

Original Research Article

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Comparative Study on Histopathological Changes caused by *Meloidogyne enterolobii* in *Psidium guajava* and *Psidium cattleianum* Guava Species

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ABSTRACT

Keywords

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Root knot nematode *Meloidogyne enterolobii* cause considerable yield losses in guava. It is now an emerging menace in guava production. Many authors reported the resistant nature of *Psidium cattleianum* against *M. enterolobii*. *P. cattleianum* is a small tree or shrub with a smooth bark and leaves are obovate elliptic and glabrous. Fruits are round, having good aroma with sweet taste. The fruits of *Psidium guajava* are big in size, round, smooth skin, white or pink flesh, soft, firm, light yellow, pleasant flavor with few seeds. Mechanisms of resistance against *M. enterolobii* in guava are studied by taking microtome sections of infected root tissue of *P. cattleianum* and *P. guajava*. The results revealed *P. cattleianum* did not developed any resistant reaction against nematode infection, giant cells are found to develop normally. The number of giant cell formed is less when compared to *P. guajava*. In *P. guajava* giant cells were thick walled, multinucleated with rich cytoplasm. The giant cells in *P. cattleianum* appeared to be disrupted and with less dense cytoplasm. No complex galls or group feeding of nematodes was observed in *P. cattleianum*. Development of nematodes seems to be hindered. Smaller females are observed in resistant species when compared to *P. guajava* species.

Introduction

Guava (*Psidium guajava* L.) is one of the important commercial fruits in India and known as poor man's fruit. It is the fourth most important fruit after mango, banana and citrus. Guava is native to South America and the West Indies but it is also grown other parts of the tropics and subtropics including

India. The cultivated area of Guava in India 261 MHa with an annual production of 3961MT with a productivity of 13.9MT/Ha (NHB Data Base 2017-18) Likewise the area and production of guava in Tamil Nadu is 9.78Mha and 77.41MT respectively.

Meloidogyne enterolobii is an emerging problem in guava and has been reported from

Tamil Nadu in recent years and wide spreading now across the Country (Poornima *et al.*, 2016). *M. enterolobii* is a species of root knot nematode having a synonym as *Meloidogyne mayaguensis*, considered that it might have spread from other countries through saplings. Major hosts are *Phaseolus vulgaris* (bean), *Coffea arabica* (coffee), *Gossypium hirsutum* L. (cotton), *Solanum melongena* (eggplant), *Psidium guajava* (guava), *Solanum quitoense* (naranjilla), *Carica papaya* L. (papaya), *Capsicum annuum* (pepper), *Solanum tuberosum* (potato), *Glycine max* (soybean), *Ipomoea batatas* (sweet potato), *Nicotiana tabacum* (tobacco), *Lycopersicon esculentum* (tomato) and *Citrullis lanatus* (watermelon) (Rammah and Hirschmann, 1988; Brito *et al.*, 2007; Bitencourt and Silva, 2010). Some of the non-hosts include grape fruit, sour orange, garlic, etc.

For the establishment of a successful host-parasite relationship, there must be a compatibility between host plant and nematode during root penetration and feeding site formation is important in order to use resistant plants efficiently in breeding programmes.

The present study has been carried out to know the anatomical changes in root tissues of resistant and susceptible species of guava.

Materials and Methods

Seeds of *P. guajava* and *P. cattleianum* were obtained from IIHR Bangalore. Pre germinated seeds were sown in a heat sterilized sandy loamy soil in tumbler pots and grown in net house. Egg mass of *M. enterolobii* was collected and kept for hatching in tap water under room temperature, from pure culture of *M. enterolobii* maintained in tomato seedlings at glass house of Horticultural College, Periyakulam and Seven days after hatching, 500 infective

juveniles of *M. enterolobii* was inoculated in to each tumbler pots.

After 30 days after inoculation roots from each treatment was uprooted gently washed free of adhering soil. Two to six root galled root segments (0.5-1.0cm) per plant were selected, excised and fixed in formalin-aceto alcohol solution (FAA). Then, the root segments were dehydrated in a graded series of ethanol (35, 50, 75 and 100%, 1hr in each series of concentration) infiltrated and embedded in paraffin wax (50-55°C). Serial Transverse cross sections of thickness 10µm were cut with rotary microtome (Weswox optic MT1090), transferred to glass slides and left for two min to dry. Paraffin wax was removed by soaking the sections xylene and rinsed with ethanol followed by distilled water (Hoppert, 2003). Sections were stained with toluidine blue (Parker *et al.*, 1982) and viewed using light microscope equipped with digital camera (Medline ML105). The presence of females and egg mass was confirmed by staining the roots with acid fuchsin. After staining the roots were observed under stereomicroscope.

Results and Discussion

Histopathology of Healthy roots

Histopathology of healthy guava roots revealed the cortex is thin walled and multilayered with circular or polygonal parenchyma cells without intercellular spaces. This is mainly used for transportation of water and salts from the root hairs to the center of root (Abbasi *et al.*, 2013). The cortex, endodermis, and pericycle surrounded by the vascular cylinder (Fig. 1a). Pericycle is the outermost layer of stele and composed single layer of parenchymatous cells without intercellular spaces. Secondary xylem and phloem tissue are arranged in linear chain similar observation was also recorded Alykhan *et al.*, (2017).

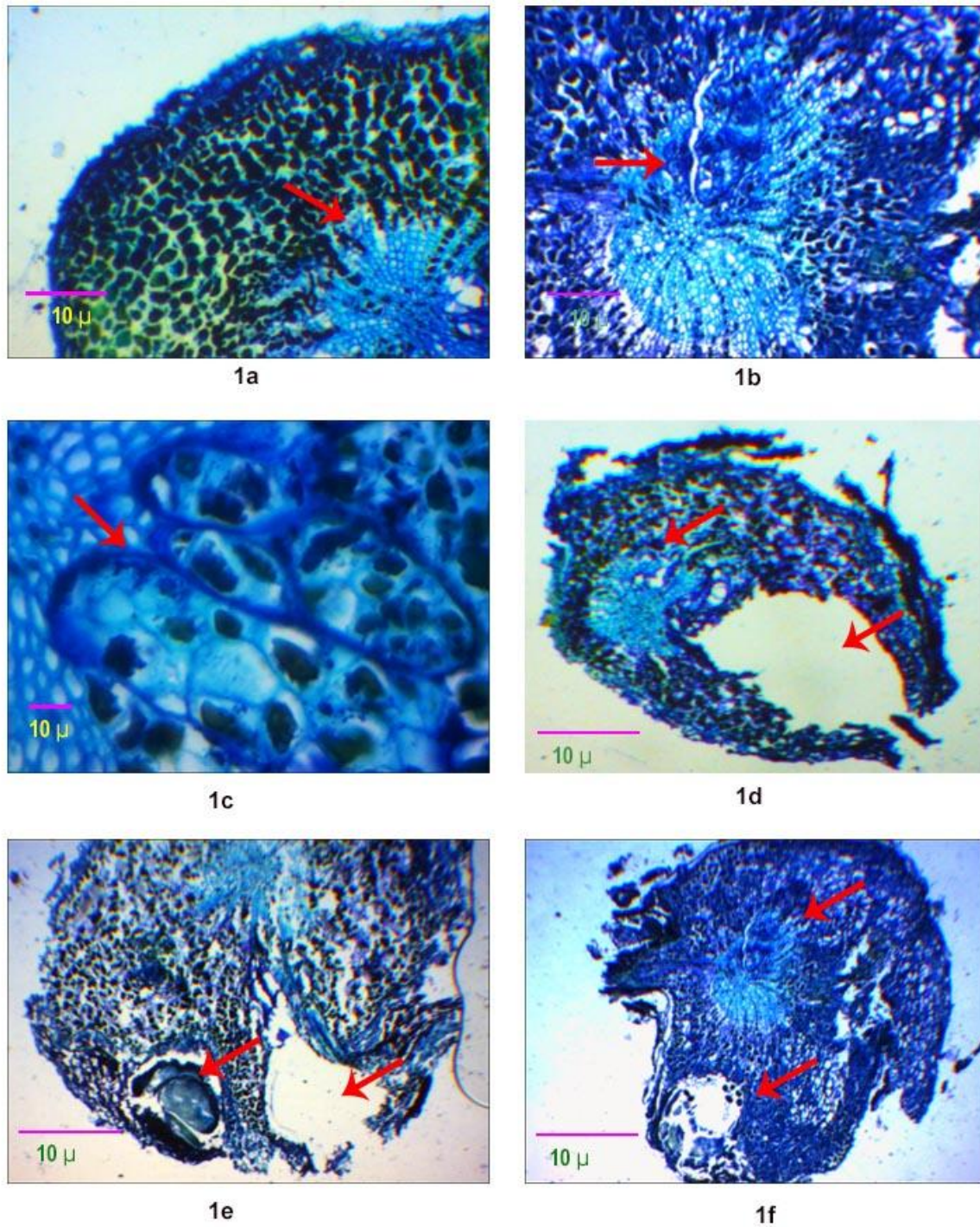


Fig 1. Histopathology of *Psidium guajava* roots infected with *Meloidogyne enterolobii*
1a. Healthy cortex with bundle sheath containing Xylem and phloem
1b. Giant cells found displacing the xylem and phloem vessels
1c. Hypertrophied cells with thick wall and rich cytoplasm
1d. Completely displaced 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

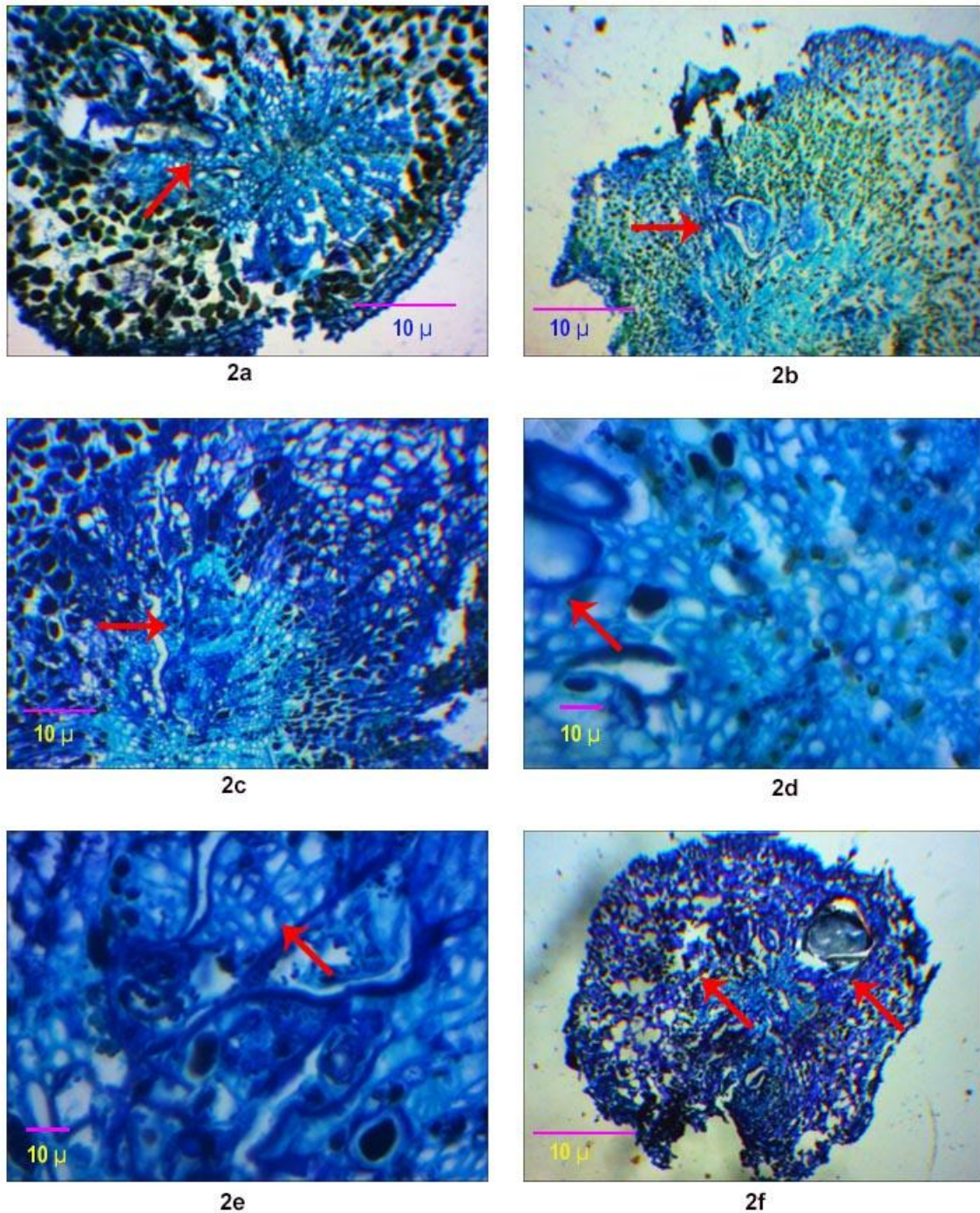


Fig 2. Histopathology of *Psidium cattleianum* roots infected with *Meloidogyne enterolobii*
2a. Head of female nematode feeding in giant cell
2b. Shriveled and poorly developed females
2c. Distorted hypertrophied cells
2d. Poorly developed giant cells
2e. Thin walled giant cells and less cytoplasm
2f. Cortex with more vacuoles and female without egg mass

Histopathology of *P. gujava* roots infected with *M. enterolobii*

Anatomical changes caused by the second stage juveniles of *M. enterolobi* revealed the absence of complex galls and group feeding of nematodes (Fig. 1b). Maximum number of giant cells range between 4-5 only (Fig. 1c). Giant cell dislocate the xylem and phloem cells hindering the transportation of water and nutrients (Fig. 1d). Thickened cell wall with granular cytoplasm and four to ten hypertrophied nuclei and nucleoli in the giant cells (Fig. 1c) was also observed, this is in concurrent with Vovlas (2004) as reported in olive root knot nematode. Hussey and Williamson, 1997 also reported the dislocation of xylem and phloem vessels due to giant cells. Egg masses and females were found to disturb the cortical region of the roots (Fig. 1e). They also distort the root surface leading to rupturing of root cortex (Fig. 1f) which is in accordance with Telizet *al.*, (2007). Fawoe, (1988) reported the deformation of xylem tissues in tomato due to giant cell formation by root knot nematode.

Histopathology of *P. cattleianum* roots infected with *M. enterolobii*

P. cattleianum showed the formation of giant cells (Fig. 2c). This confirms that this species did not provide any host reaction response to nematode penetration (Fig. 2a). There is no complex gall formation and group feeding of nematodes observed. Giant cells were found lesser in number ranges between 2-3 giant cells (Fig. 2d). Poor development of feeding sites and deteriorated giant cells were observed (Fig. 2c) by Alain denis de souse *et al.*, (2016) in some *Psidium* species. In roots infested with *M. enterolobii* more number of vacuoles (Fig. 2f) were observed it could be a type of resistant reaction of host. Similar observation was observed by Eissa *et al.*, (1988) in resistant host.

Giant cell are small and devoid of any cytoplasmic content (Fig. 2e), wall of the giant cells are also thin. These deformations in the giant cell did not support the development of

females (Fig. 2b). Cabasan *et al.*, (2013) reported the degradation of giant cells and host reaction in resistant genotypes of rice infected with root knot nematode. Size of the females was small when compared to *P. gujava*. Acid fuchsin stained roots showed that there was no egg mass formation. Das *et al.*, (2008) reported that in resistant roots nematode looks shriveled in appearance when compared to susceptible roots as there was no supply of nutrients by the giant cells. But there are healthy giant complexes with rich cytoplasm present in the susceptible roots and also nematode develops to mature female for further infection.

It is concluded in this study, the giant cells in *P. cattleianum* are found to develop normally with disrupted with less dense cytoplasm and less in number when compared to *P. gujava*. In *P. gujava* giant cells were thick walled, multinucleated with rich cytoplasm. No complex galls or group feeding of nematodes was observed in *P. cattleianum*. No much development of nematodes was seen but smaller females are observed in resistant species when compared to *P. gujava* species.

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