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MORPHO-ANATOMICAL CHANGES IN GLADIOLUS DUE TO INFECTION BY *Meloidogyne incognita*

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ABSTRACT

***Meloidogyne incognita* is emerging as a major problem in the successful cultivation of gladiolus. The present study was aimed to study the effect of *M. incognita* on some plant growth parameters of gladiolus cv. 'Sylvia' and to demonstrate the histopathological changes in gladioli due to *M. incognita* infestation. During the experiment, gladiolus crop was found severely infested with *M. incognita* and showed significant reduction in plant growth parameters and flower production. In severe infestation, no flower was produced. The histopathology of plant roots revealed that 2ndstage juveniles penetrated into root cortex and moved along the cortical layer of cells and started feeding. During feeding process, they produced some hypertrophied cells in vascular cylinder and hyperplasia in pericycle regions. The hypertrophied cells were metabolically highly active, multinucleate and contained dense cytoplasm. In gladiolus, 4-6 such hypertrophied cells were observed in vascular tissues and stellar region of roots. Though the site of infection was vascular tissue but due to the nematode development and giant cell formation, the entire nematode complex and giant cells appeared irregular in root section. The nematodes damaged complete thickness of roots and this condition lead to the destruction of feeder roots which ultimately affected plant growth by reducing water and nutrient absorption from soil.**

Key words: *Meloidogyne incognita,* multinucleate giant cells, second stage juvenile

INTRODUCTION

Gladiolus is a very popular bulbous commercial flowering plant. It is native to South Africa and has been cultivated globally. The major gladiolus producing countries are the United States, Holland, France, Poland, Italy, Bulgaria, Brazil, Australia, Israel and India. According to the National Horticulture Database published by National Horticulture Board, the area under floriculture production in India during 2014-2015 was 2,48,510 ha with a production of 16,85,000 tonnes loose flowers and 4,72,000 tonnes cut flowers. Floriculture is now commercially cultivated in several states with Tamil Nadu (17%), Karnataka (14%) and West Bengal (10%) as leading states in India. The total export of floriculture in India in 2015-2016 was ₹. 479.42 crores. There are more than 300 export-oriented units in India. More than 50% of the floriculture units are based in Karnataka, Andhra Pradesh and Tamil Nadu. With the technical collaborations from foreign companies, the Indian floriculture industry is poised to increase its share in world trade (APEDA, 2015). The major

gladiolus producing states in India are Uttar Pradesh, West Bengal, Odisha, Chhattisgarh, Haryana and Maharashtra. Gladiolus is mainly a winter season flower crop but in areas having moderate climatic conditions the crop can be raised throughout the year.

Despite the area under cut flowers in India showing increase and green house cultivation helping in growing quality flowers, yet the production is significantly lower than the domestic requirement and export demands. One of the major constrains of ornamental cultivation in green house is nematode pest problem, especially the root-knot nematodes in India (Jayaraman *et al.* 1975, Gaur *et al.,* 1995, Khan and Pal, 2001). Among the plant parasitic nematodes, root-knot nematode, *Meloidogyne incognita*, is a major nematode pest infesting almost all the vegetable and ornamental crops. The length of spike, number of spikelet and quality of spike of gladioli play important role in determining the market price. Root-knot infection deteriorates the quality and quantity of spike production in gladioli. In many instances, no flower production in plants may occur. Therefore, the present study was aimed to assess the histopathological changes caused by root-knot nematode and their effect on flower production in gladiolus.

MATERIALS AND METHODS

Nematode inoculum

The pure culture of *Meloidogyne incognita* was procured from the Department of Nematology, AAU, Jorhat (India). The culture was maintained on tomato plants cv. 'Pussa Ruby' in glasshouse of the Department of Nematology, AAU, Jorhat, Assam (India). The fresh J_2s were collected from infected tomato roots as per the method of Hussey and Barker (1973). *Invitro* study on tomato (*Solanum lycopersicum*) was conducted in the Department of Nematology, AAU, Jorhat (India).

Pot experiment

The experiment was conducted in 2 kg capacity pots. The pots were filled with sterilized mixture of soil, finely dried cow dung and sand in 2:1:1 ratio. The gladiolus corms cv. 'Sylvia' were planted in each pot and labeled. The experiment was conducted in a completely randomized design with 5 treatments and each treatment replicated 5 times. The treatments comprised of 100 J_2 1000 J_2, 5000 J_2 and 10,000 J_2 and an uninoculated control. The nematodes as per treatment were inoculated 30 days after corm planting. Watering was done regularly until harvest. The symptomatology of *M. incognita* was studied in the roots of gladioli. After harvest different plant parameters *viz.*, plant height, number of flowers, number of egg masses and galls per root system and final nematode population were observed. The experimental data were subjected to statistical analysis following the "Fischer's methods of variance (Snedecor and Cochran, 1967).

Histopathology

After 2 months of inoculation the corms were uprooted and gently washed to remove adhering soil. Galled roots were cut into bits of 1-2 cm length, then fixed in FAA (formalin acetic-alcohol solution) and processed for histological studies as per the method of Sass (1964). The root segments were dehydrated in a graded series of ethanol (50-100%, 1 h at each concentration), infiltrated with xylene and embedded in paraffin wax. Serial longitudinal and cross-sections (5 μm) were cut with a Leica RM2235 rotary microtome, transferred to the glass slides and left as such for about 40 h on a hot plate to dry. Paraffin was removed by soaking the sections in xylene and rinsed with ethanol followed by distilled water (de Neergaard *et al.*, 2000). Sections were stained with toluidine blue and viewed using a Carl Zeiss B120C light microscope. The photographs of stained roots were taken.

RESULTS AND DISCUSSION

The root-knot nematode *M. incognita* is an important pest of gladiolus. The nematode causes numerous galls on gladiolus roots. Initial infection in roots was brought about by 2nd stage juveniles (J_2). At this stage juveniles of *M. incognita* initiated the penetration by puncturing action of stylet and entered into the roots (Fig.1, 2). The transverse sections of roots exhibited severe infestation of nematode. After penetration, the 2nd stage juveniles were seen migrating towards vascular zone and then slowly settled down near the cortex (Fig. 3). After settling down, the J_2 stage moults to J_3 and J_4 stages in feeding site and finally to adult females. Voracious feeding by females in adjacent cells caused cell damage. After settling down the J_2 stage moulted to J_3 and J_4 stages and were seen at one feeding site. These were finally converted to adult females. Female feeding in adjacent cells caused cell damage thereby disintegrating cortical and vascular tissues. The nematodes in vermiform stage remained oriented parallel to the vascular strands after development into mature females then became oriented perpendicular to the vascular strands.

Development of nematode, formation of giant cells, hyperplasia and hypertrophy affected all kinds of tissues and apparently, it became difficult to locate endodermis, pericycle and structures of normal xylem and phloem. The findings are in conformity with Khan *et al*. (2010) on apple roots, Sayed *et al*. (2010) on mango roots and Robab *et al*. (2010) on soybean roots infected by *M. incognita*. The giant cell complex was induced near the nematode head but hypertrophied and hyperplastic cells were observed at a little distance surrounding the body of nematodes. The shape and size of giant cells were not uniform, some were ovoid, elongated or irregular shaped with dense cytoplasm and multinucleated condition (Fig. 4). Occasionally the common cell wall between a

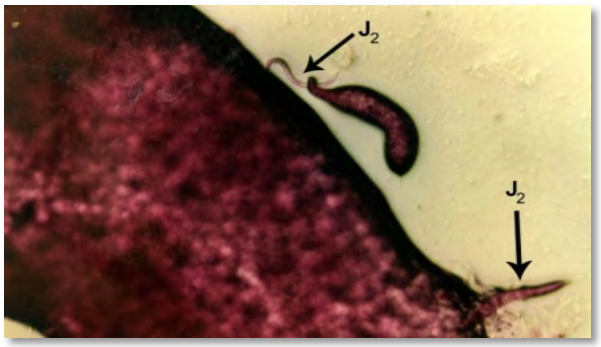

Fig. 1: Transverse section of gladiolus roots showing partial penetration of 2nd stage juvenile (J_2) of *M. incognita* into root

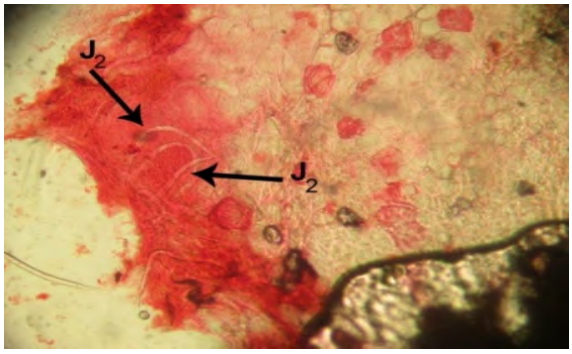

Fig. 2: Transverse section of gladiolus roots showing penetration and migration of 2nd stage juvenile (J_2) within the root tissue

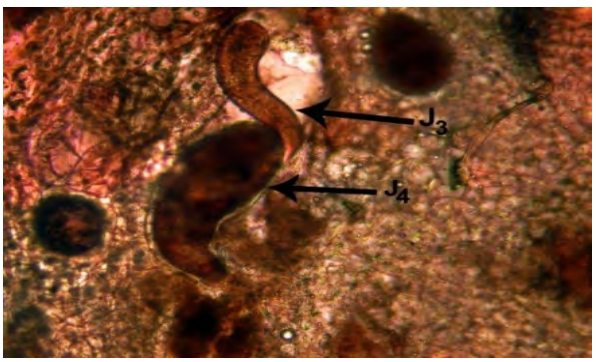

Fig. 3: Transverse section of gladiolus roots showing 3rd (J_3) and 4th stage juveniles (J_4) within root

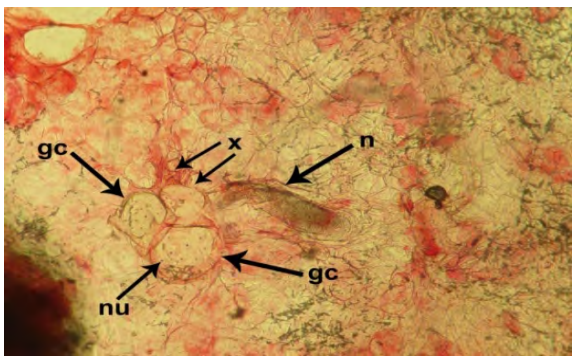

Fig. 4: Transverse section of gladiolus roots show-ing nematode (n) section, hypertrophy (ht), giant cells (gc), hyperplacia (hp) and nuclei (nu)

giant cell and parenchyma cells was dissolved and this acts as feeding sites for adult female nematode. Deformation of vascular tissues at and near the feeding sites was a common observation. Hypertrophy and hyperplasia was commonly observed in both the cortical and epidermal cells. The adult females were seen laying egg masses in gelatinous matrix embedded in cells. Fully developed females with egg mass were usually surrounded by necrotic tissues causing onset of necrosis in the roots. The eggs and hatched juveniles were found within the necrotic rings and J_2 were found moving out of the necrotic ring in the starch of healthy tissue. These observations are supported by the findings of Dipali *et al.* (2014) with similar developments in brinjal roots infested by root-knot nematodes.

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