Istituto di Nematologia Agraria, C.N.R. – 70126 Bari, Italy

## DIFFERENTIAL HISTOLOGICAL RESPONSE OF SELECTED CICER BIJUGUM AND C. ARIETINUM LINES TO HETERODERA CICERI

by

M. DI VITO and N. VOVLAS

Summary. Three lines of *Cicer bijugum* and one of *C. arietinum* were evaluated for their reaction to *Heterodera ciceri*. All lines of *C. bijugum* were resistant to the nematode but the *C. arietinum* line, was highly susceptible. Cross sections of roots of lines of *C. bijugum* infected by the nematode showed necrotic tissues, undersized or absence of syncytial cell formation surrounding the head of the nematode, and suppression of nematode development. In the roots of the susceptible line, syncytia large and well developed were associated with females of *H. ciceri*.

The chickpea cyst nematode, Heterodera ciceri Vovlas, Greco et Di Vito causes severe yield losses of chickpea (Cicer arietinum L.) and lentil (Lens culinaris Medic.) in Syria (Vovlas et al., 1985; Greco et al., 1988). Since 1986 investigations have been undertaken to identify sources of resistance to H. ciceri in lines of Cicer spp. that can be used in a breeding programme for transferring this resistance to commercial varieties. Unfortunately among the 6,000 lines of C. arietinum screened so far (Di Vito et al., 1988; ICARDA, 1989) none was found resistant to H. ciceri, but resistance was present in some lines of C. bijugum K.H. Rech. (Singh et al., 1989). Therefore further investigations were undertaken to assess the histological response to H. ciceri of some C. bijugum lines in comparison with that of a susceptible one.

## Materials and methods

Two seeds for each of the lines ILWC 7/S-15, ILWC 8-3, and ILWC 8/S-3 of C. bijugum, and of ILC 1929 of C. arietinum were sown in pots containing 1.5 dm<sup>3</sup> of steam sterilized sandy soil infested with 20 eggs and juveniles/cm<sup>3</sup> soil of a Syrian population of H. ciceri. Each line was replicated five times in a randomized block design and the pots were arranged on a bench in a glasshouse maintained at 18-23°C. Thirty days after plant emergence the chickpea roots were carefully washed free of adhering soil, and then, nematodes in the roots were extracted according to Coolen's (1979) method and counted, rating the infestation according to a 0-5 scale (Di Vito et al., 1988). Also, invaded root segments were fixed in FAA, dehydrated with ter-butyl alcohol series and embedded in parawax. They were then sectioned at 10 µm thickness with a rotary microtome and stained with safranin and fast green (Johansen, 1940). Selected sections were observed and photographed.

## **Results and discussion**

The response of chickpea lines to *H. ciceri* is reported in Table I. The *C. bijugum* lines ILWC 7/S-15, ILWC 8-3 and ILWC 8/S-3 were rated  $\leq 2$  and, therefore, considered resistant to the nematode. The numbers of *H. ciceri* in the roots of these lines were 57, 122, and 26, respectively. Roots of the control line (ILC 1929) were, however, heavily infested by the nematode they were rated 5 and contained 1,650 specimens per root (Tab. I).

The histological response of the lines ILWC 7/S-15, ILWC 8-3, ILWC 8/S-3, and ILC 1929 to *H. ciceri* inva-

TABLE I - Response of some lines of Cicer bijugum and C. arietinum to Heterodera ciceri in glasshouse.

Line	Rating index (0-5)*	Number of specimens on the roots	Reaction type
C. bijugum			
ILWC 7/S-15	1.5 A**	57 A	R***
ILWC 8-3	1.9 A	122 A	R
ILWC 8/S-3	0.9 A	26 A	R
C. arietinum			
ILC 1929	5.0 B	1,650 B	S

\* 0 = no infestation, 1 = 1-2 females per plant root, 2 = 3-5, 3 = 6-20, 4 = 21-50, and 5 = more than 50 females. \*\* Data followed by different letters in the same column indicate significant difference ( $P \le 0.01$ ). \*\*\* R = resistant, rating index  $\le 2$ ; S = susceptible, rating index > 2.



Fig. 1-6 - Differential response of selected lines of chickpea infected *Heterodera ciceri*, 30 days after inoculation: Figs 1 and 2)Longitudinal sections of *C. bijugum* line ILWC 7/S-15 roots infected by *H. ciceri* larval stages showing lack of syncytial cell formation; note the hypersensitive reaction (FIR) in Fig. 1 and the undersized syncytium in Fig. 2) N = nematode. Fig. 3) Cross section of *C. bijugum* line ILWC 8-5 roots showing necrotic stelar tissues (HR) and a dead *H. ciceri* juvenile which failed to form a functional syncytium. Fig. 4). Cross section of roots of the *C. bijugum* line ILWC 8/S-3 showing undersized syncytium (US) induced by *H. ciceri* male (N). Figs 5 and 6). Cross sections of roots of the *C. arietinum* line ILC 1929 showing females (N) feeding on normal syncytia (NS).

sion is shown in Figs. 1-4 and Figs. 5-6, respectively. Roots of resistant lines of chickpea exhibited hypersensitive reaction. In these roots syncytia were not well developed but large number of necrotic cells occurred around the nematode which suppressed nematode development (Figs. 1-4). In roots of the control line (ILC 1929) syncytia were large and the nematode juveniles developed normally to adults (Figs. 5-6).

The reaction of the resistant cultivars to the cyst nematode is evidenced by the absence of syncytia formation and necrosis of several root cells surrounding the head of the nematode juveniles. This characteristic reaction also occurred in *C. bijugum* lines attacked by *H. ciceri*, which is a further confirmation of their resistance to the nematode.

These results confirm the resistant reaction of these lines as reported previously (Singh et al., 1989).

## Literature cited

- COOLEN W.A., 1979 Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil. *In*: Root-knot Nematodes (*Meloidogyne* Species) Systematics, Biology and Control. (F. Famberti and C.E. Taylor eds), Academic Press, London, pp. 317-329.
- DI VITO M., GRECO N., SINGH K.B. and SAXENA M.C., 1988 -Response of chickpea germplasm lines to *Heterodera ciceri* attack. Nematol. medit., 16: 17-18.
- GRECO N., DI VITO M., SAXENA M.C. and REDDY M.V., 1988 -Effect of *Heterodera ciceri* on yield of chickpea and lentil and development of this nematode on chickpea. *Nematologica*, 34: 98-114.
- ICARDA, 1989 Food legumes improvement program. Studies on nematodes of food legumes. 1988/89 Progress Report. Aleppo, Syria, pp. 12.
- JOHANSEN D.A., 1940 Plant Microtechnique. McGraw Hill, New York, pp. 523.
- SINGH K.B., Di VITO M., GRECO N. and SAXENA M.C., 1989 -Reaction of wild Cicer spp. lines to Heterodera ciceri. Nematol. medit., 17: 113-114.
- Vovlas N., GRECO N. and DI VITO M., 1985 Heterodera ciceri sp. n. (Nematoda: Heteroderidae) on Cicer arietinum from Northern Syria. Nematol. medit., 13: 239-252.