Utilization of Sunflower Crop Wild Relatives for Cultivated Sunflower Improvement

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ABSTRACT
Sunflower (Helianthus annuus L.) is one of the few crops native to the United States. The current USDA–ARS National Plant Germplasm System (NPGS) crop wild relatives sunflower collection is the largest extant collection in the world, containing 2519 accessions comprising 53 species—39 perennial and 14 annual. To fully utilize gene bank collections, however, researchers need more detailed information about the amount and distribution of genetic diversity present within the collection. The wild species are adapted to a wide range of habitats and possess considerable variability for most biotic and abiotic traits. This represents a substantial amount of genetic diversity available for many agronomic traits for cultivated sunflower, which has a very narrow genetic base. Sunflower ranked fifth highest among 13 crops of major importance to global food security surveyed from the mid-1980s to 2005 in the use of traits from crop wild relatives. The estimated annual economic contribution of the wild species for cultivated sunflower is between US$267 to 384 million. Most of the value is derived from the PET1 cytoplasm from wild H. petiolaris, disease resistance genes, abiotic salt tolerance, and resistance to imidazolinone and sulfonylurea herbicides. Crop wild relatives provide a wide range of valuable attributes for traditional and molecular breeding, as well as for ecological experimentation, and have enabled rapid advances in ecological and evolutionary genetics. The wild species of Helianthus continue to contribute specific traits to combat emerging pests and environmental challenges and, at the same time, are preserved for future generations.

Crop genetic resources consist of the total genetic variability in the crop or within sexually compatible species (Holden et al., 1993). Crop wild relatives (CWR) have been undeniably beneficial to modern agriculture dating back 100 yr, providing plant breeders with a broad pool of potentially useful genetic resources (Hajjar and Hodgkin, 2007). Sunflower (Helianthus annuus L.) is one of a few crops with its center of origin in North America, where it was domesticated by Native American nations in the east central United States (Blackman et al., 2011). The crop is grown globally and performs well in most temperate climates, with significant production on all continents except Antarctica. The estimated world production of sunflower in 2014 to 2015 was 23.2 million ha in 72 different countries (Table 1; USDA, 2016b). It is the second largest hybrid crop, second only to maize (Zea mays L.), and the fifth largest oilseed crop, after soybean [Glycine max (L.) Merr.], rapeseed (Brassica napus L.), cottonseed (Gossypium hirsutum L.), and groundnut (Arachis hypogaea Fabr.). The estimated value of the global sunflower crop is US$20 billion (FAO, 2016). Sunflower is a globally important oilseed, food, and...
ornamental crop, and the seed accounts for approximately 10% of the world’s edible plant-derived oil (Kleingartner, 2004). In the United States, roughly 60% of the seed is crushed for oil, while about a quarter of the seed is used in birdseed and another 10 to 20% is sold directly for snacks and baking products, with the remaining value in the meal (Seiler and Gulya, 2015).

Sunflower, with its US origin, provides the sunflower industry with an opportunity to collect, evaluate, and preserve its CWR. This germplasm and associated information is particularly important because of the coevolution of its species and associated native insects and pathogens. The crop, wild species, and pests and abiotic stresses have coevolved in the center of origin. Knowledge of a particular habitat and adaptations of species occurring therein can often help to identify potential sources of genes for desired traits. Genetic diversity and habitat diversity go hand in hand, and where CWR grow can often provide a key to their potentially useful traits. The benefit of coevolution is that the CWR are sources of genes for resistance and tolerance because they survive in areas where abiotic and biotic challenges exist, providing the opportunity to search for these genes in the CWR. However, the sunflower crop is vulnerable to many pests and abiotic stresses, since traits of the CWR have been lost in the domestication process.

**SUNFLOWER HISTORY**

Evidence of domesticated sunflower dates back to 4625 BP, as determined from samples found in the Hayes site in middle Tennessee (Crites, 1993). Recent molecular evidence has confirmed that the eastern United States (probably the Mississippi River Valley in the vicinity of present-day Arkansas) was the single independent domestication center of cultivated sunflower (Blackman et al., 2011). Sunflower was domesticated by Native American nations, who had selected a tall, single-headed variety before European explorers reached North America in the 16th Century. Native Americans used the seed directly as food by crudely extracting the oil and the hulls as a source of dye, leaves for herbal medicines, and the pollen in religious ceremonies. While sunflower was not a staple of their diet as were maize, *Phaseolus acutifolius A. Gray*, and squash (*Cucurbita maxima* Duchesne), it nonetheless was cultivated by many Native Americans Nations from eastern North America throughout the Midwest and as far south as northern Mexico (Putt, 1997).

Historical records indicate that the Spanish were the first to introduce sunflower into Europe in the early 1500s, followed by early French and English explorers introducing it mainly as an ornamental and later as a forage crop for livestock and poultry. Sunflower spread from Western Europe along the trade routes to Egypt, Afghanistan, India, China, and Russia in the 1700s, becoming a snack food (Putt, 1997). The most significant boost for sunflower as a crop, however, came from the Lenten regulations of the Russian Orthodox Church that prohibited the consumption of many oily foods, but since sunflower was not specifically listed, the seed and oil became a dietary staple in Russia. This prompted the development of higher-oil Russian varieties exceeding 40% oil (Seiler and Jan, 2010).

Mennonite immigrants from Russia are credited with reintroducing sunflower to North America via Canada. In fact, the open-pollinated variety ‘Russian Mammoth’, sold by many garden centers, traces its lineage back to the same-named variety initially introduced in the 1880s (Seiler and Gulya, 2015). After sunflower reintroduction to the United States in the 1920s to 1940s, its primary use was livestock forage. With the increasing demand for sunflower oil, improved Russian varieties with oil concentrations of 40 to 45% were available worldwide by the second half of the 20th Century. These varieties became the basis of the North American sunflower industry as the species evolved from a forage crop to an oilseed crop in the 1950s.

A major event in sunflower history was the discovery by a French scientist of cytoplasmic male sterility (CMS) in a wild prairie sunflower, *Helianthus petiolaris* Nutt. (Leclercq, 1969), and restorer genes with recessive branching found in wild *H. annuus* populations (Kinman, 1970) and *H. petiolaris* (Leclercq, 1971) that led to the production of commercial hybrids. The higher yields, oil content, uniformity, and enhanced disease resistance of hybrid sunflower compared with open-pollinated varieties revolutionized the sunflower industry and led to the establishment of sunflower as a viable worldwide crop.

**GENETIC RESOURCES**

Crop wild relatives have been undeniably beneficial in sustaining modern agriculture by providing plant breeders with a diverse genetic pool of potentially useful traits (see Kane et al., 2013; Kantar et al., 2015). Emerging plant diseases and agricultural pests are predicted to become more
common and damaging in a warming climate (Anderson et al., 2004). Habitats of sunflower CWR are diverse, with species found in deserts, grasslands, swamps, marshes, forests, mountains, roadways, and farmers’ fields.

Taxonomically, the genus Helianthus is divided into four sections: annual sections Helianthus (includes cultivated sunflower) and Agrestis (represented by a single species), and perennial sections Ciliares and Divaricati (Timme et al., 2007). It is native to temperate North America, containing 53 species (14 annual and 39 perennial) and 19 subspecies (Schilling, 2006; Stebbins et al., 2013). The base chromosome number of Helianthus is \( n = 17 \) and contains diploid \( (2n = 2x = 34) \), tetraploid \( (2n = 4x = 68) \), and hexaploid \( (2n = 6x = 102) \) species. The 14 annual species are all diploid, and the 39 perennial species include 26 diploid, 3 tetraploid, 7 hexaploid, and 3 mixaploid species. Helianthus ciliaris DC. and Helianthus strumosus L. have both tetraploid and hexaploid forms, while Helianthus decapetalus L. and Helianthus smithii Heiser contain diploid and tetraploid forms. The different ploidy levels enhance genetic diversity but, at the same time, often complicate the incorporation into cultivated germplasms.

Germplasm resources can be categorized as in situ resources (maintained as wild populations and landraces in natural habitats) or ex situ resources (accessions preserved in gene banks). In situ conservation of CWR in natural conditions where they continue to evolve and generate novel traits in response to their environment, compared with populations maintained in gene banks, is the ideal way to maintain populations. However, habitats conducive to this type of conservation are at risk of encroachment by human activities. One advantage of ex situ conservation is that it makes the genetic resources readily available for breeding while preserving them for future generations.

The USDA–ARS National Plant Germplasm System (NPGS) maintains and manages genetic resources for agricultural crops in the United States. The NPGS sunflower CWR collection was established in Bushland, TX, in 1976 and transferred to the Ames, IA, gene bank in 1985. The sunflower CWR collection at the North Central Regional Plant Introduction Station (NCRPIS), Ames, IA, contains 2519 accessions with 1028 wild \( H. \) annuus accessions (41%), 613 accessions representing populations of 13 other wild annual species (24%), and 878 accessions representing 39 perennial species (35%). This collection is the largest and most genetically diverse ex situ collection in the world and is vital to the conservation of sunflower CWR. Approximately 90% of the CWR accessions are currently available for distribution (Marek, 2016). In addition to the CWR collection, the NCRPIS maintains and distributes about 2500 accessions of cultivated sunflower, including a significant number of lines with introgressions from CWR; 489 accessions have a CWR mentioned in their descriptive data field in the Germplasm Resources Information System (GRIN) (Marek, 2016).

Between 2011 and 2015, 775 orders from 523 recipients distributed 8600 packets of seeds of sunflower CWR within the United States and internationally (Marek, 2016). Many of the accessions have become CWR collections in countries such as Argentina, Serbia, Spain, France, Bulgaria, Romania, Hungary, India, and Mexico (Seiler, 2012).

The mission of the NPGS is to conserve genetic diversity and associated information for conducting germplasm-related research and to encourage its usage for crop improvement and product development. The Germplasm Resources Information Network (GRIN Global) serves as the database for all information related to the CWR accessions. Currently, there are approximately 40,000 data points (descriptors × accessions evaluated) describing the CWR collection in GRIN. Much of the data is related to morphological traits, but there is information for several of the major diseases and insect pests, as well as oil content and fatty acid composition of the oil. The GRIN system also serves as the portal for requesting germplasms (https://npgsweb.ars-grin.gov/gringlobal/search.aspx). The germplasm is freely available for research and educational purposes, although some restrictions are imposed by import regulations of receiving countries.

Collection of germplasms is the first step in conserving genetic resources. A history of CWR explorations covering the period from 1976 to 2004 was reviewed by Seiler and Gulya (2004). A third of the CWR accessions have been added in the last 10 yr sourced by 20 explorations directed to fill specific species and geographic gaps, including two explorations in 2015 to increase representation of species from the western, southwestern, and southern states (Marek, 2016).

The need for germplasms is driven by the priority of new traits needed for the crop. Castañeda-Álvarez et al. (2016) assessed the collection priority of sunflower CWR as medium because of the long history of the use of CWR in sunflower crop improvement, which has benefited from a relatively extensive germplasm collection. However, there are still taxa lacking comprehensive ex situ conservation, resulting in gaps in the sunflower collection. Kantar et al. (2015) used occurrence, bioclimatic, and biophysical data for 36 taxa of CWR to predict species hotspots and species gaps. This generated a gap analysis that sets the priority needs for further collection of each species and identification of possible sources of abiotic stress traits for plant breeding programs. Twenty-six CWR of sunflower were rated, with 10 high and 16 medium priorities for collection.

**GENETIC DIVERSITY OF CROP WILD RELATIVES**

The CWR of domesticated crops such as sunflower possess genetic diversity useful for productive, nutritious, and resilient crops for breeding programs, but researchers need
detailed information about the amount and distribution of their genetic diversity. Mandel et al. (2011) estimated that cultivated sunflower harbors roughly two-thirds of the total genetic diversity present in its direct wild progenitor. They further concluded that the estimated contribution of the wild genotype to the cultivated sunflower germplasm revealed that the bulk of the cultivated diversity is derived from two wild sunflower population genetic clusters that are primarily composed of individuals from the east-central United States, the same general region in which sunflower domestication occurred.

Sunflower, with its North American US origin offers the opportunity to collect and preserve its CWR. This germplasm and associated information is particularly important because of the coevolution of its species and associated native insects and pathogens. The crop, wild species, and pests and abiotic stresses have coevolved in the center of origin. Knowledge of a particular habitat and adaptations of species occurring therein can often help to identify potential sources of genes for desired traits. Genetic diversity and habitat diversity go hand in hand, and where CWR grow can often provide a key to their potentially useful traits.

**ECONOMIC IMPORTANCE**

The CWR of sunflower have been very important in the development and sustainability of sunflower as the fifth largest oilseed crop. The US sunflower production in 2015 had a farm-gate value of $559 million, concentrated in the northern Midwest (USDA, 2016a), and its economic impact has been estimated as high as $2.62 billion yr⁻¹ (Bangsund and Leistritz, 1995). The estimated losses to diseases, weeds, and insects in global sunflower production in the mid-1990s were $1.36 billion annually (Hesley, 1999). Globally, diseases account for $642 million in losses, weeds for $489 million, and insects for $229 million. Estimated losses for US production were $30 million for diseases, $26 million for weeds, and $36 million for insects. The impact of sunflower CWR has been substantial in reducing these losses and in contributing other traits to the crop. Prescott-Allen and Prescott-Allen (1986) estimated that sunflower CWR contributed 25.9% of the annual value of the crop. Tyack and Dempewolf (2015) estimated the value of sunflower CWR at $185 million yr⁻¹ in terms of 2012 dollars, based on the earlier evaluations provided by Prescott-Allen and Prescott-Allen (1986), while Hunter and Heywood (2011) estimated the values ranging from $267 to 384 million annually. The CWR trait of greatest value was the PET1 (French) CMS used as the female parent for the commercial production of sunflower hybrids. The derived traits of second highest value were rust (caused by *Puccinia helianthi* Schwein.), downy mildew (DM) [caused by *Plasmopara halstedii* (Farl.) Berl. and deToni], Verticillium wilt (caused by the soilborne fungus *Verticillium dahliae* Kleb.), Alternaria leaf spot [caused by *Alternaria helianthi* (Hansf.) Tub. and Nish.], powdery mildew [caused by *Golovinomyces cichoracearum* (DC) V. P. Heluta], Phomopsis stem canker (caused by *Diaporthe helianthi/Phomopsis helianthi* Munt.-Cvet. et al.), and Sclerotinia wilt and rot [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] resistance, and resistance to the parasitic weed broomrape (caused by *Orobanche cumana* Wallr.). These estimates do not include one very important resistance trait for imidazolinone and sulfonylurea herbicides, discovered in a wild *H. annuus* population in Kansas (Al-Khatib et al., 1998).

Hajjar and Hodgkin (2007) surveyed the introduction of genes from CWR in 13 cultivated crops of major importance to global food security from the mid-1980s to 2005. Crop wild relatives contributed seven traits for cultivated sunflower, mainly for pest and disease resistance, abiotic factors, male fertility restoration, and CMS, fifth among crops surveyed. An eighth can be added to this list: herbicide resistance to imidazolinone and sulfonylurea chemistries, which is expected to be worth millions of dollars globally when fully deployed (Hajjar and Hodgkin, 2007), with the added benefit of controlling the parasitic weed broomrape (Alonso et al., 1998; Škorić, 2012), in addition to broadleaf weed control.

**CROP WILD RELATIVES UTILIZATION**

**Interspecific Hybridization**

Interspecific hybridization is used to transfer traits, including disease and insect resistance and resistance to abiotic and biotic stresses, from CWR to cultivated sunflower, frequently using the CWR as the male parent. The use of interspecific hybridization in sunflower breeding dates back to 1916, when the Russian scientist Sazyperow produced an interspecific hybrid between *H. annuus* and *Helianthus angophyllus* T. & G. in an attempt to develop sunflower with resistance to rust (Cockerell, 1929). Interspecific hybridization in Russia continued with Galina Pustovoit’s research on perennial *Helianthus tuberosus* L. (Škorić, 1988).

Modern cultivated sunflower is grown primarily as a single-cross hybrid. Sunflower CWR and cultivated sunflower can generally be crossed, but the different breeding systems of the two, cultivated being self-compatible, and the CWR being obligate outcrossers as a consequence of a sporophytic self-incompatibility system that leads to divergence and heterogeneity, causes considerable difficulties such as cross incompatibility, embryo abortion, sterility, reduced fertility, and dormancy in interspecific hybrids. The development and application of cytogenetic methodologies have facilitated the utilization of CWR of *Helianthus* for improvement of cultivated sunflower (Jan and Seiler, 2007). In recent years, there has been greater interest in interspecific hybridization for...
transferring desirable traits from CWR into cultivated lines to develop prebreeding germplasms. One important consideration when screening the sunflower CWR for usable traits is that all wild species are open-pollinated, segregating populations, so varying degrees of gene frequencies will be found in different populations depending on the segregation ratio of the trait of interest.

All the annual *Helianthus* species, except *Helianthus petiolaris* Pollard, can be hybridized with cultivated sunflower using classical backcross methods. Direct crosses of cultivated lines with some perennial CWR are also possible using conventional methods, but the use of a two-step embryo culture procedure developed by Chandler and Beard (1983) and chromosome doubling using colchicine (Jan, 1988) greatly facilitated interspecific hybridization in sunflower. These techniques produced 53 interspecific cross combinations without multiple pollinations, with 21 of these combinations not previously reported (Chandler and Beard, 1983). Kräuter et al. (1991) obtained 33 interspecific hybrids with an overall success rate of 41%, producing many new hybrid combinations. Jan and Fernández-Martínez (2002) used amphiploids produced from perennial CWR as a bridge to improve backcross seed sets for screening for broomrape resistance.

**Cytoplasmic Male Sterility**

Much of the current germplasm used in sunflower breeding programs originated from limited genetic material, resulting in a crop with an extremely narrow genetic base. Currently, hybrid sunflower is based solely on the first CMS PET1 discovered 45 yr ago, increasing its genetic vulnerability. Cytoplasmic male sterility can arise spontaneously, from intra- and interspecific hybridization crosses, or can be induced by mutagenesis or environmental stresses (Serieys, 2005). Much of the interspecific hybridization research in sunflower has focused on the identification of additional unique CMS sources and their corresponding fertility restoration genes. Cytoplasmic male sterility segregates in the CWR due to cytonuclear interactions, possibly playing a role in ecological adaptation and selection (Sambatti et al., 2008). This may become an important model for understanding cytonuclear interactions and their role in promoting or inhibiting speciation and interspecific gene flow. Seventy-two CMS sources, 38 from wild *H. annuus*, 24 from other wild annual species, and only 10 from perennial species, have been identified in progenies of crosses between wild *Helianthus* populations and cultivated lines, or from induced mutation (Serieys, 2002; Serieys and Christov, 2005).

Twenty diverse alloplasmic cytoplasmic substitution lines from annual and perennial wild species were compared for agronomic traits with the inbred line HA89 over four environments (Jan et al., 2014b). Lines having annual species cytoplasm had no effect on agronomic traits compared with currently used PET1 cytoplasm. In general, most cytoplasm of wild annual *Helianthus* species can accommodate cultivated nuclear genes without significant adverse interactions and are potential sources of cytoplasmic diversity for sunflower breeding.

**BIOTIC TRAITS**

**Herbicide Resistance**

Gene flow between crops and wild relatives has occurred for many years and has contributed to the evolution and extinction of weed species (Ellstrand et al., 1999). A wild population of annual *H. annuus* from a soybean field in Kansas that had been repeatedly treated with imazethapyr herbicide for seven consecutive years developed resistance to the imidazolinone and sulfonylurea herbicides (Al-Khatib et al., 1998). Resistance to imazethapyr and imazamox herbicides has potential for producers in all regions of the world where this parasitic weed attacks sunflower (Alonso et al., 1998). Recently, Jacob et al. (2016) reported that *Helianthus praecox* Engelm. & A. Gray PRA-1823 plants had complete resistance to three different sulfonylurea-based herbicides and remained green regardless of the concentration used; however, the plants failed to show cross tolerance and exhibited herbicide toxicity symptoms when subjected to imazethapyr treatment.

**Disease Resistance**

Sunflower CWR have been valuable resources for resistance genes since early cultivar development and became especially important with the transition to a hybrid crop.
Host-plant resistance is one of the most durable types of resistance to the ever-evolving pathogens and is an environmentally friendly means of disease control. A recent survey of the literature for references to resistance of sunflower CWR to pathogens and parasites included 44 references for annual species and 119 for perennial species (Seiler and Marek, 2011). Twelve of the fourteen annual species were cited, with the most frequently cited species being *H. agophyllus* (32), followed by *H. petiolaris* (15), wild *H. annuus* (15), *Helianthus debilis* Nutt. (13), and *H. prae cox* (8). *Helianthus agophyllus* was also the species with the highest number of disease and parasite resistances with nine, followed by *H. debilis* (7), *H. annuus* (6), and *H. petiolaris* (5). Among the 39 perennial species, only 10 were referenced, with *H. tuberosus* cited most frequently (40 times), followed by *Helianthus pauciflorus* Nutt. (19), *Helianthus maximiliani* Schrad. (19), *Helianthus resinosus* Small (16), *Helianthus di varicatus* L. (16), and *Helianthus nuttallii* T. & G. (14). Of these referenced species, *H. tuberosus* was described as resistant to the most diseases (10), followed by *H. maximiliani* (9), *H. pauciflorus* (8), *H. resinosus* (8), and *H. di varicatus* (7). It is apparent that fewer perennial CWR have been evaluated in part due to the difficulties in overcoming dormancy and germination problems and in obtaining an adequate number of seeds and plants for testing, but also due to complicated ploidy levels in some of the perennial species causing fertility problems in recovering viable progeny.

**Alternaria**

Alternaria leaf spot causes losses in cultivated sunflower in the United States and other parts of the world in warm climates with high rainfall, where defoliation significantly reduces yield (Sackston, 1981). Several wild annual species—*H. prae cox*, *H. debilis* ssp. *cucumerifolius* T. & G., and *H. debilis* ssp. *silvestris* Heiser—had high levels of resistance to Alternaria leaf spot and also to Septoria leaf spot (Block, 1992). The perennial CWR species *Helianthus giganteus* Raf., *H. pauciflorus* ssp. *subhomoideus* Rydb., and *H. tuberosus* appear to resist infection by *A. helianthi* in field evaluations (Morris et al., 1983). Resistance to Alternaria leaf spot was also observed in interspecific hybrid derivatives of *H. di varicatus* and cultivated sunflower and two hexaploid species, *H. resinosus* and *H. tuberosus*, in field tests (Prabakaran and Sujatha, 2004). Škorić (1987) reported that *Helianthus salicifolius* Dietr. had resistance to Alternaria, while Encheva et al. (2006) reported complete resistance in interspecific hybrids of *H. salicifolius* field tests.

**Powdery Mildew**

Powdery mildew is a common foliar disease on senescing leaves of cultivated sunflower in warm regions of the world. It is seldom severe enough to warrant fungicide applications in temperate climates, whereas in tropical areas, significant yield losses can occur (Gulya et al., 1997). Annual *H. debilis* ssp. *silvestris*, *H. prae cox* ssp. *pa roc ox*, and *Helianthus bolanderi* A. Gray and 14 perennial species were tolerant of powdery mildew in both field and greenhouse tests (Saliman et al., 1982). Jan and Chandler (1985) identified a source of resistance to powdery mildew in *H. debilis* ssp. *debilis* that is incompletely dominant in the F₁ and backcross progenies. They incorporated genes from this species into a cultivated background and released a germplasm pool PM1 (Jan and Chandler, 1988). Rojas-Barros et al. (2005) observed that two subspecies, *H. debilis* ssp. *debilis* (different accession than used by Jan and Chandler, 1985) and *H. debilis* ssp. *vestitus* (E.E. Walton) Heiser, and *H. agophyllus* were completely resistant. Segregation patterns indicated that resistance in the crosses was controlled by at least two genes.

Škorić (1984) reported that interspecific hybrids with perennial species *Helianthus giganteus* L., *H. hirsutus*, *H. di varicatus*, and *H. salicifolius* had no powdery mildew symptoms in field tests. Christov (2008) indicated that there are two types of resistance to this pathogen. One is controlled by a dominant gene from perennial *H. decapetalus* and a second is controlled by multiple genes found in perennial *Helianthus glauophyllum* D.M. Smith, *H. ciliaris*, *Helianthus laevigatus* T. & G., *H. tuberosus*, and annual *H. debilis*.

**Charcoal Rot**


**Verticillium**

Verticillium wilt infects sunflower roots, causing wilting and leaf mottling, and is an important disease of cultivated sunflower, being especially severe in Argentina. Putt (1964) discovered a source of resistance to *V. dahliae* in line CM144, which was derived from an interspecific hybrid of wild *H. annuus*. *Helianthus annuus*, *H. petiolaris*, and *H. prae cox* were the major sources of the V₁ gene for Verticillium wilt resistance (Hoes et al., 1973). In field testing, only slight disease symptoms were reported on *H. hirsutus*, *Helianthus occidentalis* Riddell, and *H. tuberosus*, while populations of *H. resinosus* were free of the disease (Škorić, 1984).
A new North American strain of *V. dahliae* found in North Dakota and Minnesota in 2004 is capable of overcoming the single dominant *V-1* resistance gene used in oilseed and confectionery sunflower (Gulya, 2004). Based on the previous success of finding the *V-1* resistance gene in a wild *H. annuus* population, it is reasonable to expect that the CWR will be a source of resistance gene(s) for the new strain.

Phomopsis

In the last three decades Phomopsis brown stem canker has become the most destructive disease worldwide (Škorić, 2016). Cuk (1982) reported that wild annual *H. debilis* and perennial *H. pauciflorus* were potential sources of resistance to *P. helianthi* based on field tests. Škorić (1985) also reported tolerance in four inbred lines: two based on perennial *H. tuberosus*, and one each based on annual *H. annuus* and *H. argophyllus*. Phomopsis brown stem canker resistance has been found in perennials *H. maximiliani*, *H. pauciflorus*, *H. hirsutus*, *H. resinosis*, *H. mollis*, and *H. tuberosus* (Dozet 1990). Cultivated hybrids developed from *H. tuberosus* and *H. argophyllus* have high field tolerance to Phomopsis brown stem canker (Škorić, 1985). Nikolova et al. (2004) observed field resistance to stem canker in progenies of interspecific hybrids of perennial *Helianthus pauciflorus* Nutt. Field resistance to Phomopsis was reported in interspecific hybrids derived from *H. argophyllus*, *Helianthus deserticola* Heiser, *H. tuberosus*, and *Helianthus × lactiflorus* Pers. (Degener et al., 1999).

Christov (2008) identified annuals *H. annuus*, *H. argophyllus*, and *H. debilis* and perennials *H. pauciflorus*, *H. laevigatus*, *H. glaucophyllus*, and *Helianthus eggertii* Small as potential sources of Phomopsis brown stem canker resistance in some half-sib families based on field screening in Bulgaria. Among the perennial wild sunflowers, diploid *H. divaricatus* was identified as a source of tolerance to Phomopsis (Korrell et al., 1996). Complete resistance to Phomopsis was reported in interspecific hybrids of *H. salicifolius* by Škorić (1987) and Encheva et al. (2006).

Rust

Sunflower rust, a foliar disease, occurs in almost all sunflower-growing regions and is a constant threat due to newly emerging virulent races. Rust resistance genes from CWR have been frequently deployed as race-specific single dominant genes, and as a consequence, the commercial life of hybrids is quickly challenged as new races with increased virulence evolve. Crop wild relatives have been an important source of rust resistance genes for cultivated sunflower for many years. Resistance genes *R*₁ and *R*₂, which have been widely used in breeding programs, originated from outcrosses with CWR in Texas and are believed to be among the earliest resistance genes to genetically control a sunflower pathogen (Putt and Sackston, 1957, 1963). Hennessy and Sackston (1972) concluded that most species of wild sunflower in Texas were heterogeneous for rust resistance. An extensive survey of >200 populations of two annual species (*H. annuus* and *H. petiolaris*) and five perennial species, [*H. maximiliani*, *H. mutellii*, *Helianthus grosseserratus* Martens, *H. pauciflorus* (*rigidus*), and *H. tuberosus*) from the north-central United States was undertaken by Zimmer and Rehder (1976). Plants free of rust were observed in 190 of the populations, with widespread resistance in wild annual populations from Nebraska and Kansas. Quresh and Jan (1993) and Quresh et al. (1993) observed that the frequency of plants resistant to rust races 1 (current race 100), 2 (200), 3 (500), and 4 (700) in 78 populations of *H. annuus, H. argophyllus*, and *H. petiolaris* was 25, 28, 15, and 26%, respectively. Only 10% of the plants were resistant to all four races of rust. Immunity to rust has been reported in lines derived from *H. tuberosus* (Pogorietsky and Geshle, 1976). Resistance to the prevailing North American races of rust was identified in three wild annual species, *H. annuus, H. petiolaris*, and *H. argophyllus*, resulting in the release of seven germplasms, PH1 through PH7 (Jan et al., 2004a). Qi and Seiler (2013) released a germplasm, HA-R9, with the *R*₁₁ gene derived from CWR *H. annuus* from Oklahoma, which was previously released as germplasm RF ANN-1742 (Seiler and Jan, 1997) and identified as resistant to all known races (Gong et al., 2013a). The gene was mapped to the lower end of LG13 of the sunflower genome, tightly linked to a male fertility gene *R*₁₅₁ (Qi et al., 2012). Hulke et al. (2010b) released germplasm RHA 464 that confers resistance to race 777, the most virulent race of rust, with the source derived from a CWR, *H. annuus* population from California. The resistance gene from RHA 464 was mapped to LG11 and named *R*₁₂ using simple sequence repeat (SSR) markers (Gong et al., 2013b). Later, fine mapping using single-nucleotide polymorphism (SNP) markers defined the *R*₁₂ gene to an interval <2.3 cM in LG11 (Talukder et al., 2014). Zhang et al. (2016a) reported that the rust resistance gene named *R*₁₄ from PH3, originating from an *H. annuus* accession from South Dakota, was also mapped to LG11, close to the *R*₁₂ gene.

It appears that annual CWR populations commonly contain rust-resistant plants, but complete resistance or total susceptibility of populations is rarely found (Quresh et al., 1993). Since *P. helianthi* races are host specific, susceptible wild *Helianthus* species provide selective hosts in which virulent races of rust can develop. Because rust races are continually evolving, it is necessary to have new sources of resistance available. The rust pathogen can be effectively controlled in sunflower for long periods of time through the use of resistance genes found in the CWR. Interestingly, no perennial sunflower CWR have been identified to have resistance to rust.
Downy Mildew

Downy mildew occurs in all countries where sunflower is grown, with the exception of Australia. Forty pathotypes (races) of *P. halstedii* have been described, five of which (330, 330, 710, 730, and 770) are universally distributed (Viranyi et al., 2015). The constant evolution of new physiological races, due to pathogenic variability and selection pressure resulting from the use of resistant hybrids and seed treatment fungicides, continuously challenges breeders to identify and introduce new resistance genes or gene clusters.

Wild annual sunflower species have been a bountiful source of *Pl* genes for DM resistance. Jan et al. (2004b) released germplasms PLH1 through PLH4 resistant to DM race 730 based on annual wild *H. annuus* populations from California, New Mexico, California, and Texas, respectively. Germplasm lines HA 335 and HA 336 (*Pl* 6 gene) based on wild *H. annuus*, HA 337 through 339 based on *H. praeox* ssp. *runyonii* Heiser, and RHA 340 (*Pl* 7 gene) based on *H. argophyllus* have provided much of the historical and current resistance to DM (Miller and Gulya, 1988). *Helianthus argophyllus*-derived germplasm ARG-1575–2 (Seiler, 1991b) carries the *Pl* 7g locus conferring resistance to all known races of *P. halstedii* DM (Gascuel et al., 2015; Gilley et al., 2016). *Pl* 6 and *Pl* 7 were both previously mapped to LG8 of the sunflower genome (Bouzidi et al., 2010b), whereas *Pl* 6 was mapped to LG13 (Bachlava et al., 2011). Since *Pl* 7 was mapped to LG1, different from all other *Pl* genes previously mapped using SSRs, it was concluded that *Pl* 7g provides a new unique source of resistance to *P. halstedii* (Duflé et al., 2004; Wieckhorst et al., 2008).

Hulke et al. (2010a) released three DM-resistant genetic stocks: HA 458 based on CWR *H. annuus* from Texas, HA 459 based on CWR *H. annuus* from Idaho, and HA 460 based on *H. argophyllus* from Texas, resistant to a composite of the most common and most virulent races of *P. halstedii*. Downy mildew resistance in HA 458 is controlled by a single dominant gene, *Pl* 17g, located in LG4, the first DM gene discovered in this linkage group (Qi et al., 2015). The SNP SFW04052 and SSR ORS963 markers delimited *Pl* 17g in an interval of 3.0 cM. Zhang et al. (2016b) reported the transfer of a new DM gene, *Pl* 19g, from CWR *H. annuus* from Texas, into cultivated sunflower and mapped this gene downstream from *Pl* 17g in LG4. Line RHA 464, released by Hulke et al. (2010b) for rust resistance based on a source derived from a CWR, *H. annuus* population from California, was also resistant to DM with the *Pl* 19g gene derived from *H. argophyllus*. Recently, Qi and Seiler (2016) released germplasm HA-DM1 based on *H. argophyllus* with a single dominant *Pl* 19g gene conferring immunity to all isolates of *P. halstedii* races tested (Gilley et al., 2016), which has been mapped to LG2 of the sunflower genome (Qi et al., 2016) and is independent of all identified DM resistance genes in sunflower. Interestingly, no perennial sunflower CWR have been identified to have resistance to DM, the same as with rust.

Sclerotinia

*Sclerotinia sclerotiorum* (white mold), a necrotrophic fungus that incites sunflower basal stalk rot (BSR), mid-stalk rot (MSR), and head rot (HR), is considered the most devastating disease of sunflower in many parts of the world, in part due to the extensive host range of more than 400 broadleaf species. Sclerotinia manifests itself as three diseases on sunflower, attacking roots, stems, and heads, which requires a complex breeding strategy that involves many genes, each with small effects for each disease.

Interspecific *F* 1 progenies derived from annual *H. argophyllus* were reported to have high tolerance to Sclerotinia HR (Christov et al., 2004). Hahn (2002) reported that interspecific lines based on annual *H. argophyllus* and *H. praeox* ssp. *runyonii* were the most resistant in screening tests for HR resistance. Block et al. (2009) reported that the annual *H. argophyllus*, *H. praeox*, *H. debilis*, and *H. neglectus* had superior BSR resistance in greenhouse tests, with one accession of *H. argophyllus* (PI 649863) having 94% survival in inoculated field tests. High levels of resistance to Sclerotinia were observed in annual CWR *H. argophyllus*, *Heliannthus niveus* (Benth) Brandegee, *H. neglectus*, *H. debilis*, and *H. petiolaris* screened at the Veidelevka Institute of Sunflower, Russia (Tikhomirov and Chiryaev, 2005). In greenhouse tests, Qi et al. (2011) reported high levels of BSR resistance in interspecific *F* 1 progenies of annual *H. argophyllus*, *H. praeox* ssp. *runyonii*, and *H. petiolaris* ssp. *fallax*.

Perennial *Helianthus* species have also shown promise as a source of resistance for this disease complex. Rashid and Seiler (2004) identified 12 *H. maximiliani* and 8 *H. nuttallii* accessions from Canada that were immune to HR. Cerboncini et al. (2002) evaluated 14 wild perennial *Helianthus* species and identified *H. maximiliani* AC7 as highly tolerant to MSR. Subsequently, among interspecific progenies involving accession AC7, Sclerotinia HR resistant material rated higher than the resistant check was selected (Ronicek et al., 2004). Amplified fragment length polymorphism (AFLP) markers specific to *H. maximiliani* have been used to tentatively identify two AFLP fragments linked to Sclerotinia resistance. Mid-stalk rot resistance was reported by Micic et al. (2005) in an interspecific cross with *H. tuberosus* used to determine quantitative trait loci (QTL). Interspecific hybrids based on perennial *H. nuttallii*, *H. giganteus* and *H. maximiliani* that showed resistance to Sclerotinia stem infection were reported by Henn et al. (1997).

An extensive greenhouse BSR evaluation conducted by Block et al. (2012) suggested remarkable resistance levels for all the perennial species tested, including >90% survival for 13 populations of *H. decapetalus*, 42 of *H. maximiliani*, 30 of *H. nuttallii*, and 14 of *H. giganteus*, while 7 accessions of *Helianthus californicus* DC, 7 *H. salicifolius*, 9 *H.
tuberosus, and 13 H. grosseserratus accessions had 100% survival. Perennial H. resinous has been identified as a source for resistance to Sclerotinia HR by Mondolot-Cosson and Andary (1994). Block et al. (2009) reported that two populations of H. resinous, PI 650079 and PI 650082, had 100% BSR resistant plants in greenhouse screening.

Some progress has been made in increasing resistance to Sclerotinia BSR in cultivated sunflower. Miller and Gulya (1999) developed four maintainer and four restorer oilseed lines with improved tolerance to Sclerotinia stalk rot. Inbred line HA 410, released by Miller and Gulya (1999) and derived from a wild perennial hexaploid H. pauciflorus (= rigidus), had moderate tolerance to BSR.

Efforts are being focused on the transfer of BSR resistance from wild Helianthus species of different ploidy levels (2x, 4x, 6x) into adapted sunflower germplasms via interspecific hybridization (Jan and Seiler, 2008). Hexaploid perennial H. californicus, which had been identified as highly resistant to BSR, was crossed with the moderately tolerant line HA 410 (Miller and Gulya, 1999), followed by continuously backcrossing with HA 410 until BC1F1, and the chromosome number of the BC progeny was reduced to 2n = 34 (Feng et al., 2007a). Sclerotinia-resistant sources, including wild perennial diploid, tetraploid, and hexaploid CWR and hexaploid and tetraploid interspecific amphiploids derived from H. divaricatus, H. grosseserratus, H. maximiliani, H. mutallii, and H. stramosus, have been identified that segregated for high levels of resistance to BSR and will expand the diversity of resistance genes (Feng et al., 2007b; Liu et al., 2012). Liu et al. (2009) reported the use of genomic in situ hybridization (GISH) to track the chromosomes in interspecific and backcross progenies developed for Sclerotinia resistance screening involving four perennial wild species, H. californicus (2n = 102), Helianthus angustifolius L. (2n = 34), H. mutallii (2n = 34), and H. maximiliani (2n = 34).

Parasitic Plants: Broomrape

Broomrape, while not a disease, is a parasitic weed that infects sunflower roots, causing severe losses in drier climates in southern Europe and the Black Sea region, Australia, Mongolia, and China (Höniges et al., 2008). Five resistance genes (Or1–Or5) have been used successfully for broomrape control following the progression of races A through E. Since broomrape is a highly variable parasite, similar to infesting pathogens, the breakdown of resistance is a frequent phenomenon, and multiple sources of resistance are needed. The use of vertical resistance mechanisms controlled by single dominant genes has led to a rapid breakdown of resistance (Fernández-Martínez et al., 2012). This has led to the appearance of races F, G, and H and others that have not been assigned a race designation. Resistance, including immunity, to broomrape in 7 annuals and 32 perennial CWR provides breeders with a broad genetic base from which to search for resistance to existing and newly emerging races (reviewed by Seiler and Jan, 2014; Jan et al., 2014a).

The sources of resistance to Orobanche races found in the early sunflower breeding programs in the Former Soviet Union (FSU) originated primarily from interspecific CWR of H. tuberosus (Pustovit et al., 1976). High levels of resistance to a new race F in Spain were reported in 26 perennial species by Ruso et al. (1996). It is very interesting that broomrape has not been observed in the sunflower production regions of the Americas, yet we see a very high level of resistance (near immunity) existing in over two-thirds of the perennials and several of the annuals, without the presence of the parasite races in the United States to incite the current level of resistance.

Hladni et al. (2009) described resistance to race F in a fertility restorer line derived from annual H. desertica in Serbia, while Cvejić et al. (2012) reported resistance to race G in the same species. Interspecific amphiploids derived from perennial CWR of H. grosseserratus, H. maximiliani, and H. divaricatus were used to develop germplasm BR1 through BR4 resistant to race F (Jan et al., 2002). Resistance to race G reported by Velasco et al. (2012) was transferred from annual H. debilis ssp. tardiflorus Heiser into cultivated sunflower.

Virus

Several viruses from different continents naturally infect sunflower. In the United States, viral diseases are rarely observed and are not economically significant (Giolitti and Lenardon, 2016). There are records of sunflower mosaic virus (SuMV) occurring on wild sunflower in the lower Rio Grande Valley of Texas and on commercial fields in south Texas (Giolitti and Lenardon, 2016). Interestingly, the only sources of resistance for SuMV are from populations of wild H. annuus from extreme southern Texas. The TX16R germplasm with SuMV resistance was released and also had resistance to all known races of DM and rust (Jan and Gulya, 2006a). Three additional populations of wild H. annuus from south Texas were also identified as having high frequencies of SuMV resistance and were subsequently used to develop three genetic stocks: SuMV-1, SuMV-2, and SuMV-3 (Jan and Gulya, 2006b).

Insects

Insect pests are an acute problem in the United States but cause very limited damage in other countries. In the major production areas, there are 15 principal insect pests, and of this total, about six are considered economically important from year to year (Charlet and Brewer, 1997; reviewed by Knodel et al., 2015). Attempts to identify and transfer insect resistance from the wild species have been much less successful than the transfer of genes for disease resistance (Seiler, 2012). The reasons for the lack of success include...
are not clear but may be related to the fact that the insect resistance does not appear to be simply inherited, with multiple genes involved, and is therefore more difficult to transfer and maintain in the breeding process. Screening for insect resistance is a more complex process than disease resistance evaluations, and resources to develop reliable insect evaluation methods and screening for the major insect pests has been limited.

Sunflower Moth
Tolerance to the sunflower moth, *Homoeosoma elctellum* (Hulst), has been observed in annual *H. petiolaris* and perennial *H. maximiliani, H. ciliaris, H. strumosus,* and *H. tuberosus* (Rogers et al., 1984). These observations led to the release of three interspecific germplasm lines with resistance to sunflower moth: SFM 1 and SFM3 based on *H. petiolaris,* and SFM 2 based on *H. tuberosus.* The three released germplasms have a phytomelanin (carbon) layer that becomes extremely dense after deposition in the pericarp, making the achenes more resistant to mechanical puncture by younger sunflower moth larvae at an early stage of development (Stafford et al., 1984). Other identified forms of plant defense for the sunflower moth have a chemical basis. Sesquiterpene lactones present in capitule glandular trichomes on leaves and florets of the sunflower CWR cause mortality or delayed development for larvae of the sunflower moth (Gershenzon et al., 1985; Rogers et al., 1987).

Stem Weevil
The stem weevil, *Cylindrocopturus adesperus* (LeConte), is a particularly troublesome pest where the crop is grown west of the Mississippi River (Charlet et al., 2002). Significantly fewer larvae were recovered from stems of eight annual and all perennial CWR compared with cultivated hybrid ‘894’ (Rogers and Seiler, 1985). However, number of larvae in F₁ progenies developed from the perennial species was equivalent to the number found in cultivated sunflower. Attempts to incorporate resistance genes from CWR for stem weevil have not yet been successful. Interspecific crosses with annual *H. petiolaris* and *H. argophyllus* were tolerant in 1 yr but then failed to express tolerance in other years. This could be due to the multigenic control of the resistance, linked to other genes that are lost in the early cycles of selection. In a more recent multiple-year study of interspecific germplasms derived from the perennial *H. bisulcatum,* HIR 1734–2 (Seiler, 1991a) and HIR 828–4 (Seiler, 2000) had half the number of larvae per stem as the cultivated control (Charlet et al., 2009).

Sunflower Beetle
The sunflower beetle, *Zygogramma exclamationis* (Fabricius), has long been recognized as an important defoliator of sunflower in the northern Great Plains of the United States (Charlet et al., 1987) but is not as serious in the southern Great Plains (Rogers, 1977), nor in other production areas of the world. In laboratory studies, about half of the CWR exhibited resistance to feeding and/or reproduction (Rogers and Thompson, 1978, 1980). Antibiosis against both larvae and adults was strongly expressed in annual *H. agrestis* and *H. praeox* and perennial *H. grosseserratus, H. pauciflorus, H. salicifolius,* and *H. tuberosus* (Rogers and Thompson, 1978, 1980).

Red Seed Weevil
The red sunflower seed weevil, *Smicronyx fulvus* LeConte, is a major insect pest of sunflower in the northern Great Plains of North America, but not in other production areas (Charlet et al., 1997). Charlet and Seiler (1994) found indications of resistance to the red sunflower seed weevil in several CWR, interspecific germplasms that incorporated *H. strumosus* and *H. tuberosus* that had lower seed damage than the check hybrid ‘894’ in a single year of testing (Charlet et al., 2004). In a 3-yr field study of red sunflower seed weevil, an interspecific germplasm PAR 1673–1 (Seiler, 1991b) from *Helianthus paradoxus* Heiser had the least damaged seed per head (14%), about half of the damage observed in the hybrid control (Charlet et al., 2010).

Banded Sunflower Moth
The banded sunflower moth, *Cochylis hospes* Walsingham, has been a persistent pest of sunflower in the northern Great Plains, with populations also present in the central Great Plains but not observed elsewhere (Charlet et al., 2008). Among germplasms tested in a banded sunflower moth evaluation nursery, interspecific germplasms derived from *H. praeox ssp. hirtus* Heiser (PRA-HIR 437) (Seiler, 1991c) had <2% feeding damage, and *H. praeox ssp. prae- cox* (PRA-PRA 1142) (Seiler, 1991c) and *H. giganteus* (GIG 1616–2) (Seiler, 2000) crosses had <4% damage compared with 12% in the hybrid check (Charlet et al., 2004).

ABIOTIC TRAITS
Palmgren et al. (2015) suggested that modern crops should be developed with properties that their CWR once possessed to tolerate adverse environmental conditions that were inadvertently lost during selecting for high yield. Wild sunflower contains considerable variability for tolerance to abiotic stresses such as drought and salinity (Ortiz, 2015), and salinity tolerance has been the subject of successful interspecific germplasm development.

Salt Tolerance
Several species of *Helianthus* are native to salt-affected habitats and may possess genes for salt tolerance. The Pecos sunflower (*H. paradoxus*) is particularly well adapted to saline soils and is able to outcompete other wild sunflower species in the salt marshes of New Mexico and
western Texas, suggesting that the species is a candidate for salt tolerance genes (Seiler et al., 1981). *Helianthus paradoxus* is a unique species of homoploid origin occupying salt marshes, quite different from habitats of either of its parents, *H. annuus* (clay soils) or *H. petiolaris* (sandy soils) (Gross and Rieseberg, 2005). Hajjar and Hodgkin (2007) suggest that this species has great potential for helping to breed more salt-tolerant cultivated sunflowers, with hybrids developed using this trait potentially providing a 25% yield premium in saline soils. Chandler and Jan (1984) evaluated three wild *Helianthus* species for salt tolerance: *H. paradoxus*, *H. debilis*, and wild *H. annuus* populations native to salty desert areas. *Helianthus debilis* tolerated salt concentration at about the same level as cultivated sunflower, while the selected wild *H. annuus* populations had a higher salt tolerance than *H. debilis* and cultivated sunflower, and the *H. paradoxus* populations were highly salt tolerant, with some plants surviving at 1300 mM NaCl. Salt tolerance appeared to be a dominant trait; hybrids between *H. paradoxus* and cultivated *H. annuus* were as salt tolerant as the wild parent. Miller (1995) evaluated interspecific germplasms based on *H. paradoxus*, PAR-1084–1, PAR 1673–1 and PAR-1673–2 from Texas (Seiler, 1991b) and PAR-1671–1 and PAR-1671–2 from New Mexico (Seiler, 1991c), which withstand salt concentrations up to electrical conductivity 2.47 S m$^{-1}$. He concluded that one major gene controls salt tolerance, although a recessive modifier gene may also be present. Two salt-tolerant oilseed parental lines, HA 429 and HA 430, developed from these interspecific germplasms were released by Miller and Seiler (2003). Welch and Rieseberg (2002) grew these interspecific germplasms based on *H. paradoxus* in graduated NaCl concentrations and found them to be five times more salt tolerant than their ancestral species, *H. annuus* and *H. petiolaris*.

Salt-tolerant candidate genes that code for calcium-dependent protein kinase (CDPK3), which map to a salt tolerance QTL on LG4 in sunflower, were identified in expressed sequence tag (EST) libraries of sunflower based on homology to genes with known function and on previous QTL results (Lexer et al., 2004). *Helianthus paradoxus* constitutively under- or overexpressed genes related to potassium and calcium transport, suggesting that these genes may contribute to the adaptation of this species to salinity (Edelist et al., 2009).

**Drought Tolerance**

During the process of selecting plants for high yield, breeders may inadvertently lose some drought survival mechanisms common in CWR that maybe beneficial in a selection program to enhance drought tolerance in cultivated sunflower. Crop wild relatives provide the opportunity to study in more detail the physiological processes that are involved in the survival mechanisms of desert-inhabiting species, similar to the study by Bowsher et al. (2016) for desert-adapted *Helianthus niveus* (Benth.) Brandegee ssp. tephrades (A. Gray) Heiser, endemic to the Algodones Dunes in California, part of the Sonoran desert.

*Helianthus anomalus* has frequently been recognized as drought tolerant, with the largest achenes of any wild species and relatively high oil concentration potential (Seiler, 2007), and thus is a candidate for improving cultivated sunflower (Nabhan and Reichhardt, 1983; Seiler et al., 2006). It also appears to be more tolerant of nutrient stress than its ancestral parents, based on its slower relative growth rate and higher nutrient use efficiency (Brouillette and Donovan, 2011).

For drought tolerance breeding, *H. argophyllus* has been extensively used by sunflower breeders (Baldini et al., 1993; Belhassen et al., 1996; Baldini and Vannozzi, 1998, 1999; Griveau et al., 1998). Compared with cultivated sunflower inbred lines, interspecific lines obtained by divergent selection for physiological traits from *H. argophyllus* had higher water use efficiency, better drought susceptibility index, and higher harvest index under drought conditions (Martin et al., 1992; Baldini et al., 1993; Baldini and Vannozzi, 1998).

Sobrado and Turner (1983) compared tissue water relation characteristics and biomass productivity in two cultivars of *H. annuus* and two wild species (*H. muttallii* and *H. petiolaris*) under field conditions. Water deficits induced a major reduction in leaf area development and dry matter accumulation in all species. A water deficit also induces a significant decrease in the osmotic potential at full turgor and decreases the turgid-to-dry-weight ratio in cultivated lines, but not in wild species.

**Oil and Oil Quality**

Variability for oil concentration exists in the CWR. Seed oil concentration has been reported for most species, generally averaging about 250 g kg$^{-1}$, which is lower than in cultivated sunflower with 430 to 450 g kg$^{-1}$ (Seiler, 1985). However, when using the CWR for other traits, backcrossing to cultivated lines rapidly raises the oil concentration to an acceptable level. Annual *Helianthus anomalus* S.F Blake has the highest oil concentration of 460 g kg$^{-1}$, the highest ever observed in a wild sunflower species (Seiler, 2007). Other CWR with higher-than-normal concentrations of oil include: annual *H. niveus* ssp. canescens with 402 g kg$^{-1}$, *H. petiolaris* with 377 g kg$^{-1}$, *H. deserticola* with 343 g kg$^{-1}$, and perennial *H. salicifolius* with 370 g kg$^{-1}$ (Seiler, 1985). Cultivated sunflower generally contains 430 to 450 g kg$^{-1}$.

Fatty acid composition is an important characteristic of sunflower oil, determining its end use. Reduced concentrations of saturated palmitic and stearic fatty acids have been observed in a population of wild *H. annuus* that had a combined concentration of 58 g kg$^{-1}$, and 65 g kg$^{-1}$ was observed in a wild perennial species, *H. giganteus* (Seiler,
1998). These values are 50% lower than in the oil of cultivated sunflower (120 g kg\(^{-1}\)) providing new potential sources for reducing saturated fats in the oil.

**Crop Wild Relative Genomics**

The National Center for Biotechnology Information (NCBI) database contains nucleotide sequences and ESTs of 51 of the 53 Helianthus species with eight species responsible for 98% of data (Table 2; Kane et al., 2013). Over the past several decades, Helianthus has emerged as an excellent experimental system for studying the ecological genetics of speciation, species boundaries, hybridization, and domestication (Kane et al., 2013). With the availability of expanding genomic resources, extensively available germplasms from public gene banks, a rapidly developing genetic tool kit, and an important economic impact, Helianthus is an ideal taxon to illustrate the usage of CWR for crop improvement, and also to be used as a model crop for ecological and evolutionary studies.

Four hundred and six of the NPGS introgressed lines were recently genotyped by Baute (2015), including 351 of the most stable and fertile lines he developed with introgressions from 11 CWR. The genomic data provide information about the size and location of linkage groups of the CWR introgressions.

The genome of domesticated sunflower *H. annuus* was the primary target of two sequencing efforts, HA412-HO (a high-oleic inbred maintainer line with Sclerotinia head and stem rot resistance), sequenced by L. Rieseberg at the University of British Columbia (https://www.sunflowergenome.org/; see Renaut et al., 2013, for assembly details), and XRQ (an inbred line developed at INRA, France, from a cross between HA89 and a Russian open-pollinated variety), sequenced by the Sunrise Group at INRA (INRA, 2016). Focus has now shifted to generating a substantial number of genomics sequences for numerous CWR, and high quality annotated genomes will be assembled for several, including annual *H. argophyllus* and *H. petiolaris* and perennial *H. maximiliani* (Kane et al., 2013). To identify genes targeted by selection during the domestication and improvement of sunflower, and to detect postdomestication hybridization with wild species, Baute et al. (2015b) analyzed transcriptome sequences of 80 genotypes, including wild, landrace, and modern lines of *H. annuus*, as well as two cross-compatible wild relatives, *Helianthus argophyllus* and *Helianthus petiolaris*.

**PROSPECT OF CROP WILD RELATIVES OF SUNFLOWER**

It is the genome of a sunflower CWR that will be ultimately utilized to improve the crop and not its phenotype, collection locality, or its history of local adaptation. With this in mind, CWR germplasm resources in gene banks may eventually be best explored, surveyed, or mapped at the level of the genome as we become better at predicting the breeding value of individual accessions and/or alleles (Baute et al., 2015a). Gene banks are important suppliers of genetic resources to the genomic research community, and access to the resulting genomic information will allow traditional gene bank users to better select genetic materials for their breeding and scientific programs (Finkers et al., 2015).

The resequencing of the genomes of CWR is a rapid method to determine the likely utility of germplasms in crop improvement. The conservation of genetic resources both in situ and ex situ can be guided by information on the novelty of specific populations at the whole-genome allele level. Variation in the genomes of plants from diverse environments defines strategies that might be employed to develop climate-resistant crop varieties (Henry, 2014). Efforts to reduce deleterious effects of cross breeding with CWR through backcrossing are costly and time consuming and will no doubt affect the speed with which new cultivars are released. Molecular techniques offer a partial solution, but there will likely continue to be cases where

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**Table 2. Publically available accessions from the USDA and INRA with expressed sequence tags (ESTs) and genomic sequences data available from NCBI.†**

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<th>Taxon</th>
<th>USDA accessions§</th>
<th>INRA accessions‡</th>
<th>NCBI nucleotide sequences</th>
<th>NCBI ESTs</th>
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<tr>
<td><em>H. neglectus</em> (A)</td>
<td>28</td>
<td>3</td>
<td>132</td>
<td>0</td>
</tr>
<tr>
<td><em>H. paradoxus</em> (A)</td>
<td>2</td>
<td>2</td>
<td>268</td>
<td>30,517</td>
</tr>
<tr>
<td><em>H. petiolaris</em> (A)</td>
<td>139</td>
<td>23</td>
<td>1896</td>
<td>27,484</td>
</tr>
<tr>
<td><em>H. tuberosus</em> (P)</td>
<td>90</td>
<td>21</td>
<td>205</td>
<td>40,362</td>
</tr>
</tbody>
</table>

† Source: Kane et al. (2013).
‡ USDA–ARS–NPGS gene bank; INRA (National Institute of Agronomic Research) sunflower gene bank.
§ A, annual; P, perennial.
pleiotropic effects limit the use of genes from CWR (Hajjar and Hodgkin, 2007).

The CWR of sunflower have played a vital role in the development of a viable sunflower crop and will continue to do so into the future as one of the primary sources of genetic diversity for the sunflower crop. Traditional breeding technologies have established a basis for utilizing CWR in the improvement of cultivated sunflower. Incorporating CMS from wild sunflower facilitated the development of the globally valued sunflower crop, and incorporation of disease resistance and other biotic and abiotic traits has protected the investment in hybrid sunflower. Future developments are expected to take advantage of emerging technologies to increase the efficiencies of the breeding process of mining the genes from existing sunflower CWR.

**Conflict of Interest**

The authors declare there to be no conflict of interest.

**References**


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