

# Infection of Cultured Thin Cell Layer Roots of *Lycopersicon esculentum* by *Meloidogyne incognita*

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**Abstract:** A new aseptic culture system for studying interactions between tomato (*Lycopersicon esculentum*) and *Meloidogyne incognita* is described. Epidermal thin cell layer explants from peduncles of tomato produced up to 20 adventitious roots per culture in 4-9 days on Murashige & Scoog medium plus kinetin and indole acetic acid. Rooted cultures were transferred to Gamborg's B-5 medium and inoculated with infective second-stage juveniles. Gall formation was apparent 5 days after inoculation and egg production by mature females occurred within 25 days at 25 C in the susceptible genotypes Rutgers and Red Alert. Resistant genotypes LA655, LA656, and LA1022 exhibited a characteristic hypersensitive response. This system provides large numbers of cultured root tips for studies on the molecular basis of the host-parasite relationship.

**Key words:** aseptic culture, host resistance, host susceptibility, *Lycopersicon esculentum*, *Meloidogyne incognita*, nematode, southern root-knot nematode, tomato.

The use of aseptically grown plant tissues for propagating nematodes gnotobiotically is well established and includes alfalfa callus (7), root cultures (8), and carrot discs (11). Rearing *Meloidogyne* in aseptic culture systems on a scale suitable for germplasm maintenance (16) or molecular studies (15) is difficult because the complex nature of the host-parasite relationship (6) requires the use of root-tip cultures (5). Cultured plant roots genetically transformed by *Agrobacterium rhizogenes* (9), which causes an increased root growth response known as the hairy-root phenotype, also has been exploited to propagate root-knot and cyst nematode species (14,20).

We have investigated the use of an alternative root culture system that may have advantages for molecular studies of root-knot disease. Cultured thin cell layer (TCL) explants of epidermal tissue from floral stems of tobacco (1,18,19) or tomato (M. Compton and R. Veilleux, pers. comm.) can be induced to grow adventitious roots when cultured on an appropriate growth media. This report describes the responses of both root-knot susceptible and resistant

tomato genotypes in TCL-root cultures to infection by *Meloidogyne incognita*.

## MATERIALS AND METHODS

**Root cultures:** Tomato root cultures were established from two nematode susceptible and three resistant genotypes of *Lycopersicon esculentum* Mill. (Table 1). Red Alert (susceptible) was obtained from M. Compton, Virginia Polytechnic Institute & State University; Rutgers (susceptible) was purchased commercially; and LA655, LA656, and LA1022 (all resistant) were supplied by Dr. Charles Rick, University of California, Davis, California. Thin cell layer cultures were initiated with explants from peduncles of fully opened flowers. Tissues were surface sterilized in 1.05% NaOCl for 15 minutes and rinsed three times with sterile distilled water. Epidermal peel sections (10 × 1 × 0.2 mm) were sliced from the floral stems of 1-2-month-old plants with a sterile surgical blade and placed cut side down on 1% agar medium containing Murashige & Scoog (MS) salts (13); Nitsch vitamin mixture (12), 3% sucrose, 10<sup>-9</sup> M kinetin, and 10<sup>-5</sup> M indole acetic acid (Compton and Veilleux, pers. comm.) adjusted to pH 5.7 with 1 N NaOH before autoclaving. Cultures were incubated under 70 μE·m<sup>-2</sup>·s<sup>-1</sup> of fluorescent illumination for 16 hours per day at 25 C.

Root cultures also were established from root tips excised from aseptically germinated tomato seedlings (5). All root cul-

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TABLE 1. Root growth and *Meloidogyne incognita*-induced disease responses in thin cell layer root cultures of susceptible and resistant tomato genotypes.

Tomato genotype	Rooted cultures (%)	Roots per explant	Days to root growth	Disease response†	Roots with galls (%)
Red Alert	85	15	4	Susceptible	80
Rutgers	40	5	9	Susceptible	85
LA655	32	6	8	HR	0
LA656	30	4	8	HR	0
LA1022	35	9	8	HR	0

† HR = hypersensitive resistant response detected as localized necrosis of root tips and subsequent proliferation of secondary roots.

tures, including TCL, were grown in the dark on Gamborg's B-5 medium (4) plus 1% sucrose without plant growth regulators for at least 2 days before inoculation with nematodes.

*Inoculation of cultures:* *Meloidogyne incognita* was propagated on greenhouse-grown Rutgers tomato. Eggs were hand picked from 7–10-week-old plant and surface sterilized with 0.5% chlorhexidine diacetate for 3–4 hours at 25 C. Following several washes with sterile tap water, egg masses were recovered by centrifugation and the eggs were hatched in a moist chamber. Infective second-stage juveniles (J2) were collected with a pipet and disinfested in 0.4% chlorhexidine for 15 minutes followed by several changes of sterile tap water. Root cultures were inoculated by placing 1,000 J2 in 0.1 ml sterile tap water on the agar surface in the vicinity of the root tips. The cultures were incubated for 24 hours and the remaining juveniles were rinsed off the agar with sterile tap water.

## RESULTS

*Root culture system:* Thin cell layer root cultures were successfully initiated in each of the tomato genotypes (Table 1). The general patterns of root morphogenesis were similar in all varieties tested. Within 1–2 days of initiation of TCL cultures, the explanted tissue began to swell while maintaining a pale green appearance. The TCL explants that rooted successfully showed little or no callus growth before or during rooting. Thickness of the explant greatly influenced development of roots. Explants more than 1 mm thick typically grew more

callus and fewer roots than did explants less than 0.5 mm thick which often turned brown and died. The optimum thickness of TCL for rooting was about six cells in depth and included epidermal, subepidermal, and some of the underlying parenchymal cells.

Root initiation was usually completed within 1–2 days after the first roots appeared. Roots typically grew on the peripheral edge of each explant (Fig. 1A, B). Thereafter, additional growth was confined primarily to root elongation (Fig. 1B) yielding a mat of roots on the culture plate within 2–3 weeks. Elongation of TCL roots occurred equally well on MS or Gamborg's B-5 plant culture media; however root initiation occurred only on MS medium.

The age of plants and organs used to initiate culture of TCL influenced rooting. Rooting of TCL explants was retarded when senescing flowers or plants older than 3 months were used as the TCL source. The position on a plant from which the TCL was taken also affected rooting. Explants from vegetative stems or other plant parts, including leaves, responded poorly and often showed greater production of callus instead of roots.

*Responses to nematode inoculation:* Roots grown from TCL explants of the five tomato genotypes were observed for expression of nematode disease responses. Swelling of root tips of the susceptible genotypes due to infections was evident within 4–5 days after inoculation. In susceptible genotypes, more than 80% of the roots exhibited gall formation (Table 1). Typical susceptible responses were root-tip swell-

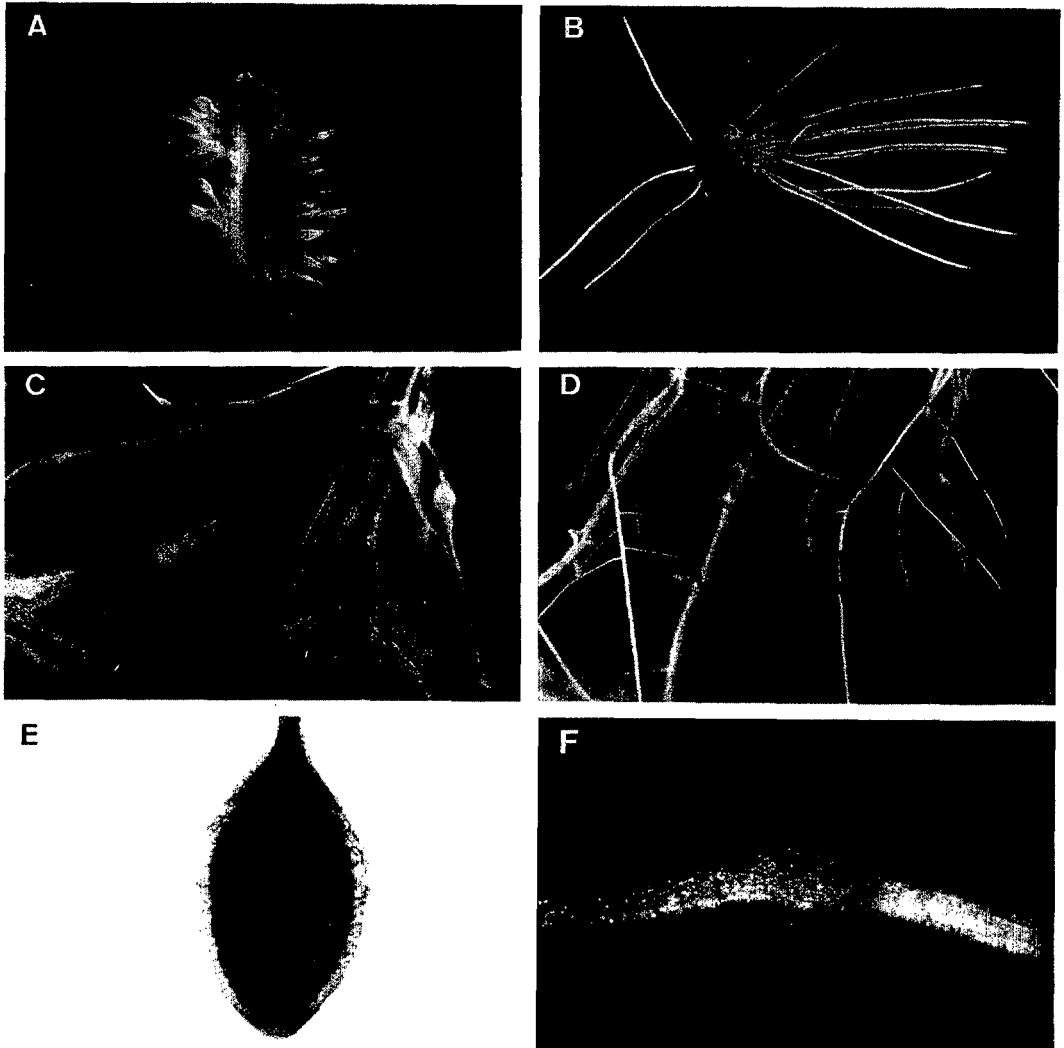


FIG. 1. Tomato thin cell layer (TCL) root cultures and in vitro expression of susceptible and resistant genotypes in response to *Meloidogyne incognita* inoculation. A) Root initiation in TCL of Red Alert after 7 days of culture. B) Root growth in TCL from genotype LA656 after 14 days of culture. Note absence of lateral roots. C) Infected roots of var. Red Alert TCL 60 days after inoculation with *M. incognita* second-stage infective juveniles. D) Root growth in TCL explants of resistant genotype LA1022 21 days after nematode inoculation. Note lateral root development. E) Micrograph of an infected root tip of Red Alert 4 days after nematode inoculation. F) Micrograph of root gall showing nematode maturation and reproduction on TCL culture 40 days after inoculation.

ing and gall formation (Fig. 1C, E). Galls in TCL roots continued to enlarge for 4–6 weeks and resulted in large, irregularly shaped galls (Fig. 1C) containing mature females and egg masses. Later disease responses also included multiple galling along the same root. Disease symptoms and nematode reproduction appeared more extensive on the Gamborg B-5 medium than on the MS medium. Initial gall for-

mation in response to nematode inoculations was similar on both media.

Galls were absent in the resistant genotypes (LA655, LA656, and LA1022) (Table 1, Fig. 1D), but microscopic examination of resistant root tips exposed to nematodes revealed localized browning and necrosis characteristic of a hypersensitive resistance response. The resistant genotypes also showed increased lateral root in-

duction within 2–3 weeks after nematode inoculation (Fig. 1D). Uninoculated resistant genotypes showed little or no growth of lateral roots (Fig. 1B).

To confirm that TCL root infections were similar to responses in roots from whole plants, nematode inoculations were done on susceptible root cultures started from aseptically germinated seedlings (5) and on roots that remained attached to the seedlings. These control experiments (data not shown) produced results similar to those observed in the TCL roots.

### DISCUSSION

An understanding of the molecular mechanisms involved in host-plant responses to nematode parasitism is a prerequisite for the rational application of genetic engineering technologies to enhance resistance in crops. Significant progress has been made in understanding the molecular mechanisms of plant diseases and resistance to viral, bacterial, and fungal pathogens (2); however, little has been done on the molecular responses of plants to nematodes. One reason has been the difficulty in achieving high levels of synchronous infections in a defined experimental system so that a sufficient amount of responding plant cells could be isolated to permit molecular analyses throughout the course of the disease process.

Whereas plants transformed by *Agrobacterium rhizogenes* provide extreme proliferation of cultured roots that may support nematode infections, the mechanism of transformed root growth unfortunately involves alterations in the plant's endogenous growth regulator levels. Because expression of genetically based resistance to root-knot nematodes (3) as well as other defense-related genes (17) can be affected in plants when phytohormone levels are altered, the use of *A. rhizogenes* may not be the best experimental system for molecular studies of plant–nematode interactions.

Our goal in developing this TCL system was to provide sufficiently large quantities of host tissue for molecular studies while

avoiding the introduction of factors that could seriously alter the infection process.

The results presented here showing rapid genotype-dependent infection of parasitic nematodes in TCL root cultures support the potential use of this experimental system for future molecular studies on the genetic regulation of root-knot disease susceptibility and resistance. Recently reported results (10) describing an analogous TCL system for a molecular study of plant apical morphogenesis lend credence to this conclusion.

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