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Scanning electron microscopy of parasitic association of soil fungus *Trichoderma* sp. with root-knot nematode *Meloidogyne incognita*

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Scanning electron microscopy of egg masses, second stage juveniles (J2) and adult females of *Meloidogyne incognita* from its laboratory culture on tomato plants (*Solanum lycopersicum*) revealed prolific fungal growth of *Trichoderma* sp. on egg masses of the nematode collected from roots of plants grown using non-sterile manure-rich soil. Fungal hyphae and conidia were found adhered to the surfaces of eggs and egg masses and the hyphae also formed trapping-rings around emerging J2 but had no contact with adult females.

Key words: *Meloidogyne incognita*, root-knot nematode, scanning electron microscopy, *Trichoderma*.

INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops. The infection starts with root penetration of second-stage juveniles (J2), hatched in soil from eggs stored in egg masses that have been laid by the females on the infected roots (Barker and Koenning, 1998).

Several approaches have been adopted for the management of plant parasitic nematodes. Some of these are the chemical, biological, cultural and physical methods. Nematicides offer a spontaneous solution to the problem but have ecological implications (Haydock et al., 2006).

Despite environmental and health concerns of nematicides, they are still widely used to control plant parasitic nematodes (Dong and Zhang, 2006). Several attempts have been made to replace pesticides with environmentally safer methods to minimize negative impact on the environment and numerous potential biocontrol agents have been reported that may have the ability to replace pesticides with environmentally safer methods, only a few have been commercialized (Barker and Koenning, 1998). Among these, *Trichoderma* species have long been recognized and highly valued as biological control agents of foliar and soil borne diseases. They possess complex chitino- and proteolytic system which makes them excellent competitors in the soil environment and by symbiotic association with roots improve plant growth and there are also reports on successful attempts to control plant-parasitic nematodes with them (Harman et al., 2004; Vinale et al., 2008; Szabo et al 2012).

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There are also other reports on successful attempts to control plant-parasitic nematodes (Windham et al., 1986; Seifullah and Thomas, 1996; Rao et al., 1998; Sharon et al., 2001; Spiegel et al., 2007, Sahebani and Hadavi, 2008; Yang et al., 2010) using *Trichoderma* spp. Apart from their antagonistic mechanisms (production of antibiotics, competing for space or nutrient), *Trichoderma* spp. excrete several lytic enzymes (glucanases, chitinases, proteases and lipases) to degrade cell wall components of other microbes (Sivan and Chet, 1989; Lorito et al., 1993; Baker and Griffin, 1995; Chet et al., 1997). The most important attribute that makes *Trichoderma* an effective antagonist of chitin containing microorganisms is their ability to produce chitinolytic enzymes. Through direct parasitism of eggs, the increase in chitinase and protease activities are thought to be responsible for the reduction in nematode infestation of plants (Sharon et al., 2001; Suarez et al., 2004).

Despite the great significance of *Trichoderma* as a biopesticide for control of phytopathogenic fungi and nematodes, little is known about the parasitic interactions between the parasitic fungus and the host organisms (Sharon et al., 2007; Vinale et al., 2008). Even basic information of morphological and physiological interacttions between the parasitic fungus and the host nematode is meagre. There has been no systematic research done to address the question of potential compatibility of *Trichoderma* spp. with various stages of root-knot nematodes. This paper describes scanning electron microscopy (SEM) of parasitic association of *Trichoderma* sp. with egg masses and juveniles of *Meloidogyne incognita* which is a widely distributed root-knot nematode (RKN) parasite of plants that causes serious economic losses to agricultural production throughout the world.

MATERIALS AND METHODS

Laboratory culture of RKN (*M. incognita*) was maintained on tomato plants (*Solanum lycopersicum*) cv. Castlerock in earthen pots using manure-rich garden soil following Den Ouden (1958). Tomato seedlings were inoculated with freshly obtained egg masses of *M. incognita* from its pre-established culture and the plants were uprooted after 45 days of inoculation. The infected roots had developed fully formed galls with adult females with numerous egg masses adhering to the root surface. Egg masses and adult females were separated in distilled water (DO) at room temperature (23±2°C) and processed immediately for SEM along with second stage juveniles (J2). For obtaining J2, some egg masses were transferred to small coarse sieve (10x10 cm size) lined with tissue paper placed before hand in a glass Petri plate containing sufficient DO so that tissue paper with egg masses remained wet and this apparatus was also maintained at room temperature (23±2°C). J2 started hatching from eggs and continued up to 3-5 days as it is expected that all the egg masses might not be infected by the fungus.

Egg masses, juveniles and adult females of *M. incognita* were fixed in 2.5% glutaraldehyde solution, washed three times in 0.2 N cacodylate buffer, post-fixed in 1% osmium tetraoxide solution followed by washing with buffer and DO and dehydrated in graded series of ethanol as per protocol given in Bozzola and Russell (1999). Because of their small size, most specimens were washed away by transferring them in different solutions and critical point drying. Therefore, after dehydration specimens were vacuum dried for 24 h before SEM observations. Dried specimens were mounted on a aluminium stubs with double sided carbon tape and gold coated in an ion sputter coater (Hitachi E-1010) and were viewed under scanning electron microscope (Hitachi S-3400N) in secondary electron mode while adjusting acceleration voltage and working distance for obtaining satisfactory images.

RESULTS AND DISCUSSION

The SEM of egg masses of *M. incognita* parasitized by soil fungus *Trichoderma* sp, revealed prolific fungal growth covering their surfaces with extensive hyphal network with numerous conidia (Figures 1 and 2). During the present studies, most of the egg masses, post-hatched juveniles and adult females showed no sign of fungal growth on their surfaces but only few egg masses collected from those tomato plants which were grown on non-sterile soil showed fungal colonization on their surfaces. Apparently, the fungus had colonized the root from its natural source in soil in the rhizosphere of the plant. Such kind of parasitic attachment of hyphae and conidia of *Trichoderma* with the surface of egg masses including that of eggs of *M. incognita* was also revealed in previous studies on *Meloidogyne javanica* (Sharon et al., 2007).

This attachment appears to be quite firm as repeated washings with water and cacodylate buffer during processing for SEM did not affect the fungal association with the host surface. The gelatinous matrix (GM) which form the bulk of the egg mass surface and also envelopes the eggs, is the major site of fungal attachment and growth and showed a thick network of extensively branched fungal hyphae which appeared adhering and anchoring the GM at some places while at other places also showed enlargements of hyphal tips and appressoria like structures (Figures 1 and 2). Separated conidia were also seen adhering to GM. Observations of eggs protruding from egg mass surface with adhering hyphae with appressoria (Figure 1) and conidia attached to the egg surface (Figure 3) without hyphae revealed that both hyphae and conidia have the ability to infect the eggs separately (Figure 3) which is similar to GM. Role of GM in establishing parasitic interaction of *Trichoderma* with nematode egg masses was also determined by using GM coated nylon fibres to which conidia and hyphae become tightly attached (Sharon et al., 2007). In fact, GM is a viscous jelly containing glycoproteins and certain enzymes (Sharon and Spiegel, 1993; Sharon et al., 2007) and it appears to provide nutrient support to the prolific fungal growth with numerous conidia as observed in the present studies. *Trichoderma* has also been shown to secrete several hydrolytic enzymes (Harman et al., 2004; Vinale et al., 2008) which may facilitate nutrition and other parasitic activities of the fungus.

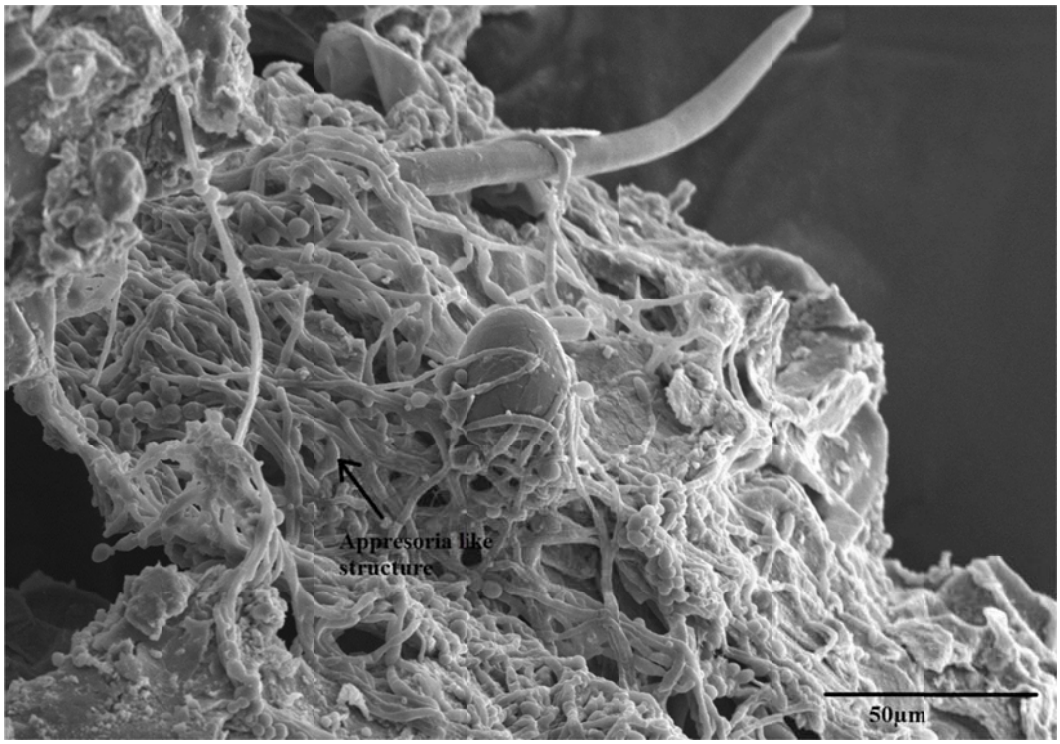

Figure 1. SEM micrograph of soil fungus *Trichoderma* sp. parasitizing the egg mass of the root-knot nematode *Meloidogyne incognita* showing fungal hyphae spread over egg mass surface, protruding egg and also forming trapping ring around an emerging second stage juvenile.

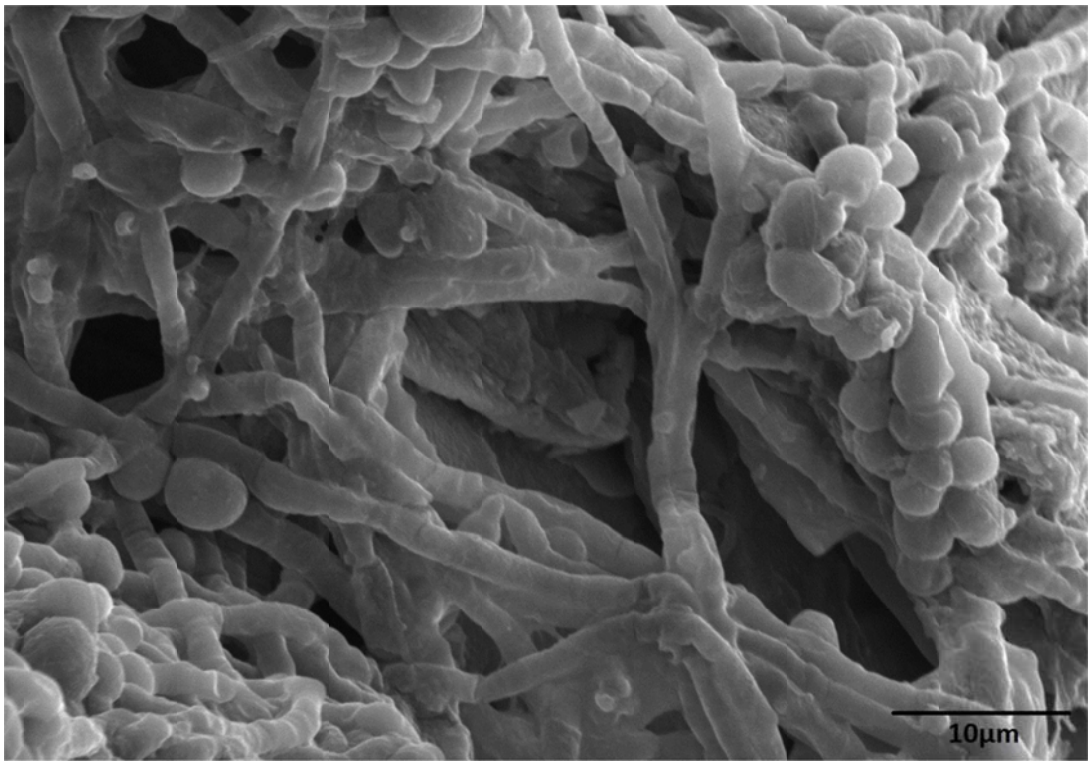

Figure 2. SEM micrograph showing prolific thick growth of the soil fungus *Trichoderma* with branching hyphae and numerous conidia on the part of the egg mass surface of *Meloidogyne incognita*.

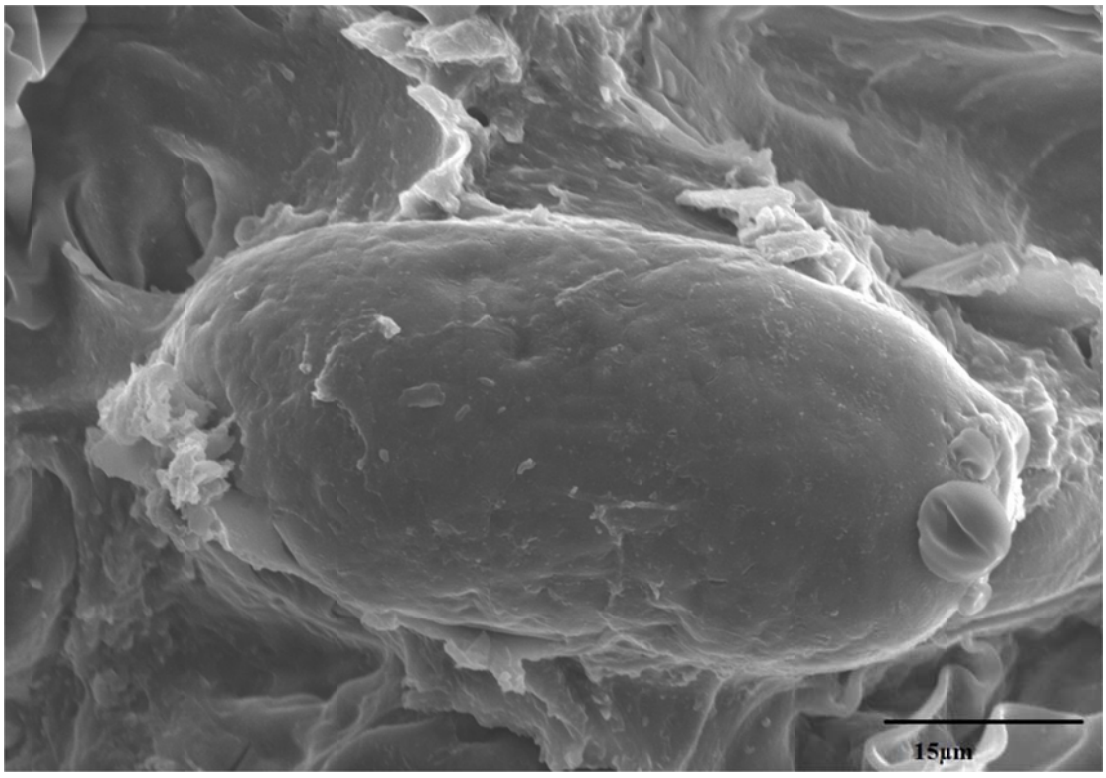

Figure 3. SEM micrograph of an egg of *Meloidogyne incognita* embedded in gelatinous matrix of the egg mass and showing the presence of an attached conidium of *Trichoderma* on the surface.

After hatching, emerging J2s were seen restrained from leaving the egg mass by the trapping rings formed by the fungus around their bodies (Figure 1). The hyphae appeared to adhere to the J2 body and grow along its length. From non-parasitized egg masses, J2 quickly leave into the medium but were seen held in place by the fungal hyphae when parasitized. J2 did not show any conidial attachment. The fungal hyphae were seen penetrating the plant roots by Yedidia et al. (1999); however, the endoparasitic sedentary adult females were not parasitized by the fungus. Not a single female show any sign of fungal attachment during the present studies. Similarly, after separation from egg masses, J2 which are free from GM, are also not affected by *Trichoderma* but those still in emerging condition, which may have GM on their surface, are restrained by the fungal hyphae by forming trapping rings. Sharon et al. (2007) also reported that J2 of *M. javanica* without GM are rarely parasitized by the fungus. The absence of conidial attachments on J2 of *M. incognita* while emerging from egg masses reveals inability of fungus to initiate growth on the surface of J2 which, somehow, get trapped by hyphae extending from GM of the egg mass.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Baker R, Griffin GJ (1995). Molecular strategies for biological control of fungal plant pathogens. In: Reuveni R (Ed.) Novel approaches to integrated pest management. FL, USA, Boca Raton. pp. 153–182.

Barker KR, Koenning SR, (1998). Developing sustainable systems for nematode management. Ann. Rev. Phytopathol. 36:165-205.

Bozzola JJ, Russell LD (1999). Electron Microscopy (Sudbury, Massachusetts: Jones and Bartlet Publishers). p. 670.

Chet I, Inbar J, Hadar Y (1997). Fungal antagonists and mycoparasites. In: Wicklow DT, Söderström B (Eds.) The Mycota IV: Environmental and Microbial Relationships. Springer-Verlag, Berlin. pp. 165–184.

Den Ouden H (1958). A new method for culturing plants enabling observation of nematodes on growing roots. Netherland J. Plant Pathol. 64:269-272.

Dong L, Zhang K (2006) Microbial control of plant parasitic nematodes: a five party interaction. Plant Soil 288:31-45.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004). *Trichoderma* sp.- opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2: 43-56.

Haydock PPJ, Woods R, Grove IG, Hare MC (2006). Chemical control of nematodes. In: Perry N R and Moens M (ed) *Plant Nematology* CAB International, Oxfordshire, U.K.

Lorito M, Harman GE, Hayes CK, Broadway RM, Tronsmo A, Woo SL, Di Pietro A (1993). Chitinolytic enzymes produced by *Trichodermaharzianum*: antifungal activity of purified endochitinase and chitobiosidase. Phytopathology 83: 302–307.

Rao MS, Reddy PP, Nagesh M (1998). Evaluation of plant based formulations of *Trichoderma harzianum*for the management of *Meloidogyne incognita* on eggplant. Nematol. Mediterr. 26:59-62.

Sahebani N, Hadavi N (2008). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Soil Biol. Biochem. 40: 2016–2020.

Seifullah P, Thomas BJ (1996). Studies on the parasitism of *Globodera rostochiensis* by *Trichoderma harzianum* using low temperature

scanning electron microscopy. Afro-Asian J. Nematol. 6: 117-122.
Sharon E, Bar-Eyal M, Chet I, Herrera-Estrella A, Kleifeld O, Spiegel Y (2001). Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*; Phytopathology 91:687-693.
Sharon E, Chet H, Viterbo A, Eyal M B, Nagan H, Samuels G J, Spiegel Y (2007). Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. Eur. J. Plant Pathol. 118: 247-58.
Sharon E, Spiegel Y (1993). Glycoprotein characterization of the gelatinous matrix in the root–knot nematode *Meloidogyne javanica*. J. Nematol. 25:585-589.
Sivan A, Chet I (1989). The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. Phytopathology 79:198-203.
Spiegel Y, Sharon E, Bar-Eyal M, Van Assche A, Van Kerckhove S, Vanachter A, Viterbo A, Chet I (2007). Evaluation and mode of action of *Trichoderma* isolates as biocontrol agents against plant-parasitic nematodes. Proceedings of IOBC/ WPRS Meeting, Spa, Belgium. IOBC/WPRS Bulletin 30:129-133.
Suarez B, Rey M, Castillo P, Monte E, Llobell A (2004). Isolation andcharacterization of PRA1, a trypsin-like protease from the biocontrol agent *Trichoderma harzianum* CECT 2413 displaying nematicidal activity. Appl. Microbiol. Biotechnol. 65:46-55.
Szabo M, Csepregi K, Galber, Viranyi F, Fekete C (2012) Control plant-parasitic nematodes with *Trichoderma* species and nematode-trapping fungi: The role of chi18-5 and chi18-12 genes in nematode egg-parasitism. Biol. Control 63:121-128.
Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008). *Trichoderma*-plant-pathogens interactions. Soil Biol. Biochem. 40:1-10.
Windham GL, Windham MT, Williams WP (1986). Effects of *Trichoderma* spp. on maize growth and *Meloidogyne arenaria* reproduction. Plant Dis. 73:493-494.
Yang ZS, Li GH, Zhao PJ, Zheng X, Luo SL, Li L, Niu XM, Zhang KQ (2010). Nematicidal activity of *Trichoderma* spp. and isolation of an active compound. World J. Microbiol. Biotechnol. 26:2297-2302.
Yedidia I, Benhamou N, Chet I (1999). Induction of defence responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 65: 1061-1070.