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Attempt to Select a Cyst Nematode Population on Soybean Plant Introduction 437654[1]

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Abstract: A method of selecting soybean cyst nematode (*Heterodera glycines* Ichinohe) on segregating soybean progeny was evaluated for developing a population capable of reproducing on PI 437654. Direct selection on PI 437654 was not possible, since no cysts developed on it. Cysts were selected for 12 nematode generations on F_3 and F_4 plants of Forrest × PI 437654. No cysts of the selected population were produced on PI 437654, but more males were produced on it by the selected population than by the base population. The number of cysts on Forrest and other soybean lines considered to have some of the same genes for resistance increased with selection as expected. The increase in number of males on these other lines with some of the same genes for resistance as Forrest was greater than anticipated, indicating that these lines may have some of the same genes as PI 437654.

Key words: Glycine max, Heterodera glycines, natural selection, soybean, soybean cyst nematode.

Selection experiments have shown that the numbers of cysts that soybean cyst nematode, *Heterodera glycines* Ichinohe (CN), populations produced on plants of resistant soybean (*Glycine max* (L.) Merr.) lines can be increased (4,11). Generally at least a few cysts developed on soybean lines reported to be resistant to some populations of CN. When the few cysts that formed on sources of resistance such as Peking or PI 88788 were put back on the same soybean line for more selection, populations that could form many cysts on those lines resulted.

The soybean line PI 437654 appears to be an exception to the normal cyst nematode–soybean selection just described. It was resistant to populations classified as races 1 and 2 (Anand, unpubl.), 3, 4, and 5 (2). No cysts of certain nematode inbreds and other populations of CN developed on PI 437654 (Luedders, unpubl.; Dale Weigelt, Asgrow Seed, Stonington, IL, pers. comm.). This absence of cysts on PI 437654 is not single gene immunity or resistance to all CN populations because F_3 plants of Forrest × PI 437654 had continuous variation of cyst numbers (Anand, unpubl.). Such continuous phenotypic distribution probably was due to the segregation of several genes for resistance.

A CN population able to reproduce on PI 437654 would show that its genes would not provide lasting protection from CN damage. Such a population also would increase our knowledge of CNs genetic variability and is necessary for genetic studies. When the occasional cysts found on PI 437654 were re-inoculated on PI 437654, no cysts formed, nor were cysts produced on Essex even when second-stage juveniles from cysts taken from PI 437654 were used as inoculum (Anand, unpubl.). Essex is often used as the susceptible standard for comparison. Thus selection of a population which matures readily on PI 437654 has not been possible by the usual methods.

An alternate procedure would be to select cysts from segregating progeny of a cross involving PI 437654, since homozygous segregates are not available. Most segregates would not have all of the genes for resistance and should "select" cysts to change some CN gene frequencies. Several generations of selection on segregates might result in a population containing individuals that could reproduce on PI 437654, which could then be used for selection. The objectives of this study were to test the method of selecting nematodes from segregating progeny of Forrest × PI 437654 and to develop a population on PI 437654.

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Materials and Methods

Both F_3 and F_4 plants of Forrest × PI 437654 were used for nematode selection. These plants were from F_2 families previously screened with a mixture of CN races 4 and 5 (Anand, unpubl.). The rating scale was 0 = 0 cysts, 1 = < 5, 2 = < 10, 3 = 10–30, 4 = > 30 cysts per plant on as many as 10 F_3 plants per family. Seven families with 1–3 ratings, 17 families with 0–2 ratings, and 16 families with only 0–1 ratings were chosen to exert different intensities of selection pressure. Equal number of seeds from each F_2 plant within each rating group were mixed and germinated. Sixteen seedlings of each group were planted singly in sandy soil in 2.5-cm-d pipes contained in crocks (5). Each pipe was infested with 1,400 eggs and juveniles (J2) of the CN base population, a combination of several diverse populations (5). Four pipes containing Essex seedlings were infested with 700 eggs and J2 and included in each crock for comparison. After 28 days the females (includes cysts) were washed from the roots, with one lot from the F_3 plants and another from the Essex plants in each crock. The cysts were ground through a 149-μm-pore sieve and the eggs were separated from the debris in a sugar gradient (1). Total numbers of eggs were determined from aliquant droplets. The numbers of eggs per F_3 plant (adjusted for the higher inoculum) were expressed as ratios of the numbers on Essex. The determination of egg numbers was an efficient method of measuring CN reproductive ability, since it allowed the calibration for inoculating the next cycle of selection.

The first selection did not produce enough eggs for the second generation of selection, so the eggs were inoculated to Essex for one generation. Then the selected eggs were used to inoculate plants of the same rating group. Most eggs were from plants with 1–3 ratings and were used to inoculate two groups of plants. The third selection generation was similar, except 25 seedlings were planted in larger crocks without pipes, and two pipes were used with two Essex plants to keep their roots separate. Also the inoculum was increased to 3,000 eggs (still 700 on Essex) to obtain larger selected populations. There were no consistent differences among the four selected populations, thus they were combined into two populations for selection generations 4 through 8, and then into one population for selection generations 9 through 12. Starting with the seventh selection generation, F_4 plants were used. Equal numbers of seed of the F_2 soybean families had been bulked and increased in the field.

The selected populations were evaluated periodically for ability to produce cysts on PI 437654 so that direct selection could be done. These were replicated tests with an inoculum level of 700 eggs and J2; the plants were maintained in a 26-C water bath for 28 days before females and cysts were harvested and counted. Nematodes from the base population and from the population selected for 12 generations on the F_3 or F_4 plants were evaluated for numbers of males and females on the soybean lines Williams, Forrest, Custer, Peking, PI 90763, PI 89772, PI 88788, and PI 437654. Seedlings were planted as described above, but the inoculum level was 1,000 eggs and J2. The shoot growing tips were removed, starting on day 6 or 7, leaving the primary leaves and cotyledons. After 12 days the plants were removed and rinsed to remove all soil particles, and two seedlings were put in each male-catching unit containing deionized water. The units were similar to those of Lung (9), essentially extensions of Baermann funnels, as suggested by R. Simmons (pers. comm.). They were made in the University of Missouri glass shop out of glass tubing (5.1-cm o.d.), with an overall length of ca. 28 cm and a volume of ca. 400 ml. Aeration was provided through a 22-gauge needle hot glued to 1-mm-d plastic spaghetti tubing. A short piece of rubber tubing with a clamp enabled easy withdrawal of the males in a small volume of water. The units were in racks and partially submerged in a waterbath. The racks were easily raised and suspended so males could

TABLE 1. Relative ability of two populations of soybean cyst nematode to become adults on eight soybean lines.

Soybean lines	Nematode population			
	Base		Selected	
	Males	Females	Males	Females
Williams	84	74	282	221
Forrest	0.50	0.17	0.92	0.90
Custer	0.26	0.12	0.96	0.64
Peking	0.24	0.08	0.99	0.54
PI 90763	0.16	0.01	0.94	0.38
PI 89772	0.17	0.03	1.16	0.46
PI 88788	1.34	0.08		0.06†
PI 437654	0.05	0	0.22	0
LSD (0.05)	0.13	0.31	0.13	0.21

Values for Williams soybean are the actual numbers of males and females. Values for the other lines and the LSD are expressed as proportions of Williams. Zeros indicate no females.

† From a different test.

be extracted for counting. The males were withdrawn at 2–3-day intervals into centrifuge tubes in 40 ml water. The males were allowed to settle to the bottom for 30 minutes and then transferred to plates for counting. The females were harvested on day 24 and counted. Data were analyzed by analysis of variance (randomized complete block), then means and LSDs were expressed as proportions of the means of Williams for easier comparison among populations (4).

RESULTS AND DISCUSSION

The initial selected population (on F_3 plants of Forrest × PI 437654) consisted of only 0.02 as many eggs as on Essex. The proportion on the F_3 plants was 0.15 in the second generation of selection and 0.06 in the third. The ratio varied within this range until the 10th generation, when it was 0.33, and it was 0.28 in the 11th. In the 12th generation of selection, however, the ratio was back to 0.15, the same as in the second. This variation seemed to be independent of the variation in the number of eggs on Essex, which varied from 10,000 to 145,000 eggs per plant. Some sampling variation is expected in selection experiments, but a greater trend of increase was expected. The sample of progeny plants from Forrest × PI 437654 was different each generation. PI 437654 appears to have many genes for resistance; thus each plant used may have had a different combination of its genes. With a different selecting–evaluating host population each generation, perhaps no great or consistent increase in nematode numbers should have been expected.

The genes of PI 88788 were not shown to affect the number of nematode males (Table 1) (7); thus, this phenotype was not determined with the selected population. Selection did increase the numbers of males relative to Williams (Table 1) on the other six lines and females on five of these. Some increases were expected, since Forrest (and Custer) had some, but not all, of the genes for resistance in Peking. Peking appears to be genetically similar to PI 89772 and PI 90763 (but not identical, since it often has more cysts), and different from PI 88788 and PI 209332 (4,11). Thus, selection on progeny from Forrest would be expected to increase numbers of cysts on Forrest, Custer, Peking, PI 89772, and PI 90763; however, the increase in numbers of males on the latter three was greater than expected. This indicates that they may have some of the same genes affecting male as well as female development as PI 437654. PI 437654, however, must have several more and different genes for resistance, because it still supports relatively few males and no females of the population selected on its segregating progeny. Several genes for resistance in Peking and PI 90763 have been postulated, but the postulations were considered to underestimate the actual numbers, since heterogeneous populations of CN were used (4,10). A reliable estimate of the number of genes for resistance in any of these lines is not available.

Selection on or by segregates from Forrest × PI 437654 increased the number of males on PI 437654, even though it did not result in any females. Response to selection is expected to be slower when it acts on many genes, which is believed to be the case here. Selection was assumed to be

against specific CN genes, although Triantaphyllou (10) stated that nematodes do not have what are often called genes for avirulence. An incompatible phenotype of a parasite–host association, such as zero cysts of CN–S (cyst nematode–soybean) (4,6), may be due to one gene-for-gene interaction of genes for avirulence-resistance (3). Such genes are alleles for incompatibility and could be designated as *a, b*—in both CN and S, with no individual *aa–aa* or *bb–bb*—CN–S resulting in a cyst, or an adult male with some alleles. Therefore, a juvenile would have to have a dominant allele at all loci to develop into a cyst. Most of the CN–S alleles for incompatibility appear to be recessive (4,10). Some may be dominant, but they will be ignored here. The selection was by many different plants; each segregate may have had a different combination of the many alleles for incompatibility in PI 437654. The selection pressure was not consistent against all of the CN alleles for incompatibility, some of which do not appear to be independent and may be linked (8,11). The frequencies of certain linkage phases may have affected the responses to selection.

Selecting nematodes on the segregating progeny of Forrest × PI 437654 was not an efficient method of increasing the numbers of adults on PI 437654. The frequency of some CN alleles apparently changed, since the number of males increased. The selected population may be useful for screening for resistance, particularly when transferring PI 437654s alleles for incompatibility into more adapted genetic backgrounds. Fewer plants might be considered to be resistant to the selected population than when screening with a given race, but the few plants would be expected to have more of PI 437654s many alleles for incompatibility. This concentration of genes is good for cultivar development but inimical to definitive genetic studies. Genetic studies may be definitive only when two discrete CN–S phenotypes are due to only two alleles at one locus in each. This condition is easier to attain with soybean lines that have just one or only a few alleles for incompatibility with CN.

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