



### **III. GENETIC RESISTANCE TO SUNFLOWER BROOMRAPE**

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# RECENT DEVELOPMENTS IN BREEDING FOR RESISTANCE TO SUNFLOWER BROOMRAPE

Leonardo VELASCO\*, José M. FERNÁNDEZ-MARTÍNEZ,  
Begoña PÉREZ-VICH

*Institute for Sustainable Agriculture, CSIC, Córdoba, Spain*

*\*Corresponding author: lvelasco@ias.csic.es*

## Abstract

Sunflower broomrape (*Orobancha cumana* Wallr.) remains as one of the most serious issues that sunflower breeders are facing nowadays. The capacity of the parasite to expand to new areas and to develop more aggressive forms poses new challenges for sunflower breeding. In addition to the well-known capacity of the parasite to overcome resistance mechanisms through mutation, a recent study in Spain has illustrated how virulence can be increased from genetic recombination between genetically distant individuals. Considering the large variability between sunflower broomrape populations and their expansion ability, sunflower breeding for resistance to broomrape must necessarily focus on pyramiding known resistance genes rather than relying on individual genes plus a “favorable” genetic background. This will require extensive research on i) genetic characterization of sunflower broomrape populations; ii) identifying novel resistance genes; iii) genetic and physiological characterization of resistance genes in sunflower; iv) genetic and physiological characterization of avirulence genes in the parasite and v) developing accurate molecular markers for avirulence genes in the parasite and for resistance genes in the sunflower. These will be central research topics in the coming years, but important advances have already been achieved in recent years. This paper reviews such recent developments in breeding research for broomrape resistance in sunflower and envisages how the availability of increasingly powerful tools such as the complete genomes of the crop and the parasite as well as the popularization of gene editing techniques can boost breeding advances in this field.

**Keywords:** avirulence genes, breeding, broomrape, genetic resistance, *Helianthus annuus*, Wild *Helianthus* sp.

# GENES EXPRESSION MEASUREMENT BY USING THE GENOME SEQUENCES OF *OROBANCHE CUMANA* AND SUNFLOWER

Jérôme GOUZY<sup>1</sup>, Nicolas POUILLY<sup>1</sup>, Johann LOUARN<sup>1</sup>,  
Xavier GRAND<sup>1,4</sup>, Marie-Claude BONIFACE<sup>1</sup>, David RENGEL<sup>1</sup>,  
Olivier BOUCHEZ<sup>2</sup>, Sébastien CARRÈRE<sup>1</sup>, Olivier CATRICE<sup>1</sup>,  
Stéphane CAUET<sup>3</sup>, Clotilde CLAUDEL<sup>4</sup>, Ludovic COTTRET<sup>1</sup>,  
Sébastien FAURE<sup>4</sup>, Álvaro Calderón GONZÁLEZ<sup>5</sup>,  
Luyang HU<sup>1</sup>, Céline JÉZIORSKI<sup>3</sup>, Marc-Marie LECHAT<sup>6</sup>,  
Ludovic LEGRAND<sup>1</sup>, Sandrine ARRIBAT<sup>3</sup>, William MARNADE<sup>3</sup>,  
Nicolas RIBIÈRE<sup>4</sup>, Erika SALLET<sup>1</sup>, Philippe SIMIER<sup>6</sup>,  
Leonardo VELASCO<sup>5</sup>, Cécile DONNADIEU<sup>2</sup>, Christophe JESTIN<sup>7</sup>, Philippe DELAVAUULT<sup>6</sup>, Hélène BERGÈS<sup>3</sup>, Marie  
COQUE<sup>4</sup>,  
Begoña PÉREZ-VICH<sup>5</sup>, Stéphane MUÑOS<sup>1\*</sup>

<sup>1</sup>Laboratoire des Interactions Plantes-Microorganismes, Université de Toulouse, INRA, CNRS, Castanet-Tolosan,  
France

<sup>2</sup>GeT-PlaGe, Genotoul, INRA, Castanet-Tolosan, France

<sup>3</sup>Centre National de Ressources Génomiques Végétales, INRA,  
Castanet-Tolosan, France

<sup>4</sup>Biogemma, Mondonville, France

<sup>5</sup>Instituto de Agricultura Sostenible, CSIC, Córdoba, Spain

<sup>6</sup>Laboratoire de Biologie et Pathologie Végétales, SFR 4207 QUASAV,  
Université de Nantes, France

<sup>7</sup>Terres Inovia, France

\*Corresponding author: [stephane.munos@inra.fr](mailto:stephane.munos@inra.fr)

## Abstract

*Orobanche cumana* (the sunflower broomrape) is an obligate parasitic plant that needs to fix to the sunflower roots for collecting water and nutrients from sunflower. For sunflower physiology, *O. cumana* can be considered as a new sink that leads to yield losses. During the early stages of the interaction, the connection of *O. cumana* to the vascular system of sunflower reprograms genes expression in both species. A better understanding of the regulation mechanisms could help in breeding for new resistant sunflower varieties. RNA-Seq experiment is the main technology for large-scale gene expression measurement. The full sequence of the sunflower genome (Badouin et al., 2017) made possible the monitoring of genes expression from sunflower during the interaction. However, the lack of the *O. cumana* genome sequence didn't allow to measure genes expression from broomrape. In a consortium with French and Spanish research institutes and by using a similar approach than for the sunflower genome sequencing, we obtained the complete genome sequence of *O. cumana*. We used the two annotated genomes to simultaneously measure genes expression from *O. cumana* and *H. annuus* during the early stages of the interaction using several sunflower genotypes.

**Keywords:** genome sequence, RNA-Seq, genes expression, interaction

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

Gerald J. Seiler\*

USDA-Agricultural Research Service, Red River Valley Agricultural Research Center, Northern Crop Science Laboratory, Fargo, ND 58102-2765, USA

\*Corresponding author: gerald.seiler@ars.usda.gov

## 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 (Pustovoi 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 Vrănceanu 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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# DILEMMAS ABOUT NEW SUNFLOWER BROOMRAPE RACES (*OROBANCHE CUMANA* WALLR.)

Dragan ŠKORIĆ<sup>1\*</sup>, Maria JOIȚA-PĂCUREANU<sup>2</sup>,  
Fyodor I. GORBACHENKO<sup>3</sup>, Oleg F. GORBACHENKO<sup>3</sup>,  
Steven MAŠIREVIĆ<sup>4</sup>

<sup>1</sup>Academy of Serbia, Novi Sad, Serbia

<sup>2</sup>National Agricultural Research&Development Institute Fundulea, Romania

<sup>3</sup>Zhdanov Don Experiment Station, All Russia Research Institute of Oil Crops,  
Rostov region, Russia

<sup>4</sup>University of Novi Sad, Novi Sad, Serbia

\*Corresponding author:draganskoric@sbb.rs

## ABSTRACT

Emergence of new broomrape populations (races) has been observed in the past 20 years in several countries (Romania, Moldova, Ukraine, Russia, Turkey, Bulgaria, Spain, Serbia, and China) where sunflower is frequently grown in the same sites without applying traditional crop rotations. Differential lines for sunflower broomrape races A to F have been secured. The new broomrape races have been identified by researchers as races G and H. The question whether the same broomrape mutations can occur in one year and affect the same countries remains unsolved. Several results of new broomrape population emergences in some of the affected will be presented in the paper.

A total of 390 genotypes were studied at four Romanian localities (Cuza Voda, Crucea-Stupina, Braila-Valea Canepii, and Tulcea-Agighiol) in 2014. At all four localities, a certain degree of sunflower broomrape infestation was observed in control hybrids and lines (Performer, LC-1093, LG-5661, and PR64LE20), which indicated the emergence of new populations higher than race H. The 390 studied genotypes had different reactions in all four localities.

In 2015, ten hybrids and controls were studied at five Romanian localities (Ciresu-Braila, Iazu-Ialomita, Stupina-Constanta, Topolog-Tulcea, and Viziru-Braila) and, according to the results, only hybrid Hy-7 was resistant in all localities. The results obtained from the three studied localities showed the emergence of new sunflower broomrape populations not controlled by gene for race H. Self-fertilization of hybrid Hy-7 produced the F<sub>2</sub> generation in 2016.

In 2017, broomrape resistance was studied at the infested (contaminated) plot at the All-Russian Research Institute of Oil Crops by the name of Pustovoit V.S – VNIIMK in Rostov on Don. The plot was found to be infested by new broomrape populations originating from Russia, Ukraine, Romania, Turkey, and Spain. The obtained results showed an infestation degree in 17.1% plants of hybrid Hy-7, 35% in the F<sub>2</sub> generation of Hy-7, control hybrids PR64LE25, LG-5580 and Donskoy-22 showed 19.4%, 23%, and 100% broomrape infestation, respectively. In conclusion, the plot contained broomrape populations which cannot be controlled by race H gene.

According to the obtained results, a permanent change in variability of broomrape populations can be confirmed practically year after year. At present, new broomrape populations found at several localities are locally dispersed. Geneticists and breeders have to make joint efforts in further detailed studies of broomrape variability.

KEY WORDS: broomrape, new variability, locality, population (race), sunflower.

## INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production for more than a century. According to Morozov

(1947) the first reports of broomrape in sunflower came from Saratov Oblast in Russia and date back to the 1890s. The same author mentions that the first sunflower varieties resistant to race A of *Orobanche* were developed by Plachek (1918) at the Saratov breeding station.

During the first half of the 20<sup>th</sup> century, cultivars resistant to the prevailing broomrape races were created in several breeding centres of the former USSR. Six broomrape races (A, B, C, D, E, and F) were determined in the second half of the 20<sup>th</sup> century. Genetics for resistance and differential lines of five races (A-E) were studied in detail by Vranceanu *et al.* (1980), while Pacureanu *et al.* (1998) studied race F.

Generally, broomrape (*Orobanche cumana* Wallr.) causes economic damage in sunflower production in a number of countries around the world, especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, and China.

At the beginning of the 21<sup>st</sup> century, sunflower cultivation area suddenly expanded in many countries while the former crop rotation practices were discontinued, which led to sudden changes in broomrape populations, especially in Spain and the Black Sea area (Russia, Ukraine, Moldova, Romania, Turkey, Bulgaria). Changes in broomrape populations occurred in many countries during the past 15 years. New variability in broomrape populations has been determined as races G and H by several authors.

In an assessment of the broomrape emergence issue at the global level, Kaya (2014) stated the following:

In addition to the development of broomrape resistance genes, research should also be performed on altering the plant anatomy of plant organs, biochemical parameters (mechanical barriers, induced germination, hormones, etc.) aspects of the parasite side such as breeding system and genetics of virulence, in order to understand the dynamics of broomrape populations and race evolution. On the other hand, Clearfield system is also an alternative and efficient control method with the use of imidazolinone (IMI) herbicide plus resistant hybrids. Combining resistance to herbicides with genetic resistance could provide a more horizontal and durable resistance and successful improvement of broomrape control in the future.

Fernandez-Martinez *et al.* (2012) concluded the following:

In recent years, several research groups have made relevant contributions on breeding for resistance and development of control strategies. Following the identification and characterization of new virulent races, new sources and mechanisms of resistance have been developed and characterized at the genetic and molecular level. There have also been important contributions to the knowledge of the biology and genetics of the parasite that will contribute to understand the dynamics of broomrape populations and race evolution.

Sunflower breeders and geneticists have achieved significant results in the use of molecular markers for identifying new broomrape races (A-F). Marker-assisted selection should be used even more in future search for *Orobanche* resistance.

The sources of resistance to broomrape which have been discovered so far mostly use the gene of resistance taken from the wild species of the genus *Helianthus*. According to the results obtained so far, there are over 20 wild species of the genus *Helianthus* which contain the gene of resistance to broomrape. Some of the breeders who used wild species of sunflower for finding the gene of resistance to broomrape are: Zhdanov (in the 1930s) – quotation Morozov (1947), Galina Pustovoit (1975), Škorić (1988, 1989, 2005), Christov *et al.* (1992, 1998, 2009), Jan *et al.* (2000 and 2002), Jan and Fernandez-Martinez (2002), Fernandez-Martinez *et al.* (2000 and 2007), Ruso *et al.* (1996), Sukno *et al.* (1998), and others.



The aim of the paper is to show the dynamics of change in broomrape populations in Romania and Russia, as countries which have experienced the most prominent changes, as well as to point out certain dilemmas over the nomenclature of the new populations (races).

## **MATERIAL AND METHOD**

Sunflower geneticists and breeders have been using different methods of testing resistance to broomrape (in open-field or greenhouse experiments, or through marker-assisted selection). Application of different methods often fails to provide confident results and adequate comparison of the obtained results.

Open-field method was used in the research, while trials were set up at the plots where a high emergence of broomrape plants had previously been observed.

Trials were set up in Romania and Russia, as countries which experienced frequent emergence of new broomrape populations (races).

Trials were set up in four Romanian locations (Cuza Voda, Crucea-Stupina, Braila-Valea Canepii and Tulcea-Agighiol) in 2014. The research included 390 sunflower genotypes and four controls (Performer, LC-1093, PR64LE20 and LG-5661) with a different degree of resistance. The main plot consisted of 2-4 rows in two replications. The final evaluation of resistance was performed during the stage of physiological maturity, while the number of broomrape-infected plants was determined at the main plot.

Ten hybrids with a different degree of broomrape resistance and five controls were studied in 2015. Unfortunately, hybrid LG-5631 was observed at only one locality. Trials were set up at five different localities in Romania. The main plot consisted of 2-4 rows. The final evaluation was carried out at physiological maturity by recording the number of broomrape plants at the main plot. The study used the results obtained from only five hybrids (among the ten tested hybrids) with a different degree of broomrape resistance.

Seed of the F<sub>2</sub> generation was produced in 2016, using only hybrid Hy-7 which exhibited complete resistance at all five localities.

Trial was set up at the experimental field of VNIIMK – Don trial station in Rostov on Don (Russia) during 2017. Besides hybrid Hy-7 (F<sub>1</sub>) and the F<sub>2</sub> generation, the trial included four control hybrids (Donskoy-22, Bella, LG-5580, and PR66LE25). The trial was carried out in two replications, and the main plot consisted of two rows. The mixture of broomrape seed collected from Russia, Ukraine, Romania, Bulgaria, Turkey and Spain was incorporated into the plot used in the previous trials. Broomrape resistance was evaluated at physiological maturity, while the percentage of broomrape infested sunflower plants and the degree of infestation were calculated.

## **RESULTS AND DISCUSSION**

The results obtained in four-year trials in different localities of Romania and VNIIMK – Trial station for breeding near Rostov on Don (Russia) revealed new variability in broomrape populations.

Trials carried out in 2014 at four localities in Romania (Cuza Voda, Crucea-Stupina, Braila-Valea Canepii, and Tulcea-Agighiol) revealed greater variability in broomrape populations compared to race F at all localities, since control line LC-1093 exhibited susceptibility. Line LC-1093 showed the highest susceptibility at the locality Braila-Valea Canepii (Table 1), followed by the localities Cuza Voda, Tulcea-Agighiol, and Crucea-Stupina. Even the susceptible hybrid Performer exhibited a high degree of infection. Also, control hybrids LG-5661 and PR64LE20 resistant to race G showed a certain degree of susceptibility at all four localities. Different degrees of susceptibility or

resistance were observed at the same localities in all 390 tested genotypes, while only the genotypes which showed resistance in at least one locality were taken for analysis. There were 10 genotypes at the locality Cuza Voda which had complete resistance to broomrape, whereas in Crucea-Stupina, Braila-Valea Canepii and Tulcea-Agighiol, genotypes showed different degrees of susceptibility. The only exception was observed in genotype No. 378, which was resistant at two localities - Cuza Voda and Tulcea-Agighiol. Among all the tested genotypes, only the genotypes No.367 and No.143 showed complete resistance at localities Crucea-Stupina and Braila-Valea Canepii, respectively.

Complete resistance was exhibited by 15 genotypes at the locality Tulcea-Agighiol, while different degrees of broomrape infestation were observed in other localities. The exception was observed in No.378 and No.386, which were completely resistant at localities Tulcea-Agighiol and Cuza Voda.

Among the tested genotypes, the following ones arouse breeders' interest: No. 185, No. 378, No. 367, No. 365, No. 348, No. 383, and No. 384 (Table 1).

Ten new hybrids and four controls were tested at five Romanian localities in 2015, as well as one line (LC-1093 gene for race F) (Table 2).

Based on the obtained results, races (populations) which cannot be controlled by gene  $Or_6$  have been confirmed. For the purpose of examining ten new hybrids, only five hybrids with a different degree of resistance to broomrape were chosen for analysis.

Hybrid Performer susceptible to broomrape was most attacked at the locality Ciresu-Braila. Hybrid LG-5542, resistant to race H, was under broomrape attack at two localities: Stupina-Constanta and Topolog-Tulcea, thus indicating the occurrence of new genetic variability in broomrape populations, which cannot be controlled by genes for race H.

Hy-7 was the only hybrid of all the tested ones which had complete resistance at all five localities, thus indicating that genes in hybrid Hy-7 can control new genetic variability of broomrape populations found in Stupina-Constanta and Topolog-Tulcea (Table 2).

Seed of the  $F_2$  generation was produced in 2016, in order to further explore broomrape resistance genetics in the hybrid Hy-7.

**Table 1:** The degree of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at various Romanian localities in 2014  
(Number of broomrape plants/main plot)

| No. | Genotype         | L o c a l i t y |                |                      |                 |
|-----|------------------|-----------------|----------------|----------------------|-----------------|
|     |                  | Cuza Voda       | Crucea-Stupina | Braila-Valea Canepii | Tulcea-Agighiol |
| 1.  | LC-1093 (rase F) | 542             | 154            | 1.136                | 267             |
| 2.  | Performer        | 1.532           | 437            | 3.397                | 432             |
| 3.  | LG-5661          | 48              | 28             | 40                   | 10              |
| 4.  | PR64LE20         | 69              | 58             | 129                  | 43              |
| 5.  | No. 97           | 0               | 46             | 70                   | 18              |
| 6.  | No. 144          | 0               | 72             | 4                    | 9               |
| 7.  | No. 185          | 0               | 3              | 1                    | 4               |
| 8.  | No. 194          | 0               | 32             | 130                  | 25              |
| 9.  | No. 242          | 0               | 64             | 17                   | 11              |
| 10. | No. 341          | 0               | 30             | 123                  | 35              |
| 11. | No. 378          | 0               | 2              | 1                    | 0               |
| 12. | No. 388          | 0               | 110            | 63                   | 10              |
| 13. | No. 367          | 38              | 0              | 1                    | 2               |
| 14. | No. 143          | -               | 38             | 0                    | 14              |
| 15. | No. 9            | 60              | 100            | 246                  | 0               |
| 16. | No. 188          | 20              | 8              | 1                    | 0               |
| 17. | No. 190          | 2               | 36             | 1                    | 0               |
| 18. | No. 191          | 3               | 33             | 5                    | 0               |
| 19. | No. 219          | 150             | 53             | 280                  | 0               |
| 20. | No. 274          | 40              | 40             | 28                   | 0               |
| 21. | No. 302          | 50              | 7              | 45                   | 0               |
| 22. | No. 348          | 1               | 9              | 3                    | 0               |
| 23. | No. 365          | 3               | 18             | 15                   | 0               |
| 24. | No. 381          | 8               | 23             | 4                    | 0               |
| 25. | No. 383          | 1               | 18             | 1                    | 0               |
| 26. | No. 384          | 2               | 7              | 2                    | 0               |
| 27. | No. 386          | 0               | 12             | 15                   | 0               |

**Table 2:** The degree of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at various Romanian localities in 2015  
(Number of broomrape plants/plot)

| No. | Genotype  | Localities            |                       |                       |                       |                       |                       |                       |                       |                       |                       |
|-----|-----------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|     |           | Ciresu-Braila         |                       | Iazu-Ialomita         |                       | Stupina-Constanta     |                       | Topolog-Tulcea        |                       | Viziru-Braila         |                       |
|     |           | Sunflower plants/plot | Broomrape plants/plot | Sunflower plants/plot | Broomrape plants/plot | Sunflower plants/plot | Broomrape plants/plot | Sunflower plants/plot | Broomrape plants/plot | Sunflower plants/plot | Broomrape plants/plot |
| 1.  | Performer | 89                    | 679                   | 48                    | 97                    | 39                    | 102                   | 46                    | 169                   | 45                    | 204                   |
| 2.  | LC-1093   | 47                    | 41                    | 43                    | 8                     | 32                    | 41                    | 43                    | 51                    | 46                    | 19                    |
| 3.  | LG-5542   | 45                    | 0                     | -                     | -                     | 38                    | 5                     | 46                    | 6                     | 46                    | 0                     |
| 4.  | LG-5631   | 46                    | 12                    | -                     | -                     | -                     | -                     | -                     | -                     | -                     | -                     |
| 5.  | PR64LE20  | 48                    | 16                    | 44                    | 0                     | 41                    | 18                    | 44                    | 26                    | 44                    | 0                     |
| 6.  | Hy-1      | 128                   | 887                   | 95                    | 136                   | 47                    | 68                    | 95                    | 113                   | 105                   | 38                    |
| 7.  | Hy-2      | 120                   | 798                   | 105                   | 87                    | 55                    | 95                    | 95                    | 233                   | 94                    | 98                    |
| 8.  | Hy-7      | 126                   | 0                     | 95                    | 0                     | 44                    | 0                     | 96                    | 0                     | 141                   | 0                     |
| 9.  | Hy-9      | 124                   | 0                     | 106                   | 1                     | 55                    | 11                    | 82                    | 18                    | 100                   | 8                     |
| 10. | Hy-10     | 132                   | 2                     | 94                    | 0                     | 46                    | 16                    | 93                    | 59                    | 95                    | 30                    |

**Table 3:** Evaluation of broomrape (*Orobanche cumana* Wallr.) resistance in different sunflower genotypes at the infested plot:  
VNIIMK – Don trial station, Rostov on Don in 2017

| No. | Genotype               | Percentage of infested sunflower plants/main plot | Degree of infestation |
|-----|------------------------|---------------------------------------------------|-----------------------|
| 1.  | Donskoy-22             | 100                                               | 100                   |
| 2.  | Bella                  | 30                                                | 3,0                   |
| 3.  | LG-5580                | 23                                                | 1.0 – 1.2             |
| 4.  | PR66LE25               | 19.4                                              | 2.6                   |
| 5.  | Hy-7 (F <sub>1</sub> ) | 17.1                                              | 1.1                   |
| 6.  | F <sub>2</sub> (Hy-7)  | 35.1                                              | 2.0                   |

Seeds of hybrid Hy-7 (F<sub>1</sub>) and its F<sub>2</sub> generation were tested in 2017, at plots infested with broomrape from Russia, Ukraine, Romania, Bulgaria, Turkey, and Spain. Trial was carried out at experimental fields of VNIIMK – Don trial station in Rostov on Don (Russia). Control hybrids were included in this trial: Donskoy-22 (susceptible control), Bella, LG-5580, and PR66LE25.

The obtained results showed the following degrees of broomrape infestation in susceptible hybrids: Donskoy-22 100%, Bella 30%, LG-5580 23%, PR66LE25 19.4%, Hy-7 17.1%, and its F<sub>2</sub> generation 35.1% (Table 3).

The results revealed that hybrid Hy-7, which contained resistance genes above race H and exhibited complete resistance in 2015 at five Romanian localities, was infested with broomrape in 17.1%

sunflower plants under the given conditions. The results indicate new variability of broomrape populations at that locality.

The results of Gorbachenko *et al.* (2018) suggest the possibility of developing new cultivars of sunflower resistant to the new variability of broomrape populations.

This method of testing broomrape resistance has its drawbacks due to the differences in broomrape distribution at the testing plots. Also, broomrape expression depends on several factors, such as soil type, distribution and amount of rainfall during the vegetation period, temperature, pH value, and so forth.

The obtained results are not precise; they are rather general results which provide a perspective on the varying degrees of resistance.

Based on exhaustive research, we can accept with high certainty the resistance genetics for races A, B, C, D, E and F with secure differential lines. However, speaking of the new races G and H, we might not be completely sure. The question is whether the same mutation can occur in broomrape populations, in various countries during the same year? Certain differences which have not yet been studied in detail surely exist. Rapid spread of the new races is also notable. In response to this question, Kaya (2014) concluded that the new races increase each year by 50% more than in the previous year. In the period from 1995 to 2002, the infested areas increased from 2% to 35%, leading to the conclusion that new *Orobanche* races occupied more than 60-70% of the sunflower production area in the Trakya Region in 2002.

The question regarding the degree of virulence in the new broomrape populations remains unanswered. Significant differences have best been illustrated by Pacureanu-Joita *et al.* (2012), stating that the most aggressive populations were found in Moldova, Romania, Russia, and Turkey. When it comes to testing variability of broomrape populations at the molecular level, we can conclude that a great number of authors have dealt with this problem. The results of Perez-Vich *et al.* (2004) who analyzed the resistance of the line P-96 to races E and F at the molecular level. Based on a linked map comprising 103 marker loci distributed on 17 linkage groups, it was determined that only five QTLS (Or<sub>1.1</sub>, Or<sub>3.1</sub>, Or<sub>7.1</sub>, and Or<sub>13.2</sub>) were responsible for resistance to race E, while only 6 QTLS (Or<sub>1.1</sub>, Or<sub>4.1</sub>, Or<sub>13.1</sub>, Or<sub>13.2</sub>, and Or<sub>16.1</sub>) controlled resistance to race F and they were found on seven of the 17 linkage groups. The results suggest that sunflower resistance to broomrape is controlled by a combination of qualitative, race-specific resistance effecting the presence or absence of broomrape and quantitative, non-race-specific resistance affecting the number of broomrape stalks per plant.

New studies worth mentioning are the ones carried out by Duca *et al.* (2017), who performed molecular characterization of 39 broomrape populations from Moldova using SSR markers.

Based on the obtained results, the authors concluded that some populations from the Southern region (especially Carabetovca, Alexanderfeld, Stefan-Voda, and Slobozia Mare) have shown major differences in the obtained profiles and presented a high degree of genetic variability. The study revealed genetic diversity of *O. cumana* populations and contributed to research by providing useful information on this economically important pathogen.

Given the different degrees of virulence observed at trial localities, the results confirm the necessity for a detailed molecular analysis of different broomrape populations found in many Romanian localities.

The results of Gorbachenko *et al.* (2018) offer the possibility of developing sunflower hybrids resistant to the new variability of broomrape populations which cannot be controlled by genes for race H.

In conclusion, the results obtained in this research partly differ from the results obtained by Rîșnoveanu *et al.* (2016), which was to be expected since the trials were carried out at various

localities (plots). However, the results concur with authors' conclusion, confirming identification of new races of *Orobanche* previously undetected at the trial localities.

## **BROOMRAPE CONTROL THROUGH DEVELOPMENT OF HYBRIDS RESISTANT TO HERBICIDES**

For over 15 years, all large breeding companies and public institutions have been breeding sunflower hybrids for resistance to imidazolinone herbicides, thus developing mass production of dominant hybrids resistant to herbicides in countries with frequent broomrape occurrence. Many companies combine genes of resistance to broomrape (Or<sub>5</sub> and Or<sub>6</sub>) with genes of resistance to imidazolinone herbicides, thereby providing secure, long-term hybrid resistance.

At the beginning of the 21<sup>st</sup> century, public resistance sources were found in the wild sunflower *Helianthus annuus* L. discovered in Kansas (USA). Resistance source named CHLA- PLUS, obtained by Sala (2008) through induced mutations, has lately been used by the company BASF.

It has been shown experimentally that the gene CHLA-PLUS a higher degree of IMI-resistance than the gene Imr<sub>1</sub>, Imr<sub>2</sub>. Breeding centers wishing to use the CHLA-PLUS gene for breeding purposes have to sign a contract on its use with the company BASF. At the same time, BASF provides a protocol for screening for resistance at the molecular level (CLEARFIELD® Protocol SF30).

Broomrape can also be managed by the development of IMI-resistant hybrids of by using biological control measures. In parallel with the search for broomrape resistance genes, efforts should be made to after the anatomy of plant organs as well as biochemical parameters (mechanical barriers, germination inhibitors, phytoalexins, etc).

## **CONCLUSION**

Based on the studies in sunflower genetics and breeding for broomrape resistance in the Black Sea area, especially in Romania and Russia, the following conclusions can be made:

There is permanent combat between breeders and geneticists on one side, and *Orobanche* on the other, and the WINNER is often found at different sides;

Methods have been developed for the evaluation of resistance to *Orobanche*;

Conventional methods and MAS (Marker Assisted Selection) have been developed in breeding for resistance to *Orobanche*;

In Romania, new virulent population (races) of broomrape have emerged, especially in the regions of Braila, Tulcea, and Constanta. At certain localities, new variability of broomrape populations has been observed, which cannot be controlled by genes for race H;

Likewise, at the trial station of VNIIMK – Don Trial Station in Russia, a new virulent broomrape population emerged, which cannot be controlled by genes for race H. Luckily, sunflower breeders have developed new genotypes resistant to the new variability of broomrape populations;

There is permanent change in populations of *Orobanche cumana* Wallr., thus hindering the success of breeding for resistance;

Sources of broomrape resistance genes are found in certain wild species of *Helianthus*, which are transferred after detection to the elite lines of cultivated sunflower using interspecies hybridization;

Besides control through genes for broomrape resistance, *Orobanche* can successfully be controlled by the development of IMI-resistance hybrids.

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# GENETIC AND BIOLOGICAL APPROACH TO DECIPHER *OROBANCHE CUMANA* RESISTANCE IN SUNFLOWER WILD RELATIVES

Xavier GRAND<sup>1</sup>, Isabelle ANDRÉ<sup>1</sup>, Sylvie LORAS<sup>1</sup>,  
Ludovic LEGRAND<sup>2</sup>, Jérôme GOUZY<sup>2</sup>, Nicolas RIBIÈRE<sup>1</sup>,  
Amandine LARIÈPE<sup>1</sup>, Bruno GREZES-BESSET<sup>1</sup>, Thierry ANDRÉ<sup>3</sup>, Sebastien CHATRE<sup>4</sup>,  
Stéphane MUÑOS<sup>2</sup>, Marie COQUE<sup>1\*</sup>

<sup>1</sup>Genetics & Genomics in Oilseeds, BIOGEMMA, Mondonville, France

<sup>2</sup>Laboratory of Plant-Microbe Interactions, INRA de Toulouse, France

<sup>3</sup>SOLTIS, Mondonville, France

<sup>4</sup>RAGT2N, Gaillac, France

\*Corresponding author: marie.coque@biogemma.com

## Abstract

Sunflower broomrape (*Orobanche cumana* Wallr.), a parasitic plant that connects to sunflower roots, became one of the most important constraints to sunflower production since it can cause complete yield loss. Breeding for resistant sunflower hybrids is an effective way to control *O. cumana*. However, sources of resistance to *O. cumana* in sunflower have been mainly based on vertical resistance mechanisms, controlled by single dominant genes. This has led to a rapid overcoming of the resistances and subsequently to the need of finding new resistance sources. Interestingly, helianthus species constitute a substantial reservoir of genes conferring resistance to new virulence broomrape races.

To elucidate the genetic control of these resistances, interspecific populations (from crosses between cultivated *H. annuus* and wild relatives) have been evaluated for broomrape resistance during two years in field trials in Spain where race F is located. One of these populations segregated for a high level of resistance. We selected the most resistant lines from this population for further analysis. At the physiological level, they have been phenotyped for the most important steps of the interaction (efficiency of the *O. Cumana* seeds germination and resistance at the earlier stage of the interaction). Resistance spectrum has also been evaluated against more aggressive *O. cumana* races. At the molecular level, a whole genome expression study by RNAseq and RT-qPCR permitted to describe large scale genes expression reprogramming in both sunflower and *O. cumana* during the interaction. At the genetic level, Genome Wide Association Study and QTL analysis highlighted new genomic regions controlling sunflower broomrape resistance.

Fine-mapping of these genomic regions are now underway using a large F2 segregating population. Combining Physiology, Genetics and functional Genomics allowed to investigate the most relevant candidate genes. Finally, this work will contribute to a better understanding of the *O. cumana* and sunflower interaction and will provide new resistance genes for future breeding programs in sunflower.

**Keywords:** wild sunflower, broomrape, genetic approach, biological approach, resistance

**A NEW SOURCE OF POSTHAUSTORIAL RESISTANCE  
TO SUNFLOWER BROOMRAPE DERIVED FROM  
*HELIANTHUS PRAECOX***

Antonio SAYAGO, Begoña PÉREZ-VICH,  
José M. FERNÁNDEZ-MARTÍNEZ, Leonardo VELASCO\*

*Institute for Sustainable Agriculture, CSIC, Córdoba, Spain*

*\*Corresponding author: lvelasco@ias.csic.es*

**Abstract**

Genetic resistance to sunflower broomrape (*Orobancha cumana* Wallr.) is crucial for sustaining sunflower production in the Old World. Because of the capacity of the parasite to overcome resistance mechanisms in the host, new sources of resistance and new mechanisms of resistance must be continuously developed to ensure sustainability of the crop. From the first observations of broomrape attacks in the 19<sup>th</sup> century, wild *Helianthus* species have played a major role as donors of resistance alleles. In this research, we report the identification of a new source of genetic resistance to *O. cumana* race G in the annual wild species *H. praecox*. Eight plants of a *H. praecox* accession from the USDA-ARS germplasm collection were evaluated with *O. cumana* race G and none of them showed emerged broomrape shoots. Pollen of the *H. praecox* plants were used to pollinate sterile heads of the GMS line P21. All F<sub>1</sub> plants showed emerged broomrape shoots, but they all showed a strongly reduced growth when compared to the susceptible control. F<sub>1</sub> plants were fertile and produced sufficient number of seeds for evaluation of the F<sub>2</sub> generation. Around one fourth of the F<sub>2</sub> sunflower plants showed normally developed broomrape shoots, whereas the rest of the population showed no emerged shoots or emerged shoots with a considerably reduced growth. It was concluded that the trait is controlled by partially dominant alleles at a single gene that we have named *Or<sub>pral</sub>*.

**Keywords:** broomrape, *Helianthus annuus*, *Helianthus praecox*, race G, posthaustorial resistance

# TOWARDS AN UNIVERSAL SET OF DIFFERENTIAL SUNFLOWER GENOTYPES FOR PRECISE BROOMRAPE RACE IDENTIFICATION

Alberto MARTÍN-SANZ<sup>1</sup>, Elena MUÑÍZ<sup>1</sup>, Enrique GONZÁLEZ<sup>1</sup>,  
Sandra RUEDA<sup>1</sup>, Begoña PÉREZ-VICH<sup>2</sup>, Leonardo VELASCO<sup>2\*</sup>

<sup>1</sup>Pioneer Hi-Bred International, Seville, Spain

<sup>2</sup>Institute for Sustainable Agriculture, CSIC, Córdoba, Spain

\*Corresponding author: lvelasco@ias.csic.es

## Abstract

Sunflower broomrape (*Orobanche cumana* Wallr.) is a holoparasitic weed that infects roots of sunflower in large areas of Europe and Asia, and it has been identified recently in North Africa. The parasitic interaction between sunflower and *O. cumana* generally follows a gene for gene model. It has been reported the existence of five pathogenic races of *O. cumana* named as A to E, controlled by resistance genes *Or1* to *Or5*. New populations overcoming *Or5* resistance and named as race F were identified from the middle 1990's in most of the areas infested by sunflower broomrape. Nowadays, increasingly virulent populations classified as races G and H are becoming predominant in countries around the Black Sea and Spain. However, there are doubts about the racial status in sunflower broomrape and research community needs imperiously of a common set of differentials to determine precisely the races present in specific countries. To clarify this situation and propose a new system of differentials we have evaluated 24 broomrape populations from different countries in which sunflower is facing broomrape. These populations were evaluated on eight inbred lines and four hybrids carrying different resistance genes, including lines used in previous studies like B117, NR5 and P96, for instance, and hybrids extensively used along Europe. The results showed that broomrape racial situation is more complex than it has been proposed before and that it is needed to include several differentials to be able to discriminate different races of what has been named before as race F, G and H. A new set of differentials will be proposed preliminarily to understand this variability and discussed with scientific community.

**Keywords:** broomrape, *Helianthus annuus* L., race, sunflower, virulence

# MOLECULAR CHARACTERIZATION OF THE MAJOR RESISTANCE GENE *Or7* CONTROLLING RESISTANCE TO *OROBANCHE CUMANA* IN SUNFLOWER

Pauline DURIEZ<sup>1,2</sup>, Sonia VAUTRIN<sup>3</sup>, Marta LOPEZ-SENDON<sup>2</sup>,  
Marie-Claude BONIFACE<sup>1</sup>, Hélène BERGÈS<sup>3</sup>, Julia BAZERQUE<sup>1</sup>, Fabienne GENTOUX<sup>2</sup>, Jean-  
Christophe ROUSSEAU<sup>2</sup>,  
Joël PIQUEMAL<sup>2</sup>, Stéphane MUÑOS<sup>1\*</sup>

<sup>1</sup>LIPM, Université de Toulouse, INRA, CNRS, Castanet-Tolosan, France

<sup>2</sup>Syngenta Seeds S.A.S., Saint-Sauveur, France

<sup>3</sup>Centre National de Ressources Génomiques Végétales, INRA-Toulouse,  
Castanet-Tolosan, France

\*Corresponding author: [stephane.munos@inra.fr](mailto:stephane.munos@inra.fr)

## Abstract

Resistant cultivars play an important role to control sunflower broomrape (*O. cumana*) in fields. Some of them carry the *Or7* locus, a major resistance gene that confers resistance to *O. cumana* race F, the main race in South of Spain. We used a combined approach to map the locus to the chromosome 7 by GWAs on 55 lines and by using a segregating population of 355 RILs. *In silico* analyses and genetic data located the *Or7* gene in a genomic region of 850kb. We then restricted the genomic region of the *Or7* gene by genotyping a large segregating population of 14,000 F2 plants and by identifying 271 F2 recombinant plants between the two markers surrounding the 850 kb genomic region. After selfing each of the 271 F2 plants, almost all F3 families were phenotyped in field, the corresponding F4 families were phenotyped in the same field, and in controlled conditions to confirm the F3 families phenotype. Finally the *Or7* gene was located in a window of around 55 kb, containing 2 predicted genes coding for Leucine Rich Repeat proteins. To obtain the parental genomic sequences, we created and screened two BAC libraries from the susceptible and resistant lines. The comparison of both genomic sequences showed a high level of divergence with large structural variations, suggesting a wild origin of the *Or7* gene. We performed a molecular diversity analysis of the region, in a panel of 170 accessions of wild relatives, wild and cultivated sunflower. Only one haplotype was found on *Or7*+ lines for a gene close to the 55 kb window. A transcriptomic analysis is underway to better understand the role of the *Or7* gene during the interaction.

**Keywords:** *Or7*, resistance, cloning, LRR protein, wild relatives

# THE ESTABLISHMENT OF INDOOR EVALUATION CRITERION AND IDENTIFICATION OF SUNFLOWER RESISTANCE LEVEL TO BROOMRAPE WITH PETRI DISH SYSTEM

Shenghua SHI<sup>1</sup>, Dongsheng XU<sup>1</sup>, Bixian SHI<sup>2</sup>, Jun ZHAO<sup>1\*</sup>

<sup>1</sup>*Agronomy Department, Innermongolia Agricultural University, China*

<sup>2</sup>*Economic Crop Department, Xinjiang Agricultural Academic Institution, China*

*\*Corresponding author: zhaojun@imau.edu.cn*

## Abstract

The sunflower broomrape is a parasitic plant that parasitizes on the roots of sunflowers. Sunflower plants show dwarf, loss of yield, and dramatic reduction of oil and protein content caused by parasitization. In this study, the Petri dish filter paper system, which was established previously, was used to build up a set of indoor criterion to evaluate the sunflower resistance level to broomrape. Through comparing the resistance level of 12 sunflower varieties between field trial and indoor petri-dish system, we set up the evaluation system of sunflower resistance to broomrape under petri-dish system. In this identification standard, no tubercles cultivar was identified as an immuned variety; the number of tubercles in each dish of sunflower root between 1 and 5 was identified as highly resistance; the number of tubercles was between 5 and 10 is moderately resistant; the number of tubercles between 10 and 15 is susceptible and the number of tubercles over 15 is a highly susceptible variety. Via using this criterion, the resistant level 80 sunflower varieties to broomrape were identified under lab condition. The results indicated that 14 sunflower varieties showed immune response to broomrape, including 11 oil and 3 confectionary varieties; twenty five were identified as highly resistant level, including 14 oil and 11 confectionary varieties. Twenty two were identified as moderately resistant level, containing 7 oil and 13 confectionary varieties. Twelve were identified as susceptible level, consisting of one oil and 11 confectionary varieties. Seven were identified as highly susceptible level and all of them were confectionary varieties. In general, oil sunflowers varieties were more resistant to broomrape comparing to confectionary varieties.

**Keywords:** sunflower broomrape, resistance identification criterion, petri dish system, resistance identification of sunflowers

# INHERITANCE OF RESISTANCE TO BROOMRAPE IN SUNFLOWER INBRED LINE LIV-17

Sandra CVEJIĆ<sup>1\*</sup>, Siniša JOCIĆ<sup>1</sup>, Boško DEDIĆ<sup>1</sup>,  
Dragana MILADINOVIĆ<sup>1</sup>, Aleksandra DIMITRIJEVIĆ<sup>1</sup>,  
Ivana IMEROVSKI<sup>2</sup>, Milan JOCKOVIĆ<sup>1</sup>, Vladimir MIKLJIĆ<sup>1</sup>

<sup>1</sup>*Institute of Field and Vegetable Crops, Novi Sad, Serbia*

<sup>2</sup>*The University of British Columbia, Vancouver, Canada*

\*Corresponding author: sandracvejic03@gmail.com

## Abstract

**Abstract:** Genetic resistance to broomrape (*Orobancha cumana* Wallr.) in sunflower is the most effective way to control the parasite. The objective of this study was to determine the inheritance and gene action for broomrape resistance in sunflower inbred line LIV-17. This line was first tested and found to be resistant to broomrape, in heavily infested locations in Spain and Turkey, where races F and G were predominant. Resistant line was crossed with susceptible line HA-26-PR and F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generation were developed. All plants in F<sub>1</sub> generation were susceptible, indicating recessive inheritance. The segregation ratios in F<sub>2</sub> generation had a goodness of fit to the expected ratio 3(S):1(R), indicating that broomrape resistance was controlled by a single recessive gene. Testing of the F<sub>3</sub> generation gave a more precise evaluation and enabled differentiation between homozygous and heterozygous susceptible plants. The respective F<sub>3</sub> families segregated, as expected, in 1 (homozygous S): 2 (heterozygous S): 1 (homozygous R) ratio, confirming that this line is resistant to broomrape when gene was present in homozygous recessive condition. From the breeding perspective, recessive nature of resistance to broomrape in LIV-17 highlights the necessity to introduce resistance genes into both parental lines in order to obtain resistant hybrid.

Key words: *sunflower, broomrape, inheritance, recessive gene*

## Introduction

The parasitic weed *Orobancha cumana* (sunflower broomrape) is an obligatory and non-photosynthetic root parasitic plant of the sunflower (*Helianthus annuus* L.) and is a substantial threat in Europe, especially in countries around the Black Sea and in Spain (Molinero-Ruiz et al., 2013, Louarn et al., 2016). Under favourable conditions, it infects the roots of sunflower plants and connects to the vascular tissue, thus depleting the nutrients and affecting host growth and yield (Heide-Jorgensen, 2008; Molinero-Ruiz et al., 2015). Broomrape seeds are very small and individual plants can produce an impressive number of seeds that remain viable in the soil for up to 20 years. They are widely disseminated by water, wind, animals, humans, machinery, or through attachment to sunflower seeds (Parker, 2013).

A major difficulty for the breeders is the fast development of new races of the parasite, which overcome the resistance of sunflower genotypes. To the present day, more than seven races of sunflower broomrape have been identified (Kaya 2014). Vranceanu et al. (1980) identified five races of *O. cumana*, designated as A, B, C, D and E. Later on, more virulent race F was identified in Spain (Alonso et al., 1996; Molinero-Ruiz et al., 2008, Martin-Sanz et al. 2016), Romania (Păcureanu-Joita et al., 1998), Turkey (Kaya et al., 2004) and some other countries. Presence of more virulent broomrape races, designated G and H, has been also reported (Shindrova and Penchev, 2012, Antonova et al., 2014, Kaya, 2014). Current racial situation of broomrape in the main infested areas is unclear, since there is a lack of information on whether races under the same

name reported in different countries are the same or differ in terms of virulence (Fernández-Martínez et al., 2012; Molinero-Ruiz et al., 2015; Martin-Sanz et al., 2016).

Breeding for genetic resistance appears to be the most appropriate and reliable measure to control the parasite. Resistance to *O. cumana* in sunflower is primarily vertical, that is, monogenic, dominant, and race specific (Škorić et al., 2010; Molinero-Ruiz et al., 2015). Therefore, *Or*<sub>1</sub>, *Or*<sub>2</sub>, *Or*<sub>3</sub>, *Or*<sub>4</sub> and *Or*<sub>5</sub>, conferring resistance to races A, B, C, D and E, respectively, are single dominant genes (Vranceanu et al., 1980). Resistance to the race F could be controlled by a single dominant gene, *Or*<sub>6</sub> (Păcureanu-Joita et al., 1998; Pérez-Vich et al., 2002), two recessive genes (Akhtouch et al., 2002) or two partially dominant genes (Velasco et al., 2007), depending on origin sunflower origin. Velasco et al. (2012) showed that the resistance (from *H. debilis* subsp. *tardiflorus*) to the race G was dominant and controlled by a single locus in their population, while Imerovski et al. (2015) found that resistance to races higher than F in a newly identified resistance source is controlled by a single recessive gene.

The changes in broomrape race composition have forced sunflower breeders to continuously search for resistance genes to new races and study their genetic control. The objective of this study was to determine the inheritance and gene action for broomrape resistance in sunflower inbred line LIV-17, which was first tested and found to be resistant to broomrape, in heavily infested fields, where races F and G were predominant.

## Materials and methods

Sunflower inbred line LIV-17, was chosen from the gene pool of Institute of Field and Vegetable Crops, Novi Sad. It originates from population developed from interspecific hybridization with *Helianthus tuberosus*. This line was found to be resistant to broomrape in consecutive trials in the field where virulent races, overcoming F, appear (Cvejić et al., 2012, 2014).

For genetic studies, LIV-17 were crossed with susceptible line HA-26-PR, which is completely susceptible to broomrape (Cvejić et al., 2014), and F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> progenies were developed. Parents, F<sub>1</sub> and F<sub>2</sub> generation were planted in the naturally infested field in Northern Serbia. To validate field results, F<sub>3</sub> generation was grown in greenhouse. Screening for resistance in greenhouse was done using modified method by Panchenko (1975). Broomrape plants were collected from hybrids resistant to race E across the heavily infested sunflower field. Broomrape plants were dried, minute seed was separated using mesh and stored on 4°C until use. Surface sterilised sunflower seed was sown in 9 dm<sup>3</sup> elongated pots containing equal quantity of sand, perlite and substrate (Klasmann-Deilmann Substrat 1). Approximately 8 mg of broomrape seed per dm<sup>3</sup> of mixture was added. Ten plants per pot in two replications were grown for seven weeks under temperature regime of 24/18°C and 16 h photoperiod. Presence of broomrape was evaluated after careful uprooting of sunflower plants and counting of - nodules and stalks. Plants were considered resistant (R) when no broomrape nodules or stalk was found within the complete pot; and susceptible (S) when plants were infested (even one broomrape nodule or/and stalk per plant). The  $\chi^2$  analyses were performed to test the goodness of fit of observed to expected ratios.

## Results and discussion

In the naturally infested field, the resistant line LIV-17 was uniformly resistant while susceptible line HA-26-PR was completely susceptible. All F<sub>1</sub> progenies from HA-26-PRxLIV-17 crosses had more than five broomrape stalks per plant when tested in the infested field, which indicates their susceptibility (Table 1). The susceptibility of F<sub>1</sub>s suggested the recessive nature of broomrape resistance. Broomrape resistance controlled by recessive genes has also been reported by Akhtouch et al. (2002) and Rodríguez-Ojeda et al. (2001) for race F, as well as for races overcoming race F (Imerovski et al., 2014). Although recessive resistance is not as exploited in breeding as dominant resistance, it appears to be generally very durable (Lecoq et al., 2004; Ssali et al., 2013). The barley

*mlo* allele, associated with resistance to powdery mildew, remains effective more than 30 years after its introduction (Lyngkjaer et al., 2000).

Table 1. Segregation of broomrape resistance in F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> progenies of HA-26-PRxLIV-17

| Pedigree        | Generation     | Total | Observed no. of plants/families |                            | Hypothesized ratio       |           | $\chi^2$    | P value |
|-----------------|----------------|-------|---------------------------------|----------------------------|--------------------------|-----------|-------------|---------|
|                 |                |       | Resistant                       | Susceptible (heterozygous) | Susceptible (homozygous) | Observed  | Theoretical |         |
| Ha-26-PR        | P <sub>1</sub> | 20    | 0                               |                            | 20                       |           |             |         |
| LIV-17          | P <sub>2</sub> | 17    | 17                              |                            | 0                        |           |             |         |
| HA-26-PRxLIV-17 | F <sub>1</sub> | 18    | 0                               |                            | 18                       |           |             |         |
|                 | F <sub>2</sub> | 99    | 26                              |                            | 73                       | 1:2.8     | 1:3         | 0.220   |
|                 | F <sub>3</sub> | 89    | 26                              | 46                         | 17                       | 1:1.7:0.6 | 1:2:1       | 0.130   |

In F<sub>2</sub> generation, segregation ratio of 3(S):1(R) was observed, indicating that resistance to broomrape in LIV-17 was controlled by a single recessive gene (Table 1). In order to deduct the genotypes of F<sub>2</sub> individual plants, their respective F<sub>3</sub> families were tested for broomrape resistance in the greenhouse. F<sub>3</sub> families segregated, as expected, in 1(homozygous S):2(heterozygous S):1 (R) ratio (Table 1). Some of previous studies revealed similar mechanisms of inheritance depending on the used material and racial composition. Păcureanu-Joița et al. (1998) reported single dominant gene (*Or*<sub>6</sub>) in inheritance of race F in Romania in differential line LC-1093. Perez-Vich et al. (2002) crossed inbred line (J1) derived from interspecific population BR4 with a susceptible genotype and found that the line had a single dominant gene for resistance to race F in the segregating generations. Furthermore, monogenic control was also reported in resistance to broomrape overcoming race F. Velasco et al. (2012) found single dominant inheritance of broomrape resistance to population classified as race G in a resistant sunflower accession of *Helianthus debilis* subsp. *trandiflorus*. However, inheritance study on F<sub>2</sub> generation with resistance donor line HA-267 showed segregation ratio 1(R): 3(S) (Imerovski et al., 2014). This new line was found to be resistant in fields where broomrape races F and G were detected and has resistance higher than *Or*<sub>6</sub>. This study is in agreement with our findings of single recessive mode of inheritance of resistance to races overcoming F in inbred line LIV17.

In conclusion, results pointed out that line LIV17 had a resistant reaction to broomrape to races overcoming F when gene was present in homozygous recessive condition. The present information will be further clarified using molecular markers in identifying *Or* genes. From the breeding perspective, recessive nature of these inbred lines highlights the necessity to introduce resistance genes into both parental lines in order to obtain resistant hybrids, but pyramidization of these two genes in both parental lines could provide better and more reliable protection to broomrape in heavily infested areas.

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# SUNFLOWER RESISTANCE TO RACE G OF BROOMRAPE: THE DEVELOPMENT OF THE LINES AND THE STUDY OF INHERITANCE

Saida GUCHETL\*, Tatyana ANTONOVA,  
Nina ARASLANOVA, Tatyana TCHELYUSTNIKOVA

*All-Russian Research Institute of Oil Crops by the name of Pustovoit V.S., Russia*

*\*Corresponding author: saida.guchetl@mail.ru*

## Abstract

**Keywords:** *Sunflower, Broomrape, Race G, Resistance, Inheritance*

## Introduction.

Malicious weed broomrape (*Orobanche cumana* Wallr.) is an obligate parasite from higher flowering herbaceous plants, which causes significant damage to the sunflower crop in the most countries where it is cultivated. The intensification of sunflower cultivation as a high-yielding crop has resulted in the emergence and rapid spread of new highly virulent races of the parasite that overcame the immunity of sunflower varieties and hybrids that were previously resistant to it.

The monitoring of the racial composition of broomrape, carried out by us every year, has showed that race G already prevails in most populations of broomrape in the southern regions of the Russian Federation [1, 2]. Therefore, the search for sources of immunity to this race in order to develop a resistant initial material of sunflower and to study the genetic control of this trait is of high priority. The aim of this work was to search for possible sources of resistance, the development on their basis sunflower lines that are not affected by race G of broomrape, and the determination of genetic control of this trait.

**Materials and methods.** Seeds of broomrape were collected on the fields of the Bokovskiy district of the Rostov region. The identification of their racial belonging with the help of the known differentiation lines: 202A (C), LC1002 (D), LC1003 (E), LC1093 and P96 showed that the seeds belong purely to the race G. The material for research were over 1000 samples of cultivated sunflower from the collection of the Kuban Experimental Station VIR, the collection of VNIIMK of cultivated sunflower of various origin, as well as the breeding lines VK 551, VK 678 B, VK 678 A, VK 1 IMI B, VK 1 IMI A, VK 301, VK 580 and PRO2, susceptible to broomrape.

Forced self-pollination and hybridization of sunflower plants were carried out using the method, which is customary for VNIIMK [3]. A greenhouse evaluation of resistance to broomrape was carried out by the method of A.Y. Panchenko [4]. To create an infectious background in the greenhouse, the broomrape seeds were added to the boxes with soil-sand mixture at the rate of 200 mg per 1 kg of mixture, distributing them evenly. Sunflower plants were grown at a temperature of 25-27 °C and a 16-hour photoperiod. The plants were dugged out in 25 days after the seedlings emergence and the broomrape specimens on their roots were counted. VNIIMK 8883 was used as a control, which is susceptible to the modern races *O. cumana*.

Analysis of variance of the received data was carried out according to the method laid down by B.A. Dospekhov [5]. Chi squared analyses were carried out to detect the deviations from the expected Mendelian ratios 1:2:1 or 1:1 [6].

**Results and discussion.** Over the last century of the joint evolution of sunflower and *O. cumana* plants there has been a constant rapid adaptation of the parasite to the mechanisms of host immunity. Consequently, the detection of resistance genes in cultivated sunflower has become problematic. Thus, most of the studied samples were affected to a high degree, some at the susceptible control level. However, samples were found that were divided into weakly affected and

non-affected genotypes, further work with which can make it possible to develop lines immune to race G. The selection of such samples of domestic and foreign sunflower from the VIR collection are listed in Table 1. Among the presented material, samples of local breeding from the Krasnodar Region attract attention: lines VIR-665, VIR-221, VIR-222, Kabardino-Balkarian (No. 667 catalog), Armenian (No. 769 catalog), Argentine (No. 3046 catalog).

Table 1. The degree of infestation by race G of broomrape (*O. cumana*) of some samples of cultivated sunflower of VIR collection in greenhouse conditions

| Catalog No.                     | Origin                             | Number of evaluated plants, pcs. | Infested plants, % | Degree* of infestation |
|---------------------------------|------------------------------------|----------------------------------|--------------------|------------------------|
| 667                             | Kabardino-Balkaria                 | 30                               | 23,3               | 2                      |
| 769                             | Armenian SSR                       | 30                               | 23,3               | 1                      |
| 1010                            | England                            | 30                               | 80,0               | 5                      |
| 3300                            | The Krasnodar region, line VIR-221 | 30                               | 33,3               | 4                      |
| 3475                            | The Krasnodar region, line VIR-665 | 30                               | 10,0               | 2                      |
| 2005                            | The Primorsk region                | 26                               | 26,9               | 2                      |
| 3109                            | Bulgaria                           | 23                               | 17,4               | 2                      |
| 3301                            | The Krasnodar region, line VIR-222 | 30                               | 50,0               | 3                      |
| 1434                            | Bulgaria                           | 28                               | 60,7               | 6                      |
| 3046                            | Argentina                          | 22                               | 9,0                | 2                      |
| 2954                            | Argentina                          | 30                               | 80,0               | 12                     |
| 2925                            | France                             | 30                               | 100                | 110                    |
| 2978                            | Spain                              | 29                               | 100                | 62                     |
| 2982                            | Spain                              | 30                               | 100                | 121                    |
| 3080                            | Mexico                             | 30                               | 100                | 115                    |
| 3015                            | Hungary                            | 30                               | 100                | 65                     |
| VNIIMK 8883 susceptible control | Russia                             | 30                               | 100                | 115                    |

\* - number of broomrape specimens per one affected plant

On the basis of the obtained non-affected forms from all the studied collections, 6 lines resistant to race G were developed using inbreeding method. Genetic control of the resistance of one of them, line RG, has been studied. In order to study the inheritance of resistance there were carried out the cross-breeds of RG with susceptible lines of sunflower of VNIIMK breeding: VK 551, VK 678 B, VK 678 A, VK 1 IMI B, VK 1 IMI A, VK 301, VK 580 and PRO2. 12 combinations of cross-breeds were obtained, 3-8 families of each hybrid. Fifty plants of each family were evaluated for resistance to broomrape. The degree of plant infestation from the F<sub>1</sub> families of the studied hybrid combinations is shown in Table 2.

Table 2. The degree of infestation by broomrape of families of hybrid combinations of sunflower in F<sub>1</sub>

| Hybrid combination | Number of evaluated families | Plants infested, % | The average number of broomrape tubercles per one plant, pcs. |              |
|--------------------|------------------------------|--------------------|---------------------------------------------------------------|--------------|
|                    |                              |                    | affected                                                      | accountable* |
| RG × VK 580        | 3                            | 43,0               | 2                                                             | 1,2          |
| RG × VK 551        | 8                            | 56,5               | 5,1                                                           | 4,7          |
| VK 551 × RG        | 6                            | 97,5               | 11,5                                                          | 11,4         |
| RG × VK 301        | 5                            | 26,0               | 1                                                             | 0,2          |
| VK 301 × RG        | 6                            | 67,0               | 4,8                                                           | 3,2          |
| RG × VK1-imiB      | 7                            | 51,0               | 2,3                                                           | 1,4          |
| VK1-imiB × RG      | 5                            | 62,0               | 2,6                                                           | 1,6          |
| VK1-imiA × RG      | 6                            | 74,0               | 3                                                             | 2,2          |
| RG × VK 678 B      | 8                            | 89,0               | 3                                                             | 2,8          |
| VK 678 B × RG      | 6                            | 88,0               | 3,8                                                           | 3,3          |
| VK 678 A × RG      | 8                            | 83,6               | 4,4                                                           | 3,6          |
| PRO2 × RG          | 3                            | 55,2               | 2,4                                                           | 1,6          |

\*accountable plant – total number of analyzed plants from the family: affected and not affected by broomrape

All hybrid combinations F<sub>1</sub> were affected by broomrape. Depending on the degree of infestation, sunflower plants were divided into 3 groups. The plants were considered susceptible when more than 5 tubercles or formed broomrape sprouts were found on their roots. The plants were considered resistant when no healthy tubercles or sprouts were found on their roots, but there were numerous necroses of cells in the area of broomrape penetration and dead tubercles. Sunflower plants having five and less broomrape tubercles on the roots have been identified as genotypes with incomplete resistance.

On the basis of their damage to the plants of each family they have showed a segregation to the unaffected and affected to a small extent in comparison with the severe lesion of the control susceptible genotype. The minimum percentage of affected F<sub>1</sub> plants (RG × VK 301) averaged 26. The average number of broomrape tubercles per the affected plant was 1 piece, per the accountable plant it was 0.2 pieces. The maximum percentage of affected plants of F<sub>1</sub> hybrid (VK 551 × RG) averaged 97.5. The average number of broomrape tubercles per the affected plant was 11.5 pieces, per the accountable plant it was 11.4 pieces. But it should be noted that this combination of cross-breeding is uncharacteristic in the general number of hybrids in terms of the number of affected plants and the number of broomrape tubercles. In general, the average number of broomrape tubercles per the affected plant did not exceed 5.1 pieces, and per the accountable plant was 4.7 pieces. The obtained data indicate an incomplete dominance of the resistance trait to race G of broomrape of the line RG in F<sub>1</sub>.

To study the influence of the reciprocal effect and the dependence of the resistance on the genetic plasma of the susceptible parent line, reciprocal cross-breeds of the line RG with sunflower lines VK 551, VK 678 B, VK 1 IMI B, and VK 301 (Table 3) were analyzed. 8 combinations of cross-breeding were received, 5-8 families of each hybrid. Fifty plants of each family were evaluated for resistance to broomrape.

Table 3. The degree of infestation by broomrape of families of reciprocal hybrid combinations

| Hybrid combination | Number of evaluated families | Plants infested, % | The average number of broomrape tubercles per one accountable plant, pcs. |
|--------------------|------------------------------|--------------------|---------------------------------------------------------------------------|
| RG × VK 1IMI B     | 7                            | 51                 | 1,4                                                                       |
| VK 1 IMI B × RG    | 5                            | 62                 | 2,3                                                                       |
| SSD <sub>05</sub>  |                              | 11,14              | 1,09                                                                      |
| RG × VK 678 B      | 8                            | 89                 | 2,80                                                                      |
| VK 678 B × RG      | 6                            | 88                 | 3,30                                                                      |
| SSD <sub>05</sub>  |                              | 3,72               | 0,89                                                                      |
| RG × VK 551        | 8                            | 56,5               | 4,7                                                                       |
| VK 551 × RG        | 6                            | 97,5               | 11,4                                                                      |
| SSD <sub>05</sub>  |                              | 11, 91             | 7,5                                                                       |
| RG × VK 301        | 5                            | 26                 | 0,2                                                                       |
| VK 301 × RG        | 6                            | 67                 | 3,2                                                                       |
| SSD <sub>05</sub>  |                              | 16,01              | 3,3                                                                       |

In terms of the "the percentage of infested plants" and "the average number of broomrape tubercles per plant," SSD<sub>05</sub> was calculated for all reciprocal cross-breeding. Differences in these characteristics were unreliable at the 5% significance level for the susceptible parental lines of VK 1 IMI B and VK 678 B. Consequently, the presence of the reciprocal effect and the dependence of resistance on the genetic plasma of susceptible parent lines participating in hybrid combinations were not proved. For the other two lines VK 551 and VK 301, differences in "the percentage of infested plants" are valid at a 5% significance level. This indicates the presence of a maternal effect in these genotypes.

The progeny F<sub>2</sub> and BC<sub>1</sub> were developed in the field conditions and were evaluated in the greenhouse for resistance and susceptibility to race G of broomrape with artificial infection. In F<sub>2</sub> populations, the segregation into 3 phenotypic classes was observed: resistant, slightly affected (intermediate class), and susceptible in a ratio of 1:2:1 ( $\chi^2 = 1.40-4.57$ ,  $P=0.10-0.50$ ) (Table 4).

Table 4. The inheritance of resistance of sunflower to the race G of broomrape in F<sub>2</sub> in cross-breeds of resistant line RG with susceptible lines

| Cross-breeding | Number of plants, pieces |              |             | Expected segregation ratio | $\chi^2$ | df | P         |
|----------------|--------------------------|--------------|-------------|----------------------------|----------|----|-----------|
|                | resistant                | intermediate | susceptible |                            |          |    |           |
| RG × VK 678 B  | 26                       | 65           | 35          | 1:2:1                      | 1.40     | 2  | 0.50-0.30 |

|                 |    |    |    |       |      |   |           |
|-----------------|----|----|----|-------|------|---|-----------|
| RG × VK 1 IMI B | 25 | 81 | 38 | 1:2:1 | 4.57 | 2 | 0.20-0.10 |
|-----------------|----|----|----|-------|------|---|-----------|

In BC<sub>1</sub>, the segregation into 2 phenotypic classes was observed: resistant and affected to a small extent and susceptible and affected to a small extent in a ratio of 1:1 ( $\chi^2 = 0.03-0.24$ ,  $P=0.70-0.90$ ) (Table 5).

Table 5. The inheritance of resistance of sunflower to the race G of broomrape in BC<sub>1</sub> in cross-breeds of resistant line RG with susceptible lines

| Cross-breeding             | Number of plants, pieces |              |             | Expected segregation ratio | $\chi^2$ | df | P         |
|----------------------------|--------------------------|--------------|-------------|----------------------------|----------|----|-----------|
|                            | resistant                | intermediate | susceptible |                            |          |    |           |
| (VK 678 B × RG) × VK 678 B | 0                        | 16           | 17          | 1:1                        | 0.03     | 1  | 0.90-0.80 |
| (VK 1 B × RG) × VK 1 B     | 0                        | 12           | 11          | 1:1                        | 0.04     | 1  | 0.90-0.80 |
| (VK 680 B × RG) × RGI      | 55                       | 59           | 0           | 1:1                        | 0.14     | 1  | 0.70      |
| (PRO2 × RG) × RG           | 36                       | 32           | 0           | 1:1                        | 0.24     | 1  | 0.70      |

The actual segregations of F<sub>2</sub> and BC<sub>1</sub> corresponded to the monohybrid inheritance model with incomplete dominance of the trait.

Inheritance of resistance to race G in the line of sunflower RG differs from the type of inheritance in sunflower samples studied by other researchers. In the line AO-548, two independent dominant genes control genetic resistance to the population of race G of broomrape from Romania [7]. The line developed as a result of interspecies cross-breeding of cultivated sunflower with wild sunflower (*H. divarticatus*) is resistant to race G and its resistance is controlled by one recessive gene *or<sub>ab-vl-8</sub>* [8]. Velasco *et al.* [9] found that resistance to race G of broomrape in *H. debilis* subsp. *tardiflorus* in cross-breeds with cultivated sunflower is controlled by the dominant allele of one gene.

The other 5 sunflower lines that we have developed and which are not affected by race G are in the process of a hybridologic analysis to determine the genetic control of their resistance. The combination of different genes of resistance to the same race of broomrape in one sunflower genotype and the cultivation of hybrids carrying genes that control different mechanisms of resistance could contribute to the long-term resistance of the crop to the parasite.

**Conclusion.** Thus, 6 sunflower lines of RG line resistant to race G were developed. It is established that the resistance of one of them (RG line) is inherited monogeneously with incomplete dominance of a trait. In cross-breeding, the presence of the reciprocal effect and the dependence of resistance on the genotype of some susceptible parental line are established. There is no evidence of a reciprocal effect for the VK 1 IMI B and VK 678 B lines. Five non-affected lines of another origin are in the process of hybridologic analysis to determine the genetic control of their resistance.

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# **miPEPiTO PROJECT: A NEW STRATEGY TO STUDY AND CONTROL THE SUNFLOWER – *OROBANCHE CUMANA* INTERACTION**

Sabine TOURNEUR<sup>1</sup>, Jean-Philippe COMBIER<sup>2</sup>, Stéphane MUNOS<sup>3</sup>, Thomas LAURENT<sup>4</sup>,  
Philippe DELAVault<sup>1\*</sup>

<sup>1</sup>Laboratory of Plant Biology and Pathology, University of Nantes, France

<sup>2</sup>Laboratory on Plant Sciences, UMR 5546 UPS-CNRS, Toulouse

<sup>3</sup>Laboratory of Plant-Microbe Interactions, UMR CNRS-INRA 2594/441, Toulouse

<sup>4</sup>MicroPEP Technologies, Toulouse

\*Corresponding author: philippe.delavault@univ-nantes.fr

## **Abstract**

The broomrape species *Orobancha cumana* causes important losses to the production of sunflower in countries surrounding the Black Sea, in Southern Europe and in growing area of France. Unfortunately, no sustainable or efficient methods to control these root parasitic weed are presently available. The objectives of the miPEP project are to develop new molecular tools to investigate the biology of the parasite, and to develop an innovative and sustainable biocontrol technology for management of this Orobanchaceae pest.

One partner of the project has recently discovered a new class of regulatory peptides, the miPEPs, which are encoded by primary transcripts of miRNAs. Each miPEP stimulates the transcription of its own encoding transcript, leading to the production of higher amount of the corresponding miRNA and consequently to a downregulation of specific target genes. This natural molecular regulation of gene expression can be obtained with synthetic miPEPs, so that specific stages of plant development can be perturbed temporally by exogenous treatment with appropriate miPEPs.

The project will consist in two main tasks:

- 1) to identify *O. cumana* miPEPs potentially involved in the regulation of seed germination/haustorium formation, to identify sunflower miPEPs most likely involved in regulating sunflower immunity, to produce the corresponding synthetic candidate peptides and to assess the activity of *O. cumana* synthetic miPEPs on seed germination and haustorium differentiation.
- 2) to select the synthetic *O. cumana* and sunflower miPEP candidates and evaluate their capacity to negatively affect parasitism by either decreasing broomrape growth and infection or improving sunflower resistance.

The expected results of the first task are to increase our knowledge on key molecular mechanisms underlying a complex parasitic interaction. The expected results of the second task, to be exploited by MicroPEP Technologies, will be to provide a new phytosanitary method to control broomrape parasitism with highly specific and biodegradable natural substances.

**Keywords:** miPEP, miRNA, germination, haustorium, resistance

## HELIANTHUS SPECIES AS A SOURCES FOR BROOMRAPE RESISTANCE

Milan JOCKOVIĆ\*, Siniša JOCIĆ, Sandra CVEJIĆ, Dragana MILADINOVIĆ, Boško DEDIĆ,  
Sreten TERZIĆ, Ana MARJANOVIĆ-JEROMELA, Vladimir MIKLIĆ

Institute of Field and Vegetable Crops, Novi Sad, Serbia

### Abstract

Broomrape (*Orobanche cumana*) is an obligate holoparasite and has become one of the most important biotic factors limiting sunflower production in Southern Europe, the Black Sea region, Ukraine and China. For more than 60 years broomrape is present in Serbia and the most affected region is Vojvodina province. The aim of this study was evaluation of resistance of some wild *Helianthus* species to broomrape populations that are highly virulent in Serbia. Experiment was carried out at the Institute of Field and Vegetable Crops, Novi Sad where is one of the biggest collection of wild *Helianthus* species worldwide. Evaluation was done in green house using buckets infested with broomrape seeds collected from naturally infested fields from Bačka region. Plant material for evaluation included 6 annual wild *Helianthus* species and in total 13 populations. Results of the experiment showed that in total 9 populations were resistant. Resistant populations were *H. debilis* 1134, *H. neglectus* 0457, *H. niveus* 0608, *H. argophyllus*, *H. petiolaris* 0338, *H. petiolaris* 1383, *H. petiolaris* 1910, *H. praecox* 1151, *H. praecox* 1340. Susceptible populations were *Helianthus debilis* 1287, 1569, 1566 and *Helianthus praecox* 0380. Further testings of resistant populations should indicate possibility of finding source for resistance for broomrape populations present in Spain, Ukraine and Black Sea region.

**Keywords:** broomrape, *helianthus* species, resistance evaluation

### INTRODUCTION

Being one of the most important oil crop, worldwide, sunflower (*Helianthus annuus* L.) is faced with many biotic factors that are limiting its production. Considering that, sunflower breeders are confronted with difficult tasks, because while breeding for higher seed and oil yield, they are also in constant race for resistance genes to different pathogens and parasites that attack sunflower. Among many of them, sunflower broomrape (*Orobanche cumana*) is considered to be the most important one. Broomrape is an obligate holoparasite and has become one of the most important biotic factor limiting sunflower production in Southern Europe, the Black Sea region, Ukraine and China. Besides sunflower, broomrape parasites many other crops such as tomatoes (*Solanum lycopersicum*), hop (*Humulus lupulus*), tobacco (*Nicotiana tabaccum*), sugar beet (*Beta vulgaris*), carrot (*Daucus carota*), hemp (*Cannabis sativa*), alfalfa (*Medicago sativa*) and others (Dedić et al., 2009; Marinković et al., 2014). It is obligatory and non-photosynthetic root parasitic plant of the sunflower (Louarn et al., 2016). Broomrape is flowering weed that is entomophily pollinated and attacks the root system of sunflower plants (Marinković et al., 2014). Once attached to a host, it reduces the vitality of the affected plants by taking their water and minerals, which in the end, depending on the severity of the attack, leads to partial or complete yield reduction. Depending on the susceptibility the infected sunflower plants are smaller, substantial decrease in head diameter is observed and significant yield losses are recorded (Alcántara et al., 2006; Duca, 2015). There are several methods of controlling the broomrape but still the most efficient one is breeding for genetic resistance (Thomas et al., 1998; Mauromicale et al., 2005; Tan et al., 2005; Echevarria-Zomeno et

al., 2006; Kaya et al., 2012; Louarn et al., 2016). Valuable source of genetic resistance to broomrape races found in wild *Helianthus* species and introgression of the resistance genes from interspecific crosses was described in previous studies (Dozet and Marinković, 1998; Jan and Fernandez-Martínez, 2002; Velasco et al., 2007; Fernandez-Martinez et al., 2008; Terzić et al. 2010; Seiler and Jan, 2014).

For more than 60 years broomrape is present in Serbia and the most affected region is Vojvodina province. In Vojvodina the severity of attack is in Bačka and diffusion of the parasite is between Bačka Topola and Subotica (Maširević, 2001). Broomrape presence in Serbia was firstly described in early 1950s (Acimović, 1998). According to Maširević and Medić-Pap (2009) significant damage to susceptible hybrids were recorded in the 1990s. The most dominant race in Serbia is E and it appeared in 1996 (Mihaljčević, 1996). For more than 20 years race E is present in Serbia and according to Miladinović et al. (2014) no new broomrape races have appeared.

The aim of this study was evaluation of resistance of some wild *Helianthus* species to broomrape populations that are highly virulent in Serbia.

## MATERIAL AND METHODS

Experiment was carried out at the Institute of Field and Vegetable Crops, Novi Sad where is one of the biggest collection of wild *Helianthus* species, worldwide. Plant material for evaluation included 6 annual wild *Helianthus* species and in total 13 accessions (Tab. 1).

As previously described by Terzić et al. (2010) seed of wild species was firstly dehulled and placed in petri pots containing filter paper moistened with water to promote germination. Petri pots were placed in a growth chamber with a constant temperature of 24°C. After root emergence seedlings were transferred to jiffy 7 pots and grown in the greenhouse at 21°C (day and night), with RH (relative humidity) around 80% and constant light until the phase of two pairs of leaves, after which they were transferred to buckets (10 L). A mixture of sand:perlite:peat in a 1:1:1 ratio which was homogeneously infested with broomrape seeds collected in a Bačka region at a rate of 70 mg/dm<sup>3</sup> soil mixture. Plants were grown in the infested soil at a photoperiod of 16/8h day/night until physiological maturity. Broomrape resistance of tested accessions was evaluated at the stage of physiological maturity. Plants with emerged or underground broomrape stalks were considered susceptible and those without a broomrape stalk as resistant (Terzić et al., 2010). Furthermore, roots were thoroughly checked and degree of broomrape nodules was observed. Degree of broomrape attack was calculated as number of broomrape plants/number of sunflower plants.

## RESULTS AND DISCUSSION

Since broomrape appearance at the end of 19<sup>th</sup> century sunflower breeders were successful in development of resistant genotypes (Seiler and Jan, 2014). Introgression of first resistance genes from wild species, mainly *H. tuberosus*, to cultivated sunflower was described by Pustovoit et al. (1966). Newer studies have also reported resistance to different races of broomrape in many other *Helianthus* species (Nikolova et al., 2000; Fernández-Martínez et al., 2010; Škorić and Pacureanu, 2011; Antonova et al., 2011; Christov, 2013).

Evaluated populations of wild *Helianthus* species showed different reaction to broomrape (Tab. 1). Out of four tested accessions of *H. debilis* only one was completely resistant (DEB1134). This result is in accordance with previous research of Terzić et al. (2010) who also reported resistance in the same population. Accession DEB1287 was found to be the most sensitive accession in experiment with degree of broomrape attack and nodules of 10.75 and 4, respectively.

In *H. praecox*, accession PRA0380 was sensitive with intensity of broomrape attack of 7, while PRA1151 and PRA-HIR1340 were resistant. Accessions of *H. petiolaris* PET0338, PET1383 and PET1910, were completely resistant to the broomrape population used in the experiment. Fernandez-Martinez et al. (2008) reported *H. praecox* and *H. petiolaris* to be 100% sensitive to broomrape race F. In a comprehensive study Christov (2013) reported that annual *Helianthus* species, *H. annuus* (wild), *H. argophyllus*, *H. debilis*, *H. petiolaris*, and *H. praecox*, were resistant to broomrape races in Bulgaria. Accession of *H. argophyllus* also showed to be resistant in this study. Petcu and Pacureanu (2011) reported that interspecific hybrid derived from *H. argophyllus* showed resistance to broomrape race F in Romania. *H. neglectus* (NEG0457) and *H. niveus* (NIV0608) showed complete resistance to broomrape population used in this study.

Table 1. Tested wild sunflower accessions and their reaction to broomrape

| No  | Population (ifvcs) | Species               | Broomrape incidence (%) | Degree of broomrape attack | Degree of broomrape nodules |
|-----|--------------------|-----------------------|-------------------------|----------------------------|-----------------------------|
| 1.  | DEB 1134           | <i>H. debilis</i>     | 0                       | 0                          | -                           |
| 2.  | DEB 1287           | <i>H. debilis</i>     | 80                      | 10.75                      | 4                           |
| 3.  | DEB 1569           | <i>H. debilis</i>     | 25                      | 9                          | 1                           |
| 4.  | DEB 1566           | <i>H. debilis</i>     | 40                      | 1                          | 14.5                        |
| 5.  | NEG 0457           | <i>H. neglectus</i>   | 0                       | 0                          | -                           |
| 6.  | NIV 0608           | <i>H. niveus</i>      | 0                       | 0                          | -                           |
| 7.  | ARG                | <i>H. argophyllus</i> | 0                       | 0                          | -                           |
| 8.  | PET 0338           | <i>H. petiolaris</i>  | 0                       | 0                          | -                           |
| 9.  | PET 1383           | <i>H. petiolaris</i>  | 0                       | 0                          | -                           |
| 10. | PET 1910           | <i>H. petiolaris</i>  | 0                       | 0                          | -                           |
| 11. | PRA 1151           | <i>H. praecox</i>     | 0                       | 0                          | -                           |
| 12. | PRA 0380           | <i>H. praecox</i>     | 20                      | 7                          | -                           |
| 13. | PRA-HIR 1340       | <i>H. praecox</i>     | 0                       | 0                          | -                           |

## CONCLUSIONS

Bearing in mind narrow genetic background in cultivated sunflower and many constraints in its production, especially evolution of broomrape populations into more virulent races, evaluation of existing germplasm is needed in order to find sources for resistance genes.

Accessions of wild *Helianthus* species showed different reaction to broomrape. Resistant accessions were *H. debilis* 1134, *H. neglectus* 0457, *H. niveus* 0608, *H. argophyllus*, *H. petiolaris* 0338, *H. petiolaris* 1383, *H. petiolaris* 1910, *H. praecox* 1151, *H. praecox* 1340. Susceptible to broomrape were *Helianthus debilis* 1287, 1569, 1566 and *Helianthus praecox* 0380. Further testing of resistant accessions of wild species should indicate possibility of finding source for resistance for more virulent broomrape populations present in Spain, Ukraine and Black Sea region.

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# USING WILD SUNFLOWER TO IMPROVE RESISTANCE OF CULTIVATED SPECIE TO THE PARASITE BROOMRAPE (*OROBANCHE CUMANA* WALLR.)

Gabriel Florin ANTON<sup>1,2\*</sup>, Maria JOIȚA-PĂCUREANU<sup>1</sup>,  
Luxița RÎȘNOVEANU<sup>3,4</sup>, Alexandru BRAN<sup>2,5</sup>, Elisabeta SAVA<sup>5</sup>

<sup>1</sup> National Agricultural Research and Development Institute, 1 Nicolae Titulescu Street, Fundulea, Călărași County, Romania

<sup>2</sup> University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

<sup>3</sup> Agricultural Research Development Station Brăila, Viziru km. 9 Street, Brăila, Brăila County, Romania

<sup>4</sup> „Dunărea de Jos” University of Galați, Engineering and Agronomy Faculty of Brăila, Agronomy Center for Research and Consultancy and Environment „Lunca”, 29 Călărași Street, Brăila, Brăila County, Romania

<sup>5</sup> The State Institute for Variety Testing and Registration, Bucharest, Romania

\*Corresponding author: gabi22mai@yahoo.com

## Abstract

The parasitic plant *Orobancha cumana* (broomrape) is the most important biotic constraint to the production of sunflower, in all counties where this crop is grown, excepting North and South America.

The aim of our study was evaluation of populations of some wild *Helianthus* species to broomrape populations that are highly virulent in Romania. Evaluation was done in green house using pots infested with broomrape seeds collected from naturally infested fields from Brăila and Constanța areas. Plant material for evaluation included 24 populations, derived from crosses of 4 wild *Helianthus* with cultivated sunflower. Results of the experiment showed that 7 populations were resistant, in case of broomrape coming from Constanța region and only one population was resistant to broomrape coming from Brăila region. All resistant populations were the result of crosses with *H. debilis* and *H. maximiliani*.

Testing of populations indicate the possibility to obtain source of resistance to broomrape populations present in Romania, but also in countries situated around Black Sea region.

**Keywords:** sunflower, broomrape, resistance, wild species, sources of resistance

# ALPIN – A NEW BULGARIAN SUNFLOWER HYBRID

Galin GEORGIEV\*, Alexander PISKOV

*Dobrudzha Agricultural Institute – General Toshevo, Bulgaria*

*\*Corresponding author: galindz@abv.bg*

## Abstract

Sunflower hybrid Alpin was developed at Dobrudzha Agricultural Institute – General Toshevo (DAI). It is a male sterile two-linear hybrid derived through inter-linear hybridization. The mother component is line 2607, which possesses cytoplasmic male sterility, and the father component is line 10681R, a branched fertility restorer. Both parental forms have excellent general and specific combining ability. Hybrid Alpin is early maturing, its vegetative growth duration is 115 – 121 days. Plant height is within the range 145 – 180 cm, and the head diameter – within 18 – 21 days. Thousand kernel weight varies within 53 – 60 g, and the oil content is 45-47 %. The oil is of linoleic type. Protein content in the whole seed is 18 – 20 %. The number of seeds per plant is 1090 – 1250, and their weight is 70 – 75 g. The duration of flowering is 12 – 13 days. In the trial fields of DAI, the hybrid went through two-year testing, showing a maximum yield of 4230 kg/ha, while in Romania the maximum seed yield obtained in the process of official testing was 4513 kg/ha. The hybrid demonstrated high field resistance to the economically important diseases and the parasite *Orobanche*. In 2008, hybrid Alpin was provided to our partners from Saaten Union – Romania; following one-year successful testing in their trial fields, it was submitted for official testing within the system of the State Institute for Variety Testing and Registration – ISTIS, Romania. Averaged for the three years of official testing at 10 locations, the new hybrid exceeded the standard with 8 % by the index seed yield. In 2012, hybrid Alpin was officially registered in Romania with certificate No 1197/10.02.2012 and was enlisted in the European Catalog of Field and Vegetable Crop Varieties.

**Key words:** Sunflower, Hybrid, Productivity, Testing, Disease Resistance

## Introduction

As a result from successful breeding work, a great number of high-yielding sunflower hybrids have been developed at Dobrudzha Agricultural Institute (DAI) (Christov et al., 2009; Encheva et al., 2011; Georgiev and Encheva, 2014; Encheva et al., 2015; Georgiev, 2015; Peevska and Georgiev, 2016; Georgiev, 2016). The breeding work at DAI is in line with the contemporary requirements for sunflower production and with the scientific achievements worldwide, it depends on the collected initial materials, the applied breeding methods and, finally yet importantly, on the potential of the staff involved in the breeding work.

Under the conditions of Bulgaria, the medium early hybrids with duration of the vegetative growth 120 – 125 days are most suitable. Such hybrids have been developed at DAI in the past decade: Valin, Veleka, Vokil, Gabi, Velko, Alpin, Mihaela, Dea, Deveda, Divna, Vyara, Tedi, Lindzi, etc. Early maturing hybrids have also been developed, such as the hybrids Sava and Maritsa, which are being successfully grown in the northern regions of Bulgaria.

Similar to the breeding programs of other field crops, the priorities are genotypes with high adaptability potential (Mihova et al., 2017) and efficiency of the production (Dimitrova-Doneva, 2016).

In the past few years, the volume of the breeding work is increasing in relation to developing herbicide-resistant sunflower hybrids (Encheva et al., 2016; Valkova et al., 2017), which are becoming dominant on the market and the demand for them is getting greater. Such hybrids have



already been developed at DAI; they are now within the system for official testing both in Bulgaria and abroad and their registration and distribution on the market is forthcoming.

The development of good sunflower hybrids is not difficult for us because our collection of parental lines is very rich and variable and is constantly being enlarged, and our breeders are qualified and experienced. Our breeding programs are very well elaborated and are being applied strictly and consistently. Our main problem is that after having developed a new high-yielding hybrid, it is difficult for us to position it well on the market, which requires serious advertising and marketing activities implying mostly serious financial resources. Therefore, we have to provide our research products to other companies with greater marketing and management possibilities.

The aim of this investigation was to present a more detailed morphological, biological and economic characterization of the Bulgarian sunflower hybrid Alpin.

### **Material and methods**

Sunflower hybrid Alpin is a male fertile simple hybrid derived through the method of inter linear hybridization. The mother line 2607 was developed by selection of lines obtained from the Russian variety Birimirets. This line participated as a mother component in the most famous hybrid of DAI Albena, which was a world standard and occupied almost 40 % of the area with sunflower crops in France. Line 2607 was involved in many of our hybrids, such as Merkurii, Magura, Mussala, Michaela, Yana, Rada, etc., as well as in many hybrids developed jointly with our foreign partners, such as San Luka, All Star, Santafe, Alliance, etc. The sunflower hybrids involving mother line 2607 remained longest on the sunflower seed market in the past few decades. Line 2607 possesses exceptionally good combining ability. It is resistant to downy mildew and lodging, tolerant is to rust and moderately susceptible to phoma and phomopsis. It does not have resistance to the parasite *Orobanche*.

The father line of hybrid Albena is the fertility restorer 10681R. It is a branched line rich in pollen and restores the fertility of the hybrid to 100 %. It was obtained by combining conventional and biotechnology breeding methods. The line is resistant to downy mildew races up to 731 and to the parasite broomrape up to race F. It is moderately resistant to phoma, phomopsis and alternaria.

The cross between the two parental lines was first made in 2002, then it went through a 3-year testing in the breeding fields of DAI – two years of control testing and one year in a unified competitive varietal trial. The testing of all sunflower hybrids was carried out according to a technology officially approved for growing of this crop (Georgiev et al., 1997). The hybrid was subjected to testing for two more years at different locations in Bulgaria, and was then provided in 2008 to our partners from company Saaten Union – Romania.

Having gone through one-year testing and demonstrating very good results, it was submitted for official testing within the system of the State Institute for Variety Testing and Registration – ISTIS, Romania. In another three years of successful tests and very good results, hybrid Alpin was officially registered with certificate No 1197/10.02.2012 in the European Catalog of field and vegetable crop varieties.

The description of the morphological traits of the hybrid was done according to the UPOV protocol (2002). The plant pathology characterization was carried out at Dobrudzha Agricultural Institute – General Toshevo. The resistance to downy mildew (*Plasmopara halstedii*) was determined by a standard methodology (Vear, F., Tourvieille, D., 1987) adapted to the working conditions at DAI. The response of the hybrid to races 700 and 731 of the pathogen was presented as percent of resistance. The resistance to grey spots on sunflower (*Phomopsis helianthi*) was evaluated by the method of Encheva, V. & I. Kiryakov, (2002) under field conditions, in an artificial infection field. The type of attack was read one week after full flowering and at stage milk maturity according to the following scale: 0 – no symptoms; 1 – necrotic spot of up to 5 cm in diameter; 2 – necrotic spot larger than 5 cm in diameter; 3 – several necrotic spots merging on the stem; 4 – stem breaking at the place of infection. The testing for black spots on sunflower (*Phoma macdonaldii*) was done under artificial infection field conditions. Inoculation was done at stage budding – full maturity according to the method of Maric et al. (1981). The response of the plants was read at stage yellow-

brown maturity according to a 4-degree scale: 0 – no symptoms; 1 – necrotic spot localized around the petiole; 2 – several necrotic spots merging on the stem; 3 – entire stem covered with necrotic spots or breaking. The attacking rate was determined on the basis of the part of the stem covered with spots of the pathogen (1/3, 2/3, 3/3). The figures in the brackets show the number of spots. The ranking was as follows: 0 – immune; 1 – resistant; 2 – moderately resistant; 3 – moderately susceptible; 4 – susceptible.

The resistance to the parasite broomrape (*Orobancha cumana*) was determined by the method of Panchenko (1975). The evaluation was carried out under greenhouse conditions using the index percent of resistance.

## Results and discussion

### Morphological description

Applying the UPOV protocol (2002), a morphological description of hybrid Alpin was made (Table 1).

**Table 1.** Morphological characteristics of sunflower hybrid Alpin

| №   | Traits                                                                                         | Expression                          | Degree |
|-----|------------------------------------------------------------------------------------------------|-------------------------------------|--------|
| 1.  | Hypocotyl:anthocyanin coloration                                                               | Present                             | 9      |
| 2.  | Hypocotyl:anthocyanin coloration                                                               | Strong                              | 7      |
| 3.  | Leaf: size                                                                                     | Medium                              | 5      |
| 4.  | Leaf: green color                                                                              | Medium                              | 5      |
| 5.  | Leaf: blistering                                                                               | Medium                              | 5      |
| 6.  | Leaf: serration                                                                                | Coarse                              | 7      |
| 7.  | Leaf: shape of cross section                                                                   | Flat                                | 3      |
| 8.  | Leaf: shape of distal part                                                                     | Acuminate                           | 7      |
| 9.  | Leaf: auricles                                                                                 | Medium                              | 5      |
| 10. | Leaf: wings                                                                                    | Absent                              | 1      |
| 11. | Leaf: angle of lowest lateral veins                                                            | Right or nearly right               | 2      |
| 12. | Leaf: height of the tip of the blade compared to insertion of petiole (at 2/3 height of plant) | Medium                              | 5      |
| 13. | Stem: intensity of hairiness at the top                                                        | Medium                              | 5      |
| 14. | Time of flowering                                                                              | Medium                              | 5      |
| 15. | Ray flower: density                                                                            | Medium                              | 5      |
| 16. | Ray flower: shape                                                                              | Narrow ovate                        | 2      |
| 17. | Ray flower: disposition                                                                        | Flat                                | 1      |
| 18. | Ray flower: length                                                                             | Medium                              | 5      |
| 19. | Ray flower: color                                                                              | Orange yellow                       | 4      |
| 20. | Disk flower color                                                                              | Orange                              | 2      |
| 21. | Disk flower: anthocyanin coloration of stigma                                                  | Present                             | 9      |
| 22. | Disk flower: intensity of anthocyanin coloration of stigma                                     | Weak                                | 3      |
| 23. | Disk flower: presence of pollen                                                                | Present                             | 9      |
| 24. | Bract shape                                                                                    | Rounded                             | 3      |
| 25. | Bract: length of the tip                                                                       | Medium                              | 5      |
| 26. | Bract: green color of the external part                                                        | Medium                              | 5      |
| 27. | Bract: attitude in relation to head                                                            | Slightly embracing                  | 2      |
| 28. | Plant: natural height                                                                          | Medium                              | 5      |
| 29. | Plant: branching                                                                               | Absent                              | 1      |
| 30. | Plant: type of branching                                                                       | -                                   | -      |
| 31. | Plant: natural position of closest lateral head to the central head                            | -                                   | -      |
| 32. | Head: attitude                                                                                 | Half-turned down with straight stem | 4      |
| 33. | Head: size                                                                                     | Medium                              | 5      |

|     |                                   |                  |   |
|-----|-----------------------------------|------------------|---|
| 34. | Head: shape of grain side         | Weakly convex    | 4 |
| 35. | Seed: size                        | Medium           | 5 |
| 36. | Seed: shape                       | Broad ovoid      | 3 |
| 37. | Seed: thickness relative to width | Medium           | 5 |
| 38. | Seed: main color                  | Black            | 7 |
| 39. | Seed: stripes on margin           | Weakly expressed | 2 |
| 40. | Seed: stripes between margin      | Weakly expressed | 2 |
| 41. | Seed: color of stripes            | Grey             | 2 |

### Biological and economic properties

Hybrid Alpin is early maturing, with duration of the vegetative growth 115 – 121 days. Plant height is within 145-180 cm, and the head diameter is 18-21 cm. Thousand kernel weight varies within 53 – 60 g, and oil content is 45-47 %. Oil is of linoleic type. The protein content in the whole seed is 18-20%. The number of seeds per plant is 1090 – 1250, and their weight – 70-75 g. The duration of flowering is 12 – 13 days. In the experimental fields of DAI, the hybrid demonstrated a maximum yield of 4230 kg/ha, and in the process of official testing in Romania, the maximum seed yield was 4513 kg/ha.

The seedproduction scheme of Alpin is 10:2 (mother line : father line). Since the mother line is early flowering, it is recommended to plant the father lines 6-7 days earlier to ensure simultaneous flowering of the two lines. Furthermore, in order to ensure more pollen, a third row of the father line can be planted during the sowing of the female line, between the two rows already planted earlier. For higher yields in seedproduction, 3-4 well developed bee colonies are recommended per hectare.

### Productivity

#### Official testing

Hybrid Alpin was provided for the first time to company Saaten Union – Romania in 2008, when its testing began there. After showing very good results during the first year of testing, it was subjected to official testing. Table 2 shows the results.

**Table 2.** Results from the official testing of hybrid Alpin

| Region       | Hybrids  | Yield<br>kg/ha | % from<br>stan<br>dard | Yield<br>kg/ha | % from<br>stan<br>dard | Yield<br>kg/ha | % from<br>stan<br>dard | relative yield<br>according to the standard,<br>averaged for 3 years |
|--------------|----------|----------------|------------------------|----------------|------------------------|----------------|------------------------|----------------------------------------------------------------------|
|              |          | <b>2009</b>    |                        | <b>2010</b>    |                        | <b>2011</b>    |                        |                                                                      |
| 1.Troian     | Standart | 2649           | 100                    | 2961           | 100                    | 3556           | 100                    |                                                                      |
|              | Alpin    | 2671           | 101                    | 3502           | 118                    | 3610           | 102                    | 107                                                                  |
| 2.Tecuci     | Standart | 3647           | 100                    | 4103           | 100                    | 3921           | 100                    |                                                                      |
|              | Alpin    | 4148           | 114                    | 3723           | 91                     | 3762           | 96                     | 100                                                                  |
| 3.Rm.Sarat   | Standart | 3988           | 100                    | 3468           | 100                    | 4418           | 100                    |                                                                      |
|              | Alpin    | 4407           | 111                    | 4188           | 121                    | 4513           | 102                    | 111                                                                  |
| 4.Portaresti | Standart | 2196           | 100                    | 3355           | 100                    | 3063           | 100                    |                                                                      |
|              | Alpin    | 2546           | 116                    | 3513           | 105                    | 3529           | 115                    | 112                                                                  |
| 5.Peciu Nou  | Standart | 2580           | 100                    | 2244           | 100                    | 3928           | 100                    |                                                                      |
|              | Alpin    | 3476           | 135                    | 3083           | 137                    | 3606           | 92                     | 121                                                                  |
| 6.Negresti   | Standart | 2761           | 100                    | 4285           | 100                    | 4174           | 100                    |                                                                      |
|              | Alpin    | 3958           | 143                    | 4156           | 97                     | 4120           | 99                     | 113                                                                  |
| 7.MirceaVoda | Standart | 3618           | 100                    | 2901           | 100                    | 3780           | 100                    |                                                                      |
|              | Alpin    | 2920           | 81                     | 3875           | 134                    | 3593           | 95                     | 103                                                                  |
| 8.Inand      | Standart | 3578           | 100                    | 3049           | 100                    | 3253           | 100                    |                                                                      |

|                            |          |      |     |      |     |      |     |     |
|----------------------------|----------|------|-----|------|-----|------|-----|-----|
|                            | Alpin    | 3782 | 106 | 3153 | 103 | 3351 | 103 | 104 |
| 9.Dalga                    | Standart | 4170 | 100 | 3162 | 100 | 3606 | 100 |     |
|                            | Alpin    | 4342 | 104 | 3841 | 122 | 3623 | 101 | 109 |
| 10.Cogealac                | Standart | 1899 | 100 | 2879 | 100 | 2898 | 100 |     |
|                            | Alpin    | 2353 | 124 | 3164 | 110 | 2388 | 82  | 105 |
|                            | Standart | 3108 | 100 | 3240 | 100 | 3659 | 100 |     |
| Averaged from 10 locations | Alpin    | 3489 | 112 | 3619 | 112 | 3609 | 99  | 108 |

The official testing was carried out for 3 years at 10 locations, using hybrid Alex as a standard during the first and second year, and hybrid Daniel, the higher-yielding of the two standards, during the second year. Table 3 presents only the data on the seed yield per ha, which is the determining index for the registration of new sunflower hybrids. The seed yield of the new hybrid Alpin varied during the three year within 2353 – 4513 kg/ha. The mean values were highest during the second year of the official testing 3619 kg/ha; in that year, the exceeding of the standard was with 12 %, averaged for all locations. Averaged for all three years, the new hybrid exceeded the standard at all 10 testing locations. During the entire 3-year period, Alpin exceeded the standard with 8 %, and this was the main reason for its official registration and enlisting in the European Catalog of field and vegetable crop varieties.

### Phytopathological characterization

The phytopathological evaluation was carried out in the infection field of DAI, where all new materials are subjected to testing for the economically important diseases and the parasite broomrape together with the commercial hybrids most widely distributed in Bulgaria, used as standards at the national Executive Agency of Variety Testing, Field Inspection and Seed Control. For the purposes of comparison, phytopathological evaluation of the most recent hybrids of DAI was also done. The results are presented in Table 3.

**Table 3.** Phytopathological evaluation of sunflower hybrids in artificial infection field at DAI – General Toshevo.

| Hybrid   | <i>Phomopsis helianthi</i> |      | <i>Phoma macdonaldi</i> |      | <i>Plasmopara helianthi</i> |                           | <i>Orobanche cumana</i>    |
|----------|----------------------------|------|-------------------------|------|-----------------------------|---------------------------|----------------------------|
|          | Attacking rate             | Rank | Attacking rate          | Rank | Resistance to race 700, %   | Resistance to race 731, % | Resistance to races A-F, % |
| San Luka | 3/3(3)                     | 3    | 1/3(1)                  | 1    | 100.0                       | 92.9                      | 100.0                      |
| Perfekt  | 1/3(1)                     | 1    | 1/3(1)                  | 1    | 84.5                        | -                         | 100.0                      |
| Diabolo  | 2/3(2)                     | 2    | 1/3(1)                  | 1    | 100.0                       | 90.5                      | 100.0                      |
| Brio     | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 100.0                     | 100.0                      |
| PR64F50  | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 100.0                     | 100.0                      |
| Meldimi  | 2/3(2)                     | 2    | 1/3(1)                  | 1    | 100.0                       | 90.0                      | 100.0                      |
| Valin    | 2/3(2)                     | 2    | 1/3(1)                  | 1    | 95.0                        | 75.0                      | 100.0                      |
| Mihaela  | 2/3(2)                     | 2    | 1/3(1)                  | 1    | 100.0                       | 100.0                     | 100.0                      |
| Gabi     | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 100.0                     | 100.0                      |
| Alpin    | 2/3(2)                     | 2    | 1/3(1)                  | 1    | 100.0                       | 100.0                     | 100.0                      |
| Veleka   | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 100.0                     | 100.0                      |
| Vokil    | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 90.0                      | 100.0                      |
| Velko    | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 100.0                     | 100.0                      |
| Dea      | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 70.0                      | 100.0                      |
| Sevar    | 1/3(1)                     | 1    | 0                       | 0    | 100.0                       | 100.0                     | 100.0                      |

Hybrid Alpin, similar to Diabolo and Meldimi, is moderately resistant to the fungal pathogen *Phomopsis helianthi*, like other more recent hybrids of DAI (Michaela and Valin). It demonstrated higher tolerance to this disease than San Luka.

To the other important leaf pathogen *Phoma macdonaldi*, Alpin exhibited resistance similar to the Bulgarian hybrids San Luka, Perfekt, Michaela and Valin, and the foreign hybrids Diabolo and Meldimi. The other genotypes presented in Table 3 showed immune type of reaction.

Hybrid Alpin demonstrated full resistance to downy mildew *Plasmopara helianthi* (to the two investigated races).

The resistance to the parasite *Orobanche cumana* the resistance was 100%, as well as the resistance of almost all other hybrids involved in the investigation.

### Conclusions

The new Bulgarian sunflower hybrid Alpin is clearly distinct, homogenous and stable;

It has very good adaptability and realizes its high potential under variable soil and climatic conditions;

It demonstrated high field resistance to the economically important diseases and the parasite *Orobanche*;

It was officially registered in Romania and was enlisted in the European catalog of field and vegetable crop varieties.

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# THE BREEDING ON THE NEW SUNFLOWER VARIETYIES AGAINST BROOMRAPE IN CHINA

Liping TAN\*, Wei XUE, Wei ZHANG, Jinwe BI,  
Qingpeng LIU, Wenwen GUAN

*Chifeng Agronomy Institution, Chifeng, Inner Mongolia, China*

*\*Corresponding author: cfinkytlp@163.com*

## Abstract

Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic seed plant and it caused the dramatically yield loss annually in China now. The most effective way to control this disease is to apply resistant varieties, however, for the lacking of the resistant varieties, sunflower broomrape has become one of the most important constraints in sunflower production in China. To create the resistant varieties against race G, our group screened the resistant inbred lines in pot and obtained 3 sterile lines and 5 restore lines. In 2016, via pollination we got several confection sunflower hybrids, such as CRD101 and CRD102, which showed the high resistant level against Race G of *O. cumana*. These hybrid showed high resistant level again race G in field in 2017. Besides the high resistance level, the average seed size of new variety is around 2.3 x 0.7 cm, the seeds coat is black and easy to be removed. The average yield is 3750 kg/hectar.

**Keywords:** sunflower breeding, *Orobanche cumana*, race G

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**INVESTIGATION ON THE EFFECT OF IMPORTANT QUALITATIVE INDICES  
RELATED TO HIGHER SEED YIELD  
IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)**

Georgi GEORGIEV\*, Nina NENOVA, Penka PEEVSKA,  
Galin GEORGIEV, Daniela VALKOVA, Valentina ENCHEVA

*Dobrudja Agricultural Institute, Sunflower Breeding Department*

*\*Corresponding author: georgi\_d4@abv.bg*

**Abstract**

The investigation was carried out during 2013-2016 at Dobrudja Agricultural Institute – General Toshevo. The indices plant height, head diameter, number of seeds per plant, 1000 kernel weight and leaf area were of primary importance and had direct effect on the increase of the seed yield per unit area. The investigations included eight hybrid combinations and parental forms involved in the crosses. The inheritance of resistance to the parasite *Orobanche* (race G) in the hybrid combinations was investigated.

The indices plant height, head diameter, number of seeds per plant, seed weight per plant and 1000 kernel weight had direct effect on most of the hybrid combinations for higher seed yield. Lower was the effect of the leaf area on the seed yield. Moderate resistance (50-75%) to the parasite *Orobanche* (race G) was found in two hybrid combinations: 217A x 97R and 2008A x 99R. This moderate resistance to the pathogen was due to the fact, that the fertility restorer lines involved in the hybridizations 97R and 99R showed respective resistance 97R (100%), 99R (50-75%) to the same race during the years of the investigation.

**Keywords:** sunflower, inheritance, heritability rate, hybrid



## NEW BULGARIAN SUNFLOWER HYBRID “LINZI”

Nina NENOVA\*, Galin GEORGIEV, Valentina ENCHEVA,  
Daniela VALKOVA, Georgi GEORGIEV, Penka PEEVSKA

*Dobrudja Agricultural Institute, General Toshevo, Bulgaria*

*\*Corresponding author: nina\_n13@abv.bg*

### Abstract

The conventional sunflower hybrid “Linzi” was developed at Dobrudja Agricultural Institute, General Toshevo. It is a single interline hybrid. The mother line 217A was created in DAI. The father line 99R was developed using the method of induced parthenogenesis, combined with embryo culture from experimental hybrid 132 x 98. In 2013, the hybrid “Linzi” was included in unified competitive variety trials (UCVT) after two years testing in competitive trials. The average seed yield of hybrid “Linzi” in two UCVT was 382.7 kg/da, which exceeded with 16.6% the mean standard of hybrids San Luka, Klarisa and PR 64F50. The seed oil content was 52.5% and the protein content was 28.1%. In 2014 and 2015 it was tested by the Executive Agency for Variety Testing, Field Inspection and Seed Control. In 2017, the Patent Office issued the certificate №11125P2 for the hybrid. The plants height is 140-145 cm. The vegetation period is 118 days. “Linzi” belongs to the group of middle early hybrids. The head diameter is 25-27 cm. The head position is vertical and it is slightly convex. The seeds are black with weakly expressed grey stripes on their edges. Thousand seeds weight is 60g. The percentage of hull content is 20%. The hybrid is resistant to downy mildew/ race 731/ and broomrape. It possesses middle resistance to phoma and phomopsis. The hybrid needs no special requirements for cultivation and the traditional scheme for seed production could be applied. The optimum plant density is 65000 -71000 per hectare. Both the parental lines flower on the same time and they could be planted together during seed production of the hybrid. The hybrid “Linzi” was tested for two years in Moldova, and registered in 2017.

**Keywords:** sunflower, hybrid, oil and protein content disease resistance

# INVESTIGATION ON THE RESISTANCE OF NEW BULGARIAN SUNFLOWER HYBRIDS TO ECONOMICALLY IMPORTANT DISEASES AND THE PARASITE *OROBANCHE*

Penka PEEVSKA\*, Miglena DRUMEVA, Galin GEORGIEV,  
Valentina ENCHEVA, Georgi GEORGIEV

*Dobrudja Agricultural Institute – General Toshevo, Bulgaria*

*\*Corresponding author: ppeevska7@abv.bg*

## Abstract

The investigation involved the new Bulgarian sunflower hybrids Sevar and Dea developed at Dobrudja Agricultural Institute – General Toshevo (DAI). After having shown excellent results in the experimental fields of DAI, these hybrids were provided to the company Saaten Union – Romania for registration. Following a three-year testing within the official variety testing system of Romania, they were included in the European varietal list with certificates №4934/09.06.2015 (Sevar) and 4935/09.06.2015 (Dea). This investigation considered the resistance of the above hybrids to the parasite *Orobanche* and to downy mildew, phoma and phomopsis. The materials were examined through field and laboratory methods in three countries: Bulgaria, Romania and Ukraine. The evaluation for resistance to *Orobanche* was carried out according to a standard methodology (Panchenko, 1975) using a 0-100% scale. Inoculation with downy mildew was done according to Vear and Tourvielle (1987), also using a 0-100% scale. The evaluation for resistance to Phoma was performed under field conditions in an artificial infection field. The attack by this pathogen was read according to a scale from 1/3 to 3/3 using the method of Fayzalla and Maric (1981). The evaluation for resistance to phomopsis was carried out in an infection field in Bulgaria. The inoculation method of Encheva and Kiryakov (2000) was applied, using a 0-4 scale. The hybrids Sevar and Dea demonstrated excellent and very good resistance to the above diseases and the parasite *Orobanche*, which is a prerequisite for their introduction in production.

**Keywords:** *Orobanche*, downy mildew, new hybrids, sunflower, diseases