

GERMPLASM RESOURCES FOR INCREASING THE GENETIC DIVERSITY OF GLOBAL CULTIVATED SUNFLOWER

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SUMMARY

Genebanks are a rich source of genetic diversity that can be readily exploited for crop improvement. The USDA-ARS established a cultivated sunflower germplasm collection at the National Plant Germplasm System, North Central Regional Plant Introduction Station at Ames, Iowa in 1948. A wild *Helianthus* germplasm collection was established at the USDA-ARS Bushland, Texas station in 1976. Presently, both collections are maintained and managed at Ames, Iowa. The genus *Helianthus* consists of 52 species and 19 subspecies, with 14 annual and 38 perennial species. Over 30 explorations in the past 35 years have resulted in the assemblage of a wild sunflower collection that is the most complete in the world. Currently, the sunflower collections contain 4087 accessions: 1886 cultivated *H. annuus* accessions and 2201 wild accessions of which 1359 accessions are annual and 842 are perennial species. This germplasm is an important current and future genetic resource to combat emerging pests and environmental challenges, helping to maintain sunflower as a viable major global oilseed crop and preserve it for future generations.

Key words: *Helianthus* species, genebank, genetic diversity, genetic resources

INTRODUCTION

Genetic resources are the biological basis of global food security. Preservation of cultivars, landraces, and wild relatives of important plant species provides the basic foundation for sustainable productive agriculture (Campbell *et al.*, 2010). Indigenous germplasm of any country evolves with genetic adaptations specific to its environment. Preserved genetic resources will supply plant breeders, farmers, and other agricultural scientists with the genetic materials to develop new crop cultivars and hybrids that secure future food, fiber, and seed. They form a pool from

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which all species draw traits that allow them to adapt to biotic and abiotic stresses, such as diseases and pests (Campbell *et al.*, 2010).

Almost a century has been spent collecting and preserving genetic diversity in plants. Genebanks (germplasm collections) are living seed and other tissue collections that serve as repositories of genetic variation that have been established as a source of genes for improving agricultural crops. The use of crop wild relatives' genes to improve crop performance is well established with important examples dating back more than 60 years. Crop wild relatives, which include the progenitors of crops, as well as other species more or less closely related to them, have been undeniably beneficial to modern agriculture, providing plant breeders with a broad pool of potentially useful genes (Hajjar and Hodgkin, 2007; Holden *et al.*, 1993). Genetic diversity contributes to long-term survival of species by allowing them to adapt quickly to changes in their environment. Wild relatives of crop plants typically are genetically much more diverse than related cultivated lineages. Mandel *et al.* (2011) found that gene diversity across cultivated sunflowers was 0.47, as compared with 0.70 for a limited set of wild *H. annuus* populations using SSR markers. This study also showed that the genetic diversity of the cultivated germplasm was roughly 67% of that observed in the selected wild populations.

Although many secondary and tertiary gene pools may appear to have no apparent use in breeding programs (Burton, 1979), they may contain genes that will protect crops against recognized pests with no or minimal host resistance or new pests in the future. Thus, if new pests or environmental stresses deviate beyond normal limits of tolerance, productivity decreases, and a search for germplasm with greater resistance to the stresses is initiated. Since we cannot predict with acceptable levels of confidence the occurrence, severity, or even the nature of future stresses, germplasms with as wide a range of genetic diversity as possible should be developed and incorporated into current breeding programs (Jones, 1983). We maintain and continue to add relevant germplasm with the expectation that the genebanks will contain resources to overcome these challenges.

The loss of variation in crops due to modernization of agriculture has been described as genetic erosion. Genetic erosion of cultivated diversity is reflected in a modernization bottleneck in the diversity levels that occurred during the history of the crop. Two stages in this bottleneck are recognized: the initial replacement of landraces by modern cultivars; and the further erosion of diversity as a consequence of modern breeding practices (Van de Wouw *et al.*, 2009). The modern sunflower crop is a result of selection for narrow uniform quality standards and almost universal use of a single source for cytoplasmic male-sterility (*cms*) or disease resistance contributing to genetic erosion.

The development and application of molecular techniques and genomics have dramatically improved the characterization and deployment of plant genetic resources (Van *et al.*, 2011). Genetic linkage maps have made it possible to locate genes on chromosomes that influence complex traits used to improve agricultural

crops. Genome research may finally unleash the genetic potential of our wild and cultivated germplasm resources for the benefit of society (Tanksley *et al.*, 1997). Presently, bioinformatics is a major bottleneck for a more complete exploitation of the information about genetic resources that is rapidly accumulating (Van de Wouw *et al.*, 2009).

Diversity in germplasm is critical for all successful crop breeding programs, but to date diverse germplasm has received little attention (Harlan, 1976). In the case of cultivated sunflower, wild species of *Helianthus* have been used to some extent, but contain a tremendous amount of genetic diversity yet to be exploited. Unlocking the full potential of sunflower germplasm collections, however, requires an understanding of the amount and distribution of the genetic variation contained within them. The genetic diversity within the wild species can make a significant contribution to the global sunflower industry by providing genes for resistance (tolerance) to pests and environmental stresses that will allow the crop to maintain and extend its economic viability.

Sunflower genetic resources

Crop genetic resources consist of the total pool of genetic variability that exists in the crop species or within species with which the crop plant is sexually compatible (Holden *et al.*, 1993). For the domesticated sunflower, this includes most species of *Helianthus*. Germplasm resources can be categorized as *in situ* resources (*i.e.*, wild populations and landraces) or *ex situ* resources (accessions preserved in seed banks).

Genetic diversity

Helianthus, a native to temperate North America, contains 52 species and 19 subspecies with 14 annual and 38 perennial species (Schilling, 2006) (Tables 1 and 2). The genus *Helianthus* has a basic chromosome number of $n=17$ and contains diploid ($2n=2x=34$), tetraploid ($2n=4x=68$), and hexaploid ($2n=6x=102$) species (Tables 1 and 2). The 14 annual species are all diploid, and the 38 perennial species include 25 diploid, 3 tetraploid, 7 hexaploid, and 3 mixaploid species. *Helianthus ciliaris* and *H. strumosus* have both tetraploid and hexaploid forms, while *H. decapetalus* and *H. smithii* contain diploid and tetraploid forms. The different ploidy levels enhance genetic diversity, but also complicate incorporation into cultivated germplasm.

Sunflower, with its likely origin in eastern USA (Blackman *et al.*, 2011), offers the opportunity to collect and preserve the ancestors of the sunflower crop. This germplasm and associated information is particularly important for the genus *Helianthus* because of the coevolution of its species and associated native insects and pathogens. The crop, wild species, and pests and abiotic stresses have all coevolved in the center of origin of the species. Knowledge of a particular habitat and adaptations of a species occurring therein can often help to identify potential sources of genes for desired traits.

A benefit of coevolution is that the wild species are sources of genes for resistance and tolerance because they are able to survive in areas where challenges exist, providing an opportunity to search for these genes in the wild species. A negative result of coevolution is that the crop is exposed to many pests and abiotic stresses for which the crop does not have genes for resistance. Genetic diversity and habitat diversity go hand in hand and where a species grows can often provide a key to its potentially useful traits.

Table 1: Infrageneric classification of annual *Helianthus* species and number of accessions in the USDA-ARS, NPGS sunflower genebank.

Section* (Chromosome number)	Species	Common name	Number accessions
<i>Helianthus</i>			
(2n=34)	<i>H. annuus</i> L.	Prairie	930
(2n=34)	<i>H. anomalus</i> Blake	Anomalous	6
(2n=34)	<i>H. argophyllus</i> T. & G.	Silver-leaf	49
(2n=34)	<i>H. bolanderi</i> A. Gray	Bolander's, Serpentine	7
(2n=34)	<i>H. debilis</i>		
(2n=34)	ssp. <i>debilis</i> Nutt.	Beach	12
(2n=34)	ssp. <i>cucumerifolius</i> (T. & G.) Heiser	Cucumber- leaf	11
(2n=34)	ssp. <i>silvestris</i> Heiser	Forest	22
(2n=34)	ssp. <i>tardiflorus</i> Heiser	Slow-Flowering	5
(2n=34)	ssp. <i>vestitus</i> (Watson) Heiser	Clothed	3
(2n=34)	<i>H. deserticola</i> Heiser	Desert	21
(2n=34)	<i>H. exilis</i> A. Gray	Serpentine	30
(2n=34)	<i>H. neglectus</i> Heiser	Neglected	28
(2n=34)	<i>H. niveus</i>		
(2n=34)	ssp. <i>niveus</i> (Benth.) Brandege	Snowy	1
(2n=34)	ssp. <i>tephrodes</i> (Gray) Heiser	Ash-Colored, Dune	12
(2n=34)	<i>H. paradoxus</i> Heiser	Pecos, Puzzle, Paradox	2
(2n=34)	<i>H. petiolaris</i> Nutt.		
(2n=34)	ssp. <i>canescens</i> (A. Gray) E.E.Schilling	Gray	19
(2n=34)	ssp. <i>fallax</i> Heiser	Deceptive	29
(2n=34)	ssp. <i>petiolaris</i>	Prairie	96
(2n=34)	<i>H. praecox</i>		
(2n=34)	ssp. <i>hirtus</i> Heiser	Texas	7
(2n=34)	ssp. <i>praecox</i> Englm. & A.Gray	Texas	10
(2n=34)	ssp. <i>runyonii</i> Heiser	Runyon's	24
<i>Agrestes</i>			
(2n=34)	<i>H. agrestis</i> Pollard	Rural, Southeastern	10
<i>Porteri</i>			
(2n=34)	<i>H. porteri</i> (A. Gray) J. F. Pruski	Confederate Daisy, Porter's	9

*Schilling and Heiser, 1981; Schilling, 2006

Table 2: Infrageneric classification of perennial *Helianthus* species and number of accessions in the USDA-ARS, NPGS sunflower genebank.

Section* (Chromosome number)	Series	Species	Common name	Number accessions
<i>Ciliares</i>				
(2n=34)	<i>Ciliares</i>	<i>H. arizonensis</i> R. Jackson	Arizona	2
(2n=68,102)		<i>H. ciliaris</i> DC.	Texas blueweed	26
(2n=34)		<i>H. laciniatus</i> A. Gray	Alkali	7
<i>Ciliares</i>				
(2n=34)	<i>Pumili</i>	<i>H. cusickii</i> A. Gray	Cusick's	20
(2n=34)		<i>H. gracilentus</i> A. Gray	Slender	6
(2n=34)		<i>H. pumilus</i> Nutt.	Dwarfish	52
<i>Atorubens</i>				
(2n=102)	<i>Corona-solis</i>	<i>H. californicus</i> DC.	California	21
(2n=34,68)		<i>H. decapetalus</i> L.	Ten-petal	30
(2n=34)		<i>H. divaricatus</i> L.	Divergent	26
(2n=102)		<i>H. eggertii</i> Small	Eggert's	12
(2n=34)		<i>H. giganteus</i> L.	Giant	25
(2n=34)		<i>H. grosseserratus</i> Martens	Sawtooth	44
(2n=68)		<i>H. hirsutus</i> Raf.	Hairy	12
(2n=34)		<i>H. maximiliani</i> Schrader	Maximilian	64
(2n=34)		<i>H. mollis</i> Lam.	Soft, Ashy	27
(2n=34)		<i>H. nuttallii</i> ssp. <i>nuttallii</i> T. & G.	Nuttall's	23
(2n=34)		<i>H. nuttallii</i> ssp. <i>rydbergii</i> (Brit.) Long	Rydberg's	12
(2n=102)		<i>H. resinosus</i> Small	Resinous	23
(2n=34)		<i>H. salicifolius</i> Dietr.	Willow leaf	19
(2n=102)		<i>H. schweinitzii</i> T. and G.	Schweinitz's	1
(2n=68, 102)		<i>H. strumosus</i> L.	Swollen, Woodland	33
(2n=102)		<i>H. tuberosus</i> L.	Jerusalem artichoke	90
<i>Atorubens</i>				
(2n=34)	<i>Microcephali</i>	<i>H. glaucophyllus</i> Smith	White leaf	11
(2n=68)		<i>H. laevigatus</i> T. and G.	Smooth	7
(2n=34)		<i>H. microcephalus</i> T. and G.	Small-headed	13
(2n=34, 68)		<i>H. smithii</i> Heiser	Smith's	6
<i>Atorubens</i>				
(2n=34)	<i>Atorubentes</i>	<i>H. atorubens</i> L.	Purple-disk	14
(2n=34)		<i>H. occidentalis</i> ssp. <i>occidentalis</i> Riddell	Few leaf, Western	1
(2n=34)		<i>H. occidentalis</i> ssp. <i>plantagineus</i> (T. & G.) Heiser	Few leaf, Western	14
(2n=102)		<i>H. xlaetiflorus</i> Pers.	Mountain	12

Table 2: Infrageneric classification of perennial *Helianthus* species and number of accessions in the USDA-ARS, NPGS sunflower genebank.

(2n=102)	<i>H. pauciflorus</i> Nutt. ssp. <i>pauciflorus</i>	Stiff	22
(2n=102)	<i>H. pauciflorus</i> ssp. <i>subrhomboides</i> (Rydb.) O. Spring	Stiff	15
(2n=34)	<i>H. silphoides</i> Nutt.	Odorous	15
<i>Atorubens</i>			
(2n=34)	<i>Angustifolii</i> <i>H. angustifolius</i> L.	Narrow leaf, Swamp	25
(2n=34)	<i>H. carnosus</i> Small	Fleshy	2
(2n=34)	<i>H. floridanus</i> A. Gray ex Chapman	Florida	8
(2n=34)	<i>H. heterophyllus</i> Nutt.	Variable leaf	18
(2n=34)	<i>H. longifolius</i> Pursh	Longleaf	3
(2n=34)	<i>H. radula</i> (Pursh) T. and G.	Scraper, Rayless	41
(2n=34)	<i>H. simulans</i> E. E. Wats.	Muck, Imitative	4
(2n=34)	<i>H. verticillatus</i> Small	Whorled	2

*Schilling and Heiser, 1981; Schilling, 2006

Plant exploration

A key component of a strong wild species germplasm collection is an active exploration program. The USDA-ARS Plant Exchange Office, National Germplasm Resources Laboratory, Beltsville, Maryland has generously provided funding for wild sunflower exploration since 1984, funding 25 explorations. Collection of germplasm not only facilitates its preservation, it also provides valuable information about the diverse habitats occupied by wild sunflowers and associated species. Thirty explorations covering 175,000 km in the USA, Canada, and Australia (where populations of naturalized wild species were sampled) have resulted in the collection of over 2,100 populations of wild sunflower over the past 35 years. Fifteen of these explorations were undertaken in the past 10 years. A more detailed history of wild sunflower explorations in the USA and Canada can be found in a review by Seiler and Gulya (2004).

NPGS sunflower collections

The mission of the USDA-ARS National Plant Germplasm System (NPGS) is to conserve genetically diverse crop germplasm and associated information, to conduct germplasm-related research, and to encourage the use of germplasm and associated information for research, crop improvement, and product development. Currently, the NPGS contains 543,203 accessions represented by 13,852 species. The collections are held at four regional plant introduction genebanks, and 17 specialized crop centers and clonal repositories across the USA, including Alaska, Hawaii, and Puerto Rico. The long-term seed storage, acting as a backup system, is located at the National Center for Genetic Resource Preservation (NCGRP) at Fort Collins, Colorado. In an ongoing process coordinated by NCGRP, many accessions,

including sunflower, are being stored in the newly opened Svalbard Global Seed Vault, sponsored by the Global Biodiversity Trust, on Spitsbergen Island near the Arctic Circle in Norway. The NPGS locations are united through the Germplasm Information Resource Network (GRIN) database which serves as the portal for requesting available germplasm from the NPGS collections and as a resource for passport data and associated information for each accession.

Cultivated sunflower collection

The NPGS sunflower collections are maintained at the USDA-ARS, North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa, USA. The cultivated sunflower germplasm collection was established at Ames, Iowa in 1948. A majority of the first accessions in this collection were from Turkey, deposited in October, 1948. These included PI 170385 from the Turkey Agricultural Farm, Bornova, Izmir; PI 170386 from the tropical Breeding Station, Antalya; PI 170387 from Bucak, Burdur; and PI 170389 from the Government Farm, Dalaman, Cigli, Mugla. Currently, the collection contains 122 cultivated accessions from Turkey. Of these, 112 accessions, almost 90% of the total collection at that time, were placed in the cultivated collection in 1948 and 1949. The remaining 10 accessions were placed in the collection in 1960. Germplasm from Turkey provided the nucleus of the cultivated sunflower collection for the NPGS.

Wild sunflower species collection

The wild *Helianthus* species collection was established at Bushland, Texas in 1976. In 1985 it was transferred to Ames, Iowa where it is currently housed. The wild species collection contains all 38 perennial species and 14 annual species (Schilling, 2006). Cultivated sunflower is represented by a single species, *Helianthus annuus*. The NPGS sunflower collection is a diverse assemblage of 4087 accessions with 1886 cultivated *Helianthus annuus* accessions (46%) from 59 countries. The number of wild species accessions is 2201 (54%), of which 930 are wild *Helianthus annuus* accessions (42%), and 429 accessions representing 13 other wild annual *Helianthus* species (20%). Thirty-eight perennial *Helianthus* species are represented by 842 accessions, or 38% of the wild collection. Currently, 92% of the cultivated accessions are available for distribution, while 95% of the wild annual species accessions and 70% of the perennial species accessions are available for distribution.

The USDA-ARS NPGS wild species collection is one of the largest and most genetically diverse sunflower seed collections in the world, and it is vital to the conservation of *Helianthus* germplasm. However, other wild species collections have been established. Notable are collections at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, which contains 39 of the 52 wild species and 447 accessions (IBPGR, 1984; Cuk and Seiler, 1985; Atlagić *et al.*, 2006), the collection of the Dobroudja Agricultural Institute (DAI) at General Toshevo, Bulgaria, containing 428

accessions representing 37 of the 52 species of *Helianthus* (Christov *et al.*, 2001), and the collection maintained at INRA, Montpellier, France, which has more than 600 accessions of 45 of the 52 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), while Sujatha (2006) established a collection of 36 wild *Helianthus* species obtained from Novi Sad, Serbia and from the USDA-ARS, NPGS at the Directorate of Oilseed Research, Hyderabad, India. Tavaljanski *et al.* (2002) reported that the Veidelevka Institute of Sunflower, Veidelevka, Russia has a wild sunflower collection containing eight annual species (208 accessions) and 27 perennial species (227 accessions). The N.I.Vavilov Research Institute (VIR), St. Petersburg, Russia also maintains 550 accessions of 29 species with 128 perennial accessions (Vera Gavrilova, personal communication).

NPGS germplasm distribution

In addition to preserving genetic diversity, germplasm collections are dynamic and available as resources for desirable genes. Between 2001 and 2011, the NPGS sunflower collections filled 1,170 requests from 944 recipients, distributing 22,514 items representing all available accessions. The cultivated collections accounted for 650 of the requests from 514 recipients and 11,831 total accession items. The wild collections had 520 orders from 430 recipients, distributing 10,603 accession items in the same time period. The germplasm was distributed both within the United States and internationally providing genetic materials to be evaluated for numerous traits, as well as to answer basic questions about species diversity, domestication, evolution, and speciation. The tremendous widespread utilization of the NPGS sunflower collections illustrates the value of the collections and the importance of the maintenance program to continue making this germplasm available for scientific and educational purposes worldwide as a resource to move the sunflower crop into the future.

Global sunflower collections

A summary of the global sunflower collections was published recently by the FAO (2010). A total of 39,380 accessions of sunflower were reported from 92 institutions. The largest collections, containing 62% of the accessions include repositories in: Serbia, USA, China, France, Brazil, Australia, Russia, India, Morocco, Poland, and Hungary. Eighty-one other institutions reported smaller collections for the remaining 38% of the accessions. Globally, sunflower is produced on 22.9 million ha in 60 countries with an estimated worth of 40 billion USD (FAO, 2008). It is the fifth largest edible oilseed crop, providing 10% of the world's edible oil and is the second largest hybrid seed crop.

Economic value of wild species

The wild sunflower species are adapted to a wide range of habitats and possess considerable variability for most agronomic and achene quality traits, and reaction to insects and disease pathogens. Hajjar and Hodgkin (2007) surveyed the use of crop wild relatives from the mid-1980s to 2005 in 13 crops of major importance to global food security and found that sunflower had incorporated seven wild sunflower species traits, fifth highest of the crops surveyed.

The economic contribution of the wild species to the cultivated sunflower industry in the USA has been estimated to range from \$269.5 million per year (Phillips and Meilleur, 1998) to \$384 million per year (Prescott-Allen and Prescott-Allen, 1986). At current crop prices, this would translate to close to one billion USD. These early estimates did not include the recently incorporated traits of salt and herbicide tolerance which would increase the wild species value substantially. The trait of greatest value was considered to be PET 1 (French) *cms* cytoplasm from *H. petiolaris*. Wild *Helianthus* species have also been a reliable source of genes for resistance to economically important diseases. Much of the value is derived from disease resistance genes for rust, downy mildew, Verticillium wilt, Alternaria leaf spot, powdery mildew, Phomopsis stem canker, Sclerotinia wilt/rot, and the parasitic weed broomrape.

Germplasm enhancement-Interspecific hybridization

Interspecific hybrids are crosses between different species of the same genus. Interspecific hybridization is usually used to transfer traits including disease and insect resistance, and resistance to abiotic stresses from wild species to cultivated sunflower. In sunflower, interspecific hybridization has facilitated the transfer of genes from the wild species into a cultivated background. Wild sunflower species and cultivated sunflower generally can be crossed, but the divergence and heterogeneity of the genus causes considerable difficulties, such as cross-incompatibility, embryo abortion, sterility, and reduced fertility in interspecific hybrids. Effective protocols have been developed to overcome these difficulties and allow gene transfer from wild species into cultivated sunflower. Immature embryos are rescued from premature abortion using a two-step method developed by Chandler and Beard (1983). Colchicine is used to double chromosomes and increase fertility of the F_1 s of diploid and tetraploid wild accessions crossed with cultivated sunflower to improve backcross and sib-pollinated seed set (Jan, 1988). Wild annual species are the most receptive to crossing with cultivated sunflower, except for *H. agrestis*. The perennial diploids are some of the most difficult to cross.

Male sterility

Sunflower is the only member of the Asteraceae family in which *cms* has been found. A single male-sterile cytoplasm, PET1 (French cytoplasm), derived from an *H. petiolaris* ssp. *petiolaris* population from the St. Louis, Missouri area originally

collected by Dr. Charles Heiser (Leclercq, 1969), and the identification of dominant fertility restoration genes (Enns *et al.*, 1970; Kinman, 1970; Vrănceanu and Stoenescu, 1971) advanced sunflower production from the use of open-pollinated cultivars to hybrid production over 40 years ago. This source of *cms* 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).

Sixty-two *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 (Serieys, 2002; Serieys and Christov, 2005). A universal coding system was proposed by Serieys (1991) to accommodate the ever-increasing number of *cms* sources. This coding system is widely accepted among sunflower researchers. A *cms* source is coded by a 3-letter abbreviation of the cytoplasm donor species and/or subspecies followed by a numeral starting from one, corresponding to the time of its discovery and its reaction to restoration testers.

Germplasm characterization-Pathogens

Diseases limit production in a majority of sunflower-producing countries. Sunflower is a host to a wide array of diseases that cause serious economic damage in terms of yield and quality, and fungal diseases are 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. Wild sunflower species have been a valuable source of resistance genes for many of these common pathogens. The relative severity of individual diseases depends upon climate and host cultivars. Breeding for resistance often is the most effective means of control.

Table 3: Citations of wild *Helianthus* species as sources of resistance for sunflower diseases and parasites.

Disease/Parasite	Number of different wild species identified with resistance	
	Annual	Perennial
Disease	Rust	5
	Downy mildew	15
	Sclerotinia	18
	Phomopsis	18
	Alternaria	9
	Powdery mildew	9
	Rhizopus	4
	Phoma	8
	Charcoal rot	5
	Verticillium	3
Parasite	Broomrape	25
Total	44	119

Sources of resistance or improved levels of tolerance for most diseases are available among the wild species of *Helianthus*. A survey of the literature for information about resistance of wild species to pathogens/parasites included 44 references to annual species and 119 to perennial species (Table 3). The number of times a species was mentioned as a source for resistance for many common sunflower pathogens is summarized in Table 3. Twelve annual species and 10 perennial species were mentioned, with some species mentioned several times; for example, annual *H. argophyllus* was mentioned 32 times, and perennial *H. tuberosus* mentioned 40 times. In general, a specific pathogen race(s) or specific species populations were not indicated. Information presented above illustrates the frequent occurrence of resistance genes for various pathogens and the diversity of the species available for many of the common pathogens.

Downy mildew

Downy mildew (DM), *Plasmopara halstedii* (Farl.) Berl. and de Toni, occurs in all countries where sunflower is grown except Australia. The global distribution of the DM pathogen has been compiled by Gulya (2007). Among the methods of control, host-plant resistance using race-specific genes designated *Pl*, of which 18 have been described, is the most effective (Gulya, 2007). 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 sunflower species have been a plentiful source of genes for DM resistance with 10 annual and 15 perennial species being identified as having resistance from one to multiple races of DM (Table 3).

Downy mildew can be controlled by single, race-specific major dominant genes. Multi-race resistant germplasm and single-race resistant germplasms from wild sunflower species have been developed (Miller and Gulya, 1988; Tan *et al.*, 1992; Jan *et al.*, 2004a). Wild *H. annuus*, *H. petiolaris*, *H. tuberosus*, and *H. praecox* ssp. *runyonii* are sources of dominant genes for single-race resistance, while *H. argophyllus* is the only known source of dominant genes for all current races of the fungus (Miller and Gulya, 1988, 1991; Jan *et al.*, 2004a; Pustovoit and Krokhn, 1977; Vrânceanu and Stoenescu, 1970; Zimmer and Kinman, 1972; Miller *et al.*, 2002; Tan *et al.*, 1992; Dušle *et al.*, 2004; Hulke *et al.*, 2010; Seiler, 1991).

Helianthus argophyllus derived germplasm ARG-1575-2, carries the *Pl_{arg}* locus conferring resistance to all known races of downy mildew (Seiler, 1991). Since *Pl_{arg}* was mapped to a linkage group different from all other *Pl* genes previously mapped using SSRs, it can be concluded that *Pl_{arg}* provides a new unique source of resistance to downy mildew (Dušle *et al.*, 2004; Wieckhorst *et al.*, 2008, 2010).

Rust

Sunflower rust, a foliar disease caused by *Puccinia helianthi* Schwein. occurs in almost all sunflower growing regions. Wild *Helianthus* species have been an important source of rust resistance genes for cultivated sunflower for several years (Table 3). Resistance genes R_1 and R_2 , which have been widely used in sunflower breeding programs, originated from outcrosses with wild sunflower in Texas and are believed to be the first resistance genes to 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 over 200 populations of seven species including two annual species, *H. annuus* and *H. petiolaris*, and five perennial species, *H. maximiliani*, *H. nuttallii*, *H. grosseserratus*, *H. pauciflorus* (*rigidus*) and *H. tuberosus* from the north central USA was undertaken by Zimmer and Rehder (1976). Plants free of rust were observed in 190 of 200 populations of wild annual and perennial *Helianthus* species. They observed a multiplicity of rust resistance, with widespread resistance in wild annual populations from Nebraska and Kansas. Quresh *et al.* (1993) and Quresh and Jan (1993) observed that resistance to rust races 1, 2, 3, and 4 in 78 populations of *H. annuus*, *H. argophyllus*, and *H. petiolaris* was 25, 28, 15 and 26% of all plants for the four races, respectively. Only 10% of the plants were resistant to all four races of rust. McCarter (1993) observed varying levels of resistance to rust in populations of *H. tuberosus*. 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 has been identified in three wild annual species, *H. annuus*, *H. petiolaris*, and *H. argophyllus* (Jan *et al.*, 2004b).

It appears that wild species populations contain rust-resistant plants, but complete resistance or total susceptibility of populations is rarely found. Since *P. helianthi* races are host specific, susceptible wild *Helianthus* species provide selective hosts in which virulent races of rust 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 specific race genes found in the wild species. 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 a single dominant gene.

Parasite-Broomrape

Broomrape, *Orobanche cumana* Wallr., is a parasitic weed that infects sunflower roots causing severe crop losses in Southern Europe and the Black Sea region (Höniges *et al.*, 2008). 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 parasite, the breakdown of resistance is a frequent phenomenon, and multiple sources of resist-

ance are needed. Sources of resistance to *Orobanche* races found in the early sunflower breeding programs in the Former Soviet Union (FSU) originated from land races of cultivated sunflower, but genetic resistance was also introduced into susceptible sunflower from wild species, mainly *H. tuberosus* (Pustovoit *et al.*, 1976). The early FSU cultivars and *H. tuberosus* were also important sources of resistance for the broomrape complex of races in Romania (Vrânceanu *et al.*, 1980). Fernández-Martínez *et al.* (2000, 2010), Nikolova *et al.* (2000), and Bervillé (2002) reported that sunflower germplasm evaluation for resistance to broomrape races has demonstrated that wild *Helianthus* species constitute the major reservoir of genes conferring resistance to new virulence races.

Recent studies identified a new broomrape race in Spain, designated Race F, capable of overcoming all previously effective resistance genes (Domínguez *et al.*, 1996). High levels of resistance to Races E and F have been found in wild *Helianthus* species by Ruso *et al.* (1996) and Fernández-Martínez *et al.* (2000). Resistance to Races E and F was found in 29 perennial wild species, while very low levels of resistance were found in annual species: only four of eight species evaluated showed some resistance to Race F. Ruso *et al.* (1996) evaluated wild annual and perennial sunflower species' reactions to Spanish races and found two annual species, *H. anomalus* and *H. exilis*, expressing some resistance. All 26 perennial species tested were resistant. Crossing perennial species with cultivated sunflower can be difficult, but with the use of embryo culture and chromosome doubling of the F_1 s, amphiploids can be created that facilitate the transfer of broomrape-resistant genes from the wild perennial species. Using these techniques, amphiploids of perennial wild species *H. grosseserratus*, *H. maximiliani*, and *H. divaricatus* were produced that were resistant to Race F (Jan and Fernández-Martínez, 2002) and led to the release of four germplasm populations resistant to Race F named BR1 through BR4 (Jan *et al.*, 2002). 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). Pérez-Vich *et al.* (2002) studied the inheritance of resistance to Race F derived from interspecific amphiploids of *H. annuus* and of two wild perennials, *H. divaricatus* and *H. grosseserratus*. They suggested that the resistance is controlled by a single dominant gene. In a re-examination by Velasco *et al.* (2006), however, the resistance of the sunflower germplasm J_1 derived from *H. grosseserratus* proved to be digenic, the second gene being influenced by environmental factors.

Abiotic constraints-salt tolerance

Several species of *Helianthus* are native to salt-impacted habitats and may possess genes for salt tolerance. Seiler *et al.* (1981) suggested that *H. paradoxus*, a species growing in saline marshes in western Texas and New Mexico, would be a likely candidate for salt tolerance genes. 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, with lethality at an NaCl concentration of 250 to 400 mM. The wild *H. annuus* populations had a higher salt tolerance, with some plants surviving at 800 mM. *Helianthus paradoxus* had the highest salt tolerance, with some plants surviving at 1300 mM. Salt tolerance appeared to be a dominant trait, with hybrids between *H. paradoxus* and cultivated *H. annuus* surviving as well as the wild *H. paradoxus* parent. Welch and Rieseberg (2002) subjected *H. paradoxus* to NaCl treatments and found it to be five times more salt tolerant than its parental progenitor species, *H. annuus* and *H. petiolaris*. Interspecific germplasm derived from *H. paradoxus* has been developed with high salt tolerances, withstanding salt concentrations up to EC 24.7 dS/m. More recently, salt tolerance genes have been identified and transferred into cultivated sunflower with the release of two salt-tolerant parental oilseed maintainer lines, HA 429 and HA 430 (Miller and Seiler, 2003). It appears that one major gene controls salt tolerance, although a recessive modifier gene may also be present (Miller, 1995).

Herbicide tolerance

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). The potential for gene flow between cultivated and wild sunflower (*Helianthus annuus*) in the United States has existed since sunflower was first cultivated (Burke *et al.*, 2002). Herbicide-resistant crops are becoming increasingly common in agricultural production. A wild population of annual *H. annuus* from a soybean field in Kansas that had been treated repeatedly with imazethapyr for seven consecutive years developed resistance to the imidazolinone and sulfonylurea herbicides (Al-Khatib *et al.*, 1998; Al-Khatib and Miller, 2000). Crop resistance to imazethapyr and imazamox herbicides has great potential for producers in all regions of the world for controlling several broadleaf weeds. A selection of wild sunflower populations (*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 from the central USA had some resistance to imazamox, while 57% had some resistance to tribenuron (Olson *et al.*, 2004). In Canada, 52% of 23 wild *H. annuus* populations had some resistance to tribenuron (Miller and Seiler, 2005). Massinga *et al.* (2003) demonstrated that gene flow of imidazolinone-resistant domesticated sunflower to imidazolinone-susceptible *H. annuus* and *H. petiolaris* occurs, but decreases with distance. Genetic stocks IMISUN-1 (oil maintainer), IMISUN-2 (oil restorer), IMISUN-3 (confection maintainer), and IMISUN-4 (confection restorer) have been 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, the two herbicides may control broomrape in areas of the world where this parasitic weed attacks sunflower (Alonso *et al.*, 1998). Thus, herbicide-resistant sunflower hybrids could be used with these herbicides to combat broomrape infection.

***Helianthus* species used as a model system**

Wild species germplasm has enabled rapid advances in ecological and evolutionary studies. Dr. Loren Rieseberg's laboratories at the University of British Columbia, Vancouver, Canada, and Indiana University, Bloomington, Indiana have been actively involved in the study of speciation, evolution, gene mapping, phylogeny, and genome sequencing of the genus *Helianthus*. Dr. John Burke, University of Georgia, Athens, Georgia has been involved in the study of domestication genes, gene mapping, association mapping, and gene flow using wild sunflower species.

An ever-expanding range of genomic tools have become available to help unlock the vast potential genetic diversity in the genus *Helianthus*. Two-thousand microsatellite or SSR markers are now available in addition to several mapping populations, genetic linkage maps such as the Sunflower CMAP database, and 323,332 Sanger ESTs available in GenBank (NCBI), and the Compositae Genome Project Database. In an effort directed by Dr. Rieseberg, expansive genomic sequencing is underway for both cultivated sunflower and a number of the wild annual species. Bioinformatics is a bottleneck for complete exploitation of genetic resources information, a situation being addressed by Dr. Rieseberg's laboratories, by Dr. Burke's research group and others.

The National Sunflower Association (NSA), Mandan, North Dakota, USA, in conjunction with the USDA-ARS and sunflower seed companies has established the **NSA Sunflower SNP (single-nucleotide polymorphism) Consortium** to help sunflower breeders create new sunflower hybrids as quickly as possible through the application of state-of-the-art marker-assisted breeding technology (NSA, 2011). They have identified over 7000 usable SNP markers and have assigned map location to about 2600 SNP markers on the sunflower genetic map.

CONCLUSIONS AND FUTURE PROSPECTS

Sunflower cultivation continues to be pushed into lower-fertility soils and other marginal environments where drought and high or low temperatures continually take their toll on yield. The challenge for the sunflower breeding community is to develop sunflowers adaptable to these marginal environments and at the same time increase seed yield.

The extremely narrow genetic base of hybrid sunflower due in part to using only a single *cms* source in the female parent for all hybrids makes the crop extremely vulnerable to an impending disaster as seen in maize in the 1970s. There remains an urgent need to increase the genetic diversity of cultivated sunflower due to the

marked reduction in genetic diversity during domestication. Wild species of sunflower have been a source of resistance to many pests, especially diseases, as well as the cytoplasm that is the basis of all hybrid sunflowers. Since wild sunflower and the sunflower crop are native to North America, associated pests have co-evolved in natural communities, thus providing the opportunity to search for pest resistance genes in the diverse wild species. Significant progress has been made in collecting and preserving wild species, increasing the genetic diversity available for crop improvement. Thus far, only a small portion of the available diversity has been exploited. Unlocking the full potential of sunflower germplasm collections, however, requires an understanding of the amount and distribution of genetic variation contained within them.

Significant advances have been made in understanding the origin, domestication, and organization of the genetic diversity, characterization, and screening methods for abiotic and biotic stresses in sunflower. The future direction will include the transfer of target genes from wild relatives into domesticated sunflower with improved genetic backgrounds adapted to local conditions. This will be facilitated by introgression of favorable alleles from alien germplasm, pyramiding favorable alleles and QTLs for specific traits, and simultaneously improving the maximum number of traits.

New sunflower hybrids will have to possess pest and disease resistance genes from distantly related or even unrelated plants and other organisms. To keep sunflower an economically viable global crop, researchers must strive to combine the best conventional and modern molecular approaches available. This will require a multidisciplinary team approach and a commitment to a long-term integrated genetic improvement program.

The future collection, preservation, and utilization of genetic resources face several challenges. The biggest challenge is the decrease in funding to maintain the current collections due to the lack of commitment of countries supporting genetic resources conservation and discovery of new traits from existing germplasm. Free and open exchange of germplasm has also changed over the past decade affecting the flow of germplasm between researchers. In the past these scientifically based exchanges have been extremely beneficial for the global sunflower community working to maintain sunflower as a viable major global oilseed crop and preserve it for future generations.

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