Foreword

The parasitic angiosperm broomrape (*Orobanche cumana* Wallr.) causes economic damage in sunflower production in a number of countries around the world, but especially in Central and Eastern Europe, Spain, Turkey, Israel, Iran, Kazakhstan, and China. For almost a century, there has been a constant tug-of-war between sunflower breeders and *Orobanche cumana*, with frequent changes in which side has the upper hand. Almost as soon as the breeders find a source of resistance to the latest race of the pathogen, broomrape responds by evolving another virulent race. The development of resistant cultivars as well as optimized managing strategies is a high priority in controlling this parasite, over the world.

The Board of the International Sunflower Association (ISA) proposed in their past meeting held in Paris in February 2017 to organize a new International Symposium on Broomrape in Sunflower in Romania in July 2018. This will be the fourth specific symposium on broomrape in sunflower, after those held in Turkey 2008, Moldova 2011 and Spain 2014.

The symposium is organized by the National Agricultural Research and Development Institute Fundulea and University of Agronomy and Veterinary Medicine Sciences in Bucharest in cooperation with the International Sunflower Association (ISA). Also, the Research Station in Brăila and Institute for Variety Testing and Registration in Bucharest, are collaborating for this. The symposium will be held in Bucharest, in the building of the Faculty of Biotechnology on July 2-4, 2018. The symposium will cover all aspects related to broomrape parasitisms in sunflower, including parasite biology, physiology, parasite-host interaction, racial status of broomrape, genetic resistance, molecular breeding, chemical control using herbicide-tolerant, integrated management.

The symposium will gather sunflower scientists around the world, presenting their recent achievements. The organizers will also invite relevant stakeholders to provide a view on broomrape situation around the world as well as prospects to overcome the limitation for sunflower production, imposed by this parasitic weed.

The Organizing Committee

C O N T E N T S

I. BIOLOGY AND GENETICS OF THE PARASITE OROBANCHE CUMANA

II. RESISTANCE MECHANISMS TO OROBANCHE CUMANA *IN SUNFLOWER*

III. GENETIC RESISTANCE TO SUNFLOWER BROOMRAPE

IV. HERBICIDE TOLERANCE AND OTHER CONTROL METHODS

INCREASE IN VIRULENCE OF SUNFLOWER BROOMRAPE IN SERBIA

Boško Dedić*, Dragana Miladinović, Siniša Jocić, Sandra Cvejić, Milan Jocković, Vladimir Miklič

Institute of Field and Vegetable Crops, Novi Sad, Serbia

*Corresponding author: bosko.dedic@ifvcns.ns.ac.rs

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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