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| Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report. |
| Project Number:  |  |
| Project Title:  | Southern Root-Knot Nematode in Maturity Group 4 Soybean: Characterization of Resistance Mechanisms and Breeding for Resistance |
| Organization:  | University of Arkansas System, Div of Ag |
| Project Lead Name: | Travis Faske |
| Report Date: | September 15th 2023 – December 15th 2023 |
| **In the Progress Summary section below, please provide a brief summary of project progress in lay language that will be shared publicly in the** [**National Soybean Checkoff Research Database**](https://www.soybeanresearchdata.com/)**. Do not include any confidential or proprietary information. If no lay language is provided, the contents of this entire report will be published in the** [**National Soybean Checkoff Research Database**](https://www.soybeanresearchdata.com/)**.** |
| Progress Summary (in non-proprietary lay language suitable to be shared publicly): |
| * The southern root-knot nematode (SRKN) is an important, yield-limiting pathogen of soybean in the Mid-Southern U.S. To better understand resistance in soybeans and develop SRKN resistant lines three objectives were designed: determine how resistance is converged in resistant lines against SRKN, utilize molecular tools to identify lines with desired traits for resistance, and developing lines with resistance to the SRKN.
* Data from a time course study suggests there is some diversity in nematode resistance among SRKN-resistant germplasm, which can be used to add new genes for resistance in breeding lines.
* To better understand what individual QTL (quality trait loci) add to protect plants from nematodes, germplasm is being selected with individual QTL.
* Breeding programs in Arkansas and Missouri continue to advance genetic germplasm with resistance to the southern root-knot nematode into their advanced germplasm with several lines at various stages of advancement. New SRKN germplasm is being introduced into the Missouri breeding program.
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| Detailed Progress Status – Expand upon the above section. What key activities were undertaken, and what were the key accomplishments during this reporting period? List each key deliverable from the proposal and describe progress made (or not made) toward achieving it, including metrics that were appropriate. |
| **Obj. 1: Characterization of the mechanism of resistance to SRKN. (Faske and Watson)*** Data were analyzed from a second time-course nematode development study (Fig. 1). Similar to the first study, resistance in these lines are related to delayed nematode maturity. Similar nematode development suggest similar genes for resistance thus, genes in PI 567305 may be similar to Forres, while those in PI 438489B and PI 567516C appear to be different. All contain the same chr. 10 QTL, but uncertain as to the other QTL present.
* Despite PI 567515C having delayed nematode development it had numerically more nematode reproduction than PI 438489B, which further suggest these lines contain different genes to suppress nematode development and reproduction.
* This information can be used to select lines that contain different mechanisms of resistance to develop breeding lines that contain multiple mechanisms for resistance and potentially different, but effective genes for resistance.

Figure 1. Nematode development at 21 days after inoculation on seven soybean germplasm lines. **Objective 2: *Genetic characterization and development of functional markers for new sources of resistance to SRKN (Nguyen)**** In this quarter, we identified and grown 38 recombinant inbred lines (RIL) generated from a cross of Magellan × PI 438489B using markers from Chr. 10 and Chr. 13. Leaf DNAs were extracted from the contrasting RILs (having combination of QTL on Chr.10 + Chr. 13, only Chr. 10 QTL and only Chr.13 QTL,). Among all RILs, QTL 10 based RILs were successfully identified using new KASP markers and currently efforts are being made to thresh the seeds of these lines and identify other RILs with Chr. 13 QTL and combination of Chr. 10 + Chr. 13 QTL. These RILs will be utilized to study mechanism for nematode reproduction and galling number.
* We have increased the seeds of the major RKN lines in the field of University of Missouri, Columbia for future genotyping and phenotyping studies, currently seed threshing is ongoing.
* Additionally, another QTL region on chromosome 13 was screened to develop markers for marker assisted selection. About 2MB genomic region from whole genome sequence of PI 567516C and Magellan were screened to identify SNPs and KASP markers were developed.

Most of the developed KASP marker efficiently distinguish RKN resistant PI 567516C and susceptible Magellan. These markers will be further used to screen and confirm various RKN resistant and susceptible genotypes based in Chr.13 QTL. **Obj. 3: Development of breeding populations and MG4 soybean varieties with resistance to SRKN.** **December 15th, 2023, Season Update**: University of Arkansas, Caio VieiraEvaluation of morphological traits such as maturity, height, and flower color and breeders’ notes were completed in all locations (Marianna, Pine Tree, Rowher, Stuttgart, DeWitt, Kibler, and Fayetteville). Yield data and harvest proceeded as physiological maturity was reached in each location. Seed plot was harvested as backup seed in at least one location for each test. Purity seed plots in Fayetteville were harvested without delay. Breeding decisions have been finalized and 2024 entries have been defined.A total of 1,364 lines across our preliminary, final, and precommercial yield trials were screened with molecular markers to determine the presence of the favorable allele conferring resistance to SRKN. Fifty lines were identified as having the SRKN trait. Based on multi-environment yield data, superior lines were advanced at different stages in the breeding pipeline.Four SRKN-resistant lines were advanced to the pre-commercial stage (Table 1). These will be tested in the 2024 USDA Southern Uniform Yield trials, the 2024 Arkansas Crop Variety Improvement Program, and our internal 2024 pre-commercial yield trials. In addition, all these four lines will undergo a backcrossing program to incorporate Enlist® and Xtend® traits. This will be conducted in off-season nurseries and is expected to be completed by Summer 2025. The ultimate objective is to have SRKN-resistant lines readily available for growers (i.e. carrying the herbicide resistance technology of their choice). R19-45980 is a potential 2024 commercial release. It was evaluated in the 2023 USDA Uniform Preliminary (data pending) and the Arkansas Variety Testing (100.5% of the test mean). Pre-foundation seed will be grown in Stuttgart, Arkansas in 2024.Additionally, pre-commercial line R19-45980 was evaluated in the 2023 USDA Uniform preliminary yield trials and the Arkansas Variety testing. This line is a potential 2024 release and will be entering the second year of evaluation at the Arkansas Crop Variety Improvement Program. Simultaneously, foundation seed will be grown in Stuttgart, AR in preparation for commercial release.**Table 1**. SRKN-resistant AR lines selected 2024 regional yield trials.

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| --- | --- | --- | --- | --- | --- | --- |
| Name | Pedigree | MG | 23 Yield | 23 % Check | 23 % Xtend | 23 % E3 |
| R21KB-06852 | R15-7016/TN11-5140 | 5E | 67.1 | 93.5 | 93.1 | 94.4 |
| R21KB-03657 | R13-13997/NCC09-200719-1-37 | 5E | 67.7 | 94.4 | 94.0 | 95.4 |
| R21KB-05522 | R16-141/R13-13997 | 5E | 66.2 | 101.5 | 101.4 | 101.7 |
| R19-45980 | Ellis/R04-357 | 5E | 67.9 | 94.5 | 95.7 | 91.1 |

Two MG-4 SRKN-resistant lines were advanced to the 2024 Finals Stage (Table 2). These will be tested across multiple environments in 2024, and pending satisfactory performance, will be advanced to the 2025 Pre-commercial Stage. R22KB-16609 will enter the trait introgression program (Enlist® and Xtend® traits) as a potential MG4-Early SRKN-resistant line.**Table 2.** SRKN-resistant lines entered into the 2024 Finals Stage

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Line | Pedigree | MG | 23 Yield | 23 % Check | 23 % Xtend | 23 % E3 |
| R22KB-02812 | SA13-1310/R17C-683 | 4L | 63.8 | 91.0 | 90.8 | 91.1 |
| R22KB-16609 | K15-1800/LD11-2170 | 4E | 62.3 | 98.0 | 100.4 | 96.5 |

**2024 Preliminary Stage**: Approximately 1,000 F4:5 progeny rows have been selected to enter the 2024 Preliminary Stage. All lines will be grown in replicated trials across locations in Arkansas and genotypically screened for SRKN resistance. Pending performance, lines may be advanced to the 2025 Finals Stage.**2023 Population Development and Generation Advancement**: Sixteen populations derived from SRKN-resistant parents are currently being advanced in Puerto Rico's off-season nursery. A total of 1,600 single plant selections will be hand-harvested and threshed. F4:5 seeds will return to the US to be planted as progeny rows in Spring 2024. Seventy-four cross-combinations with at least one parental line carrying resistance to SRKN were performed during the crossing block in the summer of 2023. EG1 seeds were sent to the off-season nursery for generation advancement. Materials will be advanced until EG4 and will return to the US to be evaluated as progeny rows in Spring 2025. |
| MSSB Missouri Nematode Report: Grover Shannon**1. 2023 Releases**: Based on excellent performance over 30 to 40 environments of yield tests, two new conventional lines maturity group V (MGV) with resistant to multiple nematodes, diseases and other favorable traits were released in 2023 as follows:

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| --- | --- | --- |
|  | S18-6013 | S19-19923HOLL(80.7% Oleic and 2.0 % Linolenic) |
| RM | 5.2 | 5.0 |
| Traits | High Oil, SCN, RKN, SC, SDS, FLS, CRT, MET | HOLL, SCN, RKN, RN, PRR, SDS, SC, CRT, BSR, MET, Salt |
| Mean  | 69.2 bu/ac | 58.8 bu/ac |
| %NXT | 117.5 | 99.7 |
| % XT  | 103.2 | 90.6 |
| # Environments | 42 | 33 |

Trait abbreviations confirmed by molecular markers are as follows: RKN, root knot nematode; SCN, soybean cyst nematode; SC, stem canker; PRR, phytophthora root rot; CRT, charcoal rot; SDS, sudden death syndrome; FLS, frogeye leaf spot; BSR, brown stem rot; Met, metribuzin tolerant. and HOLL, high oleic low linolenic acid oil. Mean: Weighted average across environments. NXT: non Xtend commercial check. XT: Xtend commercial checkA total of 11 lines with resistance to RKN, including released lines and elite lines from the University of Missouri - Fisher Delta Research, Extension and Education Center, were screened for their degree of resistance and severity towards RKN in Arkansas. These lines were planted in 3 replications on sandy soil in Hope, Arkansas. The results are shown in table 1 below. Six of the 11 MO lines showed good RKN resistance. Two group IV root knot resistant lines S19-10701C, a conventional mid- group IV (MG 4.6) and S19-7867R, a Roundup Ready 1 early group IV (4.2) are being considered for release pending positive results from 2023 tests. Both lines have shown excellent yield potential in the absence of off-target Dicamba injury and have other desirable traits.Table 1. Field performance of 13 soybean varieties against the southern root-knot nematode and frogeye leaf spot in Pulaski County Arkansas by Dr. Faske’s program. The soil texture was a sandy loam soil (59% sand, 36% silt, and 5% clay). (Pf (final population sampled at harvest) = 373 J2/100cm3 of soil). Lines with scores of 3.0 or below are considered as having good RKN resistance.

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| --- | --- |
| Variety | Percent root system galleda (severity) |
| S11-17025 | 7.9 abcb |
| S16-7922C | 3.0 bc |
| S19-10701C | 1.6 c |
| S18-6013C | 2.4 c |
| S19-6097C | 6.8 abc |
| S19-14797C | 1.0 c |
| S09-13185C | 12.9 abc |
| S19-7867RR | 2.2 bc |
| S19-12459C | 1.7 c |
| S19-12774C | 34.4 a |
| S16-11644C | 11.1 abc |
| Delta Grow DG49XF29 (Check) | 26.8 ab |
| Pioneer P43A42X (Check) | 1.3 c |

a Data are averages of four replications. Averages followed by a different letter within each column are significantly different at α = 0.05 according to Tukey’s HSD.b Root-knot nematode susceptibility was based on percent root system galled. Whereas 0-1.0% = very resistant, 1.1-4.0% = resistant, 4.1-9.0% = moderately resistant, 9.1-20.0% = moderately susceptible, 20.1-40.0% = susceptible, 40.1-100.0% = very susceptible.c Frogeye susceptibility was based on a 0-9 scale (0 = no disease and 9 = severe disease) across the entire plot in the upper 1/3 of the canopy.**2. Promising lines in regional test:** Thirty-two (32) high-yielding breeding lines were evaluated in the 2023 regional trials in early group IV, late group III and early group V tests. They were screened for several SCN Hg-types of soybean cyst nematode (SCN); reniform nematode (RN), and SKRN. Each of the 32 lines has resistance to one or more nematode species for SRKN, SCN, and RN. We are analyzing the local yield data and await results from the 2023 Southern Regional Uniform tests.**3. Advanced yield trials:** A total of 72 advanced breeding lines in our 2023 advanced yield trials (AYT) were planted in 4 local environments and 6-8 locations across different states (OH, IL, AR, LA, IN, MO, TN). Susceptible lines at the Clarkton, MO location with a history of infestations of both SCN and RKN had symptoms of severe root knot nematode injury. The 72 breeding lines were genotypically characterized using molecular markers tightly linked to genes conferring resistance to southern root-knot nematode (SRKN). Results in Table 2 below revealed that 11 lines had resistance to RKN based on DNA marker analysis. Most of the resistant lines were detected using both markers from the different institutions. Based on satisfactory yield from local environments and other states, selected lines will be entered into the regional Uniform trials trials. Table 2: Evaluation of 2023 University of Missouri advanced breeding lines for Root-knot Nematode (RKN) resistance screened with DNA markers.

|  |  |  |
| --- | --- | --- |
| **Line Name** |  | **RKN** |
| S21-11972 |  | **R** |
| S21-15672 |  | **R** |
| S21-17588 |  | **R** |
| S21-22067 |  | **R** |
| S21-23086 |  | **R** |
| S21-23528 |  | **R** |
| S21-23988 |  | **R** |
| S21-24201 |  | **R** |
| S21-5337 |  | **R** |
| S21-5391 |  | **R** |
| **S21-5901** |  | **R** |

 **4. Preliminary yield trials:** A total of 1,224 late III to Late IV soybean breeding lines in our 2023 preliminary yield tests (PYT) were planted in Portageville, MO (3 local environments) and 4 locations in AR and MO where off-target damage to Dicamba is less of a problem. More than 60% of the 1,224 lines have at least one nematode resistant parent. Approximately 130 lines were selected based on the agronomic traits and yield. These lines will be placed in our advanced trials in 2024 AYT. Also, these lines will be screened for RKN markers in January.**5. Progeny Rows:** A total of 208 bi-parental populations (~20,800 F4:5 lines) were evaluated in progeny rows and compared to widely grown commercial XtendFlex checks. Selected rows from different populations were harvested based on yield potential and other agronomic traits. In total, 214 lines were MG3L, 600 lines of MG4E and 552 lines of MG4L were selected. These 1366 lines will be advanced to 2024 preliminary yield tests (PYT). Most of these populations are derived from root knot nematode-resistant pedigrees. **6. Breeding population advancement:** We have about 100 bi-parental populations derived from nematode-resistant pedigrees are currently being advanced in winter nurseries in Puerto Rico and Costa Rica. Approximately 15,000 F:4-5 plants will be selected to evaluate for agronomic traits in 2024 F:5 progeny rows.**7. New crosses in 2023 season**:We attempted approximately 150 crosses with at least one nematode-resistant parent to develop new breeding populations from elite parents. These F1 seeds were sent to Costa Rica and Porto Rico for breeding population advancement. |
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