

Reproduction of *Globodera rostochiensis* on Transformed Roots of *Solanum tuberosum* cv. Desiree

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Abstract: Transformed roots of the susceptible potato *Solanum tuberosum* L. cv. Desiree were inoculated with second-stage juveniles (J2) of *Globodera rostochiensis* pathotype Ro1. Adult males emerged after 3-4 weeks and matings with females occurred. After 8 weeks gentle pressure on the eggs of maturing females released the J2 which were viable. Because this technique enables the production of vigorously growing roots with numerous laterals, it may be suitable for obtaining a high yield of sterile potato cyst nematodes.

Success with the sterile in vitro culture of a sedentary nematode, *Heterodera schachtii* Schmidt, was achieved many years ago (10). More recently, Murashige and Skoog (MS) medium was used to culture susceptible and resistant potato roots on which *Globodera rostochiensis* (Wollenweber) Behrens was reared (2). The use of transformed roots to rear *H. schachtii* and *Meloidogyne javanica* in vitro has opened up new possibilities for studying resistance genes against sedentary nematodes (8,13,14). The objective of this research was to determine whether *G. rostochiensis* could be cultured in vitro on transformed potato roots.

MATERIALS AND METHODS

Transformed root cultures: Susceptible *Solanum tuberosum* L. cv. Desiree tubers were soaked in absolute ethanol for 5 minutes, peeled, and surface sterilized for 15 minutes in a 10% solution of Domestos, a commercial bleach (Unilever plc, London, UK) containing 7% sodium hypochlorite. They were then washed six times in sterile distilled water and finally immersed in liquid Murashige and Skoog medium (MS powder, Flow Laboratories, Irvine, UK) supplemented with 20 g sucrose per liter, adjusted to pH 5.8 (MS20) but without hormones (12). Several columnar sections were removed from the tubers using a sterile

1-cm-d cork borer and sliced into disks 1-2 mm thick with a scalpel. The tuber disks were floated on 25 ml of MS medium containing *Agrobacterium rhizogenes* LBA 9402 carrying the Ri plasmid and binary vector pBin 19, obtained from Dr. J. Hamill (5). The bacterium had been grown overnight in YMB medium (6) containing 50 µg kanamycin/ml. After 30 minutes incubation with *Agrobacterium*, the tuber disks were transferred to the selection medium, MS20 medium solidified with 1% agar (Difco agar, Difco Laboratories, East Molesey, UK) containing 250 µg cefotaxime/ml (Roussel Laboratories, Uxbridge, UK) and 100 µg kanamycin/ml (Sigma, Poole, UK). Cefotaxime was used to kill *A. rhizogenes* and kanamycin to select transformed roots. The plates were sealed with Nesco film (Nippon Shoji-Kaisha, Osaka, Japan) and incubated at 25 C in a 16-hour photoperiod. Tuber disks were subcultured every 10 days onto the fresh selection medium. Developing roots that appeared after 4 weeks of culture were removed from the tuber disks and subcultured on fresh selection medium at least five times at 10-day intervals. The vigorously growing hairy roots free from *Agrobacterium* were then selected from the young root tips, 1 cm long, and maintained on MS20 medium with 100 µg kanamycin/ml.

Detection of neomycin phosphotransferase II (NPT II) activity: NPT II dot-blot assay was performed as previously described (11). The blot was exposed to X-ray film for 20 hours and the positive was developed and photographed.

Nematode sterilization and inoculation:

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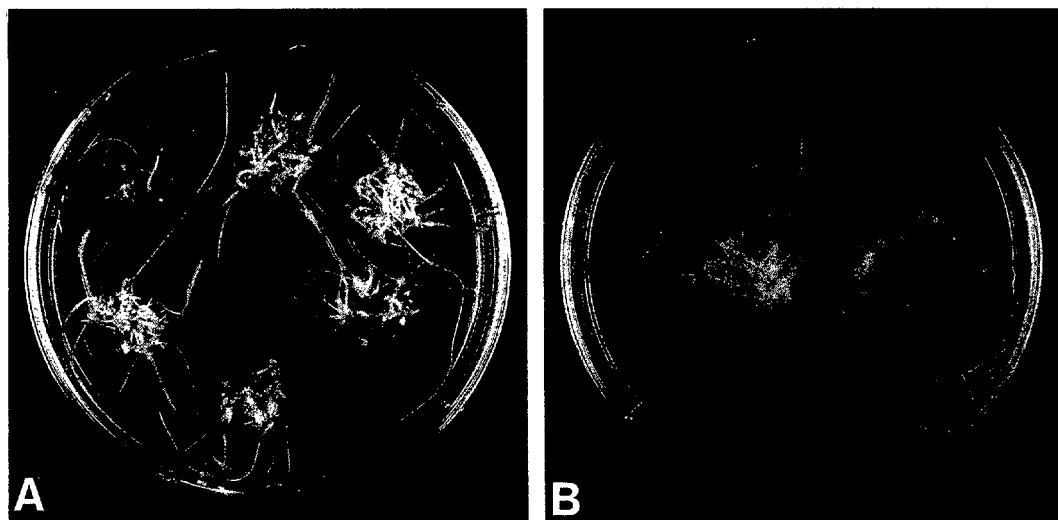


FIG. 1. Transformed roots growing from tuber disks of *Solanum tuberosum* cv. Desiree. A) Four weeks after inoculation with *A. rhizogenes* on the selection medium. B) With many vigorously growing lateral hairy roots after subculture on MS20 medium.

Hatched second-stage juveniles (J2) of *G. rostochiensis* pathotype Ro1 were obtained as previously described (1). Cysts were pre-soaked for 7 days in tap water and then in potato root diffusate. The J2 that hatched within 24 hours were placed in a polystyrene syringe fitted with polyester mesh (3) to facilitate handling and stored for 1 week at 4 C in 50 μ g streptomycin/ml water. Pieces of transformed potato root, ca. 1 cm long, were cut and transferred to nutrient agar (1.25% Davies agar with half strength MS20 medium) in petri dishes. After 3 days in antibiotic, the J2 were washed repeatedly in sterile distilled water, treated for 2 seconds with 0.01% (w/v) mercuric chloride solution, and again washed thoroughly by flushing sterile distilled water through the syringe. The volume was reduced until the nematodes formed a dense suspension. Aliquots of 300–400 J2 were drawn off and placed close to the roots with a sterile micropipet. Excess water was removed by blotting with sterile filter paper. The petri dishes were then sealed with Nesco film and placed in a dark incubator at 20 C. Root cultures were examined with a Kyowa stereomicroscope (40 \times). After 7–8 weeks golden females were removed and cut open and J2

were released from the eggs by gentle pressure. Viable juveniles were motile soon after release or on examination 3 days later.

RESULTS AND DISCUSSION

Inoculation of tuber disks of *S. tuberosum* cv. Desiree with *A. rhizogenes* LBA 9402 carrying both the plasmids Ri and Bin 19 produced vigorous root growth on the selection medium (Fig. 1A). These roots continued to grow rapidly when transferred to fresh MS20 medium lacking plant growth hormones but containing 100 μ g kanamycin/ml (Fig. 1B), demonstrating that they had been transformed by the co-transfer of the T-DNA from *A. rhizogenes* and the NPT II gene of the disarmed binary vector pBin 19 from *A. tumefaciens*, as previously reported (5). The kanamycin-resistant phenotype of the hairy roots resulting from the expression of the NPT II gene was demonstrated by the NPT II dot-blot assay. NPT II enzymatic activity was present in the transformed hairy roots and absent from the untransformed roots (not shown). Untransformed roots, which lack the gene NPT II, therefore were unable to grow on the medium containing kanamycin. Excised root pieces of transformed Desiree also grew well on MS20 medium

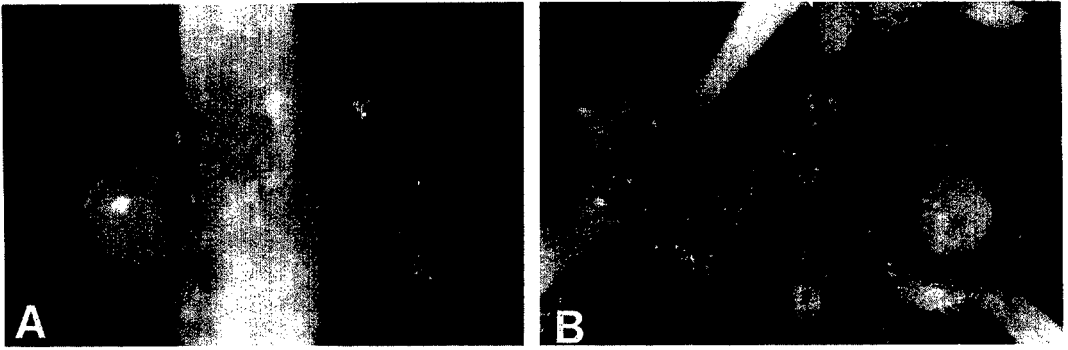


FIG. 2. *Globodera rostochiensis* Rol on transformed roots. A) Golden females 7–8 weeks after inoculation. B) Brown females 9–10 weeks after inoculation.

with or without kanamycin, producing many side roots which were densely covered with root hairs (Fig. 1B).

The J2 were able to penetrate the transformed roots and developed within them, as was reported by Mugnier (9). Males emerged after 3–4 weeks and moved across the agar to mate with expanding females which ruptured the root cortex and epidermis. After 7–8 weeks the females became golden in color (Fig. 2A). Areas of undifferentiated root growth often were found close to where the females fed (Fig. 2B). Browning of the roots also occurred. Most females contained large numbers of eggs. The nematode J2 released mechanically from the eggs were viable, as judged by their motility.

As with *H. schachtii* on beet (10), the development of *G. rostochiensis* on Desiree was not adversely affected by transformation with *A. rhizogenes*. After 7–8 weeks golden females containing many viable eggs were present in abundance, as on untransformed roots (Forrest, unpubl.). Pathogenicity of these juveniles, however, was not tested, although results with *H. schachtii* suggest changes are unlikely (12). Because of the additional advantage of many vigorously growing root tips, this system should be suitable for the large-scale culture and storage of potato cyst nematode pathotypes under sterile conditions and for in vitro screening of large numbers of susceptible and resistant mutant lines in a pro-

gram of transposon mutagenesis designed to locate resistance genes in potato.

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