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THE PARASITE BROOMRAPE (*OROBANCHE CUMANA*) IN SUNFLOWER – IDENTIFYING SOURCES FOR GENETIC RESISTANCE

Gabriel Florin ANTON[1,2], Maria JOITA-PACUREANU[2], Călina Petruţa CORNEA[1]

[1]University of Agronomic Sciences and Veterinary Medicine – Bucharest, Faculty of Biotechnologies, 59 Mărăşti Blvd, 011464 Bucharest, Romania, phone 004-021-318.36.40, fax.004-021-318.25.88, E-mail: pccornea@yahoo.com
[2]National Agricultural Research and Development Institute, Fundulea
1 N. Titulescu Street, 915200, Fundulea, E-mail: gabi22mai@yahoo.com, pacurean@ricic.ro

Corresponding author email: gabi22mai@yahoo.com

Abstract

Sunflower broomrape (Orobanche cumana Wallr.) is causing a great deal of damage to sunflower production in many countries in Europe as well as in countries from Asia and in Australia.
Extensive research on sunflower resistance to broomrape has been conducted in Russia, Romania, Bulgaria, Turkey, Serbia and Spain. New races of the parasite appear frequently. Sunflower breeders have been trying to develop sunflower genotypes resistant to all known races of this parasite.
In our research work regarding resistance to broomrape we have used different sources of resistance, the best ones being the sunflower wild species.
For obtaining results presented in this paper there have been used six cultivated sunflower genotypes which were crossed with two sunflower wild species, in order to obtain some populations. These populations are used for obtaining sunflower inbred lines which can to be used as sources of resistance (donor of genes) or directly to obtain hybrids.
There have been studied the interspecific hybrids as well as the parental forms, for the number of released seeds, for oil content, one thousand seed weight and resistance to different populations of broomrape parasite, in the artificial and natural infestation conditions.
We identified some populations full resistant to the most virulent races of broomrape in sunflower crop in Romania.

Key words*: sunflower, broomrape, genetic resources, wild species, interspecific hybrids.*

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production for more than a century. According to Morozov (1947), the first reports of broomrape in sunflower came from Saratov Oblast in Russia and date back to the 1890s. The same author mentions that the first sunflower varieties resistant to race A of *Orobanche* were developed by Plachek (1918) at the Saratov breeding station. Morozov (1947) and Pustovoit (1966) both note that Ždanov (1926) identified a new broomrape race (B) in Rostov Oblast and soon after the discovery developed a number of sunflower varieties resistant to it. In the period that followed, according to Pustovoit (1966), a number of high-oil varieties resistant to race B were developed at the VNIIMK institute in Krasnodar, Russia that thereafter played an important role in the spread of sunflower around the world. Later on, a new race that could not be controlled by the genes for resistance to races A and B was discovered in Moldova by Sharova (1968) and in Bulgaria by Petrov (1970). Through genetic research, Vrânceanu et al. (1980) established that five broomrape races (A, B, C, D, E) were detected in Romania and the dominant genes controlling resistance to them were identified. Race F was detected for the first time in Romania in 1995 (Pacureanu – Joita et al., 1998). Alonso et al. (1996) found the race (F) of the pathogen in 1996 in Spain. Papers by Alonso et al. (1996), Škorić and Jocić (2005), Fernandez-Martinez et al. (2007), Imerovski I. et al. (2015), each provide a detailed overview of the achievements of sunflower breeding for resistance to *Orobanche*.

Extensive research on broomrape resistance has been conducted in countries of the former USSR as well as in Romania, Bulgaria, Turkey, and Spain. In all these countries, broomrape causes great damage to sunflower production and new races of the pathogen appear frequently. In addition to Russia, Ukraine, Romania, Bulgaria , Turkey, and Spain, broomrape is also present in Serbia, Hungary, Moldova, Greece, Israel, Iran, Kazakhstan, China, Mongolia, and Australia (Antonova T., 2014, Batchvarova R., 2014, Pacureanu-Joita M., 2014, Pototskyi G., 2014, Molinero-Ruiz L.et al., 2015) and possibly in a few other countries as well. Sunflower breeders and geneticists have been trying to develop genotypes resistant to all known races of the parasite.

The objective of this paper was to identify new sources of sunflower resistance to *Orobanche*, useful in further breeding approaches.

MATERIALS AND METHODS

Six cultivated sunflower inbred lines belonging to NARDI Fundulea, Romania (LC 1029B, LC 991B, LC 1093B, LC1085C, LC 1095C, LC 1088C) and two sunflower wild species (*Helianthus tuberosus, Helianthus maximiliani*) have been introduced in crossing for obtaining interspecific hybrids, in order to create sunflower populations which will be used for releasing inbred lines with high resistance to broomrape (*Orobanche cumana*). These hybrids as well as the parental forms (cultivated and wild) have been studied for oil content, using the nuclear magnetic resonance (NMR) analyzer , one thousand seeds weight and resistance to the parasite broomrape.

There have been analyzed the number of sunflower heads and number of seeds/head, for each crossing.

The crossing between cultivated and wild sunflower was made by emasculation in cultivated inbred lines and making pollination with wild species pollen, as well as making emasculation in wild forms and pollination with pollen of cultivated ones (Jan and Seiler, 2008; Christov, 2008; Hristova-Cherbadzi, 2009).

For studying resistance to broomrape in different cultivated areas in Romania, there have been used some sunflower populations obtained from interspecific hybrids *(H. annuus x H. tuberosus*) after 5-6 generations of selfpollination. These populations are different regarding the level of resistance to broomrape, taking into consideration the races of the parasite which are present in each infested area. So, the populations with symbol POR, as well as some lines, having the symbol L, have been identified to be resistant to the races G or H and populations having the symbol PM and PT, as well as the differentials for these races (D1 and D2) are resistant to races F or G.

The resistance to broomrape parasite was made in natural and artificial infestation conditions. The testing in the artificial infestation conditions was made in glass house, in pots of 5 liters capacity, having inside a mixture of soil and sand (3/1) as well as broomrape seeds, races G and H, from Constanta and Braila areas (1g/pot). In natural infestation, the testing was made in four locations (Tulcea, Constanţa, Brăila, Ialomiţa) situated in different areas with different virulence of broomrape populations.

RESULTS AND DISCUSSIONS

Differences regarding the number of seeds/head obtained after the crosses between wild and cultivated sunflower were observed. When cultivated sunflower was used as pollen receptor, the number of seeds/head was higher, comparing with the case of using the wild sunflower as pollen receptor (Table 1). In this case (the second one) the number of heads was higher, taking into consideration that the wild sunflower is high branched, and so, there are many small heads.

The oil content determination for interspecific hybrids, as well as for the parental forms has shown the highest values for cultivated sunflower (fig. 1). In case of wild sunflower species, the highest level of oil content was observed in *H. maximiliani* seeds. The interspecific hybrids released by crossing between *H. annuus* and *H. tuberosus* have higher oil content, comparing with hybrids between *H. annuus* and *H. maximiliani*.

Table 1. Results regarding the number of heads and seeds obtained by hybridization of *H. maximiliani* or *H. tuberosus* with cultivated sunflower

Pollen donator / Pollen receptor	*H. tuberosus*	*H. maximiliani*	LC 1029 B	LC 991 B	LC 1093 B	LC 1085 C	LC 1095 C	LC 1088 C
	Hybridization: number of heads/number of seed							
LC 1029 B	3/640	3/250						
LC 991 B	3/520	3/120						
LC 1093 B	3/390	3/360						
LC 1085 C	3/150	3/150						
LC 1095 C	3/280	3/250						
LC 1088 C	3/130	2/110						
Helianthus tuberosus			25/15	25/7	25/5	25/14	25/23	25/12

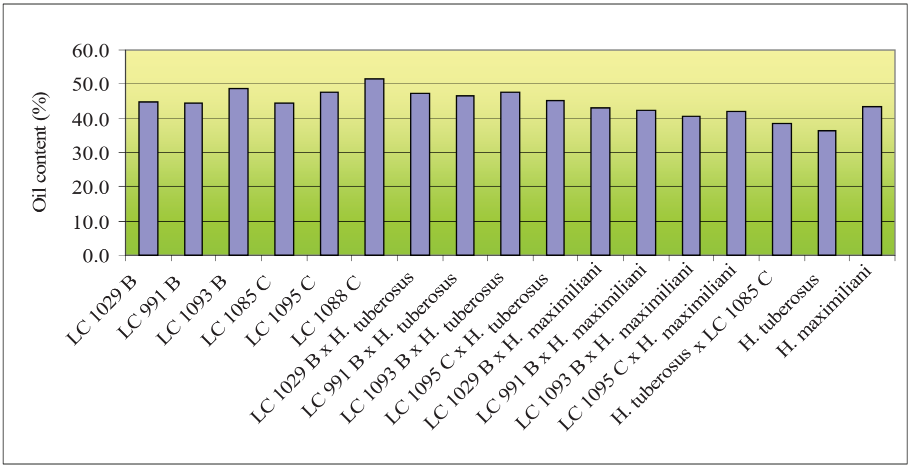

Fig. 1. The oil content for interspecific hybrids and their parental forms

Another characteristic examined for the interspecific hybrids, as well as wild parental forms and cultivated sunflower forms was the weight of one thousand seeds (fig. 2). The highest seed weight was obtained from cultivated parental forms, which have one single head. The lowest weights have the wild sunflower forms. There are some interspecific hybrids which have high seeds weight, in both cases (released with one or other wild species), this depending by the used cultivated sunflower. Some hybrids released by crossing between *H. annuus* and *H. tuberosus* have the lowest thousand seed weight.

The main objective of this paper was the selection of some interspecific hybrids of sunflower for their resistance to broomrape. In this respect, the hybrids were evaluated for the resistance to two *Orobanche* populations (race F and race G), in the artificial infestation conditions (table 2).

There are combinations released with both wild species which are resistant to both broomrape populations. The best combinations, resistant to both races are the ones obtained by crossing *H. tuberosus* with LC1085C cultivated and *H. maximiliani* with LC991B cultivated sunflower.

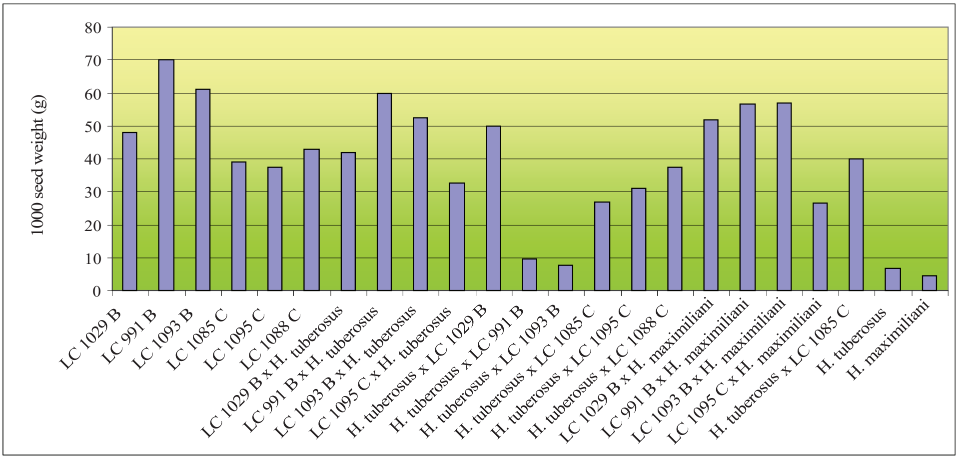

Fig. 2. One thousand seed weight for interspecific hybrids and their parental forms

Table 2. Results regarding testing of sunflower interspecific hybrids for resistance to broomrape

Combination	Variant	Race F (number of broomrapes/ sunflower plant)	Race G (number of broomrapes/ sunflower plant)
Check	1	15	25
	2	21	30
	3	17	28
LC1029 B x *H. tuberosus*	1	0	0
	2	3	2
	3	5	7
LC1029 B x *H. maximiliani*	1	2	16
	2	10	12
	3	5	24
LC991 B x *H. maximiliani*	1	0	2
	2	3	2
	3	0	0
LC1095C x *H. maximiliani*	1	2	5
	2	9	6
	3	10	4
H. tuberosus x LC1085C	1	0	0
	2	0	2
	3	0	0

Comparing the results regarding the resistance to broomrape of interspecific hybrids sunflower populations obtained in two locations situated in Tulcea and Constanta areas, differences among the populations were observed (fig. 3).

It was shown that some populations are full resistant in Tulcea area, while in Constanta area they presented a low attack degree. The sunflower differential line (LC1093B) for the race F of the parasite has a high infestation degree, in both locations.

This it means that in these locations the parasite has developed races more virulent than race F.

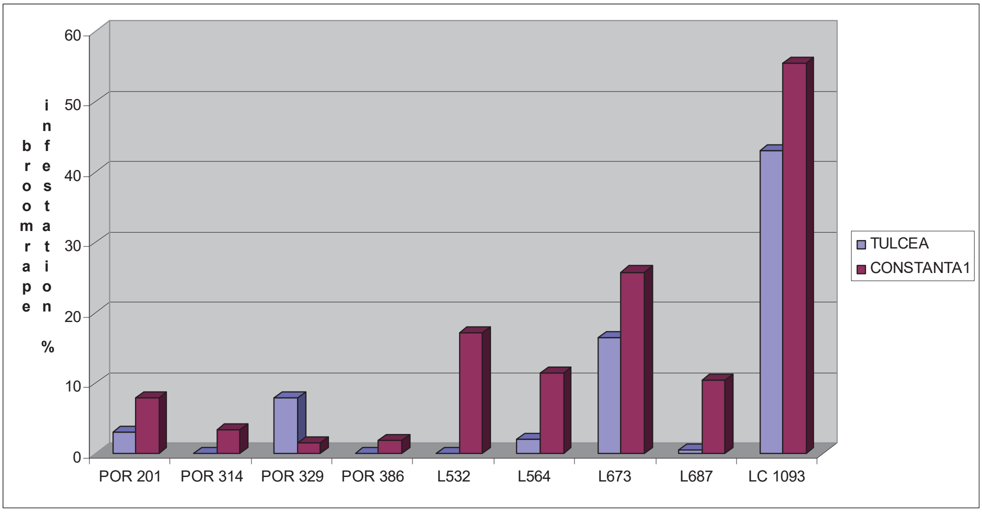

Fig. 3. Results regarding the resistance of some sunflower populations obtained from interspecific hybrids, to the broomrape parasite, in the natural infestation conditions, in two areas in Romania (average of two years, 2014 and 2015).

Among the hybrid populations of sunflower, best results were obtained with the interspecific hybrid designated as *H. tuberosus* x LC 1085 C. For this reason, the behavior of this sunflower population to the attack of broomrape parasite was examined in ten locations from five areas in Romania (Tulcea, Calarași, Brăila, Constanța, and Alexandria), in two years, 2014 and 2015 (fig.4). The results are showing that in two locations from Braila and Calarasi areas, the sunflower population is full resistant in both years. In one location from Tulcea area there is a small difference regarding the resistance in two years, in other locations having a higher difference with higher infestation degree in 2015 year.

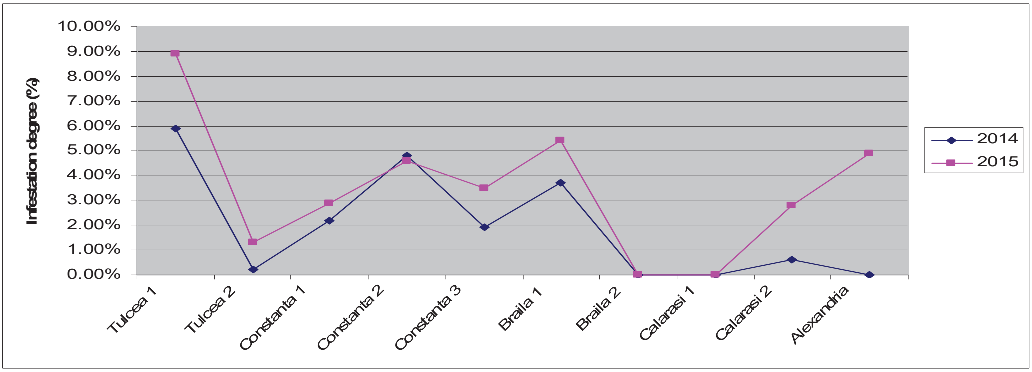

Fig. 4. Results regarding the behavior of one sunflower population obtained from an interspecific hybrid (*H. tuberosus* x LC1085C), to the attack of broomrape parasite, in ten locations from 5 areas in Romania

Moreover, the tests performed in Brăila area allowed the observation that some populations of sunflower are full resistant to broomrape, while others have a low infestation degree (fig.5). In this area, the new races of the parasite started to be present in the last years. Similar experiments were realized in Ialomița County (2014-2015) the number of full *Orobanche* resistant sunflower populations observed in this area was higher comparing with other areas (Braila, Tulcea, Constanta) (fig.6). This it means that, in this area, the broomrape parasite did not develop the new virulent races.

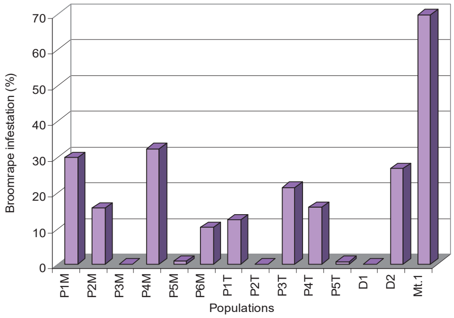

Fig. 5. Results regarding the behavior of sunflower populations obtained from interspecific hybrids, to the attack of broomrape parasite, in Braila area.

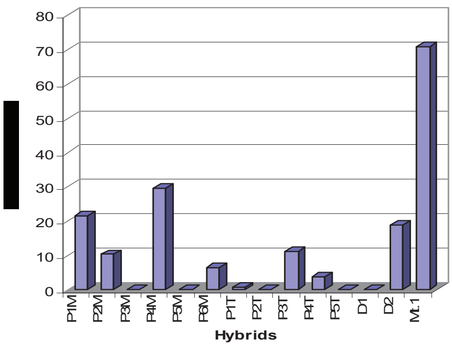

Fig. 6. Results regarding the behavior of sunflower populations obtained from interspecific hybrids to the broomrape parasite, in Ialomita area.

CONCLUSIONS

The broomrape parasite has become very dangerous for sunflower crop in almost all areas cultivated with sunflower in Europe as well as in Romania.

It is of a great importance to identify sources of resistance to the new races of broomrape. For this, the sunflower wild species are very important, they being the best source of genes for resistance.

The experiments allowed the selection of several sunflower populations obtained by crossing sunflower wild species with cultivated genotypes that have good resistance to the broomrape races which are spread in the most important areas cultivated with sunflower in Romania.

These hybrids presented also high oil contents and increased seeds weight being promising for further experiments.

REFERENCES

Alonso L.C., Fernandez-Escobar J., Lopez G., Rodriguez-Ojeda M., Sallago F., 1996. New highly virulent sunflower broomrape (Orobanche cernua Loefl.) pathotype in Spain. In: M. Moreno, J. Cubero, D. Berner, D. Joel, L. Musselman, and C. Parker (Eds), Advances in Parasitic Plant Research. Proc. 6th Int. Symp. Parasitic Weeds. Cordoba, Spain, 16-18 April 1996. pp. 639-644.

Antonova T. 2014. The history of interconnected evolution of *Orobanche Cumana* Wallr. and sunflower in the *Russian* Federation and Kazakhstan. In: Proceedings of the 3rd International Symposium on Broomrape (*Orobanche* spp.) in sunflower, Cordoba, 3–6 June 2014, pp. 57–64

Batchvarova R. 2014. Current situation of sunflower broomrape in Bulgaria. In: Proceedings of the 3rd International Symposium on Broomrape (*Orobanche* spp.) in sunflower, Cordoba, 3–6 June 2014, pp. 51–54

Christov M., 2008. *Helianthus* species in breeding research in sunflower. In: L. Velasco [ed.], Proc 17th Intl.Sunflower Conf., Cordoba, Spain, 8-12 June, 2008. Intl. Sunflower Assoc., Paris, France. pp. 709-714

Fernandez-Martinez J.M., Domingues J., Velasco L., Perez-Vich B., 2007. Update on breeding for resistance to sunflower broomrape. EUCARPIA-Oil and Protein Crops Section Meeting-„Present status and future needs in breeding oil and protein crops".(Book of Abstracts). Budapest. October 7-10. 2007. Hungary. pp.32-34.

Hristova-Cherbadzi M. 2009. Characterization of hybrids, forms and lines obtained from interspecific hybridization of cultivated sunflower *Helianthus annuus* L. with wild species of the genus *Helianthus*. Biotechnol. &Biotechnol. Eq. 23(2):112-116.

Imerovski I., Dimitrijević A., Miladinović D., Dedić B., Jocić S., Kočiš Tubić N., Cvejić S. 2015. Mapping of a new *gene* for resistance to broomrape races higher than F. Euphytica. pp 1-9.

Jan C.C. and Seiler G.J., 2008. Sunflower germplasm development utilizing wild *Helianthus* species. In: L. Velasco [ed.], Proc 17th Intl. Sunflower Conf., Cordoba, Spain, 8-12 June, 2008. Intl. Sunflower Assoc., Paris, France. pp. 29-43.

Molinero-Ruiz L., Delavault P., Perez-Vich B., Păcureanu Joiţa M., Bulos M., Altieri E., Dominguez J. 2015. Review: Joint evolution of the race structure of *Orobanche Cumana* and the breeding of sunflower for resistance to the parasitic weed. Spanish Journal of Agricultural Research. Chp. Plant Protection (Vol. 13, Nr. 4, Decembrie, 2015).

Morozov V.K., 1947. Sunflower breeding in USSR. Pishchepromizdat. Moscow. (In Russian). pp. 1-274.

Pacureanu-Joita M. 2014. Current situation of sunflower broomrape (*Orobanche Cumana* Wallr.) in Romania. In: Proceedings of the 3rd International Symposium on Broomrape (*Orobanche* spp.) in sunflower, Cordoba, 3–6 June 2014, pp. 39–43.

Păcureanu-Joiţa M., Vrânceanu A.V., Marinescu G.A., Sandu I., 1998. The evaluation of the parasite-host interaction in the system Helianthus annuus L.-Orobanche cumana Wallr. In Romania. Proc. Second Balkan Symp. On Field Crops, Novi Sad, Yugoslavia, 16-20 June, 1998, Vol. I: 153-155

Petrov D., 1970. Nova fiziološka rasa Orobanche cumana Wallroth kod nas. Plant protection in service of agriculture. Sofia. (In Bulgarian). pp. 37-48.

Pototskyi G. 2014. Current situation of sunflower broomrape in Ukraine. In: Proceedings of the 3rd International Symposium on Broomrape (O*robanche* spp.) in sunflower, Cordoba, 3–6 June 2014

Pustovoit V.S., 1966. Breeding, seed production and sunflower crop management. Kolos, Moscow. (In Russian). pp. 1-368.

Škorić D. and Jocić S. . 2005. Broomrape (Orobanche cumana Wallr.) and its possible control by genetic and chemical means. Production and Processing of Oil seeds Proc. of the 46th Oil Industry Conference. pp. 9-21. Petrovac na moru. Juny 6-10.2005. (In Serbian).

Sharova P.G., 1968. Sunflower breeding for resistance to virulent races of broomrape (In Russian). Seljskoe#hozjajstvo Moldaviji. No.5.

Vranceanu A.V., Tudor V.A., Stoenescu F.M., and Pirvu N., 1980. Virulence groups of Orobanche cumana Wallr., differential hosts and resistance source genes in sunflower. In Proc. of the 9th Int. Sunflower Conf. Vol. 2:74-82.Torremolinos. Spain. Juny 8-9. ISA. Paris.