Technique for Gnotobiotic Cultivation of *Heterodera glycines* Ichinohe on *Glycine max* (L.) Merr.[1]

J. A. Lauritis, R. V. Rebois[2], and L. S. Graney[3]

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe is one of the most destructive pests of soybean, *Glycine max* (L.) Merr., and causes soybean yield reduction that results in economic loss when fields are heavily infested. Studies by Ichinohe (1,2) have contributed significantly to our current understanding of the growth and development of the SCN. However, the results of these early reports introduced experimental variables that were inherently associated with greenhouse and field conditions. Gnotobiotic cultivation of SCN provides a controlled environment that enhances studies concerned with nematode development and host-parasite relationships. This study reports the successful gnotobiotic cultivation of SCN; preliminary results were reported earlier (3).

The SCN, Race 3, used in this study originated from infested soybean fields in western Tennessee. Nematodes were increased on the susceptible soybean cv. Essex in greenhouse pot cultiures. Cysts and egg masses from the pot cultures were provided by Dr. L. D. Young, USDA, Jackson, Tennessee. All equipment and solutions used for the isolation of SCN were sterilized by autoclaving at 15 p.s.i. for 15 min and all procedures were done aseptically. Cysts and egg masses were placed on six layers of tissues (Kimwipes[R]) supported by a wire screen in a petri dish (5) containing 20 ml of an aqueous solution of 50 ppm streptomycin sulfate (SS) and 20 ppm of 8-quinolinol sulfate (QS). After 24 h, the wire screen was removed and the aqueous solution containing second-stage larvae (L-2) which had passed through the tissues was poured onto six layers of tissues with supporting wire screen in another petri dish. The original solution was decanted and replaced with 20 ml of a fresh solution of SS plus QS. The L-2 which passed through the second set of tissues after 24 h were concentrated by centrifugation at 1,500 rpm for 5 min. The L-2 were washed three times by resuspension in distilled water. The washed L-2 were resuspended and treated three times for 20 min each with a solution of 1,000 ppm SS. The treated L-2 were collected on a 10-μm filter (Millipore[R]) and washed three times with distilled water. The L-2 were resuspended in distilled water and used immediately for inoculation of the soybean root cultures.

Axenic soybean root cultures were prepared according to the following method. Soybean seeds (cv. Kent) were sonicated in a 0.1% detergent solution (Ultrasonic Cleaner, Cavi-clean[R], Mettler Electronics

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[2]Respectively, Microbiologist and Nematologist, Nematology Laboratory Plant Protection Institute, USDA ARS NER, Beltsville, MD 20705.

[3]Agricultural Technician, Department of Botany, University of Maryland, College Park, MD 20742.

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Fig. 1. Gnotobiotic culture of excised 'Kent' soybean roots showing distribution and development of soybean cyst nematode females (arrows) 14 days after inoculation. × 1.2.

Fig. 2. Enlarged view of a 'Kent' soybean root showing a cluster of females with egg masses 20 days after inoculation. × 33.

Corp.) for 10 min. Seeds were rinsed with tap water, wrapped in cheese cloth, and sterilized by sonication in 1–1.5% sodium hypochlorite solution for 10 min. Surface sterilized seeds were placed on 1.5% water agar in petri plates (100 × 15 mm) and incubated for approximately 3 days at 25 C ± 1. Radicles were aseptically excised 2–3 cm from the root tip and transferred to petri dishes containing a modified STW agar medium (4). The STW medium was modified by increasing the concentration of $FeSO_4 \cdot 7H_2O$ from 23.0 to 27.85 mg/L (5.6 ppm Fe^{++}) and adjusting the pH of the medium to 5.8 by adding acid or base after autoclaving. At lateral root development, each root culture was inoculated with an aqueous suspension of ca. 200 treated L-2. The cultures were incubated in the dark at 25 C ± 1.

The efficacy of the described methods in establishing the SCN on Kent soybean was ca. 75%. The remaining cultures were contaminated with sundry micro-organisms, a condition attributed primarily to the inadequate sterilization of L-2 and not to seed disinfection. The medium supported substantial root growth with subsequent SCN development (Fig. 1). Mature females with eggs were often observed in clusters on the root (Fig. 2). Cultures were propagated by transferring young females with egg masses to fresh excised soybean root cultures. L-2 hatched intermittently from eggs, thus allowing the establishment and maintenance of stock cultures.

Presently, studies are in progress to simplify and improve methods of nematode sterilization, the monoxenic cultivation of other SCN races, the elucidation of the SCN life cycle, and the host-parasite interaction with susceptible and resistant soybean cultivars under gnotobiotic conditions.

LITERATURE CITED

1. Ichinohe, M. 1952. On the soy bean nematode, Heterodera glycines n. sp., from Japan. Mag. Appl. Zool. 17:1-4.

2. Ichinohe, M. 1961. Studies on the soybean cyst nematode Heterodera glycines. Hokkaido National Agric. Exp. Sta. Report 56:1-77.

3. Lauritis, J. A., R. V. Rebois, and B. Y. Endo. 1981. Monoxenic culture of Heterodera glycines propagated on root cultures of susceptible soybean. J. Nematol. 13:447 (Abstr.).

4. Orion, D., W. P. Wergin, and B. Y. Endo. 1980. Inhibition of syncytia formation and root-knot nematode development on cultures of excised tomato roots. J. Nematol. 12:196-203.

5. Schindler, A. F. 1961. A simple substitute for a Baermann funnel. Plant Dis. Reptr. 45:747-748.