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Selection and Inbreeding of *Heterodera glycines* on *Glycine max*[1]

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Abstract: Few soybean cyst nematodes (SCN), *Heterodera glycines*, of a diverse gene pool developed into females on soybeans PI 89772 or PI 209332. Nematodes surviving the selection pressure were then inbred for nine generations by single cyst transfers on the same selecting soybean line. These nematodes appeared to tolerate concurrent selection and inbreeding. Effects of selection–inbreeding, selection only, and secondary selection were evaluated by relative ability to produce cysts on 11 soybean lines. The genetic differences of PI 89772 (also Peking and Pickett 71) and PI 209332 were reaffirmed. The random effects of inbreeding indicated that Ilsoy and Williams may have genes for resistance different from those in PI 89772 or PI 209332. Egg inoculum obtained from soil resulted in very few cysts in some tests. Fresh egg inoculum (from cysts on 27–30-day-old plants) generally resulted in more cysts and more consistent results. Concurrent with the change in inoculum, there was a large increase in relative numbers of cysts on several soybean lines but no change on other lines; the true cause of this large interaction is unknown. Secondary selection of two inbreds was effective and suppressed cyst numbers on the line on which one inbred was selected initially. These results are consistent with the allelism linkage of some SCN genes reported previously.
Key words: soybeans, cyst nematodes, resistance, genes, frequencies.

Selection has been used to increase the abilities of certain populations of soybean cyst nematodes (SCN, *Heterodera glycines* Ichinohe) to reproduce on some resistant soybean lines (2,4,5,7–9). Selection changes gene frequencies, but the approach to homozygosity may be slow. Inbreeding increases the homozygosity that is necessary for reliable genetic studies but is frequently associated with inbreeding depression. SCN from one field survived nine cycles of inbreeding generated by single cyst transfers (Luedders, unpubl.). The apparent effects of inbreeding were not severely detrimental. Neither the base population nor 20 inbreds developed on Williams soybeans could form more than a few cysts on PI 89772. Numbers of cysts were somewhat higher on PI 209332, but the maximum (with one inbred) was only 0.35 as many as on Williams. High reproductive rates are necessary for genetic studies with SCN. The effects of inbreeding are random: frequencies of homozygotes vary with the frequencies of those alleles. The directed effects of selection will increase the frequencies of genes for parasitism. Thus concurrent selection and inbreeding should develop materials useful for genetic studies of both organisms. PI 89772 and PI 209332 appear to have different genes for resistance to SCN (3,9). This paper reports the effects of selection and inbreeding of SCN on PI 89772 and PI 209332.

MATERIALS AND METHODS

The SCN base population, the gene pool, was developed by mixing more than 20 samples of SCN obtained from various regions of the United States. These were put in fine sandy soil in a 91-cm-d culvert section in the greenhouse. The nematodes were allowed to intermate and reproduce on the genetically different soybean *Glycine max* (L.) Merr. cvs. Corsoy, Essex, and Williams. The soil and cysts were mixed at 30–60-day intervals to encourage more extensive recombination of SCN genes. The SCN-resistant lines PI 209332 and PI 89772 were the hosts for selection and inbreeding of gene pool SCN. Cysts recovered from the PIs had survived selection pressure but had no inbreeding. Single cysts that had developed on the PIs were transferred to seedlings of the same PI. Seedlings were grown for ca. 28 days in 2.5 × 15-cm sections of plastic pipe in crocks in a water bath maintained at ca. 27 C in the greenhouse. Subsequent single cyst transfers were performed in duplicate for each inbred. Selection preceded inbreed-

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TABLE 1. Reproduction of *Heterodera glycines* selected and inbred on PI 209332 (SI 209-) or on PI 89772 (SI 89-) on 11 soybean lines. Values for Williams are numbers of cysts, others are proportion of Williams, and zeros (0) indicate no cysts.

H. glycines	Wms	CV %	LSD (0.05)	Ess	Cor	209	89	Pic71	Pek	Yam	Kar	To6	Ils
SI 209-													
1	38	55	0.29	0.45	0.58	0.18	0	0.71	0.16				
2*	42	50	0.24	0.69	0.55	0.29	0	0.19	0.02	0.40	0.64	0.74	0.02
3	117	49	0.19	0.33	0.26	0.15	0	0.03	0.01	0.12	0.88	0.61	0.27
4	40	61	0.30	0.35	0.32	0.20	0	0.42	0.00	0.22	0.68	1.23	0.37
5	32	45	0.16	0.53	0.69	0.41	0	0.09	0.00	0.38	0.21	0.09	0.06
6	88	60	0.26	0.36	0.69	0.25	0	0.06	0	0.09	0.60	0.77	0.40
7*	70	54	0.21	0.24	0.23	0.17	0	0.67	0.01	0.16	0.54	0.60	0.09
8	69	75	0.21	0.28	0.20	0.10	0	0.32	0.02	0.17	0.22	0.41	0
9	65	48	0.14	0.48	0.25	0.22	0	0.03	0	0.25	0.17	0.18	0.08
10*	105	17	0.15	1.29	1.49	1.35	0	0.11	0.02	1.60	0.45	0.29	
11	116	20	0.17	0.89	0.97	0.76	0.01	0.66	0.17	0.90	0.83	1.05	0.81
12	67	50	0.24	0.70	0.40	0.28	0	0.04	0	0.28	0.60	0.91	0.46
13	126	17	0.17	1.24	1.29	1.32	0.01	0.13	0.03	1.39	0.94	1.02	1.24
14	180	17	0.16	1.07	1.22	1.01	0.01	0.45	0.15	1.18	1.11	1.22	0.83
SI 89-													
1	89	34	0.21	0.62	0.46	0.01	0.24	1.12	0.36				
2	34	43	0.21	0.47	0.38	0.06	0.32	0.82	0.21	0.26	0.50	0.47	0.21
3*	124	14	0.10	0.73	0.85	0.04	0.23	0.96	0.36	0.73	0.71	0.85	0.04
4*	104	38	0.20	0.51	0.52	0.01	0.22	0.83	0.24	0.38	0.37	0.58	0.35
5	41	48	0.27	0.49	0.39	0.07	0.14	1.15	0.07	0.32	0.90	0.71	0.24
6	91	13	0.14	1.08	1.15	0.04	0.68	1.20	0.93	1.26	1.13	1.14	0.70
7	101	21	0.18	0.81	0.73	0.14	0.50	0.91	0.52	0.84	1.02	1.02	0.32
8	188	20	0.15	0.69	0.63	0.01	0.37	0.86	0.37	0.70	0.84	0.77	
9	154	15	0.14	0.99	0.93	0.08	0.64	1.06	0.75	1.00	1.01	0.98	0.21
10	112	16	0.17	1.14	1.08	0.10	0.63	1.22	0.86	1.10	1.23	1.14	0.38
11	144	35	0.27	0.90	0.83	0.08	0.36	0.88	0.47	0.80	0.78	0.74	0.55
12	104	21	0.28	1.55	1.61	0.05	0.79	1.35	1.07	1.40	1.38	1.33	1.27
13	143	27	0.20	0.76	0.75	0.04	0.57	0.85	0.47	0.75	0.93	0.83	0.09
14	114	33	0.25	0.87	0.79	0.09	0.51	1.03	0.53	0.70	0.70	0.69	0.51

Identity of soybeans:

Corsoy	Karumai	Tohoku No. 6	PI 89772
Essex	Peking	Williams	PI 209332
Ilsoy	Pickett 71	Yamashiratama	

* Repeated tests in Table 2.

ing in each generation, so the 28 inbreds evaluated are designated SI 209 (PI 209332) and SI 89 (PI 89772)—1 through 14 indicating the chronological order of successful (at least 30 cysts on Williams) evaluation.

After nine generations of inbreeding, the SIs were increased on the selecting host lines PI 209332 and PI 89772. Thus, there were several additional generations of selection before their evaluation for ability to develop cysts on the 11 soybean lines Williams, Essex, Corsoy, PI 209332, PI 89772, Pickett 71, Peking, Yamashiratama (PI 379556), Karumai (PI 423885), Tohoku No. 6, and Ilsoy. Yamashiratama, Karumai, and Tohoku No. 6, considered resistant to SCN in Japan, were obtained from H. Inagaki, Agricultural Center, Kannondai 3-1-1, Yatabe-cho, Tsukuba-gun, Ibaraki 305 Japan. R. L. Bernard, USDA, Department of Agronomy, University of Illinois–Urbana, furnished the PI numbers since soybeans with the names Yamashiratama and Karumai had been added to the USDA germplasm collection.

Populations developed by selection only are designated S 209 and S 89. Secondary selection on the other host line follow the custom; e.g., SI 209-1-89 means SI 209-1 was selected for ability to reproduce on PI 89772 (4,9). Evaluation was by SCN population with soybean lines randomized in a complete block design with six replica-

TABLE 2. Repeatability of results in Table 1. Dates are of harvests.

H. glycines	Date	Wms	CV %	LSD (0.05)	Ess	Cor	209	89	Pic71	Pek	Yam	Kar	To6	Ils
SI 209-														
2	9/15/82	42	50	0.24	0.69	0.55	0.29	0	0.19	0.02	0.40	0.64	0.74	0.02
	2/11/83	131	13	0.10	0.98	0.96	0.79	0	0.40	0.10	1.01	1.06	1.02	0.11
7	11/4/82	70	54	0.21	0.24	0.23	0.17	0	0.67	0.01	0.16	0.54	0.60	0.09
	6/9/83	192	15	0.13	1.08	1.15	0.86	0.01	0.72	0.12	1.14	0.96	1.02	0.15
	6/17/83	155	20	0.13	0.82	0.86	0.54	0.01	0.46	0.10	0.79	0.73	0.64	0.12
	7/6/83	171	21	0.18	0.96	1.12	0.85	0.00	0.65	0.16	1.11	0.92	0.89	0.15
10	2/7/83	105	17	0.15	1.29	1.49	1.35	0	0.11	0.02	1.60	0.45	0.29	
	6/8/83	150	10	0.11	1.25		1.40	0.01						1.07
	7/7/83	139	25	0.25	1.23	2.03	1.96	0.00	0.17	0.02			0.37	1.21
SI 89-														
3	9/21/82	124	14	0.10	0.73	0.85	0.04	0.23	0.96	0.36	0.73	0.71	0.85	0.04
	3/3/83	124	30	0.19		0.66	0.02						0.72	0.28
	5/6/83	200	13	0.13	1.14	1.16	0.13	0.76	1.15	0.76	1.08	1.11	0.98	0.12
	6/8/83	326	11	0.10	1.10	1.02	0.16	0.77	1.05	0.79	1.13	1.10	1.07	0.07
	6/17/83	177	19	0.18	0.98	1.05	0.11	0.69	0.89	0.79	1.01	1.11	1.03	0.20
4	9/29/82	104	38	0.20	0.51	0.52	0.01	0.22	0.83	0.24	0.38	0.37	0.58	0.35
	3/3/83	139	13	0.10		0.94	0.10						1.01	0.06
	5/6/83	116	19	0.16	0.94	0.97	0.04	0.52	0.86	0.77	1.02	0.78	0.91	0.43
	6/10/83	238	11	0.11	1.03	1.09	0.08	0.72	1.08	0.79	1.14	1.03	0.91	0.57

tions. Eggs for inoculum (1,000/seedling) were obtained by the methods of Acedo and Dropkin (1). Plants were grown as described previously for selection–inbreeding (4,5); numbers of cysts were recorded after 28 days. Data were analyzed by analysis of variance and expressed as proportions of the number on Williams (3).

RESULTS AND DISCUSSION

Nematodes from the gene pool tolerated concurrent selection and inbreeding on PI 209332 and PI 89772; only a few SIs were lost during their development. Twenty-eight SIs were evaluated for their ability to produce cysts on 11 soybean lines (Table 1). Numbers of cysts in the initial tests were lower than expected on Essex, Corsoy, and the selecting lines PI 209332 and PI 89772. The repeatability of our results had been reasonably good in previous experiments and was here also, except for a few, rather large, deviations (Table 2). The greatest interaction was with SI 209-7, where the ratios of Corsoy and Yamashiratama changed drastically but PI 89772 and Ilsoy ratios were remarkably constant. Pickett 71 varied from 0.46 to 0.72, which may be a reasonable range of variation for ratios. One factor that did change was the source of eggs used as inoculum. In the earlier tests, eggs were extracted from soil and roots. Tests of several SI 209s and SI 89s resulted in few cysts at 28 days. These failures were considered to be due to inoculum quality rather than genetic incompetence. For inbreeding, single gravid females had been transferred, which resulted in moderate numbers of progeny after 28 days. Thus, fresh eggs were functional and were used as inoculum in all tests harvested 25 May 1983 (SI 209-14 and SI 89-12) and subsequently. Fresh egg inoculum required planting 27–30 days before tests were initiated, but these generally have given greater numbers of cysts and no failures. Several SIs that failed were tested again with good results.

The SCN gene pool was the base population; its success with 11 soybeans is shown in Table 3. As expected, selection (S and SI, Tables 1, 3) increased the ability of SCN to reproduce on the selecting line but with little change on the other genetically different line. Selection on PI 89772 increased numbers also on Peking and Pickett 71, which are in the same genetically similar group (3). Changes in ratios of the other lines appeared to be random effects of inbreeding. Ratios of Ilsoy with SI 209s and SI 89s vary from low to high, which may reflect the sampling of SCN

TABLE 3. Reproduction on 11 soybean lines by *Heterodera glycines* from a gene pool, selected only (S 209 and S 89), and after secondary selection on PI 89772 (e.g., SI 209-1-89). Other designations etc. as in Table 1.

H. glycines	Date	Wms	CV %	LSD (0.05)	Ess	Cor	209	89	Pic71	Pek	Yam	Kar	To6	Ils
Gene pool	11/1/82	153	23	0.14	0.93	0.74	0.15	0.02	0.18	0.05	0.67	1.04	0.92	0.47
	8/23/83	96	27	0.22	1.26	1.29	0.11	0.02	0.10	0.07	1.21	1.18	1.04	0.39
S 209	5/30/83	281	15	0.13	1.05	1.10	0.78	0.01	0.45	0.17	1.03	0.96	1.03	0.53
	8/11/83	106	21	0.18	0.97	1.29	0.82	0.02	0.27	0.13	1.08	0.97	1.07	0.51
S 89	8/10/83	66	30	0.27	0.95	1.18	0.06	0.70	0.97	0.79	1.21	0.91	0.79	0.38
SI 209-1	2/16/84	182	20	0.20	1.20	1.16	0.64	0.01	0.93	0.50	1.16	1.00	1.00	0.67
SI 209-3	2/16/84	201	18	0.15	1.12	1.03	0.96	0.00	0.02	0.00	1.19	1.25	1.09	0.57
SI 209-1-89	2/23/84	132	17	0.20	0.98	1.32	0.43	0.64	1.04	0.86	1.28	1.26	1.22	0.57
SI 209-3-89	2/24/84	178	23	0.22	1.08	1.28	0.35	0.46	1.04	0.63	1.00	1.08	1.02	0.35

genes (interacting with Ilsoy genes for resistance) to higher and lower frequencies. The high ratios of Corsoy and Yamashiratama (and other lines) with SI 209-10 and SI 89-12 could be reflecting fewer cysts on Williams due to its genes. These putative genes for resistance in Ilsoy and Williams probably are different and not in PI 209332 or in PI 89772 since their effects were with SIs of both.

Tohoku No. 6 and Yamashiratama were the parents of Karumai, which was released in Japan as resistant to SCN (6). However, they were not highly resistant to SCN in, or selected from, our gene pool. Some genetic variation exists because Tohoku No. 6 was the high parent with SI 209-4 but the low parent with SI 209-10. These large differences within tests are considered to be real, reflecting the effects of the biological systems–environments sampled. The large interaction of tests with the associations of SI 209-7 with Essex, Corsoy, and Yamashiratama causes some concern. Unfortunately, the causal factor(s), inoculum quality and (or) others, cannot be resolved here.

Secondary selection of SI 209-3 on PI 89772 increased reproduction (of SI 209-3-89, Table 3) on PI 89772, Peking, and Pickett 71 while decreasing it on PI 209332. Luedders and Dropkin (4) postulated that these SCN genes might be alleles. Young (9) obtained similar results in five of six cases with secondary selection of three different field populations. Young (9) suggested that the SCN genes might be linked when secondary selection on Bedford resulted in little change on Forrest, Peking, PI 89772, or PI 90763. Secondary selection of SI 209-1 on PI 89772 did not cause a large decrease in reproduction on PI 209332 (Table 3). The data can be explained equally well by different frequencies of the linkage phases or of three alleles at one locus. The two SI 89s being selected on PI 209332 were lost, which was unexpected since initial reproduction was greater than the SI 209s on PI 89772. Higher inoculum levels of SI 209-1 and -3 were necessary to start secondary selection on PI 89772. The data in Table 3 were obtained after 12 generations of selection. Alternating selection on PI 209332 and PI 89772 would increase the frequency of the linkage phase or of the allele for reproductive ability on both lines. Thus rotating these genetically different sources of resistance may not be an effective long-term control of SCN populations.

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