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Histopathological changes in resistant and susceptible guava (*psidium guajava*) varieties due to root knot nematode *M. enterolobi*

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Abstract

The root-knot nematode *Meloidogyne enterolobii* is an emerging and highly virulent pathogen causing severe damage in Guava (*Psidium guajava*), particularly overcoming host resistance in many commercial cultivars. This study investigates the histopathological alterations in resistant and susceptible guava genotypes following nematode infection to elucidate host-pathogen interactions and mechanisms of resistance. Root samples were collected at different stages post-inoculation and processed for microscopic examination. In susceptible guava plants, nematode infection induced extensive cellular modifications, including the formation of well-developed giant cells characterized by hypertrophy, dense cytoplasm, multinucleation, and dissolution of cell walls in the vascular cylinder. These changes facilitated the establishment of permanent feeding sites, leading to gall formation and disruption of normal vascular function. In contrast, resistant guava genotypes exhibited restricted nematode development, with limited or poorly formed giant cells, increased deposition of phenolic compounds, and localized cell necrosis around infection sites. The vascular tissues in resistant plants remained largely intact, indicating an effective defense response. Comparative histological analysis revealed that resistance in guava is associated with early defence activation, structural barriers, and inhibition of giant cell formation, thereby impairing nematode establishment and reproduction. These findings provide critical insights into the anatomical and cellular basis of resistance against *M. enterolobii* and contribute to the development of resistant cultivars and integrated nematode management strategies.

Keywords: Guava roots, root knot nematode, histopathology, resistant cultivar, perineal pattern

Introduction

The fruit which is known as the "poor man's fruit," guava (*Psidium guajava* L.) is a significant commercial fruit in India. After citrus, bananas, and mangos, it is the fourth most significant fruit. Although guava is native to South America and the West Indies, it is also produced in other tropical and subtropical regions, such as India. India's guava cultivation area is 261 MHa, producing 3961 MT annually with a yield of 13.9 MT/Ha (NHB Data Base 2017-18). Similarly, Tamil Nadu's guava production is 77.41MT and its area is 9.78Mha. A new issue with guava is *Meloidogyne enterolobii*, which has been documented from (Poornima *et al.*, 2016). *Meloidogyne mayaguensis* is a synonym for *M. enterolobii*, a species of root knot nematode that may have spread from other nations through saplings. *Phaseolus vulgaris* (bean), *Coffea arabica* (coffee), *Gossypium hirsutum* L. (cotton), *Carica papaya* L. (papaya), *Psidium guajava* (guava), *Solanum quitoense* (naranjilla), *Solanum tuberosum* (potato), *Glycine max* (soybean), *Ipomoea batatas* (sweet potato), *Nicotiana tobacum* (tobacco), *Lycopersicon esculentum* (tomato), and *Citrullus lanatus* (watermelon) are the main hosts (Rammah and Hirschmann, 1988; Brito *et al.*, 2007; Bitencourt and Silva, 2010). Garlic, grape fruit, and sour oranges are a few examples of non-hosts. In order to effectively employ resistant plants in breeding programs, the host plant and nematode must be compatible during root penetration and feeding site creation for the establishment of a successful host-parasite relationship. The goal of the current study is to understand the anatomical alterations in the root tissues of both vulnerable and resistant guava species.

Materials and methods

P. cattleianum and *P. guajava* seeds were acquired from IIHR Bangalore. Pre-germinated seeds were planted in tumbler pots with heat-sterilized sandy loamy soil and cultivated in a net house. From a pure culture of *M. enterolobii* cultivated in tomato plants, the egg mass was extracted and allowed to hatch in tap water at room temperature. Each tumbler pot received an inoculation of *M. enterolobii* juveniles. Roots from each treatment were plucked and gently cleaned of any

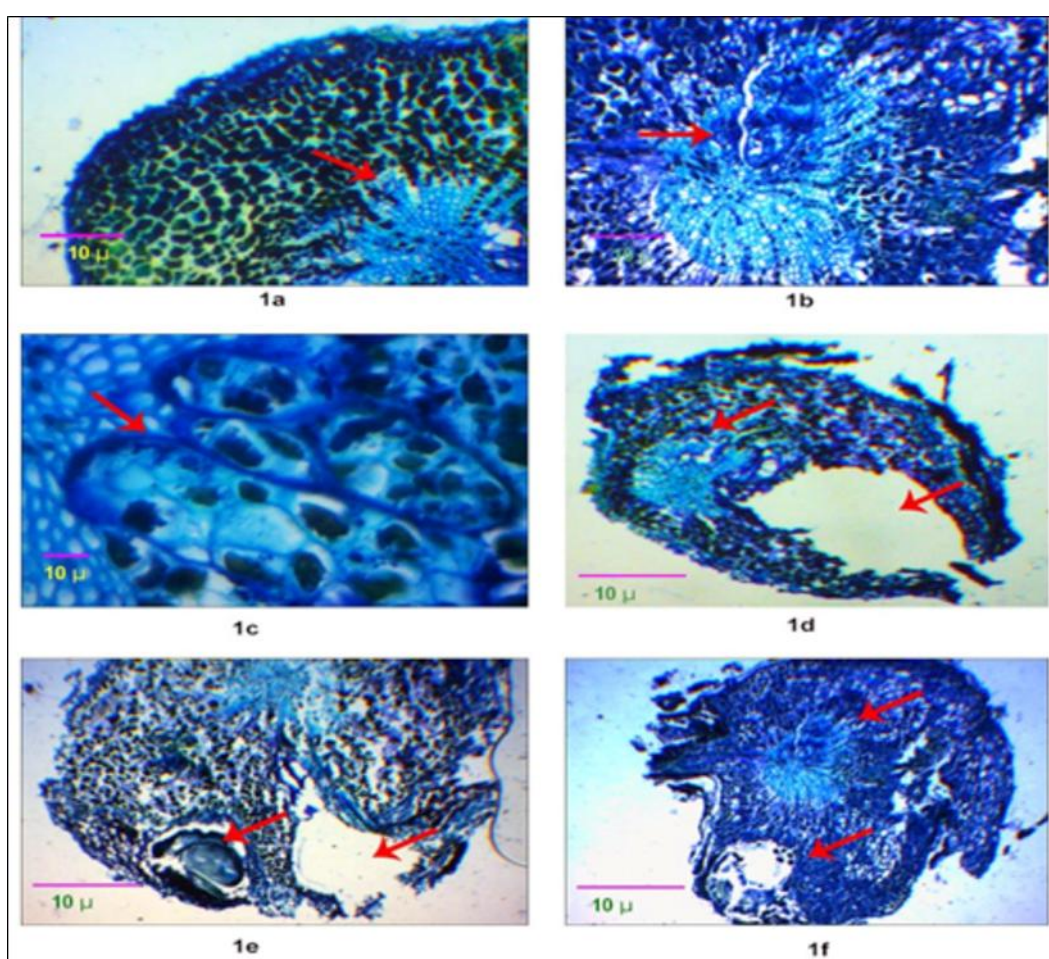
adhering soil 30 days following inoculation. Formalin-aceto alcohol solution (FAA) was used to fix two to six root galled root segments (0.5-1.0 cm) p plant. The root segments were then infiltrated and immersed in paraffin wax (50-55 °C) after being dehydrated in a graded series of ethanol (35, 50, 75, and 100%, 1 hour in each series of concentration). Using a rotary microtome (Weswox optic MT1090), serial transverse cross sections with a thickness of 10 µm were cut, placed on glass slides, and allowed to dry for two minutes. The sections were soaked in xylene to remove paraffin wax, and then they were cleaned with ethanol and distilled water (Hoppert, 2003). Sections were examined under a light microscope with a digital camera (Medline ML105) after being stained with toluidine blue (Parker *et al.*, 1982). Acid fuchsin staining of the roots verified the existence of females and egg mass. The roots were examined under a stereomicroscope following staining.

Perineal pattern identification

Perineal pattern analysis of adult females: The method created by Hartman.K *et.al.* 1985 was used to

prepare the perineal patterns of nematode females. Ten adult females were chosen from each district's water convolvulus roots, and they were placed under a stereomicroscope on a transparent plastic sheet after the anterior and posterior portions were separated using a razor. In order to remove cuticles and clean the pattern, the perineal patterns were placed in a drop of 45% lactic acid. The perineal pattern was then moved to a drop of 4% glycerine that was placed on a glass slide, sealed with nail varnish, and covered with a coverslip. After labeling the slide, it was examined under a compound microscope to determine its morphology.

Measurements for J2 and males: Using Sienhorst's approach, males taken from infected roots and J2 hatched from the single egg mass were fixed on glass slides. Under a compound microscope, the males and J2 of each isolate were examined and captured on camera.



1a. Healthy cortex with bundle sheath containing xylem and phloem.

1b. Giant cells found displacing the xylem and phloem vessels.

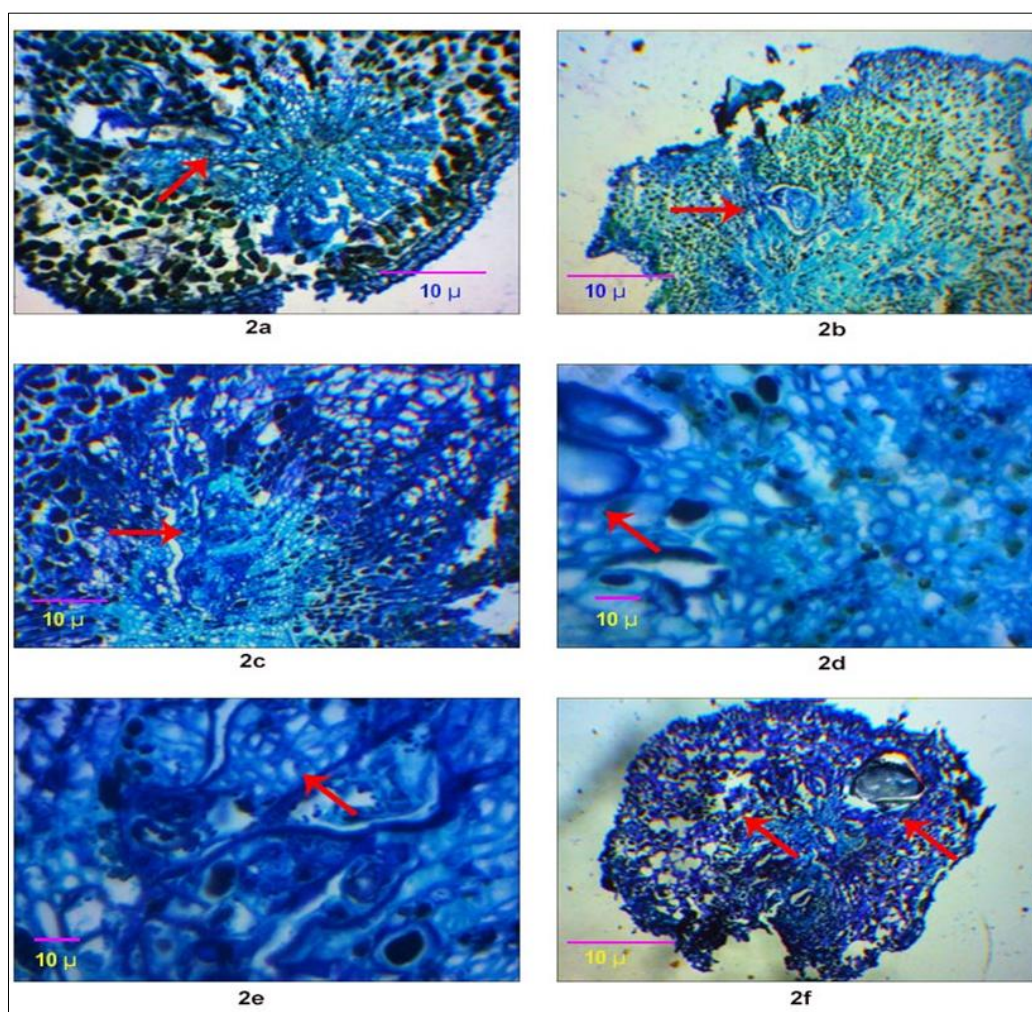
1c. Hypertrophied cells with thick wall and rich cytoplasm.

1d. Completely displaced bundle sheath and ruptured cortex due to enlargement of female.

1e. Female nematode and empty space and ruptured cortex due to release of egg mass.

1f. Hypertrophied cells and egg mass about to rupture the cortex.

Fig 1: Histopathology of *Psidium guajava* roots infected with *Meloidogyne enterolobii*



2a. Head of female nematode feeding in giant cell.

2b. Shriveled and poorly developed females.

2c. Distorted hypertrophied cells.

2d. Poorly developed giant cells.

2e. Thin walled giant cells and less cytoplasm.

2f. Cortex with more vacuoles and female without egg mass.

Fig 2: Histopathology of *Psidium cattleianum* roots infected with *Meloidogyne enterolobii*

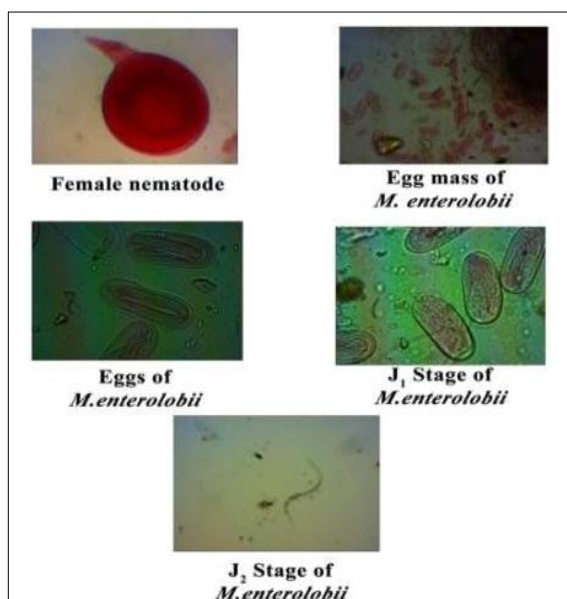


Fig 3: Life cycle of *Meloidogyne enterolobii*

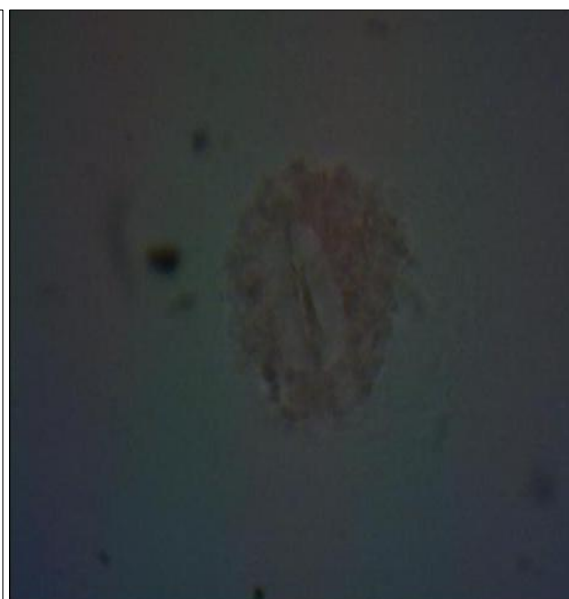


Fig 4: Perineal pattern of *M. enterolobii*

Results and discussion

Histopathology of healthy roots

The cortex of healthy guava roots has thin walls, several layers, and circular or polygonal parenchyma cells with no intercellular gaps, according to histopathology. This is mostly employed to move salts and water from the root hairs to the root's centre (Abbasi *et al.*, 2013) [19]. The vascular cylinder encircles the cortex, endodermis, and pericycle (Fig. 1a). The outermost layer of the steel is called the pericycle, and it is made up of a single layer of parenchymatous cells with no intervals between them. Aly Khan *et al.* (2017) also noted that secondary xylem and phloem tissue are arranged in a linear chain.

Histopathology of p. Gujava roots infected with m. enterolobii

The absence of complex galls and group feeding of nematodes were shown by anatomical alterations brought on by the second stage juveniles of *M. enterolobii* (Fig. 1b). The maximum number of large cells is just four or five (Fig. 1c). The xylem and phloem cells are dislocated by giant cells, impeding the movement of nutrients and water (Fig. 1d). In the large cells (Fig. 1c), thickened cell walls with granular cytoplasm and four to ten hypertrophied nuclei and nucleoli were also noted; this is consistent with Vovlas's (2004) findings about olive root knot nematodes. The displacement of xylem and phloem arteries caused by large cells was also documented by Hussey and Williamson (1997). It was discovered that females and egg masses disrupted the cortical area of the roots (Fig. 1e). According to Teliz *et al.* (2007), they also deform the root surface, which causes the root cortex to rupture (Fig. 1f). According to Fawoe (1988), the root knot nematode's large cell development caused the xylem tissues in tomatoes to distort.

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