MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 33-2015
2015 Final Report

Title of Project: Development of Reniform Nematode Resistant Lines from JTN-5203, PI 404166, and 02011-126-1-1-5-1-1 Soybean

Principal Investigator: Dr. Salliana R. Stetina (Sally.Stetina@ars.usda.gov)

BACKGROUND AND OBJECTIVES

Many soybean growers, in the Mississippi Delta region in particular, are faced with the challenge of producing a profitable crop in fields infested with reniform nematode (*Rotylenchulus reniformis*). The soybean lines JTN-5203 (PI 664903), PI 404166, and 02011-126-1-1-5-1-1, previously identified by project scientists as having resistance to Mississippi isolates of reniform nematode, were used as parents in crosses to transfer that resistance into soybean lines adapted for Mississippi.

The objectives of this project were to (1) cross resistant soybean lines JTN-5203, PI 404166, or 02011-126-1-1-5-1-1 with soybean lines agronomically adapted for Mississippi, (2) evaluate genetic populations from the above sources for determining how resistance to reniform nematode is controlled, and (3) develop improved breeding lines with resistance to reniform nematode from these populations.

REPORT OF PROGRESS/ACTIVITY

Population development from crosses between resistant and susceptible soybean lines

In 2012, the resistant soybean line JTN-5203 was crossed to two lines (R99-1613F and LG09-1459-8) with agronomic characteristics desirable for Mississippi production systems. The F_1 seeds were sent to a winter nursery in Puerto Rico in the fall of 2012, and F_2 seeds from both crosses were received at the USDA ARS in Stoneville, MS in the spring of 2013. A subset of F_2 seeds was planted in the field along with the parents to assess segregation of morphological markers (such as flower color, pod color, hilum color, pubescence color, stem termination, etc.), and progeny were confirmed to be from true crosses in July of 2013.

In 2013, reniform nematode-resistant soybean lines PI 404166 and 02011-126-1-1-5-1-1 were crossed to lines adapted for Mississippi that have desirable agronomic traits such as high yield and very good seed quality. The F_1 seeds were planted in a soybean winter nursery in Puerto Rico, and F_2 seeds were received at the USDA ARS in Stoneville, MS in the spring of 2014. A subset of F_2 seeds from each cross was planted in the field along with the parents to assess segregation of morphological markers. In July of 2014, seven true crosses were confirmed: 04030-1-4-1-1/02011-126-1-1-5-1-1, 04025-1-1-4-1-1/02011-126-1-1-5-1-1, DB04-10836/02011-126-1-1-5-1-1, DS97-94-9/PI 404166, DS880/PI 404166, LG01-5087-5/PI 404166, and DB04-18036/PI 404166.

The remaining subset of F_2 seeds from each cross was reserved to screen for reniform nematode resistance after the crosses were confirmed to be true.

Determining how resistance to reniform nematode is genetically controlled

Individual F_2 plants from selected crosses were evaluated for resistance to reniform nematode under controlled conditions. Plants were established in a growth chamber with the temperature held constant

at 28 C and the day length was set at 16 hours. Adequate soil moisture was maintained throughout each experiment with an automated watering system, with the watering interval increased as needed during the experiment to supply additional water as plants grew. Ten plants of each parent, along with known susceptible and resistant soybean genotypes used as controls, were evaluated in the test.

Each plant was inoculated with 1,000 reniform nematodes (mixed vermiform stages). Four weeks after inoculation, most of the root system was removed from each plant and the number of female nematodes attached to the roots determined at 200X magnification. The roots were gently separated from surrounding soil, then stained using a red food coloring solution to help visualize the nematodes. Root fresh weights were measured and nematode infection was expressed as number of females per gram of root. At the conclusion of the test, the plants with about 2.5 cm of root system still attached were transplanted into potting soil mix. Over the next several months, leaf tissue was collected from each parent and progeny plant for DNA extraction and plants' set seeds.

A total of 228 individual F_2 plants from the cross R99-1613F/JTN-5203 were screened for reaction to reniform nematode in the fall of 2013. Over 220 of the F_2 plants successfully set pods. In this population, the resistant and susceptible parents did not differ as much as expected with respect to infection by reniform nematode. Though the maximum infection levels for the parents were quite different, the mean and minimum infection levels were similar (Table 1). In addition to the lack of clear separation between the resistant and susceptible parents (Figure 1), the phenotypic distribution of the F_2 progeny was less than optimal and not conducive to identification of new molecular markers for reniform nematode resistance. Nonetheless, DNA was isolated from 222 of the 228 F_2 plants. Five SSR markers from regions of linkage groups D1b and G were applied to the DNA. These markers were from genomic regions our previous work has shown likely loci associated with resistance, but likely due to the less than optimal phenotypic distribution, we could not identify any reliable markers. Though the initial goal of developing molecular markers for the resistance in JTN-5203 was abandoned for this population, the screening results allowed selection and advancement of the most resistant selections in the breeding program.

A total of 136 F_2 plants from the cross 04025-1-1-4-1-1/ 02011-126-1-1-5-1-1 were evaluated in the growth chamber for infection by reniform nematode in the fall of 2014. The distribution of phenotypes of the F_2 plants (Figure 2) was skewed in favor of resistant plants; 11 plants had resistance levels comparable to or greater than the resistant parent. The plants were repotted in the greenhouse to allow production of F_3 seeds and collection of DNA from leaf tissue for molecular analysis. Seeds were successfully harvested from 135 recovered plants in December 2014.

Research from previous work done by this project team (DS97-084-1 source of resistance) and other published reports in the literature identified putative loci for resistance to reniform nematode on chromosome 18. However, when the phenotypic data from this F_2 population were analyzed, neither of the two markers from chromosome 18 was significantly associated with the resistance, regardless of whether resistance was considered a binary or continuous variable. Based on molecular marker data, the resistance identified in this population could be unique. Alternative approaches to identifying this locus were considered. The best approach would be to develop recombinant inbred lines from susceptible and resistant individuals in this population that would eventually allow replicated testing of each line for reaction to reniform nematode and improve the precision of the test. However, generating the recombinant inbred lines will take many years and therefore is beyond the scope of the current research project.

A total of 309 F_2 plants from the cross between LG01-5087-5/PI 404166 were evaluated for reniform nematode infection in growth chamber tests in the fall of 2015. All but 17 plants were repotted in the

greenhouse to allow production of F_3 seeds and collection of DNA from leaf tissue for molecular analysis. The distribution of phenotypes of the F_2 plants is shown in Figure 3. Data were collected on 13 plants of the susceptible parent (LG01-5087-5) and 20 plants of the resistant parent (PI 404166). The mean number of females per gram of root for the susceptible parent was 33.6 (ranging from zero to 80.0), whereas the mean of the resistant parent was 5.7 (ranging from zero to 19.8). As shown in Figure 3, five of the 13 plants (38.4 %) of the susceptible parent fell within the range of the values of the resistant parent and may represent escapes. The high percentage of escapes noted in the parents raises the possibility that escapes also occurred within the F_2 population, which greatly limits the interpretation of the segregation pattern and the marker-trait associations. With the data on hand, it is not possible to determine the true rate of escapes in the F_2 population or specify which plants are the escapes.

Examining the F_2 distribution in Figure 3 shows no clear break point for resistant and susceptible classes as would be expected for simple Mendelian segregation (for either a one or two gene model). There does appear to be transgressive susceptible segregation (approximately 104 F_2 plants with females per gram of root values greater than the susceptible parent mean value). Note that selecting a break point at roughly 45 females per gram of root does fit a single dominant gene very well (233:76, 3:1, χ^2 = 0.0202, P = 0.87). Nonetheless, the lack of clear delineation between classes may indicate a more quantitative resistance. However, this may also be a result of the potentially high rate of escapes for susceptible plants. If we conclude that the F_2 population is segregating in a quantitative manner, this would be of importance as the only known resistance for reniform nematodes identified to date can be explained by a single gene model. However, to accurately investigate and map quantitative inheritance will require the development, phenotyping and genotyping of a recombinant inbred line population. This would require several years and considerable cost.

To determine if the one known gene was contributing to the resistance evident in this F_2 population, we screened a selection of the most resistant and most susceptible plants in the population with molecular markers near its genomic location (near the beginning of chromosome 18). Using 44 resistant F_2 plants (females per gram of root < 6.0) and 38 susceptible F_2 plants (females per gram of root > 50.0), markers near the location of the known reniform resistance gene on chromosome 18 were tested for association with resistance. The two SNPs evaluated did not show a significant association for the Fisher's Exact statistic (P = 0.1294 and 0.0583) at a 0.05 probability threshold, although one of the markers was very close. Given the potential for escapes, it is likely that the SNP that is very nearly significantly associated with the known gene provides a strong indication that the resistance evident in PI 404166 is at or near the known gene on chromosome 18.

In our previous work we identified a potential new gene located on chromosome 2; a marker tested at this location on the resistant and susceptible plants did not show any association with resistance (P = 0.5293). Because the previously identified reniform resistance locus on chromosome 18 is at or very near a well-known SCN resistance locus, we also evaluated our population with markers near a second SCN resistance locus on chromosome 10. However, the markers tested did not show any association with reniform resistance (P > 0.5000). The lack of significant marker associations at these putative loci further suggests that the genetics of this population may be complex and require a greater investment of time, effort and funds to elucidate.

Development of improved breeding lines with resistance to reniform nematode

Two different approaches were used to identify reniform nematode-resistant plants and advance them in the breeding program. The pool of F_2 seed was divided into two subsets, and the processes for evaluating materials and selecting superior genotypes took place at the same time for each subset.

One approach is to screen for nematode resistance in the F_2 generation as previously described, recover the best plants, and use their seeds to plant the F_3 generation in the field. Individual F_2 plants were tested in the growth chamber as previously described, and plants were repotted so that F_3 seeds could be harvested from the most resistant ones. The harvested seeds were then evaluated in single rows in the field to assess agronomic suitability. Lines with superior agronomic traits were selected for advancement to the next generation.

In the second approach, the best F_2 plants from each cross were selected in the field based on agronomic characteristics and F_3 seeds were harvested. Ten F_3 plants representing as many as 20 different F_2 families were tested in the growth chamber to determine if reniform nematode-resistant lines occur in any of the $F_{2:3}$ families; concurrently, the seeds from each $F_{2:3}$ family were grown in the field for advancement to the next generation and further evaluation of agronomic properties.

For the growth chamber testing, a susceptible check, a resistant check, and 10 F_3 plants representing each F_2 family were inoculated with 1,000 reniform nematodes (mixed vermiform stages). Approximately 4 weeks later, the root system was removed from each plant and the number of nematodes infecting the root system was determined as previously described. The best $F_{2:3}$ families demonstrated to contain reniform nematode-resistant lines were selected for harvest and advancement to the F_4 generation.

Unfortunately, this research has not successfully identified molecular markers for reniform nematode resistance. Therefore, at each generation, phenotypic screenings are used to assess the reaction of the soybean lines to the reniform nematode. Results from the most recent set of phenotypic evaluations and the number of families advancing to the next generation are summarized in Table 2. These lines are not yet ready for release, and additional cycles of evaluation and selection will be needed.

Impacts and Benefits to Mississippi Soybean Producers

A survey of soybean disease losses in the midsouthern states of Missouri, Tennessee, Arkansas, Louisiana, and Mississippi compiled by the Southern Soybean Disease Workers Group reported annual losses to reniform nematode of 4.65 million bushels in 2014 and 3.42 million bushels in 2015. However, the geographic distribution of reniform nematode is not uniform across this area. The nematode is concentrated in Mississippi and Louisiana, so it is logical that most of the reported losses came from these two states. In these two states, prices averaged $10.95/bu in 2014 and $9.80 in 2015; this translates to potential annual losses of $50.9 million in 2014 and $33.5 million in 2015. Losses to reniform nematode could be drastically reduced if resistant soybean varieties adapted for Mississippi and the Mid South were available.

End Products–Completed or Forthcoming

The ultimate end products from this work will be soybean germplasm with resistance to reniform nematode derived from thee different soybean lines, though that end product is still several years in the future. Conference presentations and journal publications describing the germplasm releases will also be prepared.

Table 1. Reniform nematode infection on control genotypes, susceptible and resistant parents, and F_2 progeny of the cross R99-1613F/JTN-5203.

Line	Description	Number of observations	Females per g fresh root		
			Mean	Minimum	Maximum
PI 88788	susceptible control	10	144	17	265
PI 90763	resistant control	10	4	2	8
R99-1613F	susceptible parent	10	19	6	71
JTN-5203	resistant parent	10	9	4	18
R99-1613F/JTN-5203	F_2 progeny	228	36	2	160

Table 2. Selection of families with reniform nematode resistance from 12 different crosses based on plant infection by the nematode in growth chamber tests (mean, minimum, and maximum numbers of nematodes on up to 10 individual plants).

Pedigree	2015 generation	Number of families evaluated	Number of resistant families selected
R99-1613F/JTN5203	$F_{3:4}$	25	11
LG04-1459-8/JTN5203	$F_{3:4}$	2	2
DS30-1/02011-126-1-1-5-1-1	$F_{2:3}$	14	4
DS25-1/02011-126-1-1-5-1-1	$F_{2:3}$	2	0
DB04-10836/02011-126-1-1-5-1-1	$F_{2:3}$	1	0
DB04-10836/02011-126-1-1-5-1-1	$F_{2:3}$	5	5
DS-880/02011-126-1-1-5-1-1	$F_{2:3}$	11	3
DS97-94-1/PI 404166	$F_{2:3}$	1	0
DS-880/PI 404166	$F_{2:3}$	2	2
LG01-5087-5/PI 404166	$F_{2:3}$	31	8
DB04-10836/PI 404166	$F_{2:3}$	8	3
DS24-2//JTN-5203/DS-880	$F_{2:3}$	8	1

Figure 1. Phenotypic distribution of 228 F_2 progeny from the cross R99-1613F/JTN-5203 showing level of infection by reniform nematode. Mean responses of the resistant (JTN-5203) and susceptible (R99-1613F) parents are indicated.

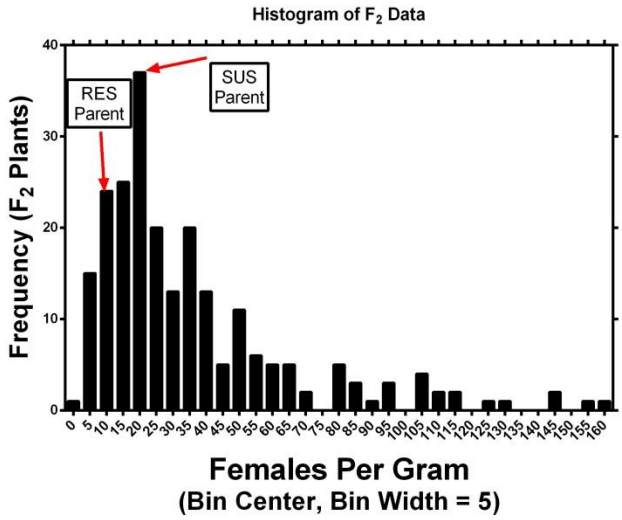

Figure 2. Frequency distribution of F_2 progeny from the cross 04025-1-1-4-1-1 (susceptible to reniform nematode) x 02011-126-1-1-5-1-1 (resistant to reniform nematode) in a growth chamber screening. R and S indicate the phenotypes of resistant and susceptible parents, respectively.

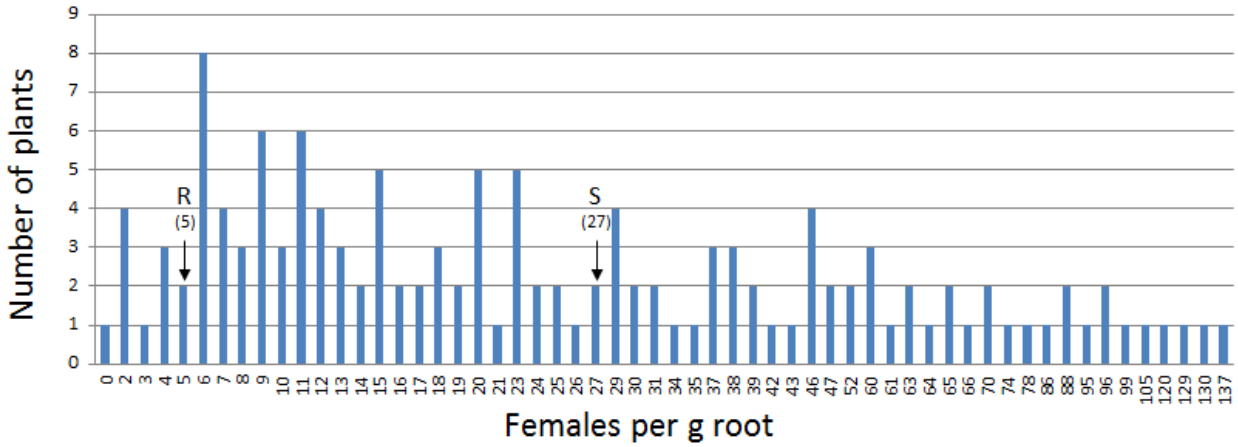

Figure 3. Frequency distribution of F_2 progeny from the cross LG01-5087-5/PI 404166. The left y-axis shows the number of F_2 plants (i.e. bar height) over the distribution of females per gram of root (in bins of 5, x-axis). Similarly the right y-axis shows the number of parental plants falling into the distribution of females per gram of root, where green filled circles are plants from LG01-5087-5 and red filled circles are plants from PI 404166.