HELIA, **25**, Nr. 36, p.p. 123-136, (2002) UDC 633.854.78:631.523.12

INTERSPECIFIC HYBRIDIZATION, GENE TRANSFER, AND THE DEVELOPMENT OF RESISTANCE TO THE BROOMRAPE RACE F IN SPAIN

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Received: November 08, 2001
Accepted: June 03, 2002

SUMMARY

The 49 North American wild *Helianthus* species have long survived extreme environments and possess resistance or tolerance genes to salt, drought, insects, diseases, as well as cytoplasmic male-sterility and fertility restoration, and are valuable genetic resources for sunflower improvement. Gene transfer from wild species into cultivated background depends on the success of interspecific hybridization, F_1 fertility, chromosome pairing for genetic recombination, efficient screening methods, and sufficient progenies for selection. Most wild annual species x sunflower crosses produce F_1 seeds and can be backcrossed easily. For the more difficult perennial x sunflower crosses where hybrid seed set is rare, a two-step embryo culture technique has been established for rescuing immature interspecific embryos prior to abortion. The chromosome homology between genomes of wild species and cultivated sunflower is high, and the meiotic chromosome pairing of wild diploid x cultivated F_1 is reasonably good, except for the multivalent formation, bridges, and fragments due to translocation and inversion differences. Chromosomal doubling by colchicine treatment substantially increases the F_1 fertility, improves backcrossing success, and leads to the production of amphiploids. The amphiploids have restored fertility and can be maintained by sib-pollination, and will serve as a bridge for gene transfer. Using this approach, we successfully transferred genes resistant to the new broomrape race F, which attacks all the cultivated sunflower in Spain. Inheritance studies suggest a single dominant gene provides resistance.

Key words: **sunflower, *Helianthus annuus* L., embryo culture, chromosome doubling, amphiploid, broomrape, *Orobanche cumana* Wallr., race F, gene transfer**

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INTRODUCTION

Domestication of wild sunflower species by the North American Indians in Arizona and New Mexico began as early as 3000 B.C. Following the spread of sunflower to Europe, it was identified as a potential oil crop and developed rapidly in Russia in the eighteenth century. Intensive breeding efforts in Russia started in the early 1900s utilizing additional wild sources, including the hexaploid perennial *Helianthus tuberosus* and a few wild annuals, and produced sunflower crops with high yield, high oil content, and resistance to diseases, insects and broomrape.

The discovery of cytoplasmic male sterility and corresponding fertility restoration genes led the sunflower crop into a hybrid production era in the early 1970s, and placed the sunflower among the four major oilseed crops of the world. However, due to its narrow genetic foundation, its continuing success as a major oilseed crop depends on the continued introgression of genes from its wild relatives for agronomic and quality characteristics, as well as tolerance to biotic and abiotic stresses.

Of the 49 sunflower species, the 12 annuals, with the exception of *H. agrestis* Pollard, can be crossed with cultivated sunflower and produce fertile F_1 plants. Conventional breeding programs have utilized these annual wild species and one hexaploid, *H. tuberosus* L., for most sunflower improvement and these will definitely play a significant role in the future. However, the rest of the wild *Helianthus* species are perennial, including diploids, tetraploids and hexaploids, and are not only much more difficult to hybridize with cultivated sunflower but also produce sterile F_1 plants. In addition, due to the limited information on genome relationships among diploid sunflower species, and between cultivated sunflower and wild species, the likelihood of gene transfer from wild perennial species into cultivated sunflower is often in question.

Wild perennial *Helianthus* species represent a huge amount of unexplored genetic variation for sunflower improvement. It is understandable that there are desirable specific genes in abundance in annual species, such as resistance to rust, downy mildew, cytoplasmic male sterility, fertility restoration genes, and salt and drought tolerance, which should be utilized before dealing with the perennials. However, the largely ignored potential of perennial species needs to be addressed and every effort is needed to bring this genetic variability into more usable forms for conventional sunflower breeding.

The recent emergence of virulent populations of broomrape (*Orobanche cumana* Wallr.) overcoming the resistance of sunflower (*Helianthus annuus* L.) hybrids with the Or_5 gene in southern Spain created an urgent need for new resistance genes. The extensive search for resistance to the new broomrape race in both cultivated sunflower collections and in wild annual species failed, however, perennial species were shown to be mostly immune, a formal cooperation with Spanish

scientists was established in 1996 to transfer broomrape race F resistance gene from the perennial species into cultivated sunflower.

This report documents the successful interspecific hybridization of annual and perennial species through the use of an improved embryo rescue technique and a colchicine treatment for chromosome doubling of the F_1 plants to restore fertility. This method led to the production of interspecific amphiploids and the transfer of genes for broomrape resistance from wild perennial *Helianthus* species into cultivated sunflower utilizing interspecific amphiploids as a bridge.

Table 1: Composition of embryo culture medium for interspecific crosses

Component	Growth medium	Germination medium
Inorganic minerals	B5 salts	B5 salts
Vitamins	mg/l	mg/l
Nicotinic acid	1.0	1.0
Thiamine-HCl	10	10
Pyridoxine-HCl	1.0	1.0
Myo-inositol	4000	100
Amino acids	mg/l	mg/l
L-alanine	1000	
L-glutamine	800	
L-serine	160	
L-tryptophane	50	
L-cysteine	10	
	g/l	g/l
NAA	0.05	
	g/l	g/l
Agar	7.0	7.0
	g/l	g/l
Sucrose	120	20

I INTERSPECIFIC HYBRIDIZATION

Embryo culture

Chandler and Beard (1983) developed a two-step embryo culture procedure which enabled them to produce 53 interspecific cross combinations without the endless effort of pollination for seed production. However, this method was not successful for crosses between the difficult-to-cross perennial diploid and the cultivated sunflower. Further modifications were made in 1983, including the addition of vitamins and the conversion from liquid to a solid medium with 0.7% agar and the use of 20 g/kg sucrose for the embryo germination medium. In addition, both growth and germination media were adjusted to pH 5.5 with 2-[N-morpholino]ethanesulfonic acid (MES) to maintain a more constant pH level. Five day old embryos were first cultured on a solid growth medium in petri dishes with Gam-

borg's B5 salts supplemented with amino acids, vitamins, NAA , and 120 g/kg sucrose. After 1 to 2 weeks, the enlarged embryos were transferred to test tubes in a germination medium with only B5 salts. Using this new culture medium, 18 perennial x cultivated hybrids were produced in 1984 with very little effort, and the use of this medium has continued to prove effective for all perennial x cultivated crosses through the years. The composition of the culture medium is listed in Table 1.

Chromosome doubling and amphiploid production

A list of selected interspecific hybrids obtained is shown in Table 2.

Table 2: The effect of chromosome doubling on seed set of diploid interspecific hybrids when crossed as seed and pollen parent with P21 and sib-pollinated

F_1 Pedigree	BC seed set				SIB seed	
	ND		D		ND Sib	D Sib
	Female	Male	Female	Male		
	Seeds/Head					
H. mollis x P21	0	—	9	60	0	0
H. mollis x P21	2.3	5.3	—	84	—	—
H. grosserratus X P21	1	—	1	33.2	—	5
H. nuttallii X P21	0.2	—	0	—	0	0.5
H. maximiliani X P21	1	—	8	30	0	—
H. maximiliani X P21	0.3	1	1	—	0.1	0.4
H. gracilentus X P21	0.9	0	7.1	8	0	5.9
H. pumilus x P21	0.8	46	24.1	185	0	12.4
H. cusickii x P21	0.3	3.3	1.8	300	0	0.2
H. arizonesis x P21	0.1	3	2.9	72	0	0
H. divaricatus X P21	0.9	3	17.5	18	0	—
	0.8	8.8	7.2	87.8	0.01	3.1

D, ND represent chromosomally doubled and non-doubled head, respectively
BC = backcross and SIB = sib-pollination

Because of the anticipated low F_1 fertility, a modified colchicine technique was used to induce chromosome doubling of the F_1 hybrids and to restore fertility. The apical meristems of the seedlings were submerged in a 1.5 g/kg colchicine solution with 2.0 g/kg DMSO (dimethyl sulfoxide) for 5 h in the dark. Seedlings were then washed and transplanted into pots. Chromosome doubling increased pollen grain size and stainability, and was verified for individual heads using Alexander's pollen stain (1969). A head was classified as chromosomally doubled when over 60% of its pollen grains were large (40 μ in diameter vs the normal 30 μ), and pollen stainability was at least 50%. Chromosome doubling increased backcrossed (BC) seed set 10-fold using the F_1 as either male or female parent. However, regardless of chromosome doubling, BC seed set was higher when the F_1 plant was the pollen parent. Part of this increase in seed per head could be due to the larger head size of the cultivated sunflower. The direction of BC should be considered when transferring

nuclear genes. Sib-pollination of chromosomally doubled heads produced an average of 3.1 seeds per head, which led to the production of interspecific amphiploids. Sib-pollination of non-doubled heads produced nearly zero seed set due to the high F_1 sterility. Intercrossing chromosomally doubled heads of each cross produced amphiploids that can be increased and maintained by sib-pollination for use in gene transfer. When amphiploids are not available, the chromosomally doubled heads of diploid hybrids can be backcrossed to cultivated sunflower to produce triploid progenies, and chromosomally doubled heads of the triploid hybrids can be backcrossed to produce tetraploid progenies for gene transfer purpose.

Selected amphiploids are shown in Table 3. The amphiploids had good germination and plant establishment in the greenhouse, with most plants grown to maturity. The amphiploids were chromosomally unstable, with a range of 2n=66 to 70 for the tetraploids and 2n=94 to 103 for the hexaploids. With a few exceptions, amphiploids produced good seed set from sib-pollination, and equally good seed set from backcrossing to cultivated sunflower. Their very low self-pollinated seed set indicated high self-incompatibility.

Table 3: Plant survival, chromosome number, and seed set of amphiploids

Parentage	Seed	Seedling	Matured plant	2n chromosome	Seeds/head		
					BC	Sib	SP
				numbers			
H. gra 1442 x P21	33	25	22	66-69	38	67	---
H. pum 773 x P21	---	---	7	67-68	3	1	---
H. hir 1126 x P21	6	6	5	96-102	50	17	0
H. mol 1531 x P21	18	8	7	68-69	18	19	0
H. str 30-002-1 x P21	19	10	7	99-103	22	19	0
H. gro x P21	13	9	9	66-68	50	6	0
H. max x P21	30	24	20	66-69	115	44	0
H. nut 730 x P21	30	29	29	66-70	44	88	0
H. cus 17-002-1 x P21	3	3	3	67-68	2	1	0
H. hir 1537 x P21	---	---	---	94-102	0.03	5.3	0.06

II GENE TRANSFER FOR BROOMRAPE RESISTANCE

INTRODUCTION

Broomrape, a parasitic weed that infects sunflower roots, is a major pest of sunflower in Southern Europe and around the Black Sea, and has been found in Western Australia, Mongolia, and China (Bulbul *et al.,* 1991; Parker, 1994; Domínguez *et al.,* 1996; Shindrova, 1994). Broomrape control with herbicide is only partially effective (García-Torres *et al.,* 1988) and soil fumigation or solarization treatments are not economically feasible (Foy and Jacobsohn, 1989). There-

fore, at the present time, genetic resistance remains the best control method for this parasite.

The current resistance to broomrape was introduced into cultivated sunflower primarily from wild *Helianthus* species (Pustovoit, 1966). The widespread use of resistant cultivars was followed by the appearance of broomrape races capable of overcoming the resistance. Following the appearance of broomrape races A through E, from the 1910s to 1970s, sunflower cultivars with resistance genes Or_1 through Or_5 were developed (Vrânceanu *et al.*, 1980).

Orobanche cumana has been observed in Spain since 1958, first on confectionery sunflower and later on oilseed sunflower in central and southern Spain (González-Torres *et al.*, 1982). However, recent studies have indicated a change in the broomrape races in Spain, with a new race, F, overcoming the widely used resistance genes (Domínguez *et al.*, 1996; Melero-Vara, 1997). All broomrape resistant hybrid sunflower in Spain and other European countries possess the Or_5 gene, and all are susceptible to race F.

Cultivated sunflower lacks resistance to race F of *O. cumana*, and a high level of resistance to race F in populations of wild perennial sunflower (Ruso *et al.*, 1996; Fernández-Martínez *et al.*, 2000). Embryo rescue and chromosome doubling techniques have improved the success in obtaining F_1 hybrids and increased F_1 fertility (Jan, 1988). In addition, meiotic studies of F_1 hybrids between *H. gracilentus, H. mollis* Lam., *H. maximiliani, H. divaricatus,* or *H. angustifolius* and P21 indicated a high degree of chromosome homology between wild perennials and the cultivated sunflower, suggesting a high probability of interspecific gene transfer when F_1 fertility was restored by chromosome doubling (Jan and Chandler, 1985).

A breeding program to transfer *O. cumana* resistance from wild perennial *Helianthus* species into cultivated sunflower was initiated in 1994. The main objective was to transfer the broomrape race F resistance from wild perennial *Helianthus* species into cultivated sunflower. This paper reports the reaction of perennial wild *Helianthus* species, interspecific amphiploids, and backcrossed progenies to two populations of *O. cumana,* including one population composed predominately of race F.

MATERIALS AND METHODS

Several sunflower germplasms were inoculated with broomrape populations, SE194 (race E) and SE296 (race F), in the greenhouse at Cordoba, Spain in 1998 and 1999. These germplasms included wild perennial accessions, amphiploids derived from crosses between cultivated *H. annuus* and diploid wild perennial species *H. angustifolius*, *H. cusickii* A. Gray, *H. divaricatus*, *H. gracilentus*, *H. grosseserratus*, *H. maximiliani,* and *H. nutallii,* and the tetraploid perennial species *H. hirsutus* and *H. strumosus,* as well as backcross progenies. SE194 was collected in 1994 near Ecija, Spain, in a field that had no history of infection with broomrape,

and was used to infect 'Florasol', a hybrid which does not possess the Or_5 gene. SE296 was collected in 1996 near Ecija in a field planted to "Ursus", a hybrid which possesses the Or_5 gene. Population SE296 overcame all the identified resistance genes, including Or_2 and Or_5 (Sukno *et al.*, 1999). Five to 10 plants of each wild parental species, 10 plants of the susceptible cultivated checks P21 and HA89, and the differential line P-1380, carrying the Or_5 gene, were also inoculated. The first backcross progenies were produced by crossing chromosomally doubled F_1 heads or amphiploids with P21 or HA89. The resulting BC_1F_1's were crossed with HA89 to produce the BC_2 F_1 progeny.

Broomrape resistant BC_2F_1 progenies involving *H. angustifolius*, *H. divaricatus*, *H. grossesserratus*, and *H. maximiliani*, 2n=34 to 51 chromosomes, were selected in Cordoba. BC_2F_2 progenies were grown in the greenhouse at Fargo, ND, to determine root-tip chromosome number. BC_2F_3 progenies from self-pollinated 2n = 34 BC_2F_2 plants were inoculated with *Orobanche* population SE296 in Cordoba.

Resistance was evaluated by transplanting 7-day-old seedlings into peat pots with 250 g of a soil mixture with sand : silt (1:1 v/v) homogeneously infested with 50 mg of broomrape seeds. The cultivated lines, amphiploids and BC_nF_1 plants were incubated at 20°C and 60% relative humidity with a 14 h photoperiod under fluorescent light (36 µmol m^{-2} s^{-1}) for 3 to 5 weeks. Plants were transferred to pots containing 3 l of a peat moss:sand:silt mixture (2 :2 : 1, v/v), and fertilized with slow-release fertilizer (N,P,K : 15,11,13 + 2% MgO plus micronutrients) at the rate of 2.5 g kg^{-1}. These plants were grown in the greenhouse at 20-25°C under natural light supplemented by high-pressure sodium lamps to obtain a 16 h photoperiod until flowering.

The number of broomrape plants infecting sunflower 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 the 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 infected plant, was calculated.

RESULTS AND DISCUSSION

Wild perennial parental species were immune to the two broomrape populations (Table 4). These results confirmed the high level of resistance previously observed in perennial species to virulent populations of *O. cumana* belonging to race E and F (Ruso *et al.*, 1996; Fernández-Martínez *et al.*, 2000). Consistent with previous reports (Fernández-Martínez *et al.*, 2000), the susceptible cultivated lines, P21 and HA89, had the highest incidence of infection (100%), with the disease severity higher in P21 than HA89. The difference in the disease severity between HA89 and P21 may be due to the existence of minor resistance genes for SE194 and

SE296. P-1380 was immune (0% incidence) to SE-194, and totally susceptible (100% incidence) to SE-296. The reactions of P-1380 indicated that races A-E were present in the broomrape population SE-194, which is avirulent in the presence of the Or_5 gene, and that SE-296 had a high frequency of the new broomrape race F.

Table 4: Reaction of wild *Helianthus* species to artificial infection with populations SE194 and SE296 of *O. cumana*

Pedigree	2n	SE194			SE296		
		Plants evaluated	Infected plants	Disease severity‡	Plants evaluated	Infected plants	Disease severity‡
		no.	%		no.	%	
H. angustifolius	34		-	-	10	0	0
H. cusickii	34	5	0	0	5	0	0
H.divaricatus	34	5	0	0	10	0	0
H. gracilentus	34	5	0	0	6	0	0
H. grossesserratus	34	5	0	0	10	0	0
H. hirsutus	68	5	0	0	10	0	0
H. maximiliani	34	5	0	0	13	0	0
H. nuttallii	34	6	33	1	9	0	0
H. strumosus	68	5	0	0	9	0	0
Cultivated checks							
P-1380 (Or_5)	34	10	0	0	10	100	4.7
HA89	34	10	100	6.3	10	100	8.2
P-21	34	10	100	13.5	10	100	15.3

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

Table 5: Reaction of interspecific amphiploids to artificial infection with population SE296 of *O. cumana*

Pedigree†	2n	SE296		
		Plants evaluated	Infected plants	Disease severity‡
		no.	%	
(*div*-830 x P21,D) (*gro* x P21,D),SIB^3	68	16	38	2.3
((*gra*-1442 x P21)D),SIB^2	68	7	0	0
(*hir*-1126 x P21,D),SIB^2	102	13	0	0
(*hir* x P21,D),SIB	102	10	0	0
(((*max* x P21,D)P21) ((*max* x P21,PD)D)),SIB	68	16	19	1.7
(*nut*-730 x P21,PD),SIB^4	68	22	18	2
(*str*-30-002-1 x P21,D),SIB^3	102	7	7	1.0
Cultivated checks				
P-1380 (Or_5)	34	10	100	4.7
HA89	34	10	100	8.2
P-21	34	10	100	15.3

† D = head with complete chromosome doubling, PD = head with partial chromosome doubling
‡ Average number of emerged shoots of *O. cumana* per infected plant

The reaction of interspecific amphiploids to SE296 is shown in Table 5. Amphiploids of *H. gracilentus* x P21 and *H. hirsutus* x P21 were immune, indicating dominant gene(s) for resistance. Amphiploids of *H. divaricatus* x P21, *H. maximiliani* x P21, *H. nutallii* x P21, and *H. strumosus* x P21 segregated for resistance, indicating either a lower frequency of the resistance gene in the parental accessions or that the resistance was partially dominant.

Table 6: Reaction of BC_nF_1 progenies derived from amphiploids to artificial infection with populations SE194 and SE296 of *O. cumana*

Pedigree†		2n	SE194			SE296		
			Plants evaluated	Infected plants	Disease severity‡	Plants evaluated	Infected plants	Disease severity‡
			no.	%		no.	%	
((*ang*-43-006 x *ann*,D)P21)HA89	BC_2F_1	34-51	14	21	1.7	10	10	3.0
((*cus* x P21,D)P21)HA89	BC_2F_1	34-51	19	16	1.7	19	11	2
((*div*-830 x P21,D)P21)HA89	BC_2F_1	34-51	10	0	0	17	59	2
(P21ms (*gro* x P21,PD))HA89	BC_2F_1	34-51	12	33	2	8	25	1
(((*gro* x P21,D),SIB)P21)HA89	BC_2F_1	34-51	8	38	2.3	10	30	1.3
((*hir*-1126 x P21,D)P21)HA89	BC_2F_1	51	46	15	1.3	16	13	2.5
((*hir* x P21,D),SIB)HA89	BC_1F_1	68	10	0	0	1	0	0
((*hir* x P21,D),SIB)HA89^2	BC_2F_1	51	20	20	4.5	12	0	0
(((*max* x P21,D)P21) ((*max* x 21,PD)D))HA89	BC_1F_1	51	11	36	2	13	39	2.2
((*max* x P21,D)P21)HA89	BC_2F_1	34-50	10	20	2	16	38	1.3
(((*max*-33-004 x P21,D)P21)HA89	BC_2F_1	34-51	9	33	2	21	52	1.8
((*nut*-730 x P21,PD),SIB^2)HA89	BC_1F_1	51	10	20	2.5	21	29	2
((*str*-30-002-1 x P21,D),SIB)HA89	BC_1F_1	68	7	0	0	14	29	2.3
((*str*-30-002-1 x P21,D)P21)HA89	BC_2F_1	51	14	36	1.8	8	8	1.0
Cultivated checks								
P-1380 (Or_5)		34	10	0	0	10	100	4.7
HA89		34	10	100	6.3	10	100	8.2
P-21		34	10	100	13.5	10	100	15.3

† D = head with complete chromosome doubling, PD = head with partial chromosome doubling
‡ Average number of emerged shoots of *O. cumana* per infected plant

All BC_2F_1 progenies with chromosome numbers from 2n=34 to 51 segregated for resistance to the two broomrape populations. The overall medium to low incidence and disease severity of most BC progenies indicated a high selective potential for resistance to the two broomrape populations (Table 6). Selection for resistance to SE194 will result in 2n=34 plants with resistance genes equivalent to Or_5. Similarly, genes for resistance to SE296 should provide protection against both the Or_5-controlled races and the most virulent new F race in SE296.

Since the SE296 population is currently the most virulent race in Spain, continuing selection has been focused on this population. A high frequency of immune

plants was obtained from the six BC_2F_3 families derived from four donor wild species, *H. grosesserratus*, *H. maximiliani*, *H. divaricatus*, and *H. angustifolius*, indicating that a major dominant gene, or genes, controls broomrape resistance to race F (Table 7). The average disease severity of 4.8 for the broomrape-infected plants is significantly lower than the severity of 10.0 found for P-1380, suggesting a contribution from minor genes.

Table 7: Reaction of BC_2F_3 progenies from selected diploid ($2n=34$) resistant BC_2F_2 plants to artificial infection with population SE296 of *O. cumana*

Pedigree†	Parental BC_2F_2 plants	Plants evaluated	Infected plants	Disease severity ‡
	Number		%	
(P21ms (*gro* x P21,PD))HA89	11	156	55	4.5
(((*gro* x P21,D),SIB)P21)HA89	3	44	64	7.2
((*max* x P21,D)P21)HA89	12	148	41	4
((*max*-33-004 x P21,D)P21^2)HA89	5	62	27	4.7
((*div*-830 x P21,D)P21)HA89	7	67	57	6.5
((*ang*-43-006 x *ann*,D)P21)HA89	2	18	44	2.1
P-1380 (Or_5)		14	100	10

† D, PD=head with complete and partial chromosome doubling, respectively. SIB=sib-pollination
‡ Average number of emerged shoots of *O. cumana* per infected plant

CONCLUSIONS

Transferring genes from perennial *Helianthus* species into sunflower has been made easy with the development of embryo culturing technique, chromosome doubling, and the creation of amphiploids. The broomrape resistance observed in the wild perennial species, amphiploids, and backcross generations is under dominant gene control. The amphiploids served as fertile bridges, overcoming the problem of F_1 interspecific hybrid sterility, and facilitated interspecific gene transfer through conventional breeding. Segregation for broomrape resistance and chromosome number (2n=34 to 51) in backcross progenies based on *H. angustifolius*, *H. cusickii*, *H. divaricatus*, *H. grossesserratus* and *H. maximiliani,* provided suitable material for selection of diploid individuals with resistance to the new *Orobanche* race. The preferential pairing between cultivated sunflower chromosomes in BC_1F_1 for the diploid x cultivated and BC_2F_1 for the tetraploid x cultivated crosses are expected to reduce the recombination between wild and cultivated chromosomes. However, our broomrape resistance gene transfer supports the use of amphiploids as a bridge, and suggests a high chromosome homology between genomes of perennial species and the cultivated sunflower.

Homozygous resistant plants in BC_2F_6 are being used for studying the inheritance of resistance to race F. Intercrosses among homozygous resistant lines derived from different wild species will be used to determine allelic relationships among the

resistance genes. F_2 populations used for inheritance studies also will be sampled for molecular mapping of the *Or* gene(s) conveying resistance to race F of broomrape.

ACKNOWLEDGMENTS

The authors wish to thank L. G. Campbell, G. J. Seiler, and B. A. Vick for thoughtful discussion and review of the manuscript. This research was supported in part by a grant from USDA/FAS/ICD/RSED.

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HIBRIDIZACION INTERSPECIES, TRANSFERENCIA DE GENES Y CREACION DE LA RESISTENCIA A LA RAZA F DE OROBANCA EN ESPANA

RESUMEN

Cuarenta especies silvestres norteamericanas del genero *Helianthus* sufrian largo tiempo las condiciones extremas de inmediaciones y obtuvieron los genes de resistencia o toelrancia a la salinidad, la sequia, los insectos, las enfermedades, asi como los genes para la esterilidad masculina citoplasmatica y la restauracion de la fertilidad, lo que los hace ser los recursos geneticos valiosos para la mejora del cultivo de girasol. La transferencia de genes a partir de las especies silvestres al fondo de cultivo depende del suceso de la hibridizacion interspecies, fertilidad de la generacion F_1, emparejamiento de cromosomas para la recombinacion de genes, eficacia de metodos de investigacion, y numero de descendientes que sirven a la seleccion. El mayor numero de cruces entre las especies silvestres de un año y el girasol cultivado crea con eficacia las semillas F_1 y facilmente se cruza retrogradamente. Para cruces un poco mas dificiles entre las especies de varios años y el girasol cultivado, donde la germinacion de semillas hibridas ocurre raramente, fue desarrollada la tecnica del cultivo de embriones, por la cual los embriones interspecies inmaturos se salvan antes de la aparicion de abortividad. La homologia de cromosomas de las especies silvestres y del girasol cultivado es muy alta, y el emparejamiento meyotico de cromosomas en la generacion F_1 de cruce de diploides silvestres y del girasol cultivado es relativamente bueno, con excepcion de multivalentes, puentes y fragmentos, que se crean como consecuencia de diferencias en la translocalizacion e inversion. La duplicacion de cromosomas por los testes con la colchicina aumenta considerablemente la fertlidad de la generacion F_1 y la eficacia del cruce retrogrado llevando a la creacion de amfiploides. Amfiploides tienen la fertlidad restaurada y pueden mantenerse por la polinizacion en semiconsaguinidad, y sirven como puente para la transferencia de genes. Utilizando este acceso, hemos transferido con exito los genes de resistencia a la nueva raza de orobanca, raza F, que ataca todo el girasol cultivado en España. La investigacion de la herencia indica que un gen predominante controla la resistencia.

HYBRIDATION INTERSPECIES, TRANSFERT GÉNÉTIQUE ET CRÉATION DE RÉSISTANCE ENVERS LA RACE F D'OROBANCHE EN ESPAGNE

RÉSUMÉ

Les 49 espèces nord américaines d'*Helianthus* sauvage ont longtemps survécu à un environnement extrême et possèdent des gènes de résistance ou de tolérance aux conditions salines, à la sécheresse, aux insectes, aux maladies ainsi que des gènes de stérilité mâle cytoplasmique et des gènes de rétablissement de la fertilité ce qui en fait une ressource génétique importante pour l'amélioration du tournesol. Le transfert de gènes d'espèces sauvages vers une base de culture dépend du succès de l'hybridation interspecies, de la fertilité de la génération F_1, de l'accouplement des chromosomes en vue d'une recombinaison génétique, de méthodes d'examen efficaces et du nombre des descendants qui serviront à la sélection. La plupart des croisements entre espèces sauvages vivaces et tournesols de culture produisent des graines F_1 et

peuvent facilement être croisées de nouveau. Une technique de culture à deux temps a été développée pour les croisements plus difficiles entre le tournesol vivace et le tournesol de culture pour lesquels on arrive rarement à la formation de graines hybrides. Cette technique a été établie dans le but de sauver les embryons interspecies qui ne sont pas encore arrivés à maturité avant qu'ils ne soient avortés. L'homologie chromosomique entre les génomes des espèces sauvages et ceux du tournesol cultivé est élevée et l'accouplement méiotique chromosomique du diploïde sauvage et de F_1 cultivé est relativement bon sauf pour ce qui concerne les multivalents, les ponts et les fragments qui apparaissent comme une conséquence des différences dans la translocation et l'inversion. Le doublement chromosomique par un traitement par la colchicine augmente significativement la fertilité de la génération F_1, améliore le croisement de retour et conduit à la production d'amphiploides. Les amphiploïdes ont une fertilité rétablie et peuvent se maintenir par pollinisation collatérale et ils serviront de pont pour le transfert de gènes. Utilisant cette approche, nous avons transféré avec succès des gènes résistants à la nouvelle race d'orobanche F qui attaque tous les tournesols de culture en Espagne. Les études de transmission suggèrent qu'un gène dominant unique contrôle la résistance.