

## Screening of Wild *Cicer* Species for Resistance to Races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*

W. J. KAISER, USDA-ARS, Western Regional Plant Introduction Station, Washington State University, Pullman 99164-6402, and A. R. ALCALÁ-JIMÉNEZ, A. HERVÁS-VARGAS, J. L. TRAPERO-CASAS, and R. M. JIMÉNEZ-DÍAZ, Instituto de Agricultura Sostenible, Consejo Superior de Investigaciones Científicas, Apartado 3048, 14080 Córdoba, Spain

### ABSTRACT

Kaiser, W. J., Alcalá-Jiménez, A. R., Hervás-Vargas, A., Trapero-Casas, J. L., and Jiménez-Díaz, R. M. 1994. Screening of wild *Cicer* species for resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris*. Plant Dis. 78:962-967.

Fifty-two accessions representing 11 wild *Cicer* spp. were screened for resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* from Andalucía, southern Spain, in pot-culture inoculations in growth chambers, and 47 accessions were tested for resistance to race 5 in inoculated field microplots in Córdoba, Spain. Resistance to *F. o. ciceris* race 5 was identified in accessions of *Cicer bijugum*, *C. cuneatum*, and *C. judaicum*, and resistance to race 0 was found in accessions of *C. bijugum*, *C. canariense*, *C. chorassanicum*, *C. cuneatum*, *C. judaicum*, and *C. pinnatifidum*. Isolations from asymptomatic plants of inoculated *Cicer* spp. indicated that the resistant reaction to races 0 and 5 occurred with or without systemic colonization of aerial tissues by the pathogen. No transmission of *F. o. ciceris* races 0 and 5 was detected in seed harvested from plants of eight *Cicer* spp. used in the artificial inoculation or microplot studies.

Fusarium wilt induced by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato is a major

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Accepted for publication 2 May 1994.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1994.

constraint to chickpea (*Cicer arietinum* L.) production in many countries (7). Yield losses up to 10% have been attributed to Fusarium wilt in India (24) and Spain (27) and up to 40% in Tunisia (2).

Seven races designated 0-6 of *F. o. ciceris* have been identified. Haware and Nene (9) first identified races 1-4 in India. Later, three additional races, namely, 0, 5, and 6, were identified in southern Spain (15). Race 0 occurs in California, Spain, and Tunisia; races 1 and 6 have been identified in California, Morocco, and Spain; and race 5 has been found only in California and Spain (12;

R. M. Jiménez-Díaz, unpublished). Race 0, the least virulent of the seven races, is not pathogenic to desi cultivar JG 62, which is susceptible to all other known races of the pathogen (9,12). Race 0 induces a progressive foliar yellowing, as compared to severe leaf chlorosis, flaccidity, and early wilt induced by races 1-6 (15,27). Race 5 is the most virulent of those occurring in California and Spain. It is pathogenic to desi cultivars JG 74 and CPS 1, which are resistant to race 1 (9,15). Fusarium wilt is a serious disease of kabuli chickpeas in both north-eastern and southern Spain (Andalucía). Race 0 is widely distributed in Andalucía and is the predominant race in north-eastern Spain, while race 1 occurs in both regions and races 5 and 6 are restricted in their distribution in southern Spain (12,15; R. M. Jiménez-Díaz, unpublished).

The most effective and economical method of controlling Fusarium wilt of chickpea worldwide is by use of resistant cultivars, the effectiveness of which is threatened by the occurrence of different pathogenic races. Chickpea cultivars with resistance to Fusarium wilt have been identified by researchers in different countries (3,14,18,20). However, this resistance is usually to one or a few races of the pathogen. At the Western

Regional Plant Introduction Station in Pullman, Washington, the *Cicer* germ plasm collection contains 3,885 plant introduction (PI) accessions. In this collection, there are 12 wild annual and perennial *Cicer* spp.

Sources of resistance to several important biotic and abiotic stresses have been found in different wild *Cicer* spp., giving them real potential for improving cultivars (4,8,23,25,26). Little is known concerning the resistance of wild *Cicer* spp. to races of *F. o. ciceris*. Wild *Cicer* spp. may prove to be sources for resistance to different races of *F. o. ciceris* that can be incorporated into wilt-susceptible, commercially desirable chickpea cultivars by conventional and new breeding techniques. The wild relatives are used in resistance breeding programs in several cultivated crops (17). The objective of this study was to determine the resistance of different wild annual and perennial *Cicer* spp. from the Pullman germ plasm collection to races 0 and 5 of *F. o. ciceris* that occur in Andalucía, southern Spain. The inoculation studies were conducted in Córdoba, Spain (13,16) in 1991 and 1992. Preliminary reports have been published (13,16).

## MATERIALS AND METHODS

**Cicer species.** Accessions of 11 wild annual and perennial *Cicer* spp. from the Pullman germ plasm collection were included in the growth chamber and microplot screening studies. The annual species were *C. bijugum* K.H. Rech., *C. chorassanicum* (Bge) M. Popov, *C. cuneatum* Hochst. ex Rich., *C. echinospermum* P.H. Davis, *C. judaicum* Boiss, *C. pinnatifidum* Juab. & Spach, *C. reticulatum* Ladiz., and *C. yamashitae* Kitamura. The perennial species were *C. anatolicum* Alef., *C. canariense* Santos Guerra & G.P. Lewis, and *C. oxyodon* Boiss & Hoh.

**Germination of seeds.** To break dormancy and promote germination, seeds of all wild *Cicer* spp. were scarified, placed in small gauze bags, and aerated for various periods in 1,000-ml flasks containing 400 ml of tap water. The tap water was changed daily. Germinated seeds were transferred to moist paper towels in small plastic moist chambers and incubated at 5–8 C until used in an inoculation experiment or transplanted to sterile soil in plastic pots and incubated in the greenhouse.

**Screening in the growth chamber.** Fifty-three accessions were screened for resistance to races 0 and 5 of *F. o. ciceris* in pot-culture inoculations (22,27) in growth chambers at Córdoba, Spain, in 1991 and 1992.

*F. o. ciceris* isolates 7802 (race 0) and 8012 (race 5) were used. These isolates were maintained in sterile soil at 4 C. Isolates of each race were transferred separately to potato-dextrose agar (PDA)

and incubated at 25 C with a 12-hr photoperiod of fluorescent and near ultraviolet light at  $36 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Inoculum was then increased in a cornmeal-sand (CMS) mixture (22) and incubated under the same conditions as the stock cultures for 2 wk. Infested CMS was mixed thoroughly (1:12, w/w) with an autoclaved soil mixture (clay loam/sand/peat, 1:1:1, v/v). Inoculum concentration in the infested soil mixture was determined by dilution plating on V8 juice-oxgall-PCNB agar (VOPA) *Fusarium*-selective medium (1) prior to sowing. Triplicate soil plates were made for each race. The plates were incubated under the same conditions as stated above for 7 days. The inoculum concentration averaged  $1.2 \times 10^5$  and  $4.9 \times 10^5$  cfu/gm of soil for races 0 and 5, respectively, in 1991 and  $3.4 \times 10^5$  and  $1.1 \times 10^6$  cfu/gm of soil for races 0 and 5, respectively, in 1992. Wilt severity in chickpea lines susceptible (P 2245) and resistant (JG 62 and 12-071/10054) to *F. o. ciceris* races 0 or 5 was not affected by inoculum concentrations of  $10^5$  to  $10^6$  cfu/gm of soil used in the 1991 and 1992 inoculation experiments (*unpublished*).

Germinated seeds were sown in 15-cm-diameter clay pots filled with the infested soil mixture. Control plants were grown in a comparable mixture of noninfested CMS and autoclaved soil. Plants were grown in a growth chamber at  $25 \pm 2$  C/ $23 \pm 2$  C (day/night) and 60–90% relative humidity, with a 14-hr photoperiod of fluorescent light at about  $252 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for 53–54 days (1991) and 63 days (1992). Surviving plants were then maintained for about 20 days more in the greenhouse. Plants were fertilized weekly with 100 ml of Hoagland's nutrient solution (10).

Plants were observed at 2-day intervals for symptom development, and disease reactions were assessed 20, 40, and 60 days after inoculation. Disease reactions were assessed on a 0–4 scale according to the percentage of foliage with yellowing or necrosis in an acropetal progression (0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100%, and 4 = dead plant). Scores <1 and >3 were considered resistant and susceptible reactions, respectively, and scores in between were considered moderately susceptible reactions. The disease rating scale was used to assess virulence of isolates of *F. o. ciceris* and to differentiate races of the pathogen (15,27). Isolations were made on VOPA from stems (1–3 cm above the crown) of symptomless and diseased plants at the termination of the experiment. Isolations were not made from roots. Experiments were carried out following a randomized complete block design with three or four replicated pots (four plants per pot) for each race-entry combination.

Three accessions of cultivated chickpea (*C. arietinum*) were included in all

growth chamber inoculation studies as controls. Accession P 2245 is susceptible to race 0 and 5, JG 62 is resistant to race 0 but susceptible to race 5, and 12-071/10054 is susceptible to race 0 but resistant to race 5 (15).

**Screening in field microplots.** Forty-seven accessions of 11 wild *Cicer* spp. included in the growth chamber tests were also screened for resistance to race 5 of *F. o. ciceris* under natural conditions in field microplots infested with the pathogen. The microplots ( $1.25 \times 1.25$  m<sup>2</sup>, 50 cm deep) were established in a fumigated sandy loam soil at the Alameda del Obispo Research Station in Córdoba in October 1986. Microplots were artificially infested with a CMS inoculum of a *F. o. ciceris* isolate of either race 0 or 5, and they have been used for epidemiological studies or resistance screening since that time. Because of limited availability of seed, screening was performed in race 5-infested microplots only.

Seeds were germinated as for the growth chamber screening studies and planted in sterile soil in 10-cm-diameter pots in the greenhouse. After 20–30 days, plants were transplanted in the microplots in rows 0.4 m apart. Each microplot contained two test entries and a central row of cultivar JG 62, which is highly susceptible to race 5, as a check. Entries were arranged in a randomized complete block design with two replicated rows of five to 25 plants, depending on seed availability.

Disease reactions were assessed by recording the incidence of dead plants. Observations on wilt development were made at 7- to 10-day intervals, and the final incidence of dead plants (total cumulative number of dead plants/total plant number at stand count) was determined 140 days after sowing. Disease reaction of an accession was classified according to the percentage of dead plants: resistant (0–20%), moderately resistant (21–50%), and susceptible (51–100%) (9,14,22). The highest disease rating in any replication was used to categorize the entry.

**Seed transmission studies.** Seeds were collected from surviving plants in the 1991 growth chamber and microplot experiments. Seeds were surface-disinfested in 0.25% NaOCl for 5 min, then plated on PDA containing 100 ppm each of penicillin G and streptomycin sulfate in 10-cm-diameter glass petri dishes. Dishes were incubated at 22–25 C with a 12-hr photoperiod of fluorescent lights at  $77 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Fungi growing from seeds were identified by colony characteristics and by sporulation.

## RESULTS

**Screening in the growth chamber.** In the first growth chamber test in 1991, accessions of *Cicer* spp. (Table 1) differed in their reactions to races 0 and 5 of

*F. o. ciceris* (Fig. 1). Tested accessions of *C. bijugum*, *C. cuneatum*, and *C. judaicum* were resistant to both races, while *C. canariense* and *C. chorassanicum* were resistant to race 0 but moderately susceptible or susceptible to race 5, respectively. The accession of *C. pinnatifidum* was resistant to race 0 and resistant to moderately susceptible to race 5. In most *Cicer* spp. with a resistant reaction to race 0 or 5, *F. o. ciceris* was not isolated from aboveground stem pieces at the termination of the experiment. However, race 0 was isolated from one of 10 resistant plants of *C. canariense* and five of 10 resistant plants of *C. chorassanicum*. Race 5 was isolated from two symptomatic plants of *C. pinnatifidum* but not from eight asymptomatic plants of this species.

In the second growth chamber experiment in 1991, nine PI accessions of *C. pinnatifidum* from Turkey were inoculated with *F. o. ciceris* races 0 and 5 (Table 2). All accessions were susceptible to race 5 but three were resistant to race 0.

In 1992, the disease reaction of six wild annual *Cicer* spp. to races 0 and 5 varied among species and among accessions within species (Table 3). Furthermore, while disease reaction was homogeneous for most accessions, some had a heterogeneous reaction to one of the races (e.g., *C. judaicum*, PI 510658), indicating that segregation was occurring. All entries of *C. echinospermum* and *C. reticulatum* tested were susceptible to races 0 and 5. The four entries of *C.*

*pinnatifidum* tested were susceptible to race 5, but two (W6-2007 and W6-10159) were resistant to race 0. Entries W6-10160 and W6-10161 were moderately susceptible to race 0, but some plants remained free of symptoms. All accessions of *C. bijugum* and *C. judaicum* were resistant to race 0, but three of six entries of *C. bijugum* and five of 10 entries of *C. judaicum* also were resistant to race 5. *C. bijugum* W6-10146 had a resistant reaction to race 5 but some plants were moderately susceptible, and *C. bijugum* W6-10147 was moderately susceptible to race 5 but some plants were resistant. Similarly, *C. judaicum* PI 510658 was susceptible to race 5 but some plants were free of disease symptoms.

Race 0 induced the foliar yellowing syndrome characteristic of this race (15,27) in all susceptible accessions except those of *C. reticulatum*, which showed a wilting reaction similar to that induced by race 5 (15,27). This might relate to the high susceptibility of this species. Race 5 induced the wilting syndrome in all susceptible entries except *C. bijugum* and *C. judaicum*, which exhibited the yellowing syndrome.

Isolations from asymptomatic plants indicated that the resistant reaction to race 0 was associated with the absence of vascular infection by the pathogen for all entries of *C. judaicum*, *C. bijugum* W6-10145 and W6-10147, and *C. pinnatifidum* W6-10159. In contrast, race 0 systemically colonized a low percentage of resistant plants of *C. bijugum*

W6-2021 and W6-10146 and a high percentage (40–100%) of resistant plants of *C. bijugum* W6-2016 and W6-10149 and *C. pinnatifidum* W6-2007. Similarly, race 5 systemically infected a moderate percentage (25–30%) of resistant plants of *C. bijugum* W6-2021, W6-10145, and W6-10146, while resistant entries of *C. judaicum* were either not infected or infected at a low percentage (PI 458558 and PI 568217).

**Screening in microplots.** In 1991, all accessions of *C. bijugum*, *C. cuneatum*, and *C. judaicum* were resistant to race 5 under natural conditions in field microplots infested with the pathogen (Table 1) and in the growth chamber tests. Some *Cicer* spp. susceptible to race 5 in the growth chamber were resistant in the microplots, including *C. anatolicum*, *C. canariense*, *C. oxyodon* PI 561084 and PI 561103, and *C. pinnatifidum*.

In 1992, the disease reaction of most *Cicer* accessions in field microplots was coincident with that of artificial inoculations in the growth chamber, except for *C. bijugum* W6-2016 and *C. judaicum* PI 510658, which had resistant reactions in the microplots. Three accessions of *C. bijugum* that were not tested in the growth chamber inoculation studies had a resistant (W6-10 and W6-10150) or a moderately resistant (W6-2014) reaction in the microplots.

**Seed transmission studies.** Seed of five and three *Cicer* spp. from the 1991 growth chamber inoculation studies were tested for infection by *F. o. ciceris* races 0 and 5, respectively. *F. o. ciceris* race 0 was not isolated from seeds of the following (number of seeds tested follows species and PI number): *C. bijugum* PI 458550, 9; *C. bijugum* PI 458552, 17; *C. chorassanicum* PI 458553, 7; *C. cuneatum* PI 458554, 31; *C. judaicum* PI 458559, 50; and *C. pinnatifidum* PI 458555, 41. *F. o. ciceris* race 5 was not isolated from *C. bijugum* PI 458550, 5; *C. bijugum* PI 458552, 7; *C. cuneatum* PI 458554, 46; and *C. judaicum* PI 458559, 27.

Seed of eight *Cicer* spp. from the 1991 microplot experiment were tested for infection by *F. o. ciceris* race 5. The fungus was not isolated from seeds of the following (number of seeds tested follows species and PI number): *C. anatolicum* PI 383626, 17; *C. bijugum* PI 458550, 20; *C. bijugum* PI 458552, 27; *C. chorassanicum* PI 458553, 20; *C. cuneatum* PI 458554, 32; *C. echinospermum* PI 489776, 3; *C. echinospermum* PI 527930, 19; *C. judaicum* PI 458559, 150; *C. pinnatifidum* PI 458555, 150; *C. pinnatifidum* PI 458557, 110; *C. yamashitae* PI 504550, 150; and *C. yamashitae* PI 510664, 150.

## DISCUSSION

Harlan and de Wet (6) formulated a systematic means of categorizing wild species according to their crossability

**Table 1.** Reaction of representatives of 12 *Cicer* species to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* in growth chamber and field inoculation tests during 1991

<i>Cicer</i> species	Accession	Origin	Growth chamber <sup>a</sup>		Microplot <sup>b</sup>
			Race 0	Race 5	Race 5
<i>anatolicum</i>	PI 383626	Turkey	S	S	R
<i>bijugum</i>	PI 458550	Turkey	R	R	R
	PI 458552	Turkey	R	R	R
<i>canariense</i>	PI 557453	Spain	R	M/S	R
<i>chorassanicum</i>	PI 458553	Afghanistan	R	S	S
<i>cuneatum</i>	PI 458554	Ethiopia	R	R	R
<i>echinospermum</i>	PI 489776	Turkey	S	S	S
	PI 527930	Turkey	S	S	S
<i>judaicum</i>	PI 458559	Lebanon	R	R	R
	PI 561084	Turkey	S	S	R
<i>oxyodon</i>	PI 561103	Turkey	S	S	R
<i>pinnatifidum</i>	PI 458555	Turkey	R	R/M	R
<i>reticulatum</i>	PI 489777	Turkey	S/R	S	S
	PI 489778	Turkey	S	S	S
<i>yamashitae</i>	PI 510664	Afghanistan	S	S	M
<i>arietinum</i> <sup>c</sup>	12-071/10054	Iran	S	R	NT
	JG 62	India	R	S	S
	P 2245	Spain	S	S	NT

<sup>a</sup>Plants were inoculated by the pot-culture method (22). Results are the mean of four seedlings per pot and two (control) or three (races 0 and 5) pots per *Cicer* accession. Disease reaction was assessed on a scale of 0–4 according to the percentage of foliage with yellowing or necrosis in an acropetal progression (0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100%, and 4 = dead plant) 54 days after inoculation. Scores of <1 = resistant (R), 1–3 = moderately susceptible (M), and >3 = susceptible (S).

<sup>b</sup>Microplots were artificially infested with race 5. Results are the mean of two replications with four to 25 plants each. R = resistant (0–20% plants killed), M = moderately susceptible (21–50% plants killed), and S = susceptible (51–100% plants killed). NT = not tested.

<sup>c</sup>The three accessions of *C. arietinum* were included as controls to identify *F. o. ciceris* races 0 and 5 (15).

with cultivated species (cultigen). Species of *Cicer* have been assigned to three crossability groups by Ladizinsky and Adler (19). In our study, we used *Cicer* spp. in the primary and tertiary gene pools (21). *C. echinospermum* and *C. reticulatum* have been placed in the primary gene pool along with the cultigen, *C. arietinum*. Crosses between wild *Cicer* spp. in the primary gene pool with *C. arietinum* are fully (*C. reticulatum*) or partially (*C. echinospermum*) fertile. In our study, no resistance was found to *F. o. ciceris* races 0 and 5 in *C. echinospermum* or *C. reticulatum*, which are in the primary gene pool. *Cicer* spp. in the tertiary gene pool are related to *C. arietinum*, but hybridization with

the cultigen has not been possible or the hybrids have been completely sterile (21). Resistance was found in the tertiary gene pool where gene transfer with the cultigen has not been possible by conventional breeding methods. Additional breeding and genetic engineering research will be required to transfer resistance to Fusarium wilt and other biotic and abiotic stresses from *Cicer* spp. in the tertiary gene pool to commercially desirable chickpea cultivars.

Reaction of *Cicer* spp. varied with the virulence of the race and among accessions within species. Resistance to the highly virulent race 5 was found only in three wild annual *Cicer* spp.: *C. bijugum*, *C. cuneatum*, and *C. judaicum*. All eight

and 11 accessions of *C. bijugum* and *C. judaicum*, respectively, were resistant to race 0, but only five and six, respectively, were resistant to race 5. The one line of *C. cuneatum* tested was resistant to both races. Of the three perennial species included in these inoculation studies, only *C. canariense* exhibited resistance to *F. o. ciceris*, and this was to race 0. Field and greenhouse inoculation studies in India with unspecified races of *F. o. ciceris* have identified lines of *C. bijugum* (8,23), *C. cuneatum* (23), *C. judaicum* (8,22,23), and *C. pinnatifidum* (8,23) with resistance to the pathogen. Some of the *Cicer* germ plasm resistant to *F. o. ciceris* in India was also resistant to races 0 and 5 in our study, e.g., *C. judaicum* PI 458559 and *C. cuneatum* PI 458554. Similarly, inoculations by means of the water-culture method (22) with a *F. o. ciceris* isolate from Italy have identified resistant lines of *C. bijugum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, and *C. reticulatum* from a collection maintained by the International Center for Research in the Dry Areas (ICARDA) in Aleppo, Syria (11). Isolates of *F. o. ciceris* from Italy have been identified as race 0, although the published disease reactions of differential lines correspond to race 1 (5).

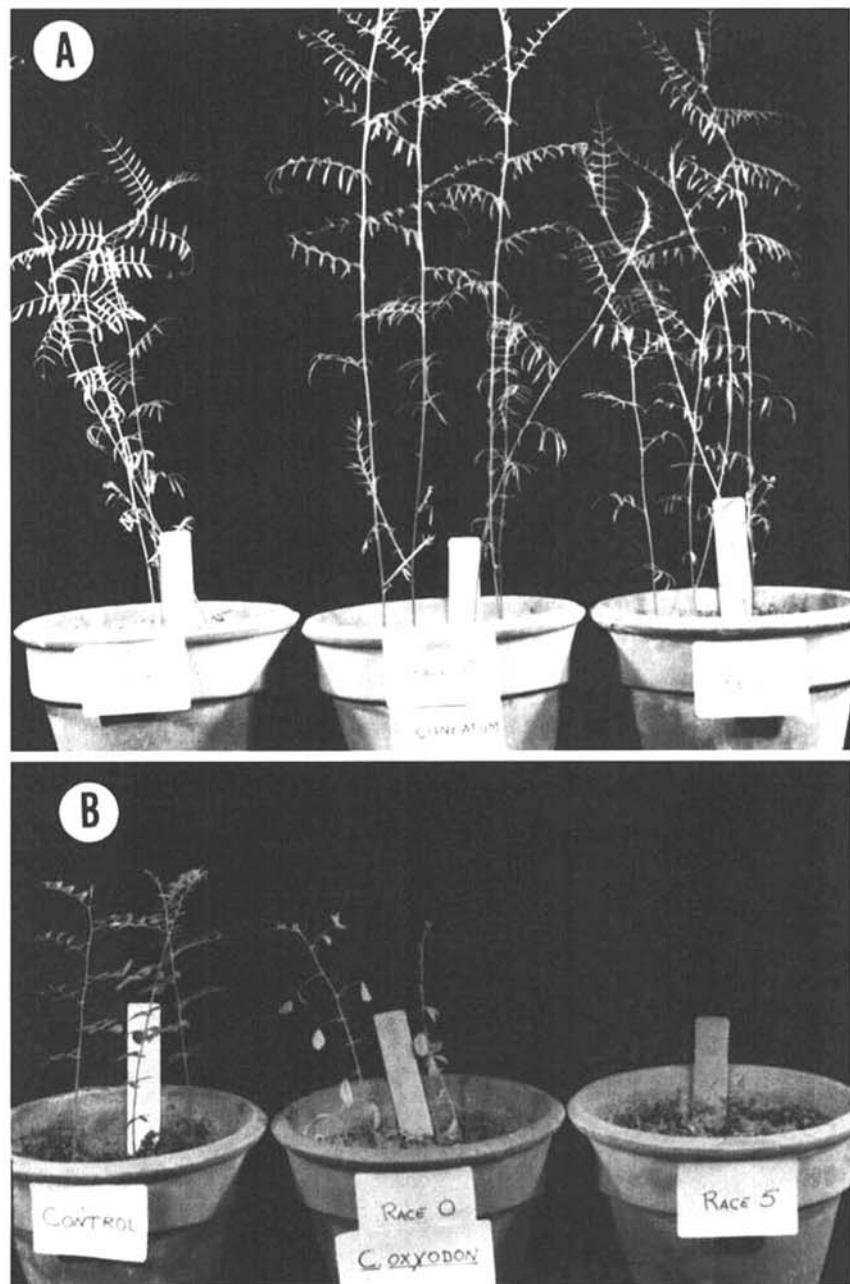
The disease reaction of most accessions to *F. o. ciceris* race 5 in the growth chamber and microplot studies in 1991 and 1992 did not differ appreciably. However, a few accessions of *C. anatolicum*, *C. bijugum*, *C. canariense*, *C. judaicum*, and *C. oxyodon* susceptible

**Table 2.** Reaction of nine accessions of *Cicer pinnatifidum* from Turkey to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* in growth chamber inoculation tests during 1991<sup>a</sup>

<i>Cicer</i> species	Accession	Race 0	Race 5
<i>pinnatifidum</i>	PI 458555	R	S
	PI 458556	R	S
	PI 458557	M/R	S
	PI 510654	R	S
	PI 510663	S	S
	PI 518860	R/M	S
	PI 518861	M/R	S
	PI 518862	S	S
	PI 518863	S	S
<i>arietinum</i> <sup>b</sup>	12-071/10054	S	R
	JG 62	R	S
	P 2245	S	S

<sup>a</sup>Plants were inoculated by the pot-culture method (22). Results are the mean of four seedlings per pot and two (control) or three (races 0 and 5) pots per *Cicer* accession. Disease reaction was assessed on a scale of 0-4 according to the percentage of foliage with yellowing or necrosis in an acropetal progression (0=0%, 1=1-33%, 2=34-66%, 3=67-100%, and 4=dead plant) 56 days after inoculation. Scores of <1 = resistant (R), 1-3 = moderately susceptible (M), and >3 = susceptible (S).

<sup>b</sup>The three accessions of *C. arietinum* were included as controls to identify *F. o. ciceris* races 0 and 5 (15).



**Fig. 1.** Two *Cicer* species 27 days after inoculation with race 0 (middle) and race 5 (right) of *Fusarium oxysporum* f. sp. *ciceris* (healthy controls on left): (A) *C. cuneatum* PI 458554 is resistant to both races and (B) *C. oxyodon* PI 561084 is susceptible to both races.

to race 5 in the growth chamber experiments were resistant to race 5 in the microplot tests. There are several possible explanations for these contradictory results. Various factors, including fungal inoculum levels and environmental conditions, are difficult to monitor and control under field conditions. Plants may have escaped infection if the inoculum density of *F. o. ciceris* race 5 was at a level lower than the threshold required for infection or if there was an uneven distribution of inoculum in the plots. The soil temperatures and/or moisture levels of the soil in the microplots may have been sub-optimal for infection to occur. In the growth chamber tests, some accessions had a heterogeneous disease reaction indicating that segregation for wilt resistance was occurring. A similar phenom-

enon may have occurred in the field plots. Additionally, it is not known what effect the ball of soil surrounding roots at the time of transplanting had on preventing or delaying root infection by *F. o. ciceris* race 5.

All of the wild *Cicer* spp. included in the growth chamber and field inoculation tests in Córdoba, Spain, were also screened for resistance to Ascochyta blight in Pullman, Washington. Ascochyta blight is an important disease of chickpea in many countries (26), including Spain and the United States. *C. bijugum* PI 458550 and PI 458552 and *C. judaicum* PI 458559 were resistant to *F. o. ciceris* races 0 and 5 and Ascochyta blight, while *C. pinnatifidum* PI 458555, PI 458556, PI 510654, and PI 518860 were resistant to *F. o. ciceris* race 0 and Ascochyta blight.

**Table 3.** Reaction of representatives of six *Cicer* species to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* in growth chamber and field inoculation tests during 1992

<i>Cicer</i> species	Accession	Origin	Growth chamber <sup>a</sup>		Microplot <sup>b</sup>
			Race 0	Race 5	Race 5
<i>bijugum</i>	W6-2014	Turkey	NT	NT	M
	W6-2016	Turkey	R	S	R
	W6-2021	Turkey	R	R	R
	W6-10145	Turkey	R	R	R
	W6-10146	Turkey	R	R(M) <sup>c</sup>	R
	W6-10147	Turkey	R	M(R)	M
	W6-10	Turkey	NT	NT	R
	W6-10149	Iraq	R	M	M
	W6-10150	Turkey	NT	NT	R
	<i>echinospermum</i>	W6-1979	Turkey	S	S
W6-1984		Turkey	S	S	S
W6-10153		Turkey	S	S	S
W6-10154		Turkey	S	S	S
PI 527932		Turkey	S	S	S
<i>judaicum</i>		W6-10156	Syria	R	S
	W6-10157	Syria	R	S	M
	W6-10158	Syria	R	S	S
	W6-4271	Syria	R	R	R
	W6-4272	Syria	R	R	R
	PI 458558	Lebanon	R	R	R
	PI 510658	Lebanon	R	S(R)	R
	PI 510661	Israel	R	R	R
	PI 510662	Israel	R	R	R
	PI 568217	Unknown	R	R	R
<i>pinnatifidum</i>	W6-2007	Turkey	R	S	S
	W6-10159	Turkey	R	S	S
	W6-10160	Syria	M(R)	S	S
	W6-10161	Syria	M(R)	S	S
<i>reticulatum</i>	PI 572537	Turkey	S	S	S
	W6-10163	Turkey	S	S	S
	PI 510655	Turkey	S	S	S
	PI 510656	Turkey	S	S	S
<i>arietinum</i> <sup>d</sup>	12-071/10054	Iran	S	R	NT
	JG 62	India	R	S	S
	P 2245	Spain	S	S	S

<sup>a</sup>Plants were inoculated by the pot-culture method (22). Results are the mean of four seedlings per pot and two (control) or four (races 0 and 5) pots per *Cicer* accession. Disease reaction was assessed on a 0-4 scale according to the percentage of foliage with yellowing or necrosis in an acropetal progression (0 = 0%, 1 = 1-33%, 2 = 34-66%, 3 = 67-100%, and 4 = dead plant) 83 days after inoculation. Scores of <1 = resistant (R), 1-3 = moderately susceptible (M), and >3 = susceptible (S). NT = not tested.

<sup>b</sup>Highest disease reaction in two replicated rows in microplots artificially infested with a race 5. R = resistant (0-20% plants killed), M = moderately resistant (21-50% plants killed), S = susceptible (51-100% plants killed). NT = not tested.

<sup>c</sup>Reaction in parentheses indicates that some plants differed from the overall reaction of the entry.

<sup>d</sup>The three accessions of *C. arietinum* were included as controls to identify *F. o. ciceris* races 0 and 5 (15).

#### ACKNOWLEDGMENTS

Research was supported by CEE ECLAIR program contract AGRE 0051 and grant AGF92-0910-C02-01 from the Comisión Interministerial de Ciencia y Tecnología, Spanish Ministry of Education and Science. The first author's sabbatical leave in 1991 at the Universidad de Córdoba in Córdoba, Spain, was supported by the Programa Sabático (SAB89-0029) of the Spanish Ministry of Education and Science.

#### LITERATURE CITED

- Bouhot, D., and Rouxel, F. 1971. Technique sélective et quantitative d'analyse des *Fusarium oxysporum* et *Fusarium solani* dans le sol. Mode d'emploi. Ann. Phytopathol. 3:251-254.
- Bouslama, M. 1980. Chickpea improvement in Tunisia. Pages 277-280 in: Proc. Int. Workshop Chickpea Improv. ICRISAT, Hyderabad, India.
- Buddenhagen, I. W., Workneh, F., and Bosque-Pérez, N. A. 1988. Chickpea improvement and chickpea diseases in California. Int. Chickpea Newsl. 19:9-10.
- Di Vito, M., Greco, N., Singh, K. B., and Saxena, M. C. 1988. Response of chickpea germplasm lines to *Heterodera ciceri* attack. Nematol. Mediterr. 16:17-18.
- Frisullo, S., Ciccarese, F., Amenduni, M., and Zamani, H. R. 1989. Wilt of chickpea (*Cicer arietinum* L.) by *Fusarium oxysporum* f. sp. *ciceri* Sclerot. in southern Italy. (In Italian.) Dif. Pianta 12:181-185.
- Harlan, J. R., and de Wet, J. M. J. 1971. Toward a rational classification of cultivated plants. Taxon 20:509-517.
- Haware, M. P. 1990. Fusarium wilt and other important diseases of chickpea in the Mediterranean area. Options Mediterr. Ser. Semin. 9:163-166.
- Haware, M. P., Narayana Rao, J., and Pundir, R. P. S. 1992. Evaluation of wild *Cicer* species for resistance to four chickpea diseases. Int. Chickpea Newsl. 27:16-18.
- Haware, M. P., and Nene, Y. L. 1982. Races of *Fusarium oxysporum* f. sp. *ciceri*. Plant Dis. 66:809-810.
- Hoagland, D. R., and Arnon, D. I. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347.
- Infantino, A., Porta-Puglia, A., and Singh, K. B. 1992. Screening wild *Cicer* species for resistance to Fusarium wilt. Pages 31-32 in: Proc. Eur. Conf. Grain Legumes Ist.
- Jiménez-Díaz, R. M., Alcalá-Jiménez, A., Hervás, A., and Trapero-Casas, J. L. 1993. Pathogenic variability and host resistance in the *Fusarium oxysporum* f. sp. *ciceris*/*Cicer arietinum* pathosystem. Proc. Eur. Sem. 3rd: *Fusarium*—Mycotoxins, Taxonomy, Pathogenicity and Host Resistance. Hodowla Rósl. Aklim. Nasienn. (spec. ed.) 37(1-4):87-94.
- Jiménez-Díaz, R. M., Kaiser, W. J., Singh, K. B., Trapero-Casas, A., and Trapero-Casas, J. L. 1992. Resistance to Fusarium wilt in *Cicer* spp. Pages 331-332 in: Proc. Eur. Conf. Grain Legumes Ist.
- Jiménez-Díaz, R. M., Singh, K. B., Trapero-Casas, A., and Trapero-Casas, J. L. 1991. Resistance in kabuli chickpeas to Fusarium wilt. Plant Dis. 75:914-918.
- Jiménez-Díaz, R. M., Trapero-Casas, A., and Cabrera de la Colina, J. 1989. Races of *Fusarium oxysporum* f. sp. *ciceri* infecting chickpeas in southern Spain. Pages 515-520 in: Vascular Wilt Diseases of Plants. Vol. H28. E. C. Tjamos and C. H. Beckman, eds. Springer-Verlag, Berlin.
- Kaiser, W. J., Alcalá-Jiménez, A. R., Hervás-Vargas, A., Trapero-Casas, J. L., and Jiménez-Díaz, R. M. 1993. Resistance to races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* in wild *Cicer* spp. Page 33 in: Program Int. *Fusarium* Workshop 7th.
- Knott, D. R., and Dvorák, J. 1976. Alien germplasm as a source of resistance to disease. Annu. Rev. Phytopathol. 14:211-235.
- Kumar, J., Haware, M. P., and Smithson, J. B. 1985. Registration of four short duration



- Fusarium wilt-resistant kabuli (garbanzo) chickpea germplasm. *Crop Sci.* 25:576-577.
19. Ladizinsky, G., and Adler, A. 1976. Genetic relationships among annual species of *Cicer* L. *Theor. Appl. Genet.* 48:197-203.
  20. Morales, G. J. A. 1986. Chickpea breeding program in Sonora, Mexico. *Int. Chickpea Newsl.* 15:11-12.
  21. Muehlbauer, F. J., Kaiser, W. J., and Simon, C. J. 1994. Potential for wild species in cool season food legume breeding. Pages 531-539 in: *Expanding the Production and Use of Cool Season Food Legumes*. F. J. Muehlbauer and W. J. Kaiser, eds. Kluwer Academic, Dordrecht, Netherlands.
  22. Nene, Y. L., and Haware, M. P. 1980. Screening chickpea for resistance to wilt. *Plant Dis.* 64:379-380.
  23. Reddy, M. V., Raju, T. U., and Pundir, R. P. S. 1990. Evaluation of wild *Cicer* accessions for resistance to Fusarium wilt and rootrots. (Abstr.) *Indian Phytopathol.* 43:243-244.
  24. Singh, K. B., and Dahiya, B. S. 1973. Breeding for wilt resistance in chickpea. Pages 13-14 in: *Symposium on Wilt Problem and Breeding for Wilt Resistance in Bengal Gram*.
  25. Singh, K. B., Malhotra, R. S., and Saxena, M. C. 1990. Sources for tolerance to cold in *Cicer* species. *Crop Sci.* 30:1136-1138.
  26. Singh, K. B., and Reddy, M. V. 1993. Sources of resistance to ascochyta blight in wild *Cicer* species. *Neth. J. Plant Pathol.* 99:163-167.
  27. Trapero-Casas, A., and Jiménez-Díaz, R. M. 1985. Fungal wilt and root rot diseases of chickpea in southern Spain. *Phytopathology* 75:1146-1151.