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A CLOSE VIEW ON HISTOLOGICAL CHANGES IN ROOTS OF GUAVA VAR. SEEDLESS IN HYDERABAD, SINDH, PAKISTAN

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ABSTRACT

Guava var. seedless naturally infected with root-knot nematode were fixed, sectioned and examined in detail. *Meloidogyne incognita* infective second stage juveniles penetrated and moved intercellularly modifying the vascular system and phloem into giant cells of various sizes. After getting mature the females start producing eggs which completely occupy the cortex while in some cells female sections were observed. Necrosis was present with hypertrophy and hyperplasia in cortex cells. Our results suggest selection of resistant guava varieties shall reduce the ability of *Meloidogyne incognita* damage. In Pakistan, most farmers are small land holders and improved varieties having multiple diseases resistance will be more useful for sustained agricultural production.

Keywords: guava var. seedless, histopathology, *Meloidogyne incognita*, root-knot nematode, Sindh

INTRODUCTION

Guava (*Psidium guajava* L.) is cultivated in many subtropical and tropical regions. The guava tree is small native of northern South America, Central America, Mexico and Caribbean Islands (Morton, 1987). Its fruit is consumed by humans, birds and animals. The central pulp seeds depend in number and hardness based on the species present. The main countries producing guava are India, China, Thailand, Indonesia, Pakistan and Brazil. Guava leaves have been intensively studied in the folk medicine and biological properties such as quercetin, myricetin, apigenin, guajivaren chlorogenic acid, caffeic acid, gallic acid etc (de Boer and Contingting, 2014; Kumar *et al.,* 2021). Increasing pace of Pakistan population expansion needs have substantially increased for fruits. Guava plantation is vulnerable to attack by many pathogens mainly fungi, algae, few bacteria and a large number of ecto- and endoparasitic nematodes (Prakash, 2012; Dawabah *et al.,* 2019; Tokeshi *et al.,* 1980).

Meloidogyne incognita are the most destructive and problematic nematode in warm moist and sandy soils around the globe. It causes yield reduction as well as diminish quality and quantity of fruits (Barker *et al.,* 1985). All the three important species (*Meloidogyne enterolobii, M*. *javanica* and *M*. *incognita*) have been reported affecting guava plantation in Pakistan (Samad *et al.,* 2012; Khan *et al.,* 2017; Sikandar *et al.,* 2022).

Root-knot nematodes are sedentary endoparasitic nematodes whose feeding produces growth of galls and knots on roots, thus disrupting normal function and formation of roots and allowing easier penetration for many bacteria and fungi causing severe decay of roots.

The symptoms caused by *Meloidogyne incognita* and yield losses are often weakened because their foliar signs are identical to those of other biotic diseases and abiotic stresses such as yellow leaves and stunted growth (Liang *et al.,* 2020). Thus, an attempt was made to collect 10 samples each from Guava (*Psidium guajava* L.) seedlings var. seedless (3-6 months old) from four nurseries of Hyderabad, Sindh.

MATERIALS AND METHODS

Surveys were conducted during October 2022 in four nurseries of Hyderabad, Sindh namely Al-madina, Dado, Khokhar and Kohsar. From each nursery 10 samples were collected. Soil

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samples (200g) with feeder roots were collected in polythene bags and properly tied to stop evaporation. Relevant data such as date of collection was tagged. The samples were brought to CDRI Laboratory for further processing to see presence of root-knot nematodes.

Perineal pattern sample preparation

Galled roots of Guava seedlings were gently washed with tap water and placed in 0.9 percent solution of sodium chloride (Elahi *et al.,* 2021). Subsequently the females were placed in 45% lactic acid on a clean slide and the posterior end cut off with a sharp optical scalpel, body tissue were gently removed. When all tissues are removed, the cuticle is transferred in a drop of pure glycerin and carefully trimmed until slightly larger than the perineal pattern which are folds around anus and vulva in adult remains which is placed in a small drop of glycerin, covered gently with a coverslip and sealed with beewax and paraffin (1:5) at 65ºC melting point.

Histopathological changes studies in the roots of Guava infected with *Meloidogyne incognita*

Histopathological studies were done with guava (*Psidium guajava* L.) var. seedless plants infected with root-knot nematodes. The infected roots were collected and washed gently in tap water, later cut into some pieces (0.5 to 0.8 cm in length), then fixed and dehydrated through ethyl alcohol series following embedding the processed root bits in paraffin wax. The sectioning was made at 6-8 µ with the aid of spencers rotary hand microtome, stained by hematoxylin and eosin, later mounted in Canada balsam. The photographs were taken using camera mounted on a research microscope Nikon (Optiphot-2).

RESULTS AND DISCUSSION

The perineal pattern studies showed that the *Meloidogyne incognita* was the species present due to presence of angularly oval structure with high dorsal arch in a typical pyriform inverted V-shape created by striae in the dorsal tail. The striae waves were not interrupted.

The main symptoms observed were browning and yellowing of leaves and wilting of the guava seedlings. In many cases sudden death of seedling was reported especially at 3-4 months. The root-knot nematode is soil borne (Ashokkumar *et al.,* 2019). The second stage juveniles present in soil invade the roots move freely and modify the vascular system and phloem into giant cells of various sizes. In our studies, the entrance point of juveniles showed impaired cell integrity of epidermis (Figure 1a), thus enhanced membrane permeability provides a solute influx from plant vascular to form giant cells of various sizes (Figure 1b). After getting mature the females start producing egg masses containing (200-400 eggs) which completely occupy cortex cells while some sections show female sections (Figure 1c). The giant cells seriously hampered solute transport in plant vessels (Al Abadiyah Ralmi *et al.,* 2016). Necrosis along with hypertrophy and hyperplasia was observed in cortex cells (Figure 1d). In previous studies conducted by Maqsood *et al.* (2020) *Meloidogyne* infection increased axial resistance of galled root segments to water flow, transpiration rate and stomatal conductance, which finally leads to restricted water transport and a low leaf water potential result in a decreased leaf chlorophyll content over time (López-Gómez *et al.,* 2015).

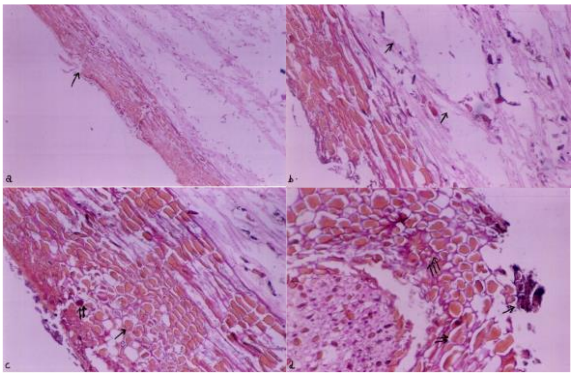

Figure 1. Histological changes in guava roots infected by root-knot nematode (*Meloidogyne incognita*)
a. The entrance point of second stage juveniles showing impaired cell integrity (arrow) (x10).
b. Giant cells of various sizes (arrows) (x100).
c. Egg masses (arrow) in cells and sections of female (double arrow) (x100).
d. Necrosis (arrow) along with hypertrophy (double arrow) and hyperplasia (triple arrow) (x100).

Kamran *et al.* (2013) suggested that severity of *Meloidogyne* depends on various factors such as nematode density, soil condition and timing of infection. Samad *et al.* (2012) observed similar changes in Guava var. Safeda roots infected with *M. javanica* from Tandojam, Sindh. The studies show that seedless guava is not resistant or tolerant to this plant parasite which was also confirmed by artificial inoculation (Gaudin *et al.,* 2023). Dwivedi and Dwivedi (2016) found two fungi *Fusarium solani* and

Fusarium oxysporum had caused severe necrosis to the epidermal layer and damage to cortex similar to our studies.

Invasion and development comparative studies of root-knot nematodes are limited (Schneider, 1991). Nematodes with a wide host range, it is advisable to understand the effect of different nematode species invasion and development.

CONCLUSION

Meloidogyne incognita invasion of guava roots produce galls, thereby disturbing the uptake of water and different nutrients by the host plant. It is recommended that those varieties must be selected which are disease resistant and having good fruit quality.

AUTHOR'S CONTRIBUTION

A. Khan: Wrote the manuscript and did identification of species.
K. A. Khanzada: Prepared slides and collected samples.
M. A. Rajput: Prepared slides and collected samples.
M. A. Samad: Collected relevant literature.
N. Khatoon: Performed histology.
S. Waheed: Did photography and typed the manuscript.

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