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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. |
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| 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 |
| Principal Investigator Name: | Travis Faske |
| Report Period: |  12/15/2023 to 3/15/2033 |
| Project Status:  |
| **Characterization of the mechanism of resistance to SRKN. (Faske and Watson)**Objective 1. (Faske and Watson) The experiment was conducted twice in Arkansas. A similar experiment was set up earlier this week to evaluate the reproduction vs galling in the PI lines that are being evaluated in this study at the Lonoke Extension Center in Arkansas.Objective 2. (Nguyen)Genetic characterization and development of functional markers for new sources of resistance to SRKNThis quarter, efforts were made to develop functional markers from another QTL region on chromosome 13 for marker-assisted selection (MAS) for the SRKN resistance program. About 2MB genomic region from the whole genome sequence of PI 567516C (resistance) and Magellan (susceptible) were screened to identify single nucleotide polymorphism and these polymorphic sequences were used to develop 15 KASP markers. Among all, six KASP markers (Gm13:29919913, Gm13:27974504, Gm13:29914173, Gm13:27880365, Gm13:28004208, Gm13:28011016) efficiently distinguish RKN-resistant PI 567516C and susceptible Magellan (Figure 1). These markers will be further used to screen and confirm various RKN-resistant and susceptible genotypes based on Chr.13 QTL. In the next quarter, we will develop accurate KASP markers from other important SRKN-resistant lines including PI 438489B which will be used in the marker-assisted selection program for SRKN. **Figure 1.** Endpoint fluorescence scatter plot of KASPar assay showing two difference plots of resistant and susceptible lines using Chr.13 based markers.**Obj. 3: Development of breeding populations and MG4 soybean varieties with resistance to SRKN.** **University of Arkansas, Caio Vieira - March 15th,2024, Season Update**: Entry lists with the finalized breeding selections and testing locations have been determined for the upcoming season. Additionally, experimental designs and seed packaging are currently underway in preparation for our 2024 planting season. Samples for all preliminary stage entries have been prepared and are being shipped for molecular marker analysis, including resistance to southern root-knot nematode.**2024 Pre-commercial Stage**: Four SRKN-resistant lines 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. Pre-commercial trials will be conducted in Marianna, Pine Tree, Rohwer, Stuttgart, and Fisk. In addition, all these four lines are undergoing a backcrossing program to incorporate Enlist® and Xtend® herbicide resistance traits. 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 2025 commercial release. It was evaluated in the 2023 USDA Uniform Preliminary MG 5 (placing 3/37) and the Arkansas Variety Testing (100.5% of the test mean). Pre-foundation seed will be grown in Stuttgart, Arkansas in 2024.**2024 Finals Stage:** Two MG-4 SRKN-resistant lines were advanced to the 2024 Finals Stage. These lines will undergo testing at four AR locations: Marianna, Pine Tree, Rohwer, Stuttgart, and Fisk, MO. Furthermore, selected lines will undergo flood testing in Stuttgart, AR, while pure seed production will occur in Fayetteville, AR. R22KB-16609 entered the trait introgression program (Enlist® and Xtend® traits) as a potential MG4-Early SRKN-resistant line.**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 three AR locations: Marianna, Pine Three, and Rohwer. Pure seed will be produced in Fayetteville, AR. Lines are being genotypically screened for SRKN resistance. Pending yield performance, lines may be advanced to the 2025 Finals Stage.**2024 Population Development and Generation Advancement**: Sixteen populations derived from SRKN-resistant parents are currently being advanced in Puerto Rico's off-season nursery. Currently, 1,600 single-plant selections are being cultivated in Puerto Rico. These selections will undergo individual hand-harvesting and threshing. F4:5 seeds are scheduled to arrive in the US during the second week of April. Upon arrival, the seed will be processed and planted as progeny rows in Kibler, AR. 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. Generation advancement is ongoing smoothly in of-season nurseries.**Identifying new sources of resistance:** To enhance genetic diversity and discover novel genetic resistance against SRKN in our breeding program, we utilized a comprehensive selection of 10,225 plant introductions (PI) from MG1 to MG4. The resistance class and galling scores were predicted for 10,225 PIs based on a previously published genomic prediction model by our group (Canella Vieira et al., 2022). A total of 26 PIs from MG I, II, and IV, originating from various regions, were predicted as resistant. Finally, to explore alternative sources of resistance, we selected 10 genetically diverse PIs exhibiting low predicted galling scores but lacking the resistant allele for the major gene on Chr. 10. Seed from selected PIs was requested at the Germplasm Resources Information Network (GRIN) and will be screened for SRKN resistance during Spring 2024 at Hope, AR.**University of Missouri RKN Report March 15 – Feng Lin**Emphasis is being placed on improving RKN in late III to early IV maturity groups. 1. **Releases:** A root-knot-resistant line S19-10701, conventional was released in 2024. This line has shown excellent yield potential in the absence of off-target Dicamba injury. It is an indeterminate mid-group IV [relative maturity (RM) 4.5] cultivar. It was tested across six states (AR, KS, MO, MS, TN, VA) in 32 environments, it yielded 94.1 to 113.8% of the non-Xtend check mean and 77 to 107.9% of the Xtend check mean, it averaged 19.4% oil, and 34.9% protein at 13% moisture. Seeds of S19-19764HOLL have been submitted to the Missouri Foundation seed program for production.
2. **Screening of Elite Lines:** In 2023,11 lines with resistance to RKN, including released lines and elite lines from the University of Missouri - Fisher Delta Center, were screened for their degree of resistance and severity towards RKN in Arkansas. Six of the 11 MO lines showed good RKN resistance.
3. **Promising lines in the regional test:** In total, we sent for RKN phenotype screening 25 lines present in the USDA Southern Regional Testes. From these, 4 promising lines carry the RKN gene of Resistance to Root-Knot nematode. These lines are listed in the table below.

Table 1: Advanced lines in the Southern Regional Testes – USDA for yield test.

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| Line | MG |
| S21-11972HP | 4-L |
| S21-17588LL55 | 4-M |
| S21-22067 | 4-E |
| S20-13179LL55 | 5-E |

1. **Advanced yield trials:** We sent 100 lines for screening with molecular markers tightly linked to genes conferring resistance to southern (SRKN) in two different institutions. We are waiting for the results.
2. **Preliminary yield trials:** Almost 1000 lines of late III to Late IV soybean breeding lines will compound our 2024 preliminary yield tests (PYT). These lines will be planted in 3 local environments, in AR and MO where off-target damage to Dicamba is lower. Most of these populations are derived from root-knot nematode-resistant pedigrees.
3. **Progeny Rows:** We have about 100 bi-parental populations derived from nematode-resistant pedigrees that are currently being advanced in winter nurseries in Puerto Rico and Costa Rica. These lines will be compared to widely grown commercial XtendFlex checks. These lines will be evaluated based on yield potential and other agronomic traits.
4. **Breeding population advancement:** In 2023, Two crosses were made with G11-7013 (elite line with a gene from PI96354) x University of Missouri RKN resistant lines. These crosses are listed in the table below. In addition, another 80 crosses were made using at least one source of Resistance to Nematode.

**Table 3:** 2023 crosses made with RKN-resistant genotypes.

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| **CROSS**  | **Female** |  | **Male** | **PEDIGREE** |
| **S23-574** | S19-14058 |  x  | G11-7013 | S19-14058 x G11-7013 |
| **S23-575** | S19-10701C |  x  | G11-7013 | S19-10701C x G11-7013 |

**8. New crosses in the 2024 season**:We are designing the crosses for RKN resistance. The crosses will be made planning to incorporate the gene from PI96354 and other sources with higher resistance to RKN. These F1 seeds will be sent to Costa Rica and Puerto Rico for breeding population advancement. 9. **Screening for Resistance:** We will screen earlier maturing soybean plant introductions (PIs) (MG 3L and 4E) with possible RKN resistance to identify new genes with greater resistance. Several PIs have been identified as possible RKN-resistant sources and will be used as Resistant Checks. A panel with 300 PIs was designed and will be screened in Clarkton, MO in sandy soil with high-pressure nematodes in 2024. Soil samples will be collected before the planting and during the scoring time to guarantee the presence of the nematode and a better phenotype. We also build a panel with 300 breeding lines from the University of Missouri Delta Center which will be screened in the same conditions cited above to evaluate the resistance present in our program.The resistance identified will be used in crosses to develop RKN resistance lines in late group III to late group IV with known RKN- genes.Presentations:Watson, T. Shared information on the time-course portion of objective 1 at Louisiana Agricultural Technical and Management Conference in Marksville, LA Feb. 8Faske, T. Soybean production meetings, Duma, AR Feb. 20 Faske, T. Soybean production meeting, McCrory, AR Feb. 27 |
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