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Histopathological Responses and Damage on the Roots of *Vigna mungo* Infected by Root Knot Nematode, *Meloidogyne incognita*

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Abstract: The plants of black gram, *Vigna mungo* were inoculated with 5, 10, 15, 20 and 25 egg masses of root-knot nematode (*Meloidogyne incognita*) per pot, under green house condition. The infected plants were significantly reduced growth of the highest inoculum levels of egg masses. The number of galls was greatly influenced by the initial population of the nematode. The second stage juveniles of root knot nematodes infected root epidermis of the roots of *Vmungo while* penetrating into the inner tissues of the young roots of the experimental plants. The juveniles migrated towards differentiating vascular tissues and their migration was done to intra into inter cellular level. In older roots, they migrated through the cortex and they caused formation of giant cells in the form of clusters. These giant cell cluster having dense cytoplasm and enlarged nuclei. All the nuclei enclosed one to few nucleoli and he giant cell clusters changed the internal morphology of the affected tissue. In addition, abnormal xylem and abnormal phloem also occupied in a major portion of the giant cells formation.

Key words: Abnormal xylem · Vascular tissues · Cortex · Giant cells · Egg masses

INTRODUCTION

Black gram is one of the important pulses, is mostly produced in Asian countries as their tropical climate and soil type suits its cultivation. India is largest producer of this pulse followed by Myanmar and Thailand. India produces 70% of worlds' black gram production and Black gram accounts for 10% of total pulse production in India. Black gram is a rich protein food. It contains about 26 percent protein, which is almost three times that of cereals. In addition, being an important source of human food and animal feed, it also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. Being a drought resistant crop, it is suitable for dry land farming and predominantly used as an intercrop with other crops [1].

Root-knot nematodes, *Meloidogyne* spp. are considered the most damaging nematode group in the world as they cause high yield losses to most cultivated plant species in subtropical and tropical regions [2]. Root-knot nematodes are among the most damaging nematodes in agriculture and consider one of the major limiting factors affecting plant growth and yield causing an estimated $100 billion loss/year worldwide [3]. Root-knot nematodes infect the roots of different fruit, vegetable and cereals and induce gall formation at the terminal or sub-terminal portions of the affected roots and other underground plant parts. Second stage juvenile of nematode cause giant cell formation, hyperplasia of protophloem and abnormal xylem proliferation [3]. These changes result in swelling at sites in stele and also surrounding cortex forming distant galls. Hence the present study was analysed to examine histopathology of infected roots of black gram, *V. mungo*.

MATERIALS AND METHODS

The healthy seeds of host plant, *Vigna mungo* were chosen and their surface was sterilized in 0.01% (w/v) mercuric chloride solution for five minutes. They were rapidly washed well with distilled water and then soaked in distilled water for two hours. *V. mungo* seeds were sown in mud pots of two liter capacity. The nematode egg masses were collected from the roots of infected tomato plants from pure culture of nematode. The egg masses

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were isolated and separated using a compound microscope (45X). The average number of eggs per egg masses < 100 eggs. The collected egg masses were separated at different levels by counting (5, 10, 15, 20 and 25 eggs) and the counted egg masses were inoculated in the experimental pots (Three replicates). For histopathological studies after 65 days of old roots of *V. mungo* were thoroughly washed with tap water and galled portion were cut and fixed in Formalin Acetic Acid-ethyl alcohol (FAA). From the fixed material, 10 mm thick sections were made by using a microtome by conventional method [4]. These slides were dried and waxed and stained in safranin fast green [5]. The sections were mounted using DPX mountant with rectangular cover slip. The slides were examined under research microscope and photographs were taken.

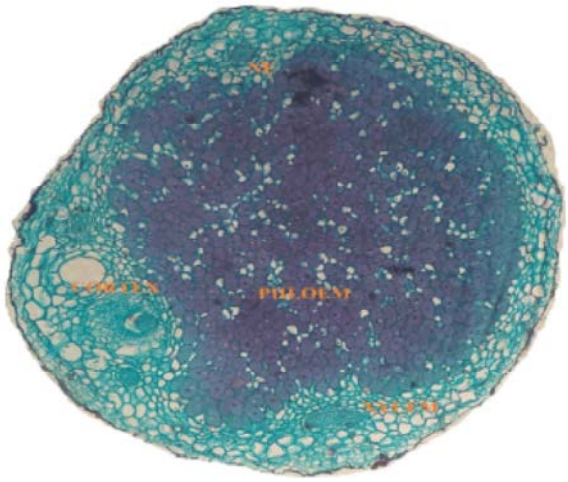

Fig. 1A: Dark colored masses of cells

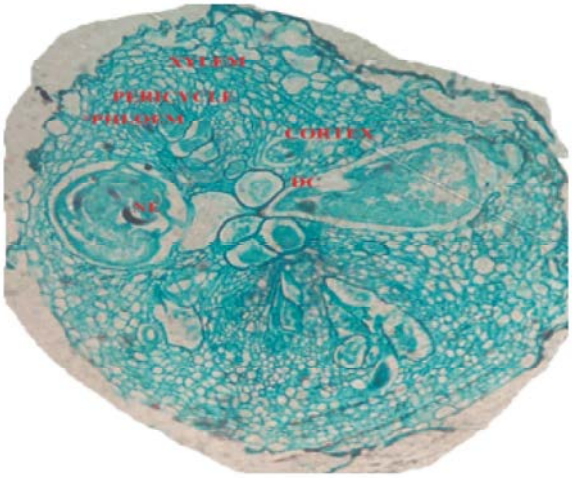

Fig. 1B: Derangement of cells

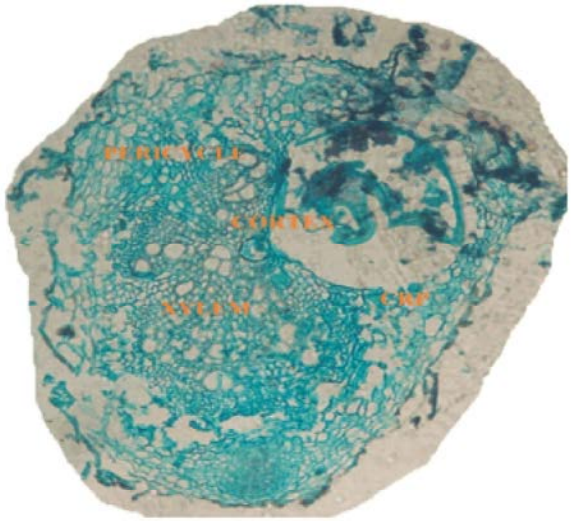

Fig. 1C: Larvae migrated and settled in cortical region

RESULTS AND DISCUSSION

In the present study, transverse sections of the roots of *V. mungo* infected with *M. incognita* exhibited severe damage to the nematode. The second stage juveniles after penetrating to the cortical cells along the pathway of nematode developed necrotic walls and appeared as dark colored masses of cells (5 egg mass inoculum). The larval penetration caused the damage and derangement of cells (10 egg mass inoculum). The larvae migrated through cortical region and finally settled in the various parts (15 egg mass inoculum). The roots early stages of infection showed destruction of phloem i.e., changes in the steler region (20 egg mass inoculum). First partially and later completely due to the pressure of accumulating piles of undifferentiated tissues in the infected zones. In the later stages of infection steler part, especially phloem parenchyma, xylem parenchyma and medullary ray parenchyma were observed as the chief sites of feeding. The cells in the infected steler region were divided further to form the abnormal xylem and phloem. Abnormal morphology of these tissues displaced the other xylem and parenchyma elements from their normal position. The cluster of giant cells was generally surrounded by a large number of abnormal xylem elements. Infected roots showing hyper-trophied cells in the pericycle with granular appearance (25 egg mass inoculum) (Fig. 1A-1E). The second-stage juveniles of *M. incognita* after migrating towards the stellar region causing extensive damage to the cortical cells. The juveniles immediate induced the formation of giant cells as well as proliferation of neighboring tissues resulting in formation of root galls.

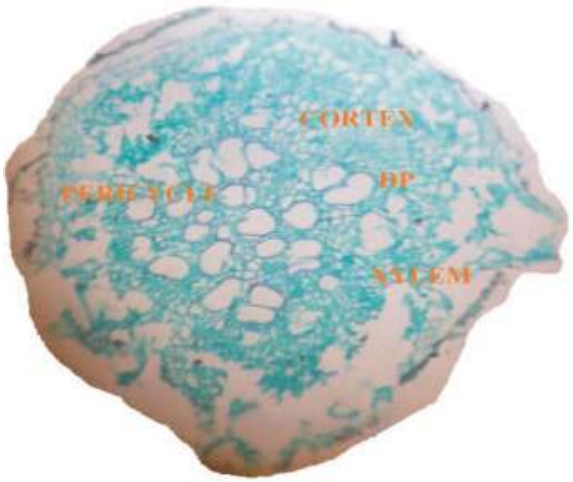

Fig. 1D: Destruction of phloem

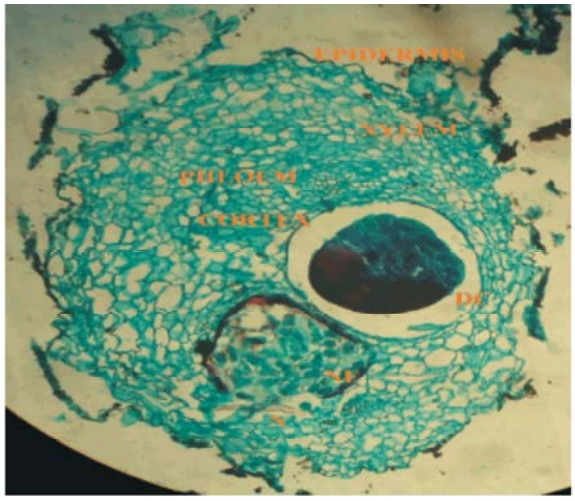

Fig. 1E: Hyper-trophied cells in the pericycle with granular appearance

N: Nematode; NE: Nematode egg; HC: Hyper trophid cells; CRP: Cortical regional phloem; DP: Differentiated phloem

Hyperplastic and hypertrophy in the tissue in the close vicinity of the nematode were clearly noticed. Our studies revealed that *M. incognita* induced 4-6 multinucleate giant cells in the vascular tissues and stellar region[6] reported 3-6 giant cells in tomato, similarly 4-9 giant cells were reported in sweet potato [7]. The site of infection is usually vascular tissue but as a result of nematode development and giant cell formation, the entire complex of nematode and giant cells appear to be located in the cortex. The nematode stimulates the giant cells to synthesize cytoplasm in enormous amount. To cope with this situation, the number of nuclei are increased which enhance the rate of metabolism at a tremendous rate. Similarly Vovlas *et al*. [8] reported that *M. javanica* induced feeding sites that consisted of three to four hypertrophied giant cells per adult female and infection of feeder roots by the nematode resulted in mature large galls which usually contained at least one mature female and egg mass. They found that the feeding sites in both tubers and roots were characterized by giant cells containing granular cytoplasm and many hypertrophied nuclei with the cytoplasm in giant cells being aggregated alongside the thickened cell walls where stelar tissues within galls appeared disorganized.

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