Screening Wild *Cicer* Species for Resistance to Fusarium Wilt

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ABSTRACT

Infantino, A., Porta-Puglia, A., and Singh, K. B. 1996. Screening wild *Cicer* species for resistance to Fusarium wilt. Plant Dis. 80:42-44.

Wilt, caused by *Fusarium oxysporum* f. sp. *ciceri*, is the most widespread soilborne disease of chickpea (*Cicer arietinum* L.). In an attempt to identify new sources of resistance to wilt, 102 accessions of six wild annual *Cicer* species were evaluated in the greenhouse. The isolate from central Italy used in this experiment has been characterized by using a set of chickpea differential lines. Highly resistant reaction to wilt was shown by all accessions of *C. bijugum* and some of *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, and *C. reticulatum*. Both accessions of *C. yamashitae* were susceptible. This evaluation has helped to identify new and diverse sources of resistance to wilt for use in chickpea breeding. Six accessions of *C. bijugum* (ILWC-64, -71, -73, -76, -80, and -83), one accession (ILWC-186) of *C. judaicum*, and two accessions (ILWC-126, and -130) of *C. reticulatum* were free from wilt damage.

Fusarium wilt (*Fusarium oxysporum* Schlechtend. emend. Snyd. et Hans. f. sp. *ciceri* (Padwick) Matuo et K. Sato) is the major soilborne disease of chickpea (*Cicer arietinum* L.). Serious yield loss has been reported from India, Myanmar, Pakistan, North Africa, East Africa, Spain, Mexico, and the United States (10). Resistance breeding programs were initiated more than 80 years ago and the first wilt resistant cultivar, Karachi, was released in 1923 in Myanmar. Later, wilt-resistant cultivars were released in many countries, but their success has been highly localized due to location-specific races of the pathogen (17). Nene and Haware (9) identified sources of resistance in "desi" chickpea (characterized by small, angular, colored seeds) and Jiménez-Díaz et al. (3) identified resistance in "kabuli" chickpea (characterized by large, ram-head-shaped, beige-colored seeds), but no resistant sources have held the resistance across locations.

Wild relatives have been used for resistance breeding in several cultivated crops (2,6). Nevertheless, wild species have not been exploited for the transfer of genes for resistance in food legumes (8). Annual *Cicer* species have been evaluated for reaction to *Ascochyta rabiei* (Pass.) Lab. (18), *Heterodera ciceri* (14), *Liriomyza cicerina* (19), and cold stress (16) at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, and a high level of resistance has been found to each stress. At ICARDA, research has been underway since 1989 to transfer genes for resistance to cyst nematode and cold from wild species to the cultigen. Other researchers, including Nene and Haware (9), have reported resistance in *C. judaicum* to race 1 of *Fusarium oxysporum* f. sp. *ciceri*. Kaiser et al. (5) reported resistance in several wild species against two different *F. oxysporum* f. sp. *ciceri* races, and Singh et al. (13) have reported resistance in *C. judaicum* and *C. pinnatifidum* to *Botrytis cinerea* Pers.:Fr. In order to expand the information base on the resistance in wild species to wilt, over 100 accessions of six *Cicer* species were evaluated (in cooperation with ICARDA) against an isolate No. 526 II of *F. oxysporum*, previously tested for virulence on a set of chickpea differential lines.

MATERIALS AND METHODS

Twenty-three accessions of *Cicer bijugum* K. H. Rech, four of *C. echinospermum* P. H. Davis, 25 of *C. judaicum* Boiss., 25 of *C. pinnatifidum* Jaub & Spach, 23 of *C. reticulatum* Ladiz., and two of *C. yamashitae* Kitam. from the world collection maintained at ICARDA, Syria, were evaluated at the Istituto Sperimentale per la Patologia Vegetale (ISPV), Rome, during 1991. The *F. oxysporum* single spore isolate used in this experiment was collected from central Italy and was the most virulent of 17 tested in pathogenicity tests on the widely cultivated Italian cultivar Calia and the ICARDA line FLIP 85-88C. This isolate was assigned No. 526 II and is maintained lyophilized in ampoules at ISPV. The following differential set of nine lines was used to characterize the isolate: JG-62, C-104, JG-74, CPS-1, BG-212, WR 315, Annigeri, Chafa, L-550, and K-850-3/27. Calia and FLIP 85-88C were used as susceptible checks.

The inoculum of the fungus was produced by liquid shake culture in potato-dextrose broth (PDB) (9). The isolate was multiplied on 100 ml of PDB in 250-ml flasks on an Orbital Shaker (Model M 49230, Thermolyne Co., Dubuque, IA) run at 160 rpm, 8 h per day, at room temperature for 15 days. The liquid culture was filtered through a double layer of cheesecloth. The spore concentration was measured by a hemacytometer and was adjusted to a final concentration of approximately 1 × 10^6 spores/ml^{-1} by the addition of sterile water.

The experiment was conducted in the greenhouse at 22 ± 3°C under natural daylight conditions supplemented by artificial illumination to obtain a 12-h photoperiod. The seeds, after treatment with sodium hypochlorite (2% active Cl for 5 min, then rinsed twice in sterile tap water), were allowed to germinate on moistened perlite in plastic trays. To facilitate germination, the seeds of wild *Cicer* species were scarified with a sterile lancet. After 2 weeks, the seedlings were transferred to 200-ml plastic glasses (five plants each), containing 150 ml of either the inoculum at 10^6 spores or sterile water as control. The seedlings were held in place against the

Table 1. Reaction of differential lines of chickpea inoculated with the Italian isolate of *Fusarium oxysporum* f. sp. *ciceri* No. 526 II used to evaluate wild *Cicer* accessions

Line	Disease score[a]	Reaction
JG-62	2.9	S
C-104	1.6	M
JG-74	2.3	M
CPS-1	3.8	S
BG-212	0.0	R
WR-315	0.0	R
Annigeri	3.6	S
Chafa	1.3	M
L-550	1.3	M
K 850-3/27	0.5	R
FLIP 85-88C	4.0	S
Calia	4.0	S

[a] Assessed on a 0 to 4 scale according to the percentage of wilting of plant canopy. Score 0 = 0%; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100%. Scores of <1 = resistant (R); >1 to 3 = moderately susceptible (M); >3 = susceptible (S).

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Accepted for publication 20 June 1995.

Publication no. D-1995-1130-07R

glass by a sterile Styrofoam disk. The glasses were kept statically on a bench and the gas exchange took place between the liquid surface and the surrounding free atmosphere, through the very permeable Styrofoam (liquid surface in the glass: 28 cm^2, liquid height: 5.3 cm). Five uninoculated seedlings for each accession were used as a check.

Starting 1 week after inoculation, the Hoagland solution was supplied each week (1 application to restore the initial level of the liquid in the glass).

All accessions were evaluated in the first experiment, using at least 14 plants. A 0 to 4 scale (0 = 0%; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100% wilted plants) was used to evaluate the plants of each accession 40 days after inoculation, according to the percentage of the plant canopy damaged by the acropetal progression of wilting.

In the second experiment, accessions rated up to 2 in the first screening, and for which seeds were available, were reevaluated on ten plants, following the same procedure described above. In both experiments, FLIP 85-88C was used as susceptible check.

In the experiment with differential lines, 15 plants per line were used.

RESULTS AND DISCUSSION

The differential lines inoculated with the Italian isolate of *F. oxysporum* f. sp. *ciceri* reacted as shown in Table 1. These results do not allow us to classify the isolate as one of the races previously described (1,4,12).

Compared with field analysis (11), in which plants could also be challenged with pathogens other than Fusarium wilt, laboratory methods allow challenging the host with selected isolates with minimum interaction from other organisms. The water culture method is suitable for screening wild *Cicer* accessions as it is reproducible and requires little space. In addition, it is simpler than the shake culture screening method described by Nene and Haware (9). All wild *Cicer* species grew well with the exception of a few *Cicer judaicum* accessions.

The results obtained from the first screening of the wild *Cicer* species accessions are summarized in Table 2. Results showed that all accessions, except one *C. bijugum* accession, had a score ≤1 (highly resistant). The highly resistant accessions in the other species were one of *C. echinospermum*, nine of *C. judaicum*, five of *C. pinnatifidum*, and seven of *C. reticulatum*. Both accessions of *C. yamashitae* were susceptible (score ≥3). The susceptible line FLIP 85-88C wilted and died 40 days after inoculation. The second experiment confirmed the reaction of the accessions rated up to 2 in the previous experiment. Comparisons between the two experiments for most representative accessions are given in Table 3.

There was high variability in resistance among the different accessions of the same species, and frequently within a single accession. This is due to heterogeneity of accessions since they are maintained as populations. The following accessions remained free of symptoms (score = 0) (Table 3): *C. bijugum* (ILWC 64, ILWC 71, ILWC 73, ILWC 76, ILWC 80, and ILWC 83); *C. judaicum* (ILWC 186); and *C. reticulatum* (ILWC 126 and ILWC 130). Among them, the two accessions of *C. reticulatum* are noteworthy because of the crossability of this species with *C. arietinum* (7). Nevertheless, it may be possible to transfer resistance present in other species to *C. arietinum* using appropriate breeding techniques, including the innovative gene-transfer techniques (8).

Cicer bijugum is an interesting candidate for use in interspecific crossing because it is also resistant to Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. (15). While most breeders have been unsuccessful to date in crossing this species with the cultigen, Verma et al. (20) have reported success. At ICARDA, the biotechnology and chickpea breeding groups have initiated research to cross *C. bijugum*, *C. judaicum*, and *C. pinnatifidum* with the cultigen.

Resistant sources in wild *Cicer* species to Fusarium wilt have not been tested internationally. Further testing is in progress at ICARDA and Rome both in greenhouse and in the field, and the Rome test has confirmed the resistance of best lines in a sick plot (data not shown). ICARDA is multiplying seed of accessions free of wilt damage to test them at multi-locations through the Chickpea International Fusarium Wilt Nursery. A small quantity of seed of resistant accessions can be obtained from ICARDA, Syria.

Table 2. Reaction of wild *Cicer* species accessions to *Fusarium oxysporum* f. sp. *ciceri* (isolate No. 526 II)

		Accessions with disease score[a]				
Cicer species	Number of accessions	0	>0 to 1	>1 to 2	>2 to 3	>3 to 4
C. bijugum	23	14	8	...	1	...
C. echinospermum	4	...	1	2	1	...
C. judaicum	25	1	8	10	6	...
C. pinnatifidum	25	...	5	10	10	...
C. reticulatum	23	2	5	7	9	...
C. yamashitae	2	...	...	...	...	2
C. arietinum[b]	1	...	...	...	...	1

[a] Assessed on a 0 to 4 scale according to the percentage of wilting of plant canopy. Score 0 = 0%; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100%.
[b] ICARDA line FLIP 85-88C.

Table 3. Reaction of ILWC (International Legume Wild *Cicer*) accessions to *Fusarium oxysporum* f. sp. *ciceri* (isolate No. 526 II) in two experiments

Cicer species	Accession ILWC	Exp. 1[a]	Exp. 2[b]
C. bijugum	32	0.0[c] ± 0.0	0.0 ± 0.2
	63	0.0 ± 0.0	0.2 ± 0.6
	64	0.0 ± 0.0	0.0 ± 0.0
	65	0.1 ± 0.5	0.2 ± 0.6
	67	0.0 ± 0.0	0.4 ± 1.3
	68	0.1 ± 0.3	0.4 ± 0.7
	69	0.3 ± 0.5	0.6 ± 0.7
	70	0.1 ± 0.3	0.0 ± 0.0
	71	0.0 ± 0.0	0.0 ± 0.0
	73	0.0 ± 0.0	0.0 ± 0.0
	74	0.5 ± 1.1	0.2 ± 0.4
	75	0.0 ± 0.3	0.1 ± 0.3
	76	0.0 ± 0.0	0.0 ± 0.0
	77	0.3 ± 0.6	0.4 ± 0.7
	79	0.2 ± 0.4	0.0 ± 0.0
	80	0.0 ± 0.0	0.0 ± 0.0
	83	0.0 ± 0.0	0.0 ± 0.0
	84	0.1 ± 0.3	0.4 ± 0.8
	177	0.0 ± 0.0	0.4 ± 1.3
C. judaicum	46	0.9 ± 1.5	0.6 ± 1.5
	93	1.8 ± 1.9	1.7 ± 2.0
	98	0.5 ± 1.4	0.4 ± 1.3
	101	1.5 ± 1.6	0.7 ± 1.4
	161	0.8 ± 1.7	1.3 ± 2.0
	168	1.0 ± 1.6	0.8 ± 1.7
	186	0.0 ± 0.0	0.0 ± 0.0
	198	0.8 ± 1.7	0.8 ± 1.3
	199	0.9 ± 1.6	1.0 ± 0.7
C. pinnatifidum	78	0.6 ± 1.3	1.0 ± 1.7
	88	1.9 ± 2.0	0.8 ± 1.7
	89	0.9 ± 1.5	0.0 ± 0.0
	96	1.0 ± 1.8	0.4 ± 1.3
	143	1.5 ± 1.9	0.8 ± 1.8
	149	1.6 ± 1.6	0.9 ± 0.7
	150	1.4 ± 1.8	1.5 ± 1.3
	155	1.7 ± 1.7	0.6 ± 0.5
	159	1.7 ± 2.0	1.4 ± 1.5
	162	1.9 ± 1.8	1.5 ± 0.7
	172	1.5 ± 1.7	0.4 ± 1.3
	203	2.0 ± 2.0	0.3 ± 0.7
C. reticulatum	104	1.9 ± 1.7	0.7 ± 0.9
	105	1.7 ± 1.7	1.7 ± 1.8
	113	1.1 ± 1.6	0.9 ± 1.3
	117	1.0 ± 1.5	0.0 ± 0.0
	120	0.4 ± 0.7	0.3 ± 0.5
	123	0.3 ± 0.8	0.7 ± 0.9
	124	2.0 ± 1.8	2.7 ± 1.7
	126	0.0 ± 0.0	0.0 ± 0.0
	129	1.2 ± 1.8	1.1 ± 1.5
	130	0.0 ± 0.0	0.0 ± 0.0
	136	0.3 ± 0.6	0.4 ± 1.3
	139	1.6 ± 1.8	1.9 ± 1.4
	183	1.3 ± 1.8	0.1 ± 0.3
C. arietinum	FLIP 85-88C	4.0 ± 0.0	4.0 ± 0.0

[a] Minimum of 14 plants tested per accession.
[b] Ten plants tested per accession.
[c] Average score ± standard deviation. Score assessed on a 0 to 4 scale, according to the percentage of wilting of plant canopy. Score 0 = 0%; 1 = 1 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100%.

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