

HISTOCHEMICAL ALTERATIONS IN NUCLEIC ACIDS BY *MELOIDOGYNE INCOGNITA* INCITED ROOT GALLS IN *TRIGONELLA FOENUM-GRAECUM*

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Trigonella foenum-graecum is severely infected by *Meloidogyne incognita*. Histochemical changes in DNA and RNA were suited in healthy and root knot infected materials collected after 60 days of nematode inoculation. There was an increase in nucleic acids in galls as compared to healthy roots. Giant cells were rich in DNA and RNA. Nema bodies and giant cells nuclei stained strongly for nucleic acids.

Keywords : DNA ; Giant Cell ; *Meloidogyne incognita* ; Nematode; RNA ; *Trigonella foenum-graecum*

Fenugreek (*Trigonella foenum-graecum*) was found severely infected by root-knot nematode *Meloidogyne incognita*¹. Infection by any pathogen leads to metabolic alterations in diseased plants due to 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. Histochemical methods are advantageous as they enabled *in situ* localization of various metabolites at the site of their synthesis or action. Trivedi and Tiagi² have reported that root galls of *Capsicum annum* incited by *Meloidogyne incognita* were richer in DNA, RNA, basic proteins and ascorbic acid as compared to healthy roots. In the present study an attempt has been made to study such histochemical changes in DNA and RNA.

Sterilized and specific *Rhizobium* culture treated seed of *Trigonella foenum-graecum* were shown in autoclaved soil. Nematode inoculation was done when seedlings were two weeks old. Egg masses were isolated from the pure culture of *Meloidogyne incognita*, maintained and multiplied on brinjal. These egg masses were allowed to incubate in a Baermann funnel for 72 hours. Hatched larvae were collected and inoculated, by pipetting and pouring larval suspension containing 1000 juveniles, around

the seedling. Plants were uproots after 60 days of inoculation. The plant material was fixed in Carnoy's fluid. Then it was dehydrated through tertiary butyl alcohol (TBA) series, infiltrated and embedded in paraffin wax. Serial transverse sections of the embedded materials were cut at a thickness of 14 μ with the help of rotatory microtome and mounted on clean slides using Haupt's adhesive. DNA was localized by Feulgen method and RNA by pyronin-Y staining³.

In healthy roots nuclei in the cells stained positive for DNA. Xylem stained non specifically. The hypertrophied parenchyma cells surrounding the giant cell complex also showed prominently stained nuclei. Walls and cytoplasm did not stain for DNA. Nematode body of all stages stained feebly while eggs were found to be Feulgen-positive. Prominent large nuclei of giant cell complex stained positively for DNA (Table1).

RNA was found to be higher in galls than in healthy roots. The nuclei in healthy roots stained pink with Pyronin. The cytoplasm also showed a positive reaction. The cell wall and xylem elements stained non-specifically. In galls, the giant cells and hypertrophied cells stained strongly. All stages of nematodes also stained positively for RNA (Table1). The developing syncytium was found to be rich in RNA which was localized

mainly in the nucleoli, nucleoplasm and cytoplasm. Giant cells associated with mature females showed a prominently stained nucleolus, a granular cytoplasm and nucleoplasm which was uniformly stained. Walls of giant cells stained non-specifically.

Nucleic acid content of *M. incognita* infected fenugreek root galls increased as compared to the healthy roots. Such nematode induced nucleic acid increase was also reported earlier^{2,4,5}. Syncytial nuclei in

infected fenugreek showed the presence of RNA and feulgen positive granules. Similar observations were made by Rubinstein and Owens⁶. They reported that DNA synthesis was dependent upon a close association of the feeding nematode with the giant cell. Such giant cells became a region of intense metabolic activity. Lobed syncytial nuclei were sites of intense nucleic acid synthesis as reported by Paulson and Webster⁷.

Table 1. Histochemical localization of nucleic acids in healthy roots and galls on fenugreek induced *M. incognita*.

Nucleic acid	Regions localized	
DNA	Healthy root	++
	Galls	+++
	Giant cell : Nuclei	+++
	Cytoplasm	—
	Nuclei of hypertrophied	
	Parenchyma cell	+
	Nematode	++
RNA	Healthy root	+
	Galls	+++
	Giant cell : Nucleoplasm	+
	Nucleoli	+++
	Cytoplasm	++
	Wall	—
	Nematode	++

Note :- + Nucleic acid present.

++,+++ Denotes increasing intensity of nucleic acid

— Nucleic acid absent.

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