Hybridization between cultivated sunflower *Helianthus annuus* L. and wild perennial species *Helianthus pumilus* Nuttall

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ABSTRACT

Helianthus pumilus Nuttall was included in hybridization programs with the cultivated sunflower *Helianthus annuus* L. The investigation encompassed the period 2000-2007. The results showed that the two species crossed, but the crossability rate was low. Seeds were obtained at both directions of crossing and hybrid plants only in the direct crosses. The F_1 plants had an intermediate type of heritability, but they resembled the wild species in their most important biomorphological characteristics. All plants showed an annual cycle of growth in contrast to the wild perennial species. It was established that *H. pumilus* carried *Rf* genes for CMS PET-1, genes controlling the resistance to diseases such as downy mildew and phomopsis and the parasite broomrape, and genes controlling quantitative seed oil content. As a result of self-pollination, sib-pollination of the F_1 plants and backcrossing with cultivated sunflower, F_2, BC_1 and the next hybrid progenies (F_3-F_7 and next to F_5BC_1) were obtained. Some of the obtained hybrid forms were included in a program for developing lines for heterosis breeding in sunflower.

Key words: *Helianthus annuus* – *Helianthus pumilus* – interspecific hybridization –b sunflower.

INTRODUCTION

H. pumilus Nuttall belongs to section *Ciliares*, series *Pumili* (Schilling and Heiser, 1981). Species of this section appear to be well isolated genetically from species of other sections (Seiler and Rieseberg, 1997). Habitats are dry, often rocky soils, from 1200 to 1800 m elevation in central Colorado northward through southeastern and central Wyoming (Rogers et al., 1982) and in 2005 covered 5150 km^2 (Seiler et al., 2007). *H. pumilus* (dwarf sunflower) is a perennial species with potential genes for oil improvement based on its xerophytic habitat. The higher concentrations of linoleic acid in *H. pumilus* could be a potential source of genes for increasing the concentration of this fatty acid in traditional sunflower oil. The *H. pumilus* populations had an average oil content of 254 g/kg, considerably lower than cultivated sunflower which has an average of 470 g/kg. The linoleic acid concentration approached 750 g/kg, much higher than the 540 g/kg /Seiler et al., 2007/. An antitumor drug, desacetyleupaserrin, has been identified from this species (Rogers et al., 1982*). H. pumilus* was hybridized with several species of genera *Helianthus*, but part of them were unsuccessful or the information from the results was insufficient (Heiser, 1965; Heiser et al., 1969; Krauter et al., 1991) used a modified embryo culture to cross *H. pumilus* with cultivated sunflower. Successful interspecific hybridization was carried out between the wild perennial diploid species *Helianthus pumilus* and cultivated *H. annuus* (Nikolova et al., 2004).

MATERIALS AND METHODS

The investigation encompassed the period 2000 - 2007. It included the cultivated sunflower *H. annuus* and the wild perennial species *H. pumilus*, accession GT-M-172. The cultivated sunflower was represented by two varieties, Peredovik and VNIIMK 6540, and by two lines, 2607 and 6116.

Hybridization was carried out through reciprocal crosses realized under field conditions. The sterile analogues of lines 2607 and 6116 (cytoplasmic male sterile lines in CMS PET-1) were used as a female parent of the cultivated sunflower in direct crosses. In the reciprocal crosses, the florets in the inflorescences of the wild species were emasculated manually and pollinated with pollen from a single line or with mixed pollen from varieties and lines. Hybrid plants were grown under field conditions, too. To obtain F_2 and BC_1, self-pollination, sib-pollination and back-crossing of F_1 to cultivated sunflower were made. Phenological observations of the F_1 hybrids were made during the vegetative period. Biometric parameters and description of the main morphologic characters and biologic peculiarities of the F_1 hybrids were performed. Similar investigations were carried out with the next hybrid generations as well. The seed set was calculated as a ratio between the seeds obtained (the number of inseminated disk flowers) and total number of disk flowers in the inflorescence. 1000 seed weight was calculated by

measuring two samples, each of 10, 25 or 50 seeds. Back-crossing with cultivated sunflower as a mother was used with the aim of confirming the presence of fertility restorer genes (*Rf* genes) in F_1 hybrids, transferred from *H. pumilus*. The reactions to diseases were studied, using standard methodologies (Panchenko, 1975; Acimovič, 1979; Vear and Tourvieille, 1987; Encheva and Kiryakov, 2002). Oil content of seeds was estimated. Cytological analyses were carried out on the mitosis, particularly on the chromosome number (Georgieva-Todorova, 1976). Pollen viability was determined by a standard methodology (Alexander, 1969; Atlagic, 1990).

RESULTS

The analysis of the results presented in Table 1 shows that the two diploid (2n = 34) sunflower species could be crossed. The crossability rate was low. Seed set per cultivated sunflower inflorescence after compulsory pollination with pollen from the wild species was low (0.52%) for the direct crosses and 3.57% for the reciprocal. The slightly higher level of this index in the cross *H. annuus x H. pumilus* was due to the difference in the inflorescence size of the cultivated and wild species, as well as to the number of the seeds. In comparison to the results obtained from the hybrids, the parental seed set after free pollination was 77.9% and 88.2% for lines 6116B and 2607B, respectively and varied from 3.3 to 8.8% for the wild species.

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

Crosses	Pollinated inflorescences			Total number of seeds	Seed set		Hybrid plants	
	total number	with seed number	with seed %		mean number	%	number	%
H. annuus x *H. pumilus*	6	4	66.67	28	7	0.52	5	17.86
H. pumilus x *H. annuus*	20	11	55.00	23	2	3.57	0	0

Seeds were obtained at both directions of crossing, and hybrid plants only in the direct crosses. A total of 28 seeds and 5 F_1 plants, 4 from the cross combination *H. annuus* line 2607A x *H. pumilus* and one from the cross *H. annuus* line 6116A x *H. pumilus* were obtained from the hybridization of *H. annuus* with *H. pumilus*. Hybrid plants were not grown in the reciprocal crosses *H. pumilus* x *H. annuus*, although mixed pollen from varieties (Peredovik and VNIIMK 6540) and lines (2607B and 6116B) was also used.

The number of chromosomes for F_1 plants ranged from 2n = 31 to 2n = 34. This was probably due to a difference between the genotypes of the parents. Five satellite chromosomes per cell were observed (Fig. 1). The karotype's formula of *H. pumilus* was 1 sat + 5 m + 5 sm + 7 st, according to Kulshreshtha and Gupta (1981), and that of *H. annuus* (cultivated sunflower) - 3 sat + 10 m + 3 sm + 4 st according to Raicu et al. (1976); 3 sat + 4 m + 8 sm + 5 st, according to Al-Allaf and Godward (1977) and 2 sat + 5 m + 8 sm + 4 st, according to Georgieva-Todorova and Bohorova (1980).

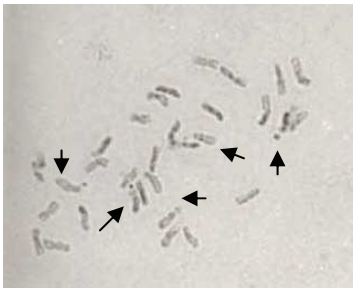
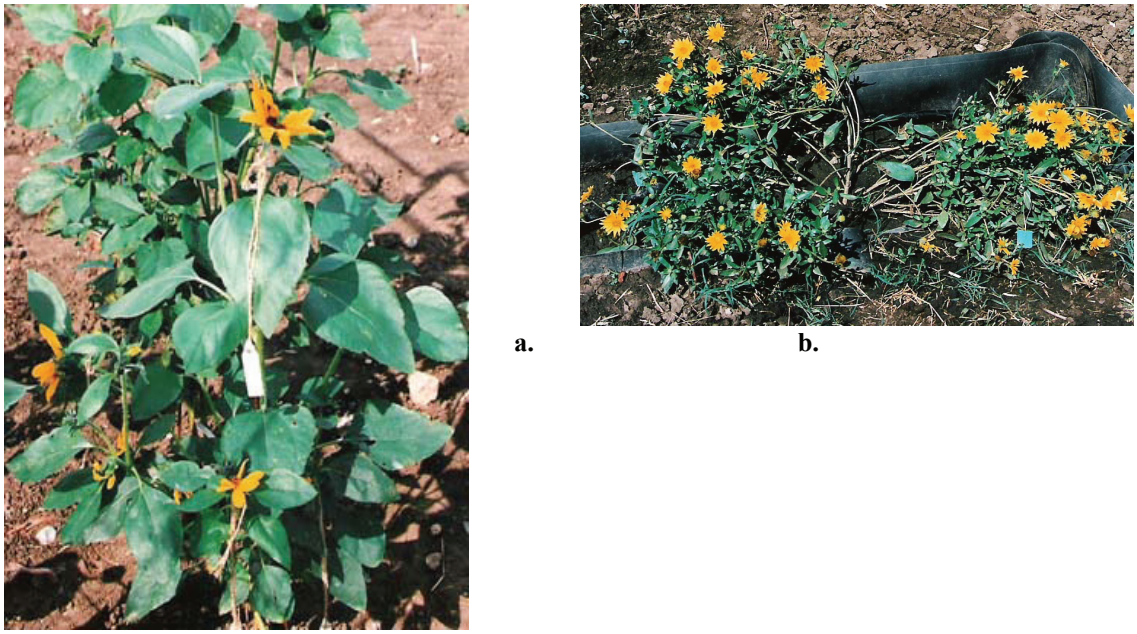


Fig. 1. Metaphase cell from F_1 plant with 5 satellite chromosomes.

The F_1 hybrid (Fig. 2a) had an annual growth habit like that of the cultivated sunflower (2 lines) in contrast to wild perennial species (Fig. 2b, Table 2).



a. **b.**

Fig. 2. Plants of: a) F_1 hybrid; b) *H. pumilus* GT M 172.

Table 2. Characterization of F_1 hybrids.

Characteristics	*H. annuus* L. 2607 A	F_1: L. 2607 A x M 172	*H. pumilus* GT M 172	F_1: L. 6116 A x M 172	*H. annuus* - L. 6116 A
Phenological characteristics					
Life cycle	annual	annual	perennial	annual	annual
Vegetation period, days	118	122 - 128	215	120	109
Morphological characteristics					
Plant height, cm	130 - 135	70 - 75	45	75	135 - 140
Number of branches	0	7 - 15	32 – 38	17	0
Leaf length, cm	24 - 27	16 - 18	4 – 15	20	21 - 27
Leaf width, cm	22 - 26	13 - 19	1 – 4	17	20 - 23
Length of leaf petiole, cm	11 - 13	2 - 3	0.7	3	14 - 17
Head diameter, cm	17 - 19	4.5 - 8	1.3	6.5	21 - 23
Technological characteristics					
1000 seed weight, g	61.4	x	2.9	x	54.2
Oil, %	43.0	x	25.1	x	45.10

The vegetation period of hybrids was similar to *H. annuus* and varied from 120 to 128 days in contrast to 215 days of *H. pumilus*.

All F_1 plants were branched along the entire stem, dark green with anthocyanin coloration and with trichomes. The stem of *H. pumilus* was low, fine, rugged, branched, green and with anthocyanin coloration at the top. The height the branches of F_1 hybrids exceeded the central stem (Table 2). This character was typical for *H. pumilus* in contrast to the cultivated sunflower, which was not branched. The presence of branches in the hybrid materials suggested dominant heritability and it was used as a

morphological marker for successful hybridization, similar to the anthocyanin coloration. They proved a transfer of genetic material from *H. pumilus* to the genotype of the F_1 plants.

The leaves of F_1 plants were green, with a glossy surface, similar to the wild species. They were distichous at the base and alternate along the other part of the stem. The lamina shape was cordate, slightly elongated, with a sharp peak. The leaf margins were serrate. The leaves presented anthocyanin coloration with time.

Heads of F_1 plants were small, like those of *H. pumilus,* with dark purple disk flowers and stigma, and orange pollen and ray flowers.

All F_1 plants were fertile. This showed that the genotype of *H. pumilus* had *Rf* genes for CMS PET-1. The mean percentage of pollen viability of F_1 hybrids was low (from 2.1 to 17.9 %), while in the wild species it varied from 75.5 to 87.2 %. After free pollination, from 1 to 11 seeds were obtained. As a result of the self-pollination of 5 central inflorescences, a total of 14 seeds were produced. After pollination of the sterile analog of line HA89 with pollen from F_1 plants, from 9 to 67 seeds were produced. The seed set after backcrossing was from 0.67 to 5.01 %.

Two fertile F_2 plants from the total of 14 seeds were obtained that differed in plant height. One of them was 85 cm high, and the other (from cross *H. annuus* line 6116A x *H. pumilus* M 172) was 112 cm high (Table 3). There were other differences in the form and size of branches, leaves, inflorescence and seeds. In F_2 plant from the cross *H. annuus* line 6116A x *H. pumilus* M 172, initially three branches occurred at the base of the stem, and another four branches developed on the main inflorescence at the beginning of flowering. These were short and situated above the middle part of stem. The lamina shape was cordate, slightly elongated, with a sharp peak and glossy surface. Seed color of F_1 was from gray-brown to anthocyanin-black.

The total number of obtained BC_1 was 198, 102 of them being fertile plants. The value of χ^2 in BC_1 generation was lower (χ^2 = 0.182) than that at level of significance of 5% (3.841). This determined an accidental nature of the differences between the observed and expected values. This result showed that *Rf* genes from *H. pumilus* were transferred in *H. annuus* and the control for recovery of male fertility at CMS Pet-1 was dominant and monogenic.

All BC_1 plants were branched with anthocyanin along the stem, branches and petiole. There were a few plants with leaves with a glossy surface. The branches were mainly in the middle and on the top part of the stem. Seeds possessed a dark brown, black and anthocyanin-black coloration.

Fig. 3. BC_1 plant.

Table 3. Characterization of plants of BC_1, F_2, and F_3 generations.

Generation	Plant height, cm	Head diameter, cm	1000 seed weight, g	Oil, %	Vegetation period, days
BC_1	95 - 130	15	36.2	44.2	126
F_2	85 and 112	12	30.3	39.9	123
F_3	145	12	31.2	45.5	125

F_3-F_7 and next to F_5BC_1 generations were produced. As a result of the selection, new forms suitable for R and B lines were developed. Genes were transferred from *H. pumilus,* which controlled the following characters: 100% resistance to downy mildew races 300 and 700, phomopsis, powdery mildew, rust and broomrape; *Rf* gene for CMS Pet-1; type of branching suitable for the R lines, high oil content in seed (from 51.25 to 59.05 %) and high combining ability.

DISCUSSION

The results obtained showed that the crossability level of the perennial diploid wild species *H. pumilus* with cultivated sunflower was low. The incompatibility was high regardless of the equal chromosome number in their genomes. This result was probably due to the more distant relationship or to some other reason, for example the higher cytoplasmic effect of the perennial species on the chromosome

conjugation, etc. Seeds were obtained from the reciprocal cross, although their percent was very low, but, however, no F_1 plants were produced. Viable plants that could reproduce were obtained only from the direct cross. The F_1 plants had an intermediate type of heritability, but they resembled the wild parent in the most important biomorphological characters. The positive result for seed set at back-crossing showed that the pollen from F_1 hybrids was viable.

Nuclear genetic material was transferred into cultivated sunflower through the direct cross. This is very important for heterosis breeding in sunflower because, besides the stability of the CMS source, the genetic potential of the nuclear material was also essential, as its material was enriched with new content in this case. According to Seiler and Gulya (2004), wild species have contributed many agronomically important traits to cultivated sunflower.

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