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HISTOLOGY OF INFECTED ALMOND ROOTS (*PRUNUS AMYGDALUS* BATSCH) SEEDLINGS WITH ROOT-KNOT NEMATODE (*MELOIDOGYNE JAVANICA* (TREUB, CHITWOOD) AND A FUNGUS *PLASMODIOPHORA BRASSICAE* WORONIN

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ABSTRACT

Histology of almond (*Prunus amygdalus* Batsch) seedlings roots was conducted for the changes by a root-knot nematode and a fungi *Plasmodiophora brassicae*. The seedlings were planted in infected soil collected from Kork, district Khuzdar, Balochistan. Sections of necrotic and galled roots were cut 6–8 µm thick using a rotary microtome. *Meloidogyne javanica* formed egg masses inside the cortical root tissue. In most cases the permanent feeding sites induced by the nematode adjacent to vascular tissue, consisted of large giant cells. While *Plasmodiophora brassicae* infected the root-cortex, reached the vascular tissue and caused hyperplasia along with hypertrophy.

Keywords: Histology, almond roots, seedlings, *Meloidogyne javanica*, *Plasmodiophora brassicae*

INTRODUCTION

Almond (*Prunus amygdalus* Batsch var. Kagazi) is commercially cultivated in Balochistan, Pakistan (Khan *et al.*, 2009). Several diseases are reported causing damage to almond cultivation worldwide (Clemmensen *et al.* 2013). Amongst plant parasitic nematodes, *Meloidogyne* spp. are considered major diseases (Khan *et al.*, 2010). Moreover, *Plasmodiophora brassicae* is an obligate parasite which causes clubroot in canola (oilseed rape), sprouts, broccoli, radish, cauliflower, turnip and black mustard (Buchwaldt and Rimmer, 2007). Since the seedlings of almond were planted in pots with soil collected from cabbage fields, although no clubroot was observed in the almond roots, howsoever the roots were blackened with high population of *P. brassicae*. Its lifecycle consists of two main phases, the first occurring in root hairs and the second in the cells of the root cortex and stele leading to gall formation and production of haploid resting spores (Navas Cortés, *et al.*, 2010). Thus affecting the ability of the infected plants to uptake nutrients and water from the soil, which leads to stunting of the seedling/plant. *Meloidogyne* spp. found associated with almond rhizosphere establish permanent feeding sites causing large giant cells (Khan *et al.*, 2010). The present study was conducted to investigate the histological changes in almond roots infected with *P. brassicae* and *M. javanica*.

MATERIALS AND METHODS

Roots and rhizosphere soil from stunted almond (*Prunus amygdalus* (Batsch var. Kagazi seedlings were collected from Kork district Khuzdar, Balochistan.

The identification of fungal pathogen *Plasmodiophora brassicae* (Fig. 1) was based on visible symptoms and microscopic identification in according with available literature (Buczacki, 1979; Mithen and Magrath, 1992). The identification of root-knot nematode species was based on Perineal patterns of females as described in Hartman and Sasser (1985). Necrotic and galled roots, having bad odour were selected for histopathological studies. Root tissues were gently washed free of adhering soil and debris and individual galls were selected. The segments were fixed in F.A.A. according to Samad *et al.* (2012) and processed for histological technique according to the standard method of Sass (1964). Dehydration was carried out in a serial concentration of known volume of ethanol alcohol. Dehydrated root tissue were then infiltrated and embedded in paraffin wax at 52°C for 12 days. During the wax infiltration process, air bubbles were removed from root tissue under vacuum. The histological sections were cut using a rotary microtome 6–8 µm thick sections of roots were cut stained by haemotoxylin and eosin. Later mounted in Canada balsam. Photographic camera mounted out on a research microscope Nickon (Optiphot-2) in the Department of Zoology, University of Karachi was employed for photography.

RESULTS AND DISCUSSION

The studies of morphometric observations of J_2's, males, perineal patterns of females were in agreement with those of *Meloidogyne javanica* and thus regarded the same species. Roots gall induced by *M. javanica* were more prominent in the lateral roots and varied in shape and size. Root-knots contained 1-3 females and eggs ranged from 2500-3500. In most cases egg mass was found inside the cortical root tissue and a few were seen protuting from the root surface. Infected cells were obvious surrounded by uninfected healthy looking cells in the histological sections of root galls induced by *M. javanica*. In certain section cell containing one or more, female and eggs mass were detected (Fig. 2). In most sections an egg mass was found inside the cortical root tissues, but in a few sections egg masses were protruding from root tissue. Furthermore, histological sections confirmed that nematode successfully established permanent feeding sites which caused alterations in the root-cortex, pericycle and vessels due to giant cells and nematode females, *P. brassicae* infected cells were seen in clusters (Fig. 3, 4). In most cases the permanent feeding sites induced by the nematode adjacent to vascular tissue, consisted of large giant cells (Fig. 5). The feeding sites caused distortion of xylem tissue. Hyperplasia of tissue was prominent which may eventually lead to the formation of root galls. Moreover, these histological sections showed that *Plasmodiophora brassicae* infected the root-cortex, reached the vascular tissue and caused increase in cell division (hyperplasia) along with enlargement (hypertrophy), that resulted in severe distortion of tissue structure (Fig. 6) and the root structure was completed destroyed, similar to the findings of Kobelt *et al.*, 2000; Ando *et al.*, 2006, Navas Cortés *et al.*, 2010. The present findings on the damage caused to almond seedlings roots indicate that these soil-borne pathogens can cause severe damage to the root tissue. Therefore, if pathogen free soil is not available to grow the seedlings, the soil must be properly treated with a fungicide and a nematicide prior to growing seedlings.

Fig. 1. *Plasmodiophora brassicae* isolated from almond seedlings.

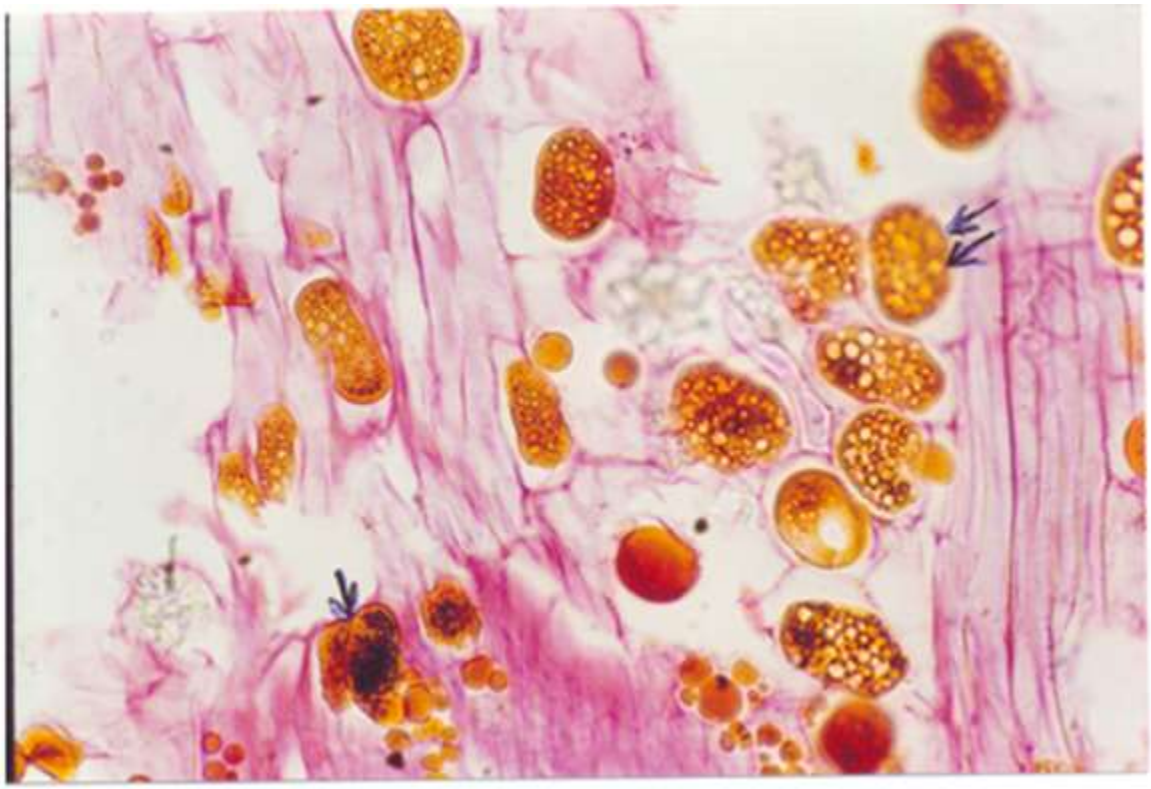

Fig. 2. Histological section showing cells with one or more female (→) and egg masses (⇉) (× 100).

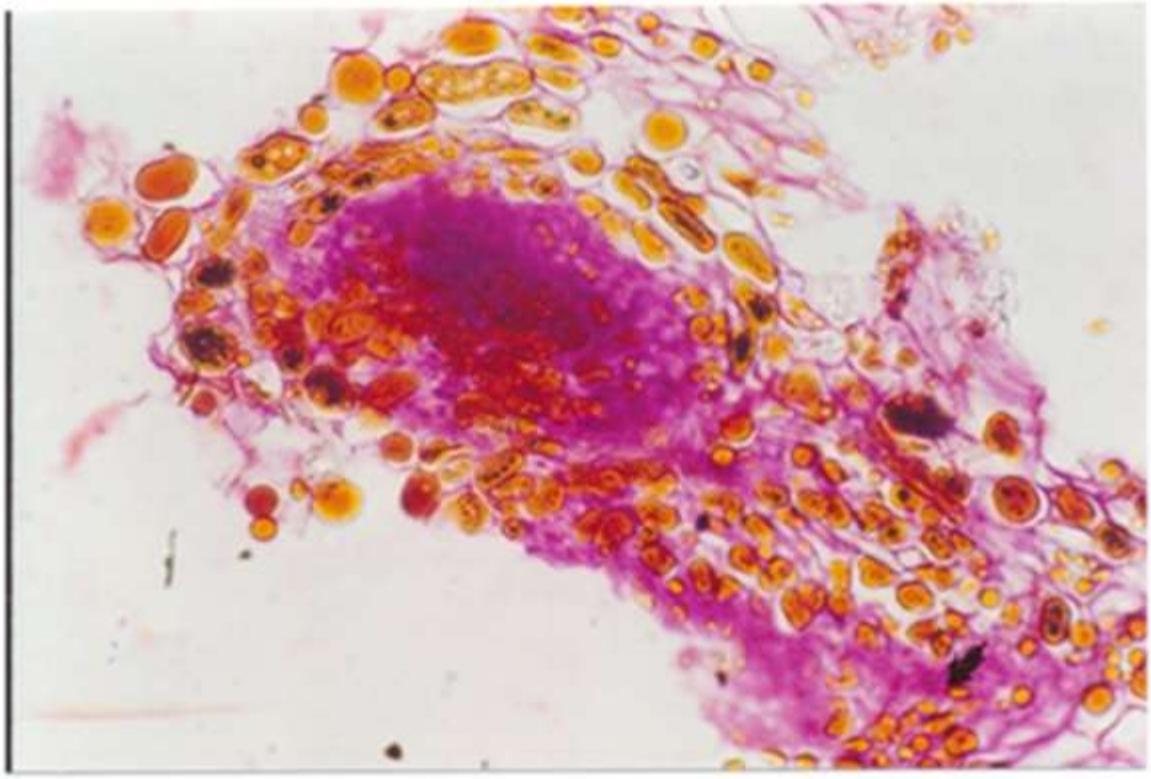

Fig. 3. Histological section showing egg masses protruding from the root tissue and *P. brassicae* occurring in clusters (× 50).

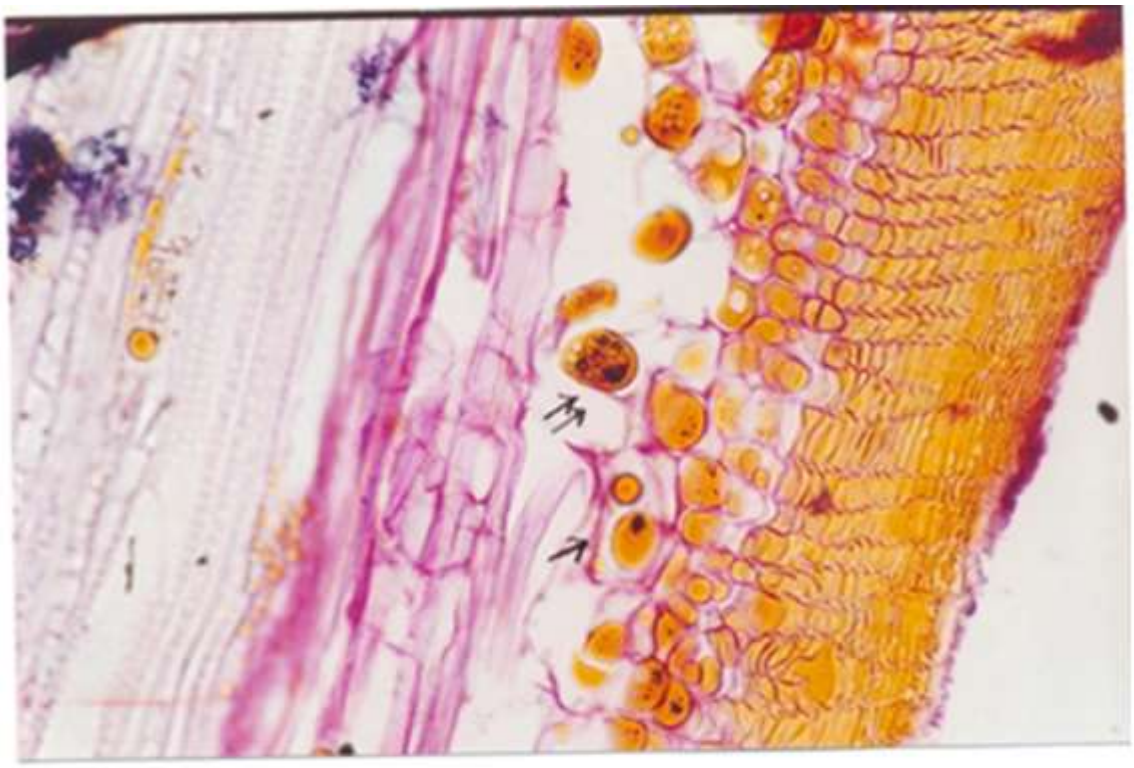

Fig. 4. Alterations observed in the root-cortex, pericycle and vessels due to giant cells (→) and nematode females (⇉) (× 100).

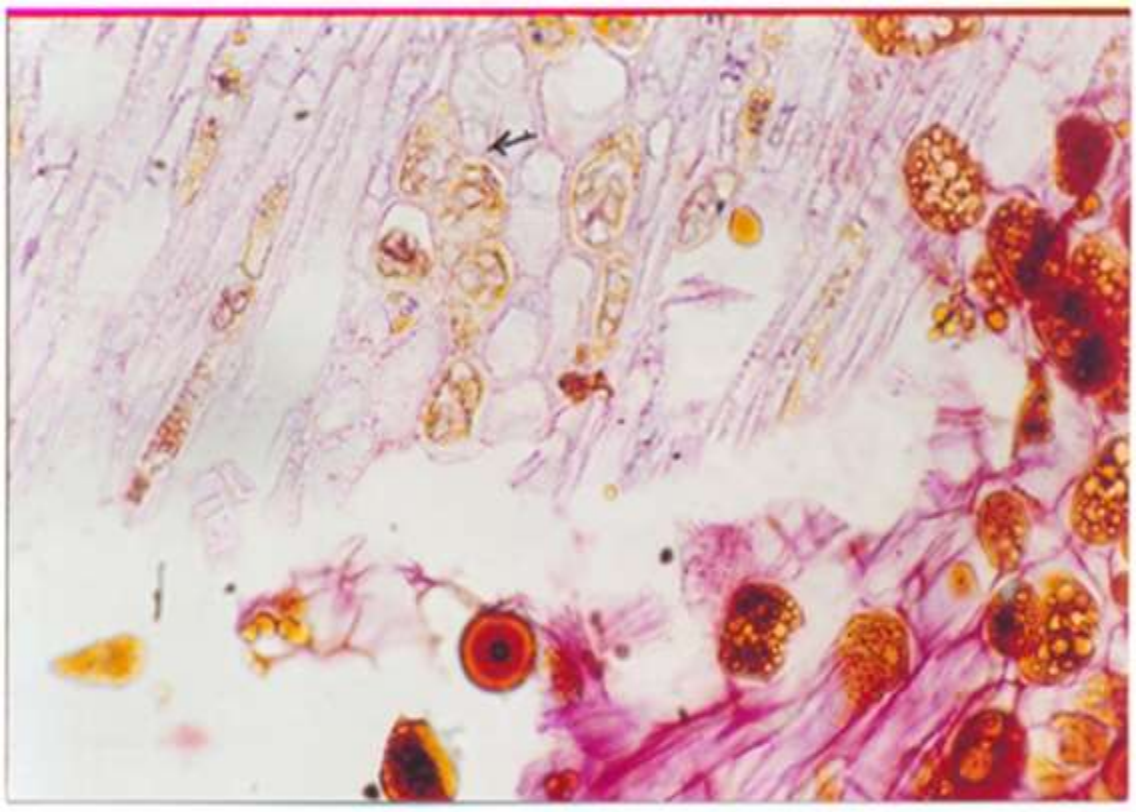

Fig. 5. Large giant cells (→) (× 100).

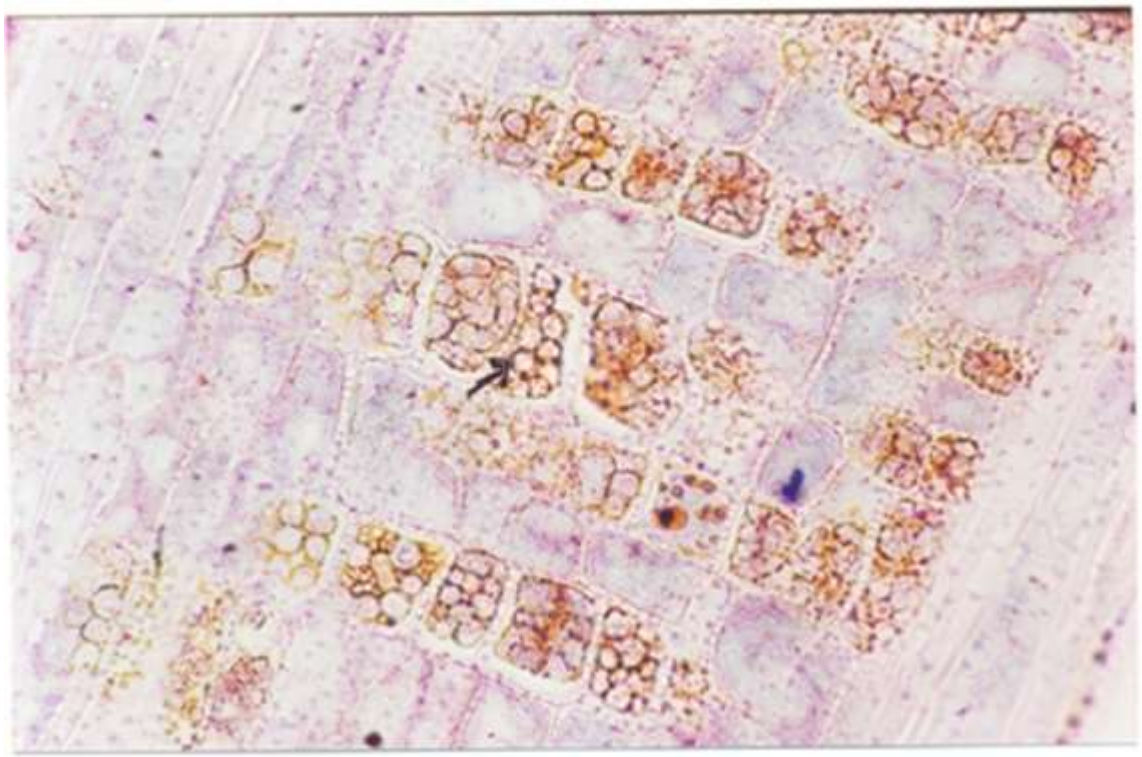

Fig. 6. Histological sections showing *P. brassicae* (→) infecting root-cortex causing hyperplasia and hypertrophy and the root structure completely destroyed (× 100).

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