

# Old and New Trends of Using Genetic Resources in Sunflower Plant Breeding With The Aim of Preserving Biodiversity

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## *Abstract*

Cultivated sunflower belongs to *Asteraceae* (*Compositae*) family or composite, genus *Helianthus*, and there are two primary types of cultivated sunflower: oil seed sunflower and non-oil seed, confectionary sunflower. The oil seed type is grown to make vegetable oil, while non-oil seed type is grown for bird food and confectionery market. Gene variability within a species presents a genetic diversity which in plant breeding enables the development of new improved varieties with improved characteristics. According to the newest taxonomy research, genus *Helianthus* comprises 51 wild species, 14 of which are annual and 37 perennial. Annual species are diploid and genetically closer to the cultivated sunflower. Perennials have different levels of ploidy (diploid, tetraploid and hexaploid), they are genetically divergent, difficult to breed with the cultivated sunflower and usually very distant from it. A large number of researchers stress the need for more frequent use of wild species, genus *Helianthus* in search of specific genes responsible for the resistance to diseases and insects, tolerance to stress and herbicides as well as means of their transfer to cultivated sunflower genotypes. Owing to the use of wild species in breeding, the genetic variability of the cultivated sunflower has been significantly improved.

**Key words:** breeding, diversity, genetic resources, sunflower

## *Introduction*

Biodiversity, biological diversity, represents a variety of species inhabiting a certain area, or a certain ecosystem. The term “biodiversity” is not only limited to “plant species richness”, but is also related to all living components in the flora and also includes the interaction of organisms with each other and the environment they inhabit (Buchs, 2003). Genetic diversity contributes to long term preservation of cultivated species by allowing them to rapidly adapt to changes in their environment (Ramanatha Rao and Hodgkin, 2002). Typically, wild relatives of crop plants are genetically much more diverse than cultivated lineages and constitute a genetic resource useful to increase the germplasm biodiversity (Harlan, 1992). The importance of genetic diversity (biodiversity) in plant breeding was recognized in the 1960s and Sir Otto Frankel coined the term “*genetic resources*” in 1967 to highlight the relevance and need to consider germplasm as natural resource for the long-term breeding of crop plants (Yunbi, 2010). Nevertheless, the global spread of commercial cultivars is reducing the genetic diversity needed to continue crop improvement. Cultivated sunflower originates from the wild annual species of sunflower. Through evolutionary time and agronomic selection, the well recognized sunflower plant has evolved. The use of classical breeding has resulted in the inter-crossing of wild and cultivated sunflower. Seiler (2012) concluded that changes in plant habitats and distribution of species occur as consequences of natural adaptation and natural selection in the genus *Helianthus*. It all affected sunflower, where it resulted in narrowing of genetic variability for many agronomical important traits. In natural ecosystems, the ability of plants to survive environmental stress is probably

more important than high achene productivity. The genetic diversity of the wild species can make a significant contribution to the global sunflower industry by providing genes for resistance (tolerance) to pests and environmental stress, allowing the crop to become and remain economically viable.

### ***Importance, distribution, history, origin and taxonomy of sunflower***

**Importance and distribution.** Cultivated sunflower (*Helianthus annuus* L.) is one of the most important oil crops in the world. Two primary types of cultivated sunflower exist: oil seed sunflower and non-oil seed confectionery sunflower (Duihua and Hoefl, 2009). Oil seed type is grown for vegetable oil and non-oil seed type to supply the bird food and confectionery market. Seeds of confectionery and oil type are distinguished by hullability, shell color, seed weight and morphology and kernel-to-pericarp weight ratio, in addition to seed oil content (Hladni et al., 2012a). Sunflower oil, according to its nutritional value belongs to the group of best plant oils used in human nutrition (Škorić et al., 2008). Sunflower utility for confectionery purpose and also for export has been realized recently. Sunflower is grown in large developed countries as well as developing countries. Today sunflower is grown on over 25,433,000 t ha<sup>-1</sup> and an average yield of 1.49 t ha<sup>-1</sup> (<http://www.sunflowerusa.com/stats/world-supply/2012>). It is grown in 62 states. The distribution of sunflower growth in the northern hemisphere is between 30° and 55° of the northern geographic latitude, and on the south hemisphere from 10° to 40° of the south geographic latitude. The main cause of the variation stagnation and diminishing of the sunflower covered surfaces is the vulnerability of the sunflower to the economically important diseases in certain parts of the world related to agro-ecological conditions (Vratarić et al., 2004). The areas under sunflower and the zeal changes annually in Serbia, and are between 160 to 210 thousand hectares, and the seed yield is 1.7-2.3 t ha<sup>-1</sup>, so by the sunflower covered area Serbia is in the 7<sup>th</sup> place in Europe, and when it comes to yield right behind France and Hungary (Miklić et al., 2007).

**Sunflower history.** The sunflower plant originates from America; according to archaeological findings the sunflower plant was known to Indians around 3,000 years B.C. It was used for nutrition, medicine and in rituals (Putt, 1997). Sunflower arrived to Europe in the 16<sup>th</sup> century, first to Spain and from there it was transferred to France, England, Germany and other European countries. The beginning of breeding on scientific bases dates back to 1912, when the first Russian populations with high oil content were created. First written data about sunflower as an oil plant culture were found in Russia in 1818. The mass production of sunflower as an oil culture began in Russia around 1830s. Sunflower in Serbia began to be cultivated at the beginning of 1930s when foreign varieties and the local populations were first grown.

Breeding in Serbia started in the sixties. One of the centres for sunflower breeding was the Institute of Field and Vegetable Crops (IFVC) in Novi Sad, where the breeding material included local varieties brought in from Romania, Bulgaria, Hungary and Russia. That is when the new varieties were created which meant the beginning of sunflower breeding in Serbia (Jocić et al., 2012). NS varieties had low oil content and no resistance to broomrape, so they were soon replaced with Russian varieties which were very productive, had high oil content and resistance to broomrape. At the IFVC, intense work on sunflower breeding had continued along with the creation of domestic hybrids which are high-oil, resistant to pathogens and to broomrape (Jocić et al., 2012). The advantages that the hybrids have over the sort populations are the larger productivity, more period of maturity and the lower humidity content harvesting, more plant height, eased gene input for tolerance to disease and vermin.

**Origin and taxonomy.** Cultivated sunflower belongs to *Asteraceae* (*Compositae*) family or composite, genus *Helianthus* which includes several species and one of them is *Helianthus annuus* L. The largest number of species can be found in Canada, USA and northern Mexico. Common sunflower, *Helianthus annuus* appears in three most important forms: *H. annuus* ssp. *lenticularis*, „wild” sunflower; *H. annuus* ssp. *annuus*, „weed” sunflower and *H. annuus* ssp. *macrocarpus*, „giant” sunflower cultivated

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for edible seed (Heiser, 1955). Genus *Helianthus* has the basic chromosome number  $n=17$ , it represents polyploid complex which consists of diploid  $2n=2x=34$ , tetraploid  $2n=2x=68$  and hexaploid  $2n=2x=108$  species (Jan, 1997). According to Schilling and Heiser (1981), the genus *Helianthus* comprises 49 species, 11 annuals and 38 perennials, divided on the basis of mutual similarity in four section and four series. According to the newest taxonomy research, genus *Helianthus* comprises 51 wild species out of which 14 annual and 37 perennial (Seiler et al., 2008). Annual species are diploid and genetically closer to the cultivated sunflower and they are easier to cross. Species have different levels of ploidity (diploid, tetraploid and hexaploid), they are genetically divergent, difficult to cross with the cultivated sunflower and usually very distant from it. Owing to the use of wild species in breeding, the genetic variability of the cultivated sunflower has significantly improved (Seiler, 1992). A large number of researchers stress the need for more frequent use of wild species genus *Helianthus* in the search for specific genes for the resistance to diseases and insects, tolerance to stress and herbicides. The genus *Helianthus* is large and polymorphic. Sunflower systematics and taxonomy have been subject to continual changes and amendments.

## ***Genetics and sunflower breeding***

The main aim of plant breeding is to develop new varieties and hybrids to meet the needs of people and domestic animals. Due to the rapid growth of the human population, loss of arable land, global climate change, and water supply problems, the production of sufficient amounts of food will be a challenge in the future. The increase of yields of cultivated plants requires not only the development of new, more productive genotypes but the advancement of growing technology as well (Jocić et al., 2011). Objectives in sunflower breeding programs vary with specific programs, but generally place emphasis on high seed yield and high oil content. For special markets, breeding objectives may be slightly different (Fick and Miller 1997). When creating confectionery hybrids it is very important to combine genes responsible for high yield potential and good technical and technological traits of the seed (Hladni et al., 2011d). For confectionery sunflower the main direction in breeding is towards increased 1000 seed weight, protein content and quality, while lowering the seed oil content and shell ratio (Hladni et al., 2011c). The major sunflower breeding objectives for all sunflower types should be high yields and quality of oil, proteins and other products for non-food industries and approaches to management of resistance genes, stability of sunflower resistance to certain pathogens (Škorić et al., 2012).

### ***Major breeding goals***

***Heterosis.*** Sunflower is a very cross-pollinated plant species with dipolar flowers. The main prerequisite for getting productive sunflower hybrids is that manifestation of heterosis, superiority of  $F_1$  hybrids compared to the parental inbred lines for agronomical important traits is confirmed in previous research (Škorić et al., 2000, 2007; Hladni et al., 2007). The occurrence of heterosis in sunflower hybrids is highly correlated with genetic distance between the parental lines. Heterosis does not appear in all hybrid combinations of the  $F_1$  generation. Heterotic effects are different for different traits (Hladni et al., 2007).

***Desirable plant architecture and direct yield components.*** The creation of productive sunflower hybrids demands a formation of a model of hybrids for certain agro-ecological conditions while determining the priority when breeding for most important traits. This can be achieved by planned creation and the use of the sunflower's genetic variability (Hladni, 2010).

Knowing the genetics of trait inheritance, the number of genes controlling the expression of a particular trait and interdependence of morphophysiological traits with yield is of utmost importance in order for their breeding programs to be successful (Škorić et al., 2012). Knowledge on seed yield

(Marinković et al., 2000; Škorić et al., 2000; Hladni et al., 2011a), seed oil content (Škorić et al., 2000; Hladni et al., 2006b), oil yield (Škorić et al., 2000; Gvozdenović, 2006; Hladni et al., 2008b), as well as important morphophysiological traits: petiole angle (Hladni et al., 2000), petiole length (Marinković et al., 1994; Hladni et al., 2002), total leaf number (Hladni et al., 2003), total leaf area per plant (Škorić, 1985; Hladni et al., 2006a), stem diameter (Marinković et al., 1994; Hladni et al., 2008a), plant height (Marinković et al., 2000; Hladni et al., 2004), head diameter (Joksimović et al., 2000; Hladni et al., 2004), 1000 seed weight (Hladni et al., 2007), total seed number per head (Marinković, 1984; Hladni et al., 2012a), seed size (Jocić et al., 2000), enable the appropriate choice of parental lines.

Fick and Miller (1997) point out that for the correlation to be of value in developing superior inbred lines the same characters (traits) in their hybrids are necessary. Correlations between different traits are aspects that should be kept in mind for better planning of breeding programs in sunflower (Hladni et al., 2010a). Studied as the seed yield, seed protein content and 1000 seed weight are the traits that have demonstrated a strong positive correlation with protein yield and can be used in improvement of seed protein yield as well as for assessment of confectionery sunflower breeding materials (Hladni et al., 2011c). The interdependence of seed yield and the direct and indirect components of seed and oil yield were examined. A positive and important interdependence was determined among morphophysiological traits like total leaf area, plant height, head diameter, mass of 1000 seeds, total number of seeds per head with seed yield (Marinković, 1992; Dušanić et al., 2004; Hladni et al., 2008c, 2010a) and oil yield Hladni et al. (2008d). In their work on early harvesting, Miklič et al. (2012) determined that the yield increased gradually until the midpoint of the observed period from flowering to maturity. The 1000 seed weight increased in an almost linear fashion until the last harvesting date. Presence or absence of correlations can contribute to the right choice of examined traits so as to enhance the efficiency of some selection criteria. The focus should be placed on traits that have a very strong positive correlation on seed yield (Hladni et al., 2011b).

### ***Tolerance to biotic and abiotic stress conditions***

***Resistance to diseases and broomrape.*** Diseases are the main limiting factor in the production of sunflower (*Helianthus annuus* L.) and they cause poor realization of genetic yield potential of sunflower hybrids (Jocić et al. 2010). Different diseases are dominant in different regions, depending on the prevailing environmental conditions. More than 30 different pathogens that attack sunflowers and cause economic loss in production have been identified so far (Škorić et al., 2012). Both vertical and horizontal genetic resistance mechanisms have been identified in wild sunflower species and determined resistance genes, especially in *Plasmopara halstedii*, *Phomopsis*, *Phoma* black stem and *Verticillium* wilt, resistance breeding overcome these diseases and resistant cultivars are planted greatly in the market (Kaya et al., 2012). Sunflower breeders have achieved significant results in finding genes for resistance or high tolerance to certain diseases in wild species and in incorporating them into the cultivated sunflower genotypes.

Broomrape (*Orobanche cernua* Loeffl.) has been the most serious problem in sunflower production in Southern and Eastern Europe, leading to considerable yield losses up to 100% and reducing sunflower seed quality. Furthermore, this parasite is developing new and more virulent races year by year which overcome the resistance of the varieties and hybrids commonly used in production (Kaya et al., 2012). Since broomrape is a highly variable parasite, the breakdown of resistance is a frequent phenomenon, and multiple sources of resistance are needed (Seiler, 2012). The sunflower breeding program at IFVC has been directed towards creating lines and hybrids which are resistant to new broomrape races. Continued work on creating new sunflower hybrids resistant to broomrape demands the screening of breeding materials for resistance in both field conditions and in controlled conditions of a greenhouse (Hladni et al. 2012b). Cultivated sunflower is genetically narrow and deficient in many desirable genes. Its genetic variability can be increased by the use of wild sunflower species and interspecific hybridization (Hladni et al., 2009). Breeding of sunflower inbred lines originating from interspecies populations DES-1474-1, DES-1474-2 and DES-1474-3, with the objective of achieving

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drought tolerance, started in 1994 at IFVC. Apart from the analysis of important agronomic traits, the new restorer lines were tested for resistance to broomrape. The testing of restorer inbred lines was conducted from 1995 to 2004, which resulted in 5 restorer inbred lines derived from interspecific population DES-1474-1, resistant to broomrape race E and possibly race F, and indicated that this population can be used for the production of new resistant sunflower hybrids. Hladni et al. (2009), Hladni et al. (2010b), Hladni et al. (2012b) conclusion that the RHA-D-7 and RHA-D-8 are Rf inbred lines developed from interspecific population with *H. deserticola* - DES-1474-1, are resistant to broomrape race E and possess gene *Or<sub>5</sub>*.

Fernandez-Martinez et al. (2008) reported that sunflower germplasm evaluation for resistance to broomrape races has demonstrated that the *Helianthus* species constitute the major reservoir of genes conferring resistance to new virulence races.

One of the approaches used in sunflower breeding for resistance to broomrape is producing IMI hybrid resistant to certain groups of herbicides. IMI-resistant hybrids are very important in regions where new races of broomrape have occurred (Škorić et al., 2012).

**Resistance to herbicides.** Herbicide-resistant crops are becoming increasingly common in agricultural production. Resistance to herbicides has great potential for producers in all region of the world for controlling several broadleaf weeds (Seiler, 2012).

Although weed control with transgenic herbicide-resistant genes have been used widely in some crops in the world, only imidazolinone (IMI) and sulfonylurea (SU) herbicide resistance which is transferred to cultivated sunflower from wild types utilizing backcross breeding is used commonly in sunflower (Kaya et al., 2012). Imidazolinone resistance in wild sunflower populations sunflower was first identified in Kansas in 1996, Miller and Al-Khatib (2000) reported that the USDA-ARS (NDSU) research team quickly transferred this genetic resistance into cultivated sunflowers and released public „IMISUN“ lines 1998. First, SU-resistant lines were developed in similar way with IMI resistance using classical backcrossing method from wild types in Kansas (Miller and Al-Khatib, 2004).

Creation and development of tribenuron-methyl resistant hybrids would enable the use of a wider palette of herbicides for sunflower, more efficient chemical control of *Cirsium arvense* and more economically profitable post-emergence control of some annual broad-leaves weeds in sunflower (Jocić et al., 2011).

**Resistance to abiotic stress.** The challenge for the sunflower breeding community is to breed sunflower adaptable to these marginal environments and at the same time to increase seed yield. Sunflower cultivation continues to be pushed into low-fertility soils and other marginal environments where drought and high or low temperatures continually take their toll on the yield per unit area (Seiler, 2012). Because of the specific structure of its main organs (root, stem leaves and head) sunflower is more resistant to abiotic stress conditions than other crops (Škorić, 2009).

Sunflower wild species grow in various habitats, some in swampy areas while others in desert habitats. One of the sunflower breeding directions is increased tolerability towards drought and high temperatures, which is why it is important for those breeding programs to use wild species like *Helianthus* (Fick and Miller, 1997; Škorić, 2009). Up to now most used species for drought resistance breeding was *H. argophyllus*. When choosing wild species it is important to use ones that inhabit desert areas, like *H. deserticola* whose name demonstrates the kind of conditions it can withstand. Apart from draught conditions, land salinity is another determining factor, although sunflower is grown in many countries that have soil of mild salinity. High tolerance to soil salinity is found in three wild species of *Helianthus* (*H. paradoxus*, *H. debilis* and wild *H. annuus*), and we can assume that they possess genes for salt tolerance (Seiler, 2012). The adaptation of these species to their harsh habitats allows the potential to exploit these traits for improving stress tolerance in the cultivated sunflower crop.

## ***The possibility of using genetic resources in sunflower breeding***

Sunflower germplasms are very important sources for plant breeding, consisting of genetic variability from cultivated ones to wild species with keeping *ex situ* (accessions preserved in seed banks) and *in situ* resources (wild populations and land races) (Kaya et al. 2012), the cultivated genetic resources in the following group: local population/landraces, obsolete cultivars, cultivars in production, breeding lines, composite varieties, synthetic varieties, wild species from genus *Helianthus* (Škorić et al., 2012). Public lines, land races and open pollinated varieties are also very important resources for sunflower breeding that comprise unique morphological and physiological traits (Kaya et al. 2012). Local populations are well adapted to local soil types, climatic conditions and other factors and they are the source of many desirable genes. Old cultivars possess certain desirable genes and are sometimes very useful in solving certain problems in sunflower production thought breeding. Wild species from the genus *Helianthus* typically possess genes for resistance to diseases (biotic stress), tolerance to abiotic stresses (drought, cold, soil salinity, certain herbicides) and high quality of proteins and oil.

Significant progress has been made in collecting and preserving wild species, understanding the origin, domestication increasing the genetic diversity and organization of the genetic diversity characterization and screening methods for abiotic and biotic stresses in sunflower so far, only a small portion of the available diversity has been exploited (Seiler, 2012).

The first step to insuring the success of a breeding program is the gathering and examining of genotypes while forming a genetic collection that enables a wide genetic variability in the selection material depending on the breeding direction (Miklić et al., 2008).

There is a continued need to collect, maintain, evaluate and enhance wild *Helianthus* germplasm for future improvement of cultivated sunflower. The genetic diversity of the wild species can make a significant contribution to the global sunflower industry by providing genes for resistance (tolerance) to the pests and environmental stresses.

***Collections of wild sunflower species.*** The main reason for establishing a collection of wild sunflower species is the reduced genetic variability of the domesticated sunflower for a number of agronomic characteristics, especially resistance to diseases. Collection of germplasm not only preserves valuable germplasm, but also provides information about the diverse habitats occupied by wild sunflowers and associated species. The wild species are adapted to a wide range of habitats and possess considerable variability for most agronomic and achene quality characters, and reaction to insects and disease pathogens. Knowledge of a particular habitat adaptation of a species often can help to identify potential sources for a desired trait (Seiler, 2012).

There are several important sunflower collections in the world, which provide breeders with sunflower germplasm possessing genetic variability necessary for successful realization of their breeding programs. The largest collection in the world in terms of genotypes of cultivated sunflower is maintained at the All-Union Scientific Research Institute of Plant Industry – N.I. Vavilov (VIR), St. Petersburg, Russia. The second largest collection belongs to USDA National Germplasm-Plant Introduction Station, Ames, Iowa, USA. Notable collections of wild sunflower species are maintained at IFVC, Novi Sad, Serbia; INRA, Montpellier, France; INTA, Pergamino, Argentina; Inta, Cordoba, Spain; VIR St. Petersburg, Russia. Regarding the germplasm of cultivated sunflower, the major collections are located at IFVC, Novi Sad, Serbia; Yurevo, Kharkov, Ukraine; VNIIMK, Krasnodar, Russia; Fundulea, Bulgarian National Institute for Field Crops, General Toshevo, Bulgaria (Škorić et al., 2012). At IFVC a collection has been comprised from annual and perennial wild species from genus *Helianthus*. A major part of the collection of wild sunflower species was collected in the course of collecting trips organized in different federal states of the USA in the period 1980-1991. Researchers from Novi Sad and Fargo took part in these trips. Additional seed samples were obtained from two more collecting trips, to Canada and Montenegro, and from other collection centres and gene banks during the 7 collecting trips conducted from 1980 to 1991, 917 accessions were collected. Currently in the collections there are 21 perennial and 7 annual species (Atlagić et al., 2006). The collection

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is intensely used in the sunflower breeding using the interspecies hybridization method from the transferral of desirable genes from the wild species to the cultivated sunflower (Miklić et al., 2009).

***Interspecific hybrids.*** The term “interspecific hybridization” implies the crossing between different species of the same genus. Interspecific hybridization is typically used for transferring traits including resistance to disease agents, soil salinity and acidity, and drought as well as for finding new sources of *cms* and *Rf* genes and the development of new sunflower ideotypes (Seiler, 2012). Breeding for seed yield components and the creation of a new sunflower ideotype require an increased use of wild *Helianthus* species in breeding programs (Hladni et al., 2011a).

Wild annuals are seldom used in interspecific hybridization programs because they are as sensitive to major diseases as the cultivated sunflower. Using conventional methods of crossing, backcrossing and selection, several pre-bred lines with altered plant architecture, high yield and oil content, maturity duration and inbuilt tolerance to major biotic stresses have been developed from crosses involving diploid annuals.

The results of the application of *interspecies* hybridization in the breeding of cultivated sunflower are of the biggest importance in the creation of hybrids resistant to the economically important diseases. The sources of the resistance were mostly perennial wild sunflower species.

Hladni et al. (2012a) used in the study 13 new divergent (A) *cms* inbred lines, 3 *Rf*-restorer lines, 39 F<sub>1</sub> hybrids developed at the IFVC in Novi Sad. Four interspecies populations, originating from three annual (*H. debilis*, *H. praecox runyonii*, *H. deserticola*) and one perennial (*H. resinosus*) wild species, were used to produce 13 new CMS inbred lines (NS-G-1, NS-G-2, NS-G-3, NS-G-4, NS-G-5, NS-G-6, NS-G-7, NS-G-8, NS-G-9, NS-G-10, NS-G-11, NS-G-12, NS-G-13). Initially the plants were selected from the interspecies population RES-1, DEB-SIL-367-2, PRA-RUN-1321 and DES-1474-2, provided by Dr Gerald Seiler (USDA-ARS, Fargo ND, USA). Studying general combining ability (GCA) in relation to the origin lines, we can observe that the lines of interspecies populations PRA-RUN the best general combiners for plant height and seed yield per plant, lines from interspecies population DES are the best general combiners for head diameter, while the lines originating interspecies populations DEB-SIL and RES the best general combiners for total seed number per head. The exception is a line from interspecies population DES NS-G-11 which is the best general combiners for total seed number per head. The worst GCA for plant height, total seed number per head and seed yield per plant have lines originating from interspecies population DES, while for plant height, head diameter and seed yield per plant have lines originating from interspecies population RES. It is very difficult to combine all positive traits in one combination (Hladni et al., 2012a).

### ***New trends of using genetic resources in sunflower plant breeding***

The wild *Helianthus* species provide a tremendous genetic diversity for sunflower breeding. Interest in using wild species in breeding programs has increased. The development and application of cytogenetic studies have been associated with the utilization of the germplasm of the genus *Helianthus* for improvement of the genome of the cultivated sunflower. Cytogenetic studies are used for determinations of chromosome number and structure and analyses of meiosis (microsporogenesis) and pollen viability, making it possible to establish phylogenetic relations between wild sunflower species and the cultivated sunflower and enabling the use of the former in sunflower breeding. Cytogenetic studies of the sunflower have evolved from cytology, through cytotaxonomy and classic cytogenetic to cytogenetic-molecular studies. Most intensive progress of cytogenetic studies has been associated with the use of interspecific hybridization in sunflower breeding (Seiler, 2012). Chromosome markers may be used for taxonomic purposes, for identification of chromosomes originating from different genomes, or for monitoring of introgression of an alien chromosome into the cultivated sunflower (Werner et al., 1992).

Wild species will be evaluated for various agronomic traits, such as insect and disease resistance, saturated fatty acid content, cytoplasmic male sterility, and fertility restoration. DNA markers will be identified and used to reveal genetic diversity in the wild *Helianthus* collection.

With the development of molecular techniques and structural and functional genomics knowledge, the research on sunflower has benefited from various advanced studies, including molecular markers and linkage maps, cDNA, libraries, BAC libraries and gene cloning (Zhao and Chao-Chien, 2012). Molecular markers are useful tools for studying the genetic diversity because of their stability and polymorphism in DNA throughout the whole genome (Škorić et al., 2012). They do not depend on the environment and could be detected in all stages of plant development (Mohan et al., 1997). Molecular markers and genetic maps provide two important tools for gene identification and genotype discrimination and thus are helpful for MAS during crossing screening and evaluation (Wenzel, 2006).

Using a combination of molecular markers, genetic maps, genomics and bioinformatics data and other developing techniques, many sunflower traits of interest can be characterized including the oil quality control, *cms* and fertility restoration genes, and quantitative trait loci (QTL) for disease, resistance and abiotic stress resistance. Some tightly linked or co-segregated markers could be used for MAS (Zhao and Chao-Chien, 2012). Molecular data on the origin and development of the cultivated sunflower are alarming, indicating that the possibility for further evolution of this economically important crop is limited (Rieseberg and Seiler, 1990).

PCR analysis could effectively classify and identify species most related to *H. annuus* which will be used for the improvement of cultivated sunflower (Sivolap and Solodenko, 1998).

Following classical genetics, it has been assumed that the controlled crosses of wild and cultivated sunflower resulted in a mixture of the genomes from the two parents. New molecular techniques now allow us to follow the genomes in these crosses and much to our surprise these interspecific crosses are not following the strict genetic rules. There appears to be much less mixing of the two species than previously thought, and less chromosomal rearrangements. It has been hypothesized that the lack of rearrangement is a mechanism to preserve the species and a prevention mechanism from cross-breeding of the different species of wild sunflowers. Armed with this knowledge and the development of newer techniques, in the future we should be able to pinpoint the desirable genes and trace their transfer from the wild species to the cultivated sunflower more efficiently (Seiler, 2012). To keep sunflower an economically viable global crop, researchers must strive to combine the best conventional and modern molecular approaches available. The combination of conventional and molecular breeding will shorten the time to obtain desired varieties for agriculture. This will require a multidisciplinary team approach and a commitment to a long-term integrated genetic improvement program. Combining all applicable methods, materials and knowledge together will help provide food security in the 21<sup>st</sup> century (Zhao and Chao-Chien, 2012).

## Conclusions

Growth of the world population and climate changes make it much more critical to improve the yield and quality of sunflower than ever before. Genetic diversity exists in wild sunflower species, including many from extreme environments, wild *Helianthus* species serve as potential sources of novel genetic variability and several desirable characteristics such as resistance to biotic and abiotic stresses, cytoplasmic male sterility, fertility restorer genes and oil quality have been successfully integrated into cultivated sunflower. The narrow genetic base of cultivated sunflower will be broadened by the infusion of genes from the wild species, which will provide a continued source of desirable agronomic traits. There is a global interest in the utilization of the wild sunflower species to improve cultivated sunflower. Armed with this knowledge and with the development of newer techniques, in the future we should be able to pinpoint the desirable genes and trace their transfer from the wild species to the cultivated sunflower more efficiently.

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