I. BIOLOGY AND GENETICS OF THE PARASITE *OROBANCHE CUMANA*

4th International Symposium
on Broomrape in Sunflower

THE BIOLOGY OF *PHELIPANCHE* AND *OROBANCHE*

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Abstract

Members of the *Orobanche* and *Phelipanche* genera, belonging to the broomrape family, are obligate root parasitic weeds. They are devoid of chlorophyll and unable to carry out photosynthesis, and then totally rely on their hosts for their water, mineral, and carbohydrate supplies. Although a fine-tuned molecular dialogue between both partners governed the underlying process of this trophic exploitation, the host unknowingly plays a crucial role. Indeed, three key development stages of the parasite are dependent of the host: 1) to germinate the seeds require the perception of molecules produced by host roots. 2) the development of a specific organ, the *haustorium*, which invades host tissues and establishes a physiological continuum between partners, is induced by host-derived molecules. 3) the establishment of a sink strength required for translocation of host resources seems to be controlled by translocated host hormones.
Given the alarming impact of *Orobanche cumana* on sunflower in countries surrounding the Black Sea, in Southern Europe and in growing area of France, deciphering the physiological and molecular events governing the parasite development and the establishment of the interaction with its host is then a necessary step toward the development of specific control methods. The knowledge of the biology of *O. cumana* will significantly progress in years to come thanks to the availability of transcriptomic and genomic data and the development of a robust system for Agrobacterium rhizogenes-mediated transformation and subsequent regeneration of the holoparasitic plant. A new technology called miPEP is in the development stage at the University of Nantes. The objectives of this new project miPEPiTO are to develop new molecular tools to investigate the biology of the parasite, and to develop an innovative and sustainable biocontrol technology for management of this pest.

Keywords: broomrape, germination, haustorium, biology, control methods

CURRENT SITUATION OF SUNFLOWER BROOMRAPE AROUND THE WORLD

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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 recent studies indicated that the new broomrape populations which have been determined in some countries were called G, H or I races. However, there is a doubt in the description of races, in different countries, especially for recent races. So, sunflower breeders and geneticists have focused on solving this problem and they have achieved significant results, using different methods, specially molecular markers, for identifying broomrape races. In order to attain their breeding goals and identify sources of broomrape resistance, the breeders must develop a breeding strategy, secure the necessary germplasm and differential lines for broomrape races identification and chose the appropriate inoculation methods. As well as developing broomrape resistance genes, some research must be developed to understand the dynamics of broomrape populations and evolution. Clearfield system which it means IMI herbicides and resistant hybrids, combined with genetic resistance could be successful for controlling broomrape parasite.

Keywords: sunflower broomrape, races distribution, resistance, breeding, control methods

Identification of Broomrape (*Orobanche Cumana* Wallr.) Biotypes in the Main Sunflower Growing Areas of China

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Abstract

In order to identify the distribution and race composition of sunflower broomrape (*Orobanche cumana* Wallr.), seed was collected from the main sunflower production areas including Inner Mongolia and Xinjiang autonomous regions, and Gansu and Heilongjiang Provinces, China in 2014, 2016 and 2017. Bulked seed samples from 55 areas collected in 2014 were evaluated against a set of differential lines. The biotypes of *O. Cumana* were Races D-F in Inner Mongolia, Races B-F in Xinjiang, predominantly Race F in Gansu, and Race E in Heilongjiang. Of the 44 areas collected in 2016, a wide distribution of broomrape Races F-G or higher was observed in Inner Mongolia, which had the highest number of biotypes, as well as the fastest spread. The distribution of broomrape biotypes varied in Xinjiang for Races D-F. Gansu and Heilongjiang were both predominantly Race E. The speed of change of Races D to F in Xinjiang accelerated in recent years. The sampling was not at the same site, but within the same general area, and our results should explain the current status of broomrape races in China. The evaluation of the 90 areas collected in 2017 is in progress. The evaluation of 16 representative broomrape samples in each of three growing areas was conducted. In Inner Mongolia, 11 of the 16 broomrape samples were Race F and the remaining were Races D-E. In Gansu, however, 3 of the 16 samples were Race D, and the others were Races E-F. In Xinjiang, the biotypes of all samples were Races C-F. The variability in biotypes observed in selected sampled areas represented the population dynamics of broomrape races in natural condition without the selection pressure of resistant sunflower hybrids.

Keywords: Sunflower, *Orobanche*, Broomrape

Introduction

Orobanche cumana is a non-photosynthetic root parasitic plant that specifically infects sunflower. It does not have roots, but only haustoria that absorb nutrition and water from the sunflower plant. The infected sunflower plants are seriously deprived of nutrition resulting in weaker plants with smaller head diameters resulting in up to an 80% yield loss. Since the discovery of Race F that attacked all the known resistance genes in the early 1980's, sunflower breeders have been extremely busy identifying newer races and their corresponding resistance genes, and closely monitoring their spread and distribution globally (Akhtouch et al. 2002, Fernandez et al. 2008; Skoric et al. 2010). The same general trend of race shifts is occurring in China, with growing areas seeing an increased presence of race F and beyond (Zhao 2014; Ma and Jan 2014; Shi et al. 2015). Considering that the Chinese sunflower production is predominantly confectionery, which lacks higher levels of broomrape resistance genes than oilseed, this rapid race shift is extremely alarming. Therefore, broomrape is listed as a quarantine requirement when importing sunflower seed into China. Currently, broomrape distribution includes Xinjiang, Gansu, Inner Mongolia, Shaanxi, and the eastern provinces of China. Monitoring the broomrape race composition in each area using bulk samples to identify the highest race(s) present will not only provide information about race dynamics and competition, but will also serve as guidelines for breeding resistant sunflower hybrids.

Materials and Methods

Broomrape seeds were collected from the main sunflower growing areas in 2014, 2016 and 2017. Bulked samples from 55 fields were collected in 2014. A total of 44 representative fields were sampled in 2016 by collecting seeds from 60 random broomrape plants from each field, including 30 fields in Inner Mongolia, one in Heilongjiang, 3 in Gansu, and 10 in Xinjiang. The 2016 collected bulked broomrape seed from each field was used to infect a set of differential lines resistant to broomrape Races A to F (71NCA101, J8281, 1532, S1358, NR5, and P96, respectively), together with the universal susceptible line B1117 to identify the highest race in each field. Of the fields sampled in 2017, 15-16 samples from one representative field from Inner Mongolia, Gansu, and Xinjiang were also evaluated for race composition of individual fields.

Small pots were prepared and filled with a mixture of soil, vermiculite and sand in a 2:1:1 ratio. About 30 mg of broomrape seeds were applied to the soil mixture for each pot. Three pots were inoculated for each area and each differential line. Sunflower seeds were planted in the small pots after germination and transplanted at the V4 stage to regular 6-inch diameter flower pots and grown under greenhouse conditions. Proper watering in the greenhouse is critical since over-watering will stunt broomrape plant growth. The scoring of the emerging broomrape plants started at the R6 stage.

Results

Identification of the highest broomrape race found in the major Chinese sunflower growing areas in 2014 and 2016 is shown in Table 1. In Inner Mongolia, the number of fields in 2014 having Races D, E, and F was 1, 2, and 16, respectively. However, in 2016 Races D and E disappeared and Race F became the predominant race in 26 fields, while Race G began to appear in 3 fields. Races B, C, D, E, and F were present in Xinjiang in 2014, but Races B and C disappeared in 2016, leaving only Races D, E, and F. In Gansu, Race F was present in 2 fields in 2014, but absent in 2016, while Race E was absent in 2014, but appeared in 3 fields in 2016. Three fields in Heilongjiang were identified with Race F in 2014 and 2 fields in 2016.

Inner Mongolia was a region with a relatively high number of broomrape races including Races D-F. Race F was the predominant biotype in 16 of the 19 sampled areas in 2014, and 26 of 29 in 2016. Race G has occurred in the last 3 years appearing in three fields in 2016. However, the frequency of Race G broomrape is still at a low level, but seed is being increased in the greenhouse for future testing.

This study clearly indicates a trend in the disappearance of lower races such as Races B and C, and the increase of higher Races D, E, and F. Overall, Race F is the predominant race in China, especially in the major sunflower production areas of Inner Mongolia.

Results of individual broomrape samples collected in 2017 from each of the three selected areas were tested against five differentials for Races A-F and summarized in Table 2. Among all samples from Inner Mongolia, 11 samples appeared to infect the NR5 differential (resistant to Race E in Spain), suggesting that a high frequency of Race F existed in the field, compared to 4 for Race E, and 1 for Race D in the same field. The four samples infected S1358 (resistant to Race D) by a low degree of infection equal to 5, 1, 3, and 2 confirmed the existence of Race E. The Race D infected 1532 (resistant to Race C) with 5 shoots per plant. In Gansu, 12 samples were Race E infecting S1358 (resistant to Race D). However, three samples had a small percentage of broomrape overcoming the Or_3 resistance gene of sunflower in the 1532 differential line, suggesting the existence of Race D. Only one sample infected NR5, suggesting Race F. In Xinjiang, the biotypes of all the samples ranged from Races C-F. Four samples overcame S1358 (resistant to Race D), 4 samples infected NR5 (resistant to Race E), suggesting Races E and F, respectively. Other samples collected showed degrees of infection among the control J8281 (resistant to Race B), 1532 (resistant to Race C), indicating the presence of Races C and D, respectively.

Discussion

In 1959, the sunflower broomrape was first discovered in Zhaozhou County, Heilongjiang Province in China. For a long time after its discovery, broomrape was not a problem in sunflower cultivation and no damage or dispersal was reported. It came to the attention of the Chinese sunflower community only in recent years due to the drastically increased activities of importing and transporting sunflower seed, and lack of attention to plant quarantine. The presence and spread of broomrape to major sunflower production areas in Inner Mongolia, Xinjiang, Heilongjiang, Jilin, Liaoning, and Shaanxi could be caused by the continuous cropping of the same fields with seeds contaminated with higher level races.

In the largest and most concentrated area of sunflower cultivation, Bayannaoer of Inner Mongolia, broomrape has infected up to 13,300 ha with a parasitic rate as high as 90% resulting in yield losses of 30 to 45%.

Currently, the planted acreage of sunflower in the main sunflower production area is about 1.3 million acres in 2017, but is expected to decrease to 1.1 to 1.2 million acres in 2018. The results in the current study were largely based on the natural coexistence of broomrape races in fields without selection pressure from growing resistant hybrids. The frequency of the different races in a single field also serves to indicate the relative competitiveness of races.

Comparing the results of 2014 and 2016, without any obvious selection pressure, a general trend moving toward higher levels of broomrape races was obvious. As time goes on, we would expect a drastic increase of Race G or higher races spreading in China as Races E and F resistant hybrids are developed and widely used in production.

Previous reports suggested an abundance of broomrape resistance genes in wild *Helianthus* species, especially the wild perennials (Jan et al.1998. 1999, 2000, 2002). Efficient resistance screening and utilization of those wild germplasms will play a critical role in the future success of sunflower when battling this most damaging parasitic weed.

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Table 1. The highest broomrape races in major Chinese sunflower growing areas in 2014 and 2016.

Region	Year	Broomrape Races						
		Race A	Race B	Race C	Race D	Race E	Race F	Race G
Inner Mongolia	2014	0	0	0	1	2	16	0
	2016	0	0	0	0	0	26	3
Xinjiang	2014	0	2	5	8	11	5	0
	2016	0	0	0	1	3	6	0
Gansu	2014	0	0	0	0	0	2	0
	2016	0	0	0	0	3	0	0
Heilongjiang	2014	0	0	0	0	0	3	0
	2016	0	0	0	0	0	2	0

Table 2. The number of broomrape samples from Inner Mongolia, Gansu, and Xinjiang infecting the differential lines resistant to specific broomrape races in 2017.

Location	Differentials Resistant to Specific Broomrape Races						
	71NCA101 A	J8281 B	1532 C	S1358 D	NR5 E	P96 F	Total sample
Inner Mongolia	0	0	1	4	11	0	16
Gansu	0	0	3	12	1	0	16
Xinjiang	0	2	5	4	4	0	15

GENETIC DIVERSITY OF SUNFLOWER BROOMRAPE POPULATIONS IN CHINA REVEALED BY GENOME RE-SEQUENCING

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Abstract

Sunflower broomrape (*Orobanche cumana*) is an obligate parasitic plant that only infects sunflower (*Helianthus annuus*) by fixing to its root. The first massively infested fields by *O. cumana* were reported in Russia in the 19th century. It is now found in the main sunflower-producing countries in Europe. *O. cumana* has been reported in China, mainly in the Inner Mongolia and Xinjiang regions located in North China, decreasing sunflower yield in the infested fields.

Five representative populations of *O. cumana* from China (Inner Mongolia) were selected for genome re-sequencing using Illumina Hiseq3000 with an average depth of 19.6X. By mapping the sequence reads on the full genome sequence of *O. cumana* (1.4 Gb), we performed SNP calling for each population. A set of few markers will be selected to characterize more Chinese populations and to follow their evolution in the future. Molecular diversity of these populations will be compared to European populations and their virulence level (race structure) will be determined in a set of differential sunflower lines.Our results will enable a better understanding of the history of *O. cumana* in China and its genetic diversity.

Keywords: *Orobanche cumana*, China, re-sequencing, genome, diversity

PARASITIC WEED *OROBANCHE CUMANA* INFESTATION ESPECIALLY ON CONFECTIONARY SUNFLOWER IN THE NORTHWEST REGIONS OF CHINA

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Abstract

Sunflower (*Helianthus annuus*) is an important oil crop in China, covering 21 provinces (regions), including Inner Mongolia, Jilin, Heilongjiang, Xinjiang and Shanxi etc. In 2015, the total cultivated area under sunflower was about one million hectares with a 1.4 million tons yield. The dominant sunflower production region is Inner Mongolia, which accounts for 52.3% of China's entire sunflower production with 50% area under sunflower cultivation. In Inner Mongolia, Northern slope of the Mount Yinshan area is known for producing high quality confectionary sunflower. In recent years, due to the increasing demand in domestic and international market, the profit of sunflower cultivation increases rapidly. On the other hand, with the popularization and promotion of hybrid sunflower, the international exchange and flow of key sunflower germplasm in China is frequent, resulting in the introduction and spread of different physiological races of sunflower broomrape (*Orobanche cumana*) in China. Especially, in the northern slope of the Mount Yinshan, due to permeable soil type and wind erosion damages which make this district best option for propagation of *O. cumana* seeds. The survey of Ulanqab in 2015 showed that more than 80% of sunflower fields of Siziwang Banner had different levels of *O. cumana* infestation, which reduced yield up to 50%, even total failure was also observed in some fields. In Wuchuan county of Hohhot, 60-70% of sunflower broomrape infestation was emerged while seriously affected fields lost 50-70% or no yield in 2016. Farming and Animal Husbandry Bureau of Siziwang Banner reported 90% sunflower cultivation area of the Da Heihe village is under the threat of heavy *O. cumana* infestation, causing about 3.5 million US dollars loss in 2017. In addition, huge seed bank of *O. cumana* in the fields of some non-host crops like potato, rapeseed and maize also exits. Once sunflower is planned to be grown in these farmlands, the potential damage of sunflower due to broomrape infection will be huge.

Keywords: sunflower, *Orobanche cumana*, parasitism, infestation, Inner Mongolia

INCREASE IN VIRULENCE OF SUNFLOWER BROOMRAPE IN SERBIA

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Abstract

Sunflower broomrape (*Orobanche cumana*) is a parasitic plant that can have significant negative impact on yield. Change in virulence of parasite is a potential threat for sunflower production, as resistant hybrids growing is an extensively used control measure. Increase in broomrape virulence has been reported frequently in a number of countries with sunflower as major crop and with a long presence of parasite. In Serbia, virulence of broomrape was described as race E. In our research, broomrape seeds were collected from infested fields and virulence was determined using sunflower lines differing in resistance. Experiments were conducted in greenhouse, by sowing sunflower seed in pots with substrate containing conditioned broomrape seeds. Sunflower roots were evaluated for parasite presence after six weeks. Results of greenhouse trial indicated presence of race F at one site, as broomrape was present on roots of lines LC 1003 and NR5 that are resistant to race E and susceptible to broomrape populations with virulence higher than E. Parasite was absent in line P96, resistant to race F. Increase in virulence was further confirmed with susceptible reaction of selected hybrids resistant to race E. To our knowledge, this is the first report on occurrence of broomrape race F in Serbia, on small infestation spot.

Keywords: sunflower, broomrape, virulence

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is a major constraint in sunflower production. Its negative impact depends on host resistance and environmental factors. This parasitic plant is present in all countries where sunflower is grown with exception of South and North America. In Serbia it was reported in early 1950s (Aćimović, 1977). Distribution area of broomrape in Serbia has expanded with time, with region of north part of Vojvodina being the most vulnerable (Miladinović *et al.,* 2014).

Races of *O. cumana* were defined based on virulence toward differential hosts (Vranceanu *et al.,* 1980; Molinero-Ruiz *et al.,* 2015). In time, number of races increased as a result of pressure from hybrids carrying vertical resistance and ability of parasite to grow and produce seeds on these genotypes (Perez-Vich *et al.*, 2013). In Serbia, after first appearance of broomrape, parasite virulence was described as race B as cultivars carrying resistance to this race were clear form parasite (Miladinović *et al.,* 2014). In early 1990s broomrape was observed on hybrids previously described as resistant. This more virulent race was described, in greenhouse tests, as race E (Mihaljčević, 1996). Following the appearance, race E soon became dominant across area of broomrape distribution. However, broomrape races higher than race E were repeatedly reported in countries bordering Serbia (Hargitay, 2014; Pacureanu, 2014). The objective of our research was to determine virulence of new broomrape populations that appeared present in sunflower growing regions of Vojvodina.

MATERIAL AND METHOD

Orobanche cumana seeds from mature broomrape plants were collected from two fields. Population OC0117 was collected from field close to town of Kula (45°40'25.6"N; 19°32'34.2"E) and population OC0317 from field close to town of Vrbas (45°35'25.2"N; 19°40'06.2"E). Broomrape plants were sampled on susceptible hybrid Labud. Broomrape seed was separated from plants using 224 µm and 500 µm sieves and kept in sealed jars on room temperature until used.

Seven sunflower inbred lines were used for determination of broomrape virulence. Line AD66 is highly susceptible to broomrape (Vranceanu *et al.,* 1980). Lines LC 215, LC 288 and LC 1002 differ in resistance as a result presence of genes *Or2, Or3* and *Or4*, respectively. Differential lines NR5 and LC 1003 used in this research have gene *Or5* thus conferring resistance to race E (Pacureanu-Joita et al., 2009; Perez-Vich *et al.,* 2002). L86 line was described as resistant to race F but susceptible to race E and differential line P96 was found to be resistant to race F (Molinero-Ruiz *et al.,* 2015). Three hybrids susceptible to race E and fourteen hybrids resistant to race E, marked as H1-3 and H4-17, respectively, were also included in the experiment.

Greenhouse study was conducted in accordance to the guidelines for broomrape pot experiments (Kroschel, 2001). Broomrape seeds were mixed with mixture of equal volumes of peat (Klasmann Deilman Substrate 1), perlite and sand. Pots were filled with 9 l of mix with 100 mg/l of parasite seeds. Broomrape seeds in the pots were conditioned for 7 days on and after this period sunflower seeds were sown. Reaction of differential sunflower lines was tested using both broomrape populations and reaction of selected hybrid using population OC0317. Each genotype was tested in two pots containing up to 7-10 sunflower plants. Each pot represented one replication. Sunflower plants were grown for six weeks at 24°C temperature with 14h/10h photoperiod. Presence of viable broomrape plants was determined on host root and counted. Reaction of sunflower differential lines was marked as susceptible or resistant based on degree of attack calculated as average number of broomrape on parasitized sunflower plants. Differential line was considered as resistant in case of absence of broomrape plants. Results of tested hybrids were presented with parameters of incidence described as percentage of sunflower plants with broomrape and degree of attack. These data were compared with the same parameters of lines AD 66, NR5 and LC 1003.

RESULTS AND DISCUSSION

Majority of differential lines were susceptible to both broomrape populations used in the research. Degree of attack differed significantly, ranged from 1.0 to 17.0 (Table 1.). As expected, the most susceptible genotype was line AD 66. Broomrape was completely absent on sunflower roots of line NR5 (resistant to race E) and line P96 (resistant to race F), grown in the presence of seeds of broomrape population OC0117, indicating presence of race E. In tests with OC0317 population, only line P96 was completely resistant, indicating presence of race F.

Table 1. Degree of attack by sunflower broomrape in differential lines from experiment conducted in greenhouse using sampled parasite population from two locations in Vojvodina

Sunflower inbred line	Broomrape population	
	OC0117	OC0317
AD 66	11.5	17.0
LC 215	5.2	4.9

LC 288	7.4	3.8
LC 1002	1.0	2.1
NR 5	0.0	1.0
L86	1.3	1.3
P96	0.0	0.0

Broomrape was observed on all hybrids resistant to race E grown in the presence of broomrape population OC0317 (Table 2.). Incidence of attack ranged from 38% to 95% and degree of attack from 1.5 to 3.0. Hybrids susceptible to race E had more sunflower plants with broomrape and in average more broomrape attachments compared with hybrids resistant to race E. Broomrape presence on hybrids resistant to race E and susceptibility of line LC 1003 resistant to race E, is another indicator of presence of race F.

In previous reports, the most virulent race of broomrape sampled in Serbia was race E (Garcia-Carneros *et al*., 2014; Miladinović *et al.,* 2014). There is number of potential reasons for observed increase in virulence. The presence of races more virulent than race E is continually observed in countries neighbouring Serbia (Batchvarova, 2014; Hargitay, 2014; Pacureanu, 2014). Races higher in virulence than race F were reported in Braila region in Romania and they are spreading (Risnoveanu *et al.,* 2016). Therefore, hypothesis of introduction from other countries should be considered. Increase in virulence could be result of admixture of broomrape populations (Martin-Sanz *et al.,* 2016). Greater variability and emergence of new physiological races of broomrape on some weedy species may also favour break of sunflower resistance (Pineda-Martos *et al.,* 2014).

Table 2. Degree of attack of sunflower broomrape in inbred lines and hybrids susceptible and resistant to race E using broomrape population OC0317

Sunflower inbred lines and hybrids	Resistance to broomrape race E*	Broomrape incidence (%)	Degree of attack (%)
AD 66	S	100	17.0
LC 1003	R	100	5.2
NR5	R	22	1.0
H1	S	99	6.8
H2	S	98	6.2
H3	S	93	4.4
H4	R	76	2.7
H5	R	77	1.9
H6	R	80	2.6
H7	R	68	2.0
H8	R	70	2.2
H9	R	95	3.0
H10	R	60	1.9
H11	R	68	2.1
H12	R	88	2.7
H13	R	78	2.1
H14	R	75	2.2
H15	R	70	2.9
H16	R	55	2.2
H17	R	38	1.5

* S - susceptible; R – resistant

To our knowledge, this is the first report on occurrence of broomrape race F in Serbia on small infestation spot. The most efficient measure to control, introduction of resistant genotypes, is available, as IFVCNS genotypes resistant to races F and G based on field testing in countries other than Serbia, are developed (Cvejić *et al.,* 2014; Jocić *et al.,* 2016).

Further research will be focused on determination of broomrape virulence across the area of broomrape distribution in Serbia and determination of its genetic diversity.

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THE RACE TEYE IDENTIFICATION AND GENETIC ANALYSIS OF SUNFLOWER BROOMRAPE IN CHINA

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Abstract

Currently, sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic seed plant and caused dramatic yield loss annually. It has become one of the most important constraints in sunflower production in China. The most effective way to control this disease is to apply resistant varieties. So, it is rather urgent to make clear the race type composition and distribution of sunflower broomrape in China, so as to facilitate the breeders to arrange the resistant varieties against different race type reasonable.

To identify the race types of *Orobanche cumana* in China, 29 samples (broomrape seeds) collected from different locations in the main sunflower production region in China. With the standard differential lines provided by Dr. Skoric, the identification tests were conducted using plastic cup under greenhouse condition. Our results showed that there are five race types of *Orobanche cumana* in China: Race A, D, E, F and G. Among them, the race G is the highest level of physiological race and all from Inner Mongolia region (Linhe, Wulateqianqi and Xixiaozhao). Race F is only found at Siwangziqi of Wulanchabu region. Race E mainly distributes in Xinjiang and Inner Mongolia. Race D is the most dominant race type, which is mainly distributed at Xinjiang, Inner Mongolia and Jinlin. Race A is mainly distributed at Shanxi, Hebei and Shaanxi and the Tuzuoqi of Inner Mongolia, where the local cultivars planted.

The genetic diversity study was performed with 96 samples from different sunflower growing areas together with 12 ISSR primers, which were screened out from 100 primers based on their high polymorphism and good reproducibility. A total of 147 bands were detected, which 90 were polymorphic bands. The percentage of polymorphic bands (PPB) was 61.22%. The genetic diversity analysis showed that the diversity of *Orobanche cumana* from Inner Mongolia and Xinjiang was relatively higher. The Shannon diversity index was 0.5560 and 0.5067 respectively. The cluster analysis showed that the *Orobanche cumana* from 6 different provinces can be divided into two subgroups, of which Shanxi, Shaanxi and Hebei provinces were clustered into one subgroup. Jilin, Inner Mongolia and Xinjiang were clustered into another subgroup. The genetic distance between Shanxi and Hebei was the closest, while distance between Hebei and Xinjiang was the longest.

Keywords: sunflower, *Orobanche cumana*, race type identification, genetic variation

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Alelopathic effect of sunflower broomrape (*Orobanche cumana* Wallr.) on the development of *Helianthus annuus* L. under the conditions of the Republic of Bulgaria

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Abstract

The allelopathic effect of dry biomass of sunflower broomrape (*Orobanche cumana*Wallr.) collected from ten regions of the Republic of Bulgaria was studied in laboratory conditions at the Institute for Forage Crops - Pleven. The allopathic effect of the experimental samples of sunflower broomrape on the seed germination and on the initial development of the sunflower, variety "Paredovik".

It was found that the studied concentrations (8.0, 16.0 and 32.0% w/v) dry weed biomass of sunflower broomrape showed a stimulatory effect (from -0.7 to -55.3%) or an inhibitory effect on the germination of seeds (from 1.8 to 43.1%) and on the initial development (from 2.4 to 21.0%) of sunflower, variety "Paredovik".

Depending on the alleopathic effect and the origin of the sunflower broomrape, they can be grouped in the following order: $P_{7(Selanovtsi)}$ (86.9%) → $P_{9(Radnevo)}$ (83.0%) → $P_{4(Kardam)}$ (73.4%) →$P_{5(Tyulenovo)}$ (70.7%) →$P_{6\ (Dyakovo)}$ (61.9%) →$P_{1\ (Kardam)}$ (54.8%) →$P_{2(DZI1,infection\ field)}$ (50.6%) →$P_{3(DZI2,\ experimental\ field)}$ (50.2%) →$P_{8(Svishtov)}$ (46.0%) → $P_{10(Radnevo)}$ (27.9%).

The highest overall allelopathic potential (OAP = 0.6) conditionally was determined for sunflower broomrape originating $P_{2\ (DZI\ infection\ field)}$ and $P_{3\ (DZI\ experimental\ field)}$. The lowest overall allelopathic potential (from 0.2 to 0.3) was determined for sunflower broomrape with the origin $P_{7\ (Selanovtsi)}$ and $P_{9\ (Radnevo)}$.

This information can be explained by the genetic differences of the studied origins.

Key words: *allelopathic effect, broomrape,sunflower, inhibition*

Parasitic plants are among the most problematic pests of agricultural crops worldwide (Runyon et al., 2009, Blagojević et al., 2014).The technological approaches and effective means for control against them are extremely limited, because the close physiological connection between the parasite plant and the host plant impedes the efficient control using traditional methods.

According to Macias et al. (2003), Reigosa et al. (2006) and Willis (2007), six families of parasitic weeds species (*Scrophulariaceae, Orobanchaceae, Santalaceae, Cuscutaceae, Viscaceae* and *Loranthaceae*) are a great economic importance and cause the highest loss of agricultural production.

The parasitic weed sunflower broomrape (*Orobanche cumana* Wallr.) is one of the major limiting factors in sunflower production in the Republic of Bulgaria (Encheva and Shindrova, 1993, Shindrova, 2006, Venkov and Shindrova, 2000, Shindrova 2006) and in many countries of the world (Miladinovic et al., 2012, Molinero-Ruiz et al., 2015, Pineda-Martos et al., 2014).

The broomrape in sunflower has a higher competitive ability than any other weed speciesas its invasion leads to an adverse effect on the natural weeds in sunflower crops.(Habimana et al.,2014).

Due to the competitive nature, the parasitic species – sunflower broomrape causes significant losses, expressed on the one hand in the reduction of yield and on the other - in the deterioration of the quality of the production obtained (Shindrova et al., 1994, Perez-de-Luque et al., 2001).

Scientific researches in recent years are focused mainly on creating resistant varieties and hybrids and on the development of highly effective systems for integrated control against parasitic weed species. (Chittapur et al., 2001, Rubiales, 2012; Joel et al., 2013).

In this respect, the search for alternative means of weed control is very important (Chauhan and Mahajan, 2014). There is a growing interest to the allelopathy in agriculture at present.This phenomenon could provide perspective alternative methods of weed control and to help for reducing of the application of synthetic herbicides (Lopez-Raez, 2008).

Although allelopathy is under study by ecologists, chemists, soil scientists, agronomists, herbologists, biologists and plant physiologists the allelopathic interrelations in the "weed – plant" system are not fully understood and in the case of "*host*plant–parasite" are extremely limited.

According to Jacobs and Rubery (1988), Serghini al., 2001, Perez-de-Luque et al., 2001, Qasem, (2006) Matusova, et al., 2005, Kalinova (2010) *Orobanche cumana* Wallr. has an alleopathic potential, probably due to the content of the allelochemicals (secondary metabolites - coumarins and others).

The discovery of main regularities in the allelopathic interaction in agrophytocenoses of *Helianthus annuus* L.and *Orobanche cumana* Wallr, appears to be a major element of the theoretical basis for sustainable plant-growing production.

The purpose of this study is to determine the alopeptic effect of some origins *Orobanche cumana* Wallr. distributed in the main agricultural areas of the country on the germination and growth of *Helianthus annuus* L.

MATERIALS AND METHODS

The study was conducted during the 2017-2018 period under laboratory conditions in the Institute of Forage Crops in Pleven, Bulgaria.

Two factors have been studied: Factor A - the location of the sunflower broomrape (*Orobanche cumana* Wallr.) (Table 1): a_1-Kardam; a_2 - DAI (infection field); a_3 - DAI (experimental field); a_4-Kardam; a_5-Tyulenovo; a_6 - Dyakovo; a_7 - Selanovtsi; a_8 - Svishtov; a_9 – Radnevo 2016 and a_{10} – Radne 2017.

Factor B - parasitic weed biomass concentration: b_1- 0.0% (control); b_2-8.0%; b_3-16.0% and b_4-32.0% w/v. Sampling of sunflower broomrape is consistent with the factor Ain a growth stage BBCH 65-69 (Hess et al., 1997).

Table 1. The race consists of sunflower broomrape (*Orobanche cumana* Wallr.)

Origin		Year	Race
Code	Location		
P_1	Kardam	2016	H
P_2	DAI(infection field)	2016	H
P_3	DAI (experimental field)	2017	E
P_4	Kardam	2017	H
P_5	Tyulenovo	2017	H
P_6	Dyakovo	2017	H
P_7	Selanovtsi	2017	D
P_8	Svishtov	2017	E
P_9	Radnevo	2016	E
P_{10}	Radnevo	2017	E

Legend: DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

To evaluate the allelopathyc potential oftested samples of sunflower broomrape (*Orobanche cumana* Wallr.) was used the adapted method called "Rhizosphere Soil Method" (RSM) of Fujii et al. (2005) under laboratory conditions.

Parasitic weed biomass from tested samples sunflower broomrape (*Orobanche cumana* Wallr.), according to factor B is placed in petri dishes (90 mm).

On the parasitic weed biomass are pipetted 20 ml 0.8% strength agar supplemented with 1 ml/l thymol $C_{10}H_{14}O$, as a chemical preservative.(Marinov-Serafimov, Golubinova, 2015).

The samples are stored for 72 h at 18 ± 2°C, after then ten seeds of sunflower, variety “Paredovik” are added.

The prepared samples are placed in an incubator at 22 ± 2°C in the dark for five days.

Distilled water is used for control. Each variant is pledged in nine repetitions.

Effect assessment. For assessing the results of the experiments were used the following parameters.
Quantitative parameters. Number of germinated seeds in each treatment: percentof germination in each treatment (%).
Biometric parameters. Length of the root, stem and seedling, cm; fresh biomassin g per root, stem and seedling, g. Length was measured using graph paperand the weight was recorded on an analytical balance.
Statistical evaluation and calculated formulas.
Germination seeds (GS%) was determined by the Equation (1) prescribed according to ISTA (1985).
GS%=(Number of seed germinated)/(Total number of seed plated).100
(1)
Percent inhibition (IR) was determined by the equation (2) (Ahn and Chung, 2000).

$$IR\% = \frac{C-T}{C} x100 \qquad (2)$$

Where, C– characteristic in the control treatment; T – characteristics in each treatment;
The index of plant development (GI) was determined by the Equation (3) (Gariglio et al., 2002).

$$GI = \left[\left(\frac{G}{G_0}\right) \cdot \left(\frac{L}{L_0}\right)\right] . 100$$

(3)
Where, G – germinated seeds in each treatment, %; G_0 – germinated seeds in the control treatment, represpectively %; L – average length (cm) of seedlings in treatment transformed into percentage as against the control treatment; L_0 – average length (cm) of the seedlings in the control treatment taken as 100%;
Seedling vigor index (SVI) was determined by the equation (4) (Islam et al. 2009).

$$SVI = \left(\frac{S.G}{100}\right) \qquad (4)$$

Where, S – seedling length in the treatments and control variant, cm; G – germinated seeds in the treatments and control variant, %;
Coefficient of allometry(CA) was determined by the equation (5) (Nasrand Mansour, 2005):

$$CA = \frac{L_s}{L_r} \quad (5)$$

Where,, Ls is shoot length and Lr is root length, cm.

Overall allelopathic potential (OAP) was determined by the equation (6) (Smith (2013):
OAP = mean (Ia + Ib)/100 (6)
where Ia, a percent inhibition of the seedling growth at the lowest applied concentration of 8.0% w/v and Ib percent inhibition of the seedling growth at the highest applied concentration of 32.0% w/v, compared to the control variants.
A score between 0.0 and 1.0 was obtained and the data were ranked according to this score. A maximum score of 1.0 would indicate that the test samples sunflower broomrape (*Orobanche cumana* Wallr.) had totally inhibited growth, while a score of 0.0 would indicate that no allelopathic inhibition had occurred.
The percentage of seed germination was calculated after preliminary arcsin-transformation following the formula, Y=arcsin√(x%/100), forwarded by Hinkelmann and Kempthorne(1994).
The collected data were analyzed using the software Statgraphics Plus for Windows Ver. 2.1 and Statistica Ver. 10.

RESULTS AND DISCUSSION

The above ground dry biomass of samples sunflower broomrape (*Orobanche cumana* Wallr.) from different regions of Bulgaria manifests an inhibitory effect (IR fom 5.0 to 55.2% w/v) on the seed germination of sunflower (*Helianthus annuus* L.) (Table 2).

By increasingthe content of the parasitic weed biomass (from 16.0 to 32.0% w/v), the germinating seeds of test plant - *Helianthus annuus* L.decreased disproportionately compared to the control variant. The differences being statistically significantly reduced at P=0.05.

An exception to the described dependence was found in variant P7 at the lowest concentration (8.0% w/v). The differences being statistically unproven at P = 0.05 compared to the control variant (Table 2).

This relationship can be explained by the presence of allelochemicals (secondary metabolite, coumarins and others) in the studied samples from sunflower broomrape (Perez-de-Luque et al., 2001).

Depending on the origin and race of the tested sunflower broomrapes samples (Table 1), the degree of inhibition of *Helianthus annuus* L. (Table2) germination seeds can be arranged in the following order: P_7 ($IR_{average}$17.1%) → P_6 ($IR_{average}$17.9%) → P_5 ($IR_{average}$20.7%) → P_8 ($IR_{average}$26.8%)→ P_4 ($IR_{average}$28.0%)→ $P_1$and P_2($IR_{average}$30.2%)→ P_{10} ($IR_{average}$30.6%)→ P_9 ($IR_{average}$30.9%)→ P_3($IR_{average}$43.1%).

The data of the biometric measurements of the length of the root, stem and seedling length growth (cm) give possibility for objective estimation of the differences at the initial growth and development stages of the *Helianthus annuus* L.depending on the type and applied concentration of the tested parasitic weed biomass from different tested sunflower broomrape (*Orobanche cumana* Wallr.) (Table 3).

The degree of reducing the length of the germ of *Helianthus annuus* L. is in the range of 5.5 to 68.5%. The most severe is the degree of inhibition of the root (from 2.9 to 71.5%), and relatively weaker of the stem (from 1.6 to 64.4%) compared to the control with distilled water (Table 3).

Concerning concentration dependencies, it is evident that with increase content parasitic weed biomass (from 16.0 to 32.0% w/v), the length of the seedlings decreased disproportionately in all treatments of *Helianthus annuus* L., compared to the control treatment. The differences are statistically proven reduced at P=0.05.

The lowest applied concentration (8.0% w/v) of parasitic weed biomass from all tested samples of sunflower broomrape has from relatively weak inhibitory to weak stimulating effect on the growth of sunflower seedling.The differences are statistically insignificant at P=0.05 (Table 3)

An exception is found at code P_9 where in all tested concentrations of parasitic weed biomas, the differences in the length of the root, shoot and seedling of the test variety Peredovik are statistically insignificant. At code P_2 and P_{10} the studied indicators are statistically significant decrease at P=0.05 compared to the control variant (Table 1 and 2).

The accumulation of fresh biomass in g for one root, one stem and one seedlingat the early growth stages of test plant - sunflower depends of the same factors (origin of sunflower broomrape and applied concentrations) and follows the established dependencies on the growth of root, shoot and seedlihg of length with this difference they are less pronounced (Table 1 and 3).

Therefore, the observed differences at tested samples of *Orobanche Cumana* Wallr. in terms of their allelopathic potential versus test plants *Helianthus annuus* L.can be probably explained by biochemical differences, because the comparisons between them are performed at equal conditions.

The variation analysis to determine the influence of the studied factos (η_2) on the seed germination and on the initial growth of *Helianthus annuus* L.shows that the largest share of the total variation was due to Factor A (η_2 from 11.8 to 34.1) - the racesof *Orobanche cumana* Wallr.

The applied concentrations of parasit weed biomas (Factor B) are factors with a significant impact on the variation of phytotoxicity (η_2 from 3.7 to 21.4).

The variants due to the "species sunflower broomrape –applied concentration" relationship are in the range from$\eta_2$11.8 to 39.8.

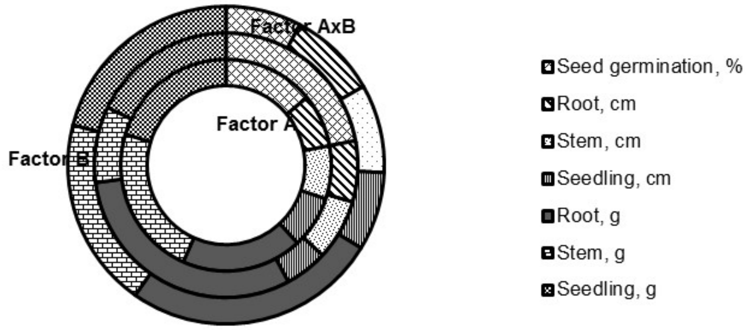

Figure 1. Projection of influence of factors on the factorial plane

The obtained experimental data confirmed the results of Ruiyu et al., 2007, Ali et al. (2013), Takemura et al. (2013), according to them the effect of the allelochemicals is manifested already during the seed germination, but it is more pronounced during the growth and accumulation biomass of seedlings of the test-plants.

The obtained results were analogous when determining seedling vigor index (SVI) and alumetry coefficient (CA) of sunflower (*Helianthus annuus* L.) (Table 4). Tested sunflower broomrapes samples provoked an inhibitory effect on sunflower vitality (from 5.2 to 82.6%) and reduces (from 0.5 to 2.8 times) alumetry coefficient (CA), as compared to the control variant.

The index of plant development (*GI*) dependsof the same factors and follows the observed relationship pattern with regard to laboratory seed germination, and accumulation of fresh biomass and growth of seedling of test plants - *Helianthus annuus* L.(Table 4).

The analyses indicated that the studied races sunflower broomrape (*Orobanche cumana* Wallr.) showsan allelopathic effect – *GI* varing from 27.9 to 144.4% and depending of the applied concentrations.It can be arranged in the following order: $P_{7(Selanovtsi)}$ (86.9%) → $P_{9(Radnevo)}$ (83.0%) → $P_{4(Kardam)}$ (73.4%) →$P_{5(Tyulenovo)}$ (70.7%) →$P_{6\ (Dyakovo)}$ (61.9%) →$P_{1\ (Kardam)}$ (54.8%) →$P_{2(DAI\text{-}Infection\ field\ )}$ (50.6%) →$P_{3(DAI\text{-}experimental\ field)}$ (50.2%) →$P_{8(Svishtov)}$ (46.0%) → $P_{10(Radnevo)}$ (27.9%).

The overall allelopathic potential (OAP) of the studied races of *Orobanche cumana*Wallr. is in the range of -0.1 to 0.9.

With the highest overall allelopathic potential (OAP) can determine conditionally the races sunflower broomrape $P_{2(DAI\text{-}infection\ field)}$, $P_{5(Tyulenovo)}$, $P_{8(Svishtov)}$ и $P_{10(Radnevo)}$, with a value from 0.7 to 0.9 OAP. With the lowest OAP- 0,1 is $P_{9(Radnevo)}$.

CONCLUSIONS

Dry weed biomass of sunflower broomrape (*Orobanche cumana* Wallr.) at concentrations of 8.0, 16.0 and 32.0% w/v has a stimulatory effect (from -0.7 to -55.3%) or an inhibitory effect on the germination of seeds (from 1.8 to 43.1%) andon the initial development (from 2.4 to 21.0%) of sunflower variety Peredovik

Depending on the alleopathic effect and the origin of the sunflower broomrape (*Orobanche cumana*Wallr.), they can be grouped in the following order: $P_{7(Selanovtsi)}$ (86.9%) → $P_{9(Radnevo)}$ (83.0%) → $P_{4(Kardam)}$ (73.4%) →$P_{5(Tyulenovo)}$ (70.7%) →$P_{6\ (Dyakovo)}$ (61.9%) →$P_{1\ (Kardam)}$ (54.8%) →$P_{2(DAI\text{-}infection\ field)}$ (50.6%) →$P_{3(DAI\text{-}experimental\ field)}$ (50.2%) →$P_{8(Svishtov)}$ (46.0%) → $P_{10(Radnevo)}$ (27.9%).

The highest overallallelopathic potential (OAP = 0.6) can be conditionally determined sunflower broomrape originating $P_{2(DAI\text{-}infection\ field)}$ 2016), $P_{3(DAI\text{-}experimental\ field)}$ and P6 (Dyakovo) and with the lowest (OAP - from 0.2 to 0.3) originating from P7(Selanovtsi) and P9(Radnevo).This fact can be explained by the genetic differences of the studied origins.

Table1.Allelopathic effect of a dry above ground biomassof different races *Orobanche cumana* Wallr. on seeds germination of sunflower variety Peredovik

Treatment	Origin		Concentration % w/v	Germination seeds, %	Germination in relative to the control variant, %	IR
	Code	Location				
1		Control	0.0	80.78d	100.00	0.0

2	P_1	Kardam	8.0 16.0 32.0	61.17a-c 53.78a-c 54.22a-c	75.72 66.57 67.12	24.3 33.4 32.9
3	P_2	DAI (infection field)	8.0 16.0 32.0	64.18bc 57.10ab 47.88ab	79.45 70.69 59.28	20.5 29.3 40.7
4	P_3	DAI (experimental field)	8.0 16.0 32.0	53.78ab 47.88ab 36.22a	66.57 59.28 44.84	33.4 40.7 55.2
5	P_4	Kardam	8.0 16.0 32.0	63.43b-c 60.11ab 50.89ab	78.53 74.41 63.00	21.5 25.6 37.0
6	P_5	Tyulenovo	8.0 16.0 32.0	76.72c-d 70.38b-c 45.00ab	94.97 87.13 55.71	5.0 12.9 44.3
7	P_6	Dyakovo	8.0 16 32	70.38bc 67.50bc 61.17a-c	87.13 83.56 75.72	12.9 16.4 24.3
8	P_7	Selanovtsi	8.0 16.0 32.0	80.78d 60.1a-c 60.11a-c	100.00 74.41 74.41	0.0 25.6 25.6
9	P_8	Svishtov	8.0 16.0 32.0	60.11a-c 60.11a-c 57.10ab	74.41 74.41 70.69	25.6 25.6 29.3
10	P_9	Radnevo	8.0 16.0 32.0	61.17a-c 55.40ab 50.89ab	75.72 68.58 63.00	24.3 31.4 37.0
11	P_{10}	Radnevo	8.0 16.0 32.0	56.79ab 57.10ab 54.22ab	70.30 70.69 67.12	29.7 29.3 32.9

Legend: Means with different letters differ at $P < 0.05$ level of probability by LSD test; IR – percent inhibitions; DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

Table 2.Allelopathic effect of dray aboveground biomass of different races of *Orobanche cumana* Wallr.on the seedling growth of sunflower variety Peredovik

Treatment	Origin		Concentration % w/v	Length, cm					
	Code	Location		root	IR	stem	IR	seedling	IR
1		Control	0.0	9.78j-m	0.0	4.50m	0.0	14.28i-j	0.0
2	P_1	Kardam	8.0	8.25h-k	15.6	4.58m	-1.8	12.83h-j	10.2
			16.0	6.21e-g	36.5	2.79d-h	71.5	9.00e-g	37.0
			32.0	5.88ef	39.9	2.94b-i	69.9	8.81ef	38.3
3	P_2	DAI (infection field)	8.0	6.14e-g	37.2	2.36a-e	75.9	8.50b-e	40.5
			16.0	5.13c-e	47.5	2.31a-e	76.4	7.44b-f	47.9
			32.0	5.25c-f	46.3	2.83d-i	71.1	8.08c-f	43.4
4	P_3	DAI (experimental field)	8.0	6.57e-h	32.8	2.37a-e	75.8	8.94e-g	37.4
			16.0	8.00g-j	18.2	3.50i-k	64.2	11.50gh	19.5
			32.0	5.20c-f	46.8	3.00d-g	69.3	8.20c-f	42.6
5	P_4	Kardam	8.0	10.38m	-6.1	2.76d-g	71.8	13.14h-j	8.0
			16.0	9.31j-m	4.8	3.44g-	64.8	12.75h-	10.7

						k		i	
			32.0	7.07f-i	27.7	3.00d-k	69.3	10.07f-h	29.5
6	P_5	Tyulenovo	8.0	10.19lm	-4.2	2.29d-e	76.6	12.48 h-i	12.6
			16.0	4.20a-d	57.1	2.00a-c	79.6	6.20a-c	56.6
			32.0	2.90ab	70.3	1.60a	83.6	4.50a	68.5
7	P_6	Dyakovo	8.0	9.50j-m	2.9	2.92d-j	70.1	12.42g-i	13.0
			16.0	8.75i-m	10.5	2.58d-e	73.6	11.33g-i	20.7
			32.0	3.90a-c	60.1	3.45h-k	64.7	7.35-e	48.5
8	P_7	Selanovtsi	8.0	10.00k-m	-2.2	3.50a-e	64.2	13.50c-f	5.5
			16.0	9.38j-m	4.1	3.75i-k	61.7	13.13h-i	8.1
			32.0	5.72c-f	41.5	2.33de	76.2	8.06e-g	43.6
9	P_8	Svishtov	8.0	8.57i-l	12.4	4.43lm	54.7	13.00h-j	9.0
			16.0	2.79a	71.5	3.71k-m	62.1	6.50a-d	54.5
			32.0	2.81a	71.3	2.69c-e	72.5	5.50ab	61.5
10	P_9	Radnevo	8.0	10.44i-l	-6.7	4.75m	51.4	15.19h-j	-6.4
			16.0	8.50m	13.1	4.58m	53.2	13.08g	8.4
			32.0	8.25h-k	15.6	3.67j-m	62.5	11.92gh	16.5
11	P_{10}	Radnevo	8.0	5.60d-f	42.7	3.25h-l	66.8	8.85b-f	38.0
			16.0	5.79d-f	40.8	1.93a	80.3	7.70ef	46.1
			32.0	4.86b-e	50.3	2.71c-f	72.3	7.57b-e	47.0

Legend: Means with different letters differ at P < 0.05 level of probability by LSD test; IR – percent inhibitions; DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

Table 3.Allelopathic effect of dray above ground biomass of different races *Orobanche cumana* Wallr. on the dynamics of the accumulation of fresh biomass of sunflower seedlings variety Peredovik

Treat ment	Origin		Concentration, % w/v	Fresh biomass, g					
	Code	Location		root	IR	stem	IR	seedling	IR
1		Control	0.0	0.115i-k	0.0	0.340mn	0.0	0.455mn	0.0
2	P_1	Kardam	8.0	0.128k-n	-11.3	0.365o	-7.4	0.493q	10.2
			16.0	0.089de	22.6	0.319kl	6.2	0.408jk	37.0
			32.0	0.094e-g	18.3	0.263de	22.6	0.357g	38.3
3	P_2	DAI (infection field)	8.0	0.086de	25.2	0.323e	5.0	0.409jk	40.5
			16.0	0.089de	22.6	0.307i-k	9.7	0.396ij	47.9
			32.0	0.085de	26.1	0.295ij	13.2	0.380h	43.4
4	P_3	DAI (experimental field)	8.0	0.136mn	-18.3	0.323e	5.0	0.459no	37.4
			16.0	0.105g-i	8.7	0.307jk	9.7	0.411kl	19.5
			32.0	0.085de	26.1	0.236c	30.6	0.321e	42.6
5	P_4	Kardam	8.0	0.167o	-45.2	0.279f-h	17.9	0.446mn	8.0
			16.0	0.123g-	-7.0	0.285f-i	16.2	0.408jk	10.7

			32.0	n 0.078cd	32.2	0.275e-g	19.1	0.353fi	29.5
6	P5	Tyulenovo	8.0	0.157o	- 36.5	0.285f-i	16.2	0.443m	12.6
			16.0	0.061b	47.0	0.215b	36.8	0.277c	56.6
			32.0	0.045a	60.9	0.181a	46.8	0.225a	68.5
7	P6	Dyakovo	8.0	0.098e-h	14.8	0.288g-i	15.3	0.386hi	13.0
			16.0	0.109g-i	5.2	0.279fg	17.9	0.388hi	20.7
			32.0	0.045a	60.9	0.205b	39.7	0.250b	48.5
8	P7	Selanovtsi	8.0	0.128k- n	- 11.3	0.349n	-2.6	0.477p	8.1
			16.0	0.120g- n	-4.3	0.338mn	0.6	0.458no	43.6
			32.0	0.090de	21.7	0.249cd	26.8	0.339f	5.5
9	P8	Svishtov	8.0	0.094e-g	18.3	0.330lm	2.9	0.424e	9.0
			16.0	0.066bc	42.6	0.293h-j	13.8	0.359j	54.5
			32.0	0.065bc	43.5	0.239c	29.7	0.303d	61.5
10	P9	Radnevo	8.0	0.120j-l	-4.3	0.326lm	4.1	0.446mn	-6.4
			16.0	0.140mn	- 21.7	0.345n	-1.5	0.485pq	8.4
			32.0	0.125f-i	-8.7	0.392p	- 15.3	0.517r	16.5
11	P10	Radnevo	8.0	0.104g-i	9.6	0.271ef	20.3	0.375h	38.0
			16.0	0.133mn	- 15.7	0.338mn	0.6	0.471op	46.1
			32.0	0.079a	31.3	0.307j-k	9.7	0.386hi	47.0

Legend: Means with different letters differ at P < 0.05 level of probability by LSD test; IR – percent inhibitions; DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria

Table 4. Developmental index in the early stages of the development of sunflower (*Helianthus annuus* L.) in dependence on the sunflower broomrape (*Orobanche cumana*Wallr.)

Treat ment	Origin		Concen-tration, % w/v	*CA*	*SVI*	*GI*	*OAP*		
	Code	Location					Germination seeds	Seedling lenght	Fresh biomass on sunflower seedling
1		Control	0.0	2.2	11.5	100.0	-	-	-
2	P1	Kardam	8.0	1.8	7.9	72.2	0.6	0.5	0.1
			16.0	2.2	4.8	46.5			
			32.0	2.0	4.8	45.7			
3	P2	DAI (infection field)	8.0	2.6	5.5	49.5	0.6	0.8	0.3
			16.0	2.2	4.2	49.7			
			32.0	1.9	3.9	52.5			
4	P3	DAI (experimental field)	8.0	2.3	6.2	62.3	0.9	0.6	0.3
			16.0	2.8	4.3	55.1			
			32.0	1.7	3.0	33.2			
5	P4	Kardam	8.0	3.8	8.3	75.9	0.6	0.4	0.2
			16.0	2.7	7.7	70.9			
			32.0	2.4	5.1	73.3			
6	P5	Tyulenovo	8.0	4.5	9.6	144.4	0.5	0.8	0.5
			16.0	2.1	4.4	46.9			
			32.0	1.8	2.0	20.7			
7	P6	Dyakovo	8.0	3.3	8.7	77.5	0.3	0.6	0.6
			16.0	3.4	7.7	63.8			
			32.0	1.1	4.5	44.4			

8	P_7	Selanovtsi	8.0 16.0 32.0	2.9 2.5 2.5	10.9 7.9 4.8	85.7 84.3 90.8	0.3	0.5	0.2
9	P_8	Svishtov	8.0 16.0 32.0	1.9 0.8 1.0	7.8 3.9 3.1	72.3 36.2 29.5	0.5	0.7	0.4
10	P_9	Radnevo	8.0 16.0 32.0	2.2 1.9 2.3	9.3 7.2 6.1	73.6 100.7 74.7	0.6	0.1	-0.1
11	P_{10}	Radnevo	8.0 16.0 32.0	1.8 2.9 1.8	5.1 4.3 4.1	53.8 16.8 13.2	0.6	0.9	0.3

Legend: CA - Coefficient of allometry*; SVI* - Seedling vigor index*GI* - index of plant development; OAP - Overall allelopathic potential; *DAI- Dobroudja Agricultural Institute, General Toshevo, Bulgaria.*

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FIRST REPORT OF SUNFLOWER BROOMRAPE (*OROBANCHE CUMANA* WALLR.) IN CHAMOMILE

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Abstract

The parasitic plant *Orobanche cumana* Wallr. is the most important biotic constraint to the production of sunflower seed in all countries where sunflower is grown except in North and South America. *Orobanche cumana* is a close relative of *Orobanche cernua*, two different *Orobanche* species that have long been considered as single one (Roman et al., 2013). In fact, *O. cumana* and *O. cernua* mainly differ in their host range pattern. *O. cernua* is able to parasite different wild species of the Compositae family, mainly the genus *Artemisia,* and a few other species, while *O. cumana* has only been described as a specific parasite of sunflower crop. However, we recently discover that *O. cumana* is able to parasite other crops of the Compositae family. In 2016, we observed for the first time *Orobanche* shoot in a field of Chamomile (*Matricaria reticutita*) close to Marchena (Seville). From these *Orobanche* shoot, we collected both tissues for genotyping and seeds for phenotyping analysis. Results from the genotyping with a kit of 200 SNP dedicated to *O. cumana* diversity study (Coque et al., 2016), indicate that *Orobanche* shoots sampled into Chamomile plants are genetically very close to *O. cumana* populations parasiting the sunflower in Andalucia. Seeds from *Orobanche* parasiting the Chamomile were collected and the race of the parasite examined according to an internal differential set of lines. Both genotyping and phenotyping results confirm that *Orobanche* shoots sampled into Chamomile field are from *Orobanche cumana* species.

Keywords: chamomile, broomrape, parasiting, genotyping, phenotyping

DISTRIBUTION AND RACE COMPOSITION OF BROOMRAPE (*OROBANCHE CUMANA* WALLR.) IN BULGARIA DURING 2008 – 2017

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Abstract

Broomrape occurred in Bulgaria with the very introduction of sunflower as a field crop. In order to determine the race composition and distribution of broomrape in the country during the ten years period samples of the parasite were collected in different locations from the main sunflower production regions. The race composition of broomrape was determined under greenhouse conditions by standard method. The broomrape population in Bulgaria includes five races (C, E, F, G and H) according to the results obtained from this investigation. Race C was estimate only one time localized on a very small area. Races E and G were most widely distributed, mainly in north-east Bulgaria. Later on, the serious expands was observe in central south regions in Bulgaria. Race F was reported in beginning of this investigation in north Bulgaria. Very soon, the race disappear. May be the strong breeding pressure and the fast changing varietal structure are reason for this fact. At the beginning broomrape race “H” was distributed in the region of north-east, in the border area with Romania and along the north Black sea coast. The last 4 years this race was estimate in central north Bulgaria and we expect expanding to new regions, and the percentage and rate of attack will increased.

Keywords: broomrape, race composition, distribution

GENETIC DIVERSITY OF *OROBANCHE CUMANA* POPULATIONS USING ISSR MARKERS

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Abstract

The parasitic plant *Orobanche cumana* Wallr. has become a limiting factor for sunflower crops. The genetic diversity of broomrape from the Republic of Moldova is still grossly under-studied. This not only leads to unclear status of taxonomically problematic species and their local intraspecific taxa, but also hampers the assessment of their relevance as actual or potential treats for agricultural.

The aim of this study was to evaluate the inter-population variability of the phytopathogen *Orobanche cumana* Wallr. in the Republic of Moldova. The study of genetic polymorphism was carried out in 39 populations of *O. cumana* through the application of 14 ISSR markers.

The assessed have demonstrated different levels of genetic variability, depending on primers. The genomic-molecular analysis has shown that populations belonged from the Central and Southern region of the Republic of Moldova are characterized by the presence of the high number of alleles and are more polymorphic comparative with those from the North. Some populations in the South region (in particular, Carabetovca, Alexanderfeld, Ştefan-Vodă and Slobozia Mare) showed the major differences in the obtained profiles and a high level of genetic variability.

Taking into account the results regarding the genetic distance, the clustering analysis and the molecular profiles, we can conclude that the populations of *Orobanche cumana* in the Republic of Moldova have major differences at the molecular level.

Keywords: *Orobanche cumana*, ISSR, genetic distance, population variability

THE STATISTICAL ANALYSIS OF DATA: STRUCTURAL AND FUNCTIONAL VARIABILITY OF BROOMRAPE POPULATIONS AND ITS GENETIC BASIS

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Abstract

The objective of this study was to evaluate the efficiency of different statistical analysis and different peculiarities in the classification of broomrape populations and, particularly, to demonstrate the benefits of the approach for races identifications.

The basic material of the study where the results of seeds morphology (length, width, length/width) of 39 populations of *Orobanche cumana,* collected from field expeditions on the Republic of Moldova and molecular data of two DNA-based fingerprinting techniques (SSR and ISSR) applied to its.

The cluster analysis of morphology data showed a clear distinction of populations in the North, Central and Southern region of the Republic of Moldova and a weak association in community clusters according to physiological breeds.

The results from cluster analyses using SSR and ISSR data by DendroUPGMA indicate that these two marker techniques provide similar but not identical phylogenetic information. In case, of ISSR, does not show a clear separation of the samples based on the races. However, the dendrogram revealed a clear genetic separation of the populations according to the geographical origin. Thus, these results confirm widely previous data based on morphological peculiarities.

In contrast to the previous results, the analysis of SSR molecular pattern showed medium association with races at the first and second level and did not reveal any direct relationship between genetic structure of populations and geographical distribution. In this analysis the races E, F, G and H form a distinct cluster and more aggressive and evaluated race H includes the genetic information characteristic to the precedent races.

This study illustrates that the application of different type of cluster analysis led to a different ranking of the genetic and environmental, which is important in identification of race composition and distribution of broomrape on the territory of Moldova - critical step for any breeding program.

Keywords: sunflower, broomrape populations, genetics, variability, statistical analysis

THE COMPARATIVE FINGERPRINTING ANALISYS OF DIFERENT *OROBANCHE* ACCESSIONS

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Abstract

Orobanche cumana Wallr. is a parasitic plant spread on Republic of Moldova territory, which significantly affects the harvest. The broomrape accessions are highly variable and quickly evolve new races. Three DNA - based fingerprinting techniques - simple sequence repeats (SSR), inter simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD) analyses, were applied in broomrape populations analysis to assess genetic diversity.

Broomrape seeds collected from 39 sunflower fields located in different parts of the Republic of Moldova and three samples belonged from Romania, Ukraine and Spain, as well as two accessions collected from *Nicotiana tabacum* L. were used in this experiment.

In fact, a total of 132 ISSR and 50 SSR products were identified by 14 and, respectively, 12 primers in the accessions collected from sunflower fields. The polymorphic information content (PIC) was 0.86 and 0.57, corresponding, in the case of ISSR and SSR analysis.

It is of great interest that broomrape accessions collected from tobacco have presented distinct profiles. Thus, one of them showed profiles similar to those of the broomrape belonged from sunflower fields, while other presented lack of amplification with all SSR and ISSR primers and different amplification with RAPD primers. Thereby, obtained data support idea that one of the populations represents another species of *Orobanche.* Following complex studies will be developed to identification of species.

Keywords: broomrape, sunflower, genetic diversity

PROTEOME AND TRANSCRIPTOME ANALYSIS OF *OROBANCHE CUMANA* WITH DIFFERENT LEVELOF VIRULENCE

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Abstract

Since 1970, when a new broomrape race, called Moldovan race or race C, was identified on the territory of Moldova, the aggressiveness of the parasite increased significantly and new, highly virulent races appeared very quickly. Actually, all the known races of *Orobanche cumana* Wallr. have been detected in our country and the areas affected by these parasitic plants expanded considerably across the territory of Moldova.

Taking into account that studies of the relation between host and pathogen are focused in particular on the plant response to the parasite and less on the molecular basis of infection process and virulence of the supposed races, the aim of this paper is the comparative analysis of transcriptomic profile of genes encoding enzymes involved in host invasion (PME, PGU, PRX and CHS) and proteomic profile of germinated broomrape seeds with different virulence.

For the comparative analysis two populations of *O. cumana*, one belonging to race E or less virulent than E and one from highly virulent race H, collected from sunflower fields located in the Central part of the Republic of Moldova were used.

The study of the relative expression of genes *PRX, PME, PG* and *CHS* at the different developmental stage (tubercle, underground and aerial shoots) of angiosperm *O. cumana* has revealed the correlation between the transcription level and virulence of parasite. Thus, all the genes were up-regulated in the most virulent population comparative to the less virulent.

To analyses differentially expressed proteins two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry (MALDI-TOF MS) were used. The comparative analysis of the proteome revealed 19 DEPs. A number of these have been identified as enzymes of the respiratory system, as well as stress-related proteins. Also, a variety of transporting, regulatory or synthetic proteins have been identified, including some proteins with unknown function that can be co-expressed at various parasitic stages and may be important in the development of haustorium. Among the proteins were also identified cell wall modifying enzymes and proteins known to be involved in the invasion of the parasite.

Keywords: broomrape, two-dimensional difference gel electrophoresis, gene expression

RACE COMPOSITION AND PHENOLOGY OF SUNFLOWER BROOMRAPE *(OROBANCHE CUMANA* WALLR.) IN UKRAINE

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SUMMARY

Sunflower broomrape (*Orobanche cumana* Wallr.) is widely common in Ukraine. There is an increase in the number and harmfulness of broomrape in the crops due to violations of the farming system and over-saturation of crop rotations with sunflower. The study was carried out (i) to investigate broomrape populations, which were collected in areas of mass affection of sunflower, in terms of their race composition; and (ii) to explore the parasite's phenology in the northeast part of Ukraine. Broomrape race composition was determined under controlled conditions in a phytotron and in the experimental fields. There were no parasite biotypes with virulence higher than that of race G in *O. cumana* population collected in the east of Ukraine. The presence of a biotype, which was more virulent than race H, was proved in *O. cumana* population collected in the southeast of Ukraine. Race H was in the population of broomrape from northeast part of Ukraine. Our data attest to rapid spread of more virulent races of the parasite in Ukraine. In the northeast of Ukraine, the onset of broomrape anthesis is within the first 10 days of July. The parasite ripens within the third 10 days of August.

Key words: broomrape races, broomrape phenology, controlled conditions, field conditions, *Helianthus annuus*

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is widely common in Ukraine. It causes considerable losses in the yield in the east and south of the country, in Odesa, Mykolaiv, Kherson, Zaporizhzhia, Dnipropetrovsk, Donetsk, Luhansk regions as well as in certain districts of Kharkiv, Kirovograd and Cherkasy regions (Kyrychenko, 2005). Saturation of crop rotations with sunflower in these areas can exceed 30% (State Statistics Service of Ukraine, 2017). So, there is an increase in the number and harmfulness of broomrape *Orobanche cumana* Wallr. in the crops due to violations of the farming system and over-saturation of crop rotations with sunflower.

For Ukraine, *Orobanche cumana* Wallr. is a plant-kenophyte. It penetrated into Ukraine after Columbus had discovered America (Ministry of Ecology and Natural Resources of Ukraine, 2014). Presumably, initially it parasitized on *Artemisia* plants; at the end of the 19th century it switched to sunflower (Novopokrovskiy and Tsvelev, 1958).

B. Yenken was the first in Ukraine who started of sunflower breeding for resistance to broomrape at Kharkiv Breeding Station (now the Plant Production Institute nd.a. VYa. Yuriev of National Academy of Agrarian Sciences of Ukraine) in 1909. As a result, variety Zelenka 76, which was resistant to race A, was created (Kyrychenko, 2008). At the end of the 1920s, mass spread of broomrape was observed in Ukraine, which almost brought sunflower cultivation to stop due to a drastic decline in yields. This happened when, in some regions of the east of the country (Donetsk region), new race B (or a group of races) affected 100% of varieties, which were considered as broomrape-resistant (Bukherovich, 1967). The well-known breeder V.S. Pustovoyt used local accessions from Donetsk, Dnipropetrovsk, Poltava and Zaporizhzhia regions of Ukraine in breeding for resistance to the group of races B (Pustovoyt, 1975).

In 1963, in Kaushany district of Moldova, near the border with Ukraine (Odesa region), every single variety was infected by a new race of broomrape called "Moldavian" (Bukherovich, 1967). At the end of the 1960s, in Donetsk, Dnipropetrovsk, Odesa regions, sunflower varieties, which were resistant to the group of races B, got affected by broomrape. It was found that the virulences of the Odesa and Moldovian populations of broomrape were identical (Burlov, 1976).

In the early 1980s, studying various geographic isolates of broomrape in relation to a collection of sunflower accessions with various resistance, Burlov and Artemenko (1983) revealed that the Odesa population of broomrape comprised least five groups of physiological races (A, B, C, D, E). It was identical to the broomrape populations, which were common in other Ukrainian regions growing sunflower, in particular in Donetsk region. It was emphasized that resistance controlled by the Or_5 gene was universal.

In the early 2000s, another change in the broomrape race composition occurred in Ukraine. Studying seeds form the broomrape population collected in the south of Odesa region, Burlov and Burlov (2010) found race G determined the population. Race G was reported in the northern steppe of Ukraine (Luhansk region) in 2013 (Khablak, 2013).

Studies of researchers of the Plant Production Institute nd.a. VYa Yuriev of NAAS, which are aimed at search and investigation of broomrape-resistant sunflower biotypes, were initiated in the 1960's. At that time, there were no sunflower varieties with resistance to new broomrape races, and this fact urged to search for sources of resistance among wild species of the genus *Helianthus*. Due to crossing sunflower with annual and perennial wild species, sources of resistance to major pathogens and broomrape were identified (Sytnik, 1972). Broomrape-resistant forms with a variety of morphological and economic traits were derived from the perennial hexaploid species *Helianthus tuberosus* L. via pair and saturating crossings with individual selection on severe infection (Sytnik and Gumeniuk, 1983; Kyrychenko and Sytnik, 1985).

In the 1980s, effects of the infection load, sunflower plant number per vessel, storage period of broomrape seeds and illumination length on damage to plants were studied under phytotron conditions (Kyrychenko *et al.*, 1987, Petrenkova *et al.*, 2012). Due to the controlled climate, new breeding material of female and male sunflower that was resistant to widespread (in that period) races of broomrape was created (Aladiina, 1991). Lines Kh1002B and Kh711V were recommended for breeding practice, and commercial sunflower hybrids were developed on their basis: Kharkivskyi 49, Kharkivskyi 58, Svitoch, Oskil, Kovcheh, Yason. These hybrids occupied large sown crops in Ukraine for a long time.

The study was carried out (i) to investigate broomrape populations, which were collected in areas of mass affection of sunflower, in terms of their race composition; and (ii) to explore the parasite’s phenology in the northeast part of Ukraine.

MATERIAL AND METHODS

Controlled conditions

Broomrape race composition under controlled conditions in a phytotron was determined during two winters: 2010/2011 and 2016/2017. Broomrape seeds for the 2010/2011 assessment were harvested from a landrace in the east of Ukraine (Amvrosiivska district, Donetsk region) in 2010. Broomrape seeds for the 2016/2017 assessment were harvested from collection sunflower accessions sown in a 4-field crop rotation of the Plant Production Institute nd.a. VYa Yuriev of NAAS (Kharkiv region, Kharkiv region) in 2014 and from experimental sunflower accessions sown as a monocrop at the Institute of Oil Crops of NAAS (Zaporizhzhia district, Zaporizhzhia region) in 2016.

Broomrape seeds were harvested during the stage of full ripeness. Capsules were separated from stems, dried outdoors in the shade and stored in paper bags in a dark dry place at room temperature. Immediately before using, broomrape capsules were ground and passed through a laboratory sieve.

Seeds of sunflower accessions - differentiators of broomrape races were received under the collection exchange program from Maria Pacureanu-Joita.

The differentiators used in 2010/2011 were as follows (names are given according to the submitted list): line LC1002V - to race D, line LC1003V and hybrid F_1 – to race E, line LC1093A – to race F, hybrid F_1 – to race G, hybrid F_1 – to race H (?) and line AD-66 that is susceptible to all races.

The differentiators used in 2016/2017 were as follows: LC1002B – to race D**,** LC1003B – to race E, LC1093B – to race F, H_1 – to races E–F, H_3 – to races G–H, H_4 – to race H.

To artificially infest sunflower with broomrape and to detect resistant lines, we used a controlled climate (phytotron). Sunflower seeds were sown in 5-liter vessels filled with infected soil mixture, at the rate of 2 g of broomrape seeds per 5 kg of soil. Ten plants were left in the vessels after sunflower germination. The controlled parameters were: air temperature + 24 ... + 28°C, relative air humidity 70 ... 80%, illumination length – 16 hours/day.

Broomrape-induced damage to sunflower plants was evaluated 40 days after seeding differentiator seeds.

Field conditions

The field studies were conducted in the experimental fields of the Plant Production Institute named after VYa Yuriev of NAAS (49°59'36"S, 36°27'17"W) in 2010-2011. The study area was forest-steppe. The soil is mainly powerful weakly-leached heavy-loamy silt chernozem on silt-loamy carbonate loess. The total soil depth is more than 75 cm with good humus content (humus content in the plowing horizon is 5.46-7.28%). The climate of the zone is moderately continental. The sum of active temperatures above 10°C amounts to 3,000°C and satisfies the sunflower's need for heat.

The conditions of 2010 were unfavorable for sunflower due to abnormally high air temperatures (up to 39.8°C) during anthesis and seed filling (July-August) on severe soil and air drought. However, in general, the rainfall exceeded the norm (average for 1960-1991) by 22% during the vegetation period of 2010 (May-September), due to precipitation in May and September. A record-breaking high temperature for August (39.8°C) was documented that year. In 2011, the precipitation amount exceeded the norm by 16.1%. The precipitation amount in the 3rd 10 days of June (121.0 mm) and the 1st 10 days of July (89.3 mm) provided enough moisture in soil before anthesis. The temperature was higher than the norm in May (+2.2°C), June (+2.2°C), July (+3.7°C), August (+1.8°C), and September (+1.6°C). In general, the conditions of 2011 were more favorable for the growth and development of sunflower compared to the 2010 conditions.

The infection background was created by seeding broomrape seeds together with sunflower seeds in each seedhole with manual planters. To facilitate the inoculation, broomrape seeds were mixed with soil at a ratio of 2 g of broomrape seeds to 2 kg of soil. Sunflower plants were seeded at a distance of 70 cm between the rows and 70 cm between the plants in a row. Broomrape affection of sunflower plants on artificial infection in the field was measured in the phase of physiological ripeness of differentiator plants.

The phenologies of sunflower and broomrape were monitored. In sunflower line Skh2111A, the development phases were determined according to the scale of Shneiter and Miller (1981): VE; R1; R5; R8; R9. The following broomrape development phases were distinguished: emergence of floriferous shoots over the ground, onset of anthesis, ripening onset.

Date analyses

Under the controlled conditions, the number of affected plants (N1) and the number of parasite tubers (B) were counted on roots of each differentiator. In the field, the number of affected plants (N1) and the number of the parasite floriferous shoots (V) were counted in the differentiator plots. In the both variants, 20 plants of each sunflower accession (N) were taken in one replicate. The percentage of affected plants (P) was calculated according to the formula: $P = N1 \times 100 / N$. The affection degree (I) was determined by the parasite tuber number (phytotron) or the parasite floriferous shoot number (field) per one affected sunflower plant according to the formula: $I = V/N1$.

RESULTS AND DISCUSSION

Affection of sunflower by an *O. cumana* population of from the east of Ukraine (Donetsk region)

Analysis of the affection of accessions – race differentiator proved high virulence of an *O. cumana* population collected in the east of Ukraine (Donetsk region) in 2010 (Table 1). The contamination of surveyed sunflower crops with broomrape exceeded the economic threshold of the parasite harmfulness (3-5 broomrape floriferous shoots per sunflower plant). Both the phytotron and field data showed that the broomrape affection degree of F_1 hybrids - differentiators of races G and H was 0.0 floriferous shoots or parasite tubers, that is, there were no parasite biotypes with virulence higher than that of race G in the population.

Table 1: Affection of Accessions - Differentiators by *O. cumana* from the East of Ukraine (Donetsk Region)

Accession - differentiator	To race	Field conditions		Controlled conditions	
		% of affected plants	Affection degree	% of affected plants	Affection degree
LC1002B	D	60.0	5.7	100.0	5.2
LC1003B	E	20.0	1.0	100.0	8.4
F_1	E	80.0	5.9	100.0	8.2
LC1093A	F	40.0	5.3	70.0	2.0
F_1	G	0.0	0.0	0.0	0.0
F_1	H (?)	0.0	0.0	0.0	0.0
AD-66	susceptible	100.0	12.0	100.0	13.6

The affection degree of differentiators of races D (LC1002B) and E (LC1003B, F_1 hybrid) in the phytotron was within 5.2 - 8.4 tubers per plant, and there were no resistant plants (100% of affected plants). In the field, the percentage of affected plants in these differentiators varied from 20 to 80%. We found no resistant plants in line AD-66, which does not have genes of resistance to broomrape, under any conditions.

The affection degree of differentiator of race F (LC1093A) in the phytotron was 2 tubers per plant, and the percentage of affected plants was 70%. The difference in the broomrape affection degree between different differentiators was small (from 2.0 to 8.4 tubers per affected plant). However, we hypothesized that race F predominated in the population (the affection degree of differentiators to race E was 8.2-8.4). According to the affection of line LC1093A, race G accounted for the lowest percentage (70.0% of affected plants, 2.0 tubers per affected plant under the controlled conditions) in the studied population

Affection of sunflower by *O. cumana* populations from the southeast (Zaporizhzhia region) and the northeast (Kharkiv region) of Ukraine

Analysis of the affection of accessions – race differentiators in the phytotron proved high virulence of *O. cumana* populations collected in the southeast of Ukraine (Zaporizhzhia region) in 2016 and in the northeast of Ukraine (Kharkiv region) in 2014 (Table 2). The contamination of surveyed sunflower crops with broomrape exceeded the economic threshold of the parasite harmfulness of (3-5 broomrape floriferous shoots per sunflower plant).

Table 2: Affection of Accessions - Differentiators by *O. cumana* from the Northeast and Southeast of Ukraine

Accession - differentiator	To race	Controlled conditions[1]		Controlled conditions[2]	
		% of affected plants	Affection degree	% of affected plants	Affection degree
LC1002B	D	100.0	2.2	25.0	2.0
LC1003B	E	100.0	5.5	100.0	1.8
LC1093A	F	100.0	4.7	100.0	3.9
H1	E-F	100.0	3.4	100.0	5.0
H3	G-H	100.0	3.3	44.4	4.5
H4	H	100.0	5.7	0.0	0.0

[1] – Broomrape seeds collected in the southeast of Ukraine (Zaporizhzhia region)
[2] – Broomrape seeds collected in the northeast of Ukraine (Kharkiv region)

Using this set of differentiators, from 100% affection of plants of all accessions by a broomrape population from the southeast of Ukraine we proved the presence of a biotype, which was more virulent than race H.

The virulence of broomrape from the northeast was weaker. There were no affected plants in the differentiator of race H (H4), suggesting absence of a biotype that is more virulent than race H in the population. 44.4% of affected plants of the differentiator to races G-H (H3) indicate that there was race H in the population.

O. cumana phenology in the northeast of Ukraine

Phenological monitoring of the development of sunflower line of Skh2111A and *O. cumana* in the northeast of Ukraine (Kharkiv region) (field infection) allowed establishing the following regularities (Table 3). Upon the optimal period of sowing in the study area, when the soil temperature at the seeding depth reaches 12°C, sunflower shoots emerged on May 15, 2010 and on May 11, 2011. Broomrape appearance over the soil surface coincided with the sunflower development phases R3-R4. In 2011, the weather conditions were more favorable for sunflower than those in 2010, broomrape anthesis preceded sunflower anthesis, and broomrape ripened during the phase R8 of sunflower. In 2010, the onset of broomrape anthesis coincided with the onset of sunflower anthesis, and broomrape ripening - with the phase R9.

Table 3: Main Phenological Phases of the Development of Sunflower Line Skh 2111 A and *O. cumana*, the northeast of Ukraine (Kharkiv region), artificial infection in the field

Year	Date	Developmental phase	
		Skh 2111 A	*O. cumana*
2010[1]	May 15	VE	–
	June 17	R1	–
	July 02	R4	Floriferous shoots emerged over the soil surface
	July 07	R5	10 % floriferous shoots flowered
	August 26	R9	Ripening
2011[2]	May 11	VE	–
	June 25	R1	–
	June 30	R3	Floriferous shoots emerged over the soil surface
	July 14	R5	90 % floriferous shoots flowered
	August 25	R8	Ripening

[1] – sowing date May 05
[2] – sowing date May 03

CONCLUSIONS

The herbaceous parasite *O. cumana* is a threat to sunflower crops in Ukraine. Affection of commercial crops by broomrape in the main regions of sunflower cultivation exceeds the economic threshold of harmfulness. Evaluation of the virulence of the broomrape populations from the east (Donetsk region), northeast (Kharkiv region) and southeast (Zaporizhia region) of Ukraine related to European accessions – race differentiators demonstrated race heterogeneity of these populations. Of the studied populations, the broomrape population from the southeast of Ukraine was the most virulent. Using the test set of differentiators, we proved the presence of a biotype, which was more virulent than race **H,** in this population. Our data attest to rapid spread of more virulent races of the parasite in Ukraine.

In the northeast of Ukraine, the onset of broomrape anthesis is within the first 10 days of July. The parasite ripens within the third 10 days of August.

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