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EVALUATION OF RESISTANCE OF NEW SUNFLOWER HYBRIDS TO BROOMRAPE IN THE BREEDING PROGRAMS IN NOVI SAD

Hladni, N.*, Dedić, B., Jocić, S., Miklič, V., Dušanić, N.

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

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SUMMARY

Broomrape (*Orobanche cumana* Wallr.) presents a serious problem in sunflower production in a number of countries, it reduces sunflower seed yield and negatively affects other sunflower traits in sunflower production. Continued work on creating new sunflower hybrids resistant to broomrape requires testing of breeding materials in both field conditions and controlled conditions of a greenhouse. The best solution is to do the testing simultaneously in order to verify the congruence of the evaluation of the broomrape resistance achieved by these two methods. The screening of chosen new 15 sunflower hybrids for broomrape resistance was done in 2009 and 2010 in infected field on two locations in Vojvodina Province and in 2010 in greenhouse. Both field and the greenhouse tests rated the hybrids exclusively on the basis of presence or absence of the parasite. The infection of hybrids with broomrape has shown that out of 15 hybrids tested, 9 hybrids were resistant. These hybrids were fully resistant in both years in field conditions and controlled greenhouse conditions during one year. Fully resistant hybrids in field conditions and in greenhouse conditions NS-H-6385 and NS-H-6396 had significantly higher seed yield, seed oil content and oil yield in relation to standard.

Key words: sunflower, hybrids, resistance, *Orobanche cumana* Wallr., broomrape

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr.) is currently regarded as one of the most important constraint in sunflower (*Helianthus annuus* L.) production (Fernandez-Martinez *et al.*, 2010). Broomrape develops on the roots of sunflower plants and uses their nutritive compounds and water thus seriously damaging the yield (Alexandrov and Dimitrov, 2007). The damage caused by broomrape to sun-

* Corresponding author: Phone: ++381 21 4898 411; Fax: ++381 21 6 413 833; e-mail: nada.hladni@nsseme.com

flower may reach up to 100%. Controlling broomrape is exceptionally difficult as it produces huge numbers of seeds that remain viable in the soil for many years (Plakhine and Joel, 2010).

The parasitic angiosperm broomrape 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 effort by sunflower breeders to control *Orobanche cumana*. There is a frequent change in the parasite population enhanced with growing of resistant sunflower hybrids (Škorić *et al*., 2010). 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). Breeding for resistance has been crucial for broomrape control, a challenging task because new races of the pathogen continually emerge and ultimately overcome known resistance genes (Tang *et al*., 2003).

Sunflower breeders and geneticists have been successful in responding to the rapid changes in the race composition of broomrape. They found genes for resistance to this pathogen and incorporated them into elite lines of cultivated sunflower, making it possible to develop *Orobanche* - resistant hybrids (Škorić *et al*., 2010). The population of broomrape has been stable in Serbia for the long period of time. 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. The first report of race E in Serbia appeared (Mihaljčević, 1996). The race E has expanded through the Bačka and Banat regions, slowly becoming a serious problem in the sunflower production. According to resent researchers E is still the most virulent race present in Serbia, (Dedić *et al*., 2009; Hladni *et al*., 2010). Continual monitoring of the broomrape populations in Serbia is very important due to changes in race composition and evolution of new more virulent races in neighboring countries and also because climate changes are favorable for the expansion of *Orobanche* species in large areas (Maširević and Medić-Pap, 2009).

The constant problem with broomrape spreading demands constant research work in the filed, searching for new sunflower materials resistant to the new races. Different ways for controlling parasite were tested (different methods of soil cultivation, herbicides use, biological agents, etc.), which gave results (Christov *et al*., 2009). The resistance to races (A, B, C, D and E) is controlled by single dominant genes (Or_1, Or_2, Or_3, Or_4 and Or_5). Virulent races overcoming the resistance gene Or_5 have been identified in Romania (Pacureanu *et al*., 2004), Russia (Goncharov *et al*., 2004), Bulgaria (Shindrova, 2006) and Turkey (Kaya *et al*., 2004). The host-parasite system of sunflower - *O. cumana* described for races A through E appears to follow the gene-for-gene model (Fernandez-Martinez *et al*., 2010).

The increasing demand from manufacturers on Serbian and the world market for more quality and higher yielding hybrids demands a quick change of assortment with better, more productive and stable sunflower hybrids Miklič *et al.* (2010).

Continued work on creating new sunflower hybrids resistant to broomrape requires testing of breeding materials in both field conditions and in controlled conditions of a greenhouse. The best solution is to do the testing simultaneously in order to verify the congruence of the evaluation of the broomrape resistance achieved by these two methods. The method of testing breeding materials in field conditions is performed in a field infested with broomrape. The reliability of results acquired by this method depends on agro-ecological conditions and the amount of broomrape seeds in the soil. Much more accurate results can be obtained by putting broomrape seeds into containers filled with a pre-prepared medium (soil with the addition of some other substances), which are then placed in the controlled environment of a growth chamber or greenhouse (Škorić, 2010).

Panchenko (1975) developed a screening method for assessing resistance to broomrape in greenhouse conditions during autumn and winter. This method was further honed by Grezes-Besset (Rustica Prograin Genetique), who made testing using plastic test tubes part of the procedure. The advantage of this technique is that it provides a higher level of reliability and makes it possible to test a large number of genotypes in a short period of time. According to Mihaljčević (1996) the advantage of Panchenkov's method of artificial induction is enabling early infection diagnostics (28-35 days after sprouting) with the complete inoculums control in droppers in controlled greenhouse environment, Mihaljčević (1996) also proposes a scale from 0-4 (five group in total) which includes all possible interaction between sunflower and broomrape.

Variants of Pancenko's (1973) method are used in all major breeding centers around the word. At the Institute of Field and Vegetable Crops a modification of Pachenkov's method is applied, the quarantine chamber for testing sunflower breeding material in greenhouse was done in plastic buckets (Dozet *et al.*,1999), plastic tubes (Maširević and Medić-Pap, 2009) and in pots Terzić *et al.* (2010).

The aim of this study was testing the new hybrids for broomrape resistance and at the same time testing them for seed and oil yield. Present the screening of chosen 15 new sunflower hybrids with broomrape resistance, resistant to race E in both field conditions and controlled conditions of a greenhouse.

MATERIAL AND METHOD

This investigation included 15 new high productive sunflower (NS-H-6284, NS-H-6287, NS-H-6385, NS-H-6289, NS-H-6721, NS-H-6396, NS-H-6255, NS-H-6249, NS-H-6382, NS-H-6395, NS-H-6242, NS-H-6253, NS-H-6397, NS-H-6250, NS-H-6506) based on the achieved seed and oil yield in production conditions in 2008 and 2009 on two locations, in comparison to the standard. Hybrids were chosen for

testing on resistance to broomrape in naturally infested field and controlled conditions in greenhouse.

The screening of the chosen 15 new sunflower hybrids for broomrape resistance was performed in 2009 and 2010 in infested field on two locations in Vojvodina Province and in a greenhouse in 2010. Both the field and the greenhouse tests rated the hybrids exclusively on the basis of presence or absence of the parasite.

The resistance of new 15 hybrids to broomrape in field was assessed by presence or absence of broomrape shoots on two locations in Vojvodina with three controls: hybrid Bačvanin, resistant to race E, hybrid NS-H-111 susceptible to the race E and line AD-66 susceptible to E broomrape race. The basic sample for the analysis of the examined trait contained 40 plants (20 plants per replication) sampled from the middle row of each block.

Figure 1: Testing of selected material in field conditions (resistant and susceptible hybrid)

Figure 2: Testing of selected material in field conditions (resistant and susceptible hybrid)

The greenhouse test was done in pots containing 10: l compost, send and perlit mixture in the proportion equal amounts of with addition of broomrape seed. Broomrape seeds were placed under sunflower seeds so that's sunflower root must penetrate into the zone of broomrape seeds. Sunflower plants were grown for 7

weeks using 16 h photoperiod. Broomrape attack was assessed by uprooting and careful observation of root system for broomrape nodules and stalks. For each hybrid incidence (percentage of parasitized sunflower plants) and severity (average number of broomrape shoots per sunflower) were calculated.

In order to measure the seed yield (SY), oil content (SOC) and oil yield (OY) in the small plot trials 15 NS-experimental hybrid was set up in 2008 and 2009 on the location of Rimski Šančevi and Šupljak, Serbia. The design of the experiment was a completely randomized complete block system with three replications, with the basic field size of 28 m^2 (4 rows). The hybrids were evaluated for SY, SOC and OY. The SY was normalized to 11% of seed moisture content. The analysis of SOC was carried out nondestructively in a nuclear magnetic resonance (NMR) analyzer. OY was calculated from the seed yield and oil content. High yield hybrids Šumadinac resistant to broomrape (race E) and Sremac non-resistant to broomrape were used as standards.

Figure 3: Testing of breeding material in greenhouse conditions

Figure 4: Testing of breeding material in greenhouse conditions

RESULTS AND DISCUSSION

Continued work in sunflower breeding program at the Institute of Field and Vegetable Crops (IFVC) on creating new sunflower hybrids resistant to broomrape demands the screening of breeding materials for resistance in both field and controlled conditions of a greenhouse. Variant of Pachenkov's (1973) method used in

the quarantine chamber for testing sunflower breeding material in the greenhouse. The broomrape attack was assessed by uprooting and careful observation of root system for broomrape nodules and stalks Terzić *et al*. (2010). Evaluation of the broomrape attack of selected hybrids in greenhouse conditions (Table 1). There was no broomrape attack on resistant control while severity of attack on susceptible control was 9.39 broomrape shoots per sunflower plant. In greenhouse test hybrids NS-H-6397, NS-H-6250 and NS-H-6506 were susceptible (Table 1).

The infection of hybrids with broomrape has shown that out of 15 tested hybrids, 9 were fully resistant in field conditions and greenhouse conditions. The remaining 6 hybrids differed in resistance to broomrape in field conditions and in greenhouse (Table 2). Hybrids NS-H-6285 and NS-H-6287 were developed from crosses of susceptible *cms* lines and *Rf* restore line resistant to broomrape RHA-D-8 while hybrids NS-H-6385, NS-H-6396, NS-H-6382 and NS-H-6395 were developed from susceptible *cms* lines and *Rf* line resistant to broomrape RHA-D-7. This results confirmed the conclusions of Hladni *et al.* (2009) and Hladni *et al*. (2010) that the RHA–D-7 and RHA–D-8 *Rf* inbred lines developed from interspecific population with *H. deserticola* - DES-1474-1, are resistant to broomrape race E and possess gene Or_5.

Table 1: Evaluation of the broomrape attack of sunflower in greenhouse conditions

No	Genotype	Incidence (%) [a]	Severity of attack [b]
1	NS-H-6284	0	0
2	NS-H-6287	0	0
3	NS-H-6385	0	0
4	NS-H-6289	0	0
5	NS-H-6721	0	0
6	NS-H-6396	0	0
7	NS-H-6255	0	0
8	NS-H-6249	0	0
9	NS-H-6382	0	0
10	NS-H-6395	0	0
11	NS-H-6242	0	0
12	NS-H-6253	0	0
13	NS-H-6397	70	2.71
14	NS-H-6250	90	4.78
15	NS-H-6506	55.55	2.2
	Resistant control	0	0
	Susceptible control	92	9.39

[a] – percentage of parasitized plants
[b] – average number of broomrape plants per sunflower plant

In order to create and introduce new sunflower hybrids resistant to broomrape in production, it is necessary for the testing to be performed simultaneously in both greenhouse conditions and field condition in several years and on more than one

Table 2: Evaluation of sunflower resistance to broomrape in field conditions and in greenhouse

No	Year	2009	2009	2010	2010	2010
	Location	I	II	I	II	Greenhouse
		Resistance to broomrape [a]				
	Hybrids	%	%	%	%	%
1	NS-H-6284	+	+	+	+	+
2	NS-H-6287	+/- (1P)	+	+/- (1P)	+	+
3	NS-H-6385	+	+	+	+	+
4	NS-H-6289	+	+	+	+	+
5	NS-H-6721	+	+	+	+	+
6	NS-H-6396	+	+	+	+	+
7	NS-H-6255	+	-	-	-	+
8	NS-H-6249	+	+	+	+	+
9	NS-H-6382	+	+	+	+	+
10	NS-H-6395	+	+	+	+	+
11	NS-H-6242	+	+	-	+	+
12	NS-H-6253	-	+	+	+	+
13	NS-H-6397	+	+	+/- (2P)	+	-
14	NS-H-6250	-	-	+	-	-
15	NS-H-6506	+	+	+	-	-
	Control					
	AD-66	-	-	-	-	
	NS-H-111	-	-	-	-	
	Bačvanin	+	+	+	+	

[a] 100% -resistant to broomrape (+); NR - not resistant to broomrape (-); few broomrape plants could be found (+/-); P- Number of plants infected by broomrape

Table 3: Mean values of seed yield, oil content and oil yield of NS sunflower hybrids tested in field conditions on plots infected by broomrape and in greenhouse of the hybrids tested during 2008 and 2009 on the location of Rimski Šančevi and Šupljak

No	Locations	RŠ 08/09		Š 08/09		Average		Average		Average	
	Traits	SY	OC	SY	OC	SY	R	OC	R	OY	R
	Hybrids	tha^{-1}	%	tha^{-1}	%	tha^{-1}		%		tha^{-1}	
1.	NS-H-6284	4757	44.9	4156	46.4	4457	2	45.7	13	2036	6
2.	NS-H-6287	4515	48.2	4026	46.6	4270	9	47.4	8	2023	8
3.	NS-H-6385	4446	49.5	4246	47.5	4346	4	48.5	2	2108	3
4.	NS-H-6289	4665	49.0	3847	44.8	4256	10	46.9	10	1996	10
5.	NS-H-6721	4600	47.1	3970	46.7	4285	6	46.9	9	2011	9
6.	NS-H-6396	4646	49.4	3944	48.1	4295	5	48.7	1	2093	4
7.	NS-H-6255	4671	43.2	3786	44.7	4229	11	44.0	16	1860	15
8.	NS-H-6249	4578	45.4	3805	44.6	4192	14	45.0	15	1886	14
9.	NS-H-6382	4611	48.4	3826	47.7	4219	12	48.1	6	2029	7
10	NS-H-6395	4658	49.2	3889	47.8	4274	7	48.5	3	2073	5
11	NS-H-6242	4818	49.0	4553	47.9	4686	1	48.4	4	2270	1
12	NS-H-6253	4791	45.9	3755	44.6	4273	8	45.3	14	1934	13
12	NS-H-6397	4545	48.5	3735	47.5	4140	15	48.0	7	1987	11
14	NS-H-6250	4666	46.4	3740	46.3	4203	13	46.4	12	1948	12
15	NS-H-6506	4760	48.7	3966	48.0	4363	3	48.4	5	2111	2
	Control										
	Šumadinac	4313	47.8	3648	45.1	3981	17	46.4	11		16
	Sremac	4406	44.2	3840	41.9	4123	16	43.1	17		17

RŠ-Rimski Šančevi ; Š-Šupljak; R-rank; SY-seed yield; OC-seed oil content; OY-oil yield

location. During 2008 and 2009 on the location Rimski Šančevi and Šupljak agronomy most important agronomy traits like seed yield, seed oil content and oil yield of new experimental sunflower hybrid were examined. Based on two-year results for the agronomically most important traits, hybrids fully resistant in field conditions and greenhouse conditions NS-H-6385 and NS-H-6396 had significantly higher seed yield, seed oil content and oil yield in relation to standard. Hybrid that stood out by seed and oil yield was NS-H-6242 and the plan is for the testing for broomrape resistance to be continued (Table 3).

Both the field and the greenhouse tests 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. Test in infected field and greenhouse have showed new sunflower hybrids resistant to broomrape race E. By testing the new hybrids for broomrape resistance and at the same time testing them for seed and oil yield, the introduction into production of new high yielding hybrids resistant to broomrape was made possible.

CONCLUSION

The infection of hybrids with broomrape has shown that out of 15 hybrids tested, presence of broomrape was not spotted on 9 hybrids. These hybrids were fully resistant in both years in both field conditions and greenhouse conditions. The remaining 6 hybrids differed in resistance to broomrape in field conditions and in greenhouse conditions. In order to create and introduce new sunflower hybrids resistant to broomrape in production it is necessary for the testing to be performed simultaneously in both controlled greenhouse conditions and field condition in various years and on more than one location.

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