RESISTANCE OF SUNFLOWER (*Helianthus)* PERENNIAL SPECIES, INTERSPECIFIC AMPHIPLOIDS, AND BACKCROSS PROGENY TO BROOMRAPE (*O. cumana Wallr*.) RACES

C. C. Jan, USDA-ARS, Northern Crop Science Laboratory, PO Box 5677, ND 58105, USA
Fax: +1 701 239 1346; email: janc@fargo.ars.usda.gov

Juan A. Ruso, USDA-ARS, Northern Crop Science Laboratory, PO box 5677, Fargo, ND 58105, USA; Fax: +1 701 239 1346; email: Juan_rusofuentes@ndsu.nodak.edu

Juan Muñoz-Ruz, Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080, Cordoba, Spain; Fax:+34 957 499 252; email: jmruz@cica.es

Jose M. Fernandez-Martinez, Instituto de Agricultura, CSIC, Apdo. 4084, 14080, Cordoba, Spain; Fax:+34 957 499 252; email: cs9femaj@uco.es

Summary: Broomrape (*Orobanche cumana* Wallr.) is one of the more important constraints for sunflower production in Eastern and Southern Europe, the Middle East, Russia and Ukraine, and it has been described in Western Australia, Mongolia, and China. The widespread use of resistant cultivars has been followed by the appearance of new races of the parasite (A-E) capable of overcoming the resistance genes already in use (Or_1-Or_5). Sources of resistance for most virulent races of *O. cumana* have been identified from wild *Helianthus* species. A breeding program to transfer *O. cumana* resistance from wild perennial *Helianthus* species into cultivated sunflower was started in 1994. Parental wild species, interspecific amphiploids and first backcross progenies derived from nine wild species with different ploidy level have been tested for broomrape resistance under greenhouse conditions and artificial infection with three Spanish populations, including one population overcoming the Or_5 resistance gene. All backcross progenies, while segregating for chromosome numbers (2n=34 to 51) also segregated for resistance to all the three broomrape populations with medium-low incidence and disease severity, which indicated a high selective potential for resistance. Resistant progenies in advanced backcrosses with diploid number of chromosomes, good seed set, and pollen stainability were selected for germplasm production.

Introduction

Broomrape (*Orobanche cumana* Wallr.), a parasitic weed that infects sunflower (*Helianthus annuus* L.) roots, is currently one of the more important constraints for sunflower production in Southern Europe and regions around the Black Sea, and it has been described in Western Australia, Mongolia, and China (Bulbul et al., 1991; Parker, 1994; Dominguez et al., 1996).
Genetic resistance to broomrape was introduced into susceptible sunflower mainly from wild *Helianthus* species. The widespread use of resistant cultivars has been followed by the appearance of new broomrape races capable of overcoming the resistance genes in use.

O. cumana is known to have been present in Spain since 1958. Studies identified races that overcame Or_1, Or_3, and Or_4 genes but not Or_2 and Or_5 (Melero Vara, 1997). However, more recent studies have shown an evolution of sunflower broomrape races in Spain, with a new race, designated F, overcoming all the known resistance genes identified thus far (Dominguez et al., 1996; Melero-Vara, 1997). Since all broomrape resistant sunflower currently registered and/or commercialized are based on the Or_5 gene, the search for new resistance sources and their incorporation into cultivated genotypes is urgently needed.

A high level of resistance to race F of *O. cumana* in populations of wild perennial sunflower has been reported (Ruso et al., 1996 and Fernandez-Martinez et al., 1999), and the development of embryo rescue and chromosome doubling has greatly improved the success of obtaining F_1 hybrids and increasing F_1 fertility (Chandler and Beard, 1978; Jan, 1988). A breeding program to transfer *O. cumana* resistance from wild perennial *Helianthus* species into cultivated sunflower was started in 1994. Results reported by Sukno et al. (1998) indicated an immune reaction of *H. giganteus*, *H. laevigatus*, *H. pauciflorus* ssp. *pauciflorus*, *H. resinosus*, and their F_1's after crossing these species with HA89. The BC_1F_1 progenies segregated for resistance, using broomrape population SE-194, which can be controlled by the Or_5 gene. This paper reports the reaction of wild *Helianthus* species, interspecific amphiploids, and backcross progenies against three populations of *O. cumana*, including one population composed predominantly of race F.

Materials and Methods

Amphiploids and their backcross progenies derived from the diploid wild species *H. angustifolius*, *H. cusickii*, *H. divaricatus*, *H. gracilentus*, *H. grosseserratus*, *H. maximiliani* and *H. nutallii*, the tetraploid species *H. hirsutus*, and *H. strumosus* were tested against three broomrape populations under greenhouse conditions from 1998 to 1999 at Cordoba, Spain.

The three broomrape populations had varying levels of virulence. SE-194 was collected in 1994 from Ecija in southern Spain. CU-996 was collected in 1996 in the field near Cuenca, central Spain, where it infected hybrids possessing the Or_5 gene. SE-296 was collected in 1996 in Ecija in a field with hybrid Ursus, which possesses the Or_5 gene.Population SE-296 overcame all the known resistance genes, including Or_2 and Or_5 (Sukno et al., 1999) Five to 10 plants of each wild parent species, and 10 plants of the susceptible cultivated lines P21 and HA89, and the differential line P-1380, carrying the Or_5 gene, were also included.

Broomrape resistant BC progenies, 2n=34 to 51, resulted from backcrossing 2n=51 plants with HA89, were selected in Cordoba, and progenies grown in the greenhouse at Fargo, ND for root-tip chromosome examination, pollen stainability (Alexander, 1969), and self-compatibility obtained as percentage of seeds/total florets at harvest. Male-fertile progenies were self-pollinated, and male-sterile progenies backcrossed by HA89. Then, self-pollinated progenies of 2n=34 BCF_2 plants were evaluated against *Orobanche* population SE-296 in Cordoba, together with P-1380 (Or_5).

Inoculations were performed by planting 7-day-old seedlings in peat pots with 250 g of a soil mixture (sand:silt, 1:1 v/v) homogeneously infested with 50 mg of broomrape seeds After 14 days of incubation at 20°C with a photoperiod of 14 h of fluorescent light (36 µ mol m^{-2} s^{-1}) and 60% relative humidity for the cultivated lines, amphiploids and BC_nF_1 plants, and 3 to 5 weeks for wild species, plants were transferred into pots containing 3 L of a fertilized soil mixture (peat moss:sand:silt, 2:2:1, v/v), and amended with slow-release fertilizer (N,P,K:15,11,13 + 2 Mg 0 and micronutrients at the rate of 2.5 g/kg). These plants were grown in the greenhouse at 20-25°C until flowering, with natural light supplemented with high pressure sodium lamps to maintain a 16-h photoperiod.

The number of broomrape plants was recorded at flowering time, which was approximately 90-120 days after sowing for cultivated lines, amphiploids, and BC_nF_1, and 120-150 days after sowing for wild species. For each entry the percentage of infected plants (incidence) and the disease severity, expressed as the average number of emerged shoots of *O. cumana* per plant, were calculated.

Results and Discussion

The evaluation results are shown in Table 1. As previously reported the susceptible cultivated lines P-21 and HA89 had the highest incidence of infection (100%), but degree of attack was significantly higher in P-21. The differences in the degree of attack between HA89 and P21 suggest the possible existence of minor resistance genes to both SE-194 and SE-296. P-1380 was immune (0% incidence) to SE-194, partially susceptible to CU-996, and totally susceptible (100% incidence) to SE-296. The reactions of P-1380 indicated that races A-E, which are controlled by the Or_5 gene, were present in broomrape population SE-194. CU-996 was composed of a low frequency of new races virulent to Or_5 gene, and SE-296 had a high frequency of the new broomrape race F.

Wild perennial parental species were completely immune to the three broomrape populations. Amphiploids of *H. gracilentus* x P21 and *H. hirsutus* x P21 were immune to the three populations, indicating likely dominant gene control of the resistance. The exceptional 20% infected plants of (*hir*-1126 x P21) amphiploids to CU-996 but not to SE-296 suggested different compositions of virulent races in these two populations. Amphiploids of *H. maximiliani* x P21, *H. nutallii* x P21, and *H. strumosus* x P21 segregated for resistance to CU-996 and SE-296, indicating either a lower resistance gene frequency in those species accessions or that the resistance was partially dominant. Amphiploids of *H. hirsutus* x P21 were also reported as all resistant to broomrape populations other than SE-296 and CU-996 (Sukno et al., 1996). The production of amphiploids involving *H. angustifolius* was not successful and the amphiploid involving *H. cusickii* did not have enough seed for this test. Backcross progenies involving those two species were from 2n=51 BC_1F_1 plants that resulted from crossing P21 with chromosomally doubled F_1 heads.

All backcross progenies, while segregating for chromosome numbers from 2n=34 to51, also segregated for resistance to the three respective broomrape populations. The overall medium-low incidence and degree of attack in most BC progenies indicated a high selective potential for resistance to all the three broomrape populations (Table 1). Selection against SE-194 will result in 2n=34 plants with resistance genes equivalent to Or_5. Selection against CU-996 will likely result in plants with genes resistant to both the predominant Or_5-controlled race and the new and more virulent race in the CU-996 population. Similarly, resistance genes selected using SE-296 should provide protection against both the Or_5-controlled races and the most virulent new F race in SE-296.

Since the race composition of CU-996 and SE-296 are not determined, selections using these two populations may result in different resistance genes. As more new virulent broomrape races overcoming the Or_5 gene are being observed in Turkey as well as other European countries, selection for another universal resistance gene beyond Or_5 is urgently needed. Since SE-296 is currently considered the most virulent race in Spain, continuing selection has been focused on this population. Progenies of resistant selections with 2n=34 to 51 were shown to have chromosome numbers reduced to 2n=34 to 38, over 95% having 2n=34 to 36, and with good seed set and pollen stainability, which will greatly increase the success of broomrape resistance gene transfer in the next generation (Table 2).

Broomrape resistance of self-pollinated progenies of resistant 2n=34 BCF_2 plants are shown in Table 3. A high frequency of immune resistant plants were obtained from the six BC_2F_3 or BC_3F_3 families from four donor wild species, *H. grosesserratus*, *H. maximiliani*, *H. divaricatus*, and *H. angustifolius*, supporting a major dominant gene control of broomrape resistance. The average disease severity of

4.8 for the segregated broomrape infected plants is significantly lower than the 10.0 for P-1380, suggesting additional minor gene contribution.

The results indicate that the resistance is dominant, which facilitates its transfer through backcrossing. These amphiploids served well as fertile bridges overcoming the common problem of F_1 interspecific hybrid sterility, and will facilitate interspecific gene transfer through conventional breeding procedures. Segregation for resistance and chromosome number (2n=34-51) in backcross progenies and the high frequency of progenies with near 2n=34 chromosomes provided suitable material for further selection of diploid individuals with resistance to the new Orobanche race derived from wild parents in the next generation.

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Table 1. Reaction of wild species, cultivated lines, amphiploids, and BC_nF_1 derived from amphiploids under an artificial infection of populations of *O. cumana* Wallr.

			SE-194			CU-996		SE-296		
Pedigree		*2n*	Plant evaluated	Infected (%)	Disease severity†	Plant evaluated	Infected (%)	Plant evaluated	Infected (%)	Disease severity
H angustifolius		34		-	-			10	0	0
((ang-43-006 x ann,D)P21)HA89	BC_2F_1	34-51	14	3(21)	1.7±0.66	28	13(46)	10	1(10)	3.0
H. cusickii		34	5	0	0		-	5	0	0
((cus x P21,D)P21)HA89	BC_2F_1	34-51	19	3(16)	1.7±0.70	17	10(59)	19	2(11)	2.0±1.00
H.divaricatus		34	5	0	0		-	10	0	0
(div-830 x P21,D) (gro x P21,D),SIB[3]	AMP	68		-	-	14	12(86)	16	6(38)	2.3±0.33
((div-830 x P21,D)P21)HA89	BC_2F_1	34-51	10	0	0	24	19(79)	17	10(59)	2.0±0.39
H. gracilentus		34	5	0	0		-	6	0	0
((gra-1442 x P21)D),SIB[2]	AMP	68		-	-		-	7	0	0
H. grossesserratus		34	5	0	0		-	10	0	0
(P21ms (gro x P21,PD))HA89	BC_2F_1	34-51	12	4(33)	2.0±0.41	9	3(33)	8	2(25)	1.0±0
(((gro x P21,D),SIB)P21)HA89	BC_2F_1	34-51	8	3(38)	2.3±0.33	8	5(63)	10	3(30)	1.3±0.33
H. hirsutus		68	5	0	0		-	10	0	0
(hir-1126 x P21,D),SIB[2]	AMP	102		-	-	10	2(20)	13	0	0
((hir-1126 x P21,D)P21)HA89	BC_2F_1	51	46	7(15)	1.3±0.18	19	14(74)	16	2(13)	2.5±0.50
(hir x P21,D),SIB	AMP	102		-	-	2	0	10	0	0
((hir x P21,D),SIB)HA89	BC_1F_1	68	10	0	0	9	2(22)	1	0	0
((hir x P21,D),SIB)HA89[2]	BC_2F_1	51	20	4(20)	4.5±0.29	7	0	12	0	0
H. maximiliani		34	5	0	0		-	13	0	0
((max x P21,D)P21) ((max x P21,PD)D),SIB	AMP	68		-	-	13	5(39)	16	3(19)	1.7±0.33
((max x P21,D)P21) ((max x P21,PD)D),HA89	BC_1F_1	51	11	4(36)	2.0±0.41	22	22(100)	13	5(39)	2.2±0.58
((max x P21,D)P21)HA89	BC_2F_1	34-50	10	2(20)	2.0±0	33	27(82)	16	6(38)	1.3±0.33
((max-33-004 x P21,D)P21)HA89	BC_3F_1	34-51	9	3(33)	2.0±0	25	24(96)	21	11(52)	1.8±0.26
H. nuttallii		34	6	2(33)	1.0±0		-	9	0	0
(nut-730 x P21,PD),SIB[4]	AMP	68		-	-	20	11(55)	22	4(18)	2.0±0.41
((nut-730 x P21,PD),SIB[2])HA89	BC_1F_1	51	10	2(20)	2.5±1.51	33	32(97)	21	6(29)	2.0±0.36
H. strumosus		68	5	0	0		-	9	0	0
(str-30-002-1 x P21,D),SIB[3]	AMP	102		-	-	9	4(44)	7	1(7)	1.0
((str-30-002-1 x P21,D),SIB)HA89	BC_1F_1	68	7	0	0	12	10(83)	14	4(29)	2.3±0.75
((str-30-002-1 x P21,D)P21)HA89	BC_2F_1	51	14	5(36)	1.8±0.84	10	0	8	1(8)	1.0
Cultivated checks										
P-1380 (Or_5)		34	10	0	0	9	2(22)	10	10(100)	4.7±0.73
HA89		34	10	10(100)	6.3±0.30	10	10(100)	10	10(100)	8.2±0.77
P-21		34	10	10(100)	13.5±0.77	10	10(100)	10	10(100)	15.3±1.69
LSD (0.05)					2.1					4.0

† Average number of emerged shoots of *O. cumana* per infected sunflower plant.

Table 2. Characterization of progenies from broomrape resistant backcross selections (BC_nF_2) segregating for chromosome number.

	Chromosome number														
	34			35			36			37			38		
Pedigree†		Pollen stainability	Self Seed set		Pollen stainability	Self Seed set		Pollen stainability	Self Seed set		Pollen stainability	Self Seed set		Pollen stainability	Self Seed set
		%			%			%			%			%	
(P21ms (gro x P21,PD))HA89	12‡	98±0.8 (10)	80±10.3	5	69±8.3 (4)	33±32.2	4	88±1.3 (2)	30±20						
(((gro x P21,D),SIB)P21)HA89	4	98±1.1	73±26.7	5	78±19.1 (4)	30±23.5	8	86±3.9 (6)	43±18.2	2	9(1)	2	1	96	90
((max x P21,D)P21)HA89	14	94±3.2 (10)	96±3.1	5	97±1.1 (4)	45±14.4	1	0							
((max-33 x P21,D)$P21^2$)HA89	5	91±4.0	79±15.6	10	90±5.3 (9)	50±14.4	6	82±10.8	29±12.1						
((cus x P21,D)P21)HA89							4	87±3.9 (3)	30±25.2						
((div x P21,D)P21)HA89	7	99±0.5 (5)	100±0.0	5	94±1.3 (3)	83±8.8	2	0		1	0				
((ang x ann,D)P21)HA89	2	96±4.0	60±40	9	93±1.4	66±12.2	5	92±3.6	60±13.8				1	100	80
Total	44			39			30			3			2		

† gro = *H. grossesserratus*, max = *H. maximiliani*, cus = *H. cusickii*, div = *H. divaricatus*, ang = *H. angustifolius*, ann = *H. annuus*
‡ Number of progenies/chromosome group

Table 3. Reaction of backcross progenies derived from selected diploid (*2n*=34) under an artificial infection of populations SE-296 of *O. cumana* Wallr.

Parental pedigree†		Plants evaluated	Infected plants(%)	Disease severity §
(P21ms (gro x P21,PD))HA89, BC_2F_3	11‡	156	86 (55)	4.5±0.45
(((gro x P21,D),SIB)P21)HA89, BC_2F_3	3	44	28 (64)	7.2±0.89
((max x P21,D)P21)HA89, BC_2F_3	12	148	61 (41)	4.0±0.54
((max-33 x P21,D)$P21^2$)HA89, BC_3F_3	5	62	17 (27)	4.7±0.77
((div x P21,D)P21)HA89, BC_2F_3	7	67	38 (57)	6.5±0.86
((ang x ann,D)P21)HA89, BC_2F_3	2	18	8 (44)	2.1±0.58
Cultivated check				
P-1380 (Or_5)		14	14 (100)	10.0±1.86
LSD (0.05)				3.0

† gro = *H. grossesserratus*, max = *H. maximiliani*, div = *H. divaricatus*, ang = *H. angustifolius*, ann = *H. annuus*
‡ Number of parental plants
§ Average number of emerged shoots of *O. cumana* per infected sunflower plant.