Broomrape (*Orobanche cumana* wallr.), the most important parasite in sunflower: virulence, race distribution, host resistance

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ABSTRACT

∗ Broomrape (*Orobanche cumana* Wallr.) has a long history of parasitism on sunflower, longer than one century, starting with Russia and followed by other countries in Europe and in Asia. During this period, this serious parasite had some times characterized by different virulence degree, number of the parasite races and developed sunflower resistant genotypes. In the 1960-1970s period, Russian researchers identified the first two races of this parasite (A and B), after that, being identified other four races (C, D, E and F) as well as the sunflower differentials carrying the dominant genes for resistance, by the researchers in Romania and Spain. In the last years, some authors have communicated the appearance of the new, very virulent populations of broomrape, in different regions cultivated with sunflower, over the world. The aim of our investigation was to compare the virulence of broomrape samples collected in different areas cultivated with sunflower and infested with broomrape, from Europe and Asia. The same, we studied the dissemination of these populations in time and territory, in relation with different sunflower resistant genotypes. It was evaluated the influence of the parasite populations on several important traits in sunflower.

∗ Fourteen populations of broomrape collected from Bulgaria, China, Moldova, Romania, Russia, Serbia, Turkey, Spain and Ukraine have been used in the artificial infestation conditions, for establishing the presence of different broomrape (*Orobanche cumana* Wallr.) races in these areas. Ten of these populations have been analyzed using RAPD markers. The broomrape samples were stored in saved conditions and used for artificial infestation in the green house. There have been tested sunflower differentials for the broomrape races until the sixth one and, different hybrids and lines with different resistance to the newest virulent populations of the parasite.

∗ Results of evaluation of sunflower differentials for different races or populations of the parasite *Orobanche cumana* have demonstrated that the new, very virulent populations of broomrape are present in sunflower crop cultivated in Moldova, Romania, Russia, Turkey and Spain as well as, in Bulgaria. The most aggressive populations are in Moldova, Romania, Russia and Turkey. In each country there are two or three different populations of broomrape. In Moldova there are two broomrape populations, spread in northern part (race F) and in southern (a more virulent population, with an attack frequency of 100% on the inbred line LC 1093). In Romania, the three more spread broomrape populations in the largest area cultivated with sunflower, are very different regarding the virulence and dissemination of the parasite. The broomrape populations in Russia are very similar with populations in Romania, excepting broomrape from Rostov this being almost similar with the most virulent population in Turkey. The population of broomrape from China has not attacked the differential for the race E, the inbred line P-1380. The population collected from Serbia has not attacked the differential for the race F, the inbred line LC 1093, as well as the population from Ukraine. The population from Bulgaria has attacked the line LC 1093, with very small infestation degree (0.7%). The population collected from Spain has attacked the line LC 1093, with 1.2 % infestation degree. RAPD markers analyze has divided the ten studied broomrape populations in three groups. The genetic distance is not correlated with the geographic distance. Some of tested sunflower hybrids, show different attack degree of the parasite, some of them having high resistance to the new virulet populations of this.

∗ It could be concluded that the virulence of broomrape populations is very different in the infested areas cultivated with sunflower over the world. The most aggressive populations of the parasite are in the Black Sea area, their dissemination being rapidly In the sunflower seed market in Europe, there are full resistant or some high tolerant sunflower hybrids to this very virulent broomrape.

∗ Knowledge on genetic structure and evolution of *Orobanche cumana* populations, using classical or molecular methods, will provide plant pathologists and breeders with information necessary for classifying them and to establish a new differential set for the parasite races. This will contribute to the sustainable strategies for the sunflower broomrape control.

Key words: sunflower; broomrape; virulence; genetic variability; resistant genotypes

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.) is one of the most dangerous parasites of sunflower which deprives the plant of water and mineral substances. This parasite has 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 in Russia and date back to the 1890s. The same author mentions that it was identified the first race of broomrape (race A) in the breeding station of Saratov area (communicated by Placek, 1918) and the second race (B), communicated by Zdanov (1926), in Rostov area. Soon, after being discovered the two races of the parasite, it was developed a number of sunflower varieties resistant to them. Later on, a new race that could not be controlled by the genes for resistance to races A and B was identified in Moldova by Sharova (1968). Through genetic research, Vranceanu et al. (1980) established that there were five broomrape races (A, B, C, D, E) in Romania and identified dominant genes controlling resistance to them. They also identified a set of differential lines that had cumulative resistance to the five successive races, conferred by the dominant genes *Or1, Or2, Or3, Or4* and *Or5*, respectively. Alonso et al. (1996) found a new, virulent race (race F) of the parasite, in Spain. The race F was found in Romania too, being identified and the dominant gene *Or6* in the inbred line LC 1093, which confers resistance to this race (Pacureanu-Joita, 1998). Changes of the broomrape race composition in Romania have been reviewed (Pacureanu-Joita et al., 2009). The findings of the study show that a new virulent population of the parasite has appeared in the country, started with 2006 year. In the Trakya region in Turkey, the race composition of broomrape changed frequently. According to Bulbul et al. (1991), race E was dominant from 1983 to 1990, after which race F appeared. Kaya et al. (2004) reported a new race in Turkey, which can not be controlled by the *Or6* gene. The same author (Kaya et al., 2009) reported that genes for resistance to the newest, virulent races of the parasite have been found in several lines and hybrids, belonging to different seed companies. In Bulgaria, Shindrova (2006) made an overview of the broomrape races. According to her findings, race E of the parasite is the most widely distributed one, race F being in spreading process. Studies by Antonova et al. (2009) and Goncharov (2009), both discuss the change of broomrape races in Russia. It is known that broomrape races change frequently in Ukraine and Moldova too, and that, although no public reports have been made yet. According to Skoric and Josic (2005) race E is dominant in Serbia. Broomrape has been present in China for a long time too, and identification has been made of race A (Baichun et al., 1996). New races have appeared in the country since, but the race composition has not been determined yet.
The aim of this study is to understand genetic variability of the broomrape parasite, in the frame of the parasitic system *Helianthus annuus* L.-*Orobanche cumana* Wallr.

MATERIALS AND METHODS

Seventeen broomrape populations collected from different fields cultivated with sunflower, in nine countries from Europe and Asia have been studied in the artificial infestation conditions, in the greenhouse or in phytotron. The seeds collected in 2008, 2009 or 2010 years were stored in refrigerator at the temperature of four degree.
In the experiments for the broomrape virulence evaluation there have been used the differential lines for the races D (S-1358 line), E (P-1380 line) and F (LC 1093), as well as some hybrids resistant to the new populations of the parasite. It was used as check for sensitivity the inbred line AD-66. These hybrids have been tested before, in the natural infested fields in Romania, In Turkey or in Spain.
Broomrape tests were performed using two methods. For test in phytotron wooden cases 1.0x0.5x0.01 m in size, have been used, in which a soil-sand mixture (50:50) is laid, this being thoroughly mixed with 5 g broomrape seeds and 500 g sand. In this case 9 sunflower rows are sown, 10 cm apart. Starting with 26 days from emergence, plants can be removed, in order to note occurrence or absence of broomrape attack on sunflower plant roots. In the greenhouse, vegetation pots of 10 l in capacity have been used, and a homogenous mixture of earth, sand and broomrape seeds is poured inside, infestation being carried out with 0.5 g broomrape seeds, mixed with sand (1:9). The broomrape attack was noted after sunflower blossoming, when broomrape plants appear on soil surface. The attack degree was calculated using McKinney formula (quoted by Acimovic, 1979).
Ten populations of broomrape have been analyzed using RAPD markers. For DNA analysis, total DNA was isolated from floral buds, following Doyle &Doyle (1991) protocol. The RAPD method was optimized to generate reproducible fingerprints from genomic DNA. All primers were synthesized by University British Colombia (Canada). Genetic distance was computed after Nei&Li (1979) formula.

RESULTS AND DISCUSSION

Seventeen populations of broomrape (*Orobanche cumana* Wallr.), collected from different areas are presented in table 1. The seeds have been collected in different three years.

Table 1. The broomrape populations collected from different infested fields

Location (area)	Country	Year	Location (area)	Country	Year
Braila	Romania	2010	Cimislia	Moldova2	2009
Constanta 1	Romania	2010	Radnevo	Bulgaria	2010
Constanta 2	Romania	2010	Zaporozhye	Ukraine	2010
Constanta 3	Romania	2009	Ecija	Spain	2008
Cuza-Voda	Romania	2010	Tekirdag	Turkey	2010
Tulcea	Romania	2010	Edirne	Turkey	2009
Rimski Sancev	Serbia	2010	Stavropol	Russia	2008
Stefan Voda	Moldova 1	2009	Rostov	Russia	2009
Jilin	China	2010			

These populations were used in two studies. The first study was made by testing in the artificial infestation condition, using differential lines for different broomrape races. The results presented in the figure 1 are showing that the differential line for the race E of the parasite was attacked by all broomrape populations used in this experiment, excepting the population from China. The attack degree on this line was higher in case of source of broomrape from Rostov- Russia and Ukraine. The differential line for the race F was attacked only in case of sources of broomrape from Moldova (one population), Spain, Turkey, Russia, Bulgaria and two sources from Romania. The populations from Ukraine, Serbia, one from Moldova, one from Romania and China did not attack this line. This it means that the new virulent populations of the parasite it is still not present in these five regions.

Taking into consideration these results, it was made a study of the five populations of broomrape, using the differential line for the race D of the parasite. The results presented in figure 2 show that this line was attacked in all cases, including broomrape population from China, the attack degree being not so high comparing with the check for sensitivity. The hybrid PR64A83, which is known as resistant to the race E of the parasite, was attacked, but the attack degree was small (between 3 and 10 percent).

The broomrape population from China attacked the differential line for the race D, this meaning that in sunflower crop in China, the parasite has developed in the last ten years, four new races (B, C, D and E), taking into consideration the authors communications, before.

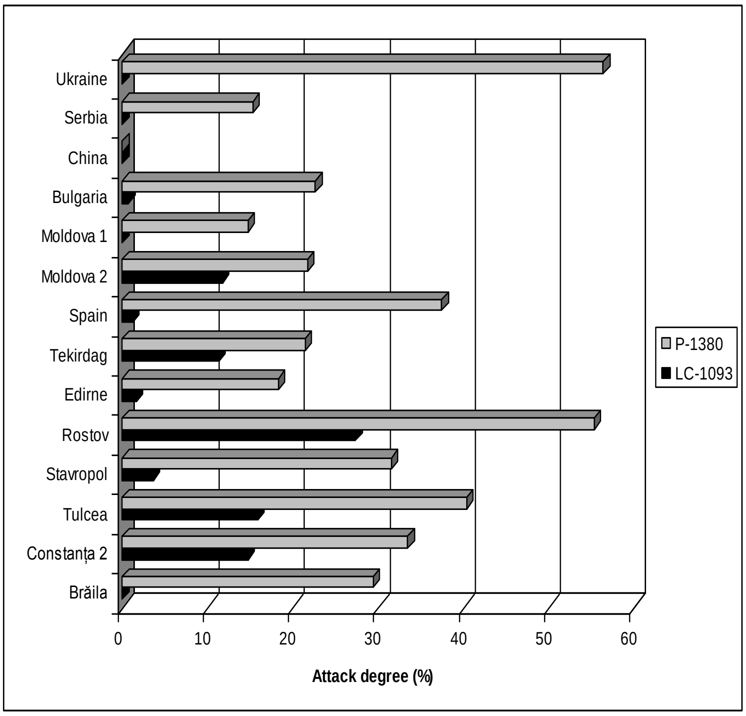

Fig. 1. Results regarding the broomrape attack, on the differential lines for the races E and F of the parasite

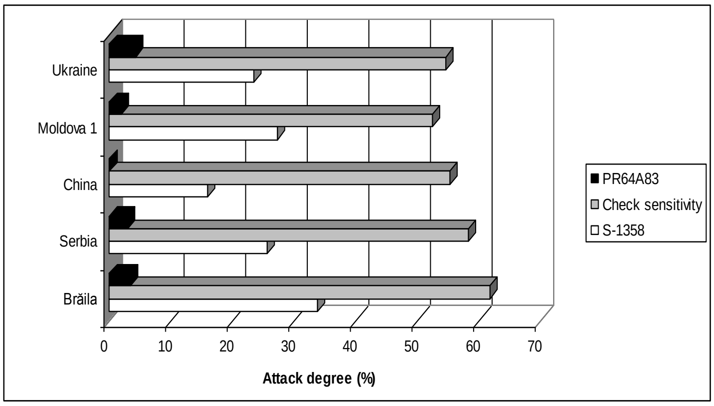

Fig. 2. Results regarding the five broomrape populations attack on the differential for the race D of the parasite

The results presented in figure 3, give the information about the virulence of the nine broomrape sources on three commercial hybrids, known as resistant to the new virulent populations of the parasite. The hybrid PR64A71 was attacked with higher degree, being not full resistant to any broomrape population. The hybrid LG-5550 was attacked in case of seven broomrape sources, excepting populations from Bulgaria and Moldova 2.

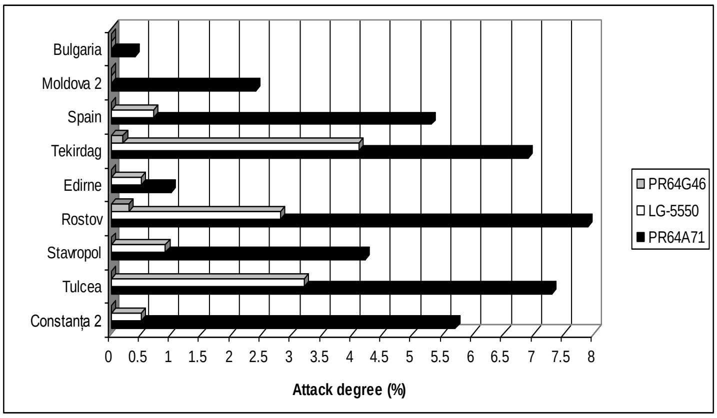

Fig. 3. Results regarding the broomrape attack on some hybrids resistant to the new populations of the parasite

The hybrid PR64G64 was attacked only in case of broomrape sources from Tekirdag – Turkey and Rostov –Russia, being full resistant to the other seven populations. These results demonstrate that the most virulent populations of broomrape are in fields situated in part of Turkey and Rostov –Russia. Anyway, taking into consideration the results obtained in our previous experiments, the virulence of these populations is increasing, year by year.

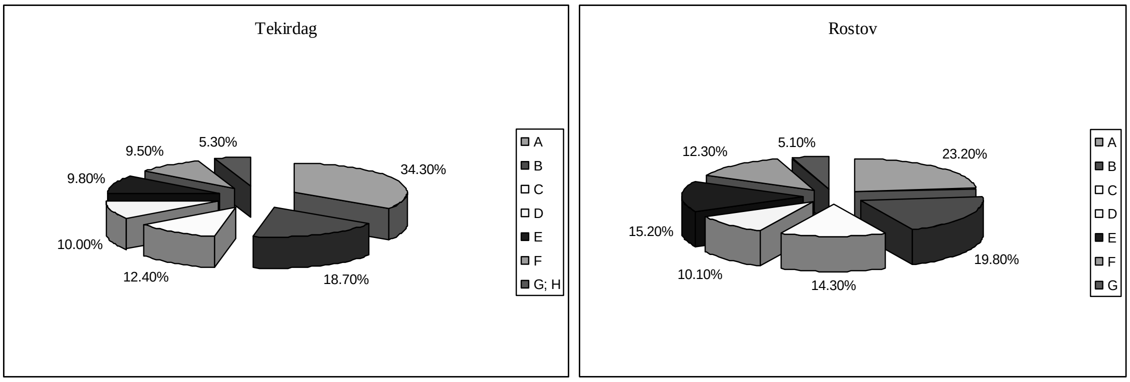

Fig. 4. Broomrape races ratio in the parasite populations from two areas, 2010 year

Taking into consideration the intensity of broomrape attack on each sunflower differential line, we established the races ratio in the parasite populations from two infested areas. The results presented in figure 4 show that all races are included in these populations, each in different percentage.

Comparing the broomrape populations from this point of view, it could be seen that there is a difference between the populations from Tekirdag –Turkey and Rostov –Russia, in the presence of the old races.

The 15 primers were used for genetic variability evaluation of ten broomrape populations. From these primers only 7 have produced polymorphic bands. Although the number of score polymorphisms yielded by the 7 primers was small, only the most consistently reproducible bands from repeated PCR amplification were considered in the study. DNA profiles showed relatively low polymorphism between these populations.

The RAPD pattern with UBC 8 primer (Fig. 5.) revealed that the population Constanta 3 (Romania) is different compared with all other populations.

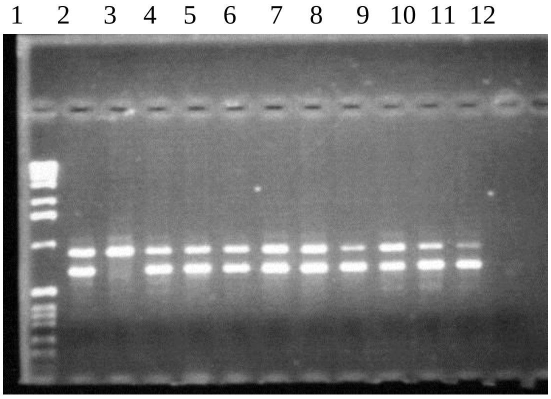

Fig. 5. RAPD pattern obtained with UBC 8. Lanes: 1-Ladder 1 kb; 2-Spain; 3-Constanta 3; 4-Serbia;5- Mold. 2; 6-Constanţa 1; 7-Moldova 1; 8-Tulcea 1; 9-Turkey ; 10-Constanţa 2; 11-Braila ; 12-C. Voda sources.

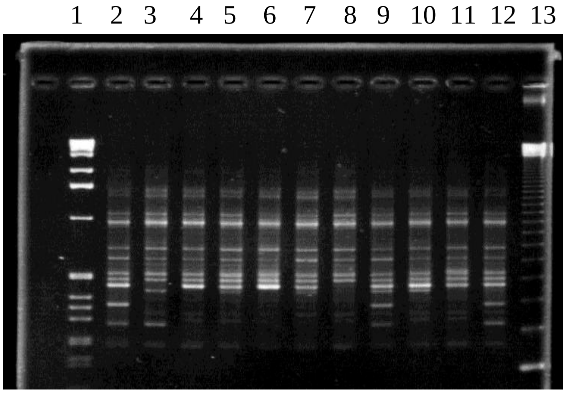

Fig.6. RAPD pattern obtained with UBC 250 Lanes: 1-Ladder 1 kb; 2-Spain; 3-Constanta 3; 4-Serbia; 5- Mold. 2; 6- Constanţa 1; 7-Mold. 1 ; 8-Tulcea ; 9-Turkey ;10-Constanţa 2 ; 11-Braila; 12-C. Voda sources; 13-Ladder 123 pb

The UBC 250 primer showed different patterns, such: similarity of Spain and Romania Cuza-Voda populations, and on the other hand, similarity of Romania – Braila and Serbia populations, which presented all polymorphic bands existent at Spain and Constanta 3 – Romania populations. (Fig. 6.)

According to our results there is a clear difference between the broomrape sources from Constanta 3 and Cuza-Voda, both from Romania, compared with all the other sources.

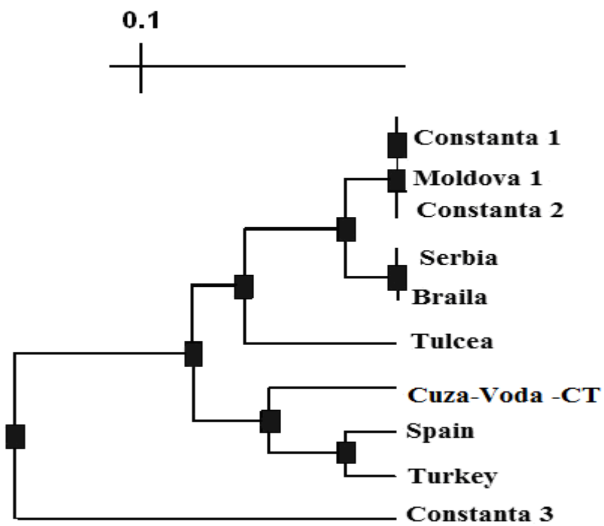

Fig. 7. UPGMA dendrogram using Nei & Li (1979) genetic distance among *Orobanche cumana* populations

The cluster analysis based on RAPD amplification of broomrape populations is given in figure 7. The dendrogram divided the ten broomrape populations in two groups: one group for Constanta 3 and the second group for all the other populations. The second group comprises other two categories. The genetic distance is not correlated with the geographic distance.

The study of the different broomrapes is of a great importance since the understanding of the variability within and between pathogenic populations is essential for developing sources of resistance.

ACKNOWLEDGEMENTS

We would like to thank to all colleagues (Aliona Glijin- UASM, Moldova; Tatiana Antonova- VNIIMK Institute, Krasnodar, Russia; Ma Ping- Academy of Agriculture Sciences, Hebei, China; Yalcin Kaya-Edirne Institute, Turkey; Rosa Gimenez- Arlesa, Spain; S. Masirevic- Novi Sad, Serbia) which provided us the broomrape samples, used in our experiments.

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