

Cytogenetic study of an F₁ sunflower interspecific hybrid (*Helianthus annuus* x *Helianthus praecox*)

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ABSTRACT

Annual species *H. praecox* (15 populations from the Novi Sad collection) has been crossed with cultivated sunflower (HA26). F₁ interspecies hybrids were obtained with three populations (1801, 1819 and 1824) and their morphology and cytology were studied. Plants of F₁ hybrids were most often more similar phenotypically to the wild parent or intermediate. The analysis of meiosis revealed that the crossed species differed for the chromosome level. Besides bivalents, multivalents (quadri- and hexavalents) were most often found in diakinesis. Trivalents and univalents were rarely observed. Frequently observed chromosome configurations in diakinesis were 15II 11V and 13II 21V. Fast and lagging chromosomes in metaphase I and anaphase, as well as chromosome bridges and fragments were found in a large number of meiocytes. Pollen viability in F₁ interspecies hybrids was low (8.3, 9.8 and 11.2%). The results suggested that the species *H. praecox* and *H. annuus* differ by 1-2 translocations and 1-2 inversions.

Key words: *H. praecox* – interspecies hybrid – meiosis – pollen viability.

INTRODUCTION

Helianthus praecox Engelm. and A. Gray (premature sunflower), is a species described by Heiser et al. (1969) and Rogers et al. (1982). It contains three subspecies, which are geographically isolated but all within the state of Texas (USA). The three subspecies *praecox* Engelm. and A. Gray, *runyonii* Heiser and *hirtus* Heiser, intercross easily and produce fertile hybrids. The flowering occurs from early summer to late fall. They are branched, with a large number of inflorescences on long peduncles and with rough to stiff hairs on slightly serrated leaves. They are very attractive as an ornamental species. Their natural and artificial hybrids with other annual species are well known. Haploid chromosome number is n=17.

According to the literature, *H. praecox* has shown resistance to two races of *Plasmopara halstedii*, to *Puccinia helianthi* and *Erysiphe cichoracearum* as well as tolerance to *Phomopsis helianthi*. High oleic acid content has also been reported for this species next to *H. argophyllus* and *H. annuus* (Seiler, 1992).

The hybridization potential of *H. praecox* has been tested by many authors, mainly to determine the similarities with other species of the *Helianthus* genus and to study their phylogenetic relations. Heiser et al. (1969) found that the species *H. praecox* is similar to species *H. debilis*, *H. petiolaris* and *H. neglectus*, which was confirmed by the results of Chandler et al. (1986).

The results of interspecific hybridization of *H. praecox* with cultivated sunflower has been published by Georgieva-Todorova (1990), Christov (1991), Jan (1997), Nikolova et al. (1998) and Iouras et al. (2002). A large hybridization program with the goal of transferring the desired traits from the species *H. praecox* has been done by Atlagić (1986) and Terzić (2006). A significant number of researchers obtained F₁, BC₁F₁ - BC₄F₁ hybrids between *H. praecox* and the cultivated sunflower. They gave a morphological description of the interspecies hybrids, but without a cytogenetic description of the hybrids.

The objective of this paper was to establish the possibility of *H. praecox* usage in cultivated sunflower breeding, through analyzing the cross compatibility and morpho-cytogenetic traits of F₁ interspecies hybrids.

MATERIALS AND METHODS

Fifteen populations of the species *H. praecox* (1145, 1151, 1168, 1181, 1142, 1333, 1340, 1341, 1342, 1801, 1819, 1812, 1824, 1826 and 1828) from the collection of wild sunflower species in Novi Sad were crossed in a classical fashion with cultivated sunflower lines (direct and reciprocal crosses). The F₁ seeds of the interspecific hybrids obtained were sown in the field. The plants of parental species and of F₁ interspecies hybrids were analyzed morphologically and samples for the analysis of meiosis and pollen viability were taken.

Meiosis was analyzed using acetocarmine method (Georgieva-Todorova, 1990) and the pollen viability with a coloring method (Alexander, 1969).

The results of meiosis are shown through chromosome configuration in diakinesis and the regularity of meiosis through other stages. Pollen viability is shown as a percentage of viable pollen grains in comparison to the total number.

RESULTS AND DISCUSSION

Of the 15 populations used in this study (71 inflorescences), 25 seeds were obtained on 11 inflorescences. Hybrid F₁ plants (2 to 12) were obtained in 3 hybrid combinations between the CMS line HA26 and the populations 1801, 1819 and 1824 of *H. praecox*.

The plants of F₁ interspecies hybrids were phenotypically similar to *H. praecox*. F₁ plants were higher than both parents; the disk diameter was similar to the wild parent; petiole length and leaf length were either similar to the cultivated sunflower or greater than both parents; leaf width was intermediate.

The analysis of meiosis of the *H. praecox* populations and the cultivated sunflower line HA26 showed that the meiosis proceeded normally with 17 bivalents in diakinesis and without irregularities in other stages. On the contrary, a high percentage of cells with irregularities was evident in F₁ plants. The analysis of diakinesis showed that the percentage of meiocytes with normal chromosome pairing (17II) was 12.5, 13.84 and 27.27%, while a significantly larger percentage of cells contained multivalent chromosomes (72.72, 73.84 and 87.50%). The most frequent multivalents were quadri- and hexavalents and the most frequent chromosome configuration in F₁ interspecies hybrids was 13II 2IV (27.69% of meiocytes in hybrids with *H. praecox* 1801), followed by 15II 1IV (31.81% of meiocytes in hybrids with *H. praecox* 1819 and 37.5% of meiocytes in hybrids with *H. praecox* 1824). Results of the meiotic analysis for each studied plant of the F₁ hybrids are shown summarized for each crossed population and given as a percentage (Table 1).

Table 1. Analysis of meiosis and pollen viability of F₁ interspecific hybrids (*H. annuus* x *H. praecox*)

	F ₁ PRA - 1801	F ₁ PRA - 1819	F ₁ PRA - 1824
Chromosome configurations in diakinesis	17 ^{II} (9); 13^{II}2^{IV}(18) ; 15 ^{II} 1 ^{IV} (16); 14 ^{II} 1 ^{VI} (6); 12 ^{II} 1 ^{IV} 1 ^{VI} (5); 12 ^{II} 2 ^{IV} 2 ^I (4); 13 ^{II} 1 ^{IV} 1 ^{III} 1 ^I (3); 11 ^{II} 3 ^{IV} (1); 11 ^{II} 1 ^{IV} 2 ^{III} 1 ^I (1); 12 ^{II} 3 ^{IV} (1); 8 ^{II} 3 ^{VI} (1)	17 ^{II} (6); 15^{II}1^{IV}(7) ; 13 ^{II} 2 ^{IV} (4); 14 ^{II} 1 ^{VI} (4); 12 ^{II} 1 ^{IV} 1 ^{VI} (1)	17 ^{II} (3); 15^{II}1^{IV}(9) ; 14 ^{II} 1 ^{VI} (6); 13 ^{II} 2 ^{IV} (4); 12 ^{II} 1 ^{IV} 1 ^{VI} (2)
most frequent configuration (%)	27.69	31.81	37.50
meiocytes with bivalents (%)	13.84	27.27	12.50
meiocytes with multivalents (%)	73.84	72.72	87.50
Percentage of meiocytes			
Metaphase I			
Normal	76.72	77.78	73.33
Fast chromosomes	23.27	22.22	26.67
Anaphase I			
Normal	64.81	66.67	69.44
Lagging chromosomes	20.72	20.51	22.22
Chromosome bridges and fragments	14.46	12.82	8.34
Telophase II			
Normal	48.71	52.63	57.14
Lagging chromosomes	14.72	21.05	9.52
Apolar with micronuclei	34.97	26.31	33.33
Pollen viability (%)	8.30	11.47	9.80

The results showed that the number of quadri- and hexavalents was variable (1-3), while tri- and univalents were rare in diakinesis. The following stages of meiosis also had a high percentage of irregularities (Table 1). The metaphase I was usually with 1 or 2 fast chromosomes (22.22, 23.27 and 26.67% of meiocytes). The anaphase I showed high percentage of meiocytes with lagging chromosomes (20.51, 20.72 and 22.22%), as well as meiocytes with chromosome bridges and fragments (8.34, 12.82 and 14.46%). In telophase II, meiocytes with lagging chromosomes were found (9.52, 14.72 and 21.05%), as well as apolar ones with micronuclei (26.31, 33.33 and 34.97%).

Plants of the F₁ interspecies hybrids were male fertile. Considering that a male sterile cultivated line HA26 was used in the crosses (PET-1 sterility source) it can be concluded that the *H. praecox* populations (1801, 1819 and 1824) contain fertility restoration genes. Pollen viability in the analyzed interspecific hybrids was extremely low (8.30, 9.80 and 11.17%) in comparison to the parental species (> 90%).

The meiotic irregularities throughout the stages cannot be discussed in relation to the origin of the hybrids because the results were not conclusive enough (PRA 1801 and PRA 1819 from the same subspecies *runyonii*, while PRA 1824 is ssp. *praecox*).

While reviewing the phylogenetic relations in the *Helianthi* section, most of the authors found that the annual species are close to each other so that their hybridization is possible. The results of the presented work show that a small number of hybrid combinations and plants of F₁ interspecies hybrids was obtained after a large number of crosses had been made. Even though *H. praecox* is an annual diploid species like the cultivated sunflower, a certain degree of cross incompatibility is present. The results of hybridization between *H. praecox* and the cultivated sunflower reported so far also pointed to this conclusion. Škorić et al. (1988a) used 30 populations and obtained interspecific hybrids with only 2 of them, developing 7 hybrid combinations in F₁, 10 in BC₁F₁ and 9 in BC₂F₁ generations. A large hybridization program done by Bulgarian researchers resulted in 6 hybrid combinations between the species *H. praecox* and *H. annuus* (Nikolova et al., 1998) and with a note that the hybridization success is greater if *H. annuus* is used as a mother. Jouras et al. (2002) published results of interspecific crosses between wild and cultivated sunflower (ten year trial period) that had the objective of transferring the resistance to *Sclerotinia* and *Phomopsis*, where the species *H. praecox* was successfully crossed, and, consequently, F₁BC₁ and BC₂ generations were obtained. Interspecific hybrids between *H. praecox* and *H. annuus* shown in the papers of the quoted authors were not analyzed cytogenetically. The similarity of the crossed species was determined on the basis of cross compatibility and morphological similarity.

The results of cytogenetic analysis of F₁ hybrids obtained in this work from the crosses between *H. annuus* and *H. praecox* pointed to the existence of large differences between these two annual species. Besides bivalents, quadri- (1-2) and hexavalents (1) were the most frequent in diakinesis, with only occasional appearance of trivalents (1-2) and univalents (1-2). These findings confirm the results of Chandler et al. (1986) who, while studying the relations between annual species of the genus *Helianthus*, found that the most frequent multivalents in interspecific hybrids between *H. annuus* and *H. praecox* were VI + IV, VI, 2 IV and a IV. A high percentage of meiocytes with non included chromosomes in metaphase I, anaphase I and telophase II, as well as a high percentage of meiocytes with chromosome bridges and fragments, also suggest the existence of non homologous chromosomes between the species *H. praecox* and *H. annuus*. The results of Chandler (1979) indicated a high frequency of chromosome bridges and fragments in the same interspecies hybrid (0/5, 1/12, 2/6, 3/4, 4/4), which is an indication of chromosomal structural differences of the inversion type. After summarizing the results of cytogenetic analysis, this author divided the annual species into two groups by the frequency of chromosome bridges and fragments: I (*H. annuus*, *H. debilis*, *H. neglectus*, *H. niveus* and *H. praecox*), II (*H. annuus* and *H. argophyllus*). On the basis of quadrivalent presence for the species *H. annuus*, he stated that this differs in 2-6 translocations from the other annual species. The species *H. praecox* is, comparing its morphology and chromosome behavior, closely related to *H. debilis* and *H. petiolaris*. Chromosome pairing suggested that the species is related closely to *H. neglectus* (Heiser et al., 1969). While analyzing the meiosis in *H. praecox* x *H. annuus* hybrids, Georgieva Todorova (1990) found a configuration of 15II, 1IV in only 9 out of 269 PMC (pollen mother cells). Previous findings (Chandler, 1979; Chandler et al., 1986; Georgieva-Todorova, 1990), together with the results shown in this paper, indicate that the species *H. annuus* and *H. praecox* differ in 1-2 translocations and 1-2 paracentric inversions.

The fertility restoration by *H. praecox* for PET-1 source of cytoplasmic sterility found in this work has previously been indicated by Škorić et al. (1988b), who reported that certain populations of the species *H. praecox* contain heterozygous genes for restoration of not only PET-1 but also of some other CMS sources (CMG-1, CMG-2, CMG-3, Indiana - 1). Low pollen viability in F₁ interspecific hybrids *H. annuus* x *H. praecox* is certainly influenced by a high percentage of meiotic irregularities and it has

previously been documented in the research performed by Chandler (1979) (2.1-4.2%) and Georgieva Todorova (1990) (11.1-61.80%).

Detailed cytogenetic analysis of F₁ interspecies hybrid *H. annuus* x *H. praecox* has shown that, even though it is an annual diploid species, *H. praecox* differs significantly chromosomally from *H. annuus*. The results of previous research together with the results given in this paper about low cross compatibility, dominant inheritance of some undesired traits, differences in chromosome structure that induce meiotic irregularities and lowered pollen viability are serious difficulties in the way of using *H. praecox* as a source of desired genes for the breeding of cultivated sunflower.

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