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Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes

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Four populations of *Pratylenchus thornei* from different locations were tested for reproductive fitness in axenic carrot disc cultures and for pathogenicity to chickpea cultivars JG 62 and UC 27 and lines K 850 and ILC 1929. Parasitism and histopathology on selected chickpea genotypes (JG 62, UC 27 and lines ILC 482, ICC 11324 and ICC 12237) were also investigated. Reproductive fitness, assessed as the ratio of the final number of nematodes per carrot disc to the number of nematodes inoculated, was similar among the populations tested and the four populations reproduced to a similar extent in a given chickpea genotype. However, the extent of reproduction was significantly affected by the chickpea genotype, JG 62 and UC 27 being the best and poorest hosts, respectively. Pathogenicity to chickpea genotypes was assessed by the difference in fresh root and dry shoot weights between infected and uninfected plants 90 days after inoculation. Plant growth was significantly reduced by the four nematode populations in all chickpea genotypes, with the exception of cv. JG 62, which was tolerant of *P. thornei*. Severity of root necrosis caused by nematode infection was similar for all populations. Histopathological studies of chickpea genotypes infected by *P. thornei* showed that all were suitable hosts according to nematode reproduction and host reaction. *P. thornei* always migrated through epidermal and cortical cells by breaking down cell walls along the nematode pathway. In the most susceptible lines (ILC 482 and JG 62), damage to endodermal cells adjacent to nematode feeding sites was occasionally observed.

Introduction

Root-lesion nematodes (*Pratylenchus* spp.) attack and damage chickpea (*Cicer arietinum*) roots in the Mediterranean basin and Indian subcontinent (Walia & Seshadri, 1985; Greco *et al*., 1992; Sharma *et al*., 1992; Di Vito *et al*., 1994; Castillo *et al*., 1996). The most important and widespread root lesion nematode attacking chickpea is *Pratylenchus thornei* (Di Vito *et al*., 1992, 1994; Greco *et al*., 1992; Castillo *et al*., 1996). However, little is known concerning host–parasite relationships in root tissues of chickpeas differing in reaction to *P. thornei* (Greco *et al*., 1988; Tiwari *et al*., 1992). Information on this subject is of importance for the use of host plant resistance as a management strategy. Management of *P. thornei* by means of crop rotation is made difficult by the wide host range of the nematode (Greco *et al*., 1988). Although soil fumigation can be effective for control of the nematode, it is nonprofitable and causes environmental pollution. For these reasons, exploitation of host plant resistance would be the most practical and cost effective control measure for these nematodes.

Reproductive fitness together with virulence are major components of pathogenicity (Shaner *et al*., 1992) and thus important for the assessment and understanding of disease reactions of plants to pathogens. Root-lesion nematodes with higher reproduction fitness may colonize more host tissue and consequently cause more damage to susceptible plants. Genetic diversity and population variability are also known to exist among these *Pratylenchidae* species (Motalaote *et al*., 1987; Griffin, 1991; Pinochet *et al*., 1993; Fallas & Sarah, 1995; France & Brodie, 1996). Differences in reproductive fitness or pathogenic capability were reported to be responsible for conflicting data among populations of *P. thornei* (Tiyagi & Parveen, 1992; Castillo *et al*., 1995b). Axenic cultures in carrot discs provide standardized conditions for production of nematode inoculum, allowing comparisons of multiplication rates among nematode populations. This article reports the reaction of selected chickpea genotypes to a population of *P. thornei* from southern Spain and examines the histopathological changes induced in the root tissue. The

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objectives of the research were: (1) to assess the reproductive fitness of four *P. thornei* populations from different locations in axenic carrot disc cultures; (2) to investigate the pathogenicity of the same four populations to selected chickpea genotypes; and (3) to describe the histopathological changes induced by the feeding and migration of the nematode in root tissues of susceptible and resistant chickpeas. Such information would be important for understanding the mechanisms of nematode damage to chickpeas, as well as possible reactions that are specific to resistant cultivars.

Materials and methods

Nematode inoculum

Three populations of *P. thornei* from southern Spain and one population from Syria were used. The Spanish populations were obtained from roots of chickpea cv. Blanco Lechoso collected from Jerez de la Frontera (Cádiz province), Cañete de las Torres and Santaella (Córdoba province). The Syrian collection was from the chickpea line ILC 482 at Tel Hadya (Aleppo). Each population was established *in vitro* on a carrot disc at 24°C for 6 weeks, and then axenically multiplied in cultures on several discs (Huettel, 1985; Castillo *et al.*, 1995a). To obtain inoculum, the infected carrot discs were placed on a Baermann funnel. The extracted nematodes were surface-sterilized with 0·02% ethoxyethyl mercuric chloride and 0·1% streptomycin sulphate solutions for 2 and 24 h, respectively, and thoroughly rinsed several times in sterilized water. Nematode inoculum concentration was determined from a 1-mL aliquot, except for the experiment on carrot discs for which gravid females were picked up with a sterile handling needle.

Plant material

'Desi' (small, angular, coloured seeds) cv. JG 62 and lines ICC 11324 (BG 212) and ICC 12237 and 'kabuli' (large, ramhead-shaped, beige seeds) cv. UC 27 and lines ILC 482, ILC 1929 and K 850 were used in this study. Chickpea seeds were surface-disinfested with 20% NaOCl solution for 3 min, washed in sterile distilled water and germinated in moist chambers at 25°C in the dark for 72 h. Germinated seeds, selected for uniformity (length of radicle 2–3 cm), were sown into clay pots (one per pot) containing 1 L (experiment II) or 0·5 L (experiment III) of an autoclaved soil potting mixture (sand:clay loam, 2:1, v/v). Plants were grown in a growth chamber adjusted to 24 ± 1°C, 60–90% relative humidity, and a 14-h photoperiod of fluorescent light at 360·5 ± 24·7 μE m^{-2} s^{-1}. Plants were watered daily with 100 mL of water and fertilized weekly with 100 mL of a nutrient solution (Hoagland & Arnon, 1950).

Experiment I. Reproductive fitness of *Pratylenchus thornei* populations in carrot disc cultures

For each of the four populations of the nematode, 10 gravid females were transferred with a sterile handling needle into a drop of sterile distilled water on the carrot disc. Inoculated discs were incubated at 25 ± 1°C in the dark for 40 days. There were 10 replicated discs in a completely randomized design for each nematode population. Upon completion of the experiment, nematodes from carrot discs were extracted by centrifugation (Coolen, 1979), and the total final nematode population (eggs, juveniles, and females) per carrot disc was determined. The experiment was repeated once.

Experiment II. Pathogenicity of *Pratylenchus thornei* populations to selected chickpea genotypes

Pathogenicity of the four populations was tested on the chickpea lines K 850 and ILC 1929, and cultivars JG 62 and UC 27. The genotypes were selected according to their known reactions to *P. thornei*. Lines ILC 1929 and K 850 were reported as susceptible to populations from India and Syria (Walia & Seshadri, 1985; Greco *et al.*, 1988), respectively; and cultivars JG 62 and UC 27 remained undamaged by one of the *P. thornei* populations from Spain 45 days after inoculation under controlled conditions (Castillo *et al.*, 1995b). Nematode inoculum consisted of the following percentages of developmental stages for each population: 'Tel Hadya' (26% females, 59% juveniles and 15% eggs), 'Jerez' (18% females, 77% juveniles and 5% eggs), 'Cañete' (23% females, 70% juveniles and 7% eggs), and 'Santaella' (19% females, 77% juveniles and 4% eggs). Germinated seeds were inoculated with 2500 nematodes in 10 mL of sterile water by applying the inoculum over the radicle at sowing. Control plants were treated similarly with 10 mL of sterile water. Treatments were replicated 10 times in a randomized complete block design. The experiment lasted for 90 days after inoculation and it was repeated once.

Experiment III. Reaction and histopathology of selected chickpea genotypes to infection by *Pratylenchus thornei*

The population from Cañete (Córdoba) was used in this experiment. The radicles of germinated seeds of chickpea genotypes JG 62, UC 27, ILC 482, ICC 11324 and ICC 12237 were inoculated with a 10 mL suspension containing 2500 nematodes (migratory life stages, 24% females and 76% juveniles). The genotypes were selected because of their known reactions to *Fusarium oxysporum* f.sp. *ciceris* (the agent of Fusarium wilt) and to *P. thornei*. Cultivars JG 62 and UC 27 and line ILC 482 represented suitable hosts for *P. thornei* (Greco *et al.*, 1988; Castillo *et al.*, 1995b, 1996). JG 62 and ILC 482 are susceptible, and UC 27 is resistant, to *F.oxysporum* f.sp. *ciceris* race 5 (Jiménez-Díaz *et al.*, 1993). Lines ICC 11324 and ICC 12237 were reported to be resistant to a population of *P. thornei* from India (Tiwari *et al.*, 1992), and were resistant and moderately resistant, respectively, to *F. oxysporum* f.sp.

ciceris race 5 (Jiménez-Díaz, unpublished data). Chickpea seedlings were sampled at 5, 10, 20 and 40 days after inoculation and their roots were carefully washed free from soil. At each sampling date, roots were assessed for severity of necrosis and for nematode reproduction. Segments of infected root from each plant were fixed in a formaldehyde chromoacetic solution for 48 h, dehydrated in a tertiary butyl alcohol series (40–70–85–90–100%) and embedded in 58°C-melting-point paraffin. Embedded tissues were sectioned with a rotary microtome. Sections 10–12 μm thick were mounted on glass slides, stained with safranin and fast-green, then mounted permanently in dammax xylene, examined microscopically and photographed (Johansen, 1940). The experimental design was a randomized complete block with eight replicated plants per treatment. The experiment was not repeated.

Assessment of plant–nematode interaction

Reproductive fitness was measured by the reproduction rate P_f/P_i (final nematode population/initial nematode population) in an inoculated disc. Severity of root necrosis was assessed on a 0–10 scale according to the percentage of the root system that was necrotic (0 = 0%, 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90% and 10 = 91–100% necrotic tissue). This rating scale has proved reliable in previous studies (Greco *et al.*, 1988; Castillo *et al.*, 1995b). Differences in pathogenicity among *P. thornei* populations were assessed by comparing the fresh root and dry shoot weights of plants with and without nematodes. Nematodes were extracted from aliquot 100 cm^3 of soil and from the entire root system of each plant by centrifugation (Coolen, 1979), and the final total nematode population was determined.

Statistical analysis

Data were analysed by ANOVA. For statistical analysis, the number of nematodes (X) was transformed into $\log_{10}(X+1)$. In the repeated experiments (I and II), similarity between experimental runs, tested by preliminary analyses of variance using experimental runs as blocks, allowed the data to be combined for analyses of variance. Treatment means were compared using Fisher's protected least significant difference test (LSD) at $P = 0{\cdot}05$.

Results

Reproductive fitness of *Pratylenchus thornei* populations in carrot disc cultures

There were no significant differences in reproduction rates among the four nematode populations tested at 40 days after inoculation. All showed high reproductive fitness (23–39-fold initial population), and no significant differences in the percentages of females (18–19%), juveniles (59–65%) and eggs (16–23%) were found in the final counts.

Pathogenicity of *Pratylenchus thornei* populations to selected chickpea genotypes

All four chickpea genotypes were infected by the *P. thornei* populations tested. There were no differences in pathogenicity, as indicated by the severity of root necrosis, among the *P. thornei* populations in any of chickpea genotypes (Table 1) and there was no significant ($P = 0{\cdot}05$) *P. thornei* population × chickpea genotype interaction. Plant growth, assessed by fresh root and dry shoot weights, was significantly ($P < 0{\cdot}05$) reduced by the four populations in all the genotypes except JG 62 where no significant differences ($P = 0{\cdot}05$) in fresh root and dry shoot weights were observed among inoculated plants and uninfected controls. There was no significant ($P = 0{\cdot}05$) *P. thornei* population × chickpea genotype interaction for nematode reproduction. The reproduction rate differed ($P < 0{\cdot}05$) among chickpea genotypes, but there were no significant differences among nematode populations for reproduction in a given genotype. Although all chickpea genotypes tested were good hosts of the *P. thornei* populations in this study, cultivars JG 62 and UC 27 supported the highest and lowest multiplication rates, respectively.

Reaction and histopathology of selected chickpea genotypes to infection by *Pratylenchus thornei*

For all five chickpea genotypes in the experiment, the severity of root necrosis as well as *P. thornei* reproduction increased steadily with time of incubation (Fig. 1). Chickpea line ICC 11324 was the genotype most severely affected from 10 days after inoculation to the end of the experiment (Fig. 1). Nematode reproduction differed significantly on different genotypes, with cv. JG 62 and line ILC 482 being the best hosts and lines ICC 11324 and ICC 12237 showing the lowest reproduction rate (Fig. 1).

Five days after inoculation, nematode females and juveniles were found penetrating chickpea roots apparently without a preferential site. Brownish elliptical necrotic lesions, 0·5–1 mm long, were observed at the penetration site. The primary cellular reaction to *P. thornei* was enlargement of the nucleus and nucleolus and cytoplasmic granulation within cortical cells of a size similar to that of noninfected cells (Fig. 2). No differences in the nematode invasion of cortical cell layers, or in host reaction, were detected among the genotypes. Ten days after inoculation, adult females, juveniles and eggs were present near the root lesions, but no, or very few nematodes were found at a distance of 2–5 mm. Nematodes were stretched out or coiled, occupying a single cell or different cell layers. Cortical cells penetrated and fed upon by the nematode were

Table 1 Pathogenicity and reproduction of four populations of *Pratylenchus thornei* in four selected chickpea genotypes[a]

Chickpea genotype	Nematode population	Root necrosis severity[b]	Fresh root weight (g)	Dry shoot weight (g)	P_f/P_i[c]
K 850					
	Control	0·0	64·4 a	5·1 a	0·0
	Syria	2·1 a	42·0 b	4·1 b	111·3 a
	Santaella	1·9 a	43·7 b	4·4 b	85·4 a
	Cañete	2·2 a	40·5 b	4·4 b	131·2 a
	Jerez	2·0 a	44·5 b	4·3 b	145·6 a
	Mean	**2·0 A**	**–**	**–**	**118·4 B**
ILC 1929					
	Control	0·0	60·5 a	5·3 a	0·0
	Syria	2·5 a	40·8 b	4·4 b	78·7 a
	Santaella	2·4 a	44·2 b	4·4 b	116·1 a
	Cañete	2·2 a	46·0 b	4·6 b	83·1 a
	Jerez	2·1 a	49·2 b	4·6 b	75·6 a
	Mean	**2·3 A**	**–**	**–**	**88·4 C**
UC 27					
	Control	0·0	58·6 a	5·4 a	0·0
	Syria	2·4 a	45·3 b	4·5 b	43·8 a
	Santaella	2·1 a	45·5 b	4·6 b	55·6 a
	Cañete	2·0 a	48·9 b	4·4 b	51·0 a
	Jerez	2·4 a	47·7 b	4·8 b	36·56 a
	Mean	**2·2 A**	**–**	**–**	**46·7 D**
JG 62					
	Control	0·0	29·6 a	3·5 a	0·0
	Syria	2·8 a	29·7 a	3·4 a	332·3 a
	Santaella	2·5 a	27·8 a	3·3 a	290·3 a
	Cañete	2·6 a	31·9 a	3·5 a	315·1 a
	Jerez	2·6 a	28·4 a	3·4 a	308·9 a
	Mean	**2·6 A**	–	–	**311·7 A**

[a]Data are the averages of two experiments with 10 replicated plants per treatment combination in each experiment. For each chickpea genotype, means followed by the same letter do not differ significantly ($P = 0·05$) according to Fisher's protected LSD test. Uppercase letters refer to mean comparisons between chickpea genotypes. Actual data are presented for each chickpea genotype, but numbers of nematodes were transformed to $\log_{10}(X+1)$ for ANOVA. [b]Assessed on a scale of 0 = 0% necrotic tissue to 10 = 91–100% necrotic tissue, 90 days after inoculation. [c]Nematode multiplication rate = final nematode population per plant/initial nematode inoculum per plant.

generally devoid of cytoplasmic content. Cortical cells adjacent to infected ones showed dense granular cytoplasm, thickened walls and a hypertrophied nucleus and nucleolus (Fig. 2). At 20 days after inoculation, many nematodes were found in cell layers deeper in the cortex, involving most of the cortical tissue (Fig. 2).

Nematodes were never seen inside the stele. However, some damaged endodermal cells were observed occasionally at 20 days in ILC 482 and JG 62, and at 40 days in all genotypes particularly these two. In roots sampled 40 days after inoculation, most of the adult females in the root cortex were gravid and eggs were frequently found in the tissue, together with all life stages of the nematode. Cavities and cells with thickened walls were very extensive in the cortex, but only occasional damage was found in endodermal cells adjacent to nematode infections, mostly in ILC 482 and JG 62 (Fig. 2).

Discussion

Although pathogenic diversity seems to be common in several *Pratylenchidae* species (Griffin, 1991; Pinochet *et al.*, 1994; Fallas & Sarah, 1995), the results showed that the populations studied here do not differ in reproductive fitness, or in pathogenicity on selected chickpea genotypes. Nematode reproduction in the chickpea line ILC 482 and cultivars JG 62 and UC 27 agreed with those from previous studies (Greco *et al.*, 1988; Castillo *et al.*, 1995b, 1996). However, results contradictory to those of Tiwari *et al.* (1992) were found in nematode reproduction on lines ICC 11324 and ICC 12237, which were reported as resistant ($P_f/P_i < 0·5$) to a population of *P. thornei* from India. In the present study, line ICC 11324 supported the lowest reproduction rate, and this was associated with extended root necrosis. Whether that could be related to chemical compounds in the chickpea roots would

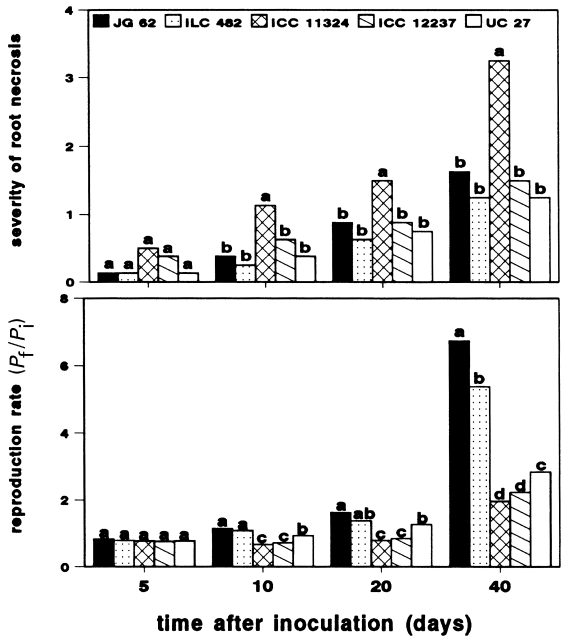

Figure 1 Reaction of chickpea genotypes to a population of the root-lesion nematode *Pratylenchus thornei* from Cañete, Córdoba, southern Spain. Data are the averages of eight replicated plants per treatment combination. For each time period, bars with the same letter do not differ significantly ($P = 0{\cdot}05$) according to Fisher's protected LSD test. Actual data are presented for each chickpea genotype, but numbers of nematodes were transformed to $\log_{10}(X+1)$ for statistical analysis. Each plant was inoculated with 2500 nematodes at migratory life stages.

require further studies. Phenolic compounds occur in roots of desi chickpeas, and chickpea root exudates have been associated with resistance to other chickpea pathogens (Kumar & Jalili, 1985; Sankuning *et al.*, 1986; Shinde & Deshnuikh, 1989; Stevenson *et al.*, 1995). Differences in reproductive fitness between the populations of the nematode from India and Spain may explain discrepant results, as reported for other *Pratylenchus* spp. (Pinochet *et al.*, 1993). In the present study, few and scarcely diverse nematode populations were used. Consequently, the possibility that the differences observed might be characteristic of *P. thornei* populations from the Mediterranean basin and the Indian subcontinent remains an open question deserving further study.

Severity of root necrosis caused by the *P. thornei* populations was similar in the four chickpea genotypes tested, and it was much higher than that occurring for a shorter period of incubation in a previous study (Castillo *et al.*, 1995b). Plant growth was significantly reduced by the four nematode populations in all chickpea genotypes except JG 62. These results agree with others (Walia & Seshadri, 1985; Tiyagi & Parveen, 1992), indicating that time for assessment of interactions between chickpeas and root-lesion nematodes under artificial conditions must be close to that under field conditions (90–115 days after inoculation with the nematode). They also support the conclusion that parasitism of chickpea roots by *P. thornei* contributes to reduced yields in field soils where large nematode populations occur. In the four populations tested, nematode multiplication was greatest on JG 62, indicating that this cultivar is a better host than the others. However, root and shoot growth of JG 62 were not impaired by nematode infection, in agreement with previous results (Castillo *et al.*, 1995b), indicating that this genotype is tolerant of *P. thornei* infections. This was further supported by histopathological observations of the plant root–nematode interactions. Further such studies should be carried out on the tolerance mechanisms.

Results from the histopathological study indicate that the five chickpea genotypes must be considered suitable hosts of the nematode as shown by reproduction rates. Histopathological responses of roots infected by *P. thornei* were similar to those in other plant–nematode interactions (Thomason *et al.*, 1976; Acosta & Malek, 1981; Townshend *et al.*, 1989). However, the results presented here suggest that *P. thornei* has no preferential site for penetration in chickpea roots, in contrast with other observations that *P. penetrans* mostly penetrates roots around and above the root elongation zone (Zunke, 1990). The damage to cells upon which the nematode has fed, and to cells adjacent to the pathway of nematode migration, might result from both physical and biochemical factors, as reported for other *Pratylenchus* spp. (Acedo & Rohde, 1971; Townshend & Stobbs, 1981; Townshend *et al.*, 1989). Although damage to endodermal cells occurred in some cultivars, *P. thornei* was not found feeding on endodermal cells, even in the most susceptible genotypes. Thomason *et al.* (1976) hypothesized that a physical barrier or poor food source protected the endodermis of snap and lima beans from parasitism by *P. scribneri*. On the other hand, *P. penetrans* is capable of invading the stele of cabbage and strawberry roots (Townshend, 1963; Acedo & Rohde, 1971). The observation that chickpea genotypes resistant or moderately resistant to *F. oxysporum* f.sp. *ciceris* are susceptible to *P. thornei*, may provide clues to the parasitism of the two organisms.

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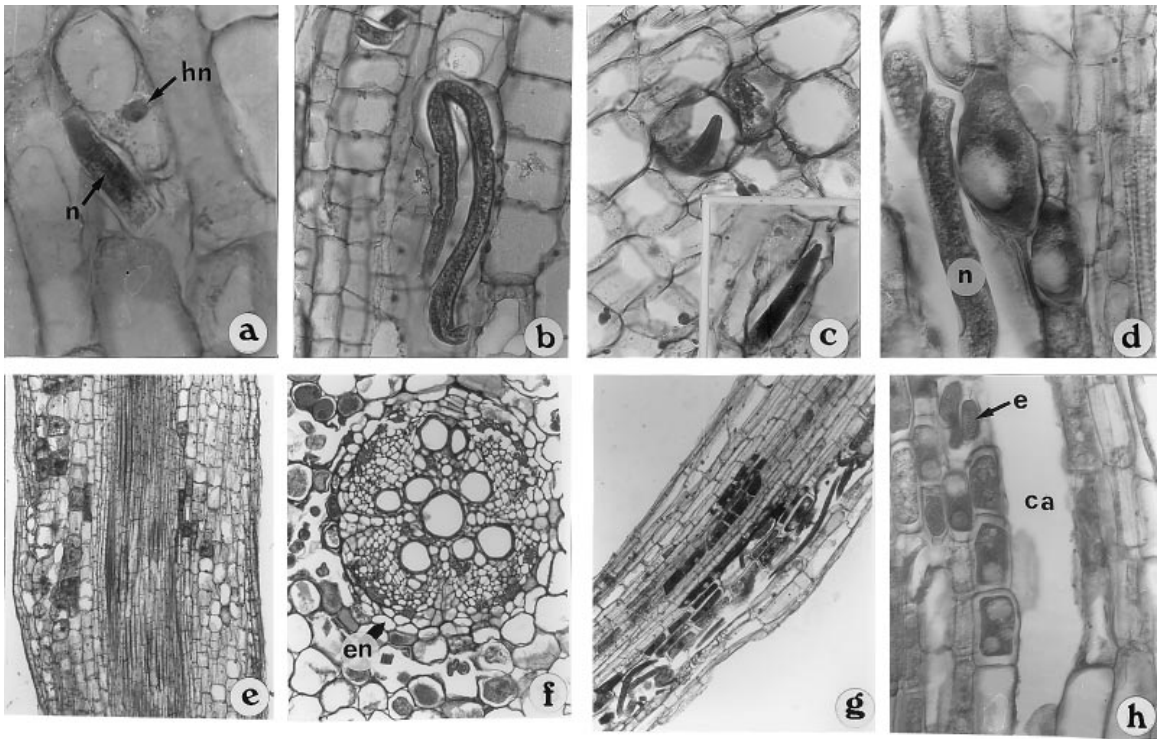

Figure 2 Histopathological changes induced by the feeding and migration of the root-lesion nematode *Pratylenchus thornei* in five selected chickpea genotypes, at 5, 10, 20 and 40 days after inoculation. (a) Primary cellular reaction to nematode infection showing enlargement of nucleus and nucleolus (line ILC 482). (b) Coiled specimens occupying single or different cells (line ICC 12237). (c) Infected cells showing the cytoplasmic content limited to the periphery of cells (line ICC 11324). (d) Thickened cell walls, hypertrophied nucleus and nucleolus, and granular cytoplasm of cells adjacent to nematode pathway (cv. UC 27). (e) Longitudinal root section showing deeper layers of cortical cells in infected areas (line ICC 12237). (f) Transverse section of secondary roots showing damaged endodermal cells (line ILC 482). (g) Extensive damage in cortical tissue (cv. UC 27). (h) Large cavity (ca) in old infections, and several deeper stained cells near the cavity (cv. JG 62). (ca, cavity; e, nematode egg; en, endodermis; hn, hypertrophic nucleus; n, nematode).

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