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HISTOLOGICAL, HISTOPATHOLOGICAL AND HISTOCHEMICAL INVESTIGATIONS ON ROOT-KNOT NEMATODE RESISTANT AND SUSCEPTIBLE LINES OF COWPEA

by
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Histopathological and histochemical investigations are of value in studies involving the physiology of parasitism and the evaluation of host-parasite relationships. However, there are not many reports in the literature on these aspects. Therefore, the present investigations were undertaken to compare histological, histopathological and histochemical defferences between nematode resistant and susceptible lines of cowpea [*Vigna unguiculata* (L.) Walp.] infected by *Meloidogyne incognita* (Kofoid *et* White) Chitwood.

Materials and Methods

Cowpea seeds of root-knot resistant lines (IC 9642-B and TVU 2430-P) and susceptible lines (S-288 and S-488) were sown singly in 20 cm clay pots filled with sterilized soil. Fifteen days after sowing, each pot was inoculated with 1,000 freshly hatched second stage larvae of *M. incognita.* Uninoculated controls were also maintained

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Table I - *The histochemical techniques employed in the localization of various metabolites.*

Metabolite localized	Test	Duration of test	Colour	Control
Total insoluble polysaccharides	Periodic acid Schiff's (PAS) method (Jensen, 1962)	Periodic acid (0.5%) for 15-25 min at 30-35 °C and staining with Schiff's reagent	Magenta	By omitting periodic acid step
Total proteins	Mercuric-bromophenol blue method (Mazia *et al.*, 1953)	Mercuric bromophenol blue for 15 min	Blue	Extraction of histones by 0.2 NHCl
Nucleic acids	Toluidine blue method (Feder and O' Brien, 1969)	1% Toluidine blue for 15 min	RNA - Violet DNA - Green	By extracting nucleic acids with 1 N perchloric acid for 12-24 hr at 4 °C

Forty-five days after inoculation, the plants were depotted, their roots washed and fixed in Carnoy's B fixative (6 parts of ethanol + 3 parts of chloroform + 1 part of glacial acetic acid). The material was processed through ethanol-n-butanol series and embedded in paraffin wax. Serial sections cut at 10 μm thickness were mounted on slides using gelatin adhesive. The methods employed in the detection of various metabolic substances are given in Table I.

Results and Discussion

Compared with susceptible lines the resistant lines had a thicker cork layer, in the form of small cells in a compact arrangement (Fig. 1 A, D). In resistant lines the cortex was narrow with small, compact cells compared with a wider cortex in susceptible lines in which the cells were large and loosely aggregated. Sclereids were present in the cortex of resistant lines (Fig. 1 A, B) but not in susceptible lines, but starch grains were more abundant in the cortex of susceptible lines (Fig. 1 D, E).

In resistant lines there were fewer giant cells around the head

Table II - *Histological characteristics of selected lines of cowpea (Mean of 10 replicates).*

Line	Cork			Cortex			Mean stelar diameter (μm)	Mean diam of starch grains (μm)
	Mean No. of layers	Mean thickness (μm)	Volume of cork cells (μm^3)	Mean No. of layers	Mean thickness (μm)	Volume of cortical cells (μm^3)		
IC 9642-B	6.0	31.10	12,100	15.9	129	1,880	516	20
TVU - 2430-P	4.5	30.20	10,900	12.9	109	2,800	551	20
S - 288	3.0	17.30	17,100	20.8	309	3,720	798	40
S - 488	2.5	20.90	25,980	27.6	321	4,200	763	30
S. Em. ±		1.708	416.186	0.187	1.689	146.894	2.694	
C. D. at 1%		6.693	1630.945	0.731	6.618	575.645	10.558	
C. V. %		2.171	7.967	3.057	2.461	8.707	1.297	

of the *M. incognita* female compared to susceptible lines (Table III) but they were smaller in resistant lines. There were fewer nuclei per giant cell or per gall in resistant lines as compared to susceptible lines as also reported by Crittenden (1958), Dropkin (1959), Fassuliotis (1970) and McClure *et al.* (1974) working with root-knot nematode resistant lines of soybean (*Glycine max*), *Cucumis* spp. and cotton (*Gossypium* spp.). In the present studies, the interference in the formation of giant cells (less in number and smaller in size with fewer nuclei) which are essential for the successful establishment of host-parasite relationship may, in part, account for the resistance to *M. incognita* in IC 9642-B and TVU 2430-P lines of cowpea.

The cytoplasm in the giant cells of the susceptible lines appeared to be dense as compared with that in adjacent cells. The cell walls of giant cells were also thickened and had dark stained finger-like projections (wall invaginations) on the inner surface (Fig. 1 G).

Death of cells (hypersensitive reaction) around infecting root-knot nematode larvae occurred in the roots of resistant cowpea line IC 9642-B (Fig. 1 C). The roots of resistant lines brown quickly and there is no nematode multiplication, so that the initial infection is isolated. Hypersensitive reaction has been postulated for root-knot nematode resistance in tobacco, tomato and *Medicago sativa* (Kurian, 1970; Paulson, 1976; Nigh, 1972). There was less degeneration of cork and cortical tissues in the infested resistant lines (Fig. 1 C) compared with that in the susceptible lines (Fig. 1 F).

Considerable differences were observed in histochemical substances between susceptible and resistant lines. The thick giant cell walls in susceptible lines infected with *M. incognita* stained intensively

Table III - *The number and volume of giant cells and number of nuclei in giant cells and galls in selected lines of cowpea infected with* M. incognita *(Mean of 5 replicates).*

Line	Mean No. of giant cells/gall	Volume of giant cell (μm^3)	Mean no. of nuclei per giant cell	Mean no. of nuclei per gall
IC 9642 - B	1.4	40827.65	2.4	3.36
TVU - 2430 - P	2.0	113977.43	2.4	4.80
S - 288	5.4	2560113.41	19.6	105.84
S - 488	7.2	7032982.36	24.0	172.80

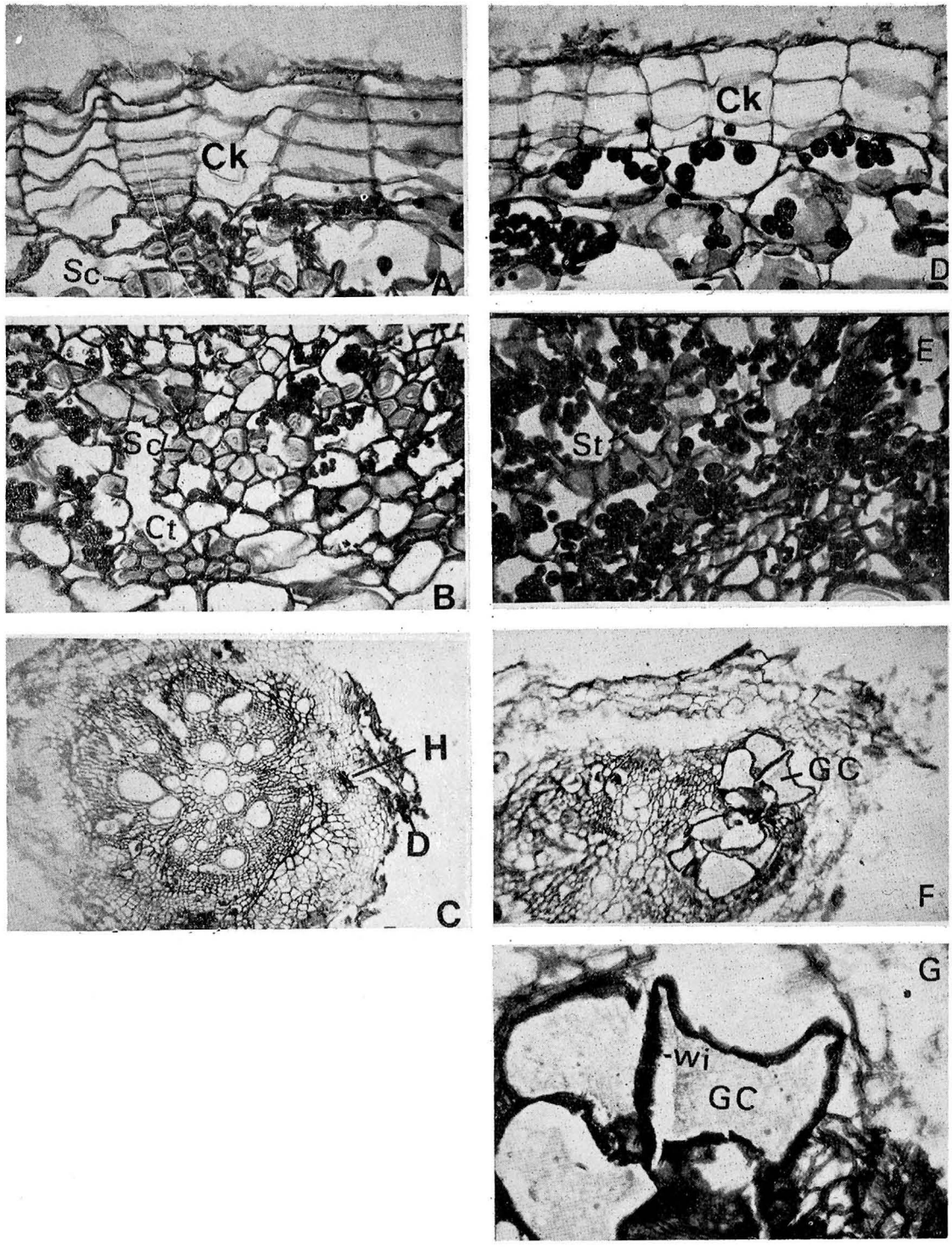

Fig. 1 - A-G. Transections of roots of resistant IC 9642-B (A, B, C) and susceptible S-288 (D, E, F, G) stained with PAS method. A (cork), B (Cortex), D (cork), and E (Cortex) are from uninoculated control and C, F and G are from inoculated plants. A, B, D, E, G - x 400; C, F - x 100 (Ck = cork; Ct = cortex; D = debris of dead nematode; H = hypersensitive zone; Sc = sclereids; St = starch; Gc = giant cell; Wi = wall invaginations).

with magenta colour compared with those in resistant lines. This indicated the presence of more insoluble polysaccharides and may be due to additional deposition of cellulose. Similar observations were recorded by Dropkin and Nelson (1960) and Trivedi and Tiagi (1980) working with soybean and chillies (*Capsicum annuum*) respectively.

The cytoplasm and nuclei of the giant cells stained intensive dark blue when treated with mercuric bromophenol blue, indicating the presence of abundant proteins in the susceptible lines. This may be due to increased protein synthesis in the giant cells. Trivedi and Tiagi (1980) also observed the abundance of basic protein in the irregularly-shaped nuclei of the giant cells of chillies infected with root-knot nematode.

Similarly increased amounts of DNA and RNA were observed in the giant cells of susceptible lines infected with *M. incognita* as compared with resistant lines of cowpea. This may be due to greater metabolic activity as reported by Trivedi and Tiagi (1980) who noted a two-fold increase in nucleic acids in galled tissues of chillies and their accumulation both in giant cells and nematodes.

Hence, the increase in number and volume of giant cells and nuclei, denser cytoplasm, greater amounts of polysaccharides, proteins and nucleic acids in susceptible lines of cowpea indicated higher metabolic activity of the giant cells which had developed faster as compared with resistant lines.

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SUMMARY

Histological, histopathological and histochemical differences in root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. resistant and susceptible lines of cowpea [*Vigna unguiculata* (L.) Walp.] have been investigated. In resistant lines the cork layer was thicker and sclereids were present in the cortex. Sclereids were absent in the susceptible lines and starch grains were more numerous in cortex than in resistant lines. There were fewer giant cells which were smaller in size and with fewer nuclei in resistant lines compared with susceptible lines. Death of cells (hypersensitive reaction) around infecting root-knot nematode larvae occurred in the roots of the resistant line IC 9642-B. More insoluble polysaccharides, proteins and nucleic acids were detected in susceptible lines compared with resistant lines of cowpea.

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