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SUNFLOWER BREEDING FOR RESISTANCE TO BROOMRAPE (*Orobanche cernua* Loefl./ *Orobanche cumana* Wallr.)

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SUMMARY

Broomrape (*Orobanche cernua* Loefl./*Orobanche cumana* Wallr.) is counted among the most detrimental parasites of sunflowers. The occurrence of a new race, race E, in Yugoslavia has moved sunflower breeders to try to develop hybrids resistant to the new race. Tests in infected fields and in a quarantine chamber in a greenhouse have produced genotypes possessing Or_5 gene, such as lines L-414, Bt analogue 2, Bt analogue 3, etc. There has been good agreement between the results obtained outdoors and indoors.

Key words: sunflower, broomrape, resistance

INTRODUCTION

The broomrape, a floriferous parasite from the family *Orobanhaceae*, is counted among the most detrimental parasites of sunflowers. It is a typical obligate parasite which deprives the sunflower plant of water and mineral substances. Although more than 100 broomrape species may be found in literature, "Flora Europeae" accepts only 42 species. The other names are considered as synonyms or temporary designations. In Yugoslavia, Pančić had already worked on broomrape classification. In a paper titled "Zur Flora des Banates", published in 1868, Pančić reported his observations on the species *Orobanche echinopsis* Pančić, which parasitized the roots of several species from *Echinops* genus in the location of Deliblato Sands. However, the parasitic species had already been described as *Orobanche major* L. Another species, *Orobanche esuleae* Pančić, described in the paper "An addendum to the flora of the Dukedom of Serbia" published in 1884 (Diklić, 1996), has been accepted by "Flora Europeae". According to "Flora Europeae", the name *Orobanche cumana* is synonymous with *Orobanche cernua*. However, Horvath (1996) states that the two species are not identical. The chromosome number in *Orobanche cernua* is 2n=38, in *Orobanche cumana* 2n=34. The two species being mutually crossable, the author proposes the names *Orobanche cernua/Orobanche cumana* group or *Orobanche cernua* Loefl. subs. *cumana* (Wallr.). The species of *Orobanche* sp. genus parasitize the families *Asteracea, Solanacea, Fabacea* etc. In addition to *Orobanche cernua,* the species *Orobanche aegyptiaca* Pers., *Orobanche ramosa* L., *Orobanche minor* Sm., *Orobanche lutea* Baumg. and *Orobanche gracilis* Sm. too are capable of causing extensive damage.

Of the species mentioned above, *Orobanche cernua* is most widely spread. It attacks annually over 7 million hectares under sunflowers. It causes heavy infestations in some regions in Russia, Ukraine, Moldavia, Turkey, Romania, Bulgaria, Spain, and Hungary but it has also been reported in other countries. Broomrape is most efficiently controlled by growing resistant hybrids, using uninfested sunflower seed, discouraging broomrape germination by deep plowing, strictly adhering to crop rotation and recommended fertilization and sowing methods, eradicating broomrape plants before they manage to produce seed, by careful harvesting of infested sunflower plots and by means of biological and chemical control (Sneyd and Petzoldt, 1994). Broomrape studies are usually focused of isolating the active analogue responsible for the germination of broomrapes on host plants. However, this analogue is difficult to isolate because it becomes active already in extremely small concentrations, it is activated in a complex substrate such as soil which makes the isolation difficult, while the already isolated and identified structures are highly unstable and they quickly transform in soil (Stewart and Press, 1990, cited by Miller, 1994). Dhanapal (1996) arrived at similar conclusions when studying methods of broomrape control *(Orobanche cernua)* on tobacco in Karnataka, southern India. In India, the third largest tobacco producer in the world, yield reductions due to broomrape range from 30 to 70%. The control is done there mostly by uprooting broomrape plants, but this is a long and difficult undertaking. That is why this author looked for alternate methods of control. For the present, the integral crop protection is the only effective method of broomrape control.

Short history of sunflower breeding for broomrape resistance

The establishment of infection stocks at the Saratov and Kruglik experiment stations in Russia marks the beginning of sunflower breeding for resistance to broomrape. Resistance tests of sunflower breeding materials conducted in the period from 1917 to 1922 resulted in the development of first broomrape-resistant varieties, Kruglik 7-15-163, Kruglik 631, Kruglik A-41, Saratovski 169, Zelenka harkovskaja, Fuksinka voronesskaja, etc. The level of broomrape resistance in these varieties was 98 to 99%. Until 1925, the broomrape problem was considered to be successfully concluded. From 1925 to 1928, however, the varieties such as Saratovski 169 and Kruglik A-41, grown in Rostov and Krasnodar regions and the Voronez district, regularly suffered heavy broomrape infestations. L.A. Zdanov found that a new broomrape race evolved, race B or "the bad race from the Don" (Pustovoit, 1966). The slightly infested or uninfested sunflower material found near the village of Andreevka was used for the development of new resistant varieties. The first varieties resistant to race B, in spite of high husk content and low oil content, produced yields which exceeded those of the susceptible varieties two to five times. Regardless of the narrow genetic base, numerous varieties resistant to race B were made before the outbreak of World War II: Ždanovski 6432, Ždanovski 8281, Ždanovski 8885, Armavirski 762, Armavirski 1813, Armavirski 1645, etc. Only in the 1950's did appear the varieties such as VNIIMK 8931 that combined high broomrape tolerance, grain yield above 2.5 t/ha, oil content above 46%, and the husk content of only 26%. These varieties were generally superior to the predominantly grown VNIIMK 1646 (Pustovoit, 1966).

Burlov and Kostjuk (1976) and Pogorelitskij and Geshele (1976) discovered that broomrape resistance is controlled by a dominant gene which was designated as *Or*. Vrânceanu *et al.* (1981) identified a series of differential lines for races C, D and E. These lines are still in wide use. Studying two lines resistant to races A-E, LC-1093 and P-1380-2, Pacureanu-Joita *et al.* (1998) found that the line P-1380-2 is not fully resistant in the regions of Constanta, Tulcea and Braila. They concluded that there must have occurred a new race, race F. The line LC-1093 evidently possesses a gene of resistance to race F as well as gene Or_6. This line is the female parent of the hybrids Turbo and Favorit. Indications of new broomrape race have been observed in Turkey and Spain (Fernández-Martínez, personal communication, 1998). Tests with the differential lines should show whether the observed races are race F or not.

Because of a narrow genetic base for broomrape resistance, new sources of resistance have been searched for in wild species. Pustovoit and Krasnokutskaja (1976) reported the species *H. scaberimus*, *H. divaricatus*, *H. tuberosus*, *H. rigidus* to be 100% resistant to broomrape. Ruso *et al.* (1996) tested 18 annual species, 26 perennial species and 29 interspecific lines highly virulent to three broomrape populations and found only *H. annuus* population 011, *H. anomalus* and *H. exilis* to be resistant to all three populations. Almost all perennials were resistant to all broomrape populations. Dozet and Marinković (1998) tested populations of *H. annuus* and *H. petiolaris* ssp. *petiolaris* for resistance to a broomrape population containing races A, B and E, and concluded that *H. petiolaris* was an excellent donor of *Or* genes.

Study of broomrape resistance in Yugoslavia

The sunflower is a traditional crop in Yugoslavia and its present acreage is about 160,000 ha. Broomrape infestation has been limited to the Vojvodina Province or, more specifically, to the region of Bačka. The study of broomrape was started by Bošković (1962), after a massive occurrence of the parasite in the late 1950's. The intensity of the attack was so high that it brought in question the entire sunflower production. The assortment grown at that time was replaced by high-oil Russian varieties such as VNIIMK 8931, Smena, Peredovik and Jenisej which were resistant to broomrape race B. The occurrence of broomrape on VNIIMK 8931 moved Aćimović (1980) to study the parasite. He came to believe that as many as 7 broomrape races are present in Yugoslavia. Following the introduction of Russian varieties in the Yugoslav commercial production, an infection stock was established at the Institute of Agricultural Research for checking sunflower breeding materials against broomrape seeds collected in all parts of the Vojvodina Province. However, the parasite did not attract large scientific interest before the late 1980's and the early 1990's when the occurrence of the parasite intensified. Broomrape again became a serious problem in the Yugoslav commercial production in 1995, on light soil types in the Vojvodina Province. Detailed studies indicated the advent of a new race of broomrape, race E (Mihaljčević, 1996). The parasite was first observed on the variegated type of sunflower which is susceptible to all broomrape races. To differentiate the local broomrape population, Mihaljčević used 5 differential lines, AD-66 without *Or* genes, Kruglik A-41 and Saratovski 169 with Or_1, VNIIMK 8931 with Or_1 and Or_2, and Record with Or_1, Or_2 and Or_5 as diffentials with for race C and the inbred line OD-3369 with Or_5 for race E. In 1996, Mihaljčević reported that the Russian varieties Progres, Jubilejnij 60 and Oktjabr 50, developed from crosses *H. annuus* x *H. tuberosus*, exhibited resistance to race E. The subsequent introduction of new hybrids resistant to race E significantly reduced the chance of further spread of the parasite in Yugoslavia (Škorić *et al.*, 1996).

MATERIAL AND METHOD

Inoculation methods

This part of the breeding process, when combined with adequate gene bank, may be considered the most important. So far, we have used three inoculation methods. From breeders' point, the most effective and reliable method is testing plant material in an infested plot, i.e., by establishing an infection stock. This procedure had been used by Pustovoit and associates at the Kruglik breeding station. It is not difficult to establish an infection stock because broomrape seeds are easy to collect. Broomrape seeds are among the smallest in the plant kingdom. Their mass ranges from 4 to 9 x 10^{-3} mg depending on species and they develop in large numbers. The seeds may remain viable for over 10 years and this is what makes them so problematic in sunflower production (Miller, 1994). This method of testing allows breeders to select resistant material effectively and reliably. The infection stock of the Institute of Field and Vegetable Crops is used to test from 1,000 to 1,500 lines each year. If the method is used only to check the reaction of breeding materials without crossing them and keeping the seed, it is too long for only a single cycle may be performed in a year. The method of Pančenko (1973) is much more suitable for testing the reaction of breeding material because it lasts only 30 do 40 days. The Institute of Field and Vegetable Crops has a quarantine chamber for early testing which is done in plastic buckets. This method allows up to 3,000 samples to be tested, at a small area (40 m^2) and in the course of the fall and winter (October-April). While the inoculum is stringently controlled and the results obtained are reliable, this method requires an experienced researcher to take into account broomrape seed dormancy, watering schedule and photoperiodism, and preparation of suitable substrates. He must be exceedingly careful not to allow broomrape seeds from being transmitted to other parts of the greenhouse of the breeding plot. Such risk is high when the size and viability of broomrape seeds are considered. The method allows a detailed analysis of the host's root system and the study of host plant - parasite interactions. The major shortcoming is that the tested plant material is discarded before flowering and it cannot be used for crossing or selfing which frequently may be necessary. The problem may be solved by increasing the amount of substrate in the bucket to at least 10 liters. Both approaches are used in the Institute's quarantine chamber. The benches covered with the substrate are used for early diagnosing, while the spaces between them accommodate buckets containing plants intended for breeding programs. The practice has shown that the quarantine chamber may be successfully used for the testing of wild sunflowers (Dozet and Marinković, 1998).

Plant material

Depending on the envisaged objective, highly diverse materials are used in sunflower breeding: inbred lines from Russian varieties, lines in the process of inbreeding derived from synthetic populations made at the Institute by crossing several inbred lines - donors of genes for resistance to *Phomopsis* sp., *Plasmopara halstedii*, and *Orobanche cernua*, lines with high self fertility, etc. The objective is to produce new inbred lines that would combine genes for all desirable characteristics. The procedure is long and arduous and it calls for a series of complementary tests involving field and laboratory methods. For the present, only classical breeding methods are available. Most of the tested lines have been included in the breeding programs on account of a large variety of different characteristics. For example, the lines HA-335, HA-336, HA-337, HA-339 and RHA-340, obtained by the courtesy of Dr. Miller (USDA, U.S.A.), have *Pl* genes, i.e., resistance to downy mildew, and they are completely homozygous for that characteristic. One of the objectives is to check these lines for resistance to broomrape race E. L-48 is a late line from Novi Sad breeding program. It has good combining abilities and high tolerance to *Phomopsis* sp. L-30 is an early line, susceptible to *Phomopsis* sp. It is intended for development of hybrids for double cropping. The line is short and attractive looking. RKS 6/1 and RKS 6/3 are prospective lines derived from the synthetic SII. The lines are presently being converted to the CMS form. The tests include also a series of new prospective restorer such as RHA V-12, which is very early, RHA Pl, which is heterozygous for resistance to downy mildew but also highly uniform, fully branched and highly tolerant to lodging, and RHA-O, which has exhibited almost 100% resistance to downy mildew. There are also lines still in the process of inbreeding such as Bt analogue 2, which is 100% resistant to downy mildew, etc. The objective of the tests was to identify the genotypes with *Or* genes, for inclusion in further breeding for resistance to broomrape.

RESULTS AND DISCUSSION

The field test conducted in the infection stock showed that only 4 lines were 100% resistant to broomrape (L-414, *Rf Or* Bt, Bt analogue 2, Bt analogue 3) (Table 1), while only two lines were 100% susceptible. The other lines were heterozygous, the number of infested plants in them varying from 10 to 60%. In the greenhouse test in tubes, which included a larger number of lines, 8 were resistant, 4 completely susceptible, and the others were heterozygous, with 10 to 71.43% of infested plants.

Table 1: Results of the 1998 field test for broomrape resistance using the available infection stock

Line or hybrid	No. of plants	No. of infested sunflower plants	Genetic constitution
L-414 A	8	0	R
L-414 B	7	0	R
L-414 x *Rf* Rum	6	0	R
Rf Or Bt	14	2	H
Bt analogue 2	9	0	R
Bt analogue 3	9	0	R
Bt analogue *Or*	6	0	R
HA-335 B	7	2	H
HA-336 B	5	3	H
HA-337 B	4	1	H
RHA-723	5	3	H
RHA-V-164	10	1	H
L-113 A	5	5	S
L-173 A	6	6	S
L-168 A	10	5	H
RHA -340	10	2	H
L-414 x *Rf*-Kr	7	0	R

R-resistant, H-heterozygous, S-susceptible

The tests indicated that the race composition of the parasitic population remained unchanged. The lines resistant in 1997 maintained their resistance in 1998. The results of the field and greenhouse test were fully compatible. The only exception was the line HA-336 which was more heavily infested in the field than in the greenhouse. It seems provident to perform both tests, or at least one of them, in two replications before proceeding to the analysis of the genetic constitution of the tested lines. The dormancy of broomrape seeds was successfully broken by keeping them in a cold chamber at +4°C for 60 days.

Table 2: Results of broomrape test in test tubes and quarantine chamber (sowing 11 February 1998, assessment 28 March 1998)

Line or hybrid	Number of plants	No. of infested sunflower plants	Genetic constitution
L-30 B	9	9	S
L-113 B	9	9	S
L-145 B	8	8	S
L-150 B	10	10	S
L-164 B	10	3	H
L-168 B	10	5	H
L-169 B	9	8	H
L-252 B	9	8	H
L-364 B	10	1	H
L-394 B	10	0	R
HA-335 B	10	6	H
HA-336 B	8	1	H
HA-337 B	8	2	H
HA-339 B	7	3	H
RHA-340	7	5	H
RHA-717	9	5	H
RHA-722	10	4	H
RHA-723	8	5	H
RHA-O	10	8	H
RHA-V-12	9	0	R
RHA-V-16	9	5	H
RHA-V-113	10	3	H
RHA-V-135	9	7	H
RHA-V-151	10	5	H
HA-1-PI B	10	4	H
HA-O-SP	10	1	H
Rf Or BT	8	0	R
Rf_1 *Or* Bt	9	2	H
Rf_2 *Or* Bt	9	0	R
Rf_1 PI	10	0	R
RHA PI	9	0	R
L-414 A	10	0	R
L-414 B	10	0	R
Bt analogue 1	10	1	H
Bt analogue 2	9	1	H
Bt analogue 3	10	0	R
Bt analogue 4	8	1	H
L-48	8	4	H
Progres	9	0	R
Novinka	8	6	H
RKS 6/1	10	0	R
RKS 6/3	10	2	H

This procedure rendered intensive infestations. It is evident that our breeding program includes both B lines and *Rf* lines which are resistant to broomrape race E. The greater part of the tested materials is heterozygous, making it a potential source of resistance to be discovered in the course of further breeding. One of the tested combinations, L-414 x *Rf* Rum, is presently in small-plot trials (Figure 1). It means that in the course of 1998 other agronomic characteristics of this hybrid combination will be available, e.g., yield potential, oil content, resistance to diseases, maturity, etc. A line that deserves attention is RKS 6/l, obtained from the synthetic SII in the breeding program of Dr. Marinković. The line is resistant to broomrape.

Figure 1: Resistant hybrid L-414 x Rf Rum (R) and susceptible control (S)

Figure 2: Heterozygous variety Novinka, susceptible (S) and resistant plant (R)

The results confirmed the conclusion of Mihaljčević (1996) that the varieties Progres and Novinka possess gene Or_5 (Figure 2). Progres exhibiting homozygous resistance in our test. As these varieties were developed from crosses between the cultivated sunflower and *H. tuberosus*, it seems clear that the resistance comes from the wild species.

The lines HA-335, HA-336, HA-337 and HA-339, which are resistant to downy mildew, produced interesting results. In our tests, these lines were heterozygous for broomrape resistance. One more check is necessary (the third one) before pronouncing these lines resistant to broomrape. If the third test produces conclusive evidence of their being resistant, it will greatly facilitate the development of hybrids resistant to the two downy mildew races (3 and 4) detected in Yugoslavia (Maširević, 1998) and broomrape.

Table 3: Infestation rates in the field and greenhouse

Line	Plants infested in field (%)	Plants infested in greenhouse (%)
L-414 A	0	0
L-414 B	0	0
Rf Or Bt	14.28	0
Bt analogue 2	0	11.11
Bt analogue 3	0	0
HA-335	28.57	60
HA-336	60	12,5
HA-337	25	25
RHA-723	60	62,5
L-113	100	100
L-168	50	50
RHA-340	20	71.43

Both the field and the greenhouse test rated the experimental material exclusively on the basis of presence or absence of the parasite. The occurrence of a single broomrape plant classified a variety as susceptible. The selection criterion was set up high, although Horvath (1988) and Mihaljčević (1996) proposed five grades that would cover all host-parasite interactions. The grouping of the materials into homozygous resistant, heterozygous and homozygous susceptible followed the criteria established by Burlova and Kostjuk (1976), Pogorelicki and Geshele (1976), Vrânceanu (1980), etc. It also reflects our own results which indicated that not a single broomrape plant develops on the resistant lines such as L-414 and that the broomrape resistance is controlled by a dominant *Or* gene, in our case by Or_1, Or_2 and Or_5. Based on the obtained results, combinations with various restorers will be made in further work aiming at the development of sunflower hybrids that would combine broomrape resistance and good agronomic characteristics, especially resistance to diseases such as *Plasmopara halstedii* and *Phomopsis* sp.

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SELECCCIÓN DE GIRASOL CON RESPECTO A LA RESISTENCIA A OROBANCA (*Orobanche cernua* Loefl./ *Orobanche cumana* Wallr.)

RESUMEN

La orobanca (*Orobanche cernua* Loefl./*Orobanche cumana* Wallr.) es considerada como uno de los parasitos de girasol más nocivos. La apariencia de nueva raza, la raza E, en Yugoslavia motivo a los seleccionadores de ensayar con la creación de híbridos resistentes a la nueva raza. Los testes en los campos infectados y en la camara de cuarentena en el invernadero han creado los genótipos que poseen el gen Or_5 como son las líneas L-414, Bt analog 2, Bt analog 3, etc. Los resultados obtenidos en el campo y en el invernadero eran en conformidad.

CULTURE DU TOURNESOL RESISTANT A L'OROBANCHE (*Orobanche cernua* Loefl./*Orobanche Cumana* Wallr.)

RÉSUMÉ

L'orobanche (*Orobanche cernua* Loefl./*Orobanche cumana* Wallr.) compte parmi les parasites les plus nuisibles aux tournesols. L'apparition d'une nouvelle espèce E en Yougoslavie a incité les chercheurs à tenter de créer des hybrides pouvant y résister. Des génotypes possédant le gène Or_5 comme les lignes L-414, Bt analogue 2, Bt analogue 3, etc., ont été obtenus à l'aide de tests effectués dans des champs infectés et dans des cellules de quarantine, en serre. Les tests effectués dans les champs et dans les serres ont eu des résultats conformes.