

Wild Sunflower Species as a Genetic Resource for Resistance to Sunflower Broomrape (*Orobanche cumana* Wallr.)

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Abstract

Broomrape (*Orobanche cumana* Wallr.) is a parasitic weed that causes economic damage in sunflower production in many countries, especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, and China. Genes for resistance to broomrape races A, B, C, D, and E are present in varietal populations of cultivated sunflower. Since broomrape is a highly variable parasitic weed, the breakdown of resistance is a frequent phenomenon, and multiple sources of resistance are needed to control the emerging races. Genes that confer resistance to races F, G, and H and others that have not been assigned a race designation have been identified in wild sunflower species and incorporated into hybrid sunflower through interspecific hybridization. The U.S. Department of Agriculture, Agricultural Research Service, National Plant Germplasm System wild sunflower collection contains 2,239 accessions with 1373 annual accessions represented by 14 species and 866 perennial accessions represented by 39 species. Sunflower germplasm evaluations for resistance to broomrape races have demonstrated that the *Helianthus* species constitute a substantial reservoir of genes conferring resistance to new virulence broomrape races. The resistance to broomrape, including immunity reported in seven annual and 32 perennial species, provides breeders a broad genetic base from which to search for resistance to existing and newly emerging races.

Keywords: [Broomrape](#); [Genebank](#); [genetic resources](#); [Helianthus](#); [parasitic weed](#); [wild species](#)

Introduction

Broomrape, caused by *Orobanche cumana* Wallr., is a parasitic weed that infects sunflower roots causing severe crop losses in Central and Eastern Europe, including the Black Sea region, and the Middle East ([Höniges et al., 2008](#); [Pricop et al., 2011](#); [Fernandez Martinez et al., 2010](#)). The parasite has also been reported in Israel, Asia ([Eizenberg et al., 2003](#)), and Tunisia ([Amri et al., 2012](#)). It has also been observed in Australia, Mongolia, and China and is generally associated with drier climates.

Broomrape was first observed in sunflower in the Saratov District in Russia in the 1890s. Traditionally five races, A through E, have been controlled using resistance genes Or_1 through Or_5 , respectively ([Vrânceanu et al., 1980](#)). The first sources of resistance to *Orobanche* found in the early sunflower breeding programs in the former USSR, Ukraine, and Romania originated from land races of cultivated sunflower. Since broomrape is a highly variable parasite, the breakdown of resistance is a frequent phenomenon, and multiple sources of resistance are needed to control the emerging races.

Historically, sunflower breeders have been successful in developing broomrape resistant cultivars but breeding programs are often based on a few dominant genes and resistance breakdown caused by the appearance of new virulent races that has occurred frequently in recent decades ([Fernández-Martínez et al., 2010](#)). Unlike other host plant-*Orobanche* systems, most sources of resistance to *O. cumana* in sunflower have been based on vertical resistance mechanisms controlled by single dominant genes ([Fernández-Martínez et al., 2012](#)). This has led to a rapid breakdown of the resistance and subsequently to a continuous need for new resistance sources. Genes that confer resistance to race E, F, G, and H and others that have not been assigned a race designation have been incorporated into hybrid sunflower through interspecific hybridization.

Genetic resources are the biological basis for global food security. Preservation of cultivars, landraces, and wild relatives of sunflower provides the basic foundation to promote and sustain sunflower production. The U.S. Department of Agriculture, Agricultural Research Service, (USDA-ARS) National Plant Germplasm System (NPGS) wild species collection is preserving the genetic diversity of the genus *Helianthus*, but at the same time making accessions available for screening for broomrape resistance. Examples of the extensive use of the wild species sources of race-specific broomrape resistance will be given.

Materials

USDA-ARS, NPGS sunflower collections

The mission of the USDA-ARS, 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. The NPGS is 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. This collection is a diverse assemblage of 1825 cultivated accessions from 59 countries. Cultivated sunflower is represented by a single species, *Helianthus annuus*. Currently, 92% of the cultivated accessions are available for distribution.

Wild sunflower species collection

The wild *Helianthus* species collection was established at Bushland, Texas in 1976. In 1985 it was transferred to NCRPIS, Ames, Iowa, where it is currently housed. The wild species collection contains all 39 perennial species and 14 annual species ([Schilling, 2006](#); [Stebbins et al., 2013](#)). The number of wild species accessions is 2239, of which 929 are wild *Helianthus annuus* accessions and 444 accessions represent 13 other wild annual *Helianthus* species ([Seiler and Marek, 2011](#)). Thirty-nine perennial *Helianthus* species are represented by 866 accessions. Currently, 95% of the annual accessions are available for distribution, while 70% of the perennial species accessions are available. The number of accessions for each species is given in [Tables 1](#) and [2](#).

[Tab.](#)

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Table 1

Infrageneric classification of annual *Helianthus* species and number of accessions in the USDA-ARS, NPGS sunflower genebank collection

[Tab.](#)

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Table 2

Infrageneric classification of perennial *Helianthus* species and number of accessions in the USDA-ARS, NPGS sunflower genebank collection

A new endemic perennial species *H. winteri* was discovered in the foothills of the Sierra Nevada Mountains, near Fresno in central California in 2012 ([Stebbins et al., 2013](#)). The new species is

distinguished from the common wild annual *H. annuus* by its shrubby perennial growth habit, woody stem, and year round flowering. It grows on dry, steep rocky slopes with granitic soils with plant heights up to 4 m.

Results and discussion

The early wild species sources were introduced into susceptible sunflower from wild species, mainly *H. tuberosus* ([Pustovoit et al., 1976](#)). The early former Soviet Union cultivars and *H. tuberosus* were also important sources of resistance for the broomrape complex of races in Romania ([Vrânceanu et al., 1980](#)). Early reports of broomrape resistance were from cultivars “Progress” and “Novinka”, which were developed using the “Group Immunity” breeding approach with germplasm derived from wild perennial *H. tuberosus* ([Pustovoit and Gubin, 1974](#)). Immunity to broomrape in lines derived from *H. tuberosus* was also described by [Pogorietsky and Geshle \(1976\)](#).

Several investigators ([Fernández-Martínez et al., 2000, 2010](#); [Nikolova et al., 2000](#); [Bervillé, 2002](#); [Škorić and Pacureanu-Joita, 2011](#); [Christov, 2013](#); [Antonova et al., 2011](#); [Terzic et al., 2010](#)) reported that sunflower germplasm evaluations for resistance to broomrape races have demonstrated that the *Helianthus* species constitute a substantial reservoir of genes conferring resistance to new virulence races. Resistance to races E, F, G and all subsequent races have been found in wild species of sunflower.

A new broomrape race, race F, discovered in Spain in 1995 that spread rapidly was capable of overcoming all previously effective resistance genes ([Alonso et al., 1996](#)). [Sukno et al. \(1998\)](#) reported that perennial *H. giganteus*, *H. laevigatus*, *H. pauciflorus*, and *H. resinous* have resistance to race SE194 from Spain. [Hladni et al. \(2009\)](#) described resistance to races E and F of broomrape in an *Rf* line derived from annual *H. deserticola* in Serbia.

High levels of resistance to races E and F have been found in the wild *Helianthus* species by [Ruso et al. \(1996\)](#) and [Fernández-Martínez et al. \(2000\)](#). They found resistance to races E and F in 29 wild perennial species, while very low levels were found in annual species, with only four of eight species evaluated showing 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*, that had resistance, and 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 that facilitate the transfer of broomrape-resistant genes from the wild perennial species can be created. 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 with *H. annuus* and with two wild perennials, *H. divaricatus* and *H. grosseserratus*. They suggested that the resistance is controlled by a single dominant gene. Upon reexamination by [Velasco et al. \(2006\)](#), however, the resistance of the sunflower germplasm J1 derived from *H. grosseserratus* proved to be digenic, the second gene being influenced by environmental factors. [Petcu and Pacureanu \(2011\)](#) reported that interspecific hybrids derived from *H. argophyllus* were resistant to races E and F in Romania.

[Christov \(2013\)](#) reported that 17 wild *Helianthus* species, perennial *H. tuberosus*, *H. pauciflorus* (= *rigidus*), *H. eggertii*, *H. x laetiflorus*, *H. decapetalus*, *H. hirsutus*, *H. divaricatus*, *H. giganteus*, *H. maximiliani*, *H. nuttallii* ssp. *r ydbergii*, *H. salicifolius*, and *H. smithii*, and annual *H. annuus* (wild), *H. argophyllus*, *H. debilis*, *H. petiolaris*, and *H. praecox* were resistant to broomrape races A to G in Bulgaria. Also in Bulgaria, resistance to broomrape (race not specified) was reported in different progenies of interspecific hybrids with *H. pumilus* by [Nikolova et al. \(2004\)](#). Diploid perennial species *H. divaricatus*, *H. giganteus*, *H. glaucophyllus*, *H. grosseserratus*, *H. mollis*, *H. nuttallii*, and *H. smithii* and their interspecific hybrids were reported to be resistant to broomrape by [Nikolova et al. \(1998\)](#).

However, a more virulent race (designated G) attacking cultivars resistant to race F was identified ([Molinero-Ruiz and Melero-Vara, 2004](#); [Škorić et al., 2010](#)). [Antonova et al., \(2011, 2013\)](#) reported a high percentage of race H in the southern regions of the Russian Federation. Recently, resistance to race G has been transferred from annual *H. debilis* ssp. *tardiflorus* ([Velasco et al., 2012](#)). [Cvejic et al. \(2012\)](#) reported a new source of resistance to race G, and unnamed more virulent races in an inbred line derived from interspecific hybridization with *H. divaricatus* in Serbia. Inbred lines possibly resistant to race G were developed from crosses with *H. tuberosus*.

The interaction between *Orobanche* and the roots of wild sunflowers has been studied by [Labrousse et al. \(2001\)](#). Roots of an interspecific hybrid derived from *Helianthus*

debilis ssp. *debilis* produced an impassable encapsulation layer that blocked the intruding parasite, which then died. Another interspecific hybrid from the same species showed reduced stimulation of broomrape seed germination and rapid necrosis at an early stage of parasite development. Resistance also occurred in an interspecific hybrid derived from *H. argophyllus* occurring mainly at stage four of the parasite development with no broomrape seed production observed, because necrosis occurred before the broomrape flowered.

Conclusions

Cultivated sunflower has a narrow genetic background and is deficient in genes for resistance to broomrape. The diversity of wild sunflower species in the USDA-ARS wild species genebank offers breeders a diverse genetic pool from which to discover unique genes for existing and emerging new races of broomrape.

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