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HISTOCHEMICAL STUDY OF *PISUM SATIVUM* L. ROOT GALLS INCITED BY *MELOIDOGNE INCOGNITA* CHITWOOD.

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The present investigation deals with the histochemical localization of carbohydrates, proteins, RNA, DNA and ascorbic acid in healthy and *Meloidogyne incognita* infected *Pisum sativum* root galls collected at various intervals after inoculation. In general, infected roots were richer in RNA, DNA, total proteins and ascorbic acid as compared to healthy roots. Syncytial cell walls with their protuberances stained strongly for insoluble polysaccharides. Starch grains were absent in giant cells and cells around them. The higher occurrence of ascorbic acid in infected roots suggested some sort of resistant response by the host plant.

Keywords : Ascorbic acid; Carbohydrate; DNA; Histochemistry; *Meloidoygyne incognita; Pisum sativum*; RNA; Total protein.

Introduction

The pea (*Pisum sativum* L.) occupies a position of considerable importance in our agricultural economy. Importance of pea as a pulse and vegetable crop in human diet needs no emphasis. It is heavily infected by *Meloidogyne incognita* in the sandy soil of North-East Rajasthan, where it is grown extensively. The development of disease syndrome is dependent on biochemical reactions taking place between substances secreted by the pathogen and those already present or produced by the host as a response to the infection. Perusal of the litereture revealed only a few reports of time bound study of biochemical quantitation in pea resulting *M. incognita* infection[1,2]. The histochemical methods are advantageous they enabled *in situ* localization of various metabolites at the site of their synthesis or action. The present study was undertaken to have a better understanding of host parasitic interaction.

Materials and Methods

Surface sterilized needs of *Pisum sativum* L. (CV Bonneville) were sown singly in 15 cm earthen pots containing autoclaved river-bed sand. The seeds were previously treated with the specific strain of *Rhizobium*. One week old seedlings were inoculated with 1000 juveniles of *M. incognita* by pipetting and pouring the larval suspension through three holes around the roots. Uninoculated plants served as control. The seedlings were fed throughout the experimental period with full strength Long Ashton nutrient solution. The healthy and infected plants were uprooted at the interval of one week after inoculation upto eight weeks and thereafter 90 days after inoculation and fixed in different fixatives (Table 1). The fixed material was dehydrated in tertiary butyl alcohol (TBA) series, infiltered and embedded in paraffin wax. The serial microtome sections, cut at 12 micron, were mounted on slides using Haupt's adhesive. The various histochemical methods for the localization of different substances in the root sections are listed in Table 1.

Observations

Carbohydrate

(i) *Healthy root* : In young root PAS-positive granules were absent, however, cell walls and phloem stained intensely. The cells of lateral root primordia were full of PAS- positive granules (Fig. 1). The starch grains were confined to phloem parenchyma.

(ii) *Infected root* :- Galls were also devoid of PAS- positive granules. However, cell

Table 1. Methods for histochemical localiztion.

Metabolite localized	Faxative	Method employed	Reagent	Colour indication	Control
Total insoluble polysaccharides	FAA	Jensen[3]	Periodic acid -Schiff's reagent	Magenta	By omitting periodic acid step
Starch	FAA	Johansen[4]	IKI solution	Blue-black	-
Total proteins	10% Neutral formalin	Mazia *et al*[5].	Murcuric-bromophenol blue (MBB)	Blue	-
DNA	Carnoy's fluid	Kallarackal[6]	1N HCl - Schiff's reagent	Magenta	By omitting hydroysis
RNA	Carnoy's fluid	Tepper and Gifford[7]	Pyronin reagent	Red	Perchloric acid treatment
Ascorbic acid	Silver nitrate	Chayen[8]	Silvernitrate reagent	Brownish-black	Oxidation with $CuSO_4$ solution

walls stained more intensely than the healthy roots. Syncytial walls with their protuberances stained strongly (Fig. 2). Starch grains were absent in giant cells and the cells around them.

Proteins

(i) *Healthy root* :- Cells in young roots showed a higher protein content in cytoplasm, nuclei and nucleoli while cell walls showed absence of protein.

(ii) *Infected root* :- Galls showed a higher amount of protein than healthy roots. Giant cells, nematode and hyperplastic parenchyma were the main sites of protein accumulation in galls (Fig. 3). There was a gradual increase in the protein content from for away-cells to the giant cells.

DNA

(i) *Healthy root* :- Nuclei in the cells of roots of all ages stained positively for DNA.

(ii) *Infected root* :- The giant cell nuclei which possessed hypertrophied nuclei showed an increased amount of DNA (Fig. 4). The cells surrounding the developing giant cells became hyperplastic and also showed prominent nuclei.

RNA.

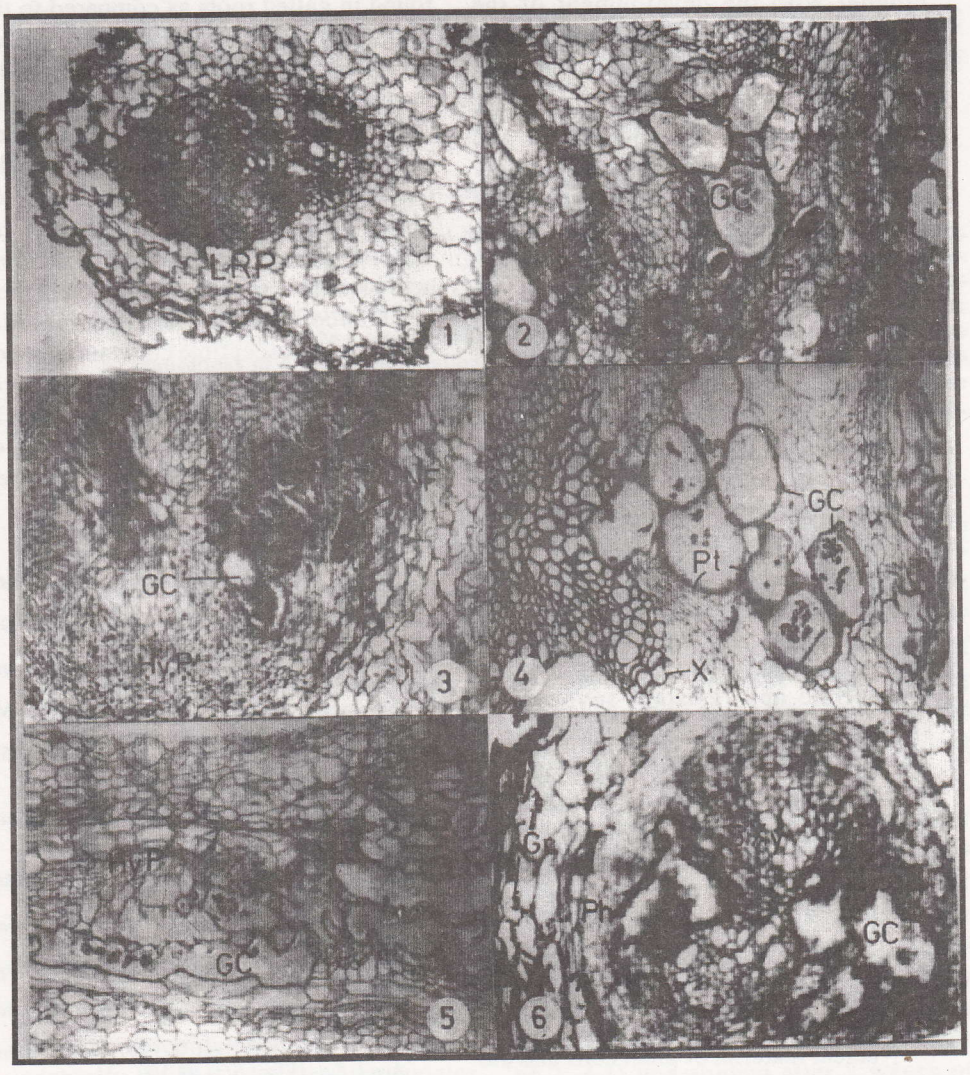
(i) *Healthy root* :- Cytoplasm and nuclei stained positively for RNA.

(ii) *Infected root* :- RNA content was higher in galls than the healthy roots. It was mainly concentrated in the cells of infection court, the cells surrounding the head of the nematode and adjacent hyperplastic parenchyma. Giant cell initials around the head of L_2S were rich in RNA and possessed hypertrophied nuclei with large nucleoli (Fig. 5).

Ascorbic acid

(i) *Healthy root* :- A few brownish-black or tan coloured granules of silver representing ascorbic acid were found in young roots in cortical and epidermal cells. In older roots the granules were localized in phloem parenchyma.

(ii) *Infected root* :- Comparatively more ascorbic acid was localized in galls than healthy roots. The frequency and size of silver grains increased from outer cortical cells to endodermis (Fig. 6). Giant cells associated with L_2S contained a few granules, however, they increased in



Figures 1-6. *Meloidogyne incognita* on *Pisum sativum*. 1-2 carbohydrate. 1. T.S. healthy root, lateral root primordium filled with PAS granules. x 100. 2. T.S. gall . x 100. 3. T.S. gall, localization of protein. x 100. 4. Localization of DNA in T.S. gall. x 100. 5. L.S. gall. RNA in nuclei and cytoplasm. x 400. 6. T.S. gall, ascorbic acid granules. x 100.

(GC-Giant cell; Gr-Granule; Hy P-Hyperplastic parenchyma; NF-Nematode fragment; LRP - Lateral root primordium; Ph - Phloem; Pt - Protuberance; SXy - Secondary xylem; T-Tail)

number with the development of the nematode and by the time the female died, the giant cells were full of ascosbic acid.

Discussion

Thick cell walls of hyperplastic and hypertrophic cells were stained intensely purplish red indicating the deposition of cellulose. Syncytial wall with its protuberances stained strongly with PAS reagent. The giant cell wall contained all the polysaccharide contents of cell wall except lignin[9,10]. The absence of starch in giant cells and cells around them might be due to secretion of amylase by the nemetode that led to the hydrolysis of starch. It seemed that soluble polysaccharides produced by the hydrolysis of starch were absorbed by the giant cells. They were further broken down and finally drawn by the nematode during feeding. Increase in hydrolytic enzymes at the feeding site in root-knot susceptible and resistant soybeans is known[11]. Absence of starch in giant cells was also reported by ealier workers[12,13].

The increase in protein content in the infected roots was also reported by many workers[10,13,14]. The gradual increase in the protein content from the cells away to the giant cells suggested a flow of proteinaceous matter towards the nematode and increased protein synthesis around the infection site.

Nuclear material of giant cells and the nematode body is abundant in DNA, whereas RNA was localized in nucleoli of giant cell nuclei, cytoplasm and in the nema bodies. greater amount of DNA and RNA inside the giant cells may be due to their greater metabolic activity. Owens and Novotny[15] reported 2-fold increase in nucleic acid content which accumulated in giant cells and nema bodies in *M. incognita* infected cucumber and tomato roots. Similar increase in nucleic acid content was also reported by other workers[16,17].

More amount of ascorbic acid was found in galled roots as compared to the healthy ones. The development of cyanide - resistant respiration was conditioned by the presence of ascorbic acid in the cells. Thus ascorbic acid could be considered as a factor of primary importance in the biological defense mechanism of plants and animals[18]. Arrigoni *et al.* demonstrated that a decrease in ascorbic acid in plants induced a reduction in their resistance to root - knot nematode. Conversely, susceptible cultivars irrigated with water solution of ascorbic acid reacted similarly to resistant cultivars. In pea plants, the higher occurrence of ascorbic acid indicated a limited resistance response.

References

1. Sharma R K and Tiagi B 1986, *Biol. Bull. India* **8** 48
2. Sharma R K, Mathur A and S Singh 1996, *J. Phytol. Res.* **9** 169
3. Jensen W A 1962, *Botanical histochemistry* W.H. Freeman Co. London.
4. Johansen D A 1940, *Plant Microtechnique* Mc Graw-Hill Book Co. Inc. New York.
5. Mazia D, Brewer P A and Alfert T M 1953, *Biol. Bull.* **104** 57
6. Kallarackal J 1974, *Curr. Sci.* **43** 120
7. Tepper H B and Gifford (Jr.) E M 1962, *Stain. Technol.* **37** 52
8. Chayen J 1953, *Int. Rev. Cytol.* **2** 78
9. Dropkin V H and Nelson P E 1960, *Phytopathology* **50** 446
10. Bird A F 1961, *J. Biophys. Biochem. Cytol.* **2** 710
11. Veech J A and Endo B Y 1970, *Phytopathology* **60** 896
12. Orion D and Bronner R 1973, *Nematologica* **19** 401
13. Trivedi P C and Tiagi B 1980, *Proc. Indian Acad. Sci.* (Plant Sci) **89** 109
14. Owens R G and Specht H N 1966, *Contrib. Boyce Thompson Inst.* **23** 181
15. Owens R G and Novotny H M 1960, *Phytopathology* **50** 650
16. Littrell R H 1966, *Phytopathology* **56** 540
17. Rubinstein J H and Owens R G 1964, *Contr. Boyce Thompson Inst.* **22** 491
18. Arrigoni O 1979, *Root-Knot nematodes (Meloidogyne species) systematics, Biology and Control* Ed. Lamberti F and Taylor CE, Academic Press, New York, 457
19. Arrigoni O, Zacheo G, Arrigoni - Liso R, Bleve - Zacheo T and Lamberti F 1979, *Root knot nematodes (Meloidogyne species). Systematics, Biology and Control* Ed. Lamberti F and Taylor C E, Academic Press, New York, 469