

# Genetic Resources of the Sunflower Crop Wild Relatives for Resistance to Sunflower Broomrape

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## Abstract

*cumana* Wallr., (sunflower broomrape), mainly distributed in the Mediterranean region and Western Asia where it exclusively parasitizes sunflowers. Sunflower broomrape, a very destructive parasitic weed causing yield losses close to 100% under high infestations, has a great capacity for dispersion and mutation. Broomrape is a highly variable parasitic weed controlled by vertical single dominant resistance genes that leads to a rapid and frequent breakdown of the resistance. This subsequently leads to the continuous need for new unique genes. One of the most threatening holoparasitic plant species is *Orobanche* and multiple sources of resistance to control new emerging virulent races. Sunflower crop wild relatives (CWR) have been a particularly valuable genetic resource with an estimated contribution of 26% of the annual value of the sunflower crop, with much of this value derived from disease resistance genes. The USDA-ARS, National Plant Germplasm System CWR collection contains 2,519 accessions of 53 species with 14 annual species (1641 accessions) and 39 perennial species (878 accessions). This diverse collection of CWR provides potential resistance sources for sunflower broomrape, especially in Central and Eastern Europe, including the Black Sea regions, and the Middle East. Sunflower CWR evaluations for resistance to broomrape races have demonstrated that the *Helianthus* species constitute a substantial reservoir of genes conferring resistance to existing and new virulence races. Resistance genes have been discovered in the sunflower CWR conferring resistance to new virulent broomrape races F, G, and H, and others that have not been assigned a race designation. Broomrape resistance genes can be incorporated into hybrid sunflower through interspecific hybridization. Resistance to sunflower broomrape, including immunity reported in seven annual and 32 perennial species, provides breeders with the prospect for durable broomrape control through exploiting genetic resistance for existing and newly emerging races.

**Keywords:** Sunflower, Broomrape, Parasitic weed, *Helianthus* species, Genetic resources

## Introduction

Sunflower broomrape, caused by *Orobanche cumana* Wallr., is an obligatory and non-photosynthetic root parasite specifically infecting sunflower (*Helianthus annuus* L.) in Central and Eastern Europe and Western Asia (Eizenberg et al., 2003; Höniges et al., 2008; Molinero-Ruiz et al., 2013) and Tunisia (Amri et al., 2012). The infected sunflower plants are smaller, and have reduced head diameters causing a reduction of up to 80-100% in yield (Alcántara et al., 2006; Duca, 2015). It has not been reported in the Americas to date (Cantamutto et al., 2014). 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) in over two-thirds of the perennial species and several of the annuals, in the absence of the parasite sunflower broomrape races in the USA to incite the current level of resistance (Seiler et al., 2017).

Genetic resistance to *O. cumana* in sunflower is in most cases qualitative or vertical, resulting in physiological races that periodically overcome all available resistance sources (Fernández-Martínez et al., 2015). The deployment of few sources of resistance to sunflower broomrape, most of them monogenic and dominant, has promoted a continuous development of increasingly virulent forms of the parasite (Velasco et al., 2016). Eight races of sunflower broomrape, A through H, have been reported thus far, with newer races F, G, and H commonly reported in several countries (Kaya, 2014). Continually emerging new races that overcome existing resistance sources requires a continuous search for new sources of resistance.

### Sunflower Crop Wild Relatives Genebank Collection as a Genetic Resource

Preservation of cultivars, landraces, and crop wild relatives (CWR) of important crop species provides a foundation for sustainable agriculture, and is the biological basis of global food security (Campbell et al., 2010). Crop genetic resources consist of the total genetic variability in the crop or within sexually compatible species (Holden et al., 1993). Crop wild relatives have been undeniably beneficial for modern agriculture, dating back 100 years, providing breeders with a broad pool of potentially useful genetic resources (Hajjar and Hodgkin, 2007). Palmgren et al. (2015) suggested that modern crops should be developed with lost properties that their ancestors once possessed in order to tolerate emerging pests and changing environmental conditions. Emerging plant diseases and agricultural pests such as broomrape are predicted to become more common and damaging in a warmer climate (Anderson et al., 2004).

Breeding for resistance seems to be the most efficient and sustainable approach to controlling broomrape. To do this, one needs additional sources of new resistance genes. Fortunately for the sunflower crop, there is a vast untapped germplasm pool of 53 species of sunflower CWR to search for resistance to emerging races of broomrape (Seiler and Marek, 2011; Jan et al., 2014; Seiler and Jan, 2014; Marek, 2016; Seiler et al., 2017). The mission of the U.S. Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (USDA-ARS, NPGS) is to conserve genetic diversity of crop germplasm and associated information, to conduct related research, and to encourage the use of germplasm for crop development. The USDA-ARS, NPGS Germplasm Resources Information Network (GRIN) database serves as the central location of information about the accessions in the sunflower CWR collection, and also serves as the portal for requesting seed of available accessions. The germplasm is freely available for research and educational purposes, although some restrictions are imposed by import regulations of receiving countries.

The sunflower CWR collection is maintained and managed at the USDA-ARS NPGS, North Central Regional Plant Introduction Station, Ames, Iowa, USA. The CWR collection contains 2519 accessions of 53 species of *Helianthus*, with 1028 annual wild *H. annuus* (41%), 613 accessions

representing accessions of the other 13 annual species (24%), and 878 accessions representing 39 perennial species (35%) (Seiler et al., 2017). CWR evaluations for resistance to broomrape have demonstrated that the species constitute a substantial reservoir of genes conferring resistance to new race-specific and emerging virulence races.

Fernández-Martínez et al. (2000, 2008, 2012); Nikolova et al. (2000); Bervillé (2002); Škorić et al. (2010); Škorić and Păcureanu-Joita (2011); Jan et al. (2014); Seiler and Jan (2014) reported that sunflower germplasm evaluation for resistance to broomrape races have demonstrated that the *Helianthus* species constitute the major reservoir of genes conferring resistance to new virulence races.

### **Identified Sources of Sunflower Broomrape Resistance in Crop Wild Relatives**

Resistance to broomrape from CWR began early in breeding programs of the Former Soviet Union, where *H. tuberosus* was introgressed into cultivated sunflower (Pustovoit et al., 1976). Immunity to broomrape in lines derived from *H. tuberosus* was also described by Pogorietsky and Geshle (1976). This source of resistance probably accounts for some of the resistance observed for the *Or<sub>1</sub>*-*Or<sub>5</sub>* genes described by Vrânceanu et al. (1980).

Antonova et al. (2011) screened accessions of five annual and 16 perennial wild species to broomrape races F, G, and H from the Rostov region of the Russian Federation. Among the annual species, only *H. petiolaris* had a high level of resistance. A majority of the perennial showed very high levels of resistance similar to the finding of Ruso et al. (1996). Antonova concluded that the potential immunity of the perennials to broomrape is not related to the absence of a stimulating effect of the host's root exudates on the seed of the parasite, but is more likely related to physiological-biochemical features of the cortex of the sunflower root.

Labrousse et al. (2001) screened wild and interspecific sunflower for race E and suggested that there are at least two mechanisms responsible for resistance in sunflower. Resistance may be characterized by the low number of broomrape attachments, or by necrosis of the attachments. In their experiment with perennial CWR, *H. resinosus* and *H. pauciflorus* had only a few attachments, while interspecific annual lines LR1 (*H. debilis* subsp. *debilis*) and 92BG1 (*H. argophyllus*) had broomrape attachments that developed, but later became necrotic. Labrousse et al. (2004) suggested that polygenic resistance could occur in LR1-derived RILs.

High levels of resistance to races E and F were observed in the wild *Helianthus* species by Ruso et al. (1996) and Fernández-Martínez et al. (2000). Resistance to races E and F was found 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, with annual species, *H. anomalus* and *H. exilis* having the highest resistance. Interspecific amphiploids derived from perennial CWR of *H. grosseserratus*, *H. maximiliani*, and *H. divaricatus* were used to develop germplasms BR1-BR4 resistant to race F (Jan and Fernández-Martínez, 2002; 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 J1 derived from *H. grosseserratus* proved to be digenic, the second gene being influenced by environmental factors. Sukno et al. (1998, 1999) reported that perennial *H. giganteus*, *H. laevigatus*, *H. pauciflorus* (= *rigidus*), and *H. resinosus* have resistance to race SE194 from Spain. Resistance to race G from an annual interspecific cross with *H. debilis* subsp. *tardiflorus* was dominant and controlled a single locus in that population (Velasco et al., 2012).

Studies by Shindrova (2006) showed that there were three broomrape races in Bulgaria: races D, E, and F. Race E was widely distributed in all sunflower growing regions, with race F being new to the country. Interspecific hybrids based on perennial *H. eggertii* and *H. smithii* were immune to broomrape in Bulgaria (Christov et al., 1998). Broomrape resistance to the local race in Bulgaria was reported in perennial *H. divaricatus*, *H. eggertii*, *H. giganteus*, *H. grosseserratus*, *H. glaucophyllus*, *H. mollis*, *H. nuttallii*, *H. pauciflorus*, *H. resinosus*, and *H. tuberosus* (Christov, 1996). Also in Bulgaria, resistance to broomrape (race not specified) was reported in different progenies of interspecific hybrids of *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 resistant to broomrape (Nikolova et al., 1998). Christov (2008) reported that several perennial *Helianthus* species showed 100% resistance including *H. tuberosus*, *H. eggertii*, *H. smithii*, *H. pauciflorus*, and *H. strumosus*. Christov (2008) concluded that resistance to broomrape in Bulgaria was controlled by one dominant gene. Christov et al. (2009) reported that annual species *H. debilis* and *H. argophyllus* were resistant, as well as an interspecific *H. petiolaris* selection. He also reported resistance in perennial accessions and interspecific crosses of *H. ciliaris*, *H. decapetalus*, *H. maximiliani*, *H. pumilus*, *H. mollis*, and *H. smithii*. Christov (2013) reported resistance to Bulgarian race G in 5 annual and 12 perennial species.

Hladni et al. (2009, 2010, 2012) identified resistance to races E and F in fertility restorer lines derived from annual *H. deserticola* in Serbia. Terzić et al. (2010) reported resistance to race E in two accessions of annual *H. debilis*, one *H. neglectus*, three *H. petiolaris*, and three *H. praecox*. They also reported high levels of resistance in F<sub>1</sub> hybrids with perennial species *H. tuberosus*, *H. pauciflorus*, *H. strumosus*, *H. divaricatus*, *H. hirsutus*, *H. eggertii*, *H. decapetalus*, *H. resinosus*, *H. laevigatus*, *H. mollis* and *H. grosseserratus*. Miladinovic et al. (2013) screened annual populations of *H. annuus* and *H. petiolaris* from Argentina to race E and found that *H. annuus* was very susceptible, while *H. petiolaris* was totally resistant. Cvejić (2012) reported resistance to race G in fertility restorer lines derived from annual *H. deserticola* in Serbia.

Anton et al. (2017) screened accessions of *H. praecox*, *H. debilis*, and *H. petiolaris* for resistance to race F in Romania with some having full resistance, while interspecific hybrids with perennial *H. tuberosus* and *H. maximiliani* and annual *H. argophyllus* had good tolerance to more than race F in the Braila region of Romania. Additionally, Anton et al. (2016) tested interspecific selections based on *H. tuberosus* and *H. maximiliani* to race G and found them to be resistant. Interspecific hybrids of *H. argophyllus* were screened for resistance to Romanian races G and H with a few lines showing good resistance to broomrape (Petcu and Păcureanu-Joița, 2012).

### **Molecular Aspects of Resistance Genes in Crop Wild Relatives**

Genes with minor effects should be also considered in any pyramiding strategy since they will contribute to the durability of resistance, particularly if the mode of action occurs at the initial stages of parasitization or if it is complementary to the mode of action of major genes (Velasco et al., 2016). There have been two reports of the molecular genetic mapping of resistance loci. The first concerns the *Or5* gene conferring resistance to race E (Lu et al., 2000; Tang et al., 2003; Perez-Vich et al., 2004). The second involved 586,955 SNPs from the SUNRISE project 8 on GeneTitan® (Affymetrix) for identification of broomrape races F and G (Dimitrijevic and Horn, 2018). Seventeen QTLs from a RIL population of HA 89 x LR1 (derived from an interspecific cross with *H. debilis*) were identified spread throughout nine LGs, among them a stable QTL on LG3 that controlled the number of broomrape emergences that explained 15-30% of the phenotypic variability controlling the number of broomrape emergences for race F in Spain and race G in Turkey (Louarn et al., 2016). This QTL was marked as the one that could be the most rapidly used. No genes have been cloned, and the molecular mechanism involved in the resistance remains unknown.

Genetic analysis and mapping of a new resistance gene for broomrape races higher than F was evaluated using a cross between the resistant sunflower inbred line AB-VL-8 and the susceptible line L-OS-1 (Imerovski et al., 2016). AB-VL-8 is a proprietary sunflower inbred line originating from the gene pool that was obtained by crossing *H. annuus* with *H. divaricatus* (Imerovski et al., 2016). Marker analysis using SSR markers revealed polymorphism only on LG3, indicating that presumably the single gene in this region conferred the resistance (Imerovski et al., 2013, 2014). The closest SSR marker to the recessive gene was ORS683, tentatively designated as *OR<sub>ab-vl-8</sub>* at a genetic distance of 1.5 centimorgans. The discovery of *Or<sub>ab-vl-8</sub>* will provide a much needed new resistance gene to broomrape races higher than F from the CWR and the associated markers will accelerate the introgression of the gene into different sunflower lines by improving breeding efficiency, thereby ensuring the protection of sunflower from the spread of new *Orobanche* pathotypes. Further studies will need to include alternative marker techniques such as SNPs, which will enable saturation of the region surrounding this resistance gene.

### **Sustainable Sunflower Broomrape Resistance**

The development of sustainable resistance will require a dedicated research strategy. The vast gene pool of the 53 species of sunflower CWR needs to be continually exploited for resistance genes for current and emerging variant races of broomrape. Broomrape resistance genes can be incorporated into hybrid sunflower through interspecific hybridization. Utilization of the CWR as a gene resource needs to shift to a strategy of pyramiding major genes controlling different mechanisms of resistance. Additionally, minor QTL and their association with resistance mechanisms is also important. Previous studies have shown that accumulations of minor QTL can result in complete resistance to broomrape in sunflower. The continued use of monogenic and dominant genes for control only promotes a continuous development of increasingly virulent forms of the parasite. Characterization of new resistance sources should include the study of the mode of inheritance, mechanism of resistance involved, and the precise tagging of the genes to facilitate the development of accurate molecular markers. The availability of genomic and transcriptomic information from ongoing projects will facilitate the development of molecular tools for a wide diversity of studies on broomrape (Muños et al., 2015).

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