated sunflower but the frequency of successful crosses was low.

The object of this investigation was to obtain hybrids, to examine the crossability and to characterize some hybrid progenies produced by crossing of *Helianthus annuus* L. with *H. maximiliani* and new inbred R lines.

MATERIALS AND METHODS

The perennial diploid species *H. maximiliani* ($2n = 34$) was included in hybridization with cultivated sunflower *H. annuus* L. Hybridization was carried out through reciprocal crosses realized under field conditions. Three accessions from *H. maximiliani* were included in hybridization with cultivated sunflower - M 175, M 176 and ND 19. The sterile analogues of four lines 2607, 6116, 6234 and HA89 (cytoplasmic male sterile lines in CMS Pet-1) were used as female parent in direct crosses. Sterile analogue of lines HA 89 and 6116 in direct cross with *H. maximiliani* M 175, sterile analogue of line 2607 in cross with *H. maximiliani* M 176 and sterile analogue of line 6234 in cross with *H. maximiliani* ND 19 were used. In the reciprocal crosses the florets in the inflorescences of the wild species were castrated manually and were pollinated with pollen from cultivated sunflower - lines: 2607B and HA 89B and variety Peredovik. The mixed pollen from lines HA 89B and 2607B were used as pollenator of *H. maximiliani* M 175 and pollen from line 2607B or variety Peredovik for *H. maximiliani* M 176.

Hybrid plants were grown under field conditions too. Self-pollination, sib-pollination and back-crossing (once) of F_1 with cultivated sunflower (cross HA 89A x F_1) were made with aim to obtain F_2 and BC_1 . $F_3 - F_{10}$ and F_1BC_1 to F_8BC_1 generations were produced.

Regular phenological observations of F_1 hybrids were carried out during the vegetation. Biometric parameters and description of morphologic characters and biologic peculiarities of all hybrids were performed during the vegetation. The presence of fertility restorer genes (*Rf* gene) for CMS Pet-1 in *H. maximiliani* was registered. 1000 seed weight was determined by measuring three samples, each of 50 seeds.

The investigation on disease resistance of hybrids and percentage of seed oil content were studied according to standard methods. Cytological analyses were carried out on the meiosis of pollen mother cells (PMC) according to Georgieva-Todorova (1976). Pollen viability was determined by the method of Alexander (Alexander, 1969).

Combining ability was tested applying diallel crosses with multivariate analyses. The general (GCA) and specific (SCA) combining ability was estimated of a set of female (as lines 6116A, 6234A and etc.) and male (the seven new inbred R lines) sunflower in hybrid combinations for seed production, oil quantity in seeds, head diameter and plant height.

RESULTS AND DISCUSSION

The species *H. maximiliani* Schrader in DAI collection is presented by 5 accessions.

Fig.1. *H. maximiliani* accession M 175

The stem of this species is from 1.5 to 2 m (Fig.1). It is smooth and slightly anthocyan colored. Leaves are alternate, simple and sessile, lanceolate, generally acuminate at both ends; mostly 14 to 30 cm long and 20 to 55 mm broad. Their margins are entire to obscurely serrate, superficially with many short hairs imparting a grayish-green color. Their heads are in a simple terminal racemose arrangement. Their ray florets are 25 to 35 mm long and bright yellow in color and the disk florets are 10 to 12 mm long. Plants flower from the second half of July till the end of August. Achenes are small, parti-coloured to light brown and crumbly. Linolic acid content in the oil is high - 79.6 % and species could be used as source of genes for developing hybrid cultivars with variable oil content. This species is resistant to the pathogens caused phomopsis, verticillium, rust, downy mildew and the parasite broomrape. It carries genes for fertility restoration of CMS - Pet-1 (Christov, 1996).



The results for crossability were presented in Table 1. The percentage of successful crosses (inseminated heads) was high, but number of seeds from one inflorescence - low. From combination *H. annuus* x *H. maximiliani* 136 seeds and only 27 F₁ hybrids were obtained:

- ✓ *H. annuus* l. HA 89A x *H. maximiliani* M 175 - 9 F₁ plants;
- ✓ *H. annuus* l. 6116A x *H. maximiliani* M 175 - 4 F₁ plants;
- ✓ *H. annuus* l. 2607A x *H. maximiliani* M 176 - 6 F₁ plants;
- ✓ *H. annuus* l. 6234A x *H. maximiliani* ND 19 - 8 F₁ plants.

From combination *H. maximiliani* x *H. annuus* only seeds were obtained.

Table 1. Crossability of cultivated sunflower *H. annuus* and wild perennial *H. maximiliani*.

Crosses	Pollinated inflorescences, n			Seed set from one inflorescence, mean		Total seeds, n	Hybrid plants	
	total	with seeds	%	seeds, n	insemination, %		number	%
<i>H.annuus</i> x <i>H.maximiliani</i>	8	8	100	18	1.32	142	27	19.9
<i>H.maximiliani</i> x <i>H.annuus</i>	12	9	75.0	11	3.98	100	0	0

It is very difficult to cross cultivated sunflower with perennial diploid species (Georgieva-Todorova, 1990; Atlagić, 1991). Although these species have the same chromosome number, it is likely that the diploid perennial species have a different genome from that of the annual diploid (Heiser and Smith, 1964). According to Christov (1990) and Hristova-Cherbadz (2007) the hybridization between cultivated sunflower and tetraploid and hexaploid wild perennial species is easier in comparison to this crossing.

The **F₁ hybrid** had annual growth habit and vegetation period similar to cultivated sunflower in contrast with wild perennials (Table2). Anthocyanin coloration was suitable morphological marker because all F₁ plants had anthocyan in contrast with cultivated sunflower. Its presence in hybrids proved transferring of genetical material from wild species. All F₁ hybrids had branched stems. The size of inflorescence of F₁ was intermediate. The number of tubular florets was near to the number of *H. maximiliani*. The tubular florets and the stigmas were colored in purple, and pollen and ray florets were orange. These characters were typical for wild species. The bracts number and coloration of F₁ were smaller of those typical for the two parents.

All F₁ plants were fertile. The mean percentage pollen viability of F₁ hybrids was low - 32.3 % (from 22.1 to 42.7 %) and thrice less of value of *H. maximiliani* (from 93.7 to 96.6 %). Reduction division of PMC in the F₁ plants occurred with deviations. Cells with 17 bivalents, cells with 15-16 bivalents and 2-4 univalents and cells with 13 bivalents and 2 quadrivalents were registered in diakinesis (8.2 %). In metaphase I there were cells with 2-3 fast chromosomes, which were situated on one side of the lamella (23.1 %). In F₁ hybrid during anaphase-I were the cells with non-included chromosomes. The number of the lagging chromosomes was to 6 (18.8 %). The cells with one chromosome bridge and one fragment were registered (14.0 %). In telophase II were observed tetrads and tetrads with to 2 lagging chromosomes (11.5 %).

According to Atlagic et al. (1995) pollen variability was low in F₁ hybrids. Meiotic abnormalities may be indicative of differences in the chromosomes between perennial and annual diploids. Meiotic analysis of the three wild species and their interspecific hybrids pointed the phylogenetic differences between “annui” and “divaricati” sections.

Table 2. Characteristics of parents and some hybrid generations.

Material	Physiological development		Morphological characteristic			Technological characteristic	
	Period of vegetation, day	Plant height, cm	Branches, n	Head diameter, cm	1000 seeds weight, g	Oil, %	
Parents							
l. HA 89	114	110	0	23	57.6	49.8	
l. 2607	118	130	0	18	61.4	43.0	
l. 6116	109	135	0	22	54.2	45.1	
l. 6234	110	110	0	23	59.3	44.9	
c. Peredovik	122	190	0	28	74.1	49.92	
<i>H. max.</i> ND 19	145	150	28	2.5	3.5	23.0	
<i>H. max.</i> M 175	160	165	25	2.5	3.4	27.5	
<i>H. max.</i> M 176	160	165	27	2.5	3.1	23.5	
<i>H. annuus</i> l. 6116A x <i>H. maximiliani</i> M 175							
F ₁	138	175	12	9.8	29.3	-	
F ₂	132	145-185	9-17	6.5-14.8	25.4-32.8	35.2	
F ₆ (№ 980)	110	130	0	21	52.2	47.1	
F ₆ (№ 1000)	126	160	6	16	48.2	49.9	
BC ₁	132	140	5	19	34.4	39.7	

As a result from self-pollination, sib-pollination of the central inflorescences:

- ✓ from 5 F₁ *H. annuus* l. HA 89A x *H. maximiliani* M 175 were obtained total 112 seeds and 97 F₂ plants,
- ✓ from 3 F₁ *H. annuus* l. 6116A x *H. maximiliani* M 175 - 35 seeds and 18 F₂ plants,
- ✓ from 3 F₁ *H. annuus* l. 2607A x *H. maximiliani* M 176 - 63 seeds and 35 F₂ plants,
- ✓ from 5 F₁ *H. annuus* l. 6234A x *H. maximiliani* ND 19 - 98 seeds and 83 F₂ plants.

The number of obtained **F₂ plants** was totally 233.

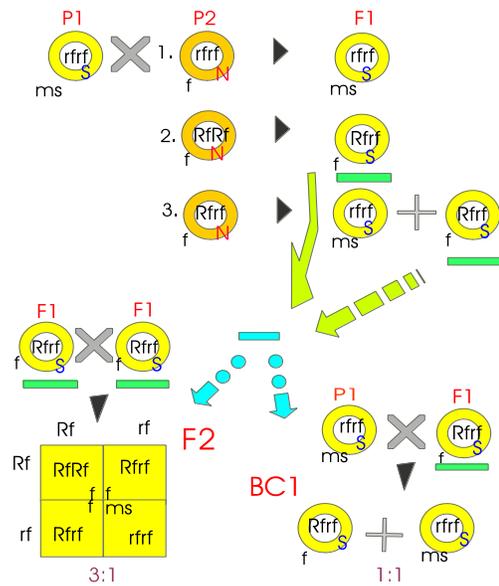
The presence of fertile plants showed that the genotype of wild species possessed *Rf* genes for CMS Pet-1 and they were transferred to F₁ hybrids. To confirm this supposition the cross HA 89A x F₁ was realised (x2 plants of line HA 89A). The total number of obtained **BC₁ plants** was 389 and for the cross:

- ✓ HA 89A x F₁ (*H. annuus* l. 6116A x *H. maximiliani* M 175) were produced 94 BC₁ plants;
- ✓ HA 89A x F₁ (*H. annuus* l. 2607A x *H. maximiliani* M 176) - 189 BC₁ plants;
- ✓ HA 89A x F₁ (*H. annuus* l. 6234A x *H. maximiliani* ND 19) - 106 BC₁ plants.

Seed color of F₂ and BC₁ plants was from grey-brown to black.

In second hybrid generation morphological difference was observed from parents that was steadily inherited and was suitable for markers. This was the coloration of the ray flowers in light lemon-yellow.

After pollination of sterile analogues of lines *H. annuus* in CMS Pet-1 with pollen from wild species, which genome did not carry *Rf* genes, all derived F₁ plants would be male sterile, as maternal line was with genotype *rfrf* (Scheme1). In this case *H. maximiliani* carried genes restorers of fertility (*Rf*) for CMS Pet-1, because all F₁ plants were fertile. Used plants from the studied accessions were homozygous dominant (with genotype *RfRf*). The presence of fertility restorer genes (*Rf* gene) for CMS Pet-1 in *H. maximiliani* was also checked after crossing HA 89A x F₁, BC₁ generation. About half of plants were fertile (196 fertile: 193 sterile plants). These results showed that *Rf* genes from wild species were transferred in *H. annuus* and the control for recovery of male fertility at CMS Pet-1 was dominant and monogenic.



All F₂ and BC₁ plants and plants from **third** generation were branched and in the next generations some unbranched plants appeared. As a result of selection were created new forms suitable for R and B lines. From *H. maximiliani* were transferred genes that controlled characteristics as: 100 % resistance to *Plasmopara helianthi*, races 700 and 731, *Phomopsis helianthi*, *Phoma macdonaldii* and *Orobanche*, *Rf* gene for CMS Pet-1, suitable type of branched for R lines.

After self-pollination, F₃ - F₁₀ (6 lines) and F₁BC₁ to F₈BC₁ (one line) generations were produced.

Line №1027 (F₈) with light lemon-yellow ray flowers was hybridized with line 2607A with normal ray flowers. It was received F₁ hybrids with yellow ray flower. This result suggested appearing of recessive gene. Skaloud and Kovacik (1978) and Secerov-Fiser and Skoric (1988) indicated a single dominant gene controlled yellow flower color with yellow dominant over orange and lemon.

Analysis of results for specific combining ability of received test hybrids showed that three from seven new inbred R lines surpassed from 0.5 to 10 % seed production in comparison with standard and the oil concentration was from 49.6 to 52.5 %.

The best three R lines (№980, №1002, №1027) were included in a sunflower breeding program for developing hybrids using heterosis effect.

Line №980 was received from crossing of *H. annuus* - line 6116 A with *H. maximiliani* M 175 with vegetation period 110 days. The stems of plants were unbranched. Plant height was 160 cm. The head diameter was 20 cm. Number of disc florets per head were 3317. Leaves were 25 cm long and 29 cm wide. Seeds obtained after self-pollination of florets of a head were 2081 (62.7 %). Seeds weight from one inflorescence was 108.6 g. The thousand seeds weight was 52.2 g. Plants lodging was not observed. This line was not distinguished with high seed oil content but with resistance to downy mildew races 700 and 731, phomopsis, phoma and broomrape. Sclerotinia, rhyzopus and botritic attacks were not observed under field conditions. Very good combining ability was reported.

Line №1002 was received from crossing of *H. annuus* - line 6234 A with *H. maximiliani* ND with vegetation period 126 days. Plants had branched stems, as the wild parent. The plant height was 160 cm. The head diameter was 16 cm. Leaves were 26 cm long and 26 cm wide. Plants' lodging was not observed. This line was distinguished with high seed oil content - 58.8 %. It was resistant to downy mildew races 700 and 731, phomopsis and broomrape. Sclerotinia, rhyzopus and botritic attacks were not observed under field conditions. Very good combining ability was reported.

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