

Screening of Wild *Helianthus* Species and Derived Lines for Resistance to Several Populations of *Orobanche cernua*

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ABSTRACT

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Twenty-six different perennial species of *Helianthus*, 18 wild annual species of the same genus, and 29 lines tracing to wild species were evaluated for resistance to three highly virulent populations of broomrape (*Orobanche cernua*). Evaluations were carried out in pots containing soil mixture infested with broomrape seeds. Most of the perennial *Helianthus* species were immune to the populations of broomrape used in the tests. Some wild annual species and wild derived lines were resistant. The resistance found in the wild species, introgressed to cultivated sunflower, could provide unique resistance to the parasite.

Sunflower broomrape (*Orobanche cernua* Loefl. syn. *O. cumana* Wallr.) is an important angiosperm holoparasite, totally devoid of chlorophyll, that infects the roots of sunflower (*Helianthus annuus* L.) plants in warm and dry regions. Broomrape is actually regarded as one of the most important constraints on sunflower production in areas of southeastern Europe, Spain, Turkey, and several Asian and North African countries. Attacks are frequently severe and yield losses have reached 50% in Turkey and 30% in Spain (20,24).

Each broomrape plant produces thousands of tiny seeds. Soilborne seeds are stimulated to germinate by host-root exudates. Seeds outside of the host rhizosphere may remain dormant and viable for longer than 10 years.

Broomrape seeds can be easily spread by wind, soil, farm machinery, and water as well as infested sunflower seeds (2). Eradication measures such as soil solarization and soil fumigation provide control (7,8) but are uneconomical for sunflower. Herbicides offer some chemical control (4). However, at present, genetic resistance is the most effective, reliable, and feasible control method against broomrape.

Genetic resistance was introduced into susceptible sunflower cultivars in early breeding work in the former USSR (24). Subsequent to the appearance of new virulent races of the pathogen, new sources of resistance were obtained mainly from germ plasm tracing to wild species *Helianthus tuberosus* L. (23,25). Since the appearance of new pathogenic races of *O. cernua* is frequent, there is a continuous search for new sources of resistance. Five race complexes of *O. cernua* have been identified, designated as A through E. Race A was originally found in Ukraine at the end of the 19th century. A newer race, B, was described in the same area in the 1920s (16). There was seven- to ninefold seed yield reduction in susceptible sunflowers due to race B. In Bulgaria, Petrov (14) identified another new race (complex M), which was also found in Moldavia (Russia). In Romania, Vrânceanu (25) identified five races (A to E) on a set of five differential hosts carrying resistance genes (Or1 to Or5) and a universal susceptible (or).

O. cernua is known to have been present in Spain since 1958, first in the areas where confectionery sunflower was traditionally grown and more recently in two areas of central and southern Spain where oilseed cultivars are widespread (5,10). Phenotypic characterization of broomrapes from different locations in Spain confirmed several races that were different from the races described in Romania (10,25). Races overcoming resistance genes Or1, Or3, and Or4, but not Or2, were identified (10). New broomrape populations found more recently in central Spain have overcome resistance of differential lines that carry Or2 and Or5 genes (20).

Although genetic resistance against Spanish races of broomrape is incorporated into recent hybrid cultivars, the increasing geographic range of the disease and the potential for new races require the search for new sources of resistance. The diverse species of the genus *Helianthus* represent considerable genetic variability, which has provided new sources of disease resistance (11,23). The objective of this study was to evaluate broomrape resistance in a *Helianthus* germ plasm collection that included 44 annual and perennial wild species as well as 29 sunflower lines derived from interspecific crosses with cultivated sunflower. A preliminary report has been published (19).

MATERIALS AND METHODS

Plant material. Accessions of 44 annual and perennial species of *Helianthus* from the sunflower germ plasm collection of Córdoba and 29 derived lines tracing to *Helianthus* subsp. from the U.S. were screened. The annual species were *H. agrestis* Pollard, *H. annuus* L., *H. anomalus* Blake, *H. argophyllus* Torr. & A. Gray, *H. bolanderi* Gray, *H. debilis* Nutt. subsp. *cucumerifolius* (T. & G.) Heiser, *H. debilis* Nutt. subsp. *silvestris* Heiser, *H. debilis* Nutt. subsp. *tardiflorus* Heiser, *H. debilis* Nutt. subsp. *vestitus* (Watson) Heiser, *H. deserticola* Heiser, *H. exilis* Gray, *H. niveus* (Benth.) subsp. *canescens* Heiser, *H. niveus* subsp. *niveus* (Benth.), *H. petiolaris* Nutt. subsp. *fallax* Heiser, *H. petiolaris* Nutt. subsp. *petiolaris* Nutt., *H. praecox* Engelman & Gray subsp. *hirtus* Heiser, *H. praecox* Engelman & Gray subsp. *praecox* Engelman & Gray, and *H. praecox* Engelman & Gray subsp. *runyonii* Heiser. The perennial species were *H. californicus* DC., *H. cusickii* Gray, *H. decapetalus* L., *H. divaricatus* L., *H. eggertii* Small, *H. glaucophyllus* Smith, *H. gracilentus* Gray, *H. grossesserratus* G. Martens, *H. hirsutus* Raf., *H. laetiflorus* Pers., *H. laevigatus* Torr. & A. Gray, *H. maximiliani* Schrad., *H. mollis* Lam., *H. nuttallii* Torr. & A. Gray subsp. *nuttallii* Torr. & A. Gray, *H. nuttallii* Torr. & A. Gray subsp. *parishii* (Gray) Heiser, *H. occidentalis* Riddell subsp. *occidentalis* Riddell, *H. occidentalis* Riddell subsp. *plantagineus* (Torr. & A. Gray) Heiser, *H. pumilus* Nutt., *H. resinusus* Small, *H. pauciflorus* (Cass.)

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Desf. subsp. *pauciflorus* (Cass.) Desf., *H. pauciflorus* (Cass.) Desf. subsp. *subrhomboides* (Rydberg) Heiser, *H. salicifolius* Dietr., *H. simulans* Watson, *H. smithii* Heiser, *H. strumosus* L., and *H. tuberosus* L. The derived lines included 26 released by the USDA (21,22) and three from the USDA germ plasm collection at Fargo, ND.

Germination of seeds. To break dormancy and promote germination, seeds of all wild *Helianthus* accessions were first surface sterilized for 10 min in a 1% aqueous solution of sodium hypochlorite to which 1 ml of Tween 20 per liter was added as surfactant. The seeds were then scarified by cutting one third off the blunt end of the seed, ensuring a cut into the embryonic tissue. The scarified seeds were subsequently soaked in a 100 mg/liter solution of gibberellic acid (GA₃) for 1 h (3), then placed in petri dishes on filter paper soaked with sterile distilled water, and incubated in the dark at 21 to 25°C. After 1 day of incubation, the hulls (pericarp) and seed coats were removed from the embryos, the petri dish was flushed three times with sterile distilled water to remove any water-soluble inhibitors, and the seeds were returned to the dark to incubate for 9 more days until planting. Wild *Helianthus*-derived lines did not require any treatment to promote germination.

Screening. One accession for every wild species and derived line was screened for resistance to *O. cernua* in pot culture inoculations in greenhouse in 1993 and 1994. One population of *O. cernua* collected in central Spain in 1992 (CU-192) and two others (SE-193 and SE-194) collected in southern Spain in 1993 and 1994, respectively, were used. In 1993, 20 germinated seedlings for wild species and 10 seeds for derived lines were planted individually in small pots (350 cm³) filled with a sand/peat mixture (1:1, vol/vol); half of them had been homogeneously infested with broomrape seeds of SE-193 and the other half with CU-192 populations, both at the rate of 200 mg per kg of soil mixture. In 1994, 10 germinated seedlings of each of the wild species and five plants of each of three new accessions from Fargo tracing to wild sunflower were tested against SE-194. Plantings in infested soil mixture (50 mg broomrape seeds per pot) were incubated for 1 month in a growth chamber at 26°C (day/night), relative humidity ≈60%, and a photoperiod of 14 h of fluorescent light (36 μE s⁻¹ m⁻²). Then, the plants were transplanted to larger pots containing 3 liters of a sand/silt/peat (2:1:2, vol/vol/vol) fertilized soil mixture. These were incubated for 1 and 2 additional months for derived lines and wild species, respectively, in a greenhouse at 20 to 25°C with natural light sup-

plemented with high-pressure sodium lamp illumination to achieve a 16-h photoperiod. For derived lines, disease assessments were made on five randomly located plants (at the two-pair-leaf stage) of each entry-population combination, 1 month after transplanting, by observing broomrape nodules attached to the root systems of uprooted plants as well as the necrotic reactions (25). Plants were regarded as resistant to broomrape when either root system was asymptomatic, had necrotic lesions, or had minute broomrape nodules that did not develop normally (1). One cultivated sunflower line (S59) susceptible to all known broomrape populations was included in inoculation studies as a control.

Accessions of wild species were screened for resistance to populations CU-192 and SE-193 in 1993 and SE-194 in 1994 after transplanting the greenhouse-grown plants to the field. For each entry-population combination, the disease was assessed on five to 10 plants that survived after transplanting from the greenhouse to the field and after 2 months incubation there. Pots were transplanted to a sandy loam soil in May 1993 and 1994 in rows 2 m apart; in each row the plants were inoculated with each one of the broomrape populations, and plant were spaced 1.5 m within the rows. Disease reactions were assessed at blooming by recording the number of emerged broomrapes per plant (degree of attack) and the percentage of plants infected (25). A low degree of attack was defined as ≤1 emerged broomrapes per plant. Data on degree of attack were transformed to $\sqrt{x+0.5}$ prior to the analysis of variance as a 15 (species) × three (broomrape) factorial design.

RESULTS

In all the experiments in 1993 and 1994, the check S59 was fully susceptible to the three populations of *O. cernua* (Tables 1, 2, and 3). Greenhouse tests on sunflower lines originating from wild sunflower indicated susceptibility except for the lines Ano.-1509-2-2, Arg.-420-1-2, and Des.-1474-1-2, which showed different proportions of plants resistant to broomrape SE-193. Only Ano.-1509-2-2 had plants resistant to CU-192 (Table 1). Lines Hir.-1537 × P21, Gra.-1442 × P21, and Nut.-730 × P21 also showed high proportions of plants resistant to SE-194 (Table 1).

In field experiments, most of the perennial wild species of sunflower were immune to the three populations of *O. cernua*. However, one accession of *H. gracilentus* was completely susceptible (100% incidence) to broomrape population SE-194, and that of *H. nuttallii* subsp. *nuttallii* had a reduced incidence of plants infected by this population, with a low degree of attack (Table 2). In contrast, only three of the 18 wild annuals (*H. anomalus*, *H. exilis*, and *H. agrestis*) showed resistance to *O. cernua* (Table 3). Some of the other an-

Table 1. Incidence (%) of plants infected by three populations of broomrape in sunflower lines tracing to wild species

Line	PI accession number ^a	Populations of broomrape		
		CU-192 ^b	SE-193 ^b	SE-194 ^c
Ano.-1509-1-2	468644	100	100	...
Ano.-1509-2-2	468644	50	0	...
Arg.-420-1-2	435630	100	50	...
Arg.-1575-1-2	468651	100	100	...
Arg.-1575-2-2	468651	100	100	...
Bol.-774-1-2	435640	100	100	...
Deb.Cuc.-1810-1-2	494583	100	100	...
Deb.Sil.-367-1-2	435652	100	100	...
Deb.Sil.-367-2-2	435652	100	100	...
Des.-1474-1-2	468701	100	75	...
Des.-1474-2-2	468701	100	100	...
Des.-1474-3-2	468701	100	100	...
Hir.-1734-2-2	539906	100	100	...
Hir.-1734-3-2	539905	100	100	...
Neg.-1255-1-2	468768	100	100	...
Par.-1084-1-2	468800	100	100	...
Par.-1671-1-2	468801	100	100	...
Par.-1671-2-2	468801	100	100	...
Par.-1673-1-2	468802	100	100	...
Par.-1673-2-2	468802	100	100	...
Pet.Pet.-1741-1-2	A-7460	100	100	...
Pra.Run.-417-3-2	435850	100	100	...
Pra.Run.-1329-1-2	468863	100	100	...
Pra.Hir.-437-1-2	435855	100	100	...
Res.-834-1-2	539987	100	100	...
Res.-834-3-2	539986	100	100	...
Hir.-1537 × P21	468739	0
Gra.-1442 × P21	468723	20
Nut.-730 × P21	435779	20
Susceptible check (S59)		100	100	100

^a Correspondence to wild parental in Fargo collection.

^b Tested in greenhouse in 1993. Values are based on 10 plants.

^c Tested in greenhouse in 1994. Values are based on five plants.

nual species (*H. annuus*, *H. debilis* subsp. *vestitus*, *H. deserticola*, *H. niveus* subsp. *canescens*, and *H. niveus* subsp. *niveus*) were completely susceptible (100% incidence) to the three populations of *O. cernua*. For the rest of the species, incidence was 65% or higher. Final degrees of attack in the susceptible annual species varied from 1.4 to 9.2, 1.4 to 16, and 1.2 to 14 for the broomrape populations CU-192, SE-193, and SE-194, respectively. There were no significant differences among these three populations of *O. cernua* but there were significant differences between species (Table 4). *H. bolanderi* was the most susceptible species to *O. cernua*, with 100% incidence and highest degrees of attack (Table 3). *H. debilis* subsp. *tardiflorus*, *H. debilis* subsp. *silvestris*, *H. deserticola*, and two tested *H. petiolaris* subsp. also showed a higher degree of attack than the other wild annuals, *H. annuus* included (Table 3). There was differential interaction between sunflower species and broomrape populations (Table 4); e.g., in *H. debilis* subsp. *silvestris* and *H. debilis* subsp. *tardiflorus* (Table 3).

DISCUSSION

The results of this investigation show that both annual and perennial wild sunflower species can be used to breed for resistance to Spanish populations of broomrape. Resistance to populations of *O. cernua* was found mainly in the perennial accessions, most of which were fully resistant. Yet, three annual species also showed resistance to the three broomrape populations tested, and four showed disease incidence lower than 100% to the three broomrape populations. Although resistant accessions were much more frequent in the perennial than in the annual species, breeders are likely to prefer annual wild sunflower accessions for the ease and efficiency of exploiting these sources of resistance. Annual species show higher relative cross compatibility with the cultivated sunflower, making easier the introgression of the resistance into the commercial lines.

Three annual species—*H. exilis*, *H. anomalus*, and *H. agrestis*—were found to show complete resistance to broomrape populations. *H. exilis* is restricted to a poor moist serpentine soil habitat in the inner coast of California (18). It is considered by some authors to be synonymous with *H. bolanderi* (18), which showed the highest degree of susceptibility in the present study. However, Heiser (6) and Olivieri and Jain (13) consider *H. bolanderi* to be the product of introgressive hybridization of *H. exilis* and *H. annuus*. Resistance genes of *H. exilis* could have been lost in the process of introgression. Moreover, wild species are usually heterogeneous and variability for resistance might occur. More accessions should be evaluated to study this variability and to find out if resistance

Table 2. Reactions of 26 perennial *Helianthus* species to three populations of *Orobancha cernua*^a

Species	2n	Access. no.	Broomrape population					
			CU-192		SE-193		SE-194	
			Incid. ^b	DA ^c	Incid. ^b	DA ^c	Incid. ^b	DA ^c
<i>H. californicus</i>	102	130	0	0	0	0	0	0
<i>H. cusickii</i>	34	138	0	0	0	0	0	0
<i>H. decapetalus</i>	34	181	0	0	0	0	0	0
<i>H. divaricatus</i>	34	184	0	0	0	0	0	0
<i>H. eggertii</i>	102	187	0	0	0	0	0	0
<i>H. glaucophyllus</i>	34	210	0	0	0	0	0	0
<i>H. gracilentus</i>	34	212	NT ^d	NT	NT	NT	100	4
<i>H. gracilentus</i>	34	213	0	0	0	0	0	0
<i>H. grosseserratus</i>	34	215	0	0	0	0	0	0
<i>H. hirsutus</i>	68	223	0	0	0	0	0	0
<i>H. laetiflorus</i>	102	224	0	0	0	0	0	0
<i>H. laevigatus</i>	68	227	0	0	0	0	0	0
<i>H. maximiliani</i>	34	230	0	0	0	0	0	0
<i>H. mollis</i>	34	238	0	0	0	0	0	0
<i>H. nuttallii</i>								
subsp. <i>nuttallii</i>	34	267	0	0	0	0	33	1
subsp. <i>parishii</i>	34	273	0	0	0	0	0	0
<i>H. occidentalis</i>								
subsp. <i>occidentalis</i>	34	276	0	0	0	0	0	0
subsp. <i>plantagineus</i>	34	280	NT	NT	NT	NT	0	0
<i>H. pumilus</i>	68	335	0	0	0	0	0	0
<i>H. resinosus</i>	102	344	0	0	0	0	0	0
<i>H. pauciflorus</i>								
subsp. <i>pauciflorus</i>	102	350	0	0	0	0	0	0
subsp. <i>subrhomboideus</i>	102	354	NT	NT	NT	NT	0	0
<i>H. salicifolius</i>	34	357	0	0	0	0	0	0
<i>H. simulans</i>	34	363	0	0	0	0	0	0
<i>H. smithii</i>	34	367	0	0	0	0	0	0
<i>H. strumosus</i>	68	373	0	0	0	0	0	0
<i>H. tuberosus</i>	102	380	0	0	0	0	0	0
Susceptible check (S59)	34	...	100	35	100	28	100	36

^a Populations CU-192 and SE-193 were used in 1993, and population SE-194 in 1994.

^b Incidence: number of infected plants / total number of sunflower plants.

^c Degree of attack: average number of broomrapes per sunflower plant.

^d Not tested.

Table 3. Reaction of 18 annual *Helianthus* species to three populations of *Orobancha cernua*^a

Species	2n	Access. no.	Broomrape population					
			CU-192		SE-193		SE-194	
			Incid. ^b	DA ^c	Incid. ^b	DA ^c	Incid. ^b	DA ^c
<i>H. agrestis</i>	34	001	NT ^d	NT	NT	NT	0	0
<i>H. annuus</i>	34	011	100	2.4	100	2.4	100	12.5
<i>H. anomalus</i>	34	075	0	0	0	0	0	0
<i>H. argophyllus</i>	34	082	67	1.8	100	2.1	100	4
<i>H. bolanderi</i>	34	125	100	9.2	100	16	100	14
<i>H. debilis</i>								
subsp. <i>cucumerifolius</i>	34	145	71	2.6	71	2.4	80	3
subsp. <i>silvestris</i>	34	160	71	2	83	1.4	80	8
subsp. <i>tardiflorus</i>	34	166	78	7.9	88	5.1	71	2
subsp. <i>vestitus</i>	34	170	100	2	NT	NT	NT	NT
<i>H. deserticola</i>	34	175	100	5	NT	NT	NT	NT
<i>H. exilis</i>	34	190	0	0	0	0	0	0
<i>H. niveus</i>								
subsp. <i>canescens</i>	34	262	100	3	100	3	100	3.2
subsp. <i>niveus</i>	34	258	100	2.7	100	2	100	2.2
<i>H. petiolaris</i>								
subsp. <i>fallax</i>	34	311	100	4	100	3	80	3
subsp. <i>petiolaris</i>	34	296	88	4.5	89	3.3	65	3
<i>H. praecox</i>								
subsp. <i>hirtus</i>	34	326	100	2	100	3	65	1.2
subsp. <i>praecox</i>	34	316	80	2.3	89	2	65	2
subsp. <i>ruyoni</i>	34	331	100	2.3	100	2.3	65	2
Susceptible check (S59)	34	...	100	46	100	27	100	26

^a Populations CU-192 and SE-193 were used in 1993, and population SE-194 in 1994.

^b Incidence: number of infected plants / total number of sunflower plants.

^c Degree of attack: average number of broomrapes per sunflower plant.

^d Not tested.

Table 4. Analysis of variance for degree of attack by three broomrape populations on a collection of annual *Helianthus* species^a

Source of variation	df	Mean squares	F value
Total	275
Species (S)	14	136.4	16.22 ^b
Populations (P)	2	16.4	1.92
Interaction S × P	28	24.6	2.93 ^b
Residual	231	8.4	...

^a *H. agrestis*, *H. debilis* subsp. *vestitus* and *H. deserticola* were evaluated against only one broomrape population (see Table 3) and were not included in this analysis. Values are based on numbers of broomrape plants attached to roots of each *Helianthus* line.

^b Significant at $P = 0.0001$.

genes of *H. exilis* are present in *H. bolanderi*. *H. anomalus* is a rare species found in isolated sites of Utah and Arizona (18). Crosses of *H. anomalus* with other species, including the cultivated *H. annuus*, have been obtained (23), indicating that the introgression of the resistance gene found in this species into cultivated sunflower is possible. The present study confirms this with the result of the evaluation of the line Ano-1509 tracing to *H. anomalus*, which showed a high proportion of resistant plants (Table 1). The occurrence of susceptible plants can be explained by the process of selfing and crossing with the susceptible cultivated parent carried out to obtain this line. *H. agrestis* is a species limited to very wet soils in Florida and Georgia and is not closely related to other annual species (18). Crosses with cultivated material have not been reported.

The occurrence of resistance to *O. cernua* in perennial *Helianthus* species was reported by Morozov (12), who identified resistance to race D of broomrape in *H. tuberosus*, *H. maximiliani*, and *H. mollis*. Pustovoit (15) reported the development of varieties resistant to a new broomrape race by backcross with *H. tuberosus*. Complete resistance to sunflower broomrape has also been found in the perennial species *H. glaucophyllus* and *H. resinusus* and in the annual *H. debilis* subsp. *debilis* (23). However, screening of broomrape resistance on such a large number of species as in the present study had not been done before. This study confirms the resistance of all perennial species reported before but many new species have been identified as resistant. The high proportion of resistant perennial species observed in this study is noteworthy and contrasts with the proportion observed in the annuals. This suggests that the perennial germ plasm is more diverse than that of the annual species. For another disease, sunflower rust, caused by *Puccinia helianthi* Schwein., Quresh et al (17) reported a general immunity of perennial *Helianthus* to the virulent races of this pathogen and variability of resistance in annual accessions. The latter was explained by the selection pressure of the disease in the natural habitat of these species. However, in the case of *O. cernua* this selection pressure and the coevolution of the host-pathogen system cannot explain

the variability of wild accessions since this parasitic angiosperm is not present in North America where wild sunflower species occur. Therefore, broomrape resistance may occur fortuitously in sunflower species.

The primary objective of this research was the identification of new sources of resistance to *O. cernua*. Therefore, efforts were concentrated on the evaluation of a high number of species, although the number of entries per species was limited. A larger number of accessions of each species would be required to make conclusive statements about intraspecific variability. The high number of species evaluated and the high proportion of resistance found, especially in the perennial species, suggest the usefulness of these species as valuable new sources of broomrape resistance, since *O. cernua* is known to be a highly variable pathogen, and breakdown of resistance is a frequent phenomenon. Resistance genes to other sunflower diseases such as rust and downy mildew (*Plasmopara halstedii* (Farl.) Berl. & De Toni in Sacc.) have been transferred from wild species, including perennials, into cultivated sunflower (9). It is likely that most of the resistance genes from these wild species will provide unique sources of resistance to sunflower broomrape. The introduction of these genes may have a great potential in breeding programs to alleviate the threat to cultivated sunflower of currently prevailing races and possible future races.

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