RESULTS OBTAINED AND FUTURE DIRECTIONS OF WILD SPECIES USE IN SUNFLOWER BREEDING

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ABSTRACT

Sunflower, of all field crops, has the largest capacity to use wild species in sunflower breeding program. This is owing to the genus *Helianthus* which contains 49 species. Contemporary, all important sunflower breeding centres in the world use wild species in their breeding programs.

The investigations realized previous years has significantly contributed to the collection of wild sunflower species, which can be used by breeders. The investigation of wild species which has been conducted in recent years, has improved our knowledge on desirable genes and their distribution in and among single species.

Significant results have been achieved in new germplasms production on the basis of interspecific hybridization. Several sources of resistance to new races of *Puccinia halianthi, Plasmopara helianthi* and wild sunflower species have been studied. The discovered genes for resistance to these pathogens were built into the genotypes of cultivated sunflower. Individual populations of *H.tuberosus* have proved to be resistant to *Phomopsis/Diaporthe helianthi,* while one of *H. maximiliani* populations have a high degree of resistance to *Sclerotinia sclerotiorum*. It has been found that some of wild species are resistant to other pathogens, as well as to *Orobanche cumana*.

In recent years, several new sources of CMS and Rf genes have been discovered at interspecific hybridization. Significant results have been achieved in determination of genetic and other differences between different CMS sources at molecular level. Recently, the highest improvement has been achieved in application of modern biotechnological techniques in studying wild species and interspecific sunflower hybrids. The establishing of differences at the level of cp DNA and rDNA has been widely used in wild species and interspecific hybrids. The application of Agrobacterium system for gene transformation from wild to cultivated sunflower has also been found. The manipulations at protoplast level have been realized as well. It can be supposed that the results achieved will set up a new phase in sunflower breeding programs, and especially in desirable genes transfer from wild species into the genotypes of cultivated sunflower.

The further investigation of wild species and their use in breeding programs should be organized by international cooperative projects.

INTRODUCTION

The domesticated sunflower has narrow genetic variability, especially regarding important agronomic characters. High-oil varietal populations and hybrids are distinguished for the narrowness of their genetic variability. The situation is similar with local populations only, in addition, they have inferior agronomic characters.

The large variety of wild *Helianthus* species and pronounced variability within the wild species offer opportunities of increasing genetic variability of the domesticated sunflower by interspecific hybridization. The validity of this assumption is confirmed by the fact that there exists a large number of natural interspecific hybrids between wild sunflowers.

The genus *Helianthus* contains 49 species and 19 subspecies, with 12 annual and 37 perennial species (Schilling and Heiser, 1981). All species are native to the North America and are adapted to a wide diversity of habitats and possess considerable variability for most economic and agronomic characteristics, insect and disease resistance, and seed quality factors (Rogers et al., 1982). Because of these, the wild species of sunflower offer a broad genetic base and considerable genetic variability for improvement of the cultivated sunflower. Genetic variability in the cultivated sunflower may be increased by crossing it with the numerous wild *Helianthus* species (Seiler, 1988).

In the last or two decades the interest in wild species of the genus *Helianthus* has risen considerably with regard to the increased requirements for the cultivated sunflower. A number of scientists focused their attention on studies of the interspecific relations of *H.annuus* with representatives of various sections of the genus *Helianthus*. Thorough investigations were made also of the species cytogenetics and interspecific hybrids (Georgieva-Todorova, 1990).

The use of wild species in sunflower breeding programs has already produced significant results. The greatest impact the wild species has made on cultivated sunflower, has been in the area of genes for disease resistance and, to a lesser extent, insects. The single most important breakthrough has been the discovery of CMS via interspecific hybridization and Rf genes, which allowed practical use of heterosis and development of hybrids.

Molecular genetics proposes new methods of manipulation for introducing genes of the wild species determining important economic characteristics and creating recombinant DNA into the genome of the cultivated sunflower. According to some authors, gene engineering as a method in sunflower breeding programs is not an utopia (Georgieva-Todorova, 1990). This has been proved by the results achieved recently.

Bohorova et al., (1991), studied regenerative ability of tissue and protoplast cultures from four explants - cotyledons, hypocotyls, leaves and buds (nodule portion) - from ten cultivated and diploid annual and perennial wild species.

Alibert et al., (1991) developed a strategy of plant improvement including somatic hybridization with wild sunflowers and direct gene transfer in protoplasts. Somatic hybrid calli were produced by electrofusion between protoplasts isolated from

H. annuus and *H. petiolaris, H. debilis and H. rigidus*.

Problems related with evolution, interspecific relations and cytogenetics of the species and hybrids in the genus *Helianthus* have certain specificity. The difficulties of solving these problems were of a complicated type. Now, by applying contemporary cytogenetic and molecular biochemical methods, these difficulties could probably be better explained and solved. Along with that, the genetic potential of the wild species should be studied, in vitro techniques elaborated and gene engineering applied. These would contribute greatly to conventional methods aimed at improving the genetical structure of cultivated sunflower (Georgieva-Todorova, 1990).

The genus *Helianthus*, besides constituting the basic genetic stock from which cultivated sunflower originated, continues to contribute specific characteristics for cultivated sunflower improvements: and there still remains much potential to be exploited. The continued need for additional genes to improve cultivated sunflower emphasizes the necessity to collect, maintain, evaluate, and enhance wild *Helianthus* germplasm for future utilization for cultivated sunflower (Seiler, 1988).

Significant contribution to the investigation of wild sunflower species has been done by Jovanka Atlagić (1991), who found that some of the studied perennial wild species exists in forms which have not been recorded in literature so far (*H.smithii* - hexaploid, *H.strumosus* - diploid, *H. laevigatus* - hexaploid).

Genetic diversity of the genus *Helianthus* is reflected by habitat diversity among the species. Also, the diversity habitats occupied by species of wild sunflower are reflections of the genetic variability present in the various populations within the species (Seiler, 1988).

Knowledge of a particular habitat and adaptations of the species occurring there can often help to identify potential sources of genes for a desired trait.

Since Seiler (1988) gave excellent review of wild species and their application in breeding programs, the objective of this paper was to present the results achieved during previous 3-4 years. Special attention will be given to germplasm exploration and collection; interspecific hybridization; biotechnological methods application; cytoplasmic male sterility; diseases; insects, and other traits. The review of further explorations, maintenance, evaluation of morphological, physiological, biochemical characters and taxonomic aspects of wild *Helianthus* species, as well as their use in breeding programs.

Germplasm exploration and collection

Explorations to locate and collect wild sunflower species represent one of the most difficult and challenging phases in the process of conserving genetic diversity in the genus *Helianthus* (Seiler, 1988). According to this author, over 2000 accession numbers are included to the entire collection until 1988, out of which 1000 annual and 500 perennial accessions are in the active collection.

Since the genetic erosion of wild species, caused by man has occurred in the nature, it is necessary to speed up the explorations with the aim to collect and maintain the existing

wild sunflower populations.

In 1989, the sunflower exploration was organized in five states of the Great Lakes Region of the United States from September 13 - 26. Eighty-four populations of wild sunflower species were collected from the Great Lakes Region of the United States. Twelve different species are represented in the populations. Helianthus tuberosus was represented by 13 accessions and *H. giganteus* by 10 accessions, significantly increased the number of accessions of *H.giganteus* in the collection. Additional accessions of *H.divericatus, H. decapetalus, H. mollis, and H. hirsutus* were also collected (Seiler, et al., 1990).

A successful sunflower exploration was conducted from September 3 to 18, 1991 in 7 states of the United States (ND, MT, WY, SD, CD, KS and NE). The team members were Dr. Gerald Seiler, Dr. Cynthia Stauffer and Dr. Surendra Duhoon, the scientists from USDA, and Dr. Radovan Marinković, whose mission was supported by IBPGR/ECP/GR. During this successful exploration, 140 accessions were collected. Wild *H.annuus* which was represented by 80 accessions dominated the collected accessions. *H.petiolaris* ssp. *petiolaris* (26) occupied the second position, and *H.maximiliani* occupied the third position with 15 accessions. Three accessions are collected from *H.grosseserratus, H. nuttallii spp. rydbergii and H. nuttallii ssp. nuttallii,* and one accession from *H.tuberosus and H.pumilis.*

It is important to mention that wild species were collected in other locations as well. Marinković and Dozet (1990) collected numerous populations of *H.tuberosus* in Monte Negro (Yugoslavia). Mexican investigators have started to collect wild sunflower species, as well. In 1988 and 1990, Gomez-Sanchez and Gonzalez explored 35 populations from the species *H. annuus, H. petiolaris, H.niveus and H.similis* in North Mexico.

Wild species collected in previous period enriched the collection of wild sunflower species. However, wild sunflower species collecting should be continued in the future, as well.

Interspecific hybridization

Numerous breeding canters use wild sunflower species in their breeding programs. In recent 3-4 years, a large number of interspecific hybrids between wild species and cultivated genotypes were developed in many research canters. Furthermore, backcrossings with cultivated genotypes were made in all canters and inbred lines based on interspecific hybrids were developed in most canters. These inbred lines may be used for different breeding purposes.

Škorić et al. (1988) reported that they bred interspecific hybrids between cultivated sunflower and 16 perennial and annual species.

The contribution of Seiler in enrichment of cultivated sunflower germplasm creating populations on the basis of interspecific hybrids should be mentioned at this occasion. Seiler (1991) registered HIR 1734-1, HIR 1734-2, HIR 1734-3, RES - 834 -1, RES-834-2 and RES-834-3. He also registered 13 interspecific sunflower germplasm lines tolerant to downy mildew: PRA-RUN-417-1, PRA-RUN-417-2, PRA-RUN-417-3, PRA-RUN-1329, PAR-1673-1, PAR-1673-2, DES-1474-1, DES-1474-2, DES-1474-3, ARG-1575-

1, ARG-1575-2, ARG-1575-3 and ARG-1575-4. The same year, Seiler registered 15 interspecific sunflower germplasm lines in which, relatively unexploited wild *H. anomolus, H. argophyllus, H. paradoxus, H.petiolaris ssp. petioalris, H. bolanderi, H. debilis ssp. silvestris, H. debilis ssp. cucumerifolius, H. neglectus, H. praexos ssp. hirtus and H. praecox ssp. praecox* were incorporated.

The results of Christov (1988) showed that the crossing of *H. eggertii, H. laevigatatus, and H. salicifolius* with cultivated sunflower is complicated but possible. The successful interspecific hybridization is proved by the results of Christov (1991). He reported numerous interspecific hybrids between cultivated and wild sunflower species *H. annuus, H. arhophyllus, H. praecox, H. eggertii, H. laevigatus, H. tuberosus, H. divaricatus, H. glaucophyllus, H. nuttallii, H. smithii and H. ciliaris)*.

Several investigators have investigated the mode of inheritance of individual traits in interspecific hybrids in F_1 and F_2 generation. The F_1 interspecific hybrids were studied for the manifestation of heterosis for various characteristics. The studied characteristics of the species and the F_1 interspecific hybrids showed some expected as well as some unexpected and unusual correlations. High correlations were found between plant height and petiole length, number of lateral branches and leaves, number of lateral branches and head diameter, and between the number of ray flowers and central head diameter.

Vannozzi et al. (1990) studied three interspecific hybrids between wild *Helianthus* species and the cultivated sunflower *(H.annuus Line A x H. argophyllus, H. annuus Line A x H. bolanderi, H. annuus Line A x H. debilis)* with the aim of examining their morphological, biometrical, biological and technological characters. Results show that these hybrids maintain several characters of wild types although the trend is to get to the cultivated ideotype. A normal desaturation activity is present; moreover, path analyses reveals that oleic acid may be increased by flowering height in *H.annuus x H. argophyllus*, by leaves number in *H.annuus x H.bolanderi* and leaf surface in *H. annuus x H. debilis*. Finally oil content may be increased by unsaturated acids in *H. annuus x H.argophyllus* and is not influenced by environmental conditions in all three hybrids.

Palla et al. (1988), investigating some wild species and their interspecific hybrids with cultivated sunflower, found that the wild parent reveals a longer flowering period, more branches and a smaller height than cultivated species. F_1 hybrid presents intermediate characters between two parents with the exception of the height, which always results definitely superior.

Utilization of many species of wild *Helianthus* is limited by poor crossability and the high degree of F_1 strility in interspecific hybrids. This was proved by the results achieved by Atlagić (1991) according to which fully sterile plants occurred when crossing most of the tested polyploid species (with hexaploid *H. tuberosus, H. rigidus and H. resinosus*, with tetraploid *H. laevigatus, H. hirsutus and H. decapetalus)*. When crossing with diploid species, sterile plants were detected only in F_1 hybrid with *H. mollis*.

Atlagić (1988) found also that sterility in BC_1F_1 hybrids was

encountered in this round of interspecific hybridization. Its presence was established by visual inspections in field and microscopic from 0 to 39.3% and from 31.3% to 100%, respectively.

According to Atlagić (1988 and 1990), pollen fertility is an important biological trait which is gratifying to study in F_1 interspecific hybrids. Eight wild species of different ploidy levels, six inbred lines and 22 F_1 hybrid combinations were screened for pollen fertility by the method of Alexander (1969). The percentage of pollen fertility was high (above 90%) in all inbred lines as well as in most wild species (only two of them were below 90%). The F_1 hybrids had lower pollen fertility than their parents, the percentages ranging from 1.9 to 70.0%.

Georgieva-Todorova (1990) claimed that incompatibility between species of the genus *Helianthus* showed that it was an often encountered phenomenon which, however, was not related to ploidy or taxonomic relations. It was conditioned by genetic and environmental factors. In investigating the species incompatibility within genus *Helianthus*, preliminary emasculation proved to be absolutely necessary.

Using a rich hybridization program and according to the successful results of standard crossing, Atlagić (1991) found that various possibilities exist in breeding different populations of the same species. Consequently, different populations should be collected and used in crossing, especially when crossing species are "cross incompatible".

Olivieri et al. (1988) studied the mechanisms of self-sterility and incompatibility related to a scarcity of the yield in the lines of sunflower developed from crosses between cultivated and *H.bolanderi* and *H.exilis*. They found that in some cases of incompatibility pollen grains did not adhere to stigma papillae as appears by scanning microscope; in other traces of pollen tubes were blocked mainly into the stigma tissues and in the upper part of the style. In compatible crosses traces were observed at the base of the style ten minutes after pollination, by fluorescence microscope, but not clear relationships were found between number of traces and achenes developed.

Species interrelations and interspecific incompatibility, evident in most cases, was of particular importance for elucidating the type of the isolating mechanisms. According to data of other scientists, several basic trends were obvious in interspecific hybridization both between cultivated sunflower and wild *Helianthus* species and between the wild species themselves. The cultivated sunflower crosses with annual diploid species easily, but with difficulty or not at all with perennial species. It could be presumed that structural differences exist between the chromosomes of perennial *Helianthus* diploid species and those of the cultivated sunflower. Investigations of species interrelations between diploid perennial species, representatives of various taxonomic sections, showed that in most cases the barriers of incompatibility were manifested even in the progamous phase. In some interspecific combinations the pollinating parent's pollen did not germinate on the stigma of the female parent. This phenomenon was observed also in species of one and the same taxonomic section (Georgieva-Todorova, 1990). According to the same author, *H. annuus* crosses with tetraploid species with difficulty, but in some cases unilateral incompatibility was

evident. The incompatibility between hexaploid species and cultivated sunflower was not of the same type and was not manifested to the same extent. For instance, hybrids between *H. annuus* and the hexaploid species *H. resinosus* can be obtained no matter which of the two species was used as female parent. The same was true for hybrids with *H. tuberosus*, although in this case incompatibility of greater degree was evident. According to their investigations on species incompatibility, the lowest one was observed between *H. annuus* and *H. rigidus*. Hybrids could be obtained, however, only in case when hexaploid was used as a female parent. Cytological investigations of hybrids between *H. annuus* and the hexaploid species *H. tuberosus, H. rigidus and H. resinosus* led to the conclusion that *H. resinosus* was most closely related to the cultivated sunflower. On the basis of this fact, they assumed that the genome constitution of the three hexaploids was not the same and their establishment in nature had not occurred in the same way.

Sterility barrier is one obstacle to interspecific hybridization which is not easily overcome. One possible answer lies in artificially doubling the number of chromosomes in the hybrid, which would give each chromosome an exact copy to pair with so that the cell division process (meiosis) can occur in an orderly fashion.

Jan (1988) found that the colchicine chromosome doubling method proved to be a simple and effective method of improving the fertility of interspecific hybrids involving wild diploid or tetraploid species and can be routinely used to improve the efficiency of interspecific gene transfer.

Using previously elaborated method of colchicine application for chromosome doubling, Jan and Chandler (1989) successfully overcame low fertility in interspecific hybrids between P21 x *H.bolanderi* and provided more seeds in backcross generations.

Regarding the wild species and interspecific sunflower hybrids, the differences in the period of flowering represent the problem very often. This can be prevented by pollen preservation for a long period. Roath et al. (1988) elaborated the method for pollen preservation in refrigerator freezer using liquid nitrogen.

USING WILD HELIANTHUS SPECIES IN BREEDING FOR RESISTANCE TO DISEASES AND PESTS

Diseases are a limiting factor of production in the majority of sunflower-growing countries. Different diseases are dominant in different regions on account of various agroecological conditions. The cultivated sunflower has a narrow genetic base and it is deficient in resistance genes. So far, sources of resistance have been sought and found in wild sunflower. Certain wild species have contributed genes of resistance to *Plasmopara helianthi, Puccinia helianthi, Verticillium albo-atrum, and Verticillium dahliae*. There is yet a large number of diseases for which resistance sources remain to be found. Among those, the most important ones are *Sclerotinia sclerotiorum, Phomopsis-Diaporthe helianthi, Macrophomina phaseoli, Phomopa sp., Alternaria helianthi, Botrytis cinerea, Rhizopus spp.*, etc. The diversity of *Helianthus* genus offers possibilities of

discovering resistance sources to all diseases.

Significant results have been achieved in recent 3-4 years with respect to wild species use in discovering sources of resistance to diseases.

Puccinia helianthi

The scientists from the North and South America were engaged in discovering the sources of resistance to rust, since in these territories, this pathogen is dominant and several races exist as well.

According to Ferreira et al. (1988), wild *H. annuus* and *H. petiolaris* may be used as resistance sources to rust.

According to Miller and Lambrides (1991), five sources for resistance to rust with diverse backgrounds were RO-20 (South Africa), NS-BCD (Yugoslavia), France R-line (PRS), PAC 308 (Australia) and ARG (Argentina). Four sources of advanced B-lines had field resistance: Bulgaria 2169, Progress (Russia), BCD85-2 (Yugoslavia) and Spain (INTA, Cordoba). Most of these genotypes were produced on the basis of wild sunflower species.

The results obtained by Quresh et al. (1991) provide strong evidence that the majority of wild species have different sources of resistance to the rust populations and can be used as donor parents for rust resistance in cultivated lines.

Wild *H. annuus, H. argophyllus and H. petiolaris* were the species most often included in the investigation. According to the same authors, these new resistant genes will broaden the genetic base of the cultivated lines and provide long-term protection against the prevailing and new entities of rust.

Jan et al. (1991) studied three wild *Helianthus* accessions, PI 451977, PI 503232 (both *H.petiolaris*), and PI 494571 (*H.argophyllus*), which were observed to have a high level of resistance to race 4 of sunflower rust.

Deviations from the one gene hypothesis of 1R to 1S segregation suggested that resistance in PI 451977 was controlled by 3 dominant complementary genes, resistance in PI 503232 by 2 dominant complementary genes, resistance in PI 494571 was controlled by a single dominant gene.

Analyzing 6 accessions, PI 413023, PI 413037, PI 413048, PI 413118, PI 413171, and PI 413175 resistant to all four rust races in crossing with a susceptible line Ha 89, Quresh and Jan (1992) found the existence of various genes for resistance.

Analyzing new germplasm produced on the basis of interspecific hybrids, Seiler (1991) also found that line ARG-1575-2 possesses genes resistant to rust.

Bauer (1991) claimed, according to the analysis of 40 years old sunflower breeding in Argentina, that two wild species, *H.annuus* and *H. argophyllus* were frequently used in crosses with the cultivated sunflower in the period 1960 - 1965, with the aim to obtain varieties resistant to *Puccinia helianthi* (Manfredi INTA, Impire INTA and Cordobes INTA).

Miller et al. (1992) reported that several new races of rust have been identified in the USA, Australia and Argentina and several accessions were tested for resistance and five were found to be resistant to race 4.

Plasmopara helianthi

Frequent exchange of breeding material, and especially hybrids brought to the occurrence of new races of this pathogen in the South and North America, Europe and Asia. The existence of genotypes obtained on the basis of interspecific hybridization gave positive results in the gene selection for resistance to this pathogen. Eight Pl genes (Pl_1 - Pl_8) were determined, and the differential lines exist which secure the success of the selection for the resistance to this pathogen.

Analyzing new germplasms produced on the basis of interspecific hybrids, Seiler (1991) found that heterogenous populations segregate for disease resistance, except one germplasm line ARG-1575-2, which showed resistance to all races of downy mildew. The potential for selecting downy mildew resistance genes from the evaluated germplasms is high.

Tan et al. (1991) found the existence of resistance genes in PI 413047, PI 413131, PI 413157, PI 413161 which were not allelic and also were different from the gene Pl6 (identified previously in inbred line Ha 336).

According to the unpublished results of Dozet, it can be concluded that the populations of wild *H. annuus* had low frequencies of genes for resistance to *Plasmopara helianthi*. It was only in the population of wild *H. annuus* 1336 that genes of resistance to *Plasmopara helianthi* races NS-1 and NS-2 were determined.

The resistance was significantly higher in the other wild sunflower species studied. In *H. argophyllus*, one population was susceptible (1681) and the other resistant to *Plasmopara helianthi* (1813). Full resistance to *Plasmopara helainthi* races NS-1 and NS-2 was shown by all populations of *H. petiolaris, H. tuberosus, H. resinosus, H. rigidus, H. nuttallii, H. mollis, H. salicifolius and H. occidentalis*.

On the basis of the results obtained a conclusion may be brought that wild species possess, to a high degree, genes of resistance to the races of *Plasmopara helianthi,* existing in Yugoslavia.

Reporting for FAO Sunflower Network, Christov (1991) claimed that the resistance to *Plasmopara helianthi* was determined in the following wild species: *H.tuberosus, H.scaberimus, H. divaricatus, H.mollis, H.salicifolius, H. giganteus, H. nuttallii, H.annuus, H. debilis and H. petiolaris*.

Phomopsis/Diaporthe helianthi

A recent discovery of genes for high tolerance to the *Phomopsis* is an excellent example of using wild species in breeding for resistance to diseases.

According to Škorić et al. (1989), several wild perennials have exhibited resistance to pathogen in field conditions. Interspecific hybrids between *H. tuberosus, H. rigidus, H. hirsutus, H. strumosus, and H. mollis* on one side and cultivated genotypes on the other are rich genetic materials for breeding for Phomopsis resistance.

Dozet (1991) claimed that mycelial test on leaf petiole proved to be most objective in testing wild species for resistance to *Phomopsis*. *H. tuberosus* populations (1704) was immune to Phomopsis, while *H. tuberosus* (1700) and *H. nuttallii*

(1996) proved to be highly resistant.

Sclerotinia sclerotiorum

The *Sclerotinia* disease complex appears to be very complicated. Looking to the wild species for resistance to this disease has not been very successful, and the prospect of finding a single dominant gene for resistance does not look too promising at the moment.

According to the results obtained by Škorić and Rajčan (1992) of two-year investigation of some wild sunflower species using three methods of artificial inoculation, the population 1631 *H.maximiliani* possesses most probably high degree of resistance to *Sclerotinia*. The inoculated stem did not bring to pathogen development only in plants of this wild species. The inoculated plants stopped by their defensive mechanisms pathogen development and produced a hard callus on the inoculation site, on stem.

On the basis of preliminary results submitted at the FAO meeting in Pisa (Italy) in 1991, a high degree of tolerance to *Sclerotinia* was found in *H. divaricatus, H. glaucophyllus, H. salicifolius and H. mollis* (General Toshevo, Bulgaria).

According to the results of Christov (1991) reported in the framework of FAO Report / FAO Subnetwork: Sunflower Genetics and Breeding (Pisa, Italy), the species of *H. sebilis ssp. silvestris, H. eggertii and H. glaucophyllus* were resistant to *Phoma* sp.

H. decapetalus and its F_1 hybrids with cultivated sunflower showed a complete resistance to *Erysiphe cichoracearum* D.C. Resistance was also exhibited by individual samples of the species *H. glaucophyllus, H. resinosus, H. tuberosus* (M-004), *H. mollis, H. giganteus and H. debilis ssp. debilis*.

A complete resistance to *Orobanche cumana* could be found mainly in the perennial species *H. divaricatus, H. glaucophyllus, H. giganteus, H. mollis, H. salicifolius, H. grosserratus, H. nuttallii and H. resinosus* (General Toshevo).

Block (1992) found tolerant populations of wild species to *Alternaria sp.*

Wild sunflower species (Helianthu spp.) as potential sources of resistance to pests

Unfortunately, wild sunflower species are rarely used in breeding programs for the resistance to pests, although they are economically significant for sunflower production in the USA, Canada and some countries of the South America and Africa.

Wild species of *Helianthus* are attacked by many species of pests in their natural environments, pests which might also mutate into biotypes which could attack the domesticated sunflower. Such a transition in pest status of species from scattered wild populations to domesticated monocultures would greatly enhance the immediate biological fitness of the pest species, causing great increases in pest population density. Insect pests represent living, evolving populations. New pests can arise from seemingly innocuous species or old pests can mutate into virulent new biotypes capable of decimating genotypes of sunflower that once were resistant (Seiler, 1990).

The use of wild species in sunflower breeding program for

the resistance to pests has not been published in recent 5 - 8 years. However, this does not mean that wild species were not used in breeding programs for the resistance to pests.

According to Seiler (1990), interspecific germplasms based on wild *H. tuberosus and H. petiolaris ssp. petiolaris* Nutt. have been developed and released for sunflower head moth (*Homoeosoma nubulellum* (Hulst)) resistance.

Significant results have been achieved in using wild species in breeding for resistance to *Cylindrocopturus adsprus* (Le Conte), *Zygogramma exclamationis* (Fab.), *Bothynus gibbosus* (De Geer), *Aphis, Mesonaphis masoni* (Knowlton), *Empoesca abrupta* (Delong), etc. (Seiler, 1990). All the results mentioned previously, are relate to work on breeding programs of Rogers et al. Unfortunately, these investigations have discontinued since he started to make other investigations.

CYTOPLASMIC MALE STERILITY

Seiler (1988) made an excellent review of the results achieved in detection of CMS sources. The results achieved in recent 3 -4 years will be presented hereafter.

A number of new CMS sources have been discovered in this period. According to Serieys (1991), nearly 25 CMS sources have been discovered so far. This number regularly increases since active interspecific programs generating alloplasmic combinations were undertaken in the world. If, for most of them, demonstration has been made that origin of the androsterility results from nucleocytoplasmic interactions, their differentiation is not still clearly established.

Serieys (1991) gave the list of the known CMS with origin and authors, as well as denomination according to FAO codification:

CLASSICAL CMS	: (H.petiolaris Nutt)	LECLERCQ	1969	= PET1
CMG1	: (H.petiolaris Nutt)	WHELAN	1980	= PET2
CMG2	: (H.giganteus)	WHELAN	1981	= GIG1
CMG3	: (H.maximiliani)	WHELAN	1980	= MAX1
KOUBAN	: (H.annuus lenticualris)	ANASCHENKO	1974	= ANL1
INDIANA 1	: (H.annuus lenticualris)	HEISER	1982	= ANL2
FUNDULEA 1	: (H.annuus texanus)	VRANCEANU	1986	= ANT1
H. ANNUUS 517	: (wild H.annuus)	SERIEYS	1984	= ANN2
H. ANNUUS 519	: (wild H.annuus)	SERIEYS	1984	= ANN3
H. ANNUUS 397	: (wild H.annuus)	SERIEYS	1984	= ANN1
H. ANNUUS 521	: (wild H.annuus)	SERIEYS	1984	= ANN4
BOLANDERI	: (H.bolanderi)	SERIEYS	1984	= BOL1
FALLAX	: (H. petiolaris fallax)	SERIEYS	1984	= PEF1
NS-ANN-81	: (wild H. annuus)	MARINKOVIĆ	1986	= ANN5
PETIOLARIS BIS	: (H.petiolaris Nutt)	LECLERCQ	1983	= PET3
VIR 126	: (H.lenticularis)	ANASCHENKO	1974	= ANL
NS-ANN-2	: (wild H. annuus)	SKORIĆ	1987	= ANN6
VULPE	: (H.rigidus)	VULPE	1972	= RIG1
EXILIS	: (H.exilis)	SERIEYS	1982	= EXI1
NEGLECTUS	: (H.neglectus)	SERIEYS	1986	= NEG1
PET/PET	: (H. petiolaris petiol.)	SERIEYS	1987	= PEP1
ANOMALUS	: (H. anomalus)	SERIEYS	1986	= ANO1
CANESCENS	: (H.niveus canescens)	SERIEYS	1982	= NIC1
ARGOPHYLLUS	: (H. argophyllus)	CHRISTOV	1990	= ARG1
ARGOPHYLLUS	: (H. argophyllus)	CHRISTOV	1990	= ARG2
PRAECOX	: (H. praecox praecox)	SERIEYS	1989	= PRP1

Six new CMS sources in wild *H. annuus*, discovered by Jan (1990) have not been included in this list.

Serieys (1991) presented the following results achieved in the framework of FAO Sunflower Network on evaluation of the CMS stability in different locations and comparison of the restoration ability of the CMS sources:

- Evaluation of the stability of 13 CMS sources by growing them in different locations. If we consider the basic material supplied by Montpelier, the CMS sources were practically sterile in all locations. Yet, some of them PET2, GIG1, ANL2 shown sterile pollen production. We can conclude that for the female lines considered the cytoplasmic sterilities were rather stable;
- Examination of the F1 hybrids (13 CMS crossed with 18 lines and observed in 2 to 3 locations) gives valuable information for the identification of restorer lines. In almost CMS, (fixed or not) restorer genes were detected. Some rare restorer factors were found into the "hard to restore" sources ANT1, ANN1, ANN2. So, we have to underline the interesting behaviour of HA291 line which contains some restorer factors for these latter two CMS;
- With the exception of weakly restored cytotypes CMS-ANN1, ANN2, ANN3, ANN4 the genetic results distinguished all the origins. In most of cases segregations were quite straightforward. Stability response was imperfect for several cytotype-nucleus combinations and 10 sources (on 13) may be easily separated using the restoration diagrams. CMS-PET1 source, appears closely related to the hight restored cytoplasms (ANL2, ANL1, BOL1), but remain distinct;
- The genetic determinism of the restoration is generally governed by serials of complementary restorer genes (several segregations suggest more than 2 genes). The case of CMS BOL1 appears singular since segregations agree with the action of 2 independent and non complementary Rf genes.

On the basis of the newest results achieved by some French authors (Crouzillat et al., 1991), Serieys (1991) concluded that RFLP of mitochondrial DNA revealed specific differences between the cytotypes studied. Three restriction enzymes and 12 probes permitted distinction of 13 cytotypes. No relationship exists between CMS cytotypes and the species from which they originated.

For genetical and mitochondrial RFLP studies, phenograms were constructed according to the similarity indexes between cytotypes. Most of the CMS defined by restoration patterns correspond with a restriction fragment pattern of mitochondrial DNA.

Contemporary approach to the detection of differences between different CMS sources using molecular techniques has become the objective of investigation of many authors, at present.

Perez et al. (1988) found that the presence of the P1T plasmid in the B lines has been established both by ethidium bromide staining technique, Leroy et al. (1985), and by hybridization with the cloned plasmid used as a probe from CANP 3 B line, Perez (1987), and from HA89 B line, Crouzillat et al., (1987). Nevertheless, some total DNA preparations of the A line gave a sporadic hybridization signal depending upon the samples. So we wonder whether the presence of P1T or any sequence

homologous to P1T can be present in total Dna from the A lines. Perez and Berville (1988) claimed that the circular plasmid called P1T found in the mitochondria of the sunflower has been used as a probe on sunflower lines used by breeders and on wild forms or wild form derived CMS. P1T cross-hybridized other various size plasmid but not all of the *H. petiolaris* species. These results allowed us to differentiate subspecies rapidly and efficiently. Hahn and Friedt (1991) found that the mtDNA of the CMS cytoplasm MAX1 differs from fertile lines in the region of the atp A gene as does the PET1 plasm. Additionally, the former cytoplasm also displays differences in the region of the atp6 gene when compared to fertile sunflower lines. Gerlach et al., (1991) made significant conclusions as well, finding that the CMS found by Leclercq (1969) is correlated with rearrangements in the mtDNA. The rearrangements consist of an 11 kb inversion and an 5 kb insertion near the atp A locus.

Belhassen (1991) mitochondrial DNA analysis had allowed interesting characterizations of the twenty CMS sources. While the modification of some sequences seems to be correlated with CMS, the presence of the two mitochondrial plasmids P1 and P2 was described to be unrelated to male-sterility.

Spassova et al. (1991) studied mitochondrial DNA from one fertile and six new cytoplasmic male sterile sunflower genotypes. CMS associated RFLP's were found, generated by various restriction enzymes in the vicinity of the atp A locus.

Laver et al. (1991) emphasising mitochondrial genome organisation and expression associated with CMS, claimed that a region of mitochondrial genome variation between the two phenotypes (fertile and CMS) has been located in the 3 flanking region of the gene encoding the alpha subunit of the F1 ATPase (atpA). The same authors found in organelle labelling of mitochondrial translation products from the two types of sunflower shows that a 15 kDa protein is synthesized by the mitochondria from sterile sunflower but not by those from fertile. The ORFc sequence could encode this 15kDa protein which may be causally related to the CMS phenotype. At the other hand, Horn and Zetsche (1991) claimed that 16 kDa polypeptide is neither expressed in the fertile lines of *H.annuus* nor of *H.petiolaris*. The 16 kDa polypeptide which is membrane-associated might be the product of the new open reading frame or fH522 which is co-transcribed with the atpA gene and also seems to be correlated to CMS.

Moreau and Berville (1991) suggested, on the basis of results obtained, that the official service for seed control will introduce the molecular assay in the licence process for CMS seed stock of sunflower.

The discovering of a large number of new CMS sources, has imposed a new task to find restorer genes for each of the discovered CMS sources, and particularly, in wild sunflower species.

According to the results obtained by Škorić et al. (1988), restorer genes for CMG-1 were registered in 19 wild species, for CMG-2 in 12 species, for CMG-3 in 11 species, for CMS-Indian-1 in 19 species, for CMS-PET-1 in 12 species, and for ANN-81 i n7 species.

The registered restorer genes were more frequently in

heterozygous than in homozygous state.

Certain populations of wild species possessed restorer genes for as much as four CMS sources. Restorer genes for three, two or one CMS source were more frequent within populations of the same species.

Studying six new CMS sources obtained on the basis of wild *H. annuus*, Jan (1990) identified relatively high frequency of fertility restoration genes in every accession. Fertility restoration genes were also identified in Ha 89, P21, and the five RHA lines for various CMS cytoplasms.

Christov (1990) concluded that the conventional CMS and the new CMS sources, ARG-1 and ARG-3, are similar in some points and different in others. Some lines known as maintainers for CMS "petiolaris" type possess the ability to restore, to a certain degree, the fertility of plants produced on the base of the new CMS. A study undertaken to clarify the differences is in progress.

Jan and Rutger (1988) indicated the occurrence of CMS in Ha 89 line using mitomycin C and streptomycin. Progeny of the 22 induced CMS mutants and CMS HA 89 crossed with fertility restorers RHA 266, RHA 274, RHA 280, and RHA 296 were all fertile. Seven nuclear gene controlled male-sterile (NMS) mutants with monogenic recessive inheritance were also verified.

Havekes et al. (1991) tested 10 different CMS sources and found that at least two new sources of CMS, CMS PET2 and CMS GIG1, were found to be potentially useful for commercial production of hybrids. Environment had an influence on fertility restoration of one CMS line, CMS MAX1. Effective restoration of male fertility for CMS RIG1, CMS ANN2, and CMS ANN3 was not found.

New sources of CMS have not been used practically by breeders. The most probable reason for these might be that conventional CMS (PET1) is still very stable and several sources of Rf genes, which are built in the lines with good GCA and SCA for agronomically important characters were discovered. Another reason is the deficiency of stable sources of fertility restoration for most of new CMS sources.

Application of biotechnologic methods in sunflower breeding

Conventional plant breeding procedures have already proven to be very well suited for successfully improving the quantity as well as the quality of oil-yield of major oil-plants, like sunflower.

Modern techniques ("biotechniques") can help to improve the efficiency of breeding, e.g. regarding adaptation to extreme environments. Specific techniques, like "haploidy-steps" or genetic engineering can help to accelerate breeding progress through avoidance of long-lasting inbred and/or backcross generations in the near future (Friedt, 1988).

The foreseeings of Friedt have started to realize already the following 2-3 years. Many authors have achieved significant results, which will open a new phase in sunflower breeding in the future.

Rieseberg et al. (1988) analyzed allozyme, chloroplast-DNA (cp DNA) and nuclear-ribosomal_DNA (rDNA) variation of *Helianthus bolanderi*. They detected a total of 37 low-frequency alleles

distinguishing the serpentine *H.bolandery* from *H. annuus*. The same authors determined a total of 17 cpDNA and five rDNA restriction-site mutations among the 19 populations examined. In addition, the weedy race of *H. bolanderi* possessed a unique cpDNA, which was outside the range of variation observed among populations of either of the presumed parental species. Mean sequence divergences between the cpDNAs of weedy *H. bolanderi* and these of serpentine *H. bolanderi and H. annuus* were 0.30% and 0.35%, respectively.

The application of molecular techniques will enable to solve certain problems which occur in the field of taxonomy. Thus, Beckstrom-Sternberg et al. (1990) decided to study *Helianthus petiolaris and H.niveus* which are morphologically distinct at the periphery of their ranges but intergrade in areas of sympathy. *Helianthus niveus* includes both annual and perennial members, whereas *H. petiolaris* is strictly annual. Chloroplast DNA and nuclear ribosomal DNA restriction site data were used to reconstruct the evolutionary history of populations of the two species. Cladistic analyses reveal the following: 1) neither species is monophyletic; 2) the annual habit is derived once in this complex; and 3) the region of morphological intergradation appears to be primary in origin.

Rieseberg and Seiler (1990) studied the origin of the domesticated sunflower at the molecular level and made important conclusions. Morphological, geographical, and archaeological evidence has led to the hypothesis that the domesticated sunflower was derived from a wild/weedy form of *H.annuus* possibly in the Midwest. Molecular evidence was concordant with this hypothesis. A high degree of enzymatic and cpDNA sequence similarity was observed between wild and domesticated *H. annuus*, and domesticated *H. annuus* contained a subset of the alleles and cpDNAs found in wild *H. annuus*. The extensive polymorphism in the wild plants and the virtual monomorphism in cultivated lines for both isozyme and cpDNA phenotypes further suggest a single origin of the domesticated sunflower from a very limited gene pool.

Spring and Schilling (1990) payed attention to the origin of natural hybrids of *H. x multiflorus* and *H. x laetiflorus* and made several conclusions: "A high degree of similarity was observed between the sesquiterpene lactone profile of *Helianthus x multiflorus* and the additive patterns of *H. annuus* and the diploid race of *H.decapetalus*. In contrast, the profile of artificial hybrids between *H. annuus* and the tetraploid race of *H. decapetalus* differed significantly from that of *H. x multiflorus*, although it was additive relative to the two parents. It is concluded that *H. x multiflorus* originated from hybridization between *H. annuus* and the diploid race of *H.decapetalus*, with one parental genome remaining unreduced. Similarity in additive sesquiterpene lactone profiles is also in agreement with previous proposals that *H.x laetiflorus* resulted from natural hybridization between *H. tuberosus* and *H. pauciflorus ssp. subrhomboideus*. The new nomenclatural combination, *H. pauciflorus ssp. subrhomboideus* (Rybd.) Spring and E. Schilling, is proposed."

A significant progress with respect to the application of techniques at the protoplasts level in sunflower has been achieved in recent 2-3 years. The results achieved announce a

new phase in sunflower breeding and solving of several problems within this important oil crop.

Bohorova (1988) established optimal culture conditions, for the respective protoplast systems. Heterokaryons (*H. annuus and H. praecox)* were also identified as protoplasts possessing chloroplasts in a rich cytoplasmic background.

Bohorova et al. (1990) concluded that an *in vitro* system for callus induction and plant regeneration from explants-bud, stem, leaf and cotyledon pieces as well as protoplasts isolation enzymatically from leaves and their culture in different media, is described using six wild diploid species (2n=2x=34) of sunflower viz., *Helianthus nuttallii* Tats., *H. mollis* Lam., *H. divaricatus L., H. debilis* Nut., *H. maximilliani* S., and *H. praecox* E & G. All explants formed calli on a modified MS medium (MSD4). Plant regeneration was obtained in all species from calli of the above explants except cotyledons on R medium. Maximum regeneration (81.25%) was obtained from stem-induced calli of *H.nuttallii* whereas it was only 4.80% from bud-induced calli of *H. mollis*. Protoplasts from all species were examined for division, colony and subsequent callus formation. Colonies were formed in all species, however, callus formation was obtained only in *H. praecox*. Freshly isolated protoplasts of these species have shown variability for yield, size and viability.

Moyne et al. (1989) found that *Helianthus annuus* protoplasts were transformed with the plasmid pCaMVNEO (Fromm et al., 1986) conferring kanamycin resistance to plant. Transformed calli were selected with a frequency of 4 calli for 10^6 treated protoplasts. DNA was extracted from kanamycin resistant calli. Analysis of this DNA shows the presence of the NPTII gene.

Kirches et al. (1991) inhibiting RNA synthesis by 3 dATP or translation of mRNA by cyclohevimide suggested that active plasmid-DNA as well as functional mRNAs are available for only short time periods in transfected protoplasts. Results indicate that DNA uptake is more efficient using PEG, while in some cases electroporation causes less damage in transfected protoplasts.

The first documented report, in the genus *Helianthus* of regeneration from protoplast to fully soil-adopted plants, was presented by Chanabe et al. (1991). They performed it as follows: Protoplasts were produced from 7-day old hypocotyls of two cultivated sunflower genotypes and three wild sunflowers. When included in agarose droplets and cultured in TL medium supplemented with 0.1 mg/l 2.4-dichlorophenoxyacetic acid, the protoplasts gave rise to loose colonies and to "embryoids". After two months the small calli emerging from the agarose were transferred to a regeneration medium on which they grew and began to differentiate. A second transfer to the same medium 40 days later induced shoot formation on one callus of *H.petiolaris*. Several shoots were successfully rooted and transferred to soil where they flowered.

Burrus et al. (1991) reported that they obtained regenerated plants from protoplast, which they transferred to the greenhouse and seed was harvested within 7 months of the initial protoplast isolation.

Friedt (1988) proclaimed, according to the results achieved recently by several authors, that functional vector-systems for gene-transfer are available: e.g. the *Agrobacterium tumefaciens*

system is an established tool for transferring genetic information into dicotyledonous plants including sunflower. Therefore, transferring agronomically important genes, encoding for resistances or quality traits, from wild *Helianthus* species to cultivated *H.annuus* and from one sunflower cultivar to another is no utopia any more, provided that entire and fertile plants can be regenerated from the manipulated cell(s).

Tassie et al. (1991) concluded that the best tissue for regeneration in our hand are hypercotyl segments from immature embryos. 3328 hypercotyl segments were transformed with *Agrobacterium* with different plasmid constructs. 1-2% of these segments were regenerating transformed shoots. Hartman (1991) worked also on *Agrobacterium* transformation in sunflower.

Today, contemporary biotechnologic methods are used to solve certain problems. Friedt (1991) claimed that substantial progress has been made in recent years with regard to, e.g., embryo culture ("embryo rescue"), meristem culture, anther and microspore culture, protoplast culture and cell fusion and molecular techniques.

In recent 2-3 years, the highest progress was achieved using molecular techniques.

The application of modern scientific methods may help better knowledge of genetic and other differences between wild species, as well as within certain species. Dry and Burdon (1986) studied the genetic structure of 11 wild populations of *H. annuus* occurring in New South Wales and Queensland (Australia) by isozyme analysis. Considerable isozyme diversity was found among loci within and between populations, with three to five alleles being identified at each of 10 loci. Rieseberg et al. (1988) achieved similar results using electrophoresis in *Helianthus bolanderi ssp. exilis, H. bolanderi ssp. bolanderi and H. annuus*. They found that most of the genetic variation in ssp. exilis resides within populations, whereas the majority of genetic variation in ssp. bolanderi and H. annuus is distributed among populations.

The application of modern methods contributes the rapid and better using of interspecific hybridization in sunflower breeding programs. Krauter and Friedt (1990) using embryo culture *in vitro* in crossing program, obtained 34 interspecific hybrids and 481 plants successfully regenerated, i.e., the average regeneration rate of 54.3% has been achieved. They also identified hybrid progenies morphologically, and cytologically by isozyme electrophoresis and/or by RFLP analysis. According to Krauter et al. (1991), RFLP analysis allows a rapid and safe characterization in early developmental stages of the hybrids.

With the purpose to obtain information on interspecific hybridization in genus *Helianthus* , Cremonini et al. (1991) studied four accessions *H. debilis ssp. debilis, H. annuus,* Ha 89 mt, F1 hybrid and F2 selfed line. According to the results obtained, microdensitometric analysis of nuclear DNA content of shoot and root apices showed variability in DNA values.

Denat and Serieys (1991) tested three basal media for the maturation and further germination of sunflower immature embryos isolated 3 days after pollination. For all of them, a high sucrose content has a positive effect on embryo development. Interspecific hybrids were obtained between species hard to cross

by classical methods, especially in crosses with *H. annuus* and several wild species *H. maximiliani, H. mollis, H. pumilis and H. salicifolius*. Rovelo et al. (1988) collected sunflower indehiscent anthers for *in vitro* culture studies. According to the results obtained they found that greater callus development was observed at increasing BA levels, however no adventitus buds were observed. Callus preparations cuts showed an uninuclear grain pollen. The pollen tube was projected along one of the pores giving origin to a group of multinuclear cells. It can be concluded that haploid sunflower callus can be obtained and the further studies are needed to regenerate complete haploid plants. Gürel et al. (1991) worked on methods for obtaining haploid plants. Guerra-Sanz worked on tissue culture method using *H. debilis*.

In recent years, large attention was given to the study of germination capacity of wild sunflower species and interspecific hybrids. Vannozzi et al. (1990) studied germination of wild species and their interspecific hybrids at different temperature levels (5, 10, 15, 20, 24 and 30° C). Germination percentage and germination energy were determined. The wild genotypes germinate better than line C at 10 and 29° C. Germination energy increases from low to high temperatures for all the accessions, particularly we can say that *H. annuus x H. arggophyllus, H. annuus x H. debilis* (at 10 and 29° C) and *H. annuus x H.bolanderi* (at 25°C) have a lower medium germination time than line C.

Seiler (1988) found that early maturing acenes of *H.annuus and H. petiolaris* had consistently lower germination at pH levels of 4 to 12 than later maturing achenes. The trend was opposite in *H. debilis* ssp. *silvestirs* and *H. argophyllus*. Storage temperature did not have a consistent influence on germination. The results of this author also proved that a 1 mM substrate solution of gibberellic acid (GA_3) or a 20 mM solution of KNO_3 significantly increased germination in the wild species tested which were at various stages of maturity and stored under different time and temperature regimes.

DISCUSSION

Sunflower has, of all field crops, the highest number of wild relatives. The genus *Helianthus* is composed of 49 species and 19 subspecies with 12 annual and 37 perennial species. These diverse species represent a considerable genetic variability which can be utilized for the improvement of cultivated sunflower (Seiler, 1988).

Significant results were achieved using wild species in sunflower breeding programs, especially regarding the resistance to diseases (*Puccinia helianthi, Plasmopara helianti* and other pathogens) and pests. The special contribution of wild species in sunflower breeding is in discovering CMS sources and Rf genes by interspecific hybridization, which enabled the production of heterosis and hybrids.

The existing variability within the cultivated sunflower does not allow the development of ideotypes for different agroecological conditions. Fortunately, variability observed in wild sunflowers opens new ways to designing different sunflower ideotypes. Unfortunately, former using of wild species was

unsatisfied. There is a small number of investigators which have consistent breeding program on the basis of wild species, with the aim to produce new idetypes of cultivated sunflower. The registered germplasms on the basis of interspecific hybridization do not offer a possibility for manipulation with respect to making new sunflower idetypes.

The existing variability within the cultivated sunflower allows the development of inbred lines with insufficient heterotic effect for grain yield. Analyzing results of the long-term FAO trial on sunflower varieties and hybrids, it was noticed that there were no cultivars which would significantly outyield the conventional varieties, e.g., Peredovic. The problem revolves around narrow genetic variability for grain yield in the cultivated sunflower. A breakthrough can be made only by increasing genetic variability of the cultivated form by means of wild *Helianthus* species.

According to Seiler (1988 and 1991), a high number of germplasm was made on the basis of interspecific hybridization. A question arises to breeders, if these germplasms or some other produced by other authors, may be used in breeding inbred lines which will have significantly higher value of SGA for seed yield. According to previous results, a small chance exists with respect to this. A small number of researchers are engaged in the phenomenon of heterosis in interspecific hybridization. For example, the only paper on this subject at the 12th International Sunflower Conference was presented by Mihaljčević (1988). Discussing this problem, the results achieved by Simmonds (1989) and other authors, which answer the question how frequent are superior genotypes in plant breeding populations, should be respected as well. Simmonds (1989) claimed that serious estimates of the rate of success are very scarce but numbers of the order of 10^{-5} or 10^{-6} are often quoted as rough frequencies of really good new cultivars in populations of lines or clones.

The future application of biotechnological methods and interspecific hybridization in sunflower breeding may produce superior genotypes with respect to seed yield. The significantly higher yields of sunflower may be achieved by building a broad spectrum of resistance to diseases and other stresses on the existing level of heterosis effect.

One of the principal targets in sunflower breeding is a change in the architecture of the photosynthetic apparatus. It is desirable to shorten the period of the forming of maximum leaf area in parent lines and hybrids alike, to prolong leaf area duration (LAD), and to increase the efficiency of NAR. It is necessary to optimize the foliar orientation towards the sun, CO_2 uptake from the air, and aeration of the crop by altering the number and position of leaves on the stem. High genetic variability in wild sunflowers warrants the desired changes of the photosynthetic apparatus feasible. If we consider the differences in the photosynthetic apparatus of *H.mollis, H. argophyllus, H. salicifolius, H. radula, H. maximiliani,* etc., we may get an idea of the extent of genetic manipulations with leaf number, form, activity and other characters.

Vranceanu (1988) discovered mutant "short petiole", which enables reconstruction of plant architecture with the view of increasing plant population per unit area and maximizing yield

capacity. On the basis of *H. mollis* and by interspecific hybridization, Dozet (unpublished results) made the lines which have leaf directly attached on stem. This source of genetic variability increases the number of plants per ha to approximately 150000.

The described genotypes will enable the production of hybrids with new plant architecture.

Wild sunflower species have recently been included in a number of research programs dealing with the determination of sources of drought resistance to sunflower. Seiler (1988) gave the review of the investigations. Besides the known parameters which determine drought resistance, it is important to reduce self-incompatibility and increase self-compatibility in future sunflower hybrids and varieties by including wild sunflowers in breeding programs. To realise this program, the most appropriate are wild species of *H. agrestis and H. radula*. However, Serieys (1988) preferred *H. argophyllus*.

The diversity of wild sunflower species and genetic differences in the composition of their seeds, heads, leaves, stems, rhizomes, and tubers offer chances of improving genetic variability of the cultivated sunflower for a number of characters, turning to better use all parts of sunflower plant.

Unfortunately, in spite of this news, breeding programs were improved slightly. Only American researchers studied the variability of these characters in wild sunflower species. Seiler et al. (1991) studied 28 taxa of *Helianthus* with respect to several characters and achieved the following results: *Helianthus ciliaris* had the highest oil yield (3.7%) and was analyzed for yield of fatty acids and unsaponifiable matter. Most taxa had low polyphenol yields (<10%), with *H.strumosus* having the highest (13.9%). *Helianthus salicifolius* yielded the most hydrocarbon (1.6%) and *H. ciliaris* had the highest protein content (13.4%). Natural rubber was present in 13 species of wild sunflowers. *Helianthus maximiliani* had the lowest Mw (29.8×10^3), while *H. laevigatus* had the highest (73.3×10^3). The MWD of rubber from sunflowers were within the range of those for commercial rubbers. The lower molecular weight rubbers may have potential as plasticizing additives in commercial processing of synthetic polyisoprenes and as hydrocarbon feedstock for a synthetic petroleum industry.

Beside these, Seiler (1990) studied 19 wild and cultivated genotypes of Jerusalem artichoke *(Helianthus tuberosus)* whose tubers are used as food, for protein and mineral content at three stages of growth. The protein content of the tubers is comparable to or higher than that of other common root-type crops. Adequate macrominerals of calcium, magnesium, and phosphorus were found in Jerusalem artichoke. Potassium and sodium concentrations were higher than other root crops. Trace elements (manganese, zinc and copper) were present in adequate amounts, with iron content higher than several other root crops. Wild and cultivated Jerusalem artichoke genotypes appear to contain adequate protein and minerals to contribute significantly toward a nutritionally balanced diet.

A PROPOSAL FOR THE PROGRAM OF INTERNATIONAL SUNFLOWER GENETIC RESOURCES NETWORK

The results obtained so far within the framework of FAO European Research Network on Sunflower are encouraging with respect to international cooperation in the use of wild sunflower species in breeding programs. However, there exist some problems which hamper a still better work. First of all, FAO European Research Network on Sunflower is joined voluntarily and each member bears costs of his own research. In this situation, junior researchers and most researchers from developing countries cannot raise sufficient funds to take part in fundamental international projects.

Participants in the FAO and other networks, from various parts of the world, have expressed a wish for the establishment of an international sunflower genetic resources network. The idea was supported by participants of ECP/GR/IBPGR, representatives of Sucrosol in Latin America, and representatives of IDRC-coordinated Sunflower Subnetwork in Africa and Asia.

At the beginning of the establishment of the international sunflower genetic resources network, it was proposed to form an international scientific committee which would include members appointed by Executive Committee of International Sunflower Association, FAO European Research Network on Sunflower, ECP/GR/IBPGR, USDA-ARS-NPGS, Sucrosol, the Sunflower Subnetwork coordinated by IDRC, and representatives of other international and national associations.

The main task of the international scientific committee would be to make a strategic plan for strengthening the international scientific effort on sunflower germplasm, especially on wild species. The plan would propose a plan of work on wild species at an international level, starting from explorations, establishment of the international collection, its maintenance, exchange, evaluation, and development of pre-breeding material. Simultaneously with those activities, it would be useful to establish an international sunflower germplasm information system which would collect all data produced within the framework of the international sunflower genetic resources network and distribute them to all participants. The scientific committee should also work out a system of work to be employed by the future network. In addition to the work on wild sunflower species, it would be necessary to plan the establishment of an international collection of cultivated genotypes.

An important activity to be realized by the international scientific committee is to contact various international associations, FAO, EC, national institutions, private companies, and individuals in order to find sponsors for the international sunflower genetic resources network.

The international sunflower genetic resources network should be organized within FAO Research Network on Sunflower because this form of international cooperation proved its advantages and produced significant results.

For the sake of efficiency, we suggest for the proposal of future cooperation submitted here to be discussed and accepted in its final form.

EXPLORATIONS AND MAINTENANCE OF THE COLLECTION OF WILD SPECIES

During the explorations carried out so far, a large portion of genetic variability of wild species of the genus *Helianthus* has been collected. Presently, the collection contains over 2,200 accession numbers. This is a rich genetic basis which is readily available to sunflower researchers.

The wild species which grow in natural conditions are threatened by genetic erosion. Because of that, the explorations should be continued according to the plan made by NPGS-USDA-ARS. The plan foresees explorations in Canada, Mexico, and selected areas of the USA. Researchers from different parts of the world should take part in these explorations. Their participation can be organized through FAO and IBPGR.

Wild species of the *Helianthus* genus are widely distributed in North America; in other parts of the world, there exists a large variability of *H. tuberosus* which had been introduced from North America. This variability should be collected too. To that end, it is necessary to make a plan of explorations to various countries and regions of the world.

Because of problems encountered in the maintenance and seed production of wild species, these activities should be given special attention. In order to carry out determination and evaluation of major traits within the framework of the activities of the international sunflower generic resources network, attempts should be made to produce at least 0.5 kg of seed of the accession numbers available in the various collections. This is a difficult and complicated task and the method of multiplication in cages with bees (Dozet et al., 1991) is recommended.

There are several collections of wild sunflower species in the world. The most complete one is the collection of NPGS-USDA-ARS, Plant Introduction Station, Ames, Iowa. The other important collections are those at the Institute of Field and Vegetable Crops, Novi Sad (Yugoslavia), INRA Station d'Amelioration des Plantes, Manquio (France), Institute of Field Crops, S. Petersburg (Russia), Institute of Genetics, Sofia (Bulgaria), Center of Agricultural Research, Cordoba (Spain), etc.

To make the maintenance of wild sunflower species more expedient, each institution mentioned above should make a list of accession numbers it intends to work on. Institutions from South America, Africa, Asia, and Australia should take part in this activity.

In the second phase of work, it would be necessary to appoint institutions for base collections, seed storage and safety duplication, and for long-term storage.

DETERMINATION OF MORPHOLOGICAL, PHYSIOLOGICAL, AND BIOCHEMICAL CHARACTERS IN ORDER TO COMPLETE BASIC DATA OF WILD SUNFLOWER SPECIES

A large number of researchers has worked on the determination of characters of wild sunflower species. Although significant results have been obtained, a complete data base with descriptions of all accessions has not been made. It is thus recommended for the international sunflower genetic resources network to define a completion of data bases within the framework

of the existing collections as one of its primary targets. Special attention should be given to exchanging data for the same accessions obtained in different research centers in order to complete the data bases at minimum extra work.

In the second phase of work, groups of characters should be studied by appointed research centers.

Molecular techniques are recommended for the determination of wild species and differences among them.

EVALUATION OF WILD *HELIANTHUS* SPECIES FOR PEST RESISTANCE

So far, the use of wild species has been most efficient in breeding for disease resistance. Wild species have been found to possess genes of resistance to *Puccinia helianthi* and *Plasmopara helianthi*, and high tolerance to *Phomopsis helianthi*. Resistance genes to *Orobanche cumana* have also been found. The resistance genes mentioned above have already been incorporated in the cultivated genotypes.

However, a comprehensive list of all wild species and populations found to possess resistance genes has not been made. Such a list should be made within the proposed network. The list should contain data on resistance sources discovered and resistance genes present in various wild species and accession numbers.

In the second phase of work, a map of resistance genes per wild species and accession numbers should be made. To do that, it is necessary to produce sufficient amounts of seed of all wild species collected so far. Research teams from various centers, using inoculation methods and differential lines, should work on specific resistance sources and specify wild species and populations that possess resistance genes. Emphasis should be placed on major, i.e., most destructive pathogens (*Phomopsis/ /Diaporthe helianthi*, *Plasmopara helianthi*, *Sclerotinia sclerotiorum*, *Puccinia helianthi*, *Alternaria helianthi*, *Macrophomina phaseoli*, *Phoma spp.*, etc.)

A similar program should be designed for testing wild species for reaction to *Orobanche cumana*.

Researchers from the countries in which insects offer problems in sunflower production should work out a project for testing wild sunflower species for resistance to dominant insects.

The following two sub-projects should also be undertaken:

1. Evaluation of wild *Helianthus* species for resistance to drought and salinity;

2. Evaluation of wild *Helianthus* species for in breeding for oil and protein content and quality.

These sub-projects should be divided in two phases. In first phase, wild species should be analyzed for the characteristics in question. In the second phase, wild species should be analyzed for the variability of the established traits. Relative the topic of drought resistance, it is important to choose and study several relevant parameters. The wild species found to possess the desirable traits should be included in programs of interspecific hybridization. A similar program should be designed for the study of salinity tolerance.

The topics of interspecific hybridization, use of biotechnology in solving problems of interspecific hybridization, and determination of CMS and Rf sources are efficiently organized within the framework of FAO European Cooperative Network on Sunflower. The network is open to institutions and individual participants from all parts of the world.

GERMPLASM POOL DEVELOPMENT

It is recommended to develop germplasm pools with wild genes which would be easily accessible to sunflower scientists. Researchers should concentrate on crossing, backcrossing, selection, and intercrossing in order to turn the interspecific hybrid progenies they are working with into easily handled working populations for breeders, and yet to maintain considerable variability.

The purpose of establishing germplasm pools is transfer of desirable traits from wild *Helianthus* into cultivated *H.annuus*. The most important quality and agronomic characteristics are disease resistance, insect resistance, drought tolerance, high heterosis for seed and oil yield, different ideotypes, salt tolerance, etc.

Germplasm pools can be established and maintained by institutions or individuals. They would serve as pre-breeding material for the development of genotypes that would satisfy the agro-climatic and economic requirements of specific regions of the world or individual countries.

Method of use of individual germplasm pools could be agreed upon among researchers, sponsors and final users of pre-breeding materials.

* * *

The ideas on the establishment of the international sunflower genetic resources network presented above should serve as a basis for a discussion which should engender the best solution. These ideas do not cover the entire scope of possible activities of the future network: for example, the problem of training of sunflower researchers from developing countries, which may become an important activity, has not been mentioned earlier.

References

Alibert,G.Chanabe, C., Burrus,M. and Bidney,D.-1991. - Protoplast biotechnology in Sunflower. Sunflower Biotechnology in Europe: p.4. Mittelwihr (France), 3-6 September.

Atlagić,Jovanka, 1988 - Occurrence of sterility and reduced fertility in F1 and BC1F1 hybrids between perennial wild species and cultivated sunflower. Proc. of the 12 th International Sunflower Conference. Vol.II: 337-339, Novi Sad (Yugoslavia). July 25-29.

Atlagić Jovanka, 1990. - Pollen fertility in some H e l i a n t h u s L. species and their F_1 hybrids with the cultivated sunflower Helia, 13. Nr. 13, p.p. 47-54.

Atlagić Jovanka 1991- Meitic features and fertility in F1 plants of interspecific sunwlower hybrids. Dectoral dissertation. Novi Sad, (1-97).

Bauer H., 1988 : Cuarenta anos en al mejoramiento del girasol (H e l i a n t h u s a n n u u s L.) en Argentina - 1947-1987... Proc. of the 12 th International in Sunflower Conference, Vol.II 392-397. Novi Sad (Yugoslavia) July 25-29.

Beckstrom-Sternberg S.M. Reiseberg L.H. and Doan K. 1991.- Gene lineage analysis in populations of H e l i a n t h u s n i v e u s and H. p e t i o l a r i s (Asteraceae). Pl.Syst. Evol. 175: 125-138.

Belhassen, E.1991: The use of molecular markers in the exploration of genetical resources. Sunflower Biotechnology in Europe: p.15. Mittelwihr (France), 3-6 September .

Block, C.C., 1992- Screeining for A l t e r n a r i a leaf blight resistance in the USDA germplasm collection. Proc.: Sunflower hesearch Workshop: p.p. 64-66 N.S.A. Bismarck N.D.

Bohorova N., 1988.- Application of tissue and protoplasts culture in the genus H e l i a n t h u s L. Proc. of the 12 th International Sunflower Conference, Vol.II. 3oo-3o5 Novi Sad, (Yugoslavia), July 25-29.

Bohorova N., Punia M.S. and Iossiftcheva C,; 1990.- Morfogenetic ability of tissue and protoplast culture of wild diploid species of sunflower (H e l i a n t h u s L.) Helia, 13, Nr 13, p.p. 35-40.

Bohorova, N., Iossiftcheva C. and Punia, M.- 1991 - A Comparative study for morphogenetical ability of H e l i a n t h u s L. genotypes of the level of tissue and protoplast cultures. Sunflower Biotechnology in Europe: p.5. Mittelwihr (France), 3-6 September .

Burrus M. Chanabe C., Alibert G., and Bidney D., 1991 -

Regeneration of fertile plants from protoplast of sunflower (H e l i a n t h u s a n n u u s) Plant Cell Reports. 9: 635-638.

Christov M., 1988- Results of the crossing of H. eggertii Smal (2n=1o2), H. l a e v i g a t u s Torrey and Gray (2n=68), H.d s l i c i f o l i u s Dietr.(2n=36) with H.a n n u u s L. (2n=34). Proc. of the 12 th International Sunflower Conference, Vol. II: 277-281. Novi Sad, (Yugoslavia), July 25-29.

Christov M. 1990 : A new source of cytoplasmic male sterility in sunflower originating from H e l i a n t h u s a r g o p h y l l u s. Helia, 13, Nr.13, p.p. 55-61.

Christov, M. 1991 - Possibilities and problems in the hybridization of cultivated sunflower with species of the genus H e l i a n t h u s L. Helia, 14, Nr. 15, p.p. 35-40.

Cremonini R., Palla S., Vannozzi G.P., 199i- Interspecific hybridization in sunflower. Helia, 14, Nr.14, p.p.43-50.

Crouzillat D.,Caual de la L., Perrault A., Ledoigt G., Vear F. and Serieys H., 1991.- Cytoplasmic male sterility in sunflower: comparison of molecular biology and genetic studies. Plant Molecular Biology 16: 415-426.

Denat,J.F., and Serieys H. 1991.- Definition of a culture medium for the development of 3 days old sunflower immature embryos: application for interspecific hybrid production. Sunflower Biotechnology in Europe: p.16. Mitterwihr (France), 3-6 September.

Dozet B., 1991.- Pronalaženje iyvora otpornosti prema Diaporthe/Phomopsis helianthi Munt.Cvet. et.al. u divljim vrstama suncokreta (H e l i a n t h u s sp.) M.Sc. Thesis. Novi Sad (1-54).

Dozet B., Mandelc S., Škorić, D., Farkaš B., 1991. Use of honey bees for controceled interpollination of wild H. a n n u u s L. and H. p e t i o l a r i s ssp. p e t i o l a r i s Nuttall. 7 th Consultation of the FAO Research Network an Sunflower. Pisa/Italy, 15-17 may.

Dry P.J. and Burdon J.J., 1986.- Genetic Structure of Natural Populations of Wild Sunflowers (H.annuus L.) in Australia. Aust. J.Biol. Sci., 39: 255-70.

Ferrira V., Reynoso L, and Szpiniak B., 1988.- Hibridos interspecificos de H e l i a n t h u s, efecto citogenetica y agronomico de las retrocruzas con girasol Proc.of the 12 th International Sunflower Conference, Vol II:271-277. Novi Sad, (Yugoslavia) July 25-29.

Friedt W., 1988.- Biotechnology in breeding of sunflower as an

industrial oil crop. Proc. of the 12 th International Sunflower Conference, Vol II: 316-322. Novi Sad, (Yugoslavia), July 25-29.

Friedt W., 1991.- Biotechnology in sunflower breeding. Sunflower Biotechnology in Europe:p.13. Mittelwihr (France), 3-6 September 1991.

Georgieva-Todorova J., and Bohorova, N. 1988.- Cytological studies on hybrids and back-cross generations of H e l i a n t h u s a n n u u s L. (2n=34) and some tetraploid H e l i a n t h u s species (2n=68). Proc. of the 12 th International sunflower Conference Vol.II, 293-3oo. Novi Sad (Yugoslavia) July 25-29.

Georgieva - Todorova J., 1990.- Genetic and cytogenetic inverstigations of the genus H e l i a n t h u s L. (In Bulgarien).BAS 1-129 Sofia.

Gerlach, J., Köhler, R. and Zetsche, K. 1991- Expression of mitochondrial genes of -CMS, male fertile, and restored sunflower lines-tissue specific investigations. Sunflower Biotechnology in Europe:p.28. Mittelwihr (France) 3-6 September.

Gomez-Sanchez, D., and Gonzalez, S., 1991.- Exploration and collection of wild species from the genus H e l i a n t h u s, from Northern Mexico. Helia, 14, Nr. 15, p.p 49-54.

Gürel, A., Kontowski, S., Nichterlein, K., Friedt, W., 1991.- Embryogenesis in microspore culture of sunflower (H e l i a n t h u s a n n u u s L.) Helia, 14, Nr. 14, p.p. 123-128.

Guerra-Sanz, J. M.,1991.- In v i t r o plant regeneration of H e l i a n t u s d e b i l i s Nutt. Sunflower Biotechnology in Europe: p.8. Mittelwihr (France), 3-6 September.

Jan C.C., Chandler J.M., and Wagner S.A., 1988.- Induced tetraploidy and trisomic production of H e l i a n t h u s a n n u u s L. Genome, Vol. 3o.:647-651.

Jan C.C., 1988.- Chromosome doubling of wild x cultivated sunflower interspecific hybrids and its direct effect on back-cross success. Proc. of the 12 th International Sunflower Conference, Vol. II: 287-293. Novi Sad, (Yugoslavia), July 25-29.

Jan C.C., and Chandler J.M., 1989 - Sunflower Interspecific hybrids and Amphiploids of Helianthus annuus x H.bolanderi. Crop Science, 29: 643-646.

Jan C.C., 1990.- In search of cytoplasmic male sterility and fertility restoration genes in wild H e l i a n t h u s

species. Proc. of Sunflower Research Workshop: 3-5 NSA. Bismarck, ND. January 8-9.

Jan C.C., Quresh, Z., and Gulya T.J., 1991.- Genetics of rust race 4 resistance derived from H. p e t i o l a r i s and H. a r g o p h y l l u s accessions. Proc. - Sunflower Research Workshop p. 112. N.S.A., Bismarck, ND.

Kirches,E. Frey-Koonen, N., and Schnabl H., 1991.-Direct gene transfer into sunflower protoplasts. Sunflower Biotechnology in Europe: p.10. Mittelwihr (France), 3-6 September 1991.

Kräuter R, and Friedt W., 1988.- Goals and results of interspecific hybridization in sunflower breeding. Proc. of the 12 th International Sunflower Conference: Vol II. 265-267. Novi Sad (Yugoslavia) July 25-29.

Kräuter R.,and Friedt, W., 1990.- Effient interspecific hybridization via embryo rescue for application in sunflower breeding. Helia, 13, Nr.13, p.p.17-20.

Krauter R., Steinmetz,A. and Friedt, W., 1991.- Efficient interspecific hybridiyation in the genus H e l i a n t h u s via "embryo rescue" and characterization of the hybrids. Sunflower Biotehchnology in Europe: p. 19. Mittelwihr (France), 3-6 September.

Laver, H., Moneger, F., Smart, C. and Leaver,C., 1991.- Mitochondrial genome organisation and expression associated with cytoplasmic male sterility in sunflower(H e l i a n t h u s a n n u u s L.) Sunflower Biotechnology in Europe: p.30. Mittelwihr (France), 3-6 September.

Miller J.F., and Lambrides C.J., 1991.- Diverse sources of rust resistrance in sunflower. Proc.Sunflower Research Workshop: p.113. N.S.A. Bismarck, ND

Miller J.F., Seiler, G.J., and Jan C.C., 1992.- Introduced germpasm use in sunflower inbred and hybrid development. Use of Plant Introductions in Cultivar Development, Part.2., CSSA Special Publication No.20. 151-165.

Mihaljčević M., 1988.- Combining ability and heterosis is H. a n n u u s x H. a n n u u s (wild) crosses. Proc. of the 12 th International Sunflower Conference, Vol. II: 494-496. Novi Sad, (Yugoslavia) July 25-29.

Moreak E. and Berville A., 1991.- A dot blot assay for the routine determination of maintainer contaminants in cytoplasmic male sterile seed stocks of sunflower (H e l i a n t h u s a n n u u s L.) Plant Varieties and Seeds-4:31-36.

Moyne A-L.,Tagu D., Thor V. Bergounioux C., Freyssinet G. and Gadal P., 1989.- Transformed c a l l i obtained by direct

gene transfer into sunflower protoplasts. Plant Cell Reports, 8:97-100.

Olivieri A.M. Luchin M., and Parrini P., 1988- Self - sterility and incompatibility in sunflower. Proc. of the 12 th International Sunflower Conference. Vol. II. 339-344. Novi Sad (Yugoslavia) July 25-29.

Palla S., Vannozzi G.P., and Cremonini R., 1988.- Cytological and agronomic characters of a hybrids: H e l i a n t h u s a n n u u s L. x H. d e b i l i s Nutt. Proc. of the 12 th International Sunflower Conference, Vol.II: 281-282. Novi Sad (Yugoslavia) July 25-29.

Perez C., Fairre - Rampant P., and Berville A.- 1988.- Is the sunflower mitochondrial plasmid PLT also present in the Leolercgs CMS plants. Proc. of the 12 th International Sunflower Conference, Vol.II. 328-331. Novi Sad (Yugoslavia) July 25-29.

Perez C., and Berville A., 1988.- The mitochondrial plasmid of sunflower: its use to establish relationships for the H e l i a n t h u s p e t i o l a r i s species. Proc. of the 12 th International Sunflower Conference, Vol II. 324-328. Novi Sad (Yugoslavia) July 25-29.

Quresh, Z., Jan C.C., and Gulya T.J., 1991.- Genetic relationship between wild and cultivated species for rust resistance in sunflower. Proc. Sunflower research Workshop: p.p. 110-111. NSA Bismarck, ND.

Quresh,Z., Jan C.C., 1992.- Linkage and allelic relationships of recently identified resistance genes to prevailing sunflower rust races. Proc. Sunflower Research Workshop: p.p. 55-56. NSA: Bismarch. N.D.

Rieseberg L.H., Soltis D.E. and Palmer J.D., 1988.- A molecular reexamination of introgression between H e l i a n t h u s a n n u u s and H. b o l a n d e r i (Compositae). Evolution, 42 (2):227-238.

Rieseberg L.H., Soltis D.E. and Soltis P.S., 1988. Genetic variation in H e l i a n t h u s a n n u u s and H. b o l n d e r i. Biochemical Systematics and Ecology Vol. 16. Nr.4.: 393-399.

Rieseberg L.H., and Seiler G.J., 1990.- Molecular evidence and the origin and development of the demesticated sunflower (H e l i a n t h u s a n n u u s, Asteraceae). Economic Botany, 44 (3 supplement): 79-91.

Roath W.W., Pomeroy J.S. and Widrlechner M.P., 1988.- Effects of sunflower (H e l i a n t h u s a n n u u s L.) pollen storage conditions on pollen viability and progeny Mahl allelic fregrency. Proc. of the 12 th International Sunflower Conference Vol.II. 337-339. Novi Sad

(Yugoslavia). July 25.-29.

Rojas R.R., Charlet L.D., and Leopold R.A., 1991. - Biochemistry and physiology of overwintering in the mature larva of the red sunflower seed weevil, S m i e r o n y x f u l v u s leconte (Coleoptere:Curculionidae). J.Insect Physiol. Vol. 32. Nr. 7: 489-496.

Rogers,C.E. Thompson T.E. and Seiler G.J., 1982.- Sunflower Species of the United States. National Sunflower Assoc. Bismarck, ND,75 p.p.

Rovelo C., Angeles-Espino A., and Suarez G., 1988.- Obtencion de callo haploide de girasol (H e l i a n t h u s a n n u u s L.) medante el cultiva de anteras in vitro. Proc. of the 12 th International Sunflower Conference, Vol.II.:309-310. Novi Sad, (Yugoslavia), July 25-29.

Seiler G.J., 1988 - Influence of pH, storage temperature, and maturity on germination of four wield annual sunflower species (H e l i a n t h u s spp.). Proc. of the 12th International Sunflower Conference, Vol.II: 269-271. Novi Sad (Yugoslavia), Jully 25-29.

Seiler G.J., 1988 - The genus H e l i a n t h u s as a source of genetic variability for cultivated sunflower. Proc. of the 12th International Sunflower Conference, Vol.I: 17-58. Novi Sad (Yugoslavia), Jully 25-29.

Seiler, G.J., Pomeroy,J.S., Dozet,B., and Vera Gavrilova, 1990-Wild sunflower germplasm collected from Great Lakes Region of the United States. Helia, 13, Nr. 13, p.p. 21-27.

Seiler, G.J., 1990 - Protein and mineral concentrations in tubers of selected genotypes of wild and cultivated Jerusalem - artickoke (H e l i a n t h u s t u b e r o s u s, Asteraceae). Economic Botany, 44(3): 322-335

Seiler G.J. 1990.- Wild sunflower species (H e l i a n t h u s spp.) as potential sources of resfstance to pests. Proc. of sixth Great Plains sunflower insect workshop: 11-15. Fargo, ND, April 17-18.

Seiler G.J., 1991.- Registration of 13 downy mildew tolerant interspecific sunflower germpasm lines derived from wild annual species. Crop Science 31: 1714-1716.

Seiler G.J., 1991.- Registration of six interspecific sunflower germplasm lines derived from wild perennial species. Crop Science 31: 1097-1098.

Seiler G.J., Carr M.E. and Bagby M.O. 1991.- Renewable Resources from Wild Sunflowers (H e l i a n t h u s spp. , Asteraceae). Economic Botany, 45(1): 4-15.

Seiler, G.J., 1991.- Evaluation of interspecific sunflower

germplasms for on disease resistance. Proc. Sunfolower Research Workshop: p.p. 119-121 N.S.A. Bismarck, ND.

Seiler, G.J., 1991.- Registration of 15 interspecific sunflower germpasm lines derived from wild annuul species. Crop. Science. 31:1389-1390.

Serieys H. Hebel P., and Vincourt P., 1988.- Selection pour la tolerance a la secheresse sur un critere de type physiologique:reponse sur le comportement agronomique de genotypes de tournesol. Proc. of the 12 th International Sunflower Conference, Vol.II. 381-387. Novi Sad,(Yugoslavia) july 25-29.

Serieys H., 1991. - Identification study and utilization in breeding programs of new CMS sources (Report on past activities 1987-1990).FAO- Consultation of the European cooperative research network on sunflower. Pisa. Itly, 13-15 May.

Simmonds N.W., 1989.- How frequent are superior genotypes in plant breeding populations. Biol.Rev. 64: 341-365.

Schilling E.E., and Heiser Ch.B., 1981.- Intrageneric clasification of H e l i a n t h u s/C o m p o s i t a e). Taxon 3o (2) 393-403.

Spassova,M. Dudov, K., Atanassov, A., Nijkamp, H.J.J., and Hille,J. - 1991.- Molecular characterization of a new CMS genotype in sunflower. Sunflower Biotechnology in Europe: p.2o. Mittelwihr (France) 3-6 September.

Spring O., and Schilling E.E., 1990.- The Origin of H e l i a n t h u s x m u l t i f l o r u s and H. x l a c t i f l o r u s (Asteraceae). Biochemical Systematics and Ecology, Vol. 18.Nr.1:19-23.

Škorić D., Atlagić Jovanka, and Dozet B., 1988.- A collection of wild sunflower species and its use in a breeding program. Proc. of the 12 th International Sunflower Conference, Vol.II: 267-269. Novi Sad, (Yugoslavia) July 25-29.

Škorić D., Marinković r., and Atlagić Jovanka, 1988.- Determination of restorer genes for sources of cytoplasmic male sterility in wild sunflower species. Proc.of the 12 th International Sunflower Conference, vol.II: 282-287. Novi Sad (Yugoslavia) 25.-29. July.

Škorić D.,Dozet B., and Marinković R., 1989.- Using wild species in the process of development of sunflower hybrids. XII EUCARPIA Congres, 12-13 Gottingen (D).

Škorić D.,1991.- Sunflower genetics and breeding (Progress report 1988-1990.). FAO- Consultation of the European cooperative research network on sunflower. Pisa. Italy, 13-15 May.

Škorić D., and Rajčan I., 1992.- Breeding for S c l e r o t i n i a tolerance in sunflower. Savetovanje o unapredjenju uljarstva Jugoslavije (In Serbien): 1-9 Belgrade.

Tan,A.S., Jan,C.C. and Gulya T.J., 1991.- Genetic analysis of resistance to race 4 of sunflower downy mildew (P l a s m o p a r a h a l s t e d i i /Farl./ Berl and de Toni) fom wild sunflowers (H e l i a n t h u s a n n u u s L.) Proc. Sunflower Research Workshop: p.p. 123-124. N.S.A. Bismarck ND.

Tassie, A., Schwall, M. and Langridge, P.,- 1991.- Tissue culture and transformation of sunflower. Sunflower Biotechnology in Europe: p.12. Mittelwihr (France), 3-6 September.

Vannozzi G.P., Megale P., and Salera E., 1990.- Germination ability of achenes of H e l i a n t h u s g e n u s wild species interspecific hybrids. Helia, 13, Nr. 13, pp. 135-142.

Vannozzi G.P., Megale P., and Salera E., 1990.- H e l i a n t h u s g e n u s wild species interspecific hybrids: morphological and technological characters. Helia, 13, Nr.13, p.p.127-133.

Vranceanu A.V., Monica Iuoras, and Stoenescu F.M., 1988.- Genetic study of short petiol trait and its use in sunflower breeding. Proc. of the 12 th International Sunflower Conference, Vol.II: 429-435. Novi Sad, (Yugoslavia) July 25.-29.