



INTERNATIONAL
SUNFLOWER ASSOCIATION
ISA

Proceedings

18th International Sunflower Conference

MAR DEL PLATA & BALCARCE - ARGENTINA

February 27 - March 1 / 2012


ASAGIR
ASOCIACION ARGENTINA DE GIRASOL

ISA Board

Carlos FEOLI, Argentina - President
André POUZET, France - CEO
Nikolai BOCHKARYOV, Russia
Maria JOITA-PACUREANU, Romania
Johan POTGIETER, South Africa
Gerald SEILER, USA
Dragan SKORIC, Serbia
Gian Paolo VANNOZZI, Italy
Felicity VEAR, France
Leonardo VELASCO, Spain
Yalcin KAYA, Turkey

ASAGIR Board

PRESIDENT

Ricardo Negri, Capelle Hnos

VICE PRESIDENT

Orlando Vellaz, Advanta Semillas

SECOND VICE PRESIDENT

Guillermo Pozzi

SECRETARY

Juan Carlos Arana, OMHSA

TREASURE

Ramiro Costa, Bolsa de Cereales

PRO SECRETARY

Carlos Sosa, Pannar SA

PRO TREASURE

Fernando Cozzi, Cargill SA

Norma Huguet

Luis Arias, Cazenave y Asociados

Alicia Rupel, Bolsa de Cereales de Bahía Blanca

José María Dodds, Nidera Semillas

Marcelo Morini, ArgenSun

Armando Casalins, Federación de Acopiadores

Antonio Hall

Raúl Tomás, SRA

Santiago Sanchez, AGD

Horacio Urpi

Hernán Busch, Banco de Galicia

Jorge Harguindeguy, AACREA

Local Organizing Committee

Antonio Hall (President) *

Carlos Feoli (ISA President) *

Ezequiel Fonseca *

Archibaldo Salvador *

José Dodds *

Pablo Paoloni

Norma Huguet

Florencia Bedacarratz

Ramiro Costa

Carlos Sosa

Facundo Quiroz

Javier Mallo

Daniel Alvarez

Daniel Funaro

Mariano Martín Sposaro

Jorge Moutous

Marcelo Morini

Arnaldo Vázquez

Marisa Della Maddalena

Maria Carolina Alegre

* Executive Committee

International Steering Committee

Dr. Antonio Hall (Argentina)

Dr. Ruth Heinz (Argentina)

Dr. Luis Aguirrezabal (Argentina)

Dr. Loren Rieseberg (Canada)

Dr. Leonardo Velasco (Spain)

Dr. Felicity Vear (France)

Program Committee

Dr. Luis Aguirrezábal (co-chair)

Dr. Miguel Cantamutto (co-chair)

Dr. Antonio Hall

Dr. Ruth Heinz

Dr. Carlos Sala

Dr. Abelardo De La Vega

Ms. Amelia Romano

Dr. Mónica Poverene

Mr. Guillermo Pozzi

Dr. Pablo Calviño

Dr. Alberto Escande

Dr. María Eugenia Bazzalo

Dr. Andrés Zambelli

Dr. Sergio Alemano

Foreword

The International Sunflower Association (ISA) and the Argentine Sunflower Association (ASAGIR) are pleased to present this guide to the 18th International Sunflower Conference.

At the time the main objectives for the meeting were defined, organizers aimed to provide a forum for the international sunflower research community with interest in any aspect of science and technology relating to the crop (in its oil-seed and confectionery variants) that would allow all involved to:

- Update knowledge in all fields of sunflower research since the previous conference held at Córdoba, Spain, June 2008;
- Review recent technological advances in sunflower production and identify knowledge gaps that require attention;
- Analyze the status and expectations for current and prospective demands for sunflower products;
- Provide a venue for workshops and special-interest meetings focusing on unresolved research, market, and production issues;
- Provide new generations with an opportunity to interact with global leaders in sunflower research.

The local Program Committee, with the help of the International Steering Committee, has developed a program covering the whole spectrum of relevant topics from genes and genomics through to field agronomy, crop protection, and industry and market issues. The program comprises 14 plenary and 13 invited presentations, 14 short oral presentations, an exhibition of 160 posters that can be visited during each of the first three days of the meeting. In addition, there will be three associated workshops (Bird Damage, Breeding, International Sunflower Genome Initiative), a special-interest presentation of the Global Crop Diversity Trust, and facilities will be available on request for small groups who wish to discuss business or scientific topics.

On the last day of the meeting, the Conference Field Day will be held at the joint INTA-Universidad de Mar del Plata facility in Balcarce. This time the traditional Conference demonstration plots of hybrids from International Sunflower Association member countries and from the host country will be complemented by a broad range of demonstrations of production and management techniques, as well as demonstrations of research techniques in current use by Argentine sunflower research teams.

This Conference has been made possible by the work of many people, by the support of sponsors from both the public and the private sector (sponsors are recognized on the back covers of this guide) and last, but certainly by no means least, those responsible for the lectures, short oral presentations, posters, associated workshops and special interest meetings, and field and laboratory demonstrations that make up the rich and varied bill of fare for this Conference, as reflected in this guide. The Organizing Committee extends their heartfelt thanks to all these individuals and organizations.

ISA and ASAGIR trust that this guide will enable all attendees to have an interesting and fruitful 18th International Sunflower Conference.

Welcome

It has been 27 years since the 11th International Sunflower Conference was held in Mar del Plata, Argentina, March 10-13, 1985. Since then, very many things have changed in the world of sunflower science, technology, and crop production and management. As the global sunflower community reconvenes once again in the same city, its members will have the opportunity to review progress in the last four years, which has been substantial in many areas.

Mar del Plata, a vibrant city located by the sea, with a fishing port, good restaurants, an unusually good choice of golf courses, and kilometers of sandy beaches, together with Balcarce, provide excellent venues for the Conference lectures and Field Day, and will allow attendees to appreciate a unique combination of seas, hills and Pampas. It is a great pleasure for the Organizing Committee to be able to host attendees to this meeting, which we hope will be both enjoyable and fruitful.

Welcome to Argentina, to Mar del Plata and Balcarce, and to the 18th International Sunflower Conference.

Feasibility of keeping F₁ interspecific sunflower hybrids

Jovanka Atlagić, Sreten Terzić, Radovan Marinković, Siniša Jocić, Vladimir Miklič

Institute of field and vegetable crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia
jovanka.atlagic@ifvcns.ns.ac.rs

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 F₁ generation. Feasibility of maintaining and using F₁ interspecific hybrids was investigated in the collection of wild sunflower species in Novi Sad.
- Three to five F₁ 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.eggertii*, *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 F₁ 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 (BC₁F₁, BC₂F₁...).

Key words: sunflower, F₁ 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 (Atlagić 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 F₁ 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 F₁ 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 F₁ 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 F₁ 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* (F₁ SAL 241 F), *H. divaricatus* (F₁ DIV 2085), *H. decapetalus* (F₁ DEC B), *H. hirsutus* (F₁ HIR 1536), *H. laevigatus* (F₁ LAE 1618), *H. strumosus* (F₁ STR 1623), *H. eggertii* (F₁ EGG 1626), *H. resinosus* (F₁ RES 1545), *H. rigidus* (F₁ RIG 1693, F₁ RIG 1696, F₁ RIG 1692, F₁ RIG 707, F₁ RIG 72272) and *H. tuberosus* (F₁TUB 6, F₁TUB 1701, F₁TUB 1698, F₁TUB 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 cut 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. F₁ 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 F₁ interspecific hybrids grown in the first year after the interspecific cross was made.

RESULTS

By phenotype, F₁ 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 (F₁ DIV 2085, F₁ HIR 1536, F₁ LAE 1618, F₁ RIG 1696) (Tab.1). Deformed and sterile pollen was found in anthers of two hybrid combinations (F₁ DEC B, F₁ EGG 1626). Deformed and sterile pollen with only

several viable pollen grains was found in anthers of one hybrid combination (F₁TUB 1701). Plants of 5 interspecific hybrids were male fertile and pollen viability ranged from 28.27% (F₁ RES 1545) to 89.96% (F₁ SAL 241 F) (Tab.1.).

Table 1. Plant phenotype, male sterility and pollen viability percentage in F₁ 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 2	89.96	F ₁ EGG 1626	Fig 14	0
Fig 1			Fig 13		
F ₁ DIV 2085	Fig 4	-	F ₁ RES 1545	Fig 16	28.27
Fig 3			Fig 15		
F ₁ DEC B	Fig 6	0	F ₁ RIG 1696	Fig 18	-
Fig 5			Fig 17		
F ₁ HIR 1536	Fig 8	-	F ₁ RIG 1692	Fig 20	68.00
Fig 7			Fig 19		
F ₁ LAE 1618	Fig 10	-	F ₁ TUB 1701	Fig 22	<1
Fig 9			Fig 21		
F ₁ STR 1623	Fig 12	54.23	F ₁ TUB 1698	Fig 24	58.33
Fig 11			Fig 23		

Table 2. Characteristics of meiosis in F₁ 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 F₁ interspecific hybrids. The table also contains references to papers which describe the results of previous cytogenetic research on the same interspecific hybrids (Tab.1).

F₁ interspecific hybrids with a diploid species *H.salicifolius* (F₁ 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 F₁ interspecies hybrid F₁ 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 F₁ DEC B was 19^{II}13^I, and in F₁ 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 itself. 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 is 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 F₁ 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 F₁ interspecific hybrids between perennial species and cultivated sunflower in long term (6-24 years).

REFERENCE

- Atlagić J. 1991. Meiotic features and fertility in F₁ plants of interspecific sunflower hybrids. PhD thesis. Faculty of agriculture. University of Novi Sad, Serbia.
- Atlagić, J. 1996. Cytogenetic studies in hexaploid *Helianthus* species and their F₁ hybrids with cultivated sunflower, *H. annuus*. *Plant Breeding*. 115:257-260.
- Atlagić, J., and D. Škorić. 1999. Cytogenetic study of *Helianthus laevigatus* and its F₁ and BC₁F₁ hybrids with the cultivated sunflower, *H. annuus*. *Plant Breeding*. 118:555-559.
- Atlagić, J., B. Dozet, and D. Škorić. 1995. Meiosis and pollen grain viability in *Helianthus mollis*, *Helianthus salicifolius*, *Helianthus maximiliani* and their F₁ hybrids with cultivated sunflower. *Euphytica*. 81:259-263.
- Atlagić, J., S. Terzić, D. Škorić, R. Marinković, Lj. Vasiljević, D. Panković-Saftić. 2006. The wild sunflower collection in Novi Sad. *Helia*. 29(44):55-64.
- Atlagić, J., S. Terzić and A. Marjanović-Jeromela. 2012. Staining and fluorescent microscopy methods for pollen viability determination in sunflower and other plant species. *Industrial Crops & Products*. 35:88-91.
- Chandler, J.M., C.C. Jan and H. Beard. 1986. Chromosomal differentiation among the annual *Helianthus* sp. *Systematic Botany*. 11(1):354-371.
- Georgieva-Todorova, J. 1990. Genetic and Cytogenetic Investigation of the Genus *Helianthus* L. (in Bulgarian). Bulgarian Academy of Sciences, Sofia.
- Jan, C.C. 1997. Cytology and Interspecific Hybridization. p.497-558. In: AA. Schneiter (ed.) *Sunflower Technology and Production*. Agronomy Monograph 35. ASA, CSSA and SSSA, Medison, WI, USA.
- Jan, C.C. and G.J. Seiler. 2007. Sunflower. p.103-165. In: Singh R.J. (Eds). *Genetic Resources, Chromosome Engineering, and Crop Improvement*. Oilseed Crops. CRC Press, Boca Raton.
- Schilling, E.E. and Ch.B. Heiser. 1981. Infrageneric classification of *Helianthus* (*Compositae*). *Taxon*. 30:393-403.
- Sukno, S., J. Ruso, C.C. Jan, J.M. Melero-Vara, and J.M. Fernandez-Martinez. 1999. Interspecific hybridization between sunflower and wild perennial *Helianthus* species via embryo rescue. *Euphytica*. 106:69-78.
- Terzić, S. 2006. The possibilities of the use of wild species of the genus *Helianthus* in sunflower breeding. M. Sc. Thesis. Faculty of agriculture. University of Novi Sad, Serbia.