Feasibility of keeping F_1 interspecific sunflower hybrids

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ABSTRACT

- *Helianthus* genus comprises 14 annual and 37 perennial species. Perennial species reproduce vegetatively (rhizomes, tubers) so that it is relatively easy to maintain them in ex situ collections in the field. Hybrid plants between perennial species and cultivated sunflower keep the ability of vegetative reproduction in F_1 generation. Feasibility of maintaining and using F_1 interspecific hybrids was investigated in the collection of wild sunflower species in Novi Sad.
- Three to five F_1 plants per hybrid combination obtained from crosses between cultivated sunflower lines and perennial species *H.divericatus*, *H.decapetalus*, *H.hirsutus*, *H.laevigatus*, *H.strumosus*, *H.eggerttii*, *H.resinosus*, *H.rigidus*, *H.tuberosus* and *H.salicifolius* were transferred to the collection of wild sunflower species. Interspecific hybrids were obtained using conventional crossing method in the period from 1987 to 2005 and are grown in a quarantine field under the same conditions as wild perennial sunflower species. Hybrid nature and genotype-clone stability was verified by morphological observations, male fertility occurrence monitoring and analysis of meiosis and pollen viability.
- It was found that the interspecific hybrid plants kept all the phenotype traits they had in the first cultivation year. The results of cytogenetic analysis were also identical to the results of pollen viability and chromosome pairing obtained on the same genotypes immediately after crossing.
- The obtained results indicate that it is possible to maintain interspecific hybrids in F_1 generation in the field for 6-24 years after hybridization under the same growing conditions (clone maintenance) as wild perennial sunflower species.
- Having in mind the difficulties in obtaining hybrids between perennial species and cultivated sunflower it is very useful to keep the existing hybrids and use them for obtaining further crossing generations (BC_1F_1, BC_2F_1...).

Key words: sunflower, F_1 interspecific hybrids, vegetative reproduction

INTRODUCTION

Of the 49 sunflower species in the genus *Helianthus* (Schilling and Heiser, 1981), the collection in Novi Sad included 43 species. Section *Helianthus* was complete and 6 species were missing from the perennial group. Regrettably, 14 species have been lost in the previous period, 4 annuals and 10 perennials. At present, the collection comprises 21 perennial and 7 annual species (Atlagic et al., 2006). The classification of Schilling and Heiser (1981) has been through six significant modifications (Jan and Seiler, 2007) and according to those, the *Helianthus* genus contains 51 sunflower species, 14 annual and 37 perennial.

Rich genetic variability in the *Helianthus* genus was mostly used to transfer useful traits, especially disease resistance, from the wild species to the cultivated sunflower. Authors that worked on interspecific hybridization usually described the difficulties in crosses between perennial wild species and cultivated sunflower. They are caused by genetic distance and the difference in chromosome number and structure. Cross incompatibility (prezygotic and postzygotic) inspired the use of embryo culture and somatic hybridization besides conventional hybridization. The obtained interspecific hybrids in F_1 generation were often male sterile, hard to self fertilize, so that majority of authors used cross pollination or back crossing to obtain further cross generations. Minority of authors observed and described vegetative reproduction in F_1 interspecific hybrids obtained by crossing perennial species and cultivated sunflower (Georgieva-Todorova, 1990; Atlagić et al, 1995; Atlagić, 1996; Atlagić and Škorić, 1999; Sukno et al, 1999). During NS-interspecific programs in the period between 1981 and 2008, several thousand crosses were made and 7 annual and 15 perennial species were crossed with cultivated sunflower lines.

The objective of this work was to evaluate the possibility of keeping F_1 interspecific hybrids in the field and considering that they are clonally propagated, to evaluate their stability based on morphological traits, the male sterility occurrence, pollen viability and characteristics of meiosis.

MATERIALS AND METHODS

Three to five F_1 plants per hybrid combination, obtained from crosses between cultivated sunflower lines and perennial species, were transferred to the collection of wild sunflower species: *H.salicifolius* (F_1 SAL 241 F), *H.divericatus* (F_1 DIV 2085), *H.decapetalus* (F_1 DEC B), *H.hirsutus* (F_1 HIR 1536), *H.laevigatus (*F_1 LAE 1618), *H.strumosus* (F_1 STR 1623), *H.eggerttii* (F_1 EGG 1626), *H.resinosus* (F_1 RES 1545), *H.rigidus* (F_1 RIG 1693, F_1 RIG 1696, F_1 RIG 1692, F_1 RIG 707, F_1 RIG 72272) and *H.tuberosus* (F_1TUB 6, F_1TUB 1701, F_1TUB 1698, F_1TUB 1699). Interspecific hybrids were obtained using conventional crossing method in the period from 1987 to 2005 and are grown in a quarantine field under the same conditions as wild perennial sunflower species. The perennials are grown in 80x120x80cm beds which are bordered with PVC foil to prevent expansion and mixing of accessions. To simulate the natural conditions of growing, the aboveground plant parts were cat each fall and new shoots allowed to grow each spring.

Morphological observations in the field comprised following traits: plant height, head diameter, leaf size and branching type. F_1 interspecific plants were photographed in the field in flowering time.

The appearance of male sterility was evaluated by visual check of flowers in the field, as well as by microscopic anther examination (pollen presence or absence). Microphotographs were made using a stereomicroscope Stemi 2000C coupled with a Canon Power Shot G5 digital camera.

Pollen viability was determined using a modified Alexander method (Atlagić et al., 2012), while analysis of meiosis was performed using acetocarmine method (Georgieva-Todorova, 1990). Both analysis were performed on a Amplival 30-G048c Carl Zeiss Jena microscope. Microphotographs were obtained using a CCD Sony DXC151AP video camera, WinFast PVR2 video card and software. Results of analysis are shown through the most frequent chromosome configuration in diakinesis or metaphase I, and the appearance of irregularities in other phases of meiosis (anaphase, telophase).

The obtained results were than compared with results obtained by analyzing the F_1 interspecific hybrids grown in the first year after the interspecific cross was made.

RESULTS

By phenotype, F_1 interspecific hybrid plants were similar to wild perennial species, but with larger leaves, larger head diameter and branched (Tab. 1).

Male sterility was frequent. Anthers of 4 interspecific hybrids completely lacked pollen production (F_1 DIV 2085, F_1 HIR 1536, F1 LAE 1618, F_1 RIG 1696) (Tab.1). Deformed and sterile pollen was found in anthers of two hybrid combinations (F_1 DEC B, F_1 EGG 1626). Deformed and sterile pollen with only

several viable pollen grains was found in anthers of one hybrid combination (F_1TUB 1701). Plants of 5 interspecific hybrids were male fertile and pollen viability ranged from 28.27% (F_1 RES 1545) to 89.96% (F_1 SAL 241 F) (Tab.1.).

Table 1. Plant phenotype, male sterility and pollen viability percentage in F_1 interspecific hybrids maintained in the collection

F_1 interspecific hybrids in 2011	Presence of pollen in anthers	Pollen viability (%)	F_1 interspecific hybrids in 2011	Presence of pollen in anthers	Pollen viability (%)
F_1 SAL 241 F Fig 1	Fig 2	89.96	F_1 EGG 1626 Fig 13	Fig 14	0
F_1 DIV 2085 Fig 3	Fig 4	-	F_1 RES 1545 Fig 15	Fig 16	28.27
F_1 DEC B Fig 5	Fig 6	0	F_1 RIG 1696 Fig 17	Fig 18	-
F_1 HIR 1536 Fig 7	Fig 8	-	F_1 RIG 1692 Fig 19	Fig 20	68.00
F_1 LAE 1618 Fig 9	Fig 10	-	F_1 TUB 1701 Fig 21	Fig 22	<1
F_1 STR 1623 Fig 11	Fig 12	54.23	F_1 TUB 1698 Fig 23	Fig 24	58.33

Table 2. Characteristics of meiosis in F_1 interspecific hybrids kept in the Novi Sad collection

F_1 hybrids	Most frequent configurations	Chro. number	Irregularities	Typical phases of meiosis	References
F_1 SAL241 F	$15^{II}1^{IV}$	34	Chromosome bridge (1-2)	Fig 1	Atlagić et al., 1995
F_1 DIV 2085	$23^{II}1^{IV}1^{I}$; $21^{II}2^{IV}1^{I}$; $17^{II}17^{I}$	51	Fast (1-6) Lagging (2-4) Chromosome bridge (1)	Fig 2	Terzić, 2006
F_1 DEC B	$19^{II}13^{I}$	51	Fast (4-12)	Fig 3	Atlagić, 1991
F_1 HIR 1536	$20^{II}2^{IV}3^{I}$	51	Fast (0-3) Lagging (0-2)	Fig 4	Atlagić, 1991
F_1 LAE 1618	$32^{II}1^{IV}$; $28^{II}2^{IV}4^{I}$; 34^{II}	68	Fast (0-4) Chromosome bridge (2) in anaphase I	Fig 5	Atlagić and Škorić, 1999; Terzić, 2006
F_1 STR 1623	$32^{II}1^{IV}$; $28^{II}2^{IV}4^{I}$; 34^{II}	68	Fast (0-4) Lagging (0-2) Chromosome bridge and fragment in telophase II	Fig 6	Atlagić, 1991; Terzić, 2006
F_1 EGG 1626	$32^{II}1^{IV}$; $30^{II}2^{IV}$; $28^{II}3^{IV}$	68	Chromosome bridge (1) in anaphase I	Fig 7	Atlagić, 1996
F_1 RES 1545	$32^{II}1^{IV}$; $32^{II}4^{I}$, $30^{II}2^{IV}$	68	Fast (0-2) Lagging (0-2) Chromosome bridge in anaphase I	Fig 8	Atlagić, 1996
F_1 RIG 1693	$20^{II}1^{IV}7^{I}$	51	Fast (2-6) Lagging (2-4)	Fig 9	Atlagić, 1996
F_1 RIG 1696, 1692, 707, 72272	$32^{II}1^{IV}$; $30^{II}2^{IV}$; $28^{II}1^{IV}8^{I}$	68	Fast (0-6) Chromosome bridge (1)	Fig 10	Atlagić, 1996
F_1 TUB 6, 1701, 1698, 1699	$32^{II}1^{IV}$; 34^{II}	68	Fast (0-2) Lagging (0-2) Chrom. bridge and fragment in telophase II	Fig 11	Atlagić et al., 1993; Terzić, 2006

Results of analysis of meiosis are shown through the most frequent configurations in diakinesis, chromosome number and irregularities in other phases of meiosis (metaphase I, anaphase I and telophase II). Typical phases of meiosis are shown in pictures for certain F_1 interspecific hybrids. The table also contains references to papers which describe the results of previous cytogenetic research on the same interspecific hybrids (Tab.1).

F_1 interspecific hybrids with a diploid species *H.salicifolius* (F_1 SAL 241F) contained one quadrivalent in diakinesis in most of the analyzed meiocytes. The most frequent configuration was $15^{II}1^{IV}$. Other phases of meiosis contained fast and lagging chromosomes and chromosome bridges (Tab.2).

Diakinesis of F_1 interspecies hybrid F_1 DIV 2085 was frequently found with configuration $23^{II}1^{IV}1^{I}$. A typical configuration was $17^{II}17^{I}$ which implies the triploid character of the hybrid (2n=51). Large number of univalents, namely fast chromosomes was found in metaphase I (1-6), as well as lagging in anaphase I (2-4) (Tab.2).

Analyzed plants from the crosses between tetraploid species *H.decapetalus* and *H.hirsutus* with cultivated sunflower were triploid (2n=51). The most frequent configuration in F_1 DEC B was $19^{II}13^{I}$, and in F_1 HIR 1536 $20^{II}2^{IV}3^{I}$. Other frequent irregularities were fast chromosomes in metaphase I (Tab.2).

Normal chromosome pairing (34^{II}) was frequently observed in diakinesis of F_1 interspecific hybrid plants obtained after crossing hexaploid species (2n=102) and cultivated sunflower (2n=34), (F_1 LAE 1618, F_1 STR 1623, F_1 TUB 6) (Tab.2). The most frequent configuration in diakinesis was $32^{II}1^{IV}$ (F_1 LAE 1618, F_1 STR 1623, F_1 EGG 1626, F_1 RES 1545, F_1 RIG 1696, F_1 RIG 1692, F_1 RIG 707, F_1 RIG 7227, F_1 TUB 6, F_1 TUB 1701, F_1 TUB 1698, F_1 TUB 1699). All interspecific hybrids were tetraploid (2n=68) except for one cross with accession RIG 1693 where F_1 hybrid was triploid (2n=51), and the configuration in diakinesis was $20^{II}1^{IV}7^{I}$. Large number of univalents (4-8) was found in some crosses (F_1 RIG 1696, F_1 RIG 1693, F_1 LAE 1618, F_1 STR 1623) (Tab.2). Other phases of meiosis in F_1 interspecific hybrids usually contained irregularities like fast chromosomes in metaphase I, lagging chromosomes in anaphase I and chromosome bridges in anaphase I and telophase II (Tab.2).

DISCUSSION

Detailed measurements were not made for phenotype traits of F_1 interspecific hybrids maintained in the collection. The phenotype was observed, noted descriptively and plants were photographed. Comparison of photographs from the first year of maintenance with the photographs from 2011 did not reveal any significant differences in plant phenotype.

The appearance of male sterility was analyzed in more detail than in the previous research on interspecific hybrids. Besides visual evaluation, anthers were analyzed using a microscope, which enabled distinction of different forms of sterility: complete pollen absence, poor or plentiful production of pollen which is deformed and sterile combined with only occasional viable and fertile pollen grain among the majority of deformed and sterile ones.

Male sterility can be a result of using CMS (Cytoplasmatic male sterility) line as a mother in crosses (sterility was introduced and fertility was not restored, because the wild parent lacked the Rf (fertility restoration) genes) or the usage of wild species as the mother (potential source of CMS, where fertility was not restored in F_1 generation because Rf genes were absent in the father line). Similar findings were described in previous research (Atlagić, 1991; Atlagić et al., 1993; Terzić, 2006). It should be emphasized that male sterility was also found in hybrid combinations where it has not been in previous research, like in F_1 EGG 1626 (Atlagić, 1996). There is a possibility that vegetative reproduction in the long period of maintenance in the field may have influenced the F_1 interspecific hybrid so that male sterility developed (sterile pollen grains were found in anthers). However, male sterility was found in a certain percentage of plants per hybrid combination in the first year of cultivation (Atlagić, 1991; Terzić, 2006) while it was not noted which plant was transferred to the collection for long term maintenance.

Besides already described causes, male sterility can also arise because of interspecific hybridization it self. Crossing phylogenetically distant species often with different chromosome number and structure, as a consequence has the development of sterility (inability to produce seeds), not only in sunflower but also in other plant species. Male sterility is more frequently described only because it easier to observe it in sunflower.

Pollen viability was similar to previously published data for the same F_1 interspecific hybrids analyzed in the first year of cultivation.

Analysis of meiosis for the interspecific hybrids grown for several years in the collection confirmed the hybrid nature of F_1 hybrids (adequate chromosome number in relation to the crossed species). The most frequent configurations in diakinesis and irregularities in other phases of meiosis confirmed the previously published results (references in Tab. 2).

Analysis of meiosis in some of the interspecific hybrids maintained for long time (F_1 STR 1623, F_1 STR 1627, F_1 TUB 1698) revealed the presence of aneuploid meiocytes (2n=44, 46, 56, 60, 64), which was not found in the previous research. Besides the configurations given in table 2 as the most frequent, others were also found. Those configurations may also be important because in this research a lower number of meiocytes was analyzed in comparison to the extent of cytogenetic research made on obtained interspecific hybrids in the earlier period.

As a difficulty in the application of interspecific hybridization, besides complete sterility, lowered pollen viability is also frequent. It is difficult to explain the direct influence of irregularities in meiosis on sterility and lowered fertility. Most of the authors consider that there is dependence, but it usually is not direct (Chandler et al., 1986; Georgieva-Todorova, 1990; Atlagić, 1991). Analyses of meiosis and pollen viability in interspecific hybrids are the most important cytogenetic observations according to many authors (Chandler et al., 1986; Georgieva-Todorova, 1990; Jan, 1997; Jan and Seiler, 2007). Besides the cross possibility, male sterility and lowered fertility, they show the possibility for the usage of the wild species and interspecific hybrids in sunflower breeding.

The obtained results on phenotype, male sterility, pollen viability and characteristics of meiosis imply that there was no significant change and no spontaneous mixing of genotypes – clones of F_1 interspecific hybrids. The literature data on interspecific hybridization and its role in cultivated sunflower breeding, as well as the results presented in this paper, show that it is possible to maintain and use the obtained F_1 interspecific hybrids between perennial species and cultivated sunflower in long term (6-24 years).

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