

USING NEW *Rf* INBRED LINES ORIGINATING FROM AN INTERSPECIFIC POPULATION WITH *H. deserticola* FOR DEVELOPMENT OF SUNFLOWER HYBRIDS RESISTANT TO BROOMRAPE

Hladni, N.^{*1}, Jocić, S.¹, Miklič, V.¹, Saftić-Panković, D.¹, Škorić, D.²

¹*Institute of Field and Vegetable Crops, Maksima Gorkog 30,
21000 Novi Sad, Republic of Serbia*

²*Serbian Academy of Sciences and Arts, Novi Sad Branch, Nikole Pašića 6,
21000 Novi Sad, Republic of Serbia*

Received: July 15, 2009
Accepted: November 10, 2009

SUMMARY

One of the directions in sunflower breeding is the selection for resistance to broomrape. The population of broomrape has been stable in Serbia for a long period of time, but the racial composition has changed in recent years, with race E being predominant in the regions of north Bačka and Banat. Cultivated sunflower is genetically narrow and deficient in many desirable genes. Its genetic variability can be increased by the use of wild sunflower species and interspecific hybridization.

The resistance to broomrape in 15 new experimental hybrids was tested on locations in Serbia (Pačir) during three years (2006, 2007 and 2008) and in Romania (Baragan, Braila) during 2008. These hybrids were produced by crossing female inbred lines (Ha-26PR-A, PH-BC₂-92-A and Ha-98-A) susceptible to broomrape race E and new *Rf* inbred lines (RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8), developed from interspecific populations originating from *H. deserticola* and resistant to race E. All examined hybrids were resistant to broomrape in both locations. Since broomrape race F is present in the location in Romania, it is expected that the new *Rf* inbred lines - male components of the examined hybrids, possess the resistance gene for race F also.

Key words: sunflower, interspecific hybridization, resistance, broomrape

INTRODUCTION

The broomrape (*Orobanche cumana* Wallr) is currently regarded as one of the most important constraints in sunflower (*Helianthus annuus* L.) production (Fernandez-Martinez *et al.*, 2008). The broomrape is an important parasitic angiosperm

* Corresponding author: Phone: +381 21 4898 411; Fax: +381 21 6413 833;
e-mail: nadah@ifvcns.ns.ac.rs

totally devoid of chlorophyll, which infects the roots of sunflower plants in warm and dry regions (Ruso *et al.*, 1996). It can significantly reduce seed and oil yields, 1000-seed weight, seed oil content, plant height and head diameter of sunflowers (Kaya *et al.*, 2004).

The sunflower plant attacked by broomrape and the seed produced by it suffer serious morphological and biochemical changes, which are directly associated with the intensity of parasite attack (Shindrova *et al.*, 1998). These attacks are frequently so severe that they diminish the yield up to 50% (Dominguez *et al.*, 1996).

The broomrape is an important limiting factor in sunflower production, especially in countries adjacent to the Black Sea as well as in Spain, Israel, Serbia, China and several other countries. It is, therefore, necessary not just to develop a highly productive hybrid but to determine genes for resistance to broomrape as well (Škorić *et al.*, 2007). The resistance to broomrape races is controlled by single dominant *Or* genes. As the sunflower acreage in the world increased, new broomrape races have developed. Until few years ago, only 5 physiological races (A, B, C, D and E) existed among the sunflower broomrape populations and five sunflower dominant genes (*Or*₁, *Or*₂, *Or*₃, *Or*₄ and *Or*₅) conferring the resistance towards these broomrape races were known. A sudden change in race composition has recently occurred. A new race, called race F, has occurred in Romania, Bulgaria, Spain and Turkey. The new broomrape race cannot be controlled by *Or*₅ gene. The changes in the broomrape population and the occurrence of the new race are new challenges for sunflower breeding, forcing the breeders to continually test the breeding material against new broomrape races while creating differential lines.

The broomrape population in Serbia was stable for a long period of time. The dominant broomrape race was race B and all hybrids were genetically resistant to it. Broomrapes started to significantly endanger the sunflower production since 1995, when a change in race composition was observed on sunflowers grown on lighter soil types in the north of Bačka and Banat, the Vojvodina Province. A detailed research showed that we were dealing with a new race, race E (Mihaljčević, 1994). Race E has expanded through the Bačka and Banat regions, slowly becoming a serious problem in the sunflower production. The sunflower breeding program at Institute of Field and Vegetable Crops (IFVC) has been directed towards creating lines and hybrids which resistant to new broomrape races. The screening for resistance to race E was conducted in broomrape-infested fields, first in the area around Bačka Topola and Svetozar Miletić, and then it was continued in the field near Pačir.

Cultivated sunflower is genetically narrow and deficient in many desirable genes but there is a large number of wild relatives of the genus *Helianthus* which have provided a continued source of desirable agronomic traits (Seiler and Rieseberg, 1997; Seiler, 1992; Seiler and Brothers, 2003).

Interspecific hybridization was applied in sunflower breeding for the resistance to broomrape. This process involves first the discovery of desirable genes in wild

Helianthus species and then their incorporation into cultivated sunflower genotypes. In this investigation, several interspecific populations with *H. deserticola* had been initially used with the intention to make *Rf* male inbred lines resistant to drought. Namely, *H. deserticola* is an annual wild species adapted to desert environments. Several agronomically important traits of new *Rf* inbred lines and their hybrids were evaluated and are discussed in this paper. Unexpectedly, resistance to broomrape race E was observed and it was consistent in all examined locations in Serbia (Pačir) during three years (2006, 2007, 2008) and in Romania (Baragan, Braila) during 2008. This indicated that some interspecific populations with *H. deserticola* were resistant to broomrape and could be used for the production of new resistant sunflower hybrids.

MATERIAL AND METHOD

New *Rf* inbred lines (RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8) were produced at IFVC from interspecific populations originating from *H. deserticola*. Initially the plants were selected from the interspecific population DES-1474-1, 2, 3 originating from *H. deserticola* and subjected to inbreeding. The testing of the material to broomrape resistance was conducted in a greenhouse and in an infested field. The greenhouse testing included the sowing of sunflower plants in test tubes filled with a mixture of sand, earth and perlite into which broomrape seeds were added. This method allows to test a large number of genotypes on a small area and in 4-5 weeks. Testing lines and hybrids in a field infested with broomrape, which was also applied in this study, is a most reliable method of checking broomrape resistance in breeding material.

The inbreeding of selected plants from interspecific populations started in 1995 (S₁). The first screening of sunflower *Rf* lines for broomrape resistance was done in glasshouse in 1999 (S₅). The seed from resistant plants was tested in infested fields, in the area of Svetozar Miletić and Bačka Topola during 2000 (S₆). Selection of resistant plants was continued in the same area from 2001 (S₇) to 2003 (S₉). The same procedure was repeated in Pačir from 2004 (S₁₀) to 2008 (S₁₄), which resulted in the development of 5 resistant restorer inbred lines. In all locations lines were sown in 3 rows (12 plants per row), but only the plants in the inside row were evaluated. In order to measure the morpho-physiological traits of resistant lines, an experiment was set at Rimski Šančevi experiment field of IFVC, in a randomized complete block system with three replications during 2004. The basic sample for the analysis of the examined traits contained 30 plants (10 plants per replication) sampled from the middle row of each block. The plant height and head diameter were measured (cm) in the field at the stage of physiological maturity. The analysis of oil content in seed was carried out nondestructively in a nuclear magnetic resonance (NMR) analyzer.

Broomrape resistance of the 15 new experimental hybrids was tested in locations in Serbia (Pačir) during three years (2006, 2007 and 2008) and in Romania (Baragan, Braila) during 2008. These hybrids were made by crossing female inbred lines (Ha-26PR-A, PH-BC₂-92-A and Ha-98-A) resistant to race D but susceptible to race E, and new *Rf* inbred lines (RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8), developed from interspecific population originating from *H. deserticola*, and resistant to broomrape race E. Control sunflower genotypes used in Serbia were the line AD66, susceptible to all broomrape races, and the hybrid Bačvanin, resistant to broomrape race E (it contains the gene *Or₅*). The experiment with the hybrids was set up in 2007 in the location of Srbobran, in a randomized complete block system with three replications, with the experimental unit size of 28 m² (4 rows). The hybrids were evaluated for plant height, head diameter, seed yield, seed oil content and oil yield. The seed yield was calculated on 11% seed moisture basis. Oil yield was calculated from the seed yield and oil content per plant.

RESULTS AND DISCUSSION

Breeding of sunflower inbred lines originating from interspecies populations DES-1474-1, DES-1474-2 and DES-1474-3, with the objective of achieving drought tolerance, started in 1994 at IFVC. Apart from the analysis of important agronomic traits, the new restorer lines were tested for resistance to broomrape. The testing of restorer inbred lines was conducted from 1995 to 2004, which resulted in 5 restorer inbred lines derived from interspecific population DES-1474-1, resistant to broomrape race E (Table 1).

Table 1: *Rf* lines resistant to broomrape developed from interspecific populations originating from *H. deserticola*

Year	2000		2001		2002		2003		2004	
Location	S.M.		S.M.		S.M.		S.M.		P.	
Origin	S ₆		S ₇		S ₈		S ₉		S ₁₀	
	TNI	RI	TNI	RI	TNI	RI	TNI	RI	TNI	RI
DES-1474-1	326	96	160	25	76	15	45	8	21	5
DES-1474-2	96	25	25	/	/	/	/	/	/	/

S.M.- Svetozar Miletić; B.T.- Bačka Topola; P. - Pačir

TNI - total number of tested inbred lines; RI - inbred lines resistant to broomrape

The resistance of the new lines was confirmed in the location of Pačir from 2005 to 2008, in tests with two controls: AD66, a line susceptible to all broomrape races, and the hybrid Bačvanin, resistant to broomrape race E (Table 2).

The new *Rf* lines were also evaluated for plant height, head diameter and seed oil content, and they exhibited significant differences among these characteristics. Plant height ranged from 134 to 173 cm, head diameter from 12.7 to 14 cm and seed oil content from 40.6 to 49.4% (Table 3).

Table 2: *Rf* lines resistant to broomrape developed from interspecific populations originating from *H. deserticola*

Population	<i>Rf</i> inbred lines	2005	2006	2007	2008	
		S ₁₁	S ₁₂	S ₁₃	S ₁₄	
1	DES-1474-1	RHA-D-2	R	R	R	R
2	DES-1474-1	RHA-D-5	R	R	R	R
3	DES-1474-1	RHA-D-6	R	R	R	R
4	DES-1474-1	RHA-D-7	R	R	R	R
5	DES-1474-1	RHA-D-8	R	R	R	R
control						
		AD-66	S	S	S	S
		Bačvanin	R	R	R	R

(R) broomrape absent

(S) broomrape present

Table 3: Mean values of plant height, head diameter, seed oil content of new broomrape-resistant *Rf* lines developed in 2004 from interspecific populations originating from *H. deserticola*

Population	<i>Rf</i>	2004		
		PH	HD	SOC
		cm	cm	%
DES-1474-1	RHA-D-2	148	13.5	47.2
DES-1474-1	RHA-D-5	165	13.2	49.3
DES-1474-1	RHA-D-6	134	13.0	40.6
DES-1474-1	RHA-D-7	143	12.7	49.0
DES-1474-1	RHA-D-8	173	14.0	48.1
	LSD 0.05	1.69	2.23	4.06
	LSD 0.01	2.48	3.50	5.14

Rf - restorer inbred line; PH - plant height

HD - head diameter; SOC - oil content

The 15 experimental hybrids were made by crossing new *Rf* lines to three female cms lines. The broomrape resistance of the new experimental hybrids was assessed in a location in Serbia (Pačir) during three years (2006, 2007, 2008), using two controls: the hybrid Bačvanin, resistant to broomrape race E, and line AD66 susceptible to all broomrape races. As race F is present in Romania, the resistance of the same hybrids was also tested and confirmed there, in the location Baragan, Braila, in 2008 (Table 4).

The new experimental hybrids resistant to broomrape differed significantly among themselves in plant height (170-210 cm), head diameter (20-24 cm), seed yield (2.60-4.88 t/ha), seed oil content (46.5-52.0%) and oil yield (1.27-2.42 t/ha). Regarding the most important agronomic traits such as seed (SY) and oil yield (OY), three resistant hybrids were significantly better than all three standard hybrids: Ha-

Table 4: Results of field testing of NS sunflower hybrids grown in broomrape-infected plots in Pačir (2006-2008) and Braila (2008)

	Origin, F ₁		Serbia, Pačir			Romania, Braila
	<i>cms</i>	<i>Rf</i>	2006	2007	2008	2008
1	Ha-26PR-A	RHA-D-2	R	R	R	R
2		RHA-D-5	R	R	R	R
3		RHA-D-6	R	R	R	R
4		RHA-D-7	R	R	R	R
5		RHA-D-8	R	R	R	R
6	PH-BC ₂ -92-A	RHA-D-2	R	R	R	R
7		RHA-D-5	R	R	R	R
8		RHA-D-6	R	R	R	R
9		RHA-D-7	R	R	R	R
10		RHA-D-8	R	R	R	R
11	Ha-98-A	RHA-D-2	R	R	R	R
12		RHA-D-5	R	R	R	R
13		RHA-D-6	R	R	R	R
14		RHA-D-7	R	R	R	R
15		RHA-D-8	R	R	R	R
control						
	AD-66		S	S	S	
	Bačvanin		R	R	R	

(R) + broomrape absent; (S) - broomrape present on every plant

Table 5: Characteristics of the 15 experimental hybrids resistant to broomrape tested in 2007 in the location of Srbobran

Female	Hybrid	PH	HD	SY	SOC	OY
		cm	cm	t/ha	%	t/ha
Ha-26PR-A	RHA-D-2	182	20.5	3.52	46.9	1.65
	RHA-D-5	200	20.5	4.82	49.7	2.37
	RHA-D-6	176	21.0	4.86	48.8	2.38
	RHA-D-7	174	22.0	3.80	48.7	1.85
	RHA-D-8	210	23.0	4.88	49.6	2.42
PH-BC ₂ -92-A	RHA-D-2	180	20.0	3.34	49.5	1.66
	RHA-D-5	190	21.0	2.95	48.5	1.43
	RHA-D-6	188	20.0	2.90	46.5	1.35
	RHA-D-7	170	23.1	2.60	49.2	1.27
	RHA-D-8	205	21.0	3.35	49.3	1.65
Ha-98-A	RHA-D-2	190	22.0	3.90	52.0	2.03
	RHA-D-5	198	22.0	3.98	49.8	1.98
	RHA-D-6	196	22.0	3.95	49.8	1.97
	RHA-D-7	194	24.0	3.95	50.0	1.89
	RHA-D-8	201	24.0	3.95	49.8	1.97
standard						
	NS-H-111	175	21.0	4.54	48.9	2.22
	Perun	155	22.0	3.77	47.6	1.79
	Pobednik	160	21.0	3.87	50.7	1.96
control						
	Bačvanin	180	21.0	3.80	50.2	1.91
	LSD 0.05	3.52	1.67	0.29	1.26	0.143
	LSD 0.01	4.71	2.24	0.39	1.69	0.191

PH - plant height; HD - head diameter; SY - seed yield; SOC - oil content; OY - oil yield

26-PR × RHA-D8 (SY 4.88t/ha; OY 2.42t/ha), Ha-26-PR × RHA-D6 (SY 4.86t/ha; OY 2.38 t/ha), Ha-26-PR × RHA-D5 (SY 4.82t/ha; OY 2.37t/ha) (Table 5).

New sources of resistance to broomrape have been extensively searched for in wild species, with focus on *H. tuberosus* (Jan *et al.*, 2002). Most of the NS hybrids resistant to broomrape produced so far have been based on interspecific populations with *H. tuberosus*, among them being the hybrid Bačvanin, used as a standard in our investigation (Škorić and Jocić, 2005). Results on the resistance in other wild sunflower populations are scarce. Seiler (1994) reported that interspecific lines derived from *H. anomalus*, *H. argophyllus* and *H. deserticola* displayed some level of resistance against broomrape race SE-192. Ruso *et al.* (1996) declared only three interspecific populations, ANO-1509-2-2, ARG-420-1-2 and DES-1474-1-2, derived from *H. anomalus*, *H. argophyllus* and *H. Deserticola*, respectively, as partially resistant to broomrape race SE-193. It is known that each wild species has a great variability among its populations, which mutually differ in values of the majority of qualities and traits (Seiler and Gulya, 2004).

Here we showed that *Rf* inbred lines developed from interspecific population with *H. deserticola* - DES-1474-1, are resistant to broomrape race E and possibly race F, and indicated that this population can be used for the production of new resistant sunflower hybrids.

CONCLUSION

Fifteen new experimental hybrids were made by crossing female inbred lines susceptible to broomrape race E with new *Rf* inbred lines, developed from the interspecific population DES-1474-1, originating from *H. deserticola* and resistant to broomrape race E. All the examined hybrids were resistant to broomrape in locations infected with race E. Since broomrape race F is present in the test location in Romania, it is expected that the new *Rf* inbred lines - male components of the examined hybrids - possess the resistance gene for race F too. Our results indicate that the interspecific population DES-1474-1 can be used for the production of new sunflower hybrids resistant to broomrape.

REFERENCES

- Dominguez J., Melero-Vara J.M. and Refoyo A., 1996. Virulence groups of *Orobanche cernua* in Andalusia (southern Spain). In: Moreno, M., Cubero, J., Berner, D., Joel, D., Musselman, L. and Parker, C. [Eds.]. Adv. Parasitic Plant Res. Proc. 6th Int. Symp. in Parasitic Weed, Cordoba, Spain, Congresos y Jornadas 36/96. Direccion General de Investigacion Agraria, Consejeria de Agricultura y Pesca, Sevilla, Spain, pp. 633-637.
- Fernandez-Martinez, J.M., Dominguez, J., Perez-Vich, B. and Velasco, L., 2008. Update on breeding for resistance to sunflower broomrape. *Helia* 31(48): 73-84.
- Jan, C.C., Fernandez-Martinez, J.M., Ruso, J. and Munoz-Ruz, J., 2002. Registration of four sunflower germplasms with resistance to *Orobanche cumana* race F. *Crop Sci.* 42: 2217-2218.
- Kaya, Y., Demerci, M., Evci, G., 2004. Sunflower (*Helianthus annuus* L.) breeding in Turkey for broomrape (*Orobanche cernua* Loeffl.) and herbicide resistance. *Helia* 27(40): 199-210.

- Mihaljčević, M., 1994. Broomrape (*Orobanche cumana* Wallr.) on sunflower in Vojvodina. Periodical of Scientific Res. on Field and Vegetable Crops, Novi Sad 25: 59-71.
- Ruso, J., Sukno, S., Dominguez-Gimenez, J., Melero-Vara, J.M. and Fernandez-Martinez J.M., 1996. Screening of wild *Helianthus* species and derived lines for resistance to several populations of *Orobanche cernua*. Plant Dis. 80: 1165-1169.
- Seiler, G.J., 1992. Utilization of wild sunflower species for the improvement of cultivated sunflower. Field Crops Res. 30: 195-230.
- Seiler, G.J., 1994. Oil concentration and fatty acid composition of achenes of North American *Helianthus* (*Asteraceae*) species. Econ. Bot. 48: 276-279.
- Seiler, G.J. and Gulya, T.J., 2004. Exploration for wild *Helianthus* species in North America: Challenges and opportunities in the search for global treasures. Proc. 16th Int. Sunf. Conf., Fargo, ND 1: 43-68.
- Seiler, G.J. and Rieseberg, L.H., 1997. Systematics, origin, and germplasm resources of wild and domesticated sunflower. In: Schneiter, A.A., [ed.], Sunflower Technology and Production. Crop Science Society of America, Madison, WI., USA, pp. 21-65.
- Seiler, G.J. and Brothers, M.E., 2003. Exploration for wild *Helianthus anomalous* and *H. deserticola* in the desert Southwest, USA. Proc. 25th Sunf. Res. Workshop, Fargo, ND, Nat. Sunf. Assoc., Bismarck, ND. 2003. (Available at: http://www.sunflowernsa.com/research/research_workshop/documents/90.pdf).
- Shindrova, P., Ivanov, P., Nikolova, V., 1998. Effect of broomrape (*Orobanche cumana* Wallr.) intensity of attack on some morphological and biochemical indices of sunflower (*Helianthus annuus* L.). Helia 21(29): 55-62.
- Škorić, D., Jocić, S., 2005. Use of wild species in sunflower species as an alternative to genetic transformations. J. Sci. Agric. Research/Arh. polj. Nauke 66(237): 115-123.
- Škorić, D., Jocić, S., Hladni N., Vannozzi, G.P., 2007. An analysis of heterotic potential for agronomically important traits in sunflower (*Helianthus annuus* L.). Helia, 30(46): 55-73.

EL USO DE NUEVAS LÍNEAS ENDOCRIADAS *Rf* ORIGINADAS DE UNA POBLACIÓN INTERESPECÍFICA CON *H. deserticola* PARA LA PRODUCCIÓN DE HÍBRIDOS RESISTENTES AL JOPO

RESUMEN

Uno de los objetivos de mejoramiento de girasol es la selección por la resistencia al jopo. La población de jopo se ha mantenido estable en Serbia durante un largo período de tiempo, pero la composición racial ha cambiado últimamente, con la raza E predominando en las regiones del norte de Ba?ka y Banat. El girasol cultivado es genéticamente estrecho y deficiente en varios genes deseables. Su variabilidad genética puede ser incrementada mediante el uso de especies silvestres de girasol e hibridación interespecífica. La resistencia de 15 nuevos híbridos experimentales al jopo se probó en localidades de Serbia (Paćir), durante tres años (2006, 2007 y 2008), y en Rumania (Baragan, Braila), durante el 2008. Estos híbridos se produjeron cruzando líneas hembras endocriadas (Ha-26PR-A, PH-BC₂-92-A y Ha-98-A), susceptibles a la raza E del jopo, y nuevas líneas endocriadas *Rf* (RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8), desarrolladas a partir de poblaciones interespecíficas originadas de *H. deserticola*, resistentes a la raza E del jopo. En ambas localidades, todos los híbridos examinados fueron resistentes al jopo. Visto que la raza F del jopo está presente en esa localidad rumana, se espera que los componentes masculinos de las nuevas líneas endocriadas *Rf* los de híbridos examinados, posean también el gen de resistencia para la raza F.

**L'UTILISATION DE NOUVELLES LIGNÉES PURES *Rf*
ISSUES DE LA POPULATION INTERSPÉCIFIQUE *H. deserticola*
EN VUE DE LA PRODUCTION DES HYBRIDES
DE TOURNESOL RÉSISTANTS À L'*Orobanche***

RESUME

L'un des objectifs majeurs dans la sélection du tournesol est la résistance à l'*Orobanche*. La population de ce parasite en Serbie est restée stable pendant une longue période, mais la composition des souches a changé ces dernières années avec la race E prédominante dans les régions nord de Bačka et de Banat.

Le tournesol cultivé est génétiquement déficient pour de nombreux gènes complémentaires. Sa variabilité génétique peut être améliorée en utilisant des espèces sauvages de tournesol et l'hybridation interspécifique.

La résistance à l'*Orobanche* de 15 nouveaux hybrides expérimentaux a été étudiée en Serbie (Pačir) durant trois ans (2006, 2007 et 2008) et en Roumanie (Baragan, Braila) en 2008.

Ces hybrides ont été produits en croisant des lignées femelles (HA-26 PR-A, PH-BC₂-92-A et HA-98-A) sensibles à la race E d'*Orobanche* et de nouvelles souches mâles restorer *Rf* (RHA-D-2, RHA-D-5, RHA-D-6, RHA-D-7, RHA-D-8), développées à partir de populations interspécifiques dérivant de *Helianthus deserticola* et résistantes à la race E.

Tous les hybrides examinés dans ces deux endroits étaient résistants à l'*Orobanche*. La race F d'*Orobanche* étant présente en Roumanie, il est espéré que les nouvelles lignées *Rf* - parents mâles des hybrides étudiés, possèdent également le gène de résistance pour la race F.