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INHERITANCE OF RESISTANCE TO A HIGHLY VIRULENT RACE F OF *Orobanche cumana* Wallr. IN A SUNFLOWER LINE DERIVED FROM INTERSPECIFIC AMPHIPLOIDS

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SUMMARY

Broomrape (*Orobanche cumana* Wallr.) populations belonging to the new race F in Spain have overcome all known resistance genes, Or_1 to Or_5 , in cultivated sunflower (*Helianthus annuus* L.) and are spreading rapidly. Resistance to race F of this parasitic weed has been found in wild perennial species of *Helianthus* and has been introgressed into cultivated sunflower. The objective of this study was to characterize the inheritance of resistance genes in cultivated sunflower derived from wild perennial species *H. divaricatus* and *H. grosseserratus*, respectively. Crosses between resistant cultivated lines and the susceptible line P21 were made, and the F_1's were resistant when evaluated for broomrape resistance using a highly virulent population of race F, indicating dominance of resistance genes. Comparison of resistance of the segregating populations, F_2 and BC_1F_1, to both parents confirmed the dominance observed in the F_1 and indicated that resistance is under the control of a single dominant gene. This dominance of resistance genes will greatly simplify the breeding for resistance.

Key words: **sunflower, *Helianthus annuus* L., inheritance, broomrape, *Orobanche cumana* Wallr., race F**

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is a parasitic angiosperm, totally devoid of chlorophyll, that infects the roots of sunflower (*Helianthus annuus* L.) plants, drawing water and nutrients from them. This parasitic plant is regarded

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as one of the most important constraints on sunflower production in areas of eastern and southern Europe, the Middle East, Russia, Ukraine and China (Parker, 1994). Broomrape attacks are frequently severe and yield losses have reached up to 50% (Domínguez, 1996a). Though several methods of control have been proposed, herbicide control is only partially effective (García-Torres *et al.,* 1988) and soil treatments by fumigation or solarization are effective but not economically feasible (Foy *et al.,* 1989; Jacobsohn *et al.,* 1987). The most economical and effective means of controlling sunflower broomrape is the use of resistant cultivars.

Genetic resistance to broomrape has been introduced into sunflower cultivars from early sunflower breeding programs in the former USSR (Pustovoit, 1966). However, the widespread use of resistant cultivars has led to the appearance of new races of the parasite that overcome the resistance genes (Škorić, 1988), and a continuous need for new resistance sources. Cultivated sunflower has a narrow genetic background and is deficient in genes for resistance to *O. cumana* and other pathogens and pests. The main sources of resistance to *O. cumana* in sunflower have been identified in wild *Helianthus* species (Pustovoit, 1966; Korell *et al.,* 1996; Ruso *et al.,* 1996; Fernández-Martínez *et al.,* 2000).

The host-parasite system of sunflower-*O. cumana* appears to follow the gene-for-gene model. Vrânceanu *et al.* (1980) established a set of five sunflower differentials carrying the five dominant resistant genes Or_1 to Or_5, each resistant to a new race and also to the previous race. These differential lines along with a universal susceptible host permitted the identification of five physiological races designated A to E. Genetic mechanisms controlling resistance to broomrape proposed more recently by other authors agreed with these results, and monogenic and dominant inheritance of resistance to sunflower broomrape was described (Pogorletsky and Geshele, 1975; Ish-Shalom-Gordon, 1993; Sukno *et al.,* 1999). Domínguez (1996b) identified one line carrying two of these dominant resistance genes.

Orobanche cumana is known to have been present in Spain since 1958. Melero-Vara *et al.* (1997) identified races that overcame Or_1, Or_3, and Or_4 genes, but not Or_2 and Or_5. However, recent studies have shown an evolution of sunflower broomrape races in Spain, with a new race, designated as F, overcoming all the resistance genes identified so far (Alonso *et al.,* 1996; Domínguez, 1999). Resistance to this new race has been found in cultivated and in wild sunflower (Sukno *et al.,* 1999; Domínguez, 1999; Fernández-Martínez *et al.,* 2000). Rodríguez-Ojeda *et al.* (2001) and Akhtouch *et al.* (2001) identified two independent recessive genes controlling resistance to race F in lines derived from cultivated germplasm.

The high number of immune entries resistant to broomrape race F observed in populations of wild perennial sunflower (Fernández-Martínez *et al.,* 2000) prompted the initiation of a program to transfer the resistance genes into cultivated sunflower. As a first result, the four germplasm populations, BR1 to BR4, resistant to race F and derived from the wild perennial sunflower species *H. maximiliani*, *H. grosseserratus*, and *H. divaricatus*, were released (Jan *et al.,* 2001). In order to use efficiently the available sources of broomrape resistance, it is necessary to determine the inheritance of resistance. The objective of this study was to determine the inheritance of resistance to broomrape race F in material derived from BR4.

MATERIALS AND METHODS

Plant materials

The plant materials used for the genetic study were:

1. an inbred line developed from BR4, which is a population resistant to race F of broomrape and derived from the sunflower wild perennial *H. divaricatus* and *H. grosseserratus* (Jan *et al.*, 2001);
2. P21, a cultivar selected from "Peredovik" carrying a recessive gene for male sterility and highly susceptible to broomrape races E and F; and
3. NR5, a cultivated line of sunflower carrying the resistance gene Or_5.

The pedigree of BR4 is as follows: *H. divaricatus*-830/P21,D//*H.grosseserratus*-001/P21,D/3/P21/4/HA89. Chromosomally doubled (D) F_1 heads of *H. divaricatus*-830 x P21 and *H. grosseserratus* x P21 were intercrossed and the resulting mixed-amphiploid maintained by sib-pollination. This mix-amphiploid was first backcrossed with P21, and the resulting triploid BC_1F_1´s backcrossed with HA89 to produce BC_2F_1 (Jan *et al.*, 2001). Evaluations for broomrape race F resistance of BC_2F_1, BC_2F_2, and BC_2F_3 were then performed (Jan *et al.*, 2000). The BR4 material was a BC_2F_4 bulk from 10 resistant BC_2F_3 families grown from selected immune BC_2F_2 plants. For the genetic study, the BC_2F_4 seeds were self-pollinated two times and checked again for resistance to race F of broomrape. All plants were breeding true for resistance. The BC_2F_6 material was designated as J1.

Genetic study

Crosses between resistant plants of J1, artificially inoculated with race F of broomrape, and male-sterile plants of the susceptible line P21 were carried out in the greenhouse in the winter of 2000. Plants of the parental lines J1 and P21, the F_1, and the check line NR5, which carries Or_5, were artificially inoculated with race F of broomrape and grown in spring-2001 at the Institute for Sustainable Agriculture at Córdoba (Southern Spain). These plants were kept outdoors until maturity. F_2 seeds were produced by self-pollinating F_1 plants, and BC_1F_1 seeds by backcrossing to both parental lines. F_2 and BC_1F_1 plants, together with plants of J1, P21, NR5 and F_1 plants, were grown in growth chambers in summer-2001 and tested for broomrape resistance (race F) in artificially infested soil.

For the spring-2001 study, artificial inoculations were performed by planting 2-d-old sunflower seedlings in small plastic pots (7 x 7 x 8 cm) containing a mixture of sand and peat (1:1, v:v) uniformly infested with 50 mg of broomrape (race F) seed. The plants were incubated in a growth chamber for 15-20 days at 28ºC/22ºC (day/night) and 14 h photoperiod. Then the plants were transplanted into larger pots containing 3 l of fertilised and uninfected sand/silt/peat (2:1:2, v:v:v) soil mixture.

For the summer-2001 study, artificial inoculations were performed by planting 2-days-old sunflower seedlings in small porex pots (4 x 4 x 7 cm) with peat uniformly infested with 35 mg of broomrape (race F) seed. The plants were kept in a growth chamber till evaluation (45 days). The incubation was conducted at 28ºC/22ºC (day/night) and 14 h photoperiod.

Evaluation for broomrape resistance was made on mature plants in the spring-2001, and on the 45-day-old plants grown in the growth chamber in the summer-2001. Plants were uprooted and their root system carefully washed to observe any established broomrape nodules or stalks. These were indicative of susceptibility to broomrape, whereas those lacking these structures were considered resistant. Number of tested plants is indicated in Table 1. Resistant to susceptible plant ratios were used to determine genetic relationships. Chi-square tests were used to test for goodness of fit to expected genetic ratios. A single dominant gene model (3:1 for the F_2 and 1:1 for the BC_1 plants) was applied to evaluate the segregation ratio for inheritance of *O. cumana* (race F) resistance.

Table 1: Resistant (R) and susceptible (S) plants of the parental lines P21 and J1, their F_1, and the F_2 and BC_1F_1 generations, segregating for resistance to race F, from their cross. NR5, which carries the Or_5 gene, served as a susceptible check to race F of broomrape

Experiment	Generation or line	Number of plants		Ratio tested	χ^2	P
		R	S			
Spring-2001	J1	15	0			
	P21	0	15			
	P21/J1, F_1	15	0			
	NR5	0	9			
Summer-2001	J1	9	0			
	P21	0	3			
	P21/J1, F_1	10	0			
	P21/J1, F_2	77	36	3:1 (R:S)	2.83	0.10
	P21/J1//P21, BC_1F_1	30	35	1:1 (R:S)	0.38	0.54
	P21/J1//J1, BC_1F_1	32	0			
	NR5	0	10			

RESULTS AND DISCUSSION

The sunflower parental line J1 was resistant to broomrape race F both in spring-2001 and summer-2001 studies, while all plants of the parental line P21 and NR5, which carries the Or_5 gene, were susceptible. This verifies the reliability of the inoculation technique, and confirmed that resistant lines were uniform and consistent for both trials. All the fifteen F_1 plants from the J1 cross to P21 tested in spring-2001, and all ten F_1 plants from the same cross tested in summer-2001 were resistant (Table 1). These results indicated a dominant gene action in the resistance to race F of broomrape in this material, which was suggested previously by Jan *et al.* (2000) in their backcrossing program to introgress resistance to race F of broomrape from wild perennial species of *Helianthus* into cultivated sunflower.

The segregation pattern of resistance to broomrape race F observed in the F_2 generation from the J1 cross to P21 fit a 3:1 (resistant:susceptible) ratio (Table 1). The data of the BC_1F_1 to the susceptible parental line P21 generation also satisfactorily fit a 1:1 (resistant:susceptible) ratio. No susceptible plants were detected in the backcrosses to the resistant parent J1 (Table 1). These data indicated that resistance to *O. cumana* race F in the J1 line is controlled by a single dominant gene. This gene was tentatively designated as Or_6.

These results are similar to those obtained in previous genetic studies with races A to E of broomrape, which evidenced monogenic dominant inheritance for resistance to broomrape (Pogorletskii and Geshele, 1975; Ish-Shalom-Gordon, 1993; Sukno *et al.,* 1999). However, in all the inheritance studies carried out to date with race F, broomrape resistance to this race has been shown to be recessive and controlled by alleles at two loci (Rodríguez-Ojeda *et al.,* 2001; Akhtouch *et al.,* 2001). The resistant germplasm tested in those studies was derived from cultivated sunflower. The results presented in the present work imply an important change in the pattern of resistance to race F recently observed. The dominance reaction of resistance genes will greatly simplify the breeding procedure for resistance to race F, as the incorporation of genes of resistance for the development of commercial resistant hybrids need to be introduced in only one parental line. On the other hand, the monogenic inheritance allows an easy and a succesful transference of the resistance into breeding lines. The different results obtained with resistance to broomrape race F in material derived from cultivated or from wild sunflower indicates that different resistance mechanisms are involved in lines derived from these different sources, and suggests the importance of the wild sunflower species as a source of unique resistant genes.

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HERENCIA DE LA RESISTENCIA A LA RAZA ALTAMENTE AGRESIVA F DE *Orobanche cumana* Wallr. EN LA LINEA DEL GIRASOL DERIVADA DE INTERSPECIES AMFIPLOIDE

RESUMEN

Las poblaciones de orobanca (*Orobanche cumana* Wallr.) que pertenecen a la raza nueva F en Espana no reaccionan a todos los genes de resistencia conocidos, de Or_1 a Or_5, siendo presentes en el girasol cultivado (*Helianthus annuus* L.) y se extienden rapidamente. La resistencia a la raza F de esta mala hierba fue encontrada en las especies silvestres del girasol y transferida en el girasol cultivado. El objetivo de este trabajo era de describir la herencia de los genes de resistencia en el girasol introducidos de las especies de girasol de varios años *H. divaricatus* y *H. grosseseratus*. Las lineas resistentes del girasol cultivado fueron cruzadas con la linea sensible P21, y los descendientes de F_1 eran considerados como resistentes cuando los testes de resistencia a la raza altamente virulente F de orobanca mostraban la presencia de los genes de resistencia predominantes. La comparacion de la resistencia de las poblaciones en separacion, F_2 y BC_1F_1, con la resistencia de los componentes paternales ha confirmado la presencia de predominacion notada en la generacion F_1, indicando al mismo tiempo que esta resistencia es controlada por un gen predominante. Esta predominacion del gen de restistencia simplificara mucho la seleccion con respecto a la resistencia.

TRANSMISSION DE RÉSISTANCE À UNE RACE TRÈS AGRESSIVE F DE l'*Orobanche cumana* Wallr. DANS UNE LIGNE DE TOURNESOL DÉRIVÉE D'AMPHIPLOÏDES INTERSPECIES

RÉSUMÉ

En Espagne, les populations d'orobanche (*Orobanche cumana* Wallr.) appartenant à la nouvelle race F ne réagissent à aucun gène de résistance connu, depuis Or_1 jusqu'à Or_5, présents dans le tournesol cultivé (*Helianthus annuus* L.) et elles se répandent rapidement. La résistance à la race F de cette herbe parasitaire a été découverte dans des espèces sauvages de tournesol et elle a été introduite dans le tournesol cultivé. Le but de cette étude était la description de la transmission des gènes de résistance dans le tournesol cultivé dérivé des espèces vivaces *H. divaricatus* et *H. grosseserratus.* Les lignes résistantes de tournesol cultivé ont été croisées avec la ligne sensible P21 et la progéniture F_1 a été considérée résistante quand les tests de résistance envers la race très agressive d'orobanche F ont montré la présence de gènes de résistance dominants. La comparaison de la résistance des populations en division, F_2 et BC_1F_1 avec celle des parents a confirmé la dominance observée dans F_1 et indiqué que la résistance était sous le contrôle d'un unique gène dominant. Cette dominance de gènes résistants simplifiera grandement la culture de plantes pour leur résistance.