

## Broomrape resistance in newly developed sunflower inbred lines

Sandra Cvejic<sup>\*</sup>, Bosko Dedic, Sinisa Jovic, Dragana Miladinovic, Vladimir Miklic

<sup>\*</sup>Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia,  
sandra.cvejic@ifvcns.ns.ac.rs

### ABSTRACT

- Broomrape (*Orobanche cumana* Wallr.) is considered to be one of the most severe constraints in sunflower (*Helianthus annuus* L.) production. Rapid appearance of new races requires continuous search for new sources of resistance in sunflower germplasm. The objective of this research was to screen new sunflower inbred lines, originating from different interspecific hybridisations or Russian open pollinated varieties, for broomrape resistance using different races of parasite.
- Newly developed sunflower *cms* and *Rf* lines were screened for resistance to broomrape in greenhouse and in field trials in two locations in Serbia, infested with the race E of the parasite, during the period 2007-2010. Research was broadened in 2011 to locations in Spain and Romania which are previously known for presence of highly virulent races F and G. Differential lines, up to race F, were planted together with selected inbred lines. Reaction of the tested sunflower lines to broomrape was evaluated by calculating incidence, i.e. ratio of infected sunflower plants per total number of plants.
- Tested inbred lines significantly differed regarding their resistance to broomrape. In the first period of research, resistant inbred lines were selected. The experiment performed in five locations in three countries (Serbia, Spain and Romania) showed different reaction of selected sunflower inbred lines to broomrape, which depended on location i.e. race composition of the broomrape. Selected inbred lines were completely resistant to broomrape race E, present in Northern Serbia. Only several inbred lines showed resistance to races present in location in Spain and Romania. Also, differential lines were mostly susceptible to parasite indicating presence of race which overcome race F. Based on broomrape incidence of tested inbred lines and differentials, race G is present in locations in Spain and Romania and maybe more aggressive race.
- Inbred lines AB VL 8, LIV 10, LIV 11, LIV 12, LIV 16, LIV 17 and LIV 18 considered being resistant to broomrape attack in heavily infested areas in Romania and Spain. Only line AB VL 8 was fully resistant to races which overcome race F, i.e. race G in Spain, and G and more virulent race in Romania.
- Results indicate presence of resistance to highly virulent races of the parasite and therefore of extreme importance for current breeding programs and sunflower hybrid production.

**Key words:** broomrape – inbred line – resistance – screening – sunflower

## INTRODUCTION

The holoparasitic plant broomrape (*Orobanche cumana* Wallr.) infects the sunflower (*Helianthus annuus* L.) roots and causes severe damage in sunflower production in many countries, especially Eastern Europe, Spain, Turkey, Israel, Iran and China (Skoric et al., 2010). It is dispersing with spreading of the sunflower growing area. Therefore, broomrape needs to be controlled by using available strategies, and breeding for resistance proved to be the most reliable way to control the parasite (Dedic et al., 2009). Breeding for broomrape resistance is a constant work. As soon as resistance to the latest race is found, broomrape responds by evolving to a more virulent race. Sources of broomrape resistance were mostly found in certain wild species of the genus *Helianthus* and have been incorporated into cultivated sunflower genotypes by interspecific hybridization (Skoric et al., 2010).

Five different races (A, B, C, D, E) of broomrape have been reported by Vranceanu et al. (1980), which are controlled by five single dominant genes *Or1* to *Or5*, respectively. The gene *Or5*, that gives resistance to all five races, was successfully introduced into inbred lines with high combining ability which were used as parents of existing and perspective hybrids (Skoric and Jovic, 2005). In Serbia, race E of broomrape has been dominant for over ten years especially in northern parts of the country. Continual monitoring of the broomrape population in Serbia is very important due to changes in race composition and evolving of more virulent races in the neighbouring countries (Hladni et al., 2010). A new race F, that overcomes the gene *Or5*, was identified in Spain (Alonso et al., 1996; Molinero-Ruiz et al., 2008), Romania (Pacureanu-Joita et al., 1998), Turkey (Kaya et al., 2004) and some other countries. Furthermore, more virulent race G that affects cultivars resistant to race F, was identified (Skoric et al., 2010). According to Dicu et al. (2011) the race G was definitely found in Tulcea and Constanta counties in Romania and latest surveys showed possible appearance of even more virulent race.

Resistance to race F was found in germplasm of both cultivated and wild sunflower (Fernandez-Martinez et al., 2000; Pacureanu-Joita et al., 1998). The resistance to races E, F, G have been found in certain wild species of the genus *Helianthus* and incorporated into cultivated sunflower genotypes by interspecific hybridization. Jan et al. (2002) crossed the wild sunflower species *Helianthus maximiliani* Schrad., *Helianthus grosseserratus* Mart., and *Helianthus divaricatus* L. with cultivated sunflower and developed four populations (BR1-BR4) resistant to race F in Spain. Great contribution was made by developing sunflower varieties through interspecific hybridisation in which *Helianthus tuberosus* was used as the donor of *Or5* and *Or6* genes (Skoric et al., 2010). The latest research conducted in Romania under field and greenhouse conditions showed that sources of resistance to the newest population of *Orobanche* that were found in Romania, Turkey and Spain are present in the lines LC-009 and AO-548. Genetics of the resistance of these sunflower lines and the race composition of broomrape populations remains to be studied (Skoric et al., 2010). The differences in sources of the resistance have led to development of lines that are resistant to the same race of broomrape but vary in their genetic constitution (Imerovski et al., 2011). Thus, different authors reported different modes of inheritance of resistance to race F; controlled by single dominant gene, *Or6* (Pacureanu-Joita et al., 1998; Perez-Vich et al., 2002) or two recessive genes (Akhtouch et al., 2002) or two partially dominant genes (Velasco et al., 2007). Preliminary results of resistance to race G indicate that it is controlled by dominant alleles at a single locus (Velasco et al., 2011).

The development of broomrape resistant inbred lines is not an easy task, since it depends on broomrape race and genetic background of sunflower. The objective of this research was to screen new sunflower inbred lines, originating from different interspecific hybridisations or Russian open pollinated varieties, for broomrape resistance using different races of parasite.

## MATERIALS AND METHODS

New sunflower inbred lines, developed at the Institute of Field and Vegetable Crops, were used for screening: 20 *cms* lines (LIV 1-20) and 20 restorer lines (R-ST 1-20), that originated from population developed from interspecific hybridization with *Helianthus tuberosus*, 20 *cms* lines (AB VL 1-20) that originated from population developed from interspecific hybridization with *Helianthus divaricatus* and 40 *cms* (SAN 1-40) lines that originated from different Russian varieties.

Screening procedure for resistance to broomrape was done in greenhouse and in field trials during the period of 2007-2010. Field trials were performed in broomrape infested area in Northern Serbia in two locations (Kula and Subotica). Each line was planted in two rows (max. 24 plants) in two replications. The greenhouse testing was performed in the winter period under artificial inoculation using broomrape seeds from infested areas of Serbia. In the greenhouse, broomrape incidence was evaluated by recording

the number of infested sunflower plants with emerged or underground broomrape plants (Terzic et al., 2010).

In year 2011, inbred lines known to be resistant to broomrape race E, were planted in two locations in Serbia (Kula and Subotica), two locations in Romania (Constanta and Tulcea) and one in Spain (Sevilla). Each inbred line was planted in two rows (24 plants) in two replications. In Romania, both locations are situated in areas with presence of highly virulent races, in Black Sea region, where race F is widely spread and race G is identified (Pacureanu-Joita et al., 2008). In Spain, inbred lines were planted in location near Sevilla, in field artificially infested with broomrape harvested from hybrids resistant to race E.

Romanian differential lines (Vranceanu, 1980) were used as checks in evaluation of inbred lines in all locations (instead of line P-1380-2, another Romanian differential line LC-1003, for race E, was used), as well as lines LC 1093 and P-96 that are differentials for race F (Pacureanu-Joita et al., 2009; Fernandez Martinez et al., 2004).

Reaction of the tested sunflower lines to broomrape was evaluated by calculating incidence, i.e. ratio of the infected sunflower plants per total number of plants. Inbred lines were considered resistant (R) when no broomrape stalk was found within the complete entry, moderately resistant (MR) when 1-50% plants had at least one broomrape stalk and susceptible (S) when more than 50 % plants were infested.

## RESULTS AND DISCUSSION

In a period of 2007-2010, among tested inbred lines in field tests in two locations in Serbia and in the greenhouse, resistant inbred lines were selected (data not shown). These lines showed complete resistance to race E, the most virulent race in Serbia, and were used for further studies or sent for testing in locations where races F and higher are present.

Selected inbred lines were planted in five locations in three countries (Serbia, Spain and Romania) in year 2011. Selected sunflower inbred lines reacted differently to broomrape, which depended on location i.e. race composition of the broomrape. Inbred lines, which showed resistance to different broomrape races existing in the five examined regions, are listed in Table 1.

**Table 1.** Broomrape incidence (%) and reaction of sunflower inbred lines in five different locations

GENOTYPE	SERBIA		ROMANIA		SPAIN
	KULA (race E)	SUBOTICA (race E)	CONSTANTA (races F, G)	TULCEA (races F, G, maybe new)	SEVILLA (race F, G)
AB VL 8	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
LIV 10	0 (R)	0 (R)	0 (R)	3 (MR)	0 (R)
LIV 11	0 (R)	0 (R)	0 (R)	5 (MR)	0 (R)
LIV 12	0 (R)	0 (R)	0 (R)	7 (MR)	0 (R)
LIV 16	0 (R)	0 (R)	0 (R)	4 (MR)	0 (R)
LIV 17	0 (R)	0 (R)	0 (R)	3 (MR)	0 (R)
LIV 18	0 (R)	0 (R)	0 (R)	9 (MR)	0 (R)
R-ST 1	0 (R)	0 (R)	0 (R)	100 (S)	100 (S)
SAN 3	0 (R)	0 (R)	<50 (MR)	<20 (MR)	100 (S)
SAN 4	0 (R)	0 (R)	<20 (MR)	<50 (MR)	100 (S)
SAN 7	0 (R)	0 (R)	<50 (MR)	<50 (MR)	100 (S)
SAN 33	0 (R)	0 (R)	<50 (MR)	<50 (MR)	100 (S)
SAN 36	0 (R)	0 (R)	<50 (MR)	<50 (MR)	100 (S)
P-96 ( <i>Or6</i> )	0 (R)	0 (R)	0 (R)	100 (S)	100 (S)
LC-1093 ( <i>Or6</i> )	0 (R)	0 (R)	50 (MR)	100 (S)	100 (S)
LC- 1003 ( <i>Or5</i> )	0 (R)	0 (R)	100 (S)	100 (S)	100 (S)
S-1358 ( <i>Or4</i> )	100 (S)	100 (S)	100 (S)	100 (S)	100 (S)
Record ( <i>Or3</i> )	100 (S)	100 (S)	100 (S)	100 (S)	100 (S)

Jdanov ( <i>Or2</i> )	100 (S)				
Kruglik A-41 ( <i>Or1</i> )	100 (S)				
AD-66	100 (S)				

R-resistant, MR-moderately resistant, S-susceptible

All inbred lines were completely resistant to broomrape attack in two locations in Serbia, as expected. This indicates that examined inbred lines were resistant to race E of broomrape present in Northern Serbia which was confirmed by differential lines carrying *Or5* and *Or6* genes (LC-1003, LC-1093 and P-96). As expected, differential lines carrying up to *Or5* gene were fully susceptible to race F of broomrape present in locations in Romania and Spain. In Constanta location, one differential line carrying *Or6* was resistant (P-96), while other line (LC-1093) had half of plants infested, indicating the presence of races which overcome race F (race G). Both these lines were susceptible in locations Sevilla and Tulcea, indicating presence of race G and maybe more virulent race. It is unknown if the race/s in Spain and Romania are the same one, since there are no differentials carrying genes over *Or6*. Pacureanu-Joita et al. (2008) tested genotypes in different location in Romania and their results showed that the differential line for the race F (LC 1093), lost resistance in the Black Sea area. Our results indicate presence of new race in this area, but severity of attack was higher in Tulcea region since all plants from differential line LC 1093 were infested. Molinero-Ruiz et al. (2008) reported that lines L86 and P96 had low values of broomrape incidence, suggesting that broomrape race higher than F is possibly present in Sevilla area (SE295). This could explain why in our research differential P-96 was totally susceptible in the location in Spain. Our results indicate that race G is present in the locations in Romania and Spain, and it is possible that a more aggressive race is present in Tulcea region. Results of Dicu et al. (2011) also suggest presence of race G and G+ in Black Sea region and Molinero-Ruiz et al. (2008) that some accessions close to Sevilla overcomes race F.

Tested inbred lines showed different level of resistance in two infested locations in Romania. Only one line, AB VL 8, showed complete resistance in both locations. In a case of six inbred lines (LIV) in Tulcea location, only 1-2 sunflower plants were infested with only one broomrape stalks, while they were completely resistant in other location in Romania. Restorer line R-ST 1 was resistant to broomrape attack in Constanta location but susceptible in Tulcea, indicating presence of more virulent race in the second location. Group of *cms* lines SAN were mainly moderately resistant, in the both locations less than half of plants were infested. Although infection occurred, these lines showed high tolerance to virulent races present in Black Sea area. In Sevilla location inbred lines AB VL 8 and LIV group of lines were completely resistant to the parasite infection while other lines were susceptible.

Newly developed sunflower inbred lines were selected as potentially resistant to highly virulent races of broomrape. Inbred lines AB VL 8 and six LIV lines are found to be resistant to broomrape attack in heavily infested areas in Romania and Spain. Only line AB VL 8 was fully resistant to races which overcome race F, i.e. race G in Spain, and G and more virulent race in Romania. A new source of resistance to the new races of the broomrape was also reported by Pacureanu-Joita et al. (2009) in genotypes LC 009 and AO-548.

According to Skoric et al. (2010) wild *Heliathus* species remain the main source of resistance to new virulent races of pathogen. Velasco et al. (2011) found resistance to race G in a wild *Heliathus debilis* subsp. *tardiflorus* and the F1 plants from the cross with cultivated sunflower were resistant indicating dominance of resistance gene(s). In our work, we have found source of resistance to race G and more virulent races in population from interspecific hybridization with *Helianthus divarticatus*. From this population, using selection and broomrape screening, inbred line AB VL 8 was developed. A lot of breeders used *Helianthus tuberosus* as the source of gene resistance to races C, D, E, F (Skoric et al., 2006). From this source, new inbred lines were developed (LIV 10, LIV 11, LIV 12, LIV 16, LIV 17 and LIV 18). These lines proved to be resistant to race G, demonstrating that different sources of sunflower germplasm are reservoir of genes conferring resistance to new virulent races of broomrape.

The new virulent race/s of broomrape spread rapidly in some regions in South and Eastern Europe. The rapid changes in the race composition of broomrape requires search for new resistant sources. In the breeding material of Institute of Field and Vegetable Crops, several sunflower lines were identified for resistance to highly virulent races of broomrape, which is of extreme importance for current breeding programs, as source of resistance for gene transferring and sunflower hybrid production. After the identification of genes controlling resistance and their action, inbred lines could be used for transferring resistance in inbred lines with good agronomic traits, or could be used directly for developing commercial hybrids.

## ACKNOWLEDGMENTS

Authors would like to thank colleagues in Romania and Spain for providing locations for testing. The research is part of project TR31025 supported by Ministry of Education and Science, Republic of Serbia.

## REFERENCES

- Dedic, B., Lacok, N., Tancic, S., Dusanic, N., and S. Jovic. 2009. Current status of broomrape (*Orobanche cumana* Wallr.) in Serbia. *Helia* 32 (51): 135-140.
- Dicu, G., Craiciu, D., Teodorescu, A., Dumitrescu, N. C., Boaghe, N., and M. Ianita. 2011. Research regarding the virulence of broomrape parasite *Orobanche cumana* Wallr. in South-eastern part of Romania. In: Proc. International Symposium on Broomrape (*Orobanche* spp.) in Sunflower. Program and Abstracts. Chisinau, Moldova, August 25-27. 18.
- Fernandez-Martinez, J., Malero-Vara, J., Munoz-Ruz, J., Ruso, J., and J. Dominguez. 2000. Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance of the *Or*<sub>5</sub> genes. *Crop Sci.* 40: 550-555.
- Fernandez-Martinez, J., Perez-Vich, B., Akhtouch, B., Velasco, L., Munoz-Ruz, J., Malero-Vara, J., and J. Dominguez. 2004. Registration of four sunflower germplasm resistant to race F of broomrape. *Crop Sci.* 44: 1033-1034.
- Hladni, N., Jovic, S., Miklic, V., Sakac, Z., and D. Skoric. 2010. Assessment of quality of new *Rf* inbred lines resistant to broomrape race E (*Orobanche cumana* Wallr.) developed from *H. deserticola* by interspecific hybridization. *Helia* 33 (53): 155-164.
- Imerovski, I., Dimitrijevic, A., Miladinovic, D., Dedic, B., Jovic, S., and V. Miklic. 2011. Molecular profiles of sunflower lines resistant to broomrape (*Orobanche cumana* Wallr.). In: Proc. International Symposium on Broomrape (*Orobanche* spp.) in Sunflower. Program and Abstracts. Chisinau, Moldova, August 25-27. 25.
- Jan, C. C., Fernandez-Martinez, J. M., Ruso, J., and J. Munoz-Ruz. 2002. Registration of four sunflower germplasm with resistance to *Orobanche cumana* race F. *Crop Sci.* 42: 2217-2218.
- Kaya, Y., Evci, G., Pekcan, V., and T. Gucer. 2004. Determining new broomrape-infested areas, resistant lines and hybrids in Trakaya region of Turkey. *Helia* 27 (40): 211-218.
- Molinero-Ruiz, M. L., Perez-Vich, B., Pineda-Martos, R., and J. M. Melero-Vara. 2008. Indigenous highly virulent accessions of the sunflower root parasitic weed *Orobanche cumana*. *Weed Research* 48: 169-178.
- Pacureanu-Joita, M., Fernandez-Martinez, J. M., Sava, E., and S. Raranciuc. 2009. Broomrape (*Orobanche cumana* Wallr.), the most important parasite in sunflower. *An I. N. C. D. A. Fundulea* 77: 49-56.
- Pacureanu-Joita, M., Vranceanu, A. V., Seare, G., Marinescu, A., and I. Sandu. 1998. The evaluation of the parasite-host interaction in the system *Helianthus annuus* L. – *Orobanche cumana* Wallr. in Romania. In: Proc. 2<sup>nd</sup> Balkan Symposium on Field Crops, Novi Sad, Yugoslavia, June 16-20. 1: 153-158.
- Pacureanu-Joita, M., Raranciuc, S., Stanciu, D., Sava, E., and D. Nastase. 2008. Virulence and aggressiveness of sunflower broomrape (*Orobanche cumana* Wallr.) populations in Romania. *Romanian Agricultural Research* 25: 47-51.
- Perez-Vich, B., Akhtouch, B., Munoz-Ruz, J., Fernandez-Martinez, J. M., and C. C. Jan. 2002. Inheritance of resistance to a highly virulent race F of *Orobanche cumana* Wallr. in a sunflower line derived from interspecific amphiploids. *Helia* 25: 137-144.

Skoric, D., and S. Jovic. 2005. Broomrape (*Orobanche cumana* Wallr.) and its possible control by genetic and chemical means. Production and Processing of Oil seeds. In: Proc. 46<sup>th</sup> Oil Industry Conference. Petrovac na Moru, Montenegro, June 6-10. (In Serbian) 9-21.

Skoric, D., Jovic S., and S. Gvozdenovic (Cvejic). 2006. Achievements in sunflower breeding for resistance to diseases, broomrape and insects. Plant doctor (In Serbian) 4-5: 299-312.

Skoric, D., Pacureanu-Joila, M., and E. Sava. 2010. Sunflower breeding for resistance to broomrape (*Orobanche cumana* Wallr.). An. I. N. C. D. A. Fundulea 78 (1): 63-79.

Terzic, S., Dedic, B., Atlagic, J., Jovic, S., and S. Tancic. 2010. Screening wild sunflower species and F<sub>1</sub> hybrids for resistance to broomrape. Helia 33 (53): 25-31.

Velasco, L., Perez-Vich, B., Jan, C. C., and J. M. Fernandez-Martinez. 2007. Inheritance of resistance to broomrape (*Orobanche cumana* Wallr.) race F in a sunflower line derived from wild sunflower species. Plant Breeding 126: 67-71.

Velasco, L., Perez-Vich, B., Yassein, A. A. M., Jan, C. C., and J. M. Fernandez-Martinez. 2011. Inheritance of resistance to sunflower broomrape (*Orobanche cumana* Wallr.) in an interspecific cross between *Helianthus annuus* and *Helianthus debilis* subsp. *tardiflorus*. Plant Breeding. DOI: 10.1111/j.1439-0523.2011.01915.x.

Vranceanu, A. V., Tudor, V. A., Stoenescu, F. M., and N. Pirvu. 1980. Virulence groups of *Orobanche cumana* Wallr. differential hosts and resistance sources and genes in sunflower. In: Proc. 9<sup>th</sup> International Sunflower Conference, Torremolinos, Spain. Int. Sunflower Assoc. Paris 74-80.