

**BROOMRAPE (*OROBANCHE CUMANA* WALLR.) IN SUNFLOWER – UPDATE ON RACIAL COMPOSITION AND DISTRIBUTION, HOST RESISTANCE AND MANAGEMENT**

***Maria Joita – Pacureanu***

*National Agricultural Research And Development Institute \_ Fundulea, Romania*

[pacurean@ricic.ro](mailto:pacurean@ricic.ro); [mariapacureanu2@yahoo.com](mailto:mariapacureanu2@yahoo.com)

**ABSTRACT**

The parasitic plant *Orobanche cumana* Wallr. is the most important biotic constraint to the production of sunflower crop, in all countries where sunflower is grown, except North and South America. The parasitism of *O. cumana* on sunflower dates back to the first half of 19<sup>th</sup> century in Russia, expanding to Moldova and Romania by the beginning of 20<sup>th</sup> century and later in others like Turkey, Spain, Serbia, Bulgaria and Ukraine. Currently, *O. cumana* is present in all countries in Southern Europe and areas around Black Sea, as well as in many countries in Asia. The first mention to different races within *O. cumana* dates back to the beginning of 20<sup>th</sup> century, in Russia, breeders developing sunflower varieties genetically resistant to races A and B. Later (1970-1980) in Romania it have been identified other three races: C,D,E as well as a set of sunflower genotypes, each of them carrying one single major gene of resistance. Races A to E were effectively controlled through genetic resistance for some decades, until race F have been identified. Along the last decades, a new break of the genetic control of *O. cumana* has occurred in Turkey, Romania, Bulgaria, Russia, Spain, as a consequence of the crop intensification and short crop rotation, together with the use of genetic material from foreign breeding programs. There have been identified new highly virulent races, as G, H or more. Because in the last years, in China sunflower was growing very much as an oil crop, broomrape parasite has developed new races. Important efforts of breeders have been devoted to the search of effective resistance against the increasingly virulent parasite populations and, as a result, resistant genotypes have been released. In each country, sunflower material identified as resistant to *O. cumana* has been used to differentiate local races of increasingly virulence and termed from A to E, or F, or G. Because no comparative studies have been conducted to test the correspondence of races among the countries, there is not knowledge of the pathogenic traits of the parasite. In spite that races A to G have been identified in many countries, few works asses the similarity of those populations from different geographic origin and characterized as belonging to the same race. Due to the diversity of *O. cumana* races identified worldwide, the use of the coded triplets as a simple and global method to internationally determine the races of the parasite seems to be imperative at this moment. Among the genotypes identified as differentials for races A to E, other inbred lines were identified by scientists as having a clearly resistance against the broomrape populations in different countries. Selection for sunflower resistance to broomrape started in the early 1910s through individual selection method, using open pollinated varieties. Extensive research was conducted by Vranceanu in Romania (1976 – 1980), being identified the dominant genes *Or1* to *Or5*. The appearance of new races considerably reduced the available sources of resistance of cultivated sunflower. A high level of resistance was found in wild *Helianthus* spp., mainly in perennial ones. The genetic resistance to *O. cumana* is more complex than previously thought, in addition to the known major dominant genes, other minor genes being identified, in different sunflower genotypes. At the start of program, breeder must determine which races are present in the region for which the hybrids are developing. The alternative breeding strategies are required to increase the durability of genetic resistance to *O. cumana*. These strategies will require QTL analysis and developing of molecular markers

linked to major and minor resistance genes, to ensure that they are simultaneously introgressed during backcross and a detailed characterization of the physiological mechanisms underlying genetic resistance. Broomrape can also be managed by development of IMI resistant hybrids or by using biological control. Efforts should be made to biochemical parameters (mechanical barriers, germination inhibitors, phytoalexins, etc.).

**Key words:** *sunflower, broomrape, races, resistance, management.*

## INTRODUCTION

Broomrape (*Orobanche cumana* Wallr. – *Orobanche cernua* Loelf.) is an angiosperm that parasitizes sunflower roots and causes economic damage to sunflower production on a worldwide scale. The parasitism of broomrape on sunflower dates back to the first half of the 19<sup>th</sup> century in Russia. It was first observed in Voronezh region in the 1890s (Satipero, 1913). Thereafter, the geographical spread of this parasite in the world followed the same pattern with some decades delay, as the expansion of the crop of sunflower in time and over different countries.

Teryokhin (1976, 1992) refers to *Orobanche cumana* Wallr. in Odessa region, as well as to *Orobanche cernua* Loefl. in Krasnodar. There have been disputed aspects regarding the distinction between *O. cumana* and *O. cernua*. Some authors consider these two forms, as variants of *O. cernua* or synonymous, some others are separated them in two distinct species. Using RAPD markers, Joel et al. (1996) have showed that *O. cumana* is an autonomous taxonomic unit, closely to *O. cernua*. In present there is a consensus in considering *Orobanche cumana* as sunflower broomrape.

From Russia, *O. cumana* significantly spread all over the crop in the former USSR and expanded to Romania, Bulgaria, Turkey, Serbia (Iliescu, 1984; Acimovic, 1988 a; Bulbul, 1991). Particularly interesting is the occurrence of infections by broomrape in Spain. This species was identified on confectionery sunflower, in Toledo province, in 1958 (Diaz-Celayeta, 1974), spreading in Cuenca and Malaga (Gonzales-Torres et al., 1980). Later, broomrape has spread on oil sunflower, specially in Andalusia (Gonzales – Carrascoza, 1992; Melero – Vara et al., 1996). Outside of Europe, *O. cumana* was identified in China, in Jilin province (Tingrui et al., 1996).

Currently, *O. cumana* is present in all the countries of Southern Europe and areas around the Black Sea where sunflowers are grown (Antonova, 2014; Batchvarova, 2014; Duca, 2014; Hargitay, 2014; Jestin et al., 2014; Kaya, 2014; Miladinovic et al., 2014; Molinero-Ruiz & Dominguez, 2014; Pacureanu, 2014; Pototskyi, 2014), as well as in North Africa (Amri et al., 2012), Israel (Eizenberg et al., 2004) and China (Baichun et al., 1996; Ma & Jan, 2014; Shi et al., 2015). The objective of this paper was to make an overview of what has been achieved in knowledge referring to sunflower broomrape race structure and distribution, host genetic of resistance and management of the parasite control.

## Broomrape (*Orobanche cumana*) contact with sunflower plants

Broomrape (*Orobanche cumana*) seed is very small and 1000 seeds weight is most often 0.001 grams. The seeds can survive in soil for up to 20 years. *Orobanche cumana* infects the roots of sunflower early in the growing season, obtaining water and inorganic compounds from the host plant through xylem to xylem contact (Heide – Jorgensen, 2008). Broomrape

seeds germination takes place in wet ground at 20-25°C temperatures . It is affected by pH value of the soil as well as by the excretion of the host roots (germination stimulators).

Pancenکو (1975) gave a detailed description of germination of broomrape seeds, the mechanism of penetration of the haustorium of susceptible genotypes of sunflower, as well as the process of dying of the haustorium in the roots of the resistant genotypes of sunflower. Haustorium penetrates the skin and parenchyma of the sunflower roots, all the way to the central cambium cylinder, only in the susceptible genotypes. In the resistant genotypes there is a layer of lignin between parenchyma and cambium which does not allow the penetration of haustoria of broomrape (Pancenکو and Antonova, 1975; Antonova, 1978).

The utilization of host photoassimilates by the parasite results in depletion of resources which are necessary for the growth of sunflower and for the optimal development of the seeds. Broomrape stems have a long underground development stage, emerging aboveground around flowering of sunflower (Melero – Vara and Alonso, 1988). By the time of broomrape emergence, most of metabolic imbalance is already produced by the parasite to sunflower (Molinero – Ruiz et al., 2015). From the first emergence of broomrape stems on, the impact of broomrape in the yield of the crop is increasingly through absent or small sized heads, low number and small seeds, even death of the plants. Yield reduction due to the infestation with broomrape depends on level of soil infestation, aggressiveness of the parasite, sunflower genotype, earliness of broomrape emergence and soil depth, among others (Jestin, 2014; Molinero – Ruiz et al., 2015).

### **Race structure of *Orobanchę cumana***

At the beginning of 20<sup>th</sup> century, broomrape spread across Russia significantly and endangered the mass production of sunflower. Efforts of soviet breeders at the Saratov experimental station resulted in the release of genetically resistant varieties of sunflower The first cultivar resistant to race A, Saratovski 169, was created by Placek (1918). In the years that followed, other cultivars resistant to race A were also produced (Kruglik A- 41; Zelenka and Fuksinka). As the mass production of sunflower spread quickly, it was followed by a new race, called B (Zhdanov, 1926). The race B was spread in Rostov and Krasnodar regions (Antonova, 2014). During 1925-1960, Pustovoit created in Russia, highly productive cultivars, resistant to race B.

After the cultivars resistant to race B, for years, nobody mentioned the occurrence of new races, even the composition of races had changed. The existing cultivars were assumed to have possessed several genes for resistance to broomrape (races C and D). Petrov (1970) announced the existence of a new race in Bulgaria (race C). At the same time, its existence was announced in Romania. Vranceanu et al. (1980) added significantly to the examination of broomrape races and helped to detect dominant genes by establishing the existence of five races which were controlled by dominant genes: A(Or1), B(Or2), C(Or3), D(Or4) and E(Or5). Pacureanu – Joita et al. (1998) announced the existence of race F (Or6).

Along the last decades a new break of the genetic control of *O. cumana* has occurred in the countries situated in Black Sea region, as a consequence of the crop intensification and short crop rotations, together with the use of genetic material from foreign breeding programs and therefore susceptible to the local ecotypes of the parasite. Current facts indicate that there are at least 7-8 ( A to G and H) broomrape races in Turkey, Romania, Russia, Ukraine, Bulgaria and Moldova ( Kaya, 2004, 2009 and 2014; Pacureanu-Joita et al., 2009 and 2012; Pacureanu-Joita, 2014; Goncharov, 2009; Antonova et al., 2009; Antonova, 2014; Pototsky, 2014; Batchvarova, 2014; Gasca et al., 2013; Duca, 2014).

Genetic variability and dynamics of changes occurring in the race composition of broomrape, in Spain, which so far has been dominated by races E and F, have been studied by Alonso et al. (1996), Dominguez et al. (1999), Sukno et al. (1999), Rodriguez-Ojeda et al. (2001), Akhtouch et al. (2002), Perez-Vich et al. (2002; 2004), Molinero-Ruiz and Melero-Vara (2005), Velasco et al. (2007), Fernandez-Martinez et al. (2009 and 2012), Molinero – Ruiz and Dominguez, 2014.

The first report of *O. cumana* in Serbia dates back to the 1950's; the populations detected were probably race B, because they were controlled by Russian oil varieties and hybrids with resistance to this race (in Miladinovic *et al.*, 2014). Economically important incidences of broomrape on sunflower were again observed in the country in the 1990's and identified as a race E (Mihaljčević, 1996 ;Skoric and Jovic, 2005). During the last 20 years, and although the parasite has spread to new sunflower production areas in the country, new races have not occurred so far (Miladinovic *et al.*, 2014).

In China, *Orobanche cumana* has been present for a long time but, the identification of race A has been done in 1990s (Baichun et al. 1996). A widespread increase in new virulent parasite races has occurred since then, so that the wide distribution of races A to F in the country, as well as the identification of race G in Inner Mongolia have been recently reported (Ma & Jan, 2014; Shi *et al.*, 2015).

Concerning other sunflower growing countries, in Hungary, populations of *O. cumana* were characterised as low to moderately virulent (races A–D) (Zoltán, 2001). Currently, race E is the most frequent one in the country (Hargitay, 2014) although race F has been also identified (Molinero-Ruiz *et al.*, 2014). ).

The presence of *O. cumana* parasitizing sunflower in France was not reported until 2007 (Jestin, 2012). It is currently located mainly in the South and also in the West of the country (Jestin *et al.*, 2014), although information about race/s is not available. Because no comparative studies have been conducted to test the correspondence of races among countries, an unbundled knowledge of pathogenic traits of the parasite is currently occurring. In spite that races A to G were or have recently been identified in many countries, very few works assess the similarity of those populations from different geographic origin and characterized as belonging to the same race (Molinero-Ruiz *et al.*, 2014).

When determining races of crop pathogens, pathotype information frequently surpasses a certain level of complexity. When this happens, mathematical codes are, by far, advantageous over the use of consecutive numbers or letters given in chronological order of pathotypes discovery. To ease communication and comparisons of results about characterization of races, a universal adoption of the coded triplets system is frequent for many plant pathogens (Liebenberg & Pretorius, 2011; Gurung *et al.*, 2013; Dreiseitl, 2014) including *Plasmopara halstedii* Farl. Berl. & de Toni, which causes sunflower downy mildew. Molinero – Ruiz et al. (2015) proposed the characterization and nomenclature of *Orobanche cumana* populations, using the coded triplets system, which is based on the use of eight sunflower differentials, grouped into three sets (table 1). In table 2, it is presented the characterization of *Orobanche cumana* populations, using the coded triplets system and its correspondence with the method based on the use of capital letters, given in chronological order of races identification (Molinero – Ruiz et al., 2015). The use of the group of differentials presented in table 1, worldwide, will facilitate comparisons of results about characterization of broomrape races in different countries. The most virulent races of the parasite and their location will be known by scientists working on the achievement of sunflower material with resistance to *Orobanche cumana* in any of the countries where the parasite occurs.

Genetic studies of the parasite are extremely important in present, in order to bring knowledge on the parasite at the level of knowledge on the genetics of resistance in sunflower. Studies of the inheritance of resistance to *Orobanche cumana* have confirmed the gene for gene interaction in the parasite – host system for races E-F, but the inheritance of genes from other sources of resistance to the most virulent races of this parasite is still unknown.

### Sources of resistance to *Orobanche cumana*

Genes for resistance to broomrape races A, B, C, D and E are present in varietal populations of sunflower developed in breeding programs from Saratov (former USSR), Krasnodar (former USSR), Odessa (Ukraine), Fundulea (Romania) and several other places. Some of these genes have been identified in certain wild species of the genus *Helianthus* and have been incorporated into cultivated sunflower genotypes by interspecific hybridization. A species of wild sunflower (*Helianthus tuberosus*) was first used as a source of *Orobanche* resistance (Vrânceanu et al., 1980; Škorić et al., 2010) as the donor of *Or* genes.

As new broomrape races were appearing, sources of resistance in cultivated sunflower became increasingly scarce. In Turkey, Gulya et al. (1994) found only 22 resistant entries in a field evaluation of 903 accessions, whereas in Spain, Domínguez et al. (1996) found 8 resistant and 33 segregating entries in the evaluation of 429 accessions of different origins for resistance to race E. Sources of resistance to the latest races have been scarce in germplasm of cultivated sunflower, although valuable resistant germplasm has been identified in breeding programs conducted in Spain (Fernández-Martínez et al., 2004; Rodríguez-Ojeda et al., 2001), Romania (Pacureanu-Joita et al., 2004, 2009b), Turkey (Kaya et al., 2009), and Russia (Gontcharov et al., 2004; Gontcharov, 2009). In most cases, the germplasm exhibited vertical or qualitative genetic resistance. In addition, genetic sources of horizontal or quantitative genetic resistance have been developed (Pérez-Vich et al., 2005). In contrast, a high level of resistance to the newest races has been found in wild *Helianthus* species. Fernández-Martínez et al. (2000) tested for race F resistance 54 wild sunflower accessions (representing 27 perennial and four annual species) and 55 cultivated sunflower accessions. Most of the perennial species proved fully resistant to this race. The only exceptions were some populations of four of the wild perennials, which had a certain percentage of susceptible plants. Among the wild annual species, *H. anomalus* and *H. agrestis* were completely resistant, while *H. debilis* ssp. *cucumerifolius* and *H. exilis* segregated with regard to *Orobanche* resistance. Jan and Fernandez-Martinez (2002) employed interspecific hybridization to incorporate genes for resistance to race F from several wild species into cultivated sunflower, and developed four populations (BR1-BR4) resistant to this race from the wild sunflower species *H. maximilianii* Schrad, *H. grosseserratus* Mart., and *H. divaricatus* L (Jan et al., 2002). Where necessary, they used embryo culture and chromosomal doubling by colchicine in order to bypass the barriers and enable the transfer of desirable genes. Christov et al. (1992, 1998, 2009) have achieved outstanding results in identifying genes for broomrape resistance in the wild species of the genus *Helianthus* and incorporating them into cultivated sunflower genotypes. Especially important are the findings reported in Christov et al. (2009), which concern the detection of *Or* genes in 11 perennial wild sunflower species and their incorporation into elite cultivated sunflower lines by means of interspecific hybridization. Hladni et al. (2009) developed five new restorer lines, to race E, from interspecific populations originating from *H. deserticola*. The resistance to a race classified as G has been transferred from *H. debilis* into cultivated sunflower by Velasco et al. (2012).

Sources of *Orobanche* resistance can also be found by the use of induced mutations. Venkov and Shindrova (1998) reported that they obtained a mutant with partial resistance to *O. cumana* using a 0.4% solution of the mutagen nitrosumethylurea.

### **Genetic of sunflower resistance to *Orobanche cumana***

In parallel with the appearance of new broomrape races and sources of broomrape resistance, the genetics of resistance to this parasitic plant has been studied. As sources of resistance to races A and B were identified, it was also determined that resistance to broomrape was controlled by dominant genes. Burlov and Kostyuk (1976) and Pogorletsy and Geshele (1976) studied the genetic basis of *Orobanche* resistance and discovered that it was controlled by a single dominant gene, which they named *Or*. Vrânceanu et al. (1980) conducted extensive genetic research as part of his study of broomrape in Romania from 1976 to 1980. They established that there were five pathogenic races of this parasite and labeled them A, B, C, D, and E. They also identified a set of differential lines that had cumulative resistance to the five successive races, conferred by the dominant genes *Or*<sub>1</sub>, *Or*<sub>2</sub>, *Or*<sub>3</sub>, *Or*<sub>4</sub>, and *Or*<sub>5</sub>, respectively. When race F subsequently appeared in Romania and resistance to it was discovered in the line LC-1093 (*Or*<sub>6</sub>) by Pacureanu-Joita et al. (1998), this cycle of genetic research was completed.

In some particular cases, the resistance seems to be controlled by a complex of genes. So, Pustovoit (1966) and Paleev (1983) refers to an intermediary inheritance of F<sub>1</sub> hybrids. Krohin (1980) concluded that the inbred line 6540 – 1M has a resistance for the race B of the parasite, controlled by two complementary genes. The same results obtained Hatnianskii (1982) and Ciriaev (1987) for some lines selected from Peredovik variety.

The appearance of new broomrape races in Spain triggered a new cycle of large-scale genetic analyses. Dominquez et al. (1996) noted that there is a low frequency of genes for resistance to race E in cultivated sunflower and that this resistance is controlled by two dominant genes. Alonso (1998) noted that, the known dominant genes notwithstanding, resistance to *Orobanche* may be more complex than previously thought and that genes other than single dominant ones may also be involved. In some cases involving cultivated sunflower germplasm, resistance to race F is controlled by recessive genes. Thus, *Orobanche* resistance found in the lines P-96 and KI-534 is controlled by recessive alleles at two loci (Rodriguez-Ojeda et al., 2001; Akhtouch et al., 2002). The same recessive genes control resistance to race E in the line KI-534 (Rodriguez-Ojeda et al., 2001). Akhtouch et al. (2002) crossed lines resistant to race F with those that are susceptible to it and found segregation ratios of 1:15 [Resistant (R): Susceptible (S)] and 1:3 (R : S) in the F<sub>2</sub>, and BC<sub>1</sub> generations, which in most cases indicates double dominant epistasis. Cases of segregation ratios of 3:13 (R : S) and 1:1 (R : S) were also recorded in the F<sub>2</sub>s and BC<sub>1</sub>s, which is indicative of dominant-recessive epistasis. Velasco et al. (2007) crossed a line resistant to race F (J1) with three susceptible lines and studied the inheritance of face F resistance, obtaining segregation ratios of 3:1, 13:3, and 15:1 (R + Moderately R : S) in the F<sub>2</sub>, generations. These results indicated incomplete dominance of the *Or*<sub>6</sub> alleles and the presence of a second gene, *Or*<sub>7</sub>, whose expression was influenced by the environment. Pacureanu-Joita et al. (2008) tested the latest, virulent race of broomrape from Romania through a cross between the resistant line AO-548 and the susceptible line AD-66 and segregation ratios of 15:1 (R:S) and 3:1 (R:S) were observed in the F<sub>2</sub> and BC<sub>1</sub> generations, respectively, indicating that the resistance in AO-548 is controlled by two independent dominant genes. In Spain, a single dominant gene controlling resistance to the most virulent race G in lines derived from interspecific crosses with *Helianthus debilis* subsp. *tardiflorus* has been reported by Velasco et al. (2012).

Most of the molecular research for characterizing broomrape resistance has been focused on mapping the *Or<sub>5</sub>* gene conferring resistance to races A to E. This gene has been mapped to a terminal, probably telomeric region of linkage group (LG) 3 of the sunflower genetic map (Lu et al., 2000; Pérez-Vich et al., 2004; Tang et al., 2003). The closest marker was identified at around 6 centimorgan downstream of *Or<sub>5</sub>* (Lu et al., 2000; Tang et al., 2003), but no flanking markers were found in the upper part of the LG. Márquez-Lema et al. (2008) identified a telomere-associated target region amplification polymorphism (TRAP) marker linked to *Or<sub>5</sub>*, probably flanking the gene in the upper telomeric side. In addition to the major role of *Or<sub>5</sub>* in race E resistance, Pérez-Vich et al. (2004) also identified a quantitative component of the race E resistance determined by four quantitative trait loci (QTL) with minor effect associated with the number of broomrape shoots per plant. Imerovski et al. (2013) demonstrated that simple sequence repeat (SSR) markers of LG 3 were also strongly associated with resistance genes *Or<sub>2</sub>*, *Or<sub>4</sub>*, and *Or<sub>6</sub>*. For race F resistance, QTL analysis in a population derived from line P-96, for which phenotypic analysis suggested the presence of two recessive loci (Akhtouch et al., 2002), revealed the presence of six QTL with small to moderate effects on reducing the number of broomrape shoots per plant, three of them being non-race specific (Pérez-Vich et al., 2004). More recently, Louarn et al. (2014) identified four QTL for broomrape (Spanish race F) resistance mechanisms in a population derived from LR1line selected from (*H. debilis* x *H. annuus*). These 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.

### **Mechanisms of sunflower resistance to broomrape**

It is very important to know all the mechanisms involved in broomrape resistance (physiological, biochemical, mechanical, etc.). Getting information on the physiological basis of different sources of resistance will have physiological based breeding and resistance genes pyramiding, underlying different resistance mechanisms (Pérez-Vich et al., 2013). The resistance mechanisms have been studied for a long time. Thus, Morozov (1947) cites the results of Richter (1924) that indicated that broomrape susceptible sunflowers had root systems with a low pH, and those of Suhorukov (1930) concerning the link between peroxidase values and sunflower susceptibility to broomrape, according to which increased soil acidity increased peroxidase activity and the susceptibility of sunflower plants to *Orobanche*. According to Morozov (1947), Barcinskiy (1932, 1935) reported that sunflower root cells contain substances that stimulate the germination and development of broomrape seeds and seedlings. Long after that, Wegmann (1998), Alonso (1998), Matusova et al. (2004), and Honiges et al. (2009) also pointed out the importance of broomrape germination stimulants. Joel et al. (2011) identified the natural broomrape germination stimulant from sunflower roots exudates as a dehydrocostus lactone. Low exudation of germination stimulants by sunflower roots has been described as a preattachment resistance mechanism (Labrousse et al., 2001). Another preattachment resistance mechanism is the exudation by sunflower roots of seed germination inhibitors and/or inhibitors of radicle exoenzymes (Höniges et al., 2008). Phytoalexins, in particular 7-hydroxylated simple coumarins, have been suggested to play a defensive role by preventing broomrape germination and subsequent connection with sunflower roots (Serghini et al., 2001).

Mechanical barriers like lignifications of the cell wall by peroxidase-catalyzed reactions have been proposed as postattachment resistance mechanisms (Höniges et al., 2008). Panchenko and Antonova (1975) concluded that the protective response of different sunflower cultivars came down to the accumulation of lignin and its precompounds in injured host cells, resulting in the

haustoria losing the ability to supply themselves with water and nutrients from the host cells. Also, a physical barrier by reinforcement of the host cell walls through suberization and protein cross-linking that prevents parasite intrusion has been described in sunflower genotypes resistant to race F (Echevarría-Zomeño et al. 2006). This mechanism was also observed for race E, but in this case cell wall was reinforced by means of callose depositions (Letousey et al., 2007).

Some of the previously mentioned studies have revealed the simultaneous occurrence of several resistance mechanisms in genotypes exhibiting complete resistance (Echevarría-Zomeño et al., 2006; Labrousse et al., 2001; Letousey et al., 2007). Labrousse et al. (2000, 2001, 2004) discuss different criteria for assessing *Orobanche* resistance and the different mechanisms by which such resistance operates. The authors were able to distinguish between three types of broomrape resistance in their work: a) resistance acting at an early stage in broomrape development (*H. debilis* ssp. *debilis*), when broomrape seedlings were present on the sunflower root, but an impassable encapsulation layer blocked the intruding parasite, which then died; b) resistance found in the resistant line LR1, which involves two types of action: decreased stimulation of broomrape germination (a three-fold reduction compared to susceptible line 2603); and rapid necrosis that appeared as early as stage 2 of parasite development; c) resistance observed at a later stage of broomrape development in the line 92B6 (necrosis developing prior to broomrape flowering). Louarn et al. (2012) found that arbuscular mycorrhizal fungi could produce inhibitors of *O. cumana* germination, and that this inhibitory effect seemed restricted to broomrape seeds.

## **Sunflower breeding for resistance to *Orobanche cumana***

### ***Methods used for evaluating broomrape resistance***

Sunflower breeders must develop a breeding strategy, decide on a breeding method, secure the necessary germplasm and differential lines for broomrape race identification, and choose the appropriate inoculation method. In the years in which races A through E were discovered, sunflower breeders tested their breeding materials in naturally infested fields, usually on plots that had been severely infested by broomrape the year before. This method is still employed by some breeders. However, this approach does not always produce reliable results due to the influence of environmental factors and an inadequate amount of broomrape seeds in the soil. In an effort to avoid this, breeders resorted to collecting broomrape seeds and to carrying out artificial infestation in the field experiments, either by incorporating the seed into the soil using basic tillage (Vrânceanu et al., 1980) or by inoculating individual plants in small pots to be transplanted into the field after 2-3 weeks in the growth chamber (Velasco et al., 2007). However, this method is prone to producing experimental errors too, caused primarily by the effects of environmental factors. Much more accurate results can be obtained by putting broomrape seeds into containers filled with a pre-prepared soil medium which are then placed in a controlled environment (growth chamber or greenhouse). Panchenko (1975) developed a screening method for assessing resistance to broomrape in greenhouse conditions during autumn and winter. This method was further honed by Grezes-Besset (1994), who made testing using plastic test tubes part of the procedure. The advantage of this technique is that it provides a higher level of reliability and makes it possible to test a large number of genotypes in a short period of time.

Different methods have been developed for evaluating sunflower physiological mechanisms of resistance, which include the evaluation of the underground broomrape development in Petri dishes assays covered with glass fiber paper (Echevarría-Zomeño et al., 2006) or in two-



layer filter paper rolls (Antonova et al., 2011; Rodríguez-Ojeda et al., 2010), or the use of hydroponic co-culture (Labrousse et al., 2004).

### **Methods of breeding for resistance to *Orobanche cumana***

Breeding programs focused on the development of broomrape-resistant hybrids of sunflower were first based on single dominant *Or* genes. To ensure their success, the best way to go is to pick out an elite line and cross it with a source of *Or* genes, which should then be incorporated into the breeding material using certain techniques (recurrent cross-breeding together with screening for resistance in all BC generations). At the start of the program, the breeder must determine which race or races are present in the region for which the hybrids are being developed. A set of differential lines for races A, B, C, D, and E has been provided by Vrânceanu et al. (1980), while Pacureanu-Joita et al. (1998) have identified such a line for race F.

There are no public differential lines for the new, virulent races of broomrape that have appeared in the last few years.

The breeding strategies have been developed in order to increase the durability of genetic resistance to broomrape. Continuous search for new sources of resistance is important. The most significant results are achieved by interspecific hybridization in which wild species of genus *Helianthus* are used as donor of the gene of resistance. Transferring resistance genes from annual wild species is accomplished rather easily with a conventional crossing scheme, but, from perennial species is generally more difficult, due to problems associated with early hybrid embryo abortion and sterility in F<sub>1</sub> and BC<sub>1</sub>F<sub>1</sub> generations. Such problem can be overcome with using of embryo rescue and chromosome doubling of the F<sub>1</sub>. Also, alternative breeding strategies involving vertical resistance should incorporate gene pyramiding, alternation of several forms of a hybrid with different *Or* genes, or mixtures of these different forms grown together. Finally, to get the best use of these major genes, they need to be backed-up by quantitative, non-race specific resistance. These strategies will require QTL analysis and development of molecular markers linked to major and minor resistance genes to ensure that they are simultaneously introgressed during backcross, and a detailed characterization of the physiological mechanisms underlying genetic resistance.

### **Alternative methods for the control of *Orobanche cumana***

The rapid changes in broomrape race composition have forced sunflower breeders and geneticists to not only search for genes for resistance to the new races of *Orobanche* but to also look for alternative solutions to the problem of broomrape control. In the past 15 years, the development of sunflower hybrids resistant to the imidazolinone herbicides has made it possible to successfully control broomrape regardless of its race composition. This option is generally used in combination with the available *Orobanche cumana* resistant genes.

Wild *Helianthus annuus* L. resistant to imidazolinones (imazethapyr, pursuit) was first identified in Kansas (USA) in 1996 in a soybean field treated for seven consecutive years with a herbicide from this group (Al-Khatib et al., 1999). The use of imidazolinone resistance in sunflower breeding through the introduction of IMI-resistance genes into cultivated sunflower genotypes provides a broad spectrum of weed control (covering over 40 broadleaf species and over 20 grass weed species) and is especially effective in controlling *Orobanche* in sunflower, as discovered by Alonso et al. (1998). The USDA-ARS (NDSU) research group quickly transferred this genetic resistance into cultivated sunflowers and released the public populations IMISUN-1 and IMISUN-2. Similar programs were developed in parallel by

Alonso et al. (1998) in Spain, by Malidža et al. (2000) and Jocić et al. (2001) in Serbia, and by several private companies in Argentina. Bruniard and Miller (2001) reported that IMI-resistance is controlled by two genes (semi-dominant type of gene action). *Imr<sub>1</sub>* is the gene responsible for imidazolinone resistance, while *Imr<sub>2</sub>* has the modifier effect when the major gene is present. Malidža et al. (2000) and Jocić et al. (2001) showed that resistance to imidazolinones is controlled by a single, partially dominant gene. These differences in the mode of inheritance could perhaps be attributed to the presence of mutations on several different loci in the original population of wild *Helianthus annuus* L.

Sala et al. (2008) obtained another gene for resistance to imidazolinones through ethyl methane-sulfonate mutagenesis of seeds and selection with the imazapyr herbicide. They labeled the gene CLHA-PLUS. Based on genetic analysis (F<sub>1</sub>, F<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub>), the authors determined that the IMI-resistance gene CLHA-PLUS is controlled by a partially dominant nuclear gene. Using the SSR marker for the AHASL1 gene, they concluded that the mutation present in CLHA-PLUS is different from *Imr<sub>1</sub>*, but that both these genes are allelic variants of the locus AHASL<sub>1</sub>.

Other chemical options have effect against *Orobanche cumana*, such as inducers of seeds germination leading to suicidal germination of the parasite in the absence of sunflower (Lachia et al., 2014), or inhibitors of the germination process (Okazawa & Benesh, 2011). Extensive information about chemical signals from hosts and their effect on parasite species can be found in other works (Smith et al., 1990; Yoneyama et al., 2008; Gomez-Roldan et al., 2008; Umehara et al., 2008). Finally, the availability of next generation sequencing technologies, metabolomics and its applications to produce continuous and massive information about parasitic weeds (Westwood et al., 2012; Pineda-Martos et al., 2014; Piednöel et al., 2012) must be exploited.

Some authors tried to find other methods for controlling this parasite, as modification of sunflower sowing date. Akhtouch et al. (2013) have showed that the modification of the SD affects differently the natural infection by *O. cumana* in susceptible and moderately resistant sunflower. Eizenberg et al. (2012) have validated a thermal time model based on the main role of temperature on the parasitism of *O. cumana* in susceptible irrigated sunflower. The effect of a shift of sowing date, on sunflower hybrids performance in fields infested by *Orobanche cumana* and under drought conditions as compared to irrigation might be investigated in the future.

## CONCLUSIONS

Sunflower breeders and geneticists have been successful in responding to the rapid changes in the race composition of broomrape (*Orobanche cumana* Wallr). They found genes for resistance to this pathogen and incorporated them into elite lines of cultivated sunflower, making it possible to develop *Orobanche*-resistant hybrids. Research so far has shown that the genes for broomrape resistance are present in some wild species of the genus *Helianthus*.

Because information about pathogenic traits of the parasite is locally obtained, its validity is restricted to particular geographical areas within Europe. Comparisons of results about characterization of races at an international level, as well as effectiveness of resistance sources in international sunflower breeding programs, will be favoured by the universal adoption of the coded triplets system for nomenclature of *Orobanche cumana* races.

On the other hand, breeding sunflowers for resistance to the AHAS-inhibiting herbicides has appeared, as an alternative for the control of *O. cumana* together with other weeds of the

crop. This alternative for the control of *O. cumana* could be implemented together with genetic resistance to races of the parasite.

Physiological and molecular mechanisms governing the *Orobanche cumana* development and the establishment of the interaction with its hosts, for example the chemical stimulation of parasite seed germination, or the role of degrading enzymes on the parasite progression between host cells, have been studied and characterized. This will help to the identification of a specific targets for new control methods.

## ACKNOWLEDGEMENTS

I wish to thank to all colleagues from the countries where sunflower crop is affected by the parasite *Orobanche cumana*, for their information and discussions on this topic.

Table 1. Proposal for standardized characterization and nomenclature of *Orobanche cumana* populations using the coded triplets system, which is based on the use of eight sunflower lines -termed differentials-, grouped into three sets. By: Molinero – Ruiz et al., 2015

| Population of <i>O. cumana</i> | Group # 1                      |                               | Group # 2                 |                               | Group # 3                                                   |                               |
|--------------------------------|--------------------------------|-------------------------------|---------------------------|-------------------------------|-------------------------------------------------------------|-------------------------------|
|                                | Differentials                  | Value if susceptible reaction | Differentials             | Value if susceptible reaction | Differentials                                               | Value if susceptible reaction |
|                                | AD66 (-)                       | 1                             | Record (Or <sub>3</sub> ) | 1                             | LC1093 (Or <sub>6</sub> )                                   | 1                             |
|                                | K A-41 (Or <sub>1</sub> )<br>b | 2                             | S1358 (Or <sub>4</sub> )  | 2                             | P96 (Or <sub>5</sub> ,<br>or <sub>6</sub> or <sub>7</sub> ) | 2                             |
|                                | J8281 (Or <sub>2</sub> )       | 4                             | P1380 (Or <sub>5</sub> )  | 4                             |                                                             |                               |
| Code <sup>a</sup> :            | Total in group # 1             |                               | Total in group # 2        |                               | Total in group # 3                                          |                               |

<sup>a</sup> Each population is identified by a code of three digits which are obtained by totals due to susceptible reactions of each of the lines into the set. Resistant reactions impart 0.

<sup>b</sup> Genetic resistance in each line according to Akhtouch *et al.* (2002), Pacureanu *et al.* (2004), Pérez-Vich *et al.* (2004) and Vrânceanu *et al.* (1980).

Table 2. Proposal for characterization of populations of *Orobanche cumana* using the coded triplets system, and its correspondence with the traditional method based on the use of consecutive capital letters (A, B, C, etc.) given in chronological order of identification of pathotypes (By: Molinero – Ruiz et al., 2015)

| Line of sunflower | Coded races of <i>O. cumana</i> |     |     |     |     |     |         |         |
|-------------------|---------------------------------|-----|-----|-----|-----|-----|---------|---------|
|                   | 100                             | 300 | 700 | 710 | 730 | 770 | 771     | 773     |
| AD66              | S                               | S   | S   | S   | S   | S   | S       | S       |
| K A-41            | R                               | S   | S   | S   | S   | S   | S       | S       |
| J8281             | R                               | R   | S   | S   | S   | S   | S       | S       |
| Record            | R                               | R   | R   | S   | S   | S   | S       | S       |
| S1358             | R                               | R   | R   | R   | S   | S   | S       | S       |
| P1380             | R                               | R   | R   | R   | R   | S   | S       | S       |
| LC1093            | R                               | R   | R   | R   | R   | R   | S       | S       |
| P96               | R                               | R   | R   | R   | R   | R   | R       | S       |
| Historical race   | A                               | B   | C   | D   | E   | F   | F or G? | F or G? |

## LITERATURE

- Acimovic, M., 1988. Sunflower diseases mapping in Europe and some countries outside Europe in the period 1984-1986. *Helia*, 11:41-49.
- Akhtouch, B., Muñoz-Ruz, J., Melero-Vara, J., Fernández-Martínez, J.M., Dominguez, J., 2002. Inheritance of resistance to race F of broomrape (*Orobanche cumana* Wallr.) in sunflower lines of different origin. *Plant Breeding* 121:266-268.
- Akhtouch, B., Molinero-Ruiz, L., Melero-Vara, J., Dominguez, J., Fernandez-Martinez, J.M., 2013. Using sowing date modification and genetic resistance to manage sunflower broomrape (*Orobanche cumana* Wallr.) *Helia*, Vol. 36, no. 59, pp. 17-34
- Al-Khatib, K., Baumgartner, J.R., Currie, R.S., 1999. Survey of common sunflower (*Helianthus annuus*) resistance to ALS-inhibiting herbicides in northeast Kansas. In: Proc. 21th Sunflower Res. Workshop, USA. p.210.
- Alonso, L.C., 1998. Resistance to *Orobanche* and resistance breeding: A review. In: K. Wegmann, L.J. Musselman, and D.M. Joel (eds.). Current problems of *Orobanche* research. Proc. 4<sup>th</sup> Int. Symp. *Orobanche*. Albena, Bulgaria. p. 233-237.
- Alonso, L.C., Fernández-Escobar, J., López, G., Rodríguez-Ojeda, M.I., Sallago, F., 1996. New highly virulent sunflower broomrape (*Orobanche cernua* Loefl.) pathotype in Spain. In: M. Moreno, J. Cubero, D. Berner, D. Joel, L. Musselman, and C. Parker (eds.), Advances in Parasitic Plant Research. Proc. 6<sup>th</sup> Int. Symp. Parasitic Weeds. Cordoba, Spain. p. 639-644.
- Amri, M., Abbes, Z., Ben, Youssef, S., Bouhadida, M., Ben, Salah, H., Kharrat, M., 2012. Detection of the parasitic plant, *Orobanche cumana* on sunflower (*Helianthus annuus* L.) in Tunisia. *Afr J Biotechnol* 11(18): 4163-4167.

- Antonova, T.S., 1978. Development of broomrape (*O. cumana* Wallr.) haustoria in roots of resistant and susceptible forms of sunflower. Bot. J. 7: 1025-1029.
- Antonova, T.S., 2014. The history of interconnected evolution of *Orobanche cumana* Wallr. and sunflower in the Russian Federation and Kazakhstan. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 57-64.
- Antonova, T.S., Araslanova, N.M., Guchetl, S.Z., Tchelustnikova, T.A., Ramazanov, S.A., Trembak, E.N., 2009. Virulence of sunflower broomrape (*Orobanche cumana* Wallr.) in some regions of Northern Caucasus. Helia. Vol. 32. NO.51: 101-110.
- Antonova, T.S., Araslanova, N.M., Strelnikov, E.A., Ramazanov, S.A., Tchelustnikova, T.A., Guchetl, S.Z., 2011. Screening of wild *Helianthus* species for resistance to high virulent *Orobanche cumana* Wallr. affecting sunflower in the Rostov Region of the Russian Federation. Helia 55:115-124.
- Baichun, D., Honglin, S., Xuejing, L., Tingrui, L., Haiyan, Y., Xinghuan, W., 1996. Identification of the races of the sunflower broomrape in the principal sunflower production area of Jilin Province. vol.2, p. 801-805. In: Proc. 14th Int. Sunflower Conf., Beijing / Shenyang. China. Int. Sunflower Assoc., Paris, France.
- Batchvarova, R., 2014. Current situation of sunflower broomrape in Bulgaria. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 51-54.
- Bulbul, A., Sahoglu, M., Sari, C. and Aydin, A., 1991. Determination of broomrape (*Orobanche cumana* Wallr.) races of sunflower in the Thrace region of Turkey. HELIA.Vol.14. Nr. 15:21-26.
- Burlov, V.V., Kostyuk, S.V., 1976. Development of counterparts restoring pollen fertility (*Rf*) and resistant to broomrape (*Orobanche cumana* Wallr.). In: Proc. 7<sup>th</sup> Int. Sunflower Conf., Krasnodar USSR. Int. Sunflower Assoc., Paris, France. vol 1, p. 322-326.
- Bruniard, J.M., Miller, J.F., 2001. Inheritance of imidazolinone herbicide resistance in sunflower. Helia 24: 11–16.
- Ciriaev, P.V., 1987. Ustoicivosti samoopilennih linii podsolnecinika k zarazihe. Naucino Tehn. Biull. VNIIMK, Vip. II(97).
- Christov, M., Batchvarova, R., Hristova-Cherbadzhi, M., 2009. Wild species of *Helianthus* L.-sources of resistance to the parasite *Orobanche cumana* Wallr. Helia 32:65-74.
- Christov, M., Shindrova, P., Encheva, V., 1992. Phytopathological characterization of wild species in the genus *Helianthus* in view of their use in breeding for resistance. Genetics and Breeding 25:45-51.
- Christov, M., Shindrova, P., Encheva, V., Bacharova, R., Christova, M., 1998. New sunflower forms resistant to broomrape. In: K. Wegmann, L.J. Musselman, and D.M. Joel (eds.). Current problems of *Orobanche* research. Proc. 4<sup>th</sup> Int. Symp. Orobanche. Albena, Bulgaria. p. 317-319.
- Dreiseitl, A., 2014. Pathogenic divergence of Central European and Australian populations of *Blumeria graminis* f. sp. *hordei*. Ann Appl Biol 165 (3): 364-372.
- Diaz-Celayeta, F., 1974. Algunas plantas parasitas de otras de interes adicicola o medical. Anales INIA, Ser. Prot. Veget., 4:143-166

- Domínguez, J., 1996. R-41, a sunflower restorer line, carrying two genes for resistance against a highly virulent Spanish population of *Orobanche cernua*. Plant Breeding 115:203-204.
- Domínguez, J., Melero-Vara, J.M., Ruso, J., Miller, J., Fernández-Martínez, J.M., 1999. Screening for resistance to broomrape (*Orobanche cernua*) in cultivated sunflower. Plant Breeding 115: 201-202.
- Duca, M., 2014. Current situation of sunflower broomrape in the Republic of Moldova. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 44-50.
- Echevarría-Zomeño, S., Pérez-de-Luque, A., Jorrín, J., Maldonado, A., 2006. Pre-Haustorial resistance to broomrape (*Orobanche cumana*) in sunflower (*Helianthus annuus*): Cytochemical Studies. J. Exp. Bot. 57:4189-4200.
- Eizenberg, H., Hershenhorn, J., Achdari, G., Ephrath, J.E., 2012. A thermal time model for predicting parasitism of *Orobanche Cumana* in irrigated sunflower-Field validation. Field Crops Res. 137:49-55
- Eizenberg, H., Plakhine, D., Landa, T., Achdari, G., Joel, D.M., Hershenhorn, J., 2004. First report of a new race of sunflower broomrape (*Orobanche cumana*) in Israel. Plant Dis 88: 1284.
- Fernández-Martínez, J.M., Velasco, L., Pérez-Vich, B., 2012. Progress in research on breeding for resistance to broomrape. In: Proc. 18<sup>th</sup> Int. Sunflower Conf., Mar del Plata, Argentina. Int. Sunflower Assoc., Paris, France.
- Fernandez-Martinez, J.M., Dominguez, J., Perez-Vich B., Velasco, L., 2009. Current research strategies for sunflower broomrape control in Spain. Helia. Vol.32. No. 51:47-56.
- Fernández-Martínez, J.M., Melero-Vara, J., Muñoz-Ruz, J., Ruso, J., Domínguez, J., 2000. Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance of the *Or5* Gene. Crop Sci. 40:550-555.
- Fernández-Martínez, J.M., Pérez-Vich, B., Akhtouch, B., Velasco, L., Muñoz-Ruz, J., Melero-Vara, J.M., Domínguez, J., 2004. Registration of four sunflower germplasms resistant to Race F of broomrape. Crop Sci 44: 1033-1034.
- Gâscă, I., Acciu, A., Glijin, A., Duca, M., 2013. Highly virulent races of sunflower broomrape in the Republic of Moldova. In: Proc. Biotech. Congress. Current Opinion in Biotech. 24(1): S132.
- Gomez-Roldan, V., Femas, S., Brewer, P.B., Puech-Pages, V., Dun, E.A., Pillot, J.P., Letisse, F., Matusova, R., Danoun, S., Portais, J.C., Bouwmeester, H., Becard G., Beveridge, C.A., Rameau, C., Rochange, S.F., 2008. Strigolactone inhibition of shoot branching. Nature 455, 189–194
- Gontcharov, S.V., 2009. Sunflower breeding for resistance to the new broomrape race in the Krasnodar region of Russia. Helia 51:75-80.
- Gontcharov, S.V., Antonova, T.S., Araslanova N.M., 2004. Sunflower breeding for resistance to the new broomrape race. Helia 40:193-198.
- Gonzales-Carrascosa, R., 1992. *Orobanche* in sunflower in Spain. Proc. 13th Intern. Sunflower Conf., Pisa, Italy Vol.I:746-749

- Gonzales-Torres, R., Jimenez-Diaz, R., Melero-Vara, J.M., 1980. Distribucion Y virulencia de *Orobanche cernua* Loef. En los cultivos de girasol en Espana. IX Conf. Intern. Del Girasol, Torremolinos, Espana, Tomo I:288-295
- Grezes-Besset, B., 1994. Evaluation de la Resistance du Tournesol a l'Orobanche. Rustica Prograin Genetique. Protocole E-16. Version No 1, p. 1-7.
- Gulya, T.J., Aydin, A., Brothers, M., 1994. Evaluation of Broomrape (*Orobanche cumana*) resistance in sunflower germplasm of the USDA Plant Introduction collection. In: G.J. Seiler (ed.), Proc. 16<sup>th</sup> Sunflower Research Workshop, Fargo, ND. National Sunflower Association: Bismarck, ND, USA. p. 53-55.
- Gurung, S., Mahto, B.N., Gyawali, S., Adhikari, T.B., 2013. Phenotypic and molecular diversity of *Cochliobolus sativus* populations from wheat. Plant Dis 97: 62-73.
- Hargitay, L., 2014. Current situation of sunflower broomrape in Hungary. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 32.
- Hatnianskii, V.I., 1982. Nasledovanie priznaka ustoicivosti podsolnecinika k porajeniu ego novim kompleksom ras zaraziha. Naucin-tehn. Biull. VNIIMK, Krasnodar, Vip.2:3-5
- Heide-Jorgensen H.S., 2008. Parasitic Flowering Plants. Koninklijke Brill NV, The Netherlands. 438 pp.
- Hladni, N., Jocic, S., Miklic, V., Saftic-Pankovic, D., Škorić, D., 2009. Using new Rf inbred lines originating from an interspecific population with *H. deserticola* for development of sunflower hybrids resistant to broomrape. Helia 51: 81-90.
- Honiges, A., Wegmann, K., Ardelean. A., 2008. *Orobanche* resistance in sunflower. Helia 31:1-12.
- Iliescu H., 1984. Annual reports on the occurrence of sunflower diseases in Romania. In Acimovic M., Sunflower diseases in Europe, the United States and Australia, 1981-1983. Helia, 7: 45-54
- Imerovski, I., Dimitrijevic, A., Miladinovic, D., Dedic, B., Jocic, S., Kovacevic B., Obreht, D., 2013. Identification of PCR markers linked to different *Or* genes in sunflower. Plant Breeding 132:115-120.
- Jan, C.C., Fernández-Martínez, J.M., 2002. Interspecific hybridization, gene transfer, and the development of resistance to the broomrape race F in Spain. Helia 25:123-136.
- Jan, C.C., Fernández-Martínez, J.M., Ruso, J., Muñoz-Ruz, J., 2002. Registration of four sunflower germplasms with resistance to *Orobanche cumana* Race F. Crop Sci. 42:2217-2218.
- Jestin, C., 2012. CETIOM. Rapport d'activité 2012. Available at: <http://www.cetiom.fr> (last accessed 30 Sept 2013).
- Jestin, C., Lecomte, V., Duroueix, F., 2014. Current situation of sunflower broomrape in France. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 28-31.
- Jocić, S., Malidža, G., Cvejic, S., Nadaa, H., Vladimira, M., Škorić D., 2011. Development of sunflower hybrids tolerant to tribenuron methyl. Genetika 43: 175-182.

- Joel, D.M., Portnoy, V., Tzuri, G., Greenberg, R., Katzir, N., 1996. Molecular markers for the identification of *Orobanche species*. In: Advances in Parasitic Plant Research; 6<sup>th</sup> Intern. Parasitic Weed Symp., Cordoba, Spain, p.152-160.
- Joel, D.M., Chaudhury, S., Plakhine, D., Ziadna, H., Steffens, J., 2011. Dehydrocostus lactone is exuded from sunflower roots and stimulates germination of the root parasite *Orobanche cumana*. *Phytochem.* 72:624-634.
- Kaya, Y., 2014. Current situation of sunflower broomrape in Turkey. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 55.
- Kaya, Y., Evci, G., Pekcan, V., Gucer, T., 2004. Determining new broomrape-infested areas, resistant lines and hybrids in Trakya region of Turkey. *Helia* 27:211-218.
- Kaya, Y., Evci, G., Pekcan, V., Gucer, T., Yilmaz, M.I., 2009. Evaluation of broomrape resistance in sunflower hybrids. *Helia* 32:161-169.
- Krohin, E.J., 1980. Nasledovanie ustoicivosti k porajeniu podsolnecnika novim kompleksom ras zarazihi. Sel-I Semenovodstvo Masl. Kultur, Krasnodar, pp.14-17.
- Labrousse, P., Arnaud, M.C., Serieyes, H., Berville, A., Thalouarn, P., 2001. Several mechanisms are involved in resistance of *Helianthus* to *Orobanche cumana* Wallr. *Ann. Bot.* 88:859-868.
- Labrousse, P., Arnaud, M.C., Griveau, Y., Fer, A., Thalouarn, P., 2004. Analysis of resistance criteria of sunflower recombined inbred lines against *Orobanche cumana* Wallr. *Crop Prot.* 23:407-413.
- Labrousse, P., Arnaud, M.C., Veronesi, C., Serieys, H., Berville, A., Thalouarn, P., 2000. Mecanismes e resistance du tournesol a *Orobanche cumana* Wallr. In: Proc. 15<sup>th</sup> Int. Sunflower Conf., Toulouse, France. Int. Sunflower Assoc., Paris, France. vol. 2, J-13.
- Lachia, M., Wolf, H.C., De Mesmaeker, A., 2014. Synthesis of strigolactones analogues by intramolecular [2+2] cycloaddition of ketene-iminium salts to olefins and their activity on *Orobanche cumana* seeds. *Bioorg Med Chem Lett* 24: 2123–212.
- Letousey, P., De Zélicourt, A., Vieira Dos Santos, C., Thoiron, S., Monteau, F., Simier, P., Thalouarn, P., Delavault, P., 2007. Molecular analysis of resistance mechanisms to *Orobanche cumana* in sunflower. *Plant Pathol.* 56:536-546.
- Liebenberg, M.M., Pretorius, Z.A., 2011. Pathogenic diversity in and sources of resistance to *Uromyces appendiculatus* in Southern Africa. *J Phytopathol* 159: 287-297.
- Louarn, J., Carbonne, F., Delavault, P., Bécard, G., Rochange, S., 2012. Reduced germination of *Orobanche cumana* seeds in the presence of arbuscular mycorrhizal fungi or their exudates. *PLoS ONE* 7:e49273.
- Louarn, J., Pouilly, N., Boniface, M.C., Blanchet, N., Pérez-Vich, B., Vincourt, P., 2014. Toward a better understanding of the genetic architecture of sunflower (*Helianthus annuus*) resistance to the parasitic plant *Orobanche cumana*. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 178-183.
- Lu, Y.H., Gagne, G., Grezes-Besset, B., Blanchard, P., 1999. Integration of molecular linkage group containing the broomrape resistance gene *Or5* into an RFLP map in sunflower. *Genome* 42:453-456.



- Ma, D.T., Jan, C.C., 2014. Distribution and race composition of sunflower broomrape (*Orobanche cumana* Wallr.) in Northern China. p. 65-69. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France.
- Malidza, G., Skoric, D., Jovic, S., 2000. Imidazolinone resistant sunflower (*Helianthus annuus* L.). Proc. 15<sup>th</sup> Intl. Sunflower Conf. Toulouse, France, June 12-15. Vol. 2 : 42-47.
- Márquez-Lema, A., Delavault, P., Letousey, P., Hu, J., Pérez-Vich, B., 2008. Candidate gene analysis and identification of TRAP and SSR markers linked to the *Or5* gene, which confers sunflower resistance to race E of broomrape (*Orobanche cumana* Wallr.). In: Proc. 17<sup>th</sup> Int. Sunflower Conf., Cordoba. Spain. Int. Sunflower Assoc., Paris, France. p. 661-666.
- Matusova, R., van Mourik, T., Bouwmeester, J., 2004. Changes in the sensitivity of parasitic seeds to germination stimulants. Seed Sci Res 14: 335-344.
- Melero-Vara, J.M., Alonso, L.C., 1988. Los enfermedades del girasol. In: Alonso, L.C., (Ed.), Enfermedades y danos de herbicidas en el cultivo del girasol. Koipesol, Madrid, pp.15-126
- Melero-Vara, J.M., Gracia-Pedrajas, M.D., Perez-Artes, E. and Jimenez-Diaz, R.M., 1996. Pathogenic and molecular characterization of populations of *Orobanche cernua* Loeffl. from sunflowers in Spain. Proc. of the 14<sup>th</sup> International Sunflower Conference. Vol.2: 677-684. Beijing / Shenyang. China. June 12-20. 1996. ISA. Paris.
- Mihaljčević, M., 1996. Distribution and virulence of *Orobanche* population in Yugoslavia. In: Proc. EUCARPIA Symp. Breed. Oil Prot. Crop, Zaporozhye, Ukraine. p. 134-136.
- Miladinovic, D., Jovic, S., Dedic, B., Cvejic, S., Dimitrijevic, A., Imerovski, I., Malidza, G., 2014. Current situation of sunflower broomrape in Serbia. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 33-38.
- Molinero-Ruiz, L., Dominguez, J., 2014. Current situation of sunflower broomrape in Spain. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanche* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 19-27.
- Molinero-Ruiz, M.L., García-Carneros, A.B., Collado-Romero, M., Raranciuc, S., Domínguez, J., Melero-Vara, J.M., 2014. Pathogenic and molecular diversity in highly virulent populations of the parasitic weed *Orobanche cumana* (sunflower broomrape) from Europe. Weed Res 54: 87–96.
- Molinero-Ruiz, L., Delavault, P., Perez-Vich, B., Păcureanu Joița, M., Bulos, M., Altieri, E., Dominguez, J., 2015. “Review: Joint evolution of the race structure of *Orobanche Cumana* and the breeding of sunflower for resistance to the parasitic weed”. Spanish Journal of Agricultural Research. Chp. Plant Protection, pg. 1 – 55, (Vol. 13, Nr. 4, Decembrie, 2015).
- Morozov, V.K., 1947. Sunflower breeding in USSR. Pishchepromizdat. Moscow. (In Russian). p.p. 1-274.
- Okazawa, A., Benesh, J., 2011. Parasitic plant control agent and parasitic plant control method. US 20110251068 A1.
- Paleev, N.G., 1983. Genetika inuniteta podsolnecinika k zarazihe. In: Genetika I Selektiia Rastenii na Donu, Rostov, Izd. Rost. Gos. Univ., pp. 85-90.

- Panchenko, A.Y., 1975. Early diagnosis of broomrape resistance in breeding and improving seed production. Vestnik-S.H.N. No.2:107-115. (In Russian).
- Panchenko, A. J., Antonova, T. S., 1975. Protective reaction of resistant forms of sunflower to new races of broomrape (In Russian). Sbornik-VNIIMK Krasnodar. p. 5-9.
- Păcureanu-Joița, M., 2014. Current situation of sunflower broomrape in Romania. In: Proc. 3rd Int. Symp. on Broomrape (*Orobanch* spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France. p. 39-43.
- Păcureanu-Joița, M., Veronesi, C., Raranciuc, S., Stanciu, D., 2004. Parasite-host plant interaction of *Orobancha cumana* Wallr. (*Orobancha cernua* Loefl) with *Helianthus annuus*. In: Proc. 16<sup>th</sup> Int. Sunflower Conf., Fargo, ND, USA. Int. Sunflower Assoc., Paris, France. p. 171-177.
- Păcureanu-Joița, M., Vrânceanu, A.V., Scare, G., Marinescu, A., Sandu, I., 1998. The evaluation of the parasite-host interaction in the system *Helianthus annuus* L. *Orobancha cumana* Wallr. in Romania., In: Proc. 2<sup>nd</sup> Balkan Symposium on Field Crops. Novi Sad, Yugoslavia. vol. 1. p. 153-157.
- Păcureanu-Joița, M., Fernández-Martínez, J.M., Sava, E., Raranciuc, S., 2009b. Broomrape (*Orobancha cumana* Wallr.), the most important parasite in sunflower. Analele I.N.C.D.A. Fundulea 77:49-56.
- Păcureanu-Joița, M., Raranciuc, S., Sava, E., Stanciu, D., Năstase, D., 2009a. Virulence and aggressiveness of sunflower broomrape (*Orobancha cumana* Wallr.) populations in Romania. Helia 51:111-118.
- Păcureanu Joița M., Ciucă, M., Sava, E., 2012. "Broomrape (*Orobancha cumana* Wallr.), the most important parasite in sunflower: virulence, race distribution, host resistance". Proceeding's of the 18<sup>th</sup> International Sunflower Conference, Mar del Plata, Argentina.
- Pérez-Vich, B., Velasco, L., Rich, P.J., Ejeta, G., 2013. Marker-assisted and physiology-based breeding for resistance to Orobanchaceae. In Parasitic Orobanchaceae; Joel, D.M.; Gressel, J.; Musselman, L.J., Eds.; Springer, New York, pp 369-391.
- Perez-Vich, B., Akhtouch, B., Muñoz-Ruz, J., Fernandez-Martinez, J.M., Jan C.C., 2002. Inheritance of resistance to a highly virulent race F of *Orobancha cumana* Wallr. in a sunflower line derived from interspecific amphiploids. HELIA. Vol.25. Nr. 36:137-144.
- Pérez-Vich, B., Akhtouch, B., Knapp, S.J., Leon, A.J., Velasco, L., Fernández-Martínez, J.M., Berry, S.T., 2004. Quantitative trait loci for broomrape (*Orobancha cumana* Wallr.) resistance in sunflower. Theor. Appl. Genet. 109:92-102.
- Pérez-Vich, B., Velasco, L., Muñoz-Ruz, J., Domínguez, J., Fernández-Martínez, J.M., 2005. Registration of three sunflower germplasms with quantitative resistance to race F of broomrape. Crop. Sci. 46:1406-1407.
- Petrov, P., 1970. Nova fiziološka rasa *Orobancha cumana* Wallroth kod nas. Plant protection in service of agriculture. Sofia, Bulgaria (In Bulgarian). p. 37-48.
- Piednoël, M., Aberer, A.J., Schneeweiss, G.M., Macas, J., Novak, P., Gundlach, H., Temsch, E.M., Renner, S.S., 2012. Next-generation sequencing reveals the impact of repetitive DNA across phylogenetically closely related genomes of *Orobanchaceae*. Mol Biol Evol 29: 3601-3611.

- Pineda-Martos, R., Velasco, L., Pérez-Vich, B., 2014. Identification, characterisation and discriminatory power of microsatellite markers in the parasitic weed *Orobancha cumana*. *Weed Res* 54: 120–132.
- Placek, E.M., 1921. Immunitet podsolnecinika k porajenniu zarazihoi (*Orobancha Cumana*). *Izvestiia Saratovskoi S. h. Opât. St. Saratov*, 3(1-2):1-20.
- Pogorletskiy, B.K., Geshele, E.E., 1976. Resistance of the sunflowers to broomrape, downy mildew and rust. In: *Proc. 7<sup>th</sup> Int. Sunflower Conf. Krasnodar. USSR. Int. Sunflower Assoc., Paris, France.* p. 238-243.
- Pototskyi, G., 2014. Current situation of sunflower broomrape in Ukraine. In: *Proc. 3rd Int. Symp. on Broomrape (Orobancha spp.) in Sunflower, Córdoba, Spain. Int. Sunflower Assoc., Paris, France.* p. 56.
- Pustovoit, G.V., 1966. Mejvidovaia ghibridizația kak mrtod selekții podsolnecinika na gruppovoi immunitet. *Ghenetika*, 1:59-69.
- Rodríguez-Ojeda, M.I., Fernández-Escobar, J., Alonso, L.C., 2001. Sunflower inbred line (KI-374) carrying two recessive genes for resistance against a highly virulent Spanish population of *Orobancha cernua* Loebl. race F. In: *Proc. 7<sup>th</sup> Int. Parasitic Weed Symp., Nantes, France.* p. 208-211.
- Rodríguez-Ojeda, M.I., Pérez-Vich, B., Alonso, L.C., Fernández-Escobar, J., 2010. The influence of flowering plant isolation on seed production and seed quality in *Orobancha cumana*. *Weed Res.* 50:515-518.
- Sala, C.A., Bulos, M., Echarte, M., 2008. Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. *Crop Sci* 48: 1817-1833.
- Sașăperov, F.A., 1913. Opîti s podsolnecinikom. *Trudi Biuro Prikl. Bot.*, 6(4):251-258
- Serghini, K., Pérez-de-Luque, A., Castejón-Muñoz, M., García-Torres, L., Jorrín, J.V., 2001. Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobancha cernua* Loebl.) parasitism: Induced synthesis and excretion of 7-hydroxylated simple coumarins. *J. Exp. Bot.* 52:2227-2234.
- Shi, B.X., Chen, G.H., Zhang, Z.J., Hao, J.J., Jing, L., Zhou, H.Y., Zhao, J., 2015. First report of race composition and distribution of sunflower broomrape, *Orobancha cumana*, in China. *Plant Dis* 99: 291.
- Škorić, D., Jocić, S., 2005. Broomrape (*Orobancha cumana* Wallr.) and its possible control by genetic and chemical means. Production and Processing of Oil seeds. In: *Proc. 46<sup>th</sup> Oil Industry Conf. (In Serbian).* p. 9-21.
- Škorić, D., Păcureanu-Joița, M., Sava, E., 2010. Sunflower breeding for resistance to broomrape (*Orobancha cumana* Wallr.). *Analele I.N.C.D.A. Fundulea* 78: 63-79.
- Smith, C.E., Dudley, M.W., Lynn, D.G., 1990. Vegetative–parasitic transition control and plasticity in *Striga* development. *Plant Physiol* 93: 208–221.
- Sukno, S., Melero-Vara, J., Fernández-Martínez, J.M., 1999. Inheritance of resistance to *Orobancha cernua* Loebl. in Six Sunflower lines. *Crop Sci.* 39:674-678.
- Tang, S., Heesacker, A., Kishore, V.K., Fernández, A., Sadik, E.S., Cole, G., Knpp, S.J., 2003. Genetic mapping of the Or5 gene for resistance to *Orobancha* Race E in sunflower. *Crop Sci.* 43:1021-1028.

- Teryokhin, E.S., 1976. Peculiarities of ontogenesis of the *Orobanche cumana* Wallr. Parasitizing sunflower roots. Proc. 7th Intern. Sunflower Conf., Krasnodar, U.S.S.R., Vol.II:312-315
- Teryokhin, E.S., 1992. Germination, invasion of *Orobanche cernua* Loebl. and its development on roots of *Helianthus annuus* L. Proc. 13th Intern. Sunflower Conf., Pisa, Italy Vol.I:854-858
- Tingrui, L., Baichun, D., Lijing, X., Haiyan, Y., 1996. Happening and controlling of broomrape. Proc. 14<sup>th</sup> Intern. Sunflower Conf., Beijing/Shenyang, China, Vol.II: 684-686
- Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., *et al.*, 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455: 195–200
- Velasco, L., Pérez-Vich, B., Jan, C.C., Fernández- Martínez, J.M., 2007. Inheritance of resistance to broomrape (*Orobanche cumana* Wallr.) race F in a sunflower line derived from wild sunflower species. *Plant Breeding* 126:67-71.
- Velasco, L., Pérez-Vich, B., Yassein, A.A.M., Jan, C.C., Fernández-Martínez, J.M., 2012. Inheritance of resistance to broomrape (*Orobanche cumana* Wallr.) in a interspecific cross between *Helianthus annuus* and *Helianthus debilis* subsp. *tardiflorus*. *Plant Breeding* 131:220-221.
- Venkov, V., Shindrova, P., 1998. Development of sunflower form with partial resistance to *Orobanche cumana* Wallr. by seed treatment with N: trisomethlurea(NMU). In: K. Wegmann, L.J. Musselman, and D.M. Joel (eds.). Current problems of *Orobanche* research. Proc. 4<sup>th</sup> Int. Symp. Orobanch. Albena, Bulgaria. p. 301-305.
- Vrâncănu, A.V., Tudor, V.A., Stoenescu, F.M, Pirvu, N., 1980. Virulence groups of *Orobanche cumana* Wallr., differential hosts and resistance source genes in sunflower. vol 2, In: Proc. 9<sup>th</sup> Int. Sunflower Conf., Torremolinos. Spain. Int. Sunflower Assoc., Paris, France. p. 74-82.
- Wegmann, K., 1998. Progress in *Orobanche* research during the past decade. In: K. Wegmann, L.J. Musselman, and D.M. Joel (eds.). Current problems of *Orobanche* research. Proc. 4<sup>th</sup> Int. Symp. Orobanch. Albena, Bulgaria. p. 13-17.
- Yoneyama, K., Xie, X., Sekimoto, H., Takeuchi, Y., Ogasawara, S., Akiyama, K., Hayashi, H., Yoneyama, K., 2008. Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. *New Phytologist* 179: 484–494.
- Ždanov, L.A., 1926. Selekcija suncokreta na Donskoj oglednoj stanici. Sunflower, pp. 219-233. (In Russian).
- Zoltán, P., 2001. Napraforgószádor - (*Orobanche cumana* Loebl.) populációk patogenitásának vizsgálata. *Növényvédelem* 37: 173–182.