Madras Agric. J., 2023; https://doi.org/10.29321/MAJ.10.200806

RESEARCH ARTICLE

Exploring the Plant Nematode Interaction of *Meloidogyne Incognita* under Okra Ecosystem

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Received: 11 May 2023

Revised: 26 May 2023

Revised: 03 June 2023

Accepted: 29 June 2023

ABSTRACT

Southern root knot nematode, *Meloidogyne incognita* is an important nematode genus infesting okra growing tracts of Tamil Nadu. The present study was carried out to understand the cytological changes that occur during *Meloidogyne incognita* interaction with okra roots by employing Scanning Electron Microscope (SEM). The results exhibited the explicit changes in cellular structures of healthy and nematode-infested roots. The healthy roots possessed normal cell size for metaxylem, protoxylem, pith and cortex region nevertheless nematode infested roots revealed coalesced cellular structures. Root cell wall thickening, cell wall ingrowth, and starch granule accumulates of varying diameter were also observed in M. incognita infested okra roots, which were found to be the factors responsible for producing above ground symptoms during host-nematode interaction. Longitudinal imaging of nematode infested roots showed the nematode entry points of varying diameter along with adhered juveniles Juveniles on root topography and this was in contrast in *M. incognita* infested okra roots. Comparatively, these structures were absent in healthy roots.

Keywords: *Host- Nematode interaction; Okra roots-; Meloidogyne incognita; Ultrastructural changes; Scanning Electron Microscope (SEM)*

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) belongs to the family Malvaceae and is an predominant vegetable crop cultivated across tropical, subtropical, and temperate countries (Singh, 2012). The okra fruit is rich in carbohydrates, proteins, vitamin A, vitamin C, vitamin K, vitamin B6, potassium, calcium, manganese, magnesium, thiamine, and folate, which account for about 31 kilo calories of energy per 100 grams of raw fruits (Badrie, 2016). Plant parasitic nematodes play key role in limiting the productivity of crops. Annual crop losses due to nematodes across the world accounts for about 80-173 billion US dollar and in India for about 242.1 billion Indian rupees with 14.6% yield loss in open field conditions and nearly 60% yield loss under protected cultivation (Gowda, Rai, & Singh, 2019). Plant parasitic nematodes also provoke other soil pathogens like fungi, bacteria, and virus. Among the different plant parasitic nematodes root-knot nematode, *Meloidogyne* spp. are regarded to be the most severe polyphagous sedentary endoparasites invading over 5500 species of crops worldwide and are considered one among three important nematode genera which are placed under most serious pests in world (Kayani, Mukhtar, Hussain, & Ul-Haque, 2013).

Symptoms of okra crops infested with root-knot nematode, *Meloidogyne incognita* include reduced plant growth yellowing of leaves, reduced fruit yield, and poor emergence of seedlings in the first few weeks after sowing. As a result of nematode feeding, hypertrophy and hyperplasia occurs in root cells leading to the formation of root galls. During the final stages, the root rots and predisposes to soil borne pathogens. The yield loss on okra due to *Meloidogyne* spp. in India accounts for about 27 percent (Gowda *et al.,* 2019).

Root knot nematode- host interaction process ramifications in formation of giant cells, as nematodes secrete the effector proteins that interacts with genes and proteins of hosts leading to successful parasitism. As nematode feeding ends up with formation of giant cells, which are consequence of hypertrophy and hyperplasia, exploring root structure changes is requisite. Hence histopathological studies play an important role in understanding the cell wall dynamics of host roots

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associated with nematodes which provide new insights in host-nematode interaction (Vilela *et al.,* 2021).

Previous studies attempted to display the ultra-structural changes in host roots due to nematode feeding, but very little literature is available in relation to okra. Hence the present study was narrowed down to observe the ultra-structural changes on okra roots under monopartite condition (host alone) and dipartite interaction (host and nematode) using Scanning Electron Microscope (SEM).

MATERIAL AND METHODS

Collection of planting materials

The okra seeds (CO-BH-4) required for the present study were obtained from Department of Vegetable Science, Tamil Nadu Agricultural University and sown in sterilized pot mixture (2:1:1 ratio of red earth: sand: FYM which were sterilized at 121psi for 15 mins). Okra roots infested with *M. incognita* was collected from the field in Coimbatore district, Tamil Nadu, India. The egg mass was collected from the roots under stereoscopic microscope and allowed for incubation at room temperature for four days.

Inoculation of nematodes

The hatched- infective stage juveniles (J2) were used for inoculation purpose for the rest of study. At 21 days after sowing (DAS), the freshly hatched juveniles were carefully pipetted out and inoculated near the rhizosphere region of plants. The plants were uprooted at 60 DAS and roots were carefully uprooted for further studies.

Egg topography

To study the egg topography, eggs of *M. incognita* were observed under SEM. The egg masses were carefully detached from the infested roots and cleaned in sterile water to remove the adhered materials. The egg mass was then placed in SEM stub and sputter coated with gold palladium and observed under SEM for studying topography.

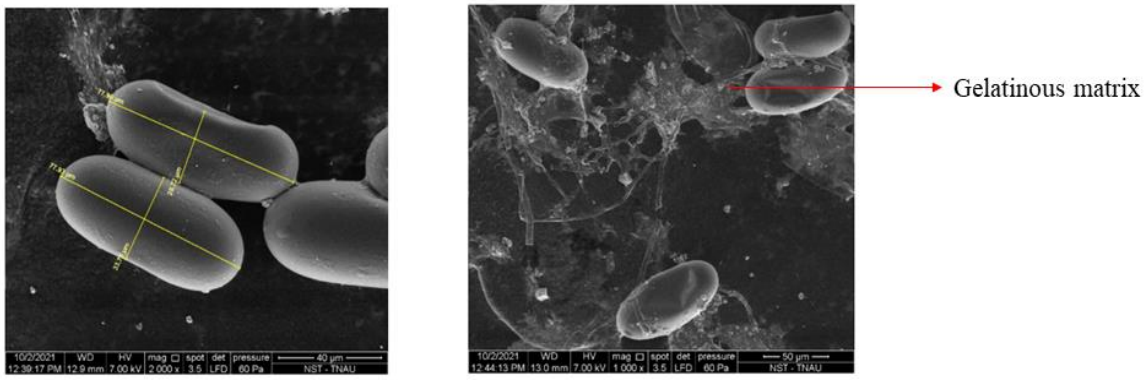
Scanning Electron Microscopic (SEM) studies

Both healthy and nematode-infested root samples were taken for studying the histopathological changes. Selected roots were washed thoroughly under running water not only to remove the adhering soil particles but also to ensure the high-quality sections. After washing the roots, they were cut into small pieces of 1 cm length using knife or blade.

The healthy and *M. incognita* infested okra roots from 60 days old plants were selected and processed for studying the histopathological changes through scanning electron microscope (SEM) by procedure explained by (Pathan, Bond, & Gaskin, 2010). The roots were stored in glass vials containing 3% glutaraldehyde with pH 6 of 0.05 M cacodylate buffer at 4° C for 22-24 hrs. Osmium tetroxide (1%) was used for post-fixation with same buffer for 12 hrs. The dehydration of samples was carried out with graded series of ethanol: 50,70,80,90,100%, and the ethanol was removed using t-butyl alcohol (100%). The galled tissues were cut into thin sections under a stereoscopic microscope to observe the internal cellular changes and then placed in stubs and coated with gold palladium of 20-30nm through a sputtering device. The coated specimens were observed in Scanning Electron Microscope (SEM) (Quanta 250, FEI, Czech Republic) having tungsten as electron source with vacuum range of 3.99 e^{-1} Pa.

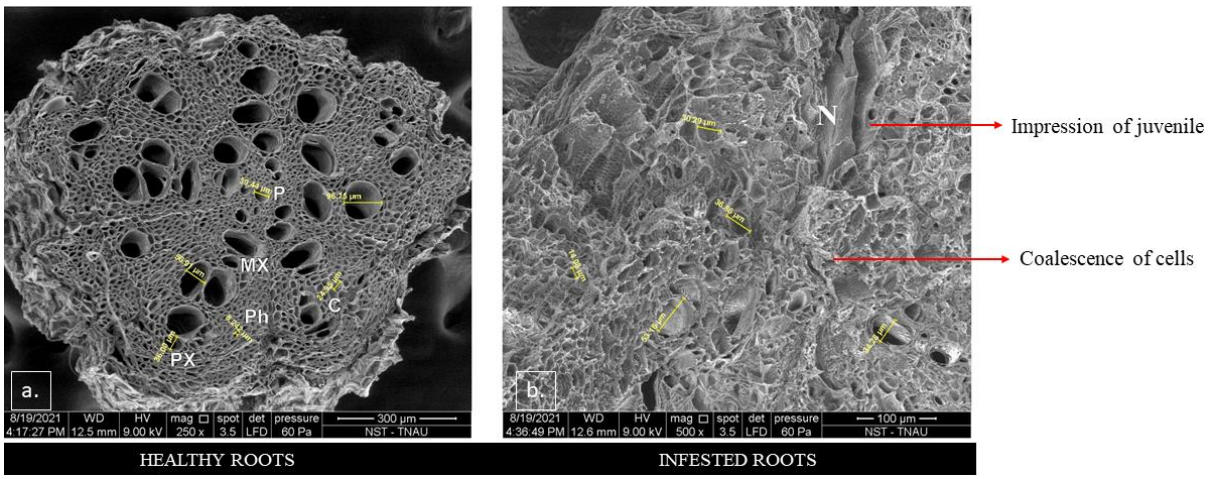
RESULTS AND DISCUSSION

The results on observation of topography of eggs revealed the dimensions of 77.93 µm, 33.7 µm from which we confirm that the egg mass was laid by *M. incognita* (Fig. 1). The observations of the study exhibited explicit cellular changes in healthy and nematode-infested roots. The cellular structure of healthy roots was visible, with internal structures such as proto-xylem, proto-phloem, pith, cortex regions with different sizes observed. The cellular size of pith was found to be 39.4 µm and the size of metaxylem, proto-xylem, phloem and cortex was found to be 56.9, 36, 8.24, 24.95 µm respectively at 250X (Fig. 2A, 4A). The 700X magnification of the cross section of roots clearly revealed the xylem and phloem vessel cells. The longitudinal imaging of healthy roots revealed the clear arrangement of cells with clear cellular compartments having cell sizes of about 50.18 to 55.4 µm with no nematode entry point (Fig. 3A). In the present study, we documented the infection process of *M. incognita* in okra roots by employing Scanning Electron Microscope (SEM). After inoculating the okra plants with nematodes, juveniles made their entry which was evident by the symptoms on the roots. Many invaded the okra roots and caused evident root gall. Wergin and Orion, 1981; Sijmons *et al.,* 1991 observed the root knot nematode invasion by using light and electron microscope. The plant's system produces enormous amount of aromatic compounds which are perceived by amphids of nematodes, eventually resulting in the attraction of soil nematodes towards the host, forming above and below ground symptoms.



M. incognita eggs *M. incognita* with gelatinous matrix

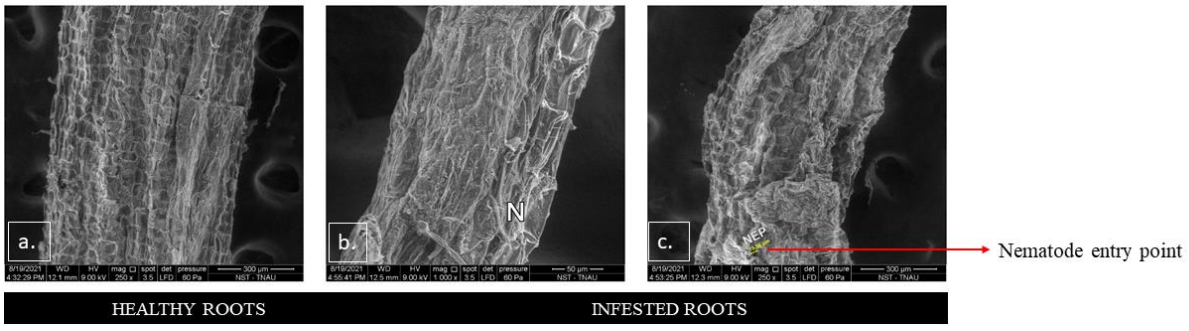
Figure 1. Scanning Electron Microscopic image of *Meloidogyne incognita* eggs



a. SEM image of healthy Okra roots showing clear cell structures *viz,* P-pith, MX-Metaxylem, Ph- Phloem, PX-Protoxylem, C-Cortex. N.

b. *M. incognita* infested roots showed total coalesced cell structures with impressions of nematode juveniles (N) on infested roots.

Figure 2. Scanning Electron Microscopic (SEM) images of healthy and *M. incognita* infested Okra roots



a. Longitudinal structure of healthy roots exhibited clear cellular compartments with no nematodes and punctures.

b. Second stage juveniles (N) adhering to external surface.

c. Entry point of J_2 {nematode entry point (NEP)} of varying diameter.

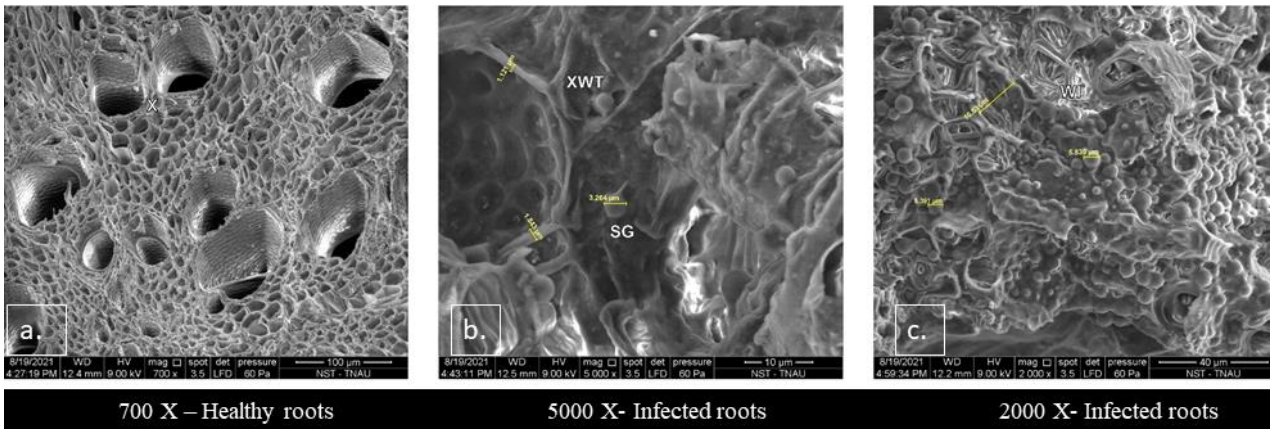
Figure 3. Scanning Electron Microscopic (SEM) view on longitudinal structures of healthy and *M. incognita* infested Okra roots

The results of SEM on *M. incognita* infested roots revealed the coalesced cellular structures root system. The traces of nematode located site were also observed with a layer of depression showing the presence of nematode feeding (Figure 2B). The cross-section of nematode infected roots also revealed the presence of circular starch grain accumulates of varying size viz.2.47 to 6.12 µm, which obscures the internal structures of plant roots. We also observed the circular depositions with varying diameters from 2.47 to 6.12 µm (Figure 4B). During host-nematode interaction, *M. incognita* produces various elicitor compounds which are been secreted by the oesophageal gl and as a result of the injection phase and eventually terminates in dissolution of cell walls. The process is then followed by ingestion phase were they draw the nutrients from host (Paulson and Webster, 1970; Hussey and Mims, 1991; Berg *et al.,* 2009; Miyashita and Koga, 2017). The present study also showcased the presence of cell wall dissolution and in growths due to nematode feeding, which may lead to symptom formation.

The findings of (Uematsu, Yabu, Yao, Kurihara, & Koga, 2020) revealed nematode penetration sites with marked cavity like structures surrounded by damaged cells. Sabella *et al.* (2019) found a similar structure in olive trees infected with *Xyllela fastidiosa* with variations in diameter and concluded that the circular depositions were starch granules. Thus, due to the enzymatic secretion of plant pathogens the host cells get degraded and malformed, which affects the normal physiology of plants.

The xylem wall thickening (XWT) was also observed in nematode-infested root sections with the formation of cellular ingrowth and modification in xylem structures nearing the giant cells. The cellular changes observed in the root system are mainly due to the enzymes secreted by nematodes during feeding, which may degrade the cell walls and helps in the feeding of nematodes (Figure 4B, 4C). The cell wall ingrowth was also observed in nematode-infested roots next to xylem cells, and the extent of wall ingrowth increased with an increase in giant cell structures with twenty times more surface area of the plasma membrane. Nevertheless, the infested roots also showed an increase in the diameter of roots with irregular arrangement of longitudinal root cells, and juveniles adhering to the root portions with their entry point was observed (Figure 3B, 3C). Jones and Dropkin (1975) findings revealed the presence of lignified cell wall of xylem in *M. incognita* infected roots, and cells exhibited the presence of cytoplasmic contents, which obscures the inner cell wall structures. (Uematsu et al., 2020) recorded root nematode, *Hirschmaniella diversa* clumped at the several external surfaces of the Indian lotus plant, which were documented under SEM.

These wall ingrowth structures lead to embolism in plants. As a result, the tension in xylem vessels becomes too high, where water column breakage can occur with xylem vessels filled with air and water vapour eventually causing above ground plant parts to dry. The reticulate cell wall ingrowth is also observed in nematode infested roots, resulting in bargain in vascular continuity exhibiting wound type vascular bundle which in symmetrical infection xylem elements forms an irregular cage-like structures around the infected site which is later transformed into giant cells. This eventually leads to the formation of above ground symptoms such as yellowing, drying and stunting of host plants.



a. 700X magnification of healthy roots showing absence of cellular changes.
b. 5000X magnification of infested roots showing xylem wall thickening and starch granules of various diameter.
c. Cell wall ingrowth of root cells and starch granules totally obscuring internal structures.

Figure 4. Scanning Electron Microscopic (SEM) view of healthy and *M. incognita* infested Okra roots at different magnifications

Thus, the SEM studies revealed the ultra-structural changes in healthy and nematode infested root sections, which aids in better understanding of plant nematode interaction which lead to understand the host- nematode interaction in okra roots.

CONCLUSION

Scanning Electron Microscope (SEM) revealed the early infestation process of root knot nematode, *Meloidogyne incognita*, on okra. The photographs revealed the destruction of metaxylem, protoxylem, pith and cortex region. Meanwhile, destruction in xylem vessels leads to abnormal translocation of water from root to apical region of okra, which resulted in the exhibition of above ground symptoms of plants. Longitudinal imaging of nematode infested roots showed the nematode entry points of varying diameter along with adhered juveniles. Hence the present study gave a preliminary idea on pathogenesis of root knot nematode, *M. incognita* in Okra.

Funding and Acknowledgment

There is no funding granted for the work.

Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

Authors declare that they have written and submitted only original work.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Author contributions

Conceptualization- AS, Experiments- SGS, AA, Guidance -AS, PK, Writing original draft - SGS, AA, Writing- reviewing &editing -AS, PK, SGS.

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