Sunflower germplasm development utilizing wild *Helianthus* species

Chao-Chien Jan[1], Gerald J. Seiler[1], Thomas J. Gulya[1], Jiuhuan Feng[2]
[1]U.S. Department of Agriculture, Agricultural Research Service,
Northern Crop Science Laboratory, 1307 18th Street North, Fargo, ND 58105, USA,
E-mail: chaochien.jan@ars.usda.gov; gerald.seiler@ars.usda.gov; thomas.gulya@ars.usda.gov
[2]Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA,
E-mail: jiuhuan.feng@ndsu.edu

ABSTRACT

The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continued source of agronomic traits for crop improvement. The genus *Helianthus* comprises 51 species (14 annual and 37 perennial), all native to North America. The available genetic diversity from the wild species is continuing to be used to broaden the genetic background of the crop. Recent advances in culturing of otherwise abortive interspecific hybrid embryos have proved to be highly effective for making the difficult-to-cross wild perennial *Helianthus* species widely available for breeding purposes, either for specific major gene transfer or for the transfer of quantitative trait genes. These techniques are discussed and illustrations are shown of how they are being used to incorporate genes from several different ploidy levels of wild perennial species into cultivated sunflower for Sclerotinia stalk rot resistance and other diseases. Significant results have been reported on the germplasm development with regard to resistance to new races of downy mildew, rust, broomrape and other major diseases. In addition, new CMS and corresponding fertility restoration genes have been continuously identified and established, together with new genes helping to improve oil quality, herbicide resistance, and salt and drought tolerance. Thus far, only a small portion of the available genetic diversity of the wild *Helianthus* species has been used globally. As a whole, there is no doubt that wild *Helianthus* species will continue to provide new genetic variability to the sunflower breeding community, helping to maintain sunflower as a viable major global oilseed crop.

Key words: amphiploids – genetic diversity – genetic resources – *Helianthus* – interspecific hybridization.

INTRODUCTION

Sunflower production continues to face challenges from both abiotic and biotic factors as well as from today's ever-changing market needs. For the most part, the crop has been doing fairly well thus far. However, the limited genetic variability in cultivated sunflower has slowed the future improvement of the crop, and has placed the crop in a vulnerable position should any major shifts of disease races or pests occur. The uniform use of a single CMS PET1 cytoplasm and a few fertility restoration genes for worldwide sunflower production makes the crop extremely vulnerable. Diversity of resistance to various diseases is strategically needed. We have seen the rapid increase in the number of rust races being identified in Australia in recent years. The continuing race shift of broomrape in Spain, Turkey and the Black Sea areas since the mid-1990s has kept researchers busy for over 10 years searching for new resistance genes. For a while, the predominant rust and downy mildew races in the USA were limited to three or four, but many new races of these two diseases have been identified in the last 10 years. All the new races have the potential of becoming the predominant races in the future in response to our introduction of resistance genes. Sunflower is not always grown on prime land, but often on marginal land with minor salt and drought problems, presenting a challenge to be productive under less than ideal conditions. An early season sunflower crop has the potential to increase production by increasing the double crop potential for producers. In order to be competitive with other premium oils in the market, the reduction of saturated fatty acids in sunflower oil will play a key role in maintaining our continuing success. Evaluations of wild species have provided information about useful genes for future sunflower improvement. However, there are still numerous genes in wild sunflower species yet to be identified and introgressed into cultivated sunflower. Extensive collection efforts for wild *Helianthus* species and the regeneration of seeds at the USDA-ARS, Regional Plant Introduction Station at Ames, Iowa have greatly increased the availability of wild *Helianthus* seed for sunflower improvement. An overall advancement of our understanding of wild *Helianthus* species and improved methods of making interspecific crosses have

increased the number of useful genes available from wild *Helianthus* species, making it possible to transfer genes that were not possible three decades ago. This report will discuss the importance of wild *Helianthus* species and their utilization for sunflower improvement in the past and present, and show examples from our current wild species breeding program, and future prospects.

DISCUSSION

Helianthus collection. The USDA-ARS National Plant Germplasm System (NPGS) sunflower collection is maintained at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. The collection contains 37 perennial species, 14 annual species, and the cultivated species, *Helianthus annuus* (Schilling, 2006). This NPGS sunflower collection is a diverse assemblage of 3850 accessions: 1708 cultivated *Helianthus annuus* accessions, 932 wild *Helianthus annuus* accessions, 437 accessions representing 11 other wild annual *Helianthus* species, and 773 accessions representing 37 perennial *Helianthus* species. This collection is the largest and most genetically diverse sunflower collections in the world and it is vital to the conservation of *Helianthus* germplasm. From 1976 to 1996, 10,000 samples of wild sunflower were distributed to 300 researchers in 30 countries. These accessions have become the basis of wild species research programs in Argentina, France, Italy, Spain, Germany, Bulgaria, Romania, Czechoslovakia, Hungary, Russia, Yugoslavia, India, China, and Mexico.

Notable is the collection at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, which contains 39 of the 51 wild species (IBPGR, 1984; Cuk and Seiler, 1985). The wild species collection of the Dobroudja Agricultural Institute (DAI) at General Toshevo, Bulgaria, is also notable, containing 428 accessions representing 37 of the 51 species of *Helianthus* (Christov et al., 2001). The wild species collection maintained at INRA, Montpellier, France has more than 600 accessions of 45 of the 51 wild sunflower species (Serieys, 1992). The Instituto de Agricultura Sostenible (CSIC) Cordoba, Spain maintains 44 annual and perennial accessions of *Helianthus* (Ruso et. al., 1996).

***Interspecific hybridization*: *The early years*.** Prior to the embryo culture method developed by Chandler and Beard (1983), nearly all the interspecific crosses were conducted in a classical fashion. All the annual *Helianthus* species, except *H. agrestis*, can be hybridized and F_1s backcrossed with cultivated lines using classical breeding methods. Direct crosses of cultivated lines with many perennial *Helianthus* species are also possible using conventional methods. Hybrids of *H. mollis* Lam. x *H. annuus* L. and *H. strumosus* L. x *H. annuus* (Heiser and Smith, 1964) and of *H. decapetalus* L. x *H. annuus* (Heiser et al., 1969; Georgieva-Todorova, 1984) have been reported. Hybrids of *H. tuberosus* L. x *H. annuus* (Heiser et al., 1969; Atlagić et al., 1993), *H. annuus* x *H. hirsutus* Raf. (Georgieva-Todorova, 1984), and *H. rigidus* (= *pauciflorus*) Nutt. x *H. annuus* (Vrânceanu and Iuoras, 1988) have also been successful. Atlagić (1990) summarized five interspecific hybrids involving crosses of perennial species *H. hirsutus*, *H. laevigatus* T. and G., *H. rigidus* (= *pauciflorus*), *H. tuberosus*, *H. maximiliani* Schrad., and *H. nuttallii* T. and G. with cultivated sunflower. Whelan (1978) used wild *H. annuus* as an "intermediate" parent or "bridge" to produce the first hybrids obtained between cultivated sunflower and *H. giganteus* L. and *H. maximiliani*.

***Interspecific hybridization*: *Utilizing embryo rescue*.** The development of a two-step embryo culture procedure by Chandler and Beard (1983) greatly facilitated interspecific hybridization. They successfully produced 53 interspecific cross combinations without the exhaustive effort of endless pollination, and 21 of these combinations had not been previously produced. Jan and Chandler (unpublished data) further modified the original procedure for culturing difficult hybrid embryos of wild perennial *Helianthus* species with cultivated *H. annuus* by adding vitamins, increasing sucrose to 20 g/kg, and the conversion from liquid to a solid medium with 0.7% agar. In addition, both growth and germination media were adjusted to pH 5.5 with 2-[N-morpholino]ethanesulfonic acid (MES) buffer. Using these modified media, 18 perennial species x *H. annuus* hybrids were established in one season, and many of them represented the first hybrid combinations ever produced (Jan, 1988).

Kräuter et al. (1991) cultured 0.2 to 1.5-mm small embryos on B5 medium with 90 g/kg sucrose, and embryos >1.5 mm on a modified MS (Murashige and Skoog, 1962) medium with 10 g/kg sucrose. When these embryos reached the size of 2 to 3 mm, they were transferred to MS medium for germination. Using this method, they obtained 33 interspecific hybrid combinations with an overall success rate of 41%. Using cultivated sunflower embryos of varying sizes, Espinasse et al. (1985) concluded that a high sucrose concentration of 90 g/kg and low nitrogen content were required for culturing small young embryos less than 2 mm in size.

As suggested by Dewey (1980), induced polyploidy could also serve as a bridge for interspecific gene transfer in sunflower. Jan and Chandler (1989) successfully doubled chromosomes of P21 x *H. bolanderi* F_1 hybrids, and increased seed set on doubled heads. Jan (1988) reported the success of a modified colchicine chromosome-doubling technique on 19 embryo-cultured wild x cultivated interspecific hybrids, and its positive effect on backcross seed set. Chromosome doubling of each head was verified by pollen grain size and stainability (Alexander, 1969). Chromosome doubling increased pollen grain size and stainability of interspecific hybrids. The increased pollen grain size directly reflected chromosome doubling and provided a reliable criterion for classifying treated plants.

Chromosome doubling restores normal fertility of amphiploids by providing an identical pairing partner for each chromosome. However, this increased fertility is likely to reduce the enforced interspecific chromosome pairing and gene exchanges during meiosis when an F_1 head is not chromosomally doubled. It would be helpful if the researchers could backcross onto both doubled and nondoubled heads, and at the same time intercross doubled heads for amphiploid production. More cytological evaluations are needed to compare the efficiency of interspecific gene transfer with or without the assistance of chromosome doubling of F_1s. Without chromosome doubling, we expect very low number of BC_1F_1 seeds and a high frequency of weak BC_1F_1 plants. With chromosome doubling, due to preferential pairing of *H. annuus* chromosomes during meiosis, we expect a reduced pairing of *H. annuus* chromosomes with chromosomes of wild *Helianthus* species.

***Interspecific hybridization*: *Introgression of genes into cultivated lines*.** In recent years, interest in interspecific hybridization has been greater for transferring useful genes from wild species into cultivated lines to develop pre-breeding germplasms for sunflower improvement. Characteristics such as disease and insect resistance, salt tolerance, drought tolerance, fatty acid variation, CMS, and fertility-restoration diversity have been emphasized.

By successful hybridization between *H. petiolaris* and *H. annuus* and backcrossing with *H. annuus*, Leclercq (1969) transferred the *H. annuus* genome into cytoplasm of *H. petiolaris* Nutt. and obtained the first cytoplasmic male sterile plants. Whelan (1980; 1981) and Whelan and Dorrell (1980) used the same technique to obtain cytoplasmic male sterility conditioned by the cytoplasm of three species, *H. petiolaris*, *H. giganteus*, and *H. maximiliani*.

Due to the use of a single male-sterile cytoplasm for worldwide hybrid sunflower production and its consequence of genetic vulnerability, a large portion of the interspecific hybridization in sunflower has focused on the identification of new CMS sources and their fertility restoration genes. Of the total 70 CMS sources resulting from interspecific hybridization, 39 were derived from wild *H. annuus* and 23 from other wild annual species, and only eight from wild perennial species. Extensive research is now focused on the identification of fertility restoration genes using both cultivated and wild species, and evaluation of their inheritance.

Rapid improvement of interspecific F_1 meiotic abnormality and low fertility was demonstrated by Whelan (1978; 1979) when he discovered CMS-PET2, G1G1, and MAX1. The differences of the parents were shown as translocations and a paracentric inversion as indicated in F_1 meiosis, which can quickly be eliminated after one or more backcrosses with cultivated lines (Whelan, 1982).

Helianthus tuberosus x *H. annuus* hybrids have been used widely in the Former Soviet Union (FSU) as a source of disease resistance. Hybrids of *H. annuus* x *H. resinosus* Small (2n=102) had stainable pollen from 0 to 50%, and meiotic diakinesis had 28 to 36 bivalents with 1 to 6 univalents (Georgieva-Todorova, 1983). The high number of bivalents suggests a high homology between the chromosomes from *H. resinosus* and those from *H. annuus*. In general, good pollen stainability is expected in the F_1s of hexaploid *Helianthus* species crossed with *H. annuus*. Atlagić (1990) reported average pollen stainability of 49.8%, 40.9%, and 64.6, respectively, for the hybrids of *H. annuus* with *H. pauciflorus*, *H. tuberosus* and *H. laevigatus.* Seiler's (1991a; 1993) release of 12 interspecific germplasm lines derived from perennial accessions of *H. hirsutus*, *H. resinosus*, and *H. tuberosus* also supports the reasonably good fertility of *H. annuus* x hexaploid accessions and some selected *H. annuus* x tetraploid accessions.

An unusual cytoplasmic-nuclear interaction causing plants with reduced vigor has been observed, and a single dominant gene was needed to restore normal plant growth (Jan, 1992). With continuous backcrossing with HA 89 as the recurrent parent into the cytoplasms of five diploid perennial species, *H. mollis*, *H. maximiliani*, *H. grosseserratus* Martens, *H. divaricatus* L., and *H. angustifolius* L. and selection for normal segregants, Jan (1992) discovered the vigor-reducing effects of these cytoplasms and a single nuclear vigor-restoration gene was needed to restore the vigor. The vigor-reducing cytoplasmic effects also have been observed in progenies when backcrossing HA 89 into cytoplasms of *H. hirsutus*, *H. occidentalis* Riddell, and *H. giganteus*. A considerable number of cultivated lines were found to possess

the same vigor restoration gene, and it was suspected to have been derived from *H. tuberosus* because of that species' popular use in early breeding programs in the FSU. Our recent discovery of a different vigor restoration gene derived from *H. giganteus* suggested the existence of different vigor restoration genes in varying perennial *Helianthus* species compensating for specific cytoplasmic effects causing reduced vigor (Jan, 2003).

Transferring genes from wild annual species into cultivated lines can be accomplished rather easily with conventional crossing and backcrossing. Seiler (1991b, c) released 15 interspecific germplasm lines having genes from wild annual species, and 13 tolerant to sunflower downy mildew, using the conventional method of crossing and backcrossing. Jan and Chandler (1985a) transferred resistance genes for powdery mildew (*Erysiphe cichoracearum* DC.) from *H. debilis* Nutt. and rust (*Puccinia helianthi* Schwein.) and downy mildew resistance genes from wild *H. annuus* into cultivated sunflower (Quresh et al., 1993; Quresh and Jan, 1993; Tan et al., 1992).

Crossing cultivated sunflower with wild perennial *Helianthus* species often results in serious problems of early hybrid embryo abortion, as well as high levels of sterility in the F_1 or BC_1F_1 generation. However, utilizing an embryo-culturing technique, 26 interspecific hybrids of wild perennials x cultivated line P21 were produced. Subsequent chromosome doubling of the F_1s of diploid and tetraploid wild accessions crossed with P21 improved backcross and sib-pollinated seed set drastically (Jan, 1988). Amphiploids of wild species utilizing *H. gracilentus* A. Gray, *H. pumilus* Nutt., *H. hirsutus*, *H. strumosus*, *H. maximiliani*, *H. nuttallii*, *H. mollis*, and *H. grosseserratus* crossed with cultivar P21 have been produced by sib-pollination of chromosomally doubled heads of each cross. These amphiploids can be maintained by sib-pollination, have improved pollen stainability and larger pollen grains, and have improved backcross seed set (Jan and Fernández-Martínez, 2002).

Interspecific gene transfer facilitated by the chromosome doubling of extremely difficult diploid perennials x *H. annuus* and tetraploid x *H. annuus* crosses has been demonstrated. Positive results of gene transfer from *H. hirsutus* into cultivated sunflower have been obtained (Jan and Zhang, 1995). By monitoring the rust resistance genes of *H. hirsutus*, which is immune to the four North American (NA) rust races, the hexaploid amphiploid was backcrossed with *H. annuus* twice. The resulting triploid BC_2F_1s had a complete set of 34 chromosomes of *H. annuus*, plus 17 chromosomes from *H. hirsutus*, and were all resistant to the four NA rust races. Several BC_3F_1 plants had 2n=36 or 37 chromosomes and were resistant to NA rust races 1 and 2, and further backcrossing resulted in many BC_4F_1 race 1- and 2-resistant plants with 2n=34. More recently, Jan et al. (2002) produced four sunflower germplasms with resistance to broomrape (*Orobanche cumana* Wallr.) race F, with resistance genes transferred from wild perennial *Helianthus* via interspecific amphiploids. In addition, interspecific amphiploids of perennial x cultivated have provided fertility restoration genes for the new CMS cytoplasms derived from *H. giganteus* (Jan, 2004) while no *Rf* genes were identified in cultivated lines. Surprisingly, *Rf* genes for this CMS were identified in four out of the seven amphiploids tested.

Chandler (1991) reviewed sunflower genomic relationships and came to the conclusion that there is little evidence of the existence of distinct genomes in *Helianthus*. The author's observation of many interspecific hybrids agrees with Chandler's statements. Even the most sterile interspecific hybrids involving diploid perennial species and cultivated *H. annuus* had satisfactory chromosome pairing (Jan and Chandler, 1985b). In order to utilize this high degree of chromosome similarity between cultivated lines and wild *Helianthus* species for interspecific gene transfer, the best approach would be to backcross without F_1 chromosome doubling. Without chromosome doubling, maximum chromosome pairing between cultivated lines and the wild species will be achieved. With chromosome doubling, preferential chromosome pairing of identical chromosomes in each parent will reduce the interspecific chromosome pairing and gene exchanges. However, the latter approach may have the advantage of having improved backcross fertility, and the reduced degree of gene exchange will enhance the quick recovery of a recurrent parent genotype carrying the specific selected gene. This was demonstrated with the rust resistance gene transfer from *H. hirsutus* into cultivated line HA 89 via amphiploidization, where chromosomes from *H. hirsutus* demonstrated their ability to challenge the perfect pairing of *H. annuus* chromosomes and to incorporate the resistance genes into the *H. annuus* genome (Jan and Zhang, 1995).

***Interspecific hybridization*: *Amphiploids*.** Colchicine treatment of interspecific F_1 hybrids resulted in high frequencies of chromosome doubling and the production of amphiploids (Jan and Fernández-Martínez, 2002). The tetraploid amphiploids produced included crosses of P21 x *H. bolanderi* (Jan and Chandler, 1989), *H. gracilentus* x P21, *H. grosseserratus* x P21, *H. cusickii* A. Gray x P21, *H. mollis* x P21, *H. maximiliani* x P21, and *H. nuttallii* x P21. These amphiploids have restored fertility, and provide easily available genetic diversity for the improvement of cultivated sunflower. The first hexaploid

amphiploids in sunflower have also been produced from crosses of *H. hirsutus* x P21 and *H. strumosus* x P21.

The interspecific amphiploids will enable the establishment of a number of chromosome addition lines for genetic studies of specific chromosomes of both cultivated and wild *Helianthus* species. With the available amphiploids and some specific interspecific crosses, the potential exists to establish additional lines with HA 89 chromosome pairs in *H. californicus*, and the chromosome pairs of *H. hirsutus*, *H. angustifolius*, *H. cusickii*, *H. gracilentus*, *H. grosseserratus*, *H. nuttallii, H. strumosus*, and *H. giganteus* in HA 89.

Male sterility. A single male-sterile cytoplasm, PET1, derived from *H. petiolaris* subsp. *petiolaris* (Leclercq, 1969) and the identification of dominant fertility restoration genes (Enns et al., 1970; Kinman, 1970; Vrânceanu and Stoenescu, 1971) advanced sunflower production from the use of open-pollinated cultivars to hybrid production 40 years ago. This source of cytoplasmic male sterility and a few fertility restoration genes, including the widely used Rf_1 and Rf_2 genes, have been used exclusively for sunflower hybrid production worldwide (Fick and Miller, 1997).

A total of 70 CMS sources have been identified from progenies of crosses between wild *Helianthus* accessions and cultivated lines, from wild accessions grown in observation nurseries, or from induced mutation. Fertility restoration genes have been reported for 34 CMS sources, and detailed inheritance studies have been conducted for only 19 of the CMS sources (Serieys, 2002). In general, it is relatively easy to isolate stable CMS cytoplasms, but the identification of simple and completely dominant fertility restoration genes has been far less successful.

Many CMS sources from wild *H. annuus* (ANN1 through ANN9) were discovered in field-grown populations. All these CMS lines except ANN8 were completely male-sterile with degenerated anthers. Restoration genes were found for ANN2, 3, 4, and 7 using a set of 20 fertility-restoration testers, plus male-fertile plants of each respective wild species accession. Inheritance studies of fertility restoration of ANN2 and ANN3 indicated complete fertility restoration by single dominant genes (Jan, 1991). Serieys (1994) also reported complete male sterility and full fertility restoration by single dominant genes for CMS-ANO1, CMS-NEG1, and CMS-PRP1. The utilization of these CMS sources for potential hybrid production should be pursued.

Diseases. Diseases limit production in a majority of sunflower producing countries. Sunflower is a host to a wide array of diseases that can cause serious economic damage in terms of yield and quality, with the fungal diseases the most numerous and economically serious. In the USA, the major diseases of concern are downy mildew, rust, Sclerotinia head and stalk rot, and Phoma black stem. Verticillium wilt, Phomopsis stem canker, Alternaria leaf spot, Septoria leaf spot, charcoal stem rot, and Rhizopus head rot occur to a lesser degree. In Europe and adjacent Mediterranean countries, downy mildew, Sclerotinia head rot, Phomopsis, Botrytis gray rot, and charcoal rot are considered the most important diseases. Some diseases are important in only a few countries, such as Verticillium wilt in Argentina and white rust (*Albugo*) in South Africa. Genetic resistance to the prevailing North American races of rust has been identified in three wild annual species, *H. annuus*, *H. petiolaris*, and *H. argophyllus* T. and G. (Jan et al., 2004a). Genes for rust resistance are frequent in the wild progenitors of the cultivated sunflower (Quresh et al., 1993). In most cases rust resistance appears to be conditioned by single dominant genes.

Downy mildew can be controlled by single, race-specific dominant resistance genes. Multi-race resistant germplasm and single-race resistant germplasms have been developed from wild sunflower species (Miller and Gulya, 1988; Tan et al., 1992; Jan et al., 2004b). Wild *Helianthus annuus, H. petiolaris* and *H. praecox* Engelm. and A. Gray are sources of single dominant genes for single race resistance, while *H. argophyllus* is the source of dominant genes for all known races of the fungus (Miller and Gulya, 1988; Miller et al., 2002).

Sclerotinia wilt (white mold) causes the greatest losses to sunflower on a global basis. This is in part due to the wide host range of *Sclerotinia sclerotiorum* (Lib.) de Bary being a facultative parasite that attacks 360 species of plants. It appears that Sclerotinia resistance is complex and controlled polygenically involving many genes, each with small effects. This means that the breeding strategy using wild species as a source of resistance needs to be quite different than for other diseases. A detailed approach and strategy for developing Sclerotinia stalk rot resistance will be discussed later in this section.

There are reports of identification of cultivated sunflower genotypes with low susceptibility or moderate resistance to Sclerotinia white mold. Wild species have also been identified as a potential source of genes for Sclerotinia tolerance. Interspecific hybrids with perennial *H. maximiliani* (Maximilian's sunflower) exhibited higher levels of resistance than head rot resistant inbred lines

(Cerboncini et al., 2002; Ronicke et al., 2004). Rashid and Seiler (2004) identified potential sources of Sclerotinia head and stem rot resistance in populations of perennial *H. maximiliani* and *H. nuttallii* from Canada. Perennial *H. resinosus* has been identified as a good source for resistance to Sclerotinia head rot by Mondolot-Casson and Andary (1994). The Sclerotinia disease complex appears to be very complicated. The prospect of finding a single dominant gene for resistance does not look promising, but progress is being made in the development of germplasm with increased tolerance to Sclerotinia head rot. Currently there are no commercial hybrids which possess a satisfactory level of resistance to Sclerotinia rot.

Some progress has been made in increasing the resistance to midstalk Sclerotinia rot in cultivated sunflower. Kohler and Friedt (1999) indicated that progenies of interspecific crosses involving *H. mollis* and *H. tuberosus* had increased levels of tolerance to midstalk white mold infection. Miller and Gulya (1999) developed four maintainer and four restorer oilseed lines with improved tolerance to midstalk Sclerotinia rot.

Sclerotinia sclerotiorum generates substantial quantities of oxalic acid, which has been identified as one of the key components in the infection process. One strategy for resistance is to obtain plants that are resistant to free oxalic acid by engineering them to degrade it. A wheat (*Triticum aestivum* L.) oxalate oxidase gene (OXO) has been identified and transferred into sunflower via transformation (Scelonge et al., 2000). A transgenic sunflower line, *H. annuus* cv. SMF3, constitutively expressed the wheat OXO gene (Hu et al., 2003) and exhibited enhanced resistance against the oxalic acid-generating fungus Sclerotinia. This approach to white mold resistance in sunflower awaits further testing and commercialization.

Phomopsis brown stem canker was first discovered in sunflower in Yugoslavia in 1980 and now is considered a serious problem in much of Europe (Mihaljcevic et al., 1982; Acimovic, 1984; Škorić, 1985). Cuk (1982) reported that wild *H. debilis* and *H. pauciflorus* are potential sources of resistance to *Phomopsis helianthi* Munt-Cvet. et al. Kurnik and Walcz (1985) reported resistance to stem canker in *H. argophyllus*, tolerance in two other wild species, and susceptibility in local populations of *H. tuberosus.* Dozet (1990) observed a high degree of resistance in two populations of *H. tuberosus*. Cultivated hybrids developed from *H. tuberosus* and *H. argophyllus* have high field tolerance to Phomopsis brown stem canker (Škorić, 1985). Škorić (1985) hypothesized that the resistance may be controlled by two or more complementary genes.

Alternaria leaf spot causes losses in cultivated sunflower in the USA and other parts of the world. In warm climates with high rainfalls, it causes defoliation and reduces yield significantly (Sackston, 1981). All 21 annual taxa and 18 of 21 perennial species evaluated were susceptible to *A. helianthii* (Hansf.) Tub. and Nish. spores applied in a suspension. Perennial species *H. hirsutus, H. pauciflorus* subsp. *subrhomboideus*, and *H. tuberosus* appear to resist infection by *Alternaria helianthi* (Morris et al., 1983). Lipps and Herr (1986) showed that 13 accessions of *H. tuberosus* had significantly less Alternaria leaf spot than commercial hybrids and concluded that the species is a potential source of resistance to leaf spot. Several wild annual species, *H. praecox, H.* x *laetiflorus* Pers., *H. debilis* subsp. *cucumerifolius*, and *H. debilis* subsp. *silvestris*, had high levels of resistance to Alternaria and *Septoria helianthi* Ellis and Kellerm. in field evaluations (Block, 1992). Although potential sources of resistance to *Alternaria* have been identified, resistance genes have not been transferred to cultivated lines.

Powdery mildew is a widely distributed pathogen of cultivated sunflower in warmer regions of the world (Zimmer and Hoes, 1978). This foliar disease is found mostly on senescing leaves, and is generally not of major economic concern. *Helianthus debilis* subsp. *silvestris, H. praecox* subsp. *praecox*, *H. bolanderi* A. Gray and 14 perennial species exhibited powdery mildew tolerance in both field and greenhouse tests (Saliman et al., 1982). Not all populations of some perennial species are resistant; populations of *H. grosseserratus* and *H. maximiliani* showed differential reactions. Jan and Chandler (1985a) characterized resistance to powdery mildew from *H. debilis* subsp. *debilis* as incompletely dominant. They incorporated genes from this species into a cultivated background and have released a germplasm pool PM1 having the resistance genes (Jan and Chandler, 1988).

Currently, cultivated sunflower does not possess resistance to Rhizopus head rot. Yang et al. (1980) reported that four out of 32 wild species and subspecies tested were resistant when inoculated with *R. arrhizus* A. Fischer and *R. oryzae* Went. The resistant sources were: *H. divaricatus*, *H. hirsutus*, *H.* x *laetiflorus*, and *H. resinosus*. Further breeding will be needed to transfer the identified sources of resistance into cultivated sunflower.

So far, most genotypes of sunflower have exhibited susceptibility to the pathogen *Phoma macdonaldii* Boerema. Under natural infection, wild sunflower species *H. maximiliani*, *H. argophyllus*, *H. tuberosus*, and *H. pauciflorus* possess excellent resistance to Phoma black spot (Škorić, 1992).

Interspecific lines based on *H. tuberosus* have resistance to charcoal rot. Wild species *H. mollis*, *H. maximiliani*, *H. resinosus*, *H. tuberosus*, and *H. pauciflorus* have also shown resistance. The number of genes and the inheritance of resistance to the pathogen have not been ascertained, although resistance appears to be dominant.

Broomrape (*Orobanche cumana*) is a parasitic weed that infects sunflower roots causing severe crop losses in Southern Europe and the Black Sea region. It has also been observed in Australia, Mongolia, and China and is generally associated with drier climates. Five resistance genes (Or_1 through Or_5) have been used successfully for broomrape control following the progression of races A through E. Since broomrape is a highly variable pathogen, the breakdown of resistance is a frequent phenomenon, and multiple sources of resistance are needed. Ruso et al. (1996) evaluated wild annual and perennial sunflower species reaction to Spanish races and found two annual species, *H. anomalus* Blake and *H. exilis* Gray that had resistance and all 26 perennial species were resistant.

Recent studies indicated the development of a new broomrape race in Spain, designated race F, which attacks all commercial sunflower hybrids, overcoming the previously effective resistance genes (Domínguez et al., 1996). High levels of resistance to race F have been observed in populations of wild perennial sunflower (Fernández-Martínez et al., 2000). Jan et al. (2002) released four race F resistant germplasms, BR1 through BR4, which were derived from wild perennial sunflowers *H. maximiliani*, *H. grossesserratus*, and *H. divaricatus*. Fernández-Martínez et al. (2004) released four sunflower germplasms, K-96, L-86, P-96 and R-96, with resistance to race F based on cultivated sunflower from Eastern Europe. Resistance to race F appears to be controlled by dominant-recessive epistasis, complicating the breeding by requiring the genes to be incorporated into both parental lines of a resistant hybrid (Akhtouch et al., 2002). Other germplasms have been released which have resistance to various races (other than race F) of broomrape including seven germplasms based on cultivated sunflower from the FSU, Romania, and Turkey (Miller and Domínguez, 2000).

Diseases*: *Current progresses in developing Sclerotinia stalk rot resistant germplasm utilizing wild perennial Helianthus. Sclerotinia stalk and head rot caused serious economic loss for more than 50 percent of the seed yield. Cultivated sunflower lacks resistance to *Sclerotinia,* although some differences in susceptibility exist. However, the over 51 species of *Helianthus*, consisting of diploid, tetraploid and hexaploid, represent a diverse potential source of Sclerotinia resistance genes. Evaluation of wild germplasm indicated that several wild perennial species possess high levels of resistance to Sclerotinia head rot and stalk rot.

Since 2005, a program focusing on the transfer of Sclerotinia stalk rot resistance from wild *Helianthus* species of different ploidy levels (2x, 4x, 6x) into adapted sunflower germplasm via interspecific hybridization was started at the Sunflower Research Unit in Fargo. In our initial experiment, hexaploid perennial *H. californicus* DC. was identified to be highly resistant to Sclerotinia stalk rot, and was crossed with the moderately tolerant line HA 410 (Miller and Gulya, 1999) followed by continuously backcrossing with HA 410 until BC_4F_1 (Feng et al., 2006).

At the same time, to expand the diversity of the resistance gene sources, interspecific amphiploids were identified that segregated for high levels of resistance to Sclerotinia stalk rot. These amphiploids had high crossability and played a critical role as bridges for interspecific gene transfer, avoiding the direct crossing of HA 410 with those wild *Helianthus* species known to cross with extreme difficulty. Thus, in 2006, amphiploids involving six wild diploid or tetraploid species were crossed with HA 410 and further backcrossed twice to transfer stalk rot resistance (Jan et al., 2006). Furthermore, based on two years of information, an additional project was started to transfer Sclerotinia stalk rot resistance from three diploid perennial species to HA 410 in 2007.

Hexaploid *H. californicus* was crossed with HA 410 in 2005 resulting in F_1 plants with 2n=68 chromosomes, which were backcrossed with HA 410 from BC_1F_1 through BC_4F_1. The chromosome numbers of the BC progeny were gradually reduced to 2n=34 (Table 1). As a result, their pollen fertility increased from 4.6, 31.3, and 38.5, to 73.9% in the BC_4F_1 generation, suggesting the continuing improvement of fertility as more *H. californicus* chromosomes were eliminated. Consistent with the improvement of pollen fertility, seed sets increased from 0.05% in BC_1F_1 up to 35.3% in BC_4F_1. It was noticed that the variation of pollen fertility was high among the BC progenies, for example, 4.6 to 62.1% in BC_2F_1, 5.0 to 95.6% in BC_3F_1, and 10 to 96.9% in BC_4F_1. This wide range of pollen fertility was expected primarily due to the variation in chromosome numbers of the individual BC progenies. Currently, of the 79 BC_4F_1 plants, 14 plants with 2n=34 have produced sufficient seed for field testing. Also, progenies derived from advanced backcross generations (BC_4) would be ideal genetic stocks for identifying chromosome segments of wild species in the cultivated background.

Table 1. Chromosome number, pollen fertility and seed set of F_1 and backcrossed progenies of *H. californicus* with HA 410 in 2005-2007.

	F_1	BC_1F_1	BC_2F_1	BC_3F_1	BC_4F_1
2n	68	50-53	40-49	35-44	34-40
Fertile pollen %	37.8	4.6 (0.9-10.2)	31.3 (4.6-62.1)	38.5 (5.0-95.6)	73.9 (10-96.9)
Seed set % (Seeds/florets)	2.71	0.05 (48/99,900)	3.35 (183/5,460)	11.9	35.3

However, the BC_1F_1 generation with 2n=51 had the most unbalanced genome relationship, obviously corresponding to the low backcross seed set. Since we only started to observe 2n=34 plants in the BC_4F_1 generation, it is obvious that deriving genes from hexaploid species often takes a long time, but the resulting germplasm will be much more of the cultivated type than that resulting from using other faster approaches. The disadvantage of this approach is less genetic variability at the 2n=34 stage for the selection of QTL as in the case for Sclerotinia resistance.

For interspecific amphiploids, a sufficient number of F_1 hybrids between the five amphiploids and HA 410 were produced in 2006 (Jan et al., 2006), which was followed with two more cycles of backcrosses with HA 410. The chromosome number, pollen fertility and seed set of crosses of interspecific amphiploids crossed with HA 410 and the backcrossed progenies are summarized in Table 2.

Table 2. Chromosome number, pollen fertility and seed set of F_1 and backcrossed progenies of interspecific amphiploids with HA 410 in 2006 and 2007.

	F_1			BC_1F_1			BC_2F_1
Parentage	2n	Fertile pollen (%)	×HA 410 seed set (%) (seeds/florets)	2n	Fertile pollen (%)	×HA 410 seed set (%) (seeds/florets)	2n
H. strumosus × P21 2n=102	68	89.4 (74.3-97.9)	19.7 (755/3,800)	49-51	26.0 (6.6-42.5)	1.9 (282/11,900)	34-41
H. grosseserratus × P21 2n=68	51	43.3 (2.4-72.8)	9.1 (165/1,818)	37-44	35.3 (6.2-84.6)	3.5 (77/4,240)	34-38
H. maximiliani × P21 2n=68	51	49.9 (2.4-66.3)	13.7 (711/5,190)	37-47	29.9 (2.4-70.9)	2.0 (97/7,920)	34-37
H. nuttallii 730 × P21 2n=68	51	29.7 (3.7-57.1)	1.1 (32/2,800)	36-43	41.0 (1.3-84.1)	8.3 (460/7,610)	34-37
H. divaricatus × P21)× (*H. grosseserratus* × P21) 2n=68	51	27.3 (1.0-48.4)	18.1 (835/4,620)	36-46	21.3 (1.4-85.1)	6.0 (434/6,020)	34-37

A total of 145 BC_2F_1 plants from five crosses between selected amphiploids and HA 410 were obtained. Because the amphiploids had a full set of 2n chromosomes from the cultivated sunflower, the elimination of the wild species chromosomes after each backcross was faster than that of the backcrosses of *H. californicus* x HA 410, and the 2n=34 progenies also had slightly higher pollen fertility and seed set (Table 1). After two backcross cycles, of the 145 BC_2F_1 plants, 47 plants had 2n=34 chromosomes and have produced sufficient seed for field testing. With continuous selection of target traits, amphiploids are expected to be extremely efficient in selecting the trait while eliminating the other undesirable wild species genes. As for the use of hexaploid wild species, the rapid elimination of wild species genes may prove amphiploids less efficient for transferring QTL.

For the diploid resistance source, *H. maximiliani*, *H. giganteus* and *H. grosseserratus* were used to pollinate NMS HA 89, and the resulting F_1 hybrids were obtained by rescuing the 5-day-old immature embryos on artificial medium as described by Feng et al. (2006). For the crosses of diploid perennials and HA 410, a total of 181 embryos were obtained from the interspecific crosses of NMS HA 89 with *H.*

maximiliani, *H. giganteus*, and *H. grosseserratus*, respectively (Table. 3). By using embryo rescue, 67 hybrid seedlings were established in the greenhouse, suggesting that the interspecific hybridization using wild species as the pollen donor was successful. Pollen fertility of the F_1 hybrids from NMS HA 89 crossed by diploid wild perennials was very low (around 1%) (Table 3). Consequently, only 155 BC_1F_1seeds were produced from 64,618 florets pollinated with HA 410. This result was consistent with the conclusion that diploid perennial species could be crossed with cultivated sunflower, but the frequency of successful crosses was low (Atlagić et al., 1995). The extremely low backcross seed set of the F_1 plants is the most limiting stage for transferring genes from diploid perennials. However, since the F_1 plants are generally perennial, sufficient BC_1F_1 seeds can be obtained by repeated pollination. The forced chromosome pairing between the cultivated and the wild diploid perennials will promote chromosome recombination and result in BC_1F_1 plants with a large number of wild species traits for the selection of QTL.

Table 3. Pollen fertility of F_1s between NMS HA 89 and wild diploid *H. maximiliani*, *H. giganteus* and *H. grosseserratus,* and backcross seed set with HA 410 in 2007.

Parentage	No. F_1 embryo/florets	No. seedlings	Fertile pollen %	BC seeds/florets
NMS HA 89 × *H. maximiliani*	10/8083	9	1.7 (0-1.7)	21/11408
NMS HA 89 × *H. giganteus*	23/5480	15	0.6 (0.3-0.8)	26/6750
NMSHA89 × *H. grosseserratus*	148/14200	43	1.0 (0-1.6)	108/46460
Total	181/ 27763	67	--	155/ 64618

In conclusion, potential interspecific pre-breeding Sclerotinia resistance lines from diploid, tetraploid and hexaploid germplasm have been produced during the past three years. Evaluation of these pre-breeding lines for their reaction to Sclerotinia stalk rot will verify the effectiveness of each approach for the selection of QTLs. The effectiveness of using each of the above approaches will also be verified by tracking of the wild species' specific molecular markers in progeny plants when they first reach the 2n=34 stage and are ready for seed increase for the field evaluation. Ultimately, we expect to identify and release germplasms with improved resistance to Sclerotinia stalk rot within the shortest time period possible.

***Insects*.** North America has the greatest problems with insect pests because the insect pests of sunflower have co-evolved with their native sunflower hosts in natural communities. In the major production area of North America, there are about 15 principal insect pests of cultivated sunflower, and of this total about six are considered of major importance as potential economic pests from year to year (Charlet and Brewer, 1997). The insects of main concern include: the sunflower beetle, the sunflower stem weevil, the red and gray seed weevils [*Smicronyx fulvus* (LeConte), and *S. sordidus* (LeConte)], the banded sunflower moth, *Cochylis hospes* Walsingham, the sunflower moth, *Homoeosoma electellum* (Hulst), and the sunflower midge, *Contarinia schulzi* Gagne.

Host-plant resistance is a pest management method that utilizes the plant's own defense mechanisms against the insect. Since wild sunflower are native to North America where their associated herbivores and entomophages co-evolved, there is an opportunity to search for insect resistance genes in the diverse wild species. Sunflower moth tolerance was observed in annual *H. petiolaris* and perennials *H. maximiliani*, *H. ciliaris* DC., *H. strumosus*, and *H. tuberosus* (Rogers et al., 1984). Stem weevil tolerance was found in perennials *H. grosseserratus*, *H. hirsutus*, *H. maximiliani*, *H. pauciflorus*, *H. salicifolius* Dietr., and *H. tuberosus* (Rogers and Seiler, 1985). Sunflower beetle tolerance was observed in annuals *H. agrestis* Pollard and *H. praecox,* and in perennials *H. grosseserratus*, *H. pauciflorus*, *H. salicifolius*, and *H. tuberosus* (Rogers and Thompson, 1978; 1980). Charlet and Seiler (1994) found indications of resistance to the red sunflower seed weevil in several native *Helianthus* species.

Interspecific germplasm using wild species as resistance sources have been created. In preliminary testing, Charlet et al. (2004) noted that germplasm derived from *H. petiolaris* had the lowest number of stem weevils. Among material tested in a banded sunflower moth evaluation nursery, germplasm derived from *H. praecox* subsp. *hirtus* had less than 2% damage. Germplasm that incorporated *H. strumosus* and *H. tuberosus* had very little red sunflower seed weevil damage in test plots. Breeding populations of promising germplasms are being developed for further testing.

Oil and oil quality. Variability for oil concentration exists in the wild species. Annual *H. anomalus* has the highest oil concentration of 460 g/kg, the highest ever observed in a wild sunflower species, followed by *H. niveus* (Benth.) Brandegee subsp. *canescens* with 402 g/kg, *H. petiolaris* with 377 g/kg, and *H. deserticola* Heiser with 343 g/kg (Seiler, 2007). Perennial *H. salicifolius* had a concentration of 370 g/kg (Seiler, 1985; Seiler and Brothers, 2003). Cultivated sunflower generally contains 450 to 470 g/kg. Reduced concentrations of saturated palmitic and stearic fatty acids have been observed in a population of wild *H. annuus* that had a combined palmitic and stearic acid concentration of 58 g/kg (Seiler, 1998). This is 50% lower than in oil of cultivated sunflower. A combined palmitic and stearic acid concentration of 65 g/kg was observed in a wild perennial species, *H. giganteus* L. (Seiler, 1998).

Salt and drought tolerance. Several species of *Helianthus* are native to salt-impacted habitats. Interspecific germplasm derived from *H. paradoxus* Heiser has been identified with high salt tolerance, withstanding salt concentrations up to EC 24.7 d/Sm. It appears that one major gene controls salt tolerance, although a modifier gene may also be present, possibly recessive in control (Miller, 1995). Two salt-tolerant parental oilseed maintainer lines, HA 429 and HA 430, have been released (Miller and Seiler, 2003). Blanchet and Gelfi (1980) evaluated stomatal resistance, leaf-water potential, photosynthetic activity, leaf structure, and number of stomata. They concluded that *H. argophyllus* is the best candidate source for drought tolerance genes because its pubescent leaves reflect sunlight, reduce water loss, and exhibit low transpiration rates. *Helianthus niveus* subsp. *canescens* was their second choice.

Herbicide tolerance. A wild population of annual *H. annuus* from a soybean field in Kansas that had been repeatedly treated with imazethapyr for seven consecutive years developed resistance to the imidazolinone and sulfonylurea herbicides (Al-Khatib et al., 1998). Resistance to imazethapyr and imazamox herbicides has great potential for producers in all regions of the world for controlling several broadleaf weeds. Several populations of wild sunflower (*H. annuus* and *H. petiolaris*) from the USA and Canada have been screened for resistance to these two herbicides. Eight percent of 50 wild sunflower populations had some resistance to imazamox and 57% had some resistance to tribenuron in the central U.S (Olson et. al., 2004). In Canada, 52% of 23 wild *H. annuus* populations had some resistance to tribenuron (Miller and Seiler, 2005). Genetic stocks IMISUN-1 (oil maintainer), IMISUN-2 (oil restorer), and IMISUN-3 (confection maintainer) have been developed and released (Al-Khatib and Miller, 2000). Miller and Al-Khatib (2002) also released one oilseed maintainer and two fertility restorer breeding lines with imidazolinone herbicide resistance. Genetic stocks SURES-1 and SURES-2 with resistance to the sulfonylurea herbicide tribenuron have been developed and released by Miller and Al-Khatib (2004). Additionally, two oilseed germplasm lines, HA 442 and RHA 443 have been released with imidazolinone resistance (Miller et al., 2006). The imidazolinone and sulfonylurea herbicides may control broomrape in areas of the world where this parasitic weed attacks sunflower (Alonso et al., 1998).

CONCLUSIONS AND PROSPECTS

Significant progress has been made in increasing the number of accessions in the wild sunflower species collection to preserve the wild species and increase the available genetic diversity for improvement of the crop. Interspecific gene transfer for sunflower improvement has been practiced since the very early years by breeders in the FSU and it has continued to play a key role as the crop developed into a major global oilseed crop. Recent advances in culturing of otherwise abortive interspecific hybrid embryos have proved to be highly effective for making the difficult-to-cross wild perennial *Helianthus* species crosses widely available for breeding purposes, either for specific major gene transfer or for the transfer of quantitative trait genes. Significant results have been reported on the germplasm development with regard to resistance to new races of downy mildew, rust, broomrape and other major diseases. In addition, new CMS and corresponding fertility restoration genes have been continuously identified and established, together with new genes helping to improve oil quality, herbicide resistance, and salt and drought tolerance. Thus far, only a small portion of the available genetic diversity of the wild *Helianthus* species

has been used globally. As a whole, there is no doubt that wild *Helianthus* species will continue to provide new genetic variability to the sunflower breeding community, helping to maintain sunflower as a viable major global oilseed crop.

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