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Histochemical localization of lipids, and lignin in healthy and *Meloidogyne incognita*, infected sunflower (*Helianthus annuus* Linn.)

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Abstract
The root knot nematodes, belonging to the genus Meloidogyne, which have a wide host range, are the deadliest enemy of vegetable crops and a pest of crucial economic importance. These produce conspicuous galls on the roots and the infestations can be recognized easily in fields. It is very common and abundant in the sandy soils of Jaipur and adjoining areas. In Jaipur district, Sunflower an economically important vegetable crop was found infested with highly pathogenic *Meloidogyne incognita*. Histochemical tests were applied to localize lipids and lignins in the diseased and healthy root tissues. The metabolites viz. lipids and lignins were found more in galled roots as compared to healthy roots.

Keywords: *Meloidogyne incognita*, histochemical localization, root knot nematode, sunflower, metabolites

Introduction

Human kind depends in a myriad ways on plant and plant products. The quest for new plant products and new methods of using them to satisfy the emerging needs is an ever expanding enterprise. Unfortunately, these plants become the victim of many disease causing micro-organisms including the plant parasitic nematodes which can bring about disruption in the physiological equilibrium of the attacked plants. The plants, in turn, react in a number of ways to off-set these disturbances and the overall plant response determines, to a large extent, the success or failure of the interactions. For a better understanding of various histopathological changes that occurred as a result of nematode infection, in situ localization of various metabolites was helpful. Although, some pathogens used mechanical force to penetrate plant tissues but subsequent development of disease syndrome was dependent on histochemical alterations and biochemical reactions taking place between substances secreted by the pathogens and those already present or produced by the host as a response to the infection.

Histochemical techniques were advantageous as they enabled in situ localization of various metabolites at the site of their synthesis or action. While information is available on morphological and biochemical changes that occur in plants invaded by endoparasitic nematodes, little work has been done with a histochemical technique. Since the anatomical area that is biochemically affected by such nematode infections may be quite small, it is imperative that histochemical techniques be employed, lest the effects of infection be lost by dilution from non-affected cells.

After histochemically studying the soybean roots infected by *Meloidogyne* sp., it was reported that giant cell walls contained cellulose and pectin but lacked lignin, suberin, starch or ninhydrin positive substances (Dropkin and Nelson, 1960) [9]. It was observed that *M. javanica* induced giant cell in tomato contained traces of carbohydrates and fats but it was particularly rich in protein and RNA. The large irregularly shaped nuclei contained a large nucleolus and a number of feulgen-positive bodies, scattered irregularly along the nuclear envelope (Bird, 1961) [2].

It was reported that in ginger infected with root-knot nematode, giant cell nuclei and cytoplasm were rich in nucleic acids. Starch was absent in the giant cells and in the cells of the infected region of the rhizome.

Giant cells showed the presence of minute protein granules. The outer side of the egg sac consisted of a thick layer of insoluble polysaccharides (Shah and Raju, 1977) [20].

The nucleic acid changes in three tomato cultivars infected with *M. incognita* were estimated; the observations showed that the amount of DNA and RNA was higher in roots of inoculated plants as compared to healthy ones in all the three cultivars (Masood and Saxena, 1980) [17].

In the present investigation histochemical tests were applied to localize total lipids, lignins, cellulose and ascorbic acid in the diseased and healthy root tissues. It will prove helpful for a clear understanding of host parasite interaction.

Material and Method

Collection of root samples

All the experiments were carried out in the Botanical Garden, Department of Botany, University of Rajasthan, Jaipur. Earthen pots were washed, cleaned and disinfested before use, by rinsing them through four percent formalin solution. The formalin was allowed to evaporate before their use for experiments. Sandy soil were steam sterilized. Earthen pots were filled with sand and soil mixture (1:1). Standard doses of NPK were used in the sand soil mixture and mixed thoroughly before filling the pots. Three random samples of sterilized soil samples were processed by modified sieving and Bearmann funnel technique [6] and examined to ensure that the sterilized soil was free from nematodes.

Isolation of nematodes (Whitehead & Hemming, 1965)

Isolation of nematodes was done by Whitehead and Hemming (1965) tray method. A sieve to support was made from a plastic covered letter basket (22 x 32 cm) or other large plastic basket inside, which was placed a course plastic mesh or seed tray and on top of this a double layer of tissue paper or milk filters. The basket was placed in collecting tray with 100 gm of finally crumbled soil was evenly spread in a thin layer over filter in the basket. Water was carefully added inside edge of the collecting tray until soil layer looked wet. To obtain edge of the collecting tray until soil layer looked wet. To obtain a clean extract it was important not to move the tray once the water had been added.

Evaporation was lessened by covering polyethylene sheeting. The nematodes were collected on floor of tray after 24 h. The basket was then slowly and carefully removed and nematode suspension from the tray was poured in the beaker and allowed to settle for 2-4 h then the supernatant water was decanted out or siphoned off.

Mass culturing of *M. incognita*

For mass culturing and preparation of nematode suspension brinjal was used as host plant. Plants were inoculated with isolated second stage juveniles. After three weeks when brinjal plants started showing the symptoms were carefully lifted from micro plots in the in the Botanical Garden, Department of Botany, University of Rajasthan, Jaipur. Roots were washed carefully in running tap water. Then roots were placed on the tissue paper for extraction of 2nd stage juveniles by Whitehead and Hemming Tray method. After 24 h water was collected in a beaker and nematode suspension was prepared for the inoculation of sunflower plants.

Screening of sunflower genotypes against *M. incognita*

Genotypes of sunflower were obtained from various AICRP – sunflower centers for the purpose of evaluating response against M. incognita inoculation. The seeds were sown in 15cm earthen pots containing 500 g of sterilized soil and sand mixture (1:1). Inoculation of freshly hatched J2s of M. incognita was done @ two J2s /g of soil at 10 days after sowing. Each treatment was replicated thrice and arranged in completely randomized design and given light irrigation as and when required.

Histochemical Techniques

Localization of Total Lipids

Lignin

Lignin was localized by phloroglucinol-HCL test (Johansen, 1940; Siegel, 1953) [14, 23].

Procedure-Freshly cut sections were placed on a slide and a drop of saturated aqueous solution of phloroglucinol made in 20% hydrochloric acid is added to it.

Lipids

Total lipids were localized by the methods given by Chiffelle and Putt (1951) [7].

Preparation of Sudan III dye - 0.7 g of the dye was dissolved in 100 ml of propylene or ethylene glycol. The solution was heated to 100-101C and stirred thoroughly. The dye was used after filtering through Whatman No.1 filter paper.

Procedure - Freshly cut sections of the tissue were placed in ethyl glycol for 3-5 minutes and subjected to occasional shaking. The sections were then transferred to a Sudan III dye and stained for 5-7 minutes. Sections were then transferred to ethyl glycol and water, and shaken for 2-3 minutes. After a through washing for 3-5 minutes in distilled water, the sections were mounted in glycerine and observed.

Observations and Results

Results are presented in Table and Plate 1 and 2, Fig. A-B.

Lignin appears orangish-violet in colour. In cortex of normal root deposition of lignin was found. Epidermis also took stain for lignin. Xylem region of both gall and normal root showed intense violet colour of lignin (Plate-1, Fig-A). Xylem region of gall was lignified as compared to normal root (Plate-1, Fig-B). Lignin was not observed in medullary rays. In gall, nematode also took stain for lignin.

Fat, oil and waxes stained orange. Epidermis, cell walls of cortical cells and vascular bundle of normal root showed presence of lipids (Plate-2, Fig-A). In root gall, large lipid globules were found in hypertrophied region of cortex. Parenchymatous cells near the mature female were also having lipid droplets. Cells of cortex and vascular bundle also took stain for lipid. Eggs and egg matrix were rich in lipid (Plate-2, Fig-B).

Discussion

Plant tissues are extremely complex, both morphologically as well as physiologically. Infection of any kind brings about a further change in the morphology and physiology of the host. Plant gall is one such example where, gall morphogenesis is the result of interaction between host and pathogen. Thus, galls are pathological structures, characterized by various structural and physiological changes in the host tissue.

To understand the physiological changes in terms of morphology certain histochemical studies have been done. An attempt has been made to interpret the biochemical data in terms of cells, tissues and tissue systems. Fresh hand cut sections were used to study the distribution and the changes brought in the lignin and lipid of the normal and galled tissue of Helianthus annuus.

Lipids are heterogenous group of compound that are insoluble in water but soluble in non-polar organic solvents. Sudan dye

stain orange fats, oil and waxes. These dyes are more soluble in the lipids than in the solvent in which they are initially dissolved.

In the root gall of sunflower, lipid globules were localized mainly in the Eggs and egg matrix. In root gall, large lipid globules were found in hypertrophied region of cortex. Parenchymatous cells near the mature female were also having lipid droplets. Cells of cortex and vascular bundle also took stain for lipid. Eggs and egg matrix were rich in lipid.

The amount of lipids increased with the development of giant cells and the nematode. Bird (1961) [2] noted traces of fat in syncytial cytoplasm and presence of fat. This accumulation of lipids was supposed to be the storage material for the nematode. Survival and infectivity of second stage larvae were known to be dependent on the food stored in the eggs (Van Gundy *et al*., (1967) [27]. Gelatinous matrix and eggs were found to be rich in lipids as reported by Bird and Rogers (1965) [4] and Trivedi and Tiagi (1983) [26].

Lignin

Giebel (1970) [11] observed that necroses formed in roots of *Heterodera rostochiensis* resistant potatoes had lignin like Fat, oil and waxes stained orange. Epidermis, cell walls of cortical cells and vascular bundle of normal root showed presence of lipids. In root gall, large lipid globules were found in hypertrophied region of cortex. Parenchymatous cells near the mature female were also having lipid droplets. Cells of cortex and vascular bundle also took stain for lipid. Eggs and egg matrix were rich in lipid. This indicated that substances secreted by the nematode were involved in the process of lignification.

Histochemical Analysis

S.N.	Localization	Normal root	Galled root
1.	Lignin	+++	++++
2.	Lipid	++	+++

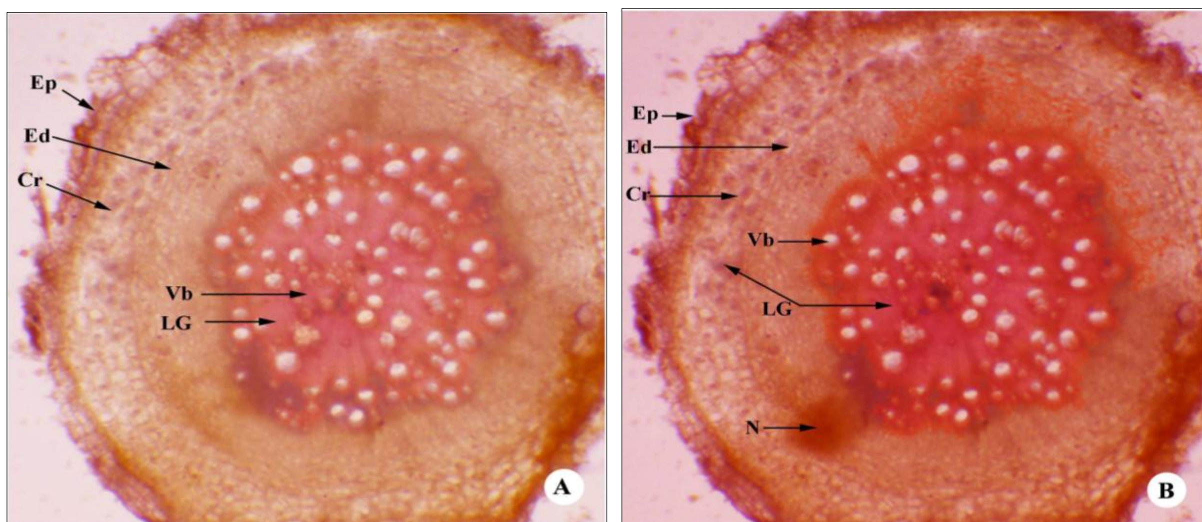

Plate 1: Localization of lignin

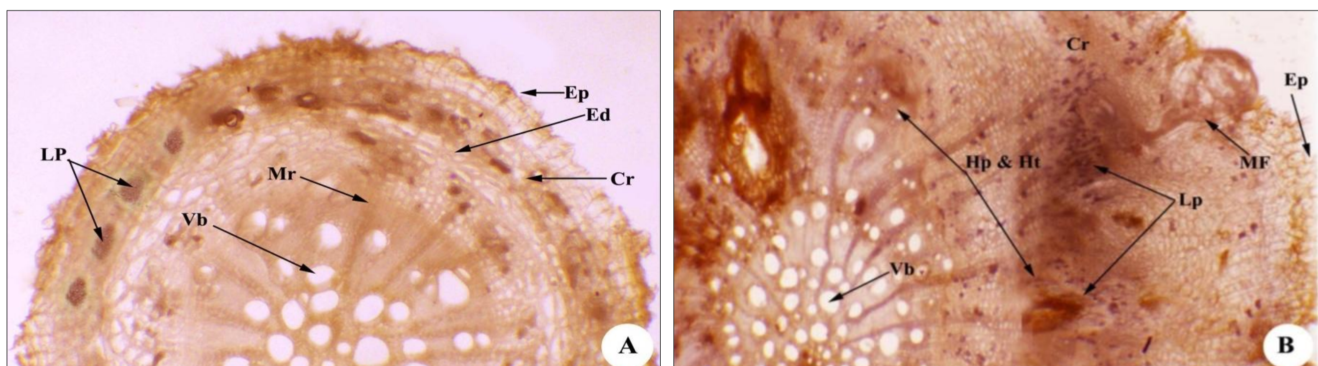

Plate 2: Localization of lipid

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References

1. Arrigoni O. A biological defence mechanism in plants. In: Root-knot nematodes (*Meloidogyne* species) systematic, Biology and Control, Ed. F. Lamberti and C.E. Taylor, Academic Press, New York, 1979, 457-467.
2. Bird AF. The Ultrastructure and histochemistry of a nematode induced giant cell. Journal Biochemistry Cytology. 1961; 11:701-715.
3. Bird AF. Plant response to root knot nematods. Annual Review of Phytopathology. 1974; 12:69-85.
4. Bird AF, Rogers GE. Ultrastructural and histochemical studies of the cells producing the gelationous matrix in Meloidogyne. Nematological. 1965; 11:231-238.
5. Bird AF, Saurer W. Changes associated with parasitism in nematode II Histochemical and micro spectrophotometric analysis of pre-parasitic and parasitic larvae of *Meloidogyne javanica.* Journal Parasitology.

1967; 53:1262-1269.

6. Chayen J. Ascorbic acid and its intracellular localization with special reference to plants. International Review of Cytology. 1953; 2:78-132.
7. Chiffelle TL, Putt A. Propylene and ethylene glycol as solvents for sudan IV and Black B; Stain tech. 1951; 26:51-56.
8. Chinoy NJ. On the specificity of the alcoholic acidic $AgNO_3$ reagent for the histochemical localization of ascorbic acid. Histochemic. 1969; 20:205-207.
9. Dropkin VH, Nelson PE. The histopathology of root-knot nematode infection in soybeans. Phytopathology. 1960; 50:442-447.
10. Erickson RO, Sax KB, Ogur M. Perchloric acid in cytochemistry of pentose nucleic acid. Science. 1949; 110:472-473.
11. Giebel J. Phenolic content in roots of some solanaceae and its influence on IAA-oxidase activity as indicator of resistance to *Heterodera restochinensis Nematologica*. 1970; 16:22-32.
12. Gomori G. Microscopic histochemistry Microscopic histochemistry Principles and practice Univ. Press, Chicago, 1952, 83.
13. Jensen WA. Botanical Histochemistry. W H Freeman Co. London, 1962.
14. Johansen DA. Plant micro technique McGraw Hill Book Co. New York, 1940, 523.
15. Kallarackal J. A modified schiff's reagent for use in Feulgen reaction. Curr Science. 1974; 43:120-121.
16. Kannan S. Studies in nenatode infected root knots of the tomato plants. Indian Journal Expt Biol. 1968; 6:153-154.
17. Masood A, Saxena SK. Nucleic acid changes in three tomato varieties infected with *Meloidogyne incognita*. Indian Journal Nematology. 1980; 10:102-104.
18. Mazia D, Brewer PA, Alfert TM. The Cytochemical staining and measurement of protein with mercuric bromophenol blue. Biological Bulletin. 1953; 104:57-67.
19. Rubinstein JH, Ovens RG. Thymidine and uridine incorporation in relation to the ontogeny of root knot syncytia. Contr Boyce Thompson Inst. 1964; 22:491-502.
20. Shah JJ, Raju EC. Histopathology of ginger infected by *Meloidogyne* sp. Phyton. 1977; 16:79-84.
21. Sharma A, Trivedi PC. Control of root-knot nematode on *Trigonella foenum-graecum* by *Paecilomyces lilacinus*. Nematol Medit. 1989; 17:131-133.
22. Sharma RK, Sarna NT, Tiagi B. Effect of foliar application of growth regulators on pea plants infected by *Meloidogyne incognita*. Journal Indian Botany Society. 1989; 68:84-86.
23. Siegel SM. Biosynthesis of lignins. Physiol. Plantarum. 1953; 6:134-139.
24. Tepper HB, Gifford (Jr.) EM. Detection of ribonucleic acid with pyronin. Stain Technology. 1962; 37:52-53.
25. Trivedi PC, Tiagi B. Histochemical study of *Capsicum annum* L. root galls incited by *Meloidogyne incognita* chitwood, Proc Indian Alad Science. (Plant Science). 1980; 89:109-115.
26. Trivedi PC, Tiagi B. Control of root-knot on chilli with nematicides. Journal of Res Assam Agri Univ. 1983; 4:65-66.
27. Van Gundy SD, Bird AF, Wallace HR. Aging and Starvation in larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. Phytopathology. 1967; 57:559-571.
28. Veech JA, Endo BY. Comparative morphology and enzyme histochemistry in root knot resistant and Suscepible Soybeans. Phytopathology. 1970; 60:896-902.
29. Whithead AG, Hemming JR. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol, 1965, 38.