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Racial status of *Orobanche cumana* Wallr. in some countries other the world

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Abstract: The study represents a review of the evolution of the parasitic plant *Orobanche cumana* Wallr. and current racial status in some sunflower cultivating countries. Identification of racial status of broomrape populations belonging to eight sunflower cultivating countries from Europe and Asia (Republic of Moldova, Romania, Bulgaria, Ukraine, Spain, Turkey, Serbia and China) revealed the presence of high virulent races G and H in the majority of countries, except Serbia, where accessions belonging to race E or less virulent than E were found. The most virulent race H was identified particularly in the Black Sea area (Romania, Ukraine and Turkey). Additionally, the emergence of new biotypes characterized by high virulence, overcoming the resistance genes to race H was observed in some areas of the Republic of Moldova, Romania and Turkey, which signifies the importance of periodic evaluations of racial status for the prevention of occurrence and dissemination of new races. Analyzing the occurrence chronology of broomrape races in different countries we conclude that by the mid-1990s, the state of play was relatively stable, the most aggressive race being E. After this period the process of *O. cumana* development and appearance of new more aggressive and virulent biotypes became faster, which may be explained by the intensification of sunflower breeding activities and, respectively by the selection pressure exerted on the parasite by new resistant hybrids.

Keywords: broomrape races; differentials; *Orobanche cumana*; sunflower; virulence.

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Introduction

Sunflower broomrape (*Orobanche cumana* Wallr.) is an obligate root parasite that uses water and nutrients from the host plant. This affects significantly the quality and quantity of the harvest and causes considerable economic damage. *O. cumana* is known to grow under semi-dry conditions in various regions of Europe, especially in southern Europe and the Balkans (Vranceanu 2000). The first-ever report of broomrape infestation on sunflower dates back to the second half of the XIX century (1860–1865), in the central Russian region of Voronezh (Oldamov 1866). In a few decades, the parasite spread rapidly practically in all sunflower production areas of the USSR (Plachek 1913). Broomrape attacks became more intense at the beginning of the 20th (Pustovoit 1937) and by the second half of the century, the parasite migrated to the South Eastern Mediterranean (Spain in 1958) (Díaz-Celayeta 1977) and East Asia (China in 1959) (Li et al. 1982). In recent years broomrape has spread to new areas in Portugal (González-Cantón et al. 2019), France (Jestin et al. 2014), Israel (Eizenberg et al. 2004) and Tunisia (Amri et al. 2012). Until now, *O. cumana* has been identified in about 60 countries around the world (CABI 2021). Broomrape is a highly variable pathogen and the virulence of the parasite populations has changed over the years. Currently, eight races of *O. cumana* are known (from A to H), the most virulent of them being F, G and H, which spread rapidly, occupying new areas (Molinero-Ruiz et al. 2015). The first two broomrape races (A and B) have been identified in 1930–1935 in Russia and Ukraine (Antonova 2014). In the 1960s some new pathotypes of broomrape were reported in the South-West of the Soviet Union and it was found that broomrape populations belonging from Chisinau (Republic of Moldova) were more aggressive than those observed in Krasnodar (Buherovici 1967; Sharova 1968). Thus, a new race of pathogen, named race C, has been identified. Later, this race has been revealed in new areas of the Soviet Union (Burlov and Kostyuk 1976), in the South-Eastern part of Romania (Vranceanu et al. 1981), as well as in Bulgaria (Petrov 1968). In 1976–1979 races D and E appeared in Eastern Europe (Vranceanu et al. 1981) and then (in the 1980s) in Spain (Melero-Vara et al. 1989). At the beginning of the 1990s a new pathogenic form, race F, was identified in Turkey (Bulbul et al. 1991), being subsequently described in Spain (Alonso et al. 1996) and Romania (M. Pacureanu-Joita, personal communication). Later (in 2005), a new highly virulent population of broomrape, named race G, was detected in Romanian sunflower fields, which over 9 years was overtaken by a more aggressive biotype – race H (M. Pacureanu-Joita, personal communication). This chronological analysis suggests that the appearance of the highly aggressive biotypes and broomrape races is associated with the centers of intensive breeding of sunflower, as established for other pathogens and crops (Stubbs 1988). So, the first three races (from A to C) initially were identified and mentioned on the territory of the former USSR, where the most important centers for sunflower breeding were located (L.A. Jdanov in Rostov, V.S. Pustovoit in Krasnodar). These races have been appeared over a period of almost 100 years from the first mention and dominated the areas where Soviet sunflower varieties were grown until the 1970s and 1980s. Subsequently, with the use of the heterosis phenomenon in plant breeding and the introduction of commercial hybrids, new broomrape races have been identified in European countries. The evolutionary dynamics of the parasite is faster in the last years (five races appeared in about 50 years), which reflects the intensity of the breeding process.

Despite this extensive information, it is still unclear whether these eight races are the same in all sunflower growing countries since there is no universal set of differential lines. The set of sunflower inbred lines proposed by Vranceanu et al. (1980) is suitable only for the determination of the racial status of the first five races. Moreover, the identification of the last broomrape races is more difficult due to the more complex genetic determinism of resistance against the parasite (two recessive genes, two partially dominant genes, partial dominance etc.) (Cvejic et al. 2020), comparative to the monogenic and dominant inheritance of resistance to races A to E. Different inbred lines and commercial hybrids are currently used to discriminate races, that create confusion regarding the current racial status of broomrape populations from different countries. Additionally, there are no universally accepted criteria for the appreciation of resistance or susceptibility of sunflower genotypes against broomrape. Thus, the comparative analysis of *O. cumana* populations belonging to different countries or regions in the same conditions using the same set of differentials is important.

In this context, this paper aimed to evaluate the virulence of broomrape populations belonging to eight sunflower cultivating countries from Europe and Asia (Republic of Moldova, Romania, Bulgaria, Ukraine, Spain, Turkey, Serbia and China).

Materials and methods

Tests were performed under artificial infestation conditions using broomrape seeds from 39 populations, as follow: 15 populations from the Republic of Moldova (Soroca, Balti, Prepelita, Cazanesti, Izbiste, Holercani, Chisinau, Sarata Mereseni, Gura Galbenei, Grigorievca, Popeasca, Congaz, Svetlii, Taraclia, Alexanderfield); four from Bulgaria (Radnevo, Rosenovo, Selanovtsi, Debovo); two from Romania (Tulcea and Braila); two from Ukraine (Odesa and Izmail); one from Spain (Sevilla); five from Turkey (Trakya, Merkez, Keșan, Luleburgaz, Adana); seven belonging to Serbia (conventionally noted as ORSR 04, ORSR 07, ORSR 11, ORSR 14, ORSR 25, ORSR 26 and ORSR 43) and three from China (Hohhot, Inner Mongolia; Bayanuur, Inner Mongolia and Hebei).

The virulence of *O. cumana* populations was evaluated on a set of differentials, including one susceptible (Performer) and six resistant genotypes, offered by the National Agricultural Research and Development Institute Fundulea, Romania (NARDI) and Limagrain Company: LC1003A (from NARDI) – resistant to race E; line LC1093A and hybrid Favorit (NARDI) – resistant to race F; hybrids H_1E (NARDI) and LG-5661 (Limagrain) – resistant to race G and H_2Lg (Limagrain) – resistant to race H.Thirty plants for each sunflower genotype, separated into two replications of 15 plants each, have been planted in boxes, which contained a mix of sand and peat in 1:1 vol ratio, uniformly infected with broomrape seeds (30 mg seeds at 200 g of mix) from each population. After the cultivation period, the plant root system was removed from the substrate and washed. To establish the aggressiveness and virulence of broomrape populations the total number of infected plants, as well as the number of broomrape attachments (tubercles, aerial and underground shoots) per host, were quantified. The incidence of broomrape (attack frequency – *F*), the average number of broomrape attachments per plant (attack intensity – *I*) and attack rate (AR) have been established using the following formulas: $F_{(\%)} = (N/Nt) \times 100$, where *N* – number of infected plants, *N*t – total number of observed plants; $I = a/N$, where *a* – total number of broomrape attachments, *N* – number of infected plants and $AR = (F \times I)/100$. The plants that had 0–5% frequency and 0–1 AR values were considered resistant ones (Vranceanu et al. 1980).

Results and discussions

Analysis of the racial status of broomrape populations from the Republic of Moldova

Broomrape has been firstly observed on the territory that is the today Republic of Moldova, from 1860 to 1870, in the same time frame such as in Voronezh. Subsequently, Afanasev and Arhanghelschi (1937) described the different levels of infestation of agricultural fields, depending on the cultivated varieties, that suggest the presence of two races with different virulence (A and B), similar to those reported in Russia and Ukraine (Plachek 1913; Zhdanov 1927). Research of the Experimental Station of Oil Plants (Chisinau branch of VNIIMK) during 1960–1970 on populations of *O. cumana* belonging from different regions of the country showed the existence of more than 60 biotypes of broomrape in Moldova. According to virulence evaluation results, most of them have been attributed to race A (65%) and B (25%). Furthermore, more virulent biotypes (race C) were identified, which overcame genetic resistance and infested the sunflower varieties resistant to race A and B (Buherovici 1967; Sharova 1968).

The studies conducted at the beginning of the 2000s in five districts of the country revealed the presence preferentially of the race of A (30–50%), B and C (18–25%). Also, race D and E were identified in a small percentage (8–18% and

2–5%, respectively) and race F – sporadically (Petcovici et al. 2009). Later Rotarenco (2010) confirmed the presence of race F in Moldova. In a few years, a new more aggressive race G was identified in some fields simultaneously with the already reported races D, E and F (M. Duca, personal communication). The research focused on race identification were extended in 2014, when more than 90 sunflower fields from different regions of Moldova (north, central and south) were analyzed and 39 broomrape populations were collected. During expeditions, it has been established that *O. cumana* was mostly present in the central and southern parts of the country and practically absent in the north. According to the results of virulence evaluation, the physiological race E or less virulent than E (35.9%), F (15.4%), G (25.6%) and H (23.1%) was identified in Moldovan sunflower fields. In the central part of the country race ≤ E prevailed (65%), while in the south the more aggressive races G and H were predominant (in around 63% of fields) (M. Duca, personal communication).

In 2019–2020 a new evaluation was performed and 15 broomrape populations from the same locations (not the same fields) as in 2014 were collected and analyzed. According to the data, six of the analyzed populations belong to the same races as in 2014 (Figure 1). Thus, the populations from Balti, Sarata Mereseni, Svetlii, Taraclia, Alexanderfield were attributed to race H or even to a more virulent pathotype in the case of the last three populations that parasitized including the sunflower hybrid H_2Lg resistant to race H (Figure 1D). The most aggressive one was the population belonging to Taraclia, it showed a moderate frequency of attack (40.0%), with a low number of broomrape attachments per host plant (2.0), followed by the population from Svetlii with one attachment per plant and low level of parasite incidence (13.3%). Similarly, as in previous studies the population belonging to Cazanesti affected only the susceptible genotype and can be attributed to race E or less virulent than E.

In other cases, the results differed from those reported in 2014, the majority of *O. cumana* populations being attributed to more virulent races. The populations from Izbiste and Holercani previously determined as race E or less virulent than E affected the hybrid H_1E resistant to race G (both populations), as well as the hybrid H_2Lg resistant to H (Izbiste). Population from Holercani showed a moderate frequency of attack (57.9%) and a high number of broomrape shoots (5.0) on hybrid H_1E (Figure 1C). In the case of Izbiste a moderate incidence of attack (13.3 and 6.7%), with a low number of *O. cumana* attachments was observed on hybrid H_1E and H_2Lg, respectively (Figure 1C and D). Similarly, the populations from Chisinau and Stefan-Voda (Popeasca) known as belonging to race ≤ E and F, respectively, affected inclusively LC1003 and LC1093 lines resistant to race E and F, being identified as race G. The Popeasca population parasitized the H_1E hybrid too, but the attack severity level was very low (frequency – 6.7%, attack rate – 0.07).

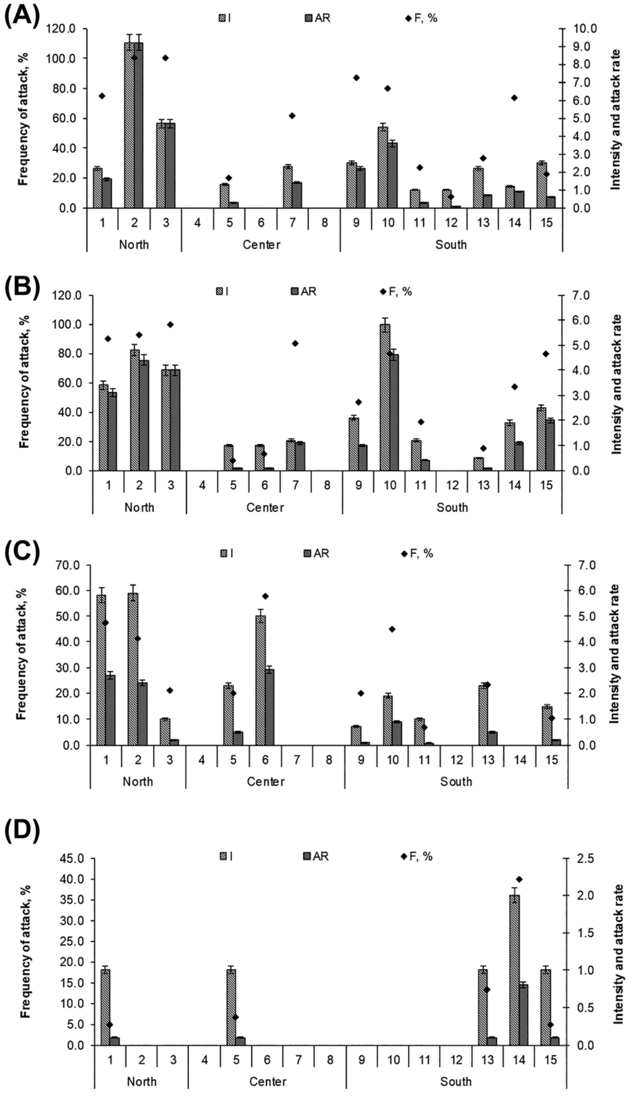

Figure 1: The frequency, intensity and attack rate of broomrape populations from the *Republic of Moldova* on the differential genotypes.
(A – LC1003A resistant to race E; B – LC1093A resistant to race F; C – hybrid H_1E resistant to race G; D – hybrid H_2Lg resistant to race H). 1 – Soroca; 2 – Balti; 3 – Prepelita; 4 – Cazanesti; 5 – Izbiste; 6 – Holercani; 7 – Chisinau; 8 – Gura Galbenii; 9 – Sarata Mereseni; 10 – Grigorievca; 11 – Popeasca; 12 – Congaz; 13 – Svetlii; 14 – Taraclia; 15 – Alexanderfield.

Populations belonging to Prepelita and Grigorievca infested all sunflower genotypes excepting the hybrid H_2Lg, subsequently being attributed to race H, comparative to race F in the previous study. They showed a high level of incidence (80.0–100.0%), with a high number of broomrape attachments per host on differential lines and moderate incidence (21.0–45.0%), with a low number of attachments (1.0–1.9) on hybrid H_1E. Also, the population from Soroca, previously determined as race G, was attributed to a more virulent race. According to results, broomrape collected from Soroca affected all sunflower genotypes, including the hybrid H_2Lg resistant to race H line (F – 5.0%; I – 1.0) that suggests an occurrence of new biotypes characterized by higher virulence as race H. The population from Gura Galbenei infested only the susceptible genotypes and was classified as race E or less virulent than E, while in previous analysis race G was identified in this location.

These results suggest the existence of pathotypes with different virulence in the same or nearly situated fields, as well as the appearance of new highly aggressive biotypes (in Soroca, Izbiste, Svetlii, Taraclia and Alexanderfield) able to infest even the hybrids considered resistant to the most virulent race H. High heterogeneity in virulence of broomrape and presence in the same field of an admixture of biotypes (races), where one or other type is dominant, was reported by Antonova et al. (2020).

The chronological analysis highlights the slow evolution of the parasite in the early stages of its appearance and more rapid evolution in the later years, which can lead to virulence explosion in a relatively short time. The new, more aggressive broomrape races have obtained a position of dominance over the old ones in the populations, replacing them.

Analysis of the racial status of broomrape populations belonging from different sunflower cultivating countries

Along with the extension of sunflower production, the parasite has spread to new areas. In Bulgaria *O. cumana* was first detected on sunflower in 1935 (Encheva and Shindrova 1994), being distributed especially in the Black Sea Coast of eastern Bulgaria. In the 1950s, Knyazkov reported the presence of race A and B and in 1966, the emergence of race C, which highly affected the Peredovik variety (Petrov 1968). After 1990, a massive attack of hybrids and varieties resistant to first races was observed in several regions, suggesting the appearance of new, more virulent biotypes of the parasite – race D and E (Shindrova et al. 1998). At the beginning of the 2000s, in individual sectors, sporadically, *O. cumana* that belonged to physiological race F was found (Shindrova 2006). According to Shindrova, this race has limited distribution and there is no danger of mass occurrence. In the timeframe of 2006–2007, in the north-eastern part of Bulgaria, near the border with Romania, a very aggressive population of broomrape was noticed, identified as race H, which was not sighted in later investigations (Shindrova and Penchev 2012). As Batchvarova (2014) reported the most common races of broomrape occurrences in Bulgarian sunflower growing areas are races E (35%), F (16%) and G (80%).

In our studies, four populations of *O. cumana* from Bulgaria were included. According to results, two populations belong to race G (Debovo and Selanovtsi) and the other two (Radnevo and Rosenovo) to race H. Thus, broomrape from Radnevo and Rosenovo have shown a moderate level of incidence 21.1% and, respectively, 47.6% with a low or moderate number of attachments per plant on the hybrid H_1E resistant to race G (Figure 2A and B), while those from Selanovtsi and Debovo have shown a low level of attack on the same genotype (Figure 2C and D).

In Romania sunflower broomrape was firstly reported in 1940–1941, the highest frequency and intensity of attack occurred in the South-eastern part of the country in Moldova, Dobrogea and east of Muntenia-Baragan (Pricop and Cristea 2012; Risnoveanu et al. 2016). The VNIIMK 8931 variety, known to be resistant to race B, has started to be infested in this area in 1964–1966, while the Romanian variety Record was attacked lower (Vranceanu et al. 1981). In 1976–1979 the presence of races C, D and E has been reported in Romania (Craiciu et al. 2010) and over 18–19 years (1996–1999) race F was identified. The broomrape problem became more stringent in the last 20 years when new, more virulent biotypes of parasites appeared. The highest level of infestation degree was found in the regions near the Black Sea. Analysis of broomrape populations (2009–2010) belonging to five localities from Tulcea and Constanta showed that most of them parasitized even the differential for race G and was named race G+ (Craiciu et al. 2010). Following these events, the most virulent biotype of broomrape, race H, has been identified (M. Pacureanu-Joita, personal contribution).

Our results are following the above mentioned, broomrape populations from Tulcea and Braila showed a high level of parasite incidence (77.8%) on hybrid H_1E resistant to race G (Figure 3).

The population from Braila infested even the hybrid H_2Lg, which is resistant to race H, suggesting the emergence of a new broomrape biotype more virulent than race H. A low level of incidence 6.7%, with a low number of attachments per plant (1.0) was observed in this case.

A high incidence of *O. cumana* was also reported in Ukraine, which is one of the largest producers of sunflower in the world. About 70% of the cultivated areas are currently infested with broomrape, driven by the intensification of sunflower cultivation and short crop rotations. The most affected are the southern and southeastern parts of the country (Pototskyi 2014). In Ukraine, as well as in the

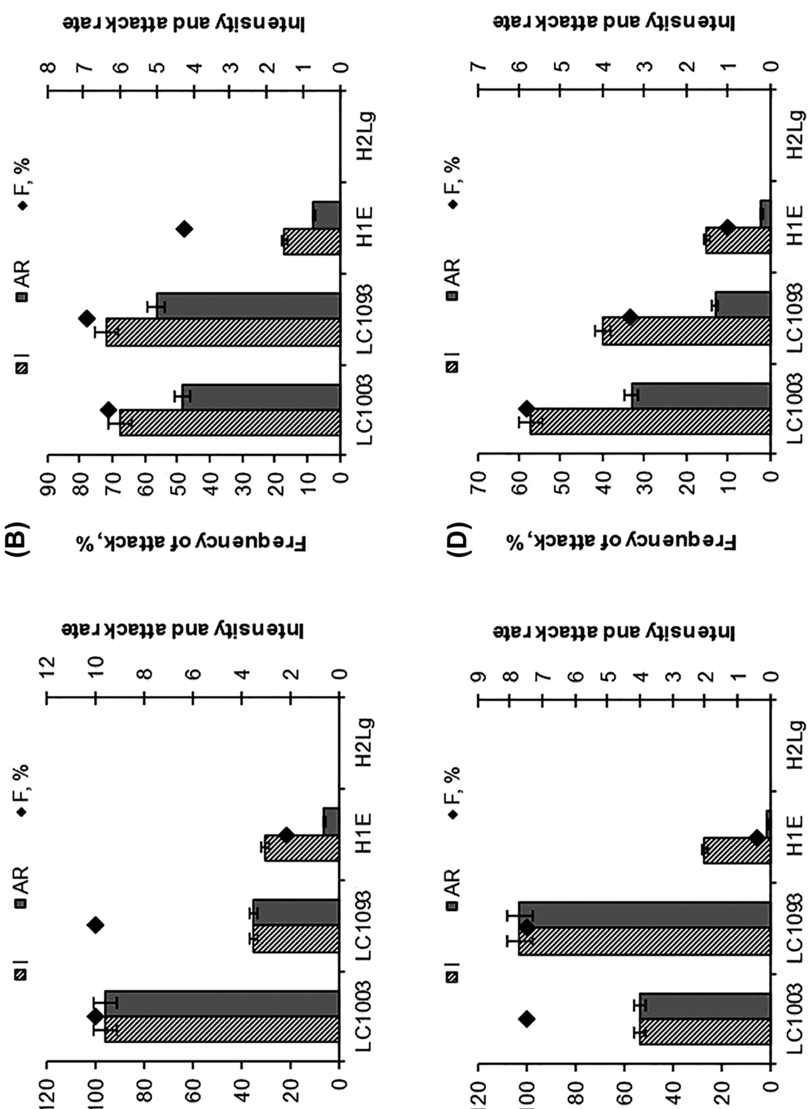

Figure 2: The frequency, intensity and attack rate of broomrape populations from *Bulgaria* on the differential genotypes. (A – Radnevo; B – Rosenovo; C – Selanovtsi; D – Debovo).

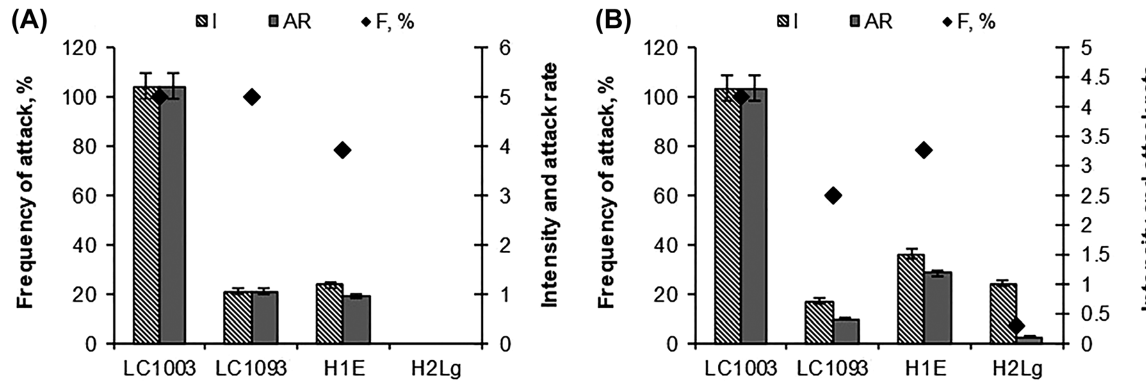

Figure 3: The frequency, intensity and attack rate of broomrape populations from *Romania* on the differential genotypes. (A – Tulcea; B – Braila).

Republic of Moldova, both parts of the former USSR, where the same sunflower varieties were cultivated and which are characterized by similar climatic conditions, the emergence and distribution of parasite races ran a similar course. Since the beginning of the 1930s, when the first races of the parasite (A and B) were detected, to nowadays all currently known races of broomrape were identified in Ukrainian sunflower cultivating areas, especially in the region of the Black Sea (Burlov and Burlov 2010).

In 1965–1970, race C was identified, which dominated in the period of 1980s–1990s in most of the sunflower growing areas (Hablak and Abdullaeva 2013). It should be noted that in the next 10 years, the virulent biotypes D and E were dominant in broomrape populations. Subsequently, more virulent races E, F and G were detected (Hablak and Abdullaeva 2013; Pototskyi 2014). The most spread races in Ukraine were race E (around 70% of sunflower surface) and race G (around 29%), the highly virulent pathotypes being identified especially in Donetsk, Odesa, Zaporozhe and Lugansk regions (Pototsky 2014). Currently, in the northeastern part of Ukraine the presence of populations belonging to race H, as well as a biotype with higher virulence than race H ware observed (Maklyak et al. 2018).

In our studies conducted in 2014 on two populations of broomrape collected from infested areas in Izmail and Odessa, it was established that both populations belong to race H (Figure 4A and B). The Izmail population was more virulent and presented a high frequency of attack (100%) and a moderate number of *O. cumana* shoots per host (4.9) even on hybrid LG-5661 resistant to race G of the parasite, while the population collected from the experimental fields of the All-Union Plant Breeding and Genetics Institute (VSGI), Odessa have shown a low number of attachments (2.13). It has been observed that the hybrid Favorit was more infested compared to its maternal line LC 1093A, in which the resistance to race F of broomrape is controlled by a single dominant gene *Or6*. Similar results were also obtained in the case of the Spanish population belonging to Seville (Figure 4C). Thus, the sample from Seville manifested a high level of attack (100.0%) with a low number of attachments (1.54) on line LC 1093 and moderate (3.25) on hybrid Favorit. At the same time, this population did not affect the hybrid LG-5661, suggesting that it belongs to race G.

In Spain, broomrape was first identified in 1958. Initially detected especially in sunflower cultivating areas of Cuenca and Guadalquivir Valley (Fernandez-Escobar et al. 2009), after two decades the parasite spread over these areas. In 1980, two biotypes of *O. cumana* were characterized (one – dominant in the southern part of the country and another – in the center), identified as race D and E, respectively (Melero-Vara et al. 1989). Later (1995), a new more virulent race F has been detected (Alonso et al. 1996). In the early 2000s, this biotype has spread

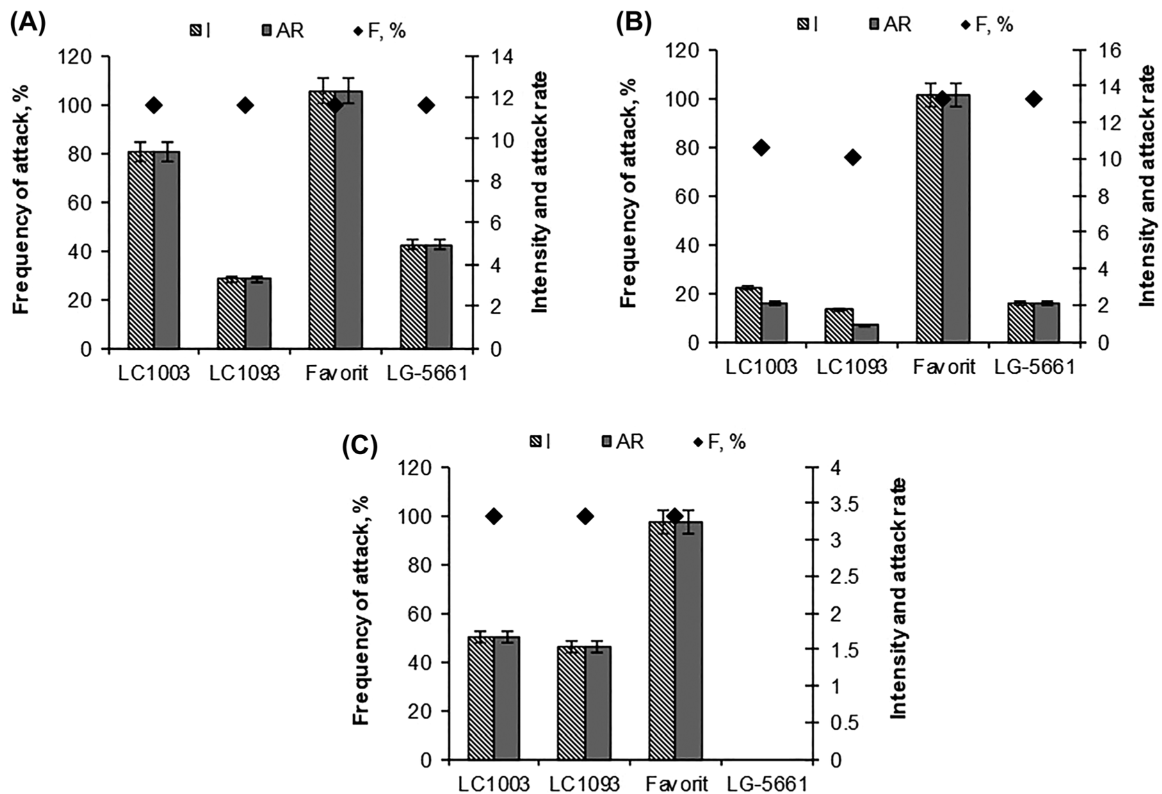

Figure 4: The frequency, intensity and attack rate of broomrape populations from *Ukraine* and *Spain* on the differential genotypes. (A – Izmail; B – Odessa, C – Sevilla).

widely in the southern part of the country (Molinero-Ruiz et al. 2009), as well as in some regions of northern and central Spain (Molinero-Ruiz and Dominguez 2014). Recently Martin-Sanz et al. (2016) described new broomrape accession belonging to Guadalquivir Valley, which was more virulent than race F, but different from race G identified in other areas (named race G_{GV}). The results of Molinero-Ruiz et al. (2008) suggested the presence of *O. cumana* race higher than F inclusive in the Seville area, these data being later confirmed by Cvejić et al. (2012). Our results are following the aforementioned reports.

Furthermore, the holoparasite *O. cumana* is a significant constrain for sunflower production in Turkey, especially in the Thrace region. First issues regarding broomrape occurred in Turkey in 1956 and already from 1983 to 1990 five-race complexes (A, B, C, D and E) existed within the Turkish population (Bulbul et al. 1991). From the middle of the 1990s new populations overcoming the *Or5* resistance gene and named race F were identified in Turkey. Turkish race F population was more virulent than those in other countries and a few years after its first documentation, it could be detected in more than 60–70% of the sunflower growing areas in the Thrace region (Kaya et al. 2004). Moreover, new virulent broomrape races have spread to other areas of Turkey, such as Cukurova, Middle Anatolia, and the Black Sea regions (Kaya 2014). According to a report of Ziadi et al. (2018) *O. cumana* collected from different regions located in the Thrace and Mediterranean region of Turkey belonged to six different races (A, D, E, F, H and H-I). The most virulent was the population from Adana, which infested even the differential for race H.

In our studies five Turkish broomrape populations originated from the Thrace region, province Edirne (Merkez and Keșan), Kirklareli (Luleburgaz) and Adana were evaluated. In contrast, to the results of Ziadi et al. (2018) the population from Adana did not affect the hybrid H_2Lg resistant to race H (Figure 5). It infested the differentials for race E, F and G, showing a low to moderate incidence of broomrape (11.1–75.05%), being classified as race H (Figure 5E). A similar pattern of virulence was found in the case of the population belonging from Merkez and Luleburgaz (Figure 5B and D), which presented a higher level of parasite incidence (44.4–90.0%) and several broomrape shoots per host plant (2.7–5.9). *O. cumana* samples from Trakya manifested a lower level of aggressiveness (attack frequency – 30.8–33.3% and intensity – 1.0–2.7). This population did not attack the genotype H_1E, being attributed to race G. The population belonging from Keșan, which affected inclusive the hybrid H2Lg resistant to race H with a low incidence of attack (10%) and broomrape attachments per plant (1.0), was the most aggressive and virulent (Figure 5C). These results suggest the emergence of a new more virulent biotype.

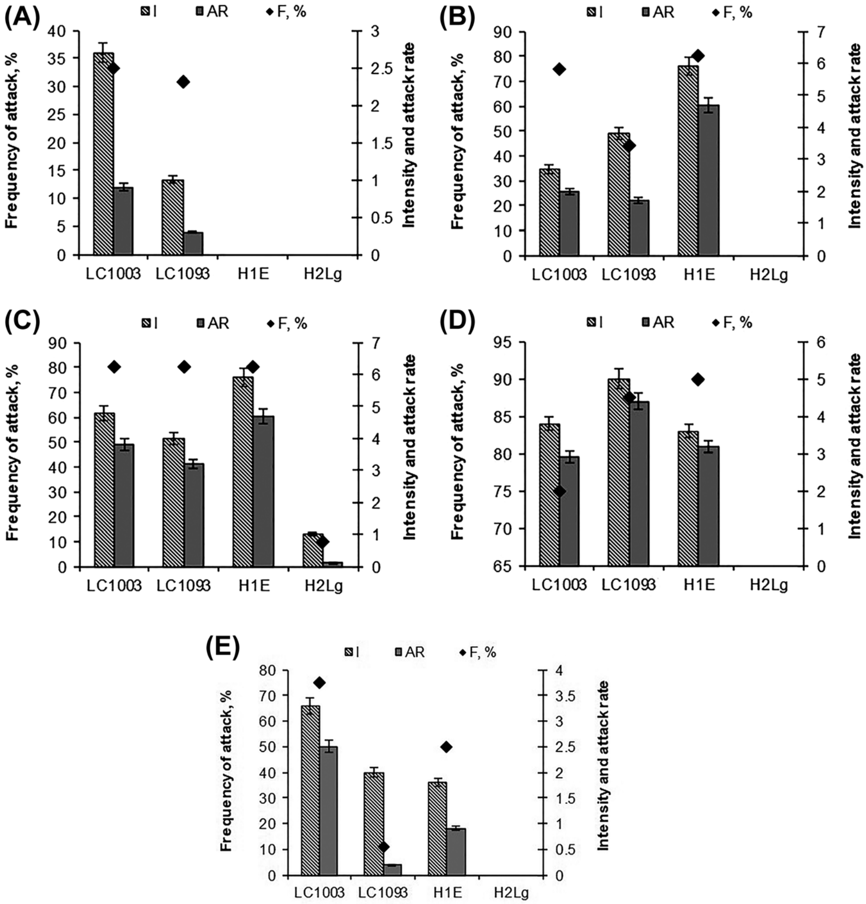

Figure 5: The frequency, intensity and attack rate of broomrape populations from *Turkey* on the differential genotypes.
(A – Trakya; B – Merkez, C – Kesan; D – Luleburgaz; E – Adana).

In contrast to the above-mentioned countries, Serbia is characterized by the presence of less virulent races. The first report on the presence of broomrape in Serbia, particularly in the Vojvodina region, dates back to the 1950s, assuming that the parasite was introduced into the country concurrent with the seeds of Soviet Union sunflower varieties imported from Ukraine. Although there are no data on the racial composition of these broomrape populations, they are considered to belong to race B because Russian varieties resistant to race C were not affected (Aćimović 1977). Later (the early 1990s), the emergence of the more virulent race E was confirmed (Mihaljčević 1996), without the identification of less virulent races C and D.

According to our studies carried out on seven broomrape populations from Serbia, these populations severely infested only the Performer hybrid (Figure 6), used as a susceptible control (S. Clapco, personal communication). The most aggressive population was ORSR 11, with an attack rate of 12.1, followed by ORSR 14 (AR – 8.7) and ORSR 26 (AR – 7.0).

Results suggest that the populations from Serbia belong to race E or less virulent than E, being following the data reported by Serbian researchers. Thus, according to Mihaljčević (1996) and Miladinović et al. (2014), the most dominant race in Serbia for more than 20 years is E. New, more virulent *O. cumana* races have not appeared. The presence of race F has been identified recently, sporadically, on a small infestation spot (Dedić et al. 2018).

Opposed to researchers from other countries that support the conception of the rapid emergence of broomrape races, Serbian scientists endorse the opinion that the evolution of the physiological races of this parasitic plant is quite slow, resulting from the mutations in virulence genes in the *O. cumana* populations, which occur with a very low rate. According to Miladinović et al. (2014), the primary inoculum of broomrape could be introduced from geographically distant regions, concurrent with infected sunflower seeds, during uncontrolled import, subsequently being subjected to selection pressure that favors races with different virulence genes.

As in other sunflower-producing countries, in China broomrape has become a serious constraint for sunflower production. Being first observed in 1959 in an

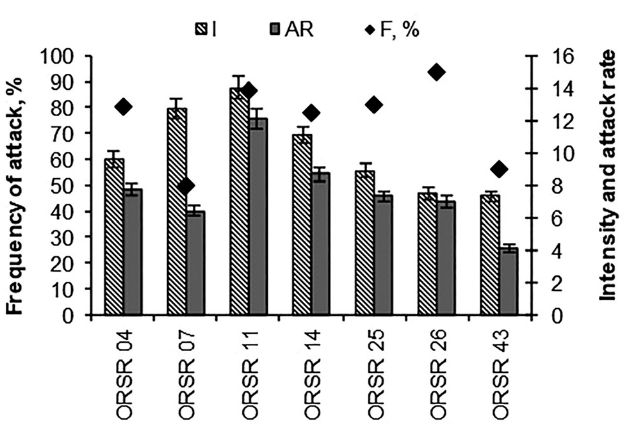

Figure 6: The frequency, intensity and attack rate of broomrape populations from *Serbia* on the sunflower susceptible hybrid Performer.

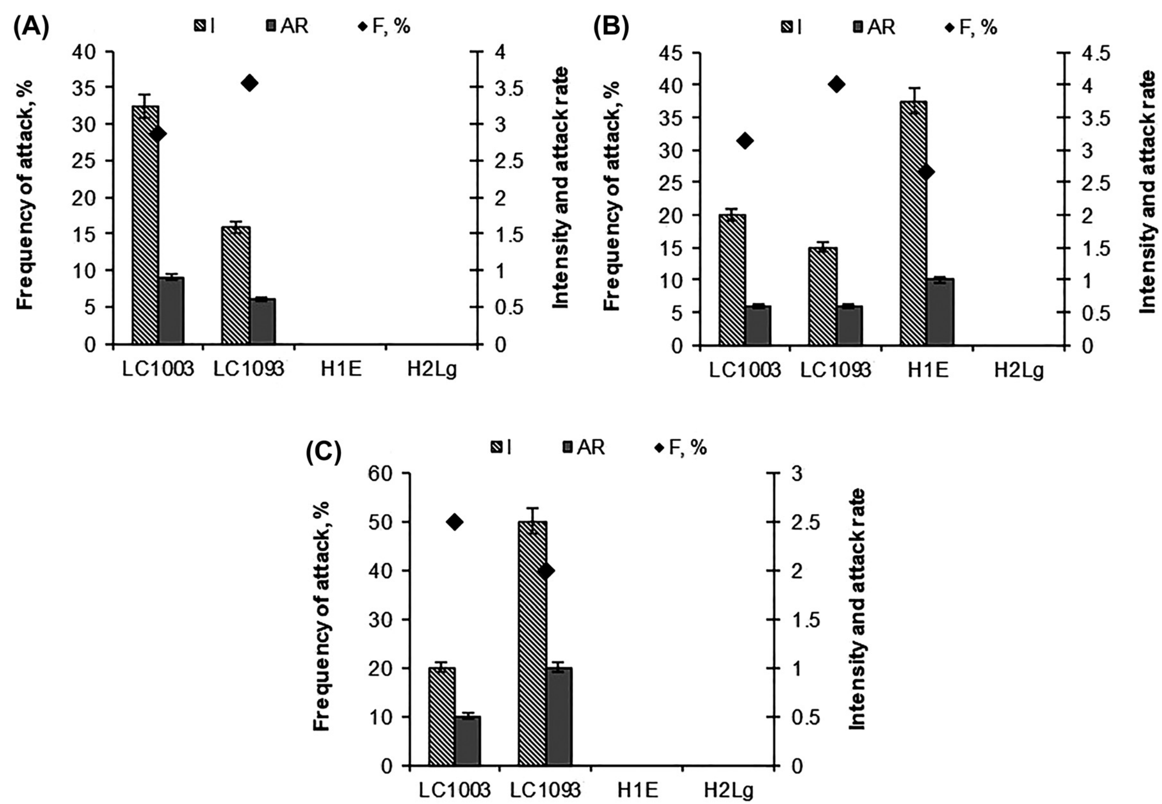

Figure 7: The frequency, intensity and attack rate of broomrape populations from *China* on the differential genotypes. (A – Inner Mongolia, Hohhot; B – Inner Mongolia, Bayanuur; C – Hebei).

individual sector in Heilongjiang Province (Li et al. 1982), subsequently, *O. cumana* spread widely in the north-eastern part of the country and now infests up to 40% of areas planted with sunflower, with mean production losses ranging from 25.0 to 40.0% (Jing et al. 2015). Seven broomrape races (A-G), as well as a biotype more virulent than G (named G+), were consecutively identified in China. The presence of race A was firstly confirmed in 1996 in Jilin Province and, later (Ma and Wan 2015), other races were found in different regions. According to Ma and Wan, less virulent races were detected in Jilin and Heilongjiang provinces (A, C, D, E and, respectively, A and E), races A, B, E and F were observed in Gansu province; B, D and F – in Inner Mongolia region and E, F – in Xinjiang region. The results of current studies performed by Shi et al. (2015) have confirmed the dominance of race A, D, E and G of *O. cumana* in sunflower cultivation areas of China, the most virulent race (G) being mainly limited to the western part of the Inner Mongolia region (Shi and Zhao 2020). It is considered that the existence of higher virulent races in the Inner Mongolia region is due to the introduction of broomrape seeds along with the imported sunflower seeds, as well as by crop rotations failing (Ma and Jan 2014).

The holoparasite is mainly found in the northwestern and northeastern parts of the country, where the largest areas cultivated with sunflowers are located. Thus, the Inner Mongolia region, the largest sunflower producing area in the country, is characterized by a wide distribution of *O. cumana*, with severe infestations in Wulanhot, Tongliao, Chifeng, Wulanchabu, Hohhot, Baotou, Bayannur and Erdos. Broomrape is a serious limiting factor of sunflower production also in Hebei province, where around 1/3 of cultivating areas are infested (Shi and Zhao 2020). In our research, two populations from the Inner Mongolia region (city Hohhot and Bayanuur) and one belonging to Hebei province were included. According to results (Figure 7), broomrape samples from Hohhot and Hebei infested the differentials for race E and F, showing a moderate incidence of attack (28.6–50.0%), with a low number of parasite shoots per host plant and can be attributed to race G (Figure 7A and C). The other Inner Mongolia population (Bayanuur) attacked including the hybrid H_1E resistant to race G (F – 26.7%; I – 3.75), suggesting that it belongs to a more virulent race (G+ or H).

Our data are in agreement with those reported by Chinese researchers. Thus, previous studies on the racial status and broomrape distribution showed the presence of a wide spectrum of races in Inner Mongolia (A, B, D, E, F, G and G+), race G being dominant as is race A in Hebei. Additionally, it was found that broomrape populations from Wuyuan county and Shuanghe town of Bayannur city infested even the genotypes carrying *Or7* genes that suggest the presence of pathotypes characterized by higher virulence than G (Shi and Zhao 2020). Distinct to previous reports, the occurrence of high virulent race G was identified in Hebei province. Because the analyzed broomrape population was collected from a location close to the Hebei-Inner Mongolia border, it can be assumed that broomrape seeds of this pathotype were brought together with sunflower seeds from the Inner Mongolia infested areas.

Conclusions

Identification of racial status of broomrape populations belonging to eight sunflower cultivating countries from Europe and Asia (Republic of Moldova, Romania, Bulgaria, Ukraine, Spain, Turkey, Serbia and China) revealed the presence of high virulent races G and H in the majority of countries, except Serbia, where accessions belonging to race E or less virulent than E were found. The most virulent race H was identified especially in the Black Sea area (Romania, Ukraine, Turkey). Additionally, the emergence of new high virulent biotypes, which overcome resistance genes to race H was observed in some areas of the Republic of Moldova (Soroca, Izbiste, Svetlii, Taraclia and Alexanderfield), Romania (Braila) and Turkey (Keșan), which underlines the importance of periodic evaluations of racial status to prevent the occurrence and dissemination of new races.

Also, analyzing the occurrence chronology of broomrape races in different countries we conclude that by the mid of 1990s, the state of play was relatively stable, the most aggressive race being E. After this period the process of *O. cumana* development and appearance of new more aggressive and virulent biotypes became faster, which may be explained by the intensification of sunflower breeding activities and, respectively, by the selection pressure exerted on the parasite by new resistant hybrids.

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