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SCREENING WILD SUNFLOWER SPECIES AND F_1 INTERSPECIFIC HYBRIDS FOR RESISTANCE TO BROOMRAPE

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SUMMARY

Broomrape has become one of the most important threats to the cultivated sunflower. New sources of resistance genes are needed to maintain sunflower production. The objective of this research was to evaluate accessions of wild sunflower species and their F_1 interspecific hybrids with cultivated sunflower for resistance to race E.

Plant material consisted of 6 accessions of perennial species and 14 F_1 accessions between perennial species and cultivated sunflower as well as 42 accessions of annual species. Cultivated line JM8 was used as the sensitive check. Accessions were screened in the greenhouse in the season of 2008/09 and in a field trial during 2009. Plants were grown in the infested soil and evaluated for reaction at the end of the vegetation.

The cultivated check was susceptible in the greenhouse and in the field trial.

The annual species showed varying resistance with *H. annuus* and *H. argophyllus* as the most sensitive with an average of 6.6 and 8.5 broomrape plants per sunflower plant. Only 2 of the 7 *H. petiolaris* accessions were susceptible. *H. neglectus* performed well in the greenhouse with only one broomrape plant infecting one plant of the four tested accessions, but accession NEG1363 was infected in the field trial leaving NEG457 and 1183 as resistant. All accessions of perennial species except for an F_1 hybrid of DEC B and F_1 RIG 707 showed complete resistance.

New potential sources of broomrape resistance genes have been found among wild species and their interspecific hybrids.

Key words: ***Helianthus annuus* L., *Orobanche cumana* Wallr., broomrape, resistance, sunflower, wild species**

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INTRODUCTION

Cultivated sunflower was obtained through a selection process where seed yield and oil content were mostly emphasized (Škorić, 1988). Its quality as a cultivated plant was greatly improved in a relatively short period between 1930 and 1970 where it started from the tenth and eventually reached the second place as an oil cultivar right behind soybean. Oil content was raised from 28% (1920) to almost 50% (1955), but the disease problem was always present (Heiser, 1976). Broomrape has now become one of the most important threats to sunflower cultivation. At present, this parasitic angiosperm can be found in Eastern Europe, Spain, the Middle East and India. The yield loss depends on the severity of infection and can be very high.

Wild species of sunflower represent the source of resistance to many pathogens that attack cultivated sunflower. Because of that, it was reasonable to use wild species as a source of resistance genes in sunflower breeding programs through interspecific crosses. Genetic resistance to broomrape has been introduced to cultivated sunflower from the wild species, mainly *H. tuberosus*, into early cultivars in the former USSR (Pustovoit, 1966). The wide use of the same resistance type led to the appearance of new races (Škorić, 1988). By continuing the broomrape race nomenclature given by Vranceanu *et al.* (1980) for five physiological races (A, B, C, D and E), a total of seven races with race G as the newest one is described (Molinero-Ruiz and Melero-Vara, 2004), even though the last one could only be a population of race F (Molinero-Ruiz *et al.*, 2009).

According to Aćimović (1977), broomrape was described for the first time in Serbia in 1951, and it started causing significant damage in susceptible hybrids in the 1990s (Maširević and Medić-Pap, 2009). The first report of race E in Serbia appeared in 1996 (Mihaljčević). It was determined using a set of differential sunflower lines and broomrape seed collected in the fields in the northern part of Vojvodina. According to recent research, race E is still the most virulent race present in Serbia (Dedić *et al.*, 2009).

Because of the constant increase in selection pressure against broomrape races through introduction of new resistance genes and cultivation of resistant hybrids, new broomrape races are also occurring in shorter intervals and limit the durability of introduced resistance. The strategy of broomrape control is therefore changing, with the aim to make resistance more durable by including the use of herbicide tolerant IMI cultivars, quantitative / horizontal resistance, and by understanding the genetic structure and variability of *O. cumana* populations (Fernandez-Martinez *et al.*, 2009).

Genetic resistance is still the main component in sunflower broomrape control and resistance genes originating from wild sunflower species can have an important role in providing durable resistance. A high level of resistance to broomrape races was previously reported in wild species like *H. tuberosus*, and H. *annuus* (Fernandez-Martinez *et al.*, 2008). The objective of this research is to distinguish accessions that are potential carriers of resistance gene/genes for race E by testing the wild species and their F_1 generations with cultivated sunflower. Although the resistance is more frequently found in the perennial species, the annual species are included in the test because they are much easier to cross with cultivated sunflower.

MATERIALS AND METHODS

Plant material consisted of 42 accessions of annual species (Table 1) as well as 6 accessions of perennial species and 14 F_1 accessions between perennial species and cultivated sunflower (Table 2). Cultivated line JM8 with no resistance genes was used as the sensitive check in the evaluated material. Accessions were screened in the greenhouse in the season of 2008/09 and in a field trial in 2009 for resistance to broomrape.

To promote germination the seed hull of wild species was removed (Chandler and Jan, 1985). Dehulled seeds were first kept in the dark in a growth chamber for 24 h and after the root emergence transferred to jiffy 7 pots. Seedlings were planted in jiffy 7 pots and grown in the greenhouse at 21°C (day and night), with relative humidity at around 80%, and constant light until the phase of two pairs of leaves, after which they were transferred to buckets (10 dm^3) on October 15^{th} 2008 or planted in the field for the field trial in 2009. The buckets were filled with a mixture of sand:perlit:peat in a 1:1:1 ratio which was homogeneously infested with broomrape seeds of race E at a rate of 70 mg/dm^3 soil mixture. Broomrape seeds were extracted from the stalks collected in August 2007 from locations in Vojvodina where race E was previously determined. Plants were grown in the infested soil for 90 days at a photoperiod of 16/8h day/night with natural light supplemented with metal halide lamps. In the field trial, the seed material was sawn at the same time as the rest of the cultivated sunflower. Reaction of plants was evaluated when they reached physiological maturity.

Plants with emerged or underground broomrape stalks were considered susceptible and those without a broomrape stalk as resistant. Severity was calculated as the number of broomrape plants per sunflower plant. Accessions that were susceptible in the greenhouse test were not included in the field trial.

RESULTS AND DISCUSSION

The cultivated check was susceptible in the greenhouse and in the field test. The annual species showed varying resistance with *H. annuus* and *H. argophyllus* as the most sensitive with an average of 6.6 and 8.5 broomrape plants per sunflower plant, respectively. All accessions of those two species were found susceptible in the greenhouse trial. First broomrape stalks emerged after 70 days in two accessions of *H. annuus* and one of *H. niveus* and the rest emerged by day 93 of growing in the greenhouse. Broomrape emergence could be taken as additional information on broomrape resistance, especially for heterogeneous material like the accessions of wild species (Tab. 1).

Table 1: Accession identifiers and reaction of annual species to race E of *Orobanche cumana* Wallr.

Genotype/ wild species	IFVCNS population	PI number	Greenhouse		Field trial	
			Intensity of attack	Broomrape emergence (DAS§)	Intensity of attack	Broomrape emergence (DAS)
H. debilis	1134	PI 468678	0	-	-	-
H. debilis	1136	PI 468680	0	-	2.4	79
H. debilis	1290	PI 468687	8	*	-	-
H. debilis	1675	PI 468692	0	-	-	-
H. debilis	1810	PI 494583	3.5	93	-	-
H. debilis	1848	PI 494589	0	-	3.5	97
H. annuus	1970	PI 531015	1.5	86	-	-
H. annuus	2034	PI 547165	8	74	-	-
H. annuus	2104	PI 586809	11.5	74	-	-
H. annuus	2150	PI 586839	9	70	-	-
H. annuus	2191	PI 586866	0.5	-	-	-
H. annuus	2206	PI 586877	3.5	70	-	-
H. annuus	2220	PI 586883	12	74	-	-
H. argophyllus	1317	PI 468649	4	93	-	-
H. argophyllus	1575	PI 468651	6.5	93	-	-
H. argophyllus	1807	PI 494573	15	86	-	-
H. petiolaris	338	PI 435803	0	-	0	-
H. petiolaris	722	PI 435831	0	-	0	-
H. petiolaris	815	PI 435845	0	-	2.1	93
H. petiolaris	1383	PI 468811	1.5	80	-	-
H. petiolaris	1910	PI 503232	0	-	0	-
H. petiolaris	2146	PI 586918	0	-	0	-
H. petiolaris	2208	PI 586931	0	-	0	-
H. praecox	1142	PI 468851	0	-	7.1	79
H. praecox	1151	PI 468854	0.5	*	-	-
H. praecox	1801	PI 494606	1.5	*	1.8	79
H. praecox	1333	PI 468865	0	-	5.2	86
H. praecox	1341	PI 468849	0	-	0	-
H. praecox	1168	PI 468847	0	-	7	90
H. praecox	1821	PI 494610	0,5	*	0	-
H. niveus	1403	PI 468784	0	-	3.9	86
H. niveus	1452	PI 468788	6,5	70	-	-
H. neglectus	1181	PI 468765	0,5	*	-	-
H. neglectus	1363	PI 468775	0	-	5.6	86
H. neglectus	457	PI 435761	0	-	0	-
H. neglectus	1183	PI 468767	0	-	0	-
JM 8	Cultivated		2,3	86	7.8	79

* All broomrape stalks were underground at the moment of evaluation

§ DAS – Days after sowing

Similar findings in regard to the resistance of *H. annuus* and *H. argophyllus* were presented by Fernandez-Martinez *et al.* (2008), who also described the species *H. petiolaris* and *H. praecox* as 100% susceptible. *H. petiolaris* performed differently in this trial with only 2 out of the 7 accessions susceptible. Accession PET1383 had 1.5 broomrape plants per sunflower in the greenhouse and was not tested in the field and accession PET815 was not infected in the greenhouse but had 2.1 broomrape plants per sunflower of infected plants in the field trial. Different resistance could be simply a result of different accessions that were used or different broomrape race composition.

H. debilis had two resistant accessions (DEB1134 and DEB 1675) out of the six tested. The rest were infected either in the greenhouse (DEB1290, 1810) or in the field trial (DEB1136, 1848). *H. neglectus* performed very well in the greenhouse, with only one broomrape plant infecting one plant of the four tested accessions, but accession NEG1363 was infected in the field trial leaving NEG457 and 1183 as resistant.

Table 2: Accession identifiers and reaction of perennial species and their F_1 hybrids with cultivated sunflower to race E of *Orobanche cumana* Wallr.

Genotype /wild species	IFVCNS population	(Wild parent) PI number	Greenhouse		Field trial	
			Intensity of attack	Broomrape emergence (DAS§)	Intensity of attack	Broomrape emergence (DAS)
F_1 *H. rigidus*	1693	-	0	-	0	-
F_1 *H. rigidus*	1696	-	0	-	0	-
F_1 *H. rigidus*	707	-	0	-	2.1	79
F_1 *H. tuberosus*	1698	-	0	-	0	-
F_1 *H. tuberosus*	1701	-	0	-	0	-
F_1 *H. tuberosus*	6	-	0	-	0	-
F_1 *H. strumosus*	1927	PI 503249	0	-	0	-
F_1 *H. strumosus*	1623	PI 468894	0	-	0	-
F_1 *H. divaricatus*	2085	PI 547174	0	-	0	-
F_1 *H. hirsutus*	1536	PI 468738	0	-	0	-
F_1 *H. eggertii*	1626	PI 468712	0	-	0	-
F_1 *H. decapetalus*	B	-	0.5	*	0	-
F_1 *H. resinosus*	1545	PI 468879	0	-	0	-
F_1 *H. laevigatus*	1618	PI 468740	0	-	0	-
H. tuberosus	675	PI 468829	0	-	-	-
H. tuberosus	26	-	0	-	-	-
H. grossesseratus	56	-	0	-	-	-
H. mollis	1530	PI 468760	0	-	-	-
H. nuttalii	239	PI 435779	0	-	-	-
H. tuberosus	CG 56	-	0	-	-	-
JM 8	Cultivated		2,3	86	7.8	79

* All broomrape stalks were underground at the moment of evaluation

§ DAS – Days after sowing

All evaluated accessions of perennial species showed complete resistance. Similar results for perennial species was obtained by other authors (Fernandez-Martinez *et al.,* 2000) which is why they were not included in the field trial to limit the possibility of unwanted propagation. It is worth noting that only two out of the 14 tested F_1 crosses between cultivated sunflower and perennial species were susceptible. An F_1 hybrid with DEC B accession reached physiological maturity in the greenhouse after approximately 90 days with an attack intensity of only one underground broomrape stalk per sunflower. An F_1 hybrid with RIG 707 was not infected with broomrape in the greenhouse, but in the field trial it had 2.1 broomrape plants per sunflower (Table 2). It has been shown that the wild species and especially perennials have a great potential in providing resistance for the future broomrape races. Although the resistance was higher in perennial species and their F_1 progenies, resistant plants found among the annual species are very important in regard to the short-term breeding programs. It is always better to use the resistance genes from the gene pool of cultivated sunflower, but in some case, like with the newest races of broomrape, it is fortunate that the interspecific crosses are possible inside the genus *Helianthus*.

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