Journal of Nematology 18(2):200–203. 1986.

Inbreeding and Hybridizing Cyst Nematodes on Pruned Soybeans in Petri Plates[1]

VICTOR H. DROPKIN AND JOHN M. HALBRENDT[2]

Abstract: Inbred nematodes propagated on a selecting host are likely to have homozygous genes of interest for investigating the genetics of host–parasite associations. A technique is presented to inbreed soybean cyst nematodes, by sibling matings at each generation, and to cross inbred lines. Soybean seedlings with severely trimmed cotyledons survive well on 0.8% agar. Eggs from a single female are incubated in water in a microtiter well. Virgin as well as mated females result from inoculation of two juveniles per root. Sibling males from the same source are produced by mass inoculations of eggs. Males are added individually to unmated females. Overall success for fertile females was 14% in 1,368 isolations. Three generations of inbreeding by siblings were achieved using nematodes from two populations that differ in their ability to reproduce on differential soybeans. Hybrids from crosses of the two inbred lines tested on differential hosts showed that the influence of Population 1 (selected and inbred on PI 209332) is greater than that of Population 2 (selected and inbred on PI 89772). Reciprocal crosses suggest that the influence of males is stronger than that of females in determining host specificity of F_1 offspring in these crosses. Our technique is simple and effective for inbreeding and crossing soybean cyst nematodes.

Key words: genetics, *Heterodera glycines*, soybean cyst nematode, hybridizing, inbreeding, technique, water agar.

Genetics of the association between soybean, *Glycine max* (L.) Merrill, and *Heterodera glycines* Ichinohe, the soybean cyst nematode (SCN), is poorly understood. Plant breeders have traditionally used pot tests with infested field soil for bioassays of resistance. Genes that regulate the complex symbiosis of plant and parasite can best be found when genetically pure populations of both associates are available. Because SCN is bisexual, its reproductive biology permits genetic manipulation by controlled matings. Near homozygous genes for virulence can be attained by strict inbreeding on a selecting host for repeated generations. Previous attempts in our laboratory to purify nematode populations through single cyst passage were not completely successful (2).

This paper reports a technique by which strict inbreeding of the nematodes was maintained for three generations and reciprocal crosses were made between two inbred nematode lines that are distinguishable on differential hosts. The technique developed from experience with pruned soybean seedlings (1).

Materials and Methods

Seeds of soybean PI 209332, PI 89772, and cv. Bedford were obtained from V. D. Luedders and H. Minor of the University of Missouri, Columbia, Department of Agronomy. L. Young, USDA ARS, Jackson, Tennessee, supplied seeds of USDA Soybean breeding line J74-88.

After treatment with 95% ethanol for 3 minutes, seeds were immersed for 10 minutes in an aqueous solution containing 1.05% NaOCl obtained by diluting commercial bleach (Clorox). They were then transferred to 0.8% water agar in plastic petri plates (9 × 1.5 cm). After germination at room temperature, cotyledons of seedlings with radicles 2–3 cm long were trimmed to ¼ size to retard root elongation. The trimmed seedlings were transferred to 0.8% water agar in petri plates.

The nematodes were isolated originally by taking eggs from greenhouse populations. These were surface sterilized with 0.5% chlorhexidine diacetate for 15 minutes and incubated in deionized water. Juveniles that hatched during the first 3 days of incubation were discarded. Juveniles collected on days 4 and 5 were centrifuged three times in sterile deionized water and added to plates of pruned seedlings. Thirty days postinoculation, females with eggs in the egg sacs were transferred individually

Received for publication 30 August 1985.

[1] Paper No. 9943 of the Agriculture Experiment Station, University of Missouri, Columbia, Missouri. Supported in part by the Missouri Soybean Merchandizing Council Agreement No. 021.

[2] Professor and Research Associate, respectively, Department of Plant Pathology, University of Missouri, Columbia, MO 65211. Requests for reprints should be addressed to the senior author.

We thank Robert Heinz for technical assistance.

TABLE 1. Fertile inbred females of soybean cyst nematodes on pruned host soybean seedlings inoculated with two juveniles each, with addition of males to unmated females.

Generation	Population 1		Population 2	
	Inoculated plants (N)	Fertile females (%)	Inoculated plants (N)	Fertile females (%)
Parental*	136	10	198	18
I-1	229	17	254	18
I-2	127	15	267	9
I-3	77	13	80	9

* Parental signifies the first generation resulting from inoculations with two juveniles from a single female. I-1 is the first inbred generation resulting from the same procedure, etc.

TABLE 2. Comparison of numbers of soybean cyst females on pruned differential soybeans inoculated with five juveniles each from inbred and from greenhouse populations.

Nematodes*	Soybean cultivar or line			
	PI 209332	Bedford	PI 89772	J 74-88
P-1 I-2	69/64	4/11	7/57	0/20
P-1 I-3	25/16			
Total P-1	94/80	4/11	7/57	0/20
P-1 greenhouse	29/20	19/13	0/20	0/20
P-2 I-2	3/68	3/25	109/72	67/36
P-2 I-3	4/26	1/20	46/40	28/25
Total P-2	7/94	4/45	155/112	95/61
P-2 greenhouse	16/20	4/14	25/20	35/20

Numerator = number of adult females, denominator = number of pruned plants.

* P-1 I-2 signifies population 1, second generation of inbreeding on PI 209332, the selecting host. P-1 greenhouse signifies population 1 maintained continuously on PI 209332. P-2 nematodes were selected and inbred on PI 89772.

to water in microtiter wells and crushed to liberate eggs. Emerged juveniles were taken up in micropipettes drawn from Pasteur pipettes. After the initial sterilizations, nematodes were transferred with care to avoid gross contamination but without strict asepsis. Petri plates were maintained at or below 30 C on laboratory benches under fluorescent light.

Inbreeding: Approximately 50 pruned seedlings each received two juveniles from one female. Remaining juveniles from the same female were added to three more seedlings for production of males. After 10 days, mass-inoculated plants were transferred to test tubes containing aerated water. Males were retrieved by daily sieving and stored at 10 C. At about 15 days after inoculation, the roots were examined at 8× magnification with a dissecting microscope with fiber optic illumination and the positions of white females were marked. Plates with two females were supplied with a sibling male transferred with a bamboo pick. This provided efficient use of the males, as often both females were inseminated. Single females were judged to be virgins if no eggs appeared by day 18–20 and were then supplied with a male. Eggs were usually extruded 3–5 days after addition of males.

Two populations of cyst nematodes were inbred. P-1 denotes nematodes selected and maintained continuously on PI 209332. P-2 denotes nematodes selected and maintained continuously on PI 89772. The original selection is described in McCann et al. (3). These populations have been in soil in a greenhouse for 7 years on their selecting hosts. In repeated tests (1) P-1 was compatible with PI 209332 and not with PI 89772; P-2 was compatible with PI 89772 and not with PI 209332, although the degree of resistance in this combination was less complete than in the combination of P-1 with PI 89772.

Hybridization: This was accomplished by adding a male of inbred line P-1 to a virgin female of a similar inbred line of P-2, obtained from cultures of pruned plants inoculated with one juvenile each. Twenty-three percent of 307 cultures contained mature females. Males were obtained as described for inbreeding. Juveniles from mated females of these crosses were tested on differential hosts.

Differential tests: Two independent experiments tested both inbred lines and the F_1 offspring of reciprocal crosses. Each test consisted of replications of two pruned seedlings in one plate of water agar inoculated with five juveniles per seedling. The first test consisted of PI 209332 and PI 89772. The second utilized Bedford and breeding line J 74-88. Bedford incorporates resistance from Peking and PI 88788; J 74-88 came from a cross between PI 89772 and Forrest. These were included to demonstrate alternatives to PI 209332 and PI 89772 for which seed may be difficult to obtain.

TABLE 3. Comparison of the numbers of females of inbred and hybrid soybean cyst nematodes from two populations on differential hosts.

	Experiment I				Experiment II			
	PI 209332		PI 89772		Bedford		J 74-88	
Nematodes*	Total	Per plant	Total	Per plant	Total	Per plant	Total	Per plant
P-1 I-3	10/8	1.25	0/8	0.0	14/10	1.4	0/12	0.0
P-2 I-3	0/12	0.0	39/20	1.95	0/8	0.0	17/12	1.4
P-1 I-2 female × P-2 I-2 male	60/31	1.9	25/32	0.78	8/4	2.0	2/10	0.2
P-1 I-2 male × P-2 I-2 female	21/16	1.3	0/18	0.0	19/9	2.1	0/13	0.0

Numerator = number of adult females, denominator = number of plants. Each was inoculated with five juveniles.
* P-1 nematodes selected and inbred for two or three generations on PI 209332. P-2 nematodes selected and inbred for two or three generations on PI 89772.

RESULTS AND DISCUSSION

At the outset, the inbreeding program was designed to carry several lines of each nematode population. This required one full-time operator to prepare seeds, pour plates, and collect data. Another concentrated on the nematodes, making inoculations and isolating eggs. After experience was gained we reduced the scale of operations; labor diminished to 1.5 full-time equivalents.

Inoculation with two sibling juveniles to the root of a pruned seedling yielded mature females in about 40% of the plates. Without the addition of males, the system was too costly because only 5% of the 1,368 plates had fertile females. Addition of males to 279 unmated females yielded 39% successful matings. Table 1 summarizes the success of inbreeding. Fourteen percent of 1,368 seedlings had mated females with eggs.

The success achieved with different batches of juveniles varied, in part because seed germination, collection of fertile females, and hatching of eggs were not always perfectly synchronized. Germinated seeds could be held for several days at 10 C without affecting results of inoculation. The emergence of juveniles was also somewhat variable. We generally inoculated with nematodes that had emerged within the preceding 24 hours. When the work was properly coordinated, 75 plants were inoculated each day.

Table 2 presents the results of differential host trials comparing laboratory inbred with selected greenhouse populations. From inoculations of five nematodes per plant, P-1 inbred females averaged just over one female per pruned plant of PI 209332 soybeans and 0.4 per Bedford plant (Table 2). There were 0.1 females per plant on PI 89772 and none on J 74-88. Results with the greenhouse population were similar, showing that Bedford and J 74-88 were similar to PI 209332 and PI 89772, respectively. As expected, P-2 nematodes were compatible with PI 89772 and J 74-88, but not with PI 209332 and Bedford (Table 2).

Differential tests with the reciprocal crosses showed that F_1 hybrid offspring yielded fewer females on PI 89772 and J 74-88 than on PI 209332 and Bedford. All four crosses, however, were highly compatible with PI 209332 and Bedford (Table 3). Offspring of the cross P-1 females × P-2 males produced fewer females on PI 89772 and J 74-88 than did those of the inbred P-2 on the same hosts. But in the reciprocal crosses between P-2 females and P-1 males, no females developed on either of these hosts. Thus genes from P-1 appear to dominate those from P-2 in determining the phenotype we measured. Further, the reciprocal crosses indicate a more effective role of the male.

We do not attempt analysis of genes for virulence from these limited data but rather present them to show that the technique employed permits the production of inbred populations and of hybrids between them. Others have mated cyst nematodes, but the populations employed were not completely homogeneous (4,5,6). SCN lines carried for many generations of inbreeding should prove useful for identification of genes for

resistance to SCN in soybeans of diverse genetic backgrounds. Because the technique is simple and convenient, it permits work to be done on a large scale in the laboratory.

Literature Cited

1. Halbrendt, J. M. 1985. The *Heterodera glycines*–soybean association: A rapid assay using modified seedlings. Ph.D. thesis, University of Missouri, Columbia.

2. Luedders, V. D. 1985. Selection and inbreeding of *Heterodera glycines* on *Glycine max*. Journal of Nematology 17:400–404.

3. McCann, J., V. D. Luedders, and V. H. Dropkin. 1982. Selection and reproduction of soybean cyst nematodes on resistant soybeans. Crop Science 22: 78–80.

4. Parrott, D. M. 1971. Mating of *Heterodera rostochiensis* pathotypes. Proceedings of the Association of Applied Biologists. Pp. 18–20.

5. Potter, J. W., and J. A. Fox. 1965. Hybridization of *Heterodera schachtii* and *H. glycines*. Phytopathology 55:800–801.

6. Price, M., C. E. Caviness, and R. D. Riggs. 1978. Hybridization of races of *Heterodera glycines*. Journal of Nematology 10:114–118.