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HISTOPATHOLOGICAL CHANGES CAUSED BY *Meloidogyne graminicola* IN RICE ROOTS

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ABSTRACT

Infection and development of *Meloidogyne graminicola* cause histopathological changes in rice roots in the form of giant cells and galls. The histopathological changes of rice roots of the variety Bg 94-1 caused by *M. graminicola* were observed at different times after inoculation at Horticultural Crop Research and Development Institute at Peradeniya. Microscope slides of root sections, 15µm thickness were prepared and micrographs were taken. Microtome sections of infected rice roots were observed 10, 20, 30 and 40 days after the nematode inoculation. Ten days after inoculation all the penetrated juveniles were found congregated near the root tip. A transverse section of rice root 30 DAI, showed many female nematodes in the intercellular space or among adjacent to the giant cells. The formation of giant cells has caused aberrations in the vascular region and resulted in curved swollen root tips. A large number of eggs inside the root knots were observed about 40 DAI. Giant cell formation was observed in the vascular region of the root and not in the cortical region.

KEYWORDS: *Oryza sativa*, Root knot nematode infection, Histopathology

INTRODUCTION

Paddy root knot nematode *Meloidogyne graminicola* is distributed mainly in the rice growing countries in South East Asia (Bridge *et al.*, 1990). In Sri Lanka the nematode was first observed in 1990 at Ranna in Hambantota district (Ekanayake and Toida, 1997). In the past, frequency of detection of *M. graminicola* in rice was very low in Sri Lanka and the nematode has been considered as a minor problem in rice, restricted to a few scattered places in Hambantota district. Recently, the nematode has been reported in major paddy growing areas in Galle, Udawalawe, Rathnapura, Polonnaruwa, Kurunagala, Moneragala, Ampara, and Mahaweli System B. The infestation level varied from mild through moderate to severe. In most of the locations, infestation level is so low that damage symptoms are not noticeable except for the presence of few root knots found at random in root systems. At present, *M. graminicola* has become a serious pest in the main paddy growing areas in Kurunagala and Ampara districts. The nematode can cause economic yield losses in upland and deep-water rice (Ibrahim *et al.*, 1972). Yields can decrease to 72% when 4000 eggs and juveniles of *M. graminicola*/plant occur in deep water rice plants by drowning out (Bridge and Page, 1982). Due to the excessive production of eggs and the easy means of spread along with water and soil, there is a high potential to expand the infested area in the country causing a serious damage on rice yields, particularly, under sub optimal management conditions.

The most distinguishable symptom of rice root knot nematode is the presence of root nodules, slightly curved inwards, at root tips. If the infestation level is high, the function of roots become seriously impaired leading to poor absorption of water and nutrients from the soil. This leads to exhibit deficiency symptoms. Infection of *M. graminicola* causes severe growth reduction, unfilled spikelets, reduced filtering, and chlorosis of young plants (Babatola, 1984).

Development of *M. graminicola* inside the root tissues stimulates the formation of giant cells, which become their feeding sites. There is hardly any information on histopathological changes that result from the infection of root knot nematodes in rice. The current study was designed to investigate histopathological changes caused by *M. graminicola* on a susceptible rice variety' Bg-94-1.

MATERIALS AND METHODS

Roots of rice variety Bg-94-1 infected with *M. graminicola* were collected from a paddy field at Udawalawe in Hambantota district to obtain the inoculum for the study. Three weeks old seedlings of the rice variety Bg 94-1were transplanted and inoculated with 2500 eggs of *M. graminicola* extracted by Hussey and Barker's technique (1973) 6 days after transplanting. One set of un-inoculated paddy plants was also maintained in the plant house for comparison. Segments of infested roots sampled 10, 20, 30 and 40 days after inoculation of the nematode were fixed in TAF (Triethanolamine, 2ml + formalin, 7ml + distilled water, 9ml) at 70°C for 24 hours. The root segments were dehydrated through the ethyl alcohol series and embedded in paraffin wax for microtomy sectioning. Fixed root segments were then sectioned at 10-15µm, stained in safranin and fast green on gelatin-coated glass slides and mounted in Dummar xylene according to Johansen's method (1940). Temporary mounts were prepared for microscopic – photographs. Root sections of infected roots were observed at 10, 20, 30, and 40 days after inoculation (DAI).

RESULTS AND DISCUSSION

At 10 DAI, second stage juveniles had penetrated the root tips and orientated in various directions' vertically and horizontally to the axis of the root (figure.1)[1]. At 10 DAI microscopic observations revealed that many juveniles were congregated near the tip of the roots.

[1] Figures are provided in plates at the end of the paper.

At 20DAI, swollen root galls were observed on 90% of the small roots of the root systems. Several developing nematodes and groups of abnormally enlarged cells were observed in the vascular region of the root tips (figure 2). Groups of giant cells were common in vascular region of many root sections (figures 2, 3). This was evidence that the penetrant juveniles had migrated towards the vascular region and stimulated the formation of giant cells. They had established in vascular area in order to feed and develop. In healthy rice root, vascular bundles are arranged in the normal way (figures.3, 4). From the time of establishment till maturity, the nematode remains in the same locality. Transverse sections of paddy root 30DAI, showed many female nematodes embedded in the intercellular spaces among or adjacent to the giant cells (figure 5). Females continued to live as sedentary endoparasites, while mature males left the roots. At this stage many females carry a large number of eggs inside their body (figure 6). In highly infected roots clusters of giant cells and several mature females were found established in the vascular region at the same location. The formation of giant cells had caused aberrations of the vascular region and resulted curved swollen root tips (figure 7). It was observed that the females developed within the root and eggs were laid mainly in the cortex (figure 8). Juveniles of *M. graminicola* can either remain in the gall or migrate through the parenchyma tissue of the cortex to new feeding sites within the same root (Bridge *et al.*, 1990). This is an adaptation of this nematode species to flood condition.

In the current study, histological changes of the roots were confined to the vascular bundles. Occurrence of giant cells was observed in the vascular region of the root, and not in the cortical region. Groups of 4 –9 giant cells were observed and these cells had thick walls and multinucleated dense cytoplasm (figures 2, 5) while some cells were vacuolated (figure 5). Taylor (1976) observed giant cells mostly in the phloem and a few in the xylem and cortical tissue and observed thin walls in giant cells. But the results of this study in were agreement with those of Scddiqui and Taylor (1970) who observed thick walled giant cells only in the vascular region of wheat and oat roots infected by *M. nassi*. Tandon and Praveen Kumar (1979) and Ekanayake (1985) observed clusters of giant cells in the vascular region and not in the cortical tissue of tomato roots infected by *M. lucknowica* and *M. incognita* respectively.

CONCLUSIONS

The feeding and development of one or more nematodes at the same locus as sedentary endoparasites cause histological changes. Deformity of rice roots and their consequent inefficiency leads to stunted growth, unfilled spikelets,

reduced filtering, and chlorosis of young plants leading to substantial yield reduction in *M. graminicola* infested paddy fields.

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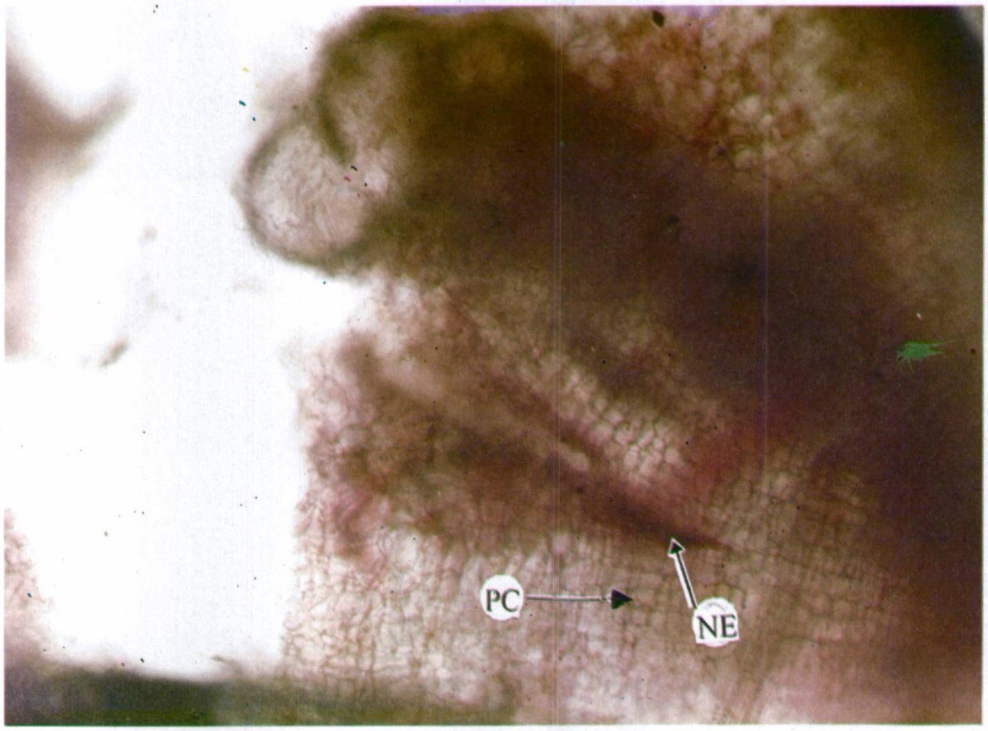

Figure 1. Longitudinal section of a rice root of the variety Bg. 94-1, 10 days after inoculation of Meloidogyne graminicola, showing migration of 2nd stage juvenile (NE) through parenchyma cells (PC). (2.5 X 20)

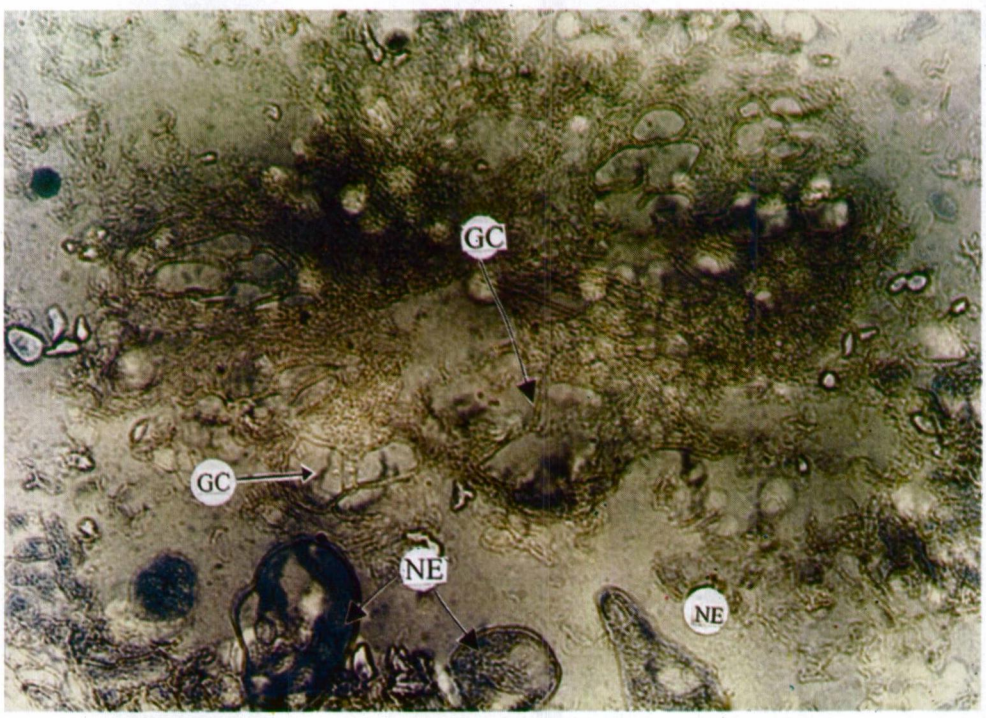

Figure 2. Longitudinal section of a rice root of the variety Bg. 94-1, 10 days after inoculation of Meloidogyne graminicola, showing developing female nematodes (NE) and groups of giant cells (GC). (2.5 X 20)

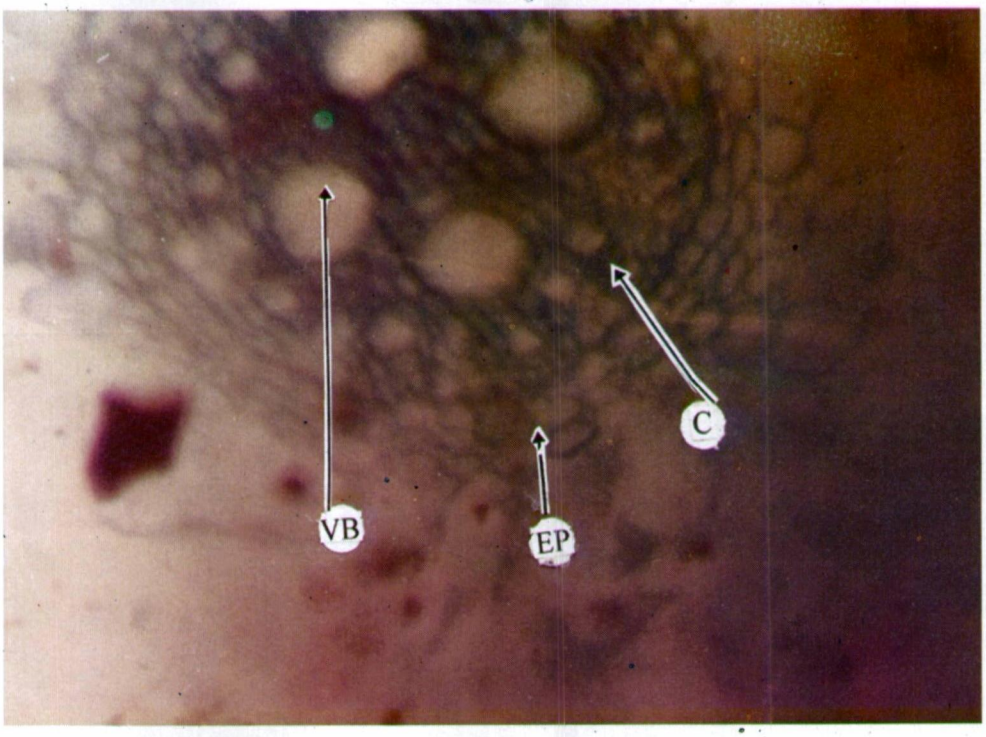

Figure 3. A portion of a transverse section of healthy (uninfected) rice roots of the variety Bg. 94-1, showing undamaged vascular bundles (VB), epidermal cells (EP) and cortex (C). (2.5 X 20)

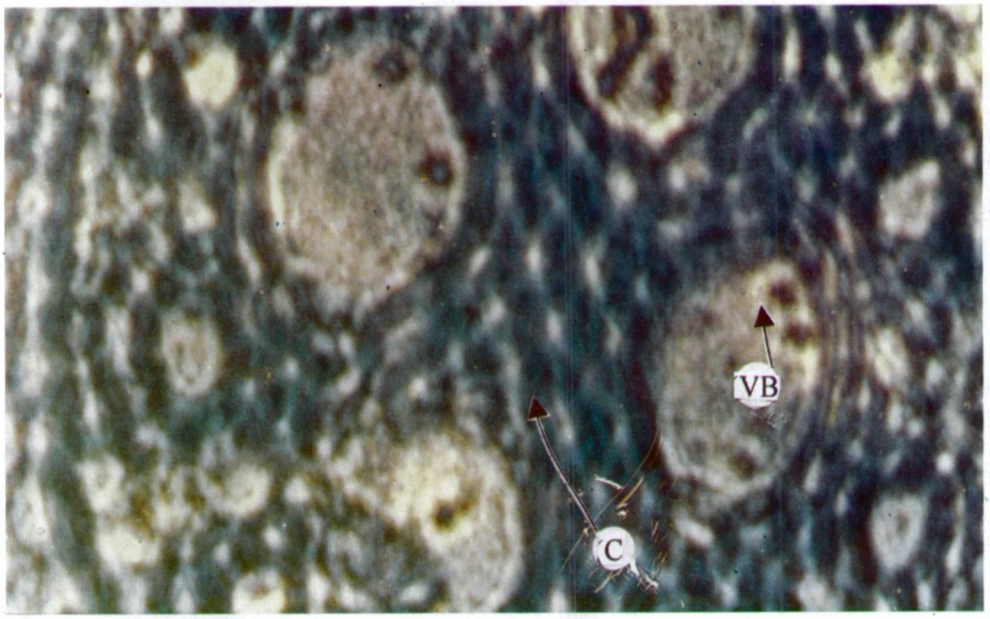

Figure 4. Enlarged sector of Transverse section of healthy (uninfected) rice roots of the variety Bg. 94-1, showing undamaged cortex and vascularbundles (VB) (2.5 X 40)

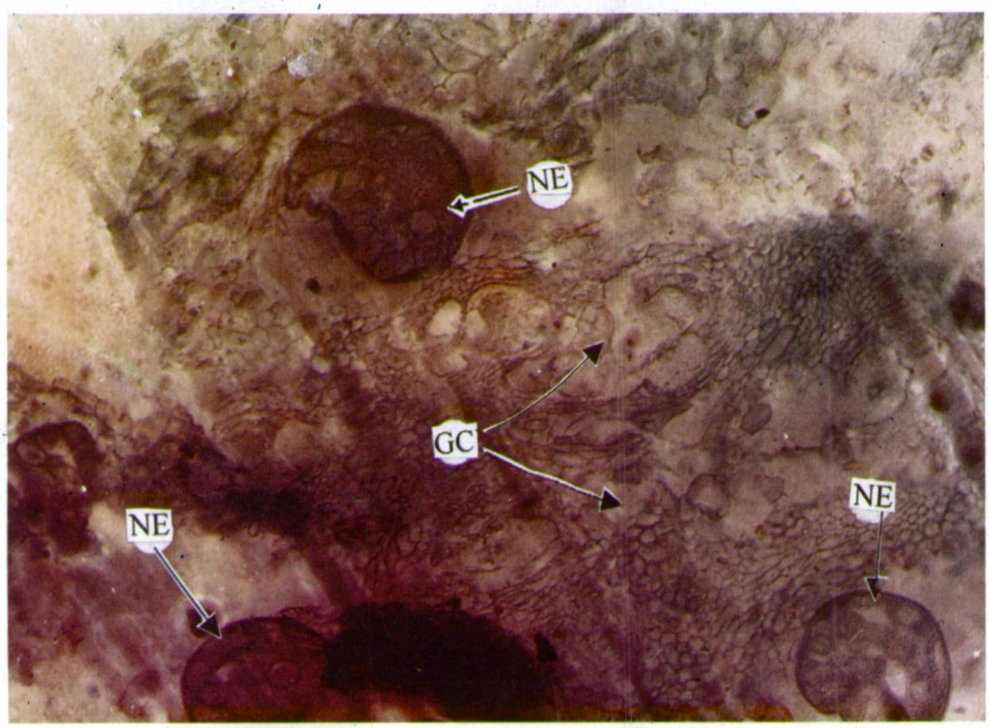

Figure 5. Transverse section of a rice roots of the variety Bg. 94-1, showing female nematodes (NE) inside root tissue and groups of vacuolated, dense cytoplasmic giant cells (GC). (2.5 X 20)

Figure 6. A female (FE) body inside an infected rice roots of the variety Bg. 94-1, 30 days after inoculation of M. graminicola. (2.5 X 40)

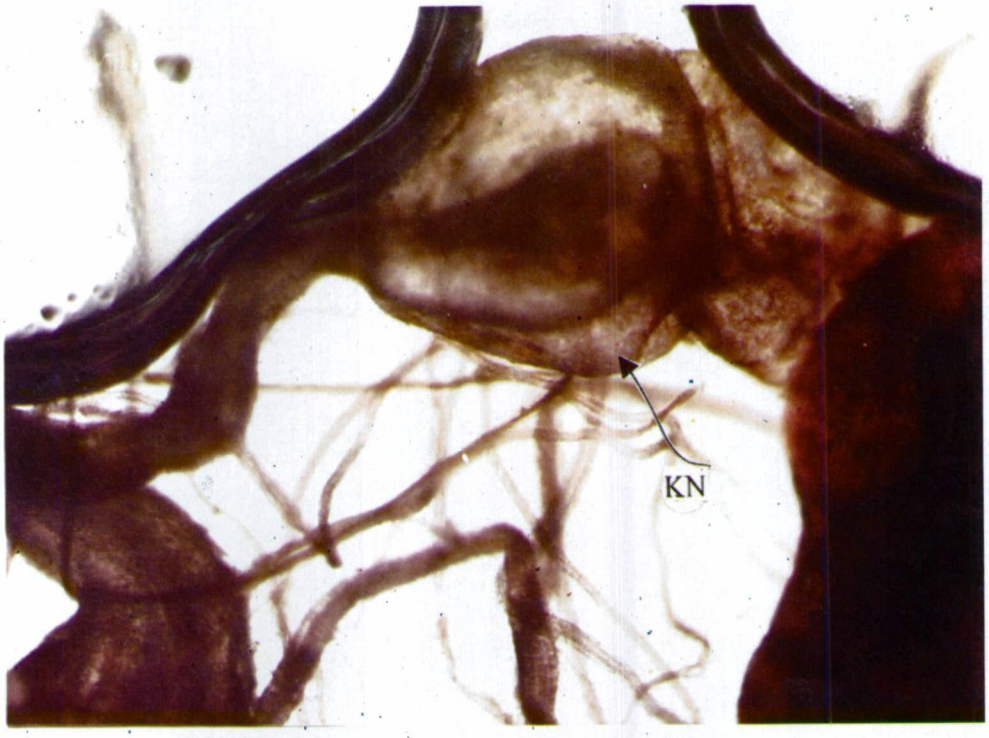

Figure 7. Infected rice roots of the variety Bg. 94-1, showing globular root knot (KN) 40 days after inoculation of M. graminicola (2.5 X 10)

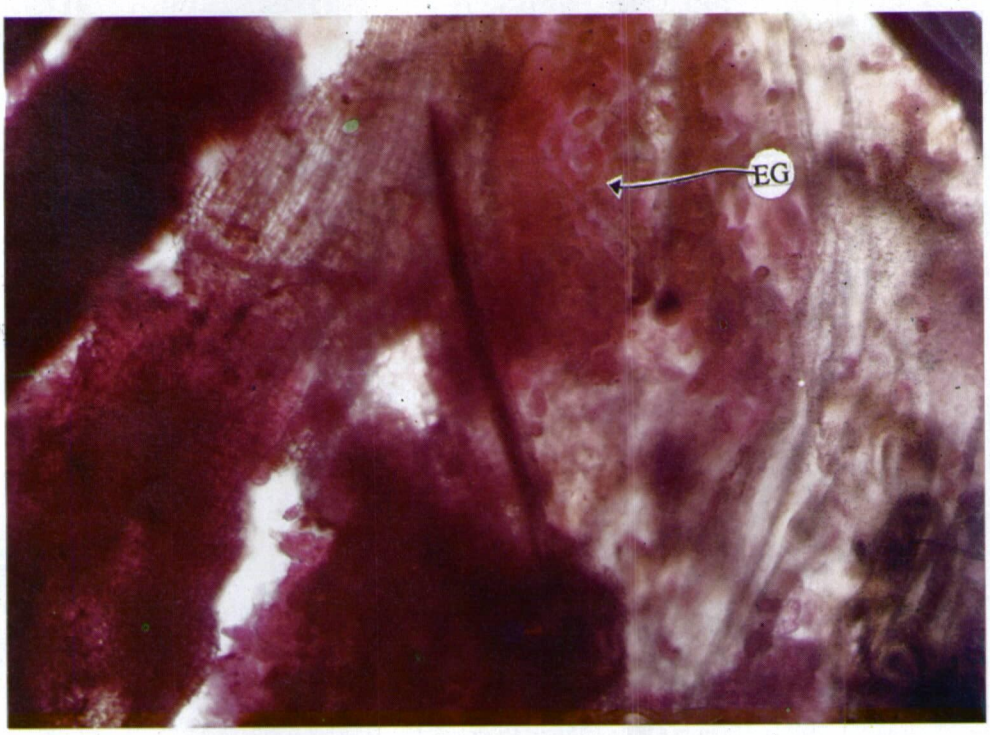

Figure 8. Longitudinal section of a rice roots of the variety Bg. 94-1, with galls showing nematode eggs (EG) inside the root tissue, 40 days after inoculation of M. graminicola (2.5 X 10)