1:50 pm, 07 Feb 23

Nebraska Soybean Board
FINAL Research/Extension Education Report Form

Note: Submit this report no later than 60 days after the NSB-funded project officially terminates.

This post-project 60-day time-frame will allow the Lead PI/Extension Educator time to complete any final data analysis and a final technical report, plus the drafting of any articles for submission to scientific journals.
This completed report will be provided to the National Soybean Checkoff Research Database: soybeanresearchdata.com.

Project # and Title:

Creating Five High-Yield Soybean Variety Pairs with Contrasting Biological Nitrogen Fixation Capabilities

PI / Extension Educator:

George L. Graef

Co-PI's / Co-Extension Educator's:

David Hyten

Project Date ***(Including No-Cost Extension)*****:** 10/1/19 **to** 9/30/22

Total Budget for Project: $134,565

1. Briefly State the Rationale for the Research.

There are no modern high-yielding varieties in maturity groups (MG) 0, I, II, III and IV zones of adaptation in the North Central US developed as isolines carrying the mutant non root-nodule phenotype that can be compared to their normal root-nodulating counterparts in order to measure the impact of their nitrogen (N) fixing capability. The only existing non-nodulating mutant isolines are two old varieties (Harosoy and Clark) that were developed in the 1960s. To facilitate ongoing research aimed at understanding the current contributions of Biological Nitrogen Fixation (BNF) and nitrate availability (soil residual or fertilizer) that is representative of production environments of MG II and III in Nebraska and the North Central growing region, it is imperative to develop and utilize modern isoline pairs that were bred for modern high-yielding environments. Thus, the key research objective in this proposal is to create a BNF contrasting pair of isolines that are representative of modern high-yielding genetic backgrounds in each MG. In addition, the genetic resources created will be available to soybean breeders, physiologists, and agronomists across the 12 states of the NCSRP region and will represent a valuable resource for research that is now pursuing optimization of both, BNF and soil Nitrate N uptake on high-yielding production environments.

2. Research Objectives: (copy from original proposal, but keep in a brief bullet format).

The Primary Objective to this Proposal is the creation of a contrasting pair of Rj1Rj1 (BNF+) and rj1rj1 (BNF-) NILs in five different varieties – one in each MG 0, I, II, III, or IV - thus spanning the MG adaptation range in the 12-state NCSRP region. Five isoline pairs in total were in the original objectives, however, at the end of the experiment, we believe we will be actually deliver 6 pairs total (see attached word document table for reference).

3. General Approach Used and (if applicable) the Nebraska Test Location.

The marker-facilitated, fast-track, backcrossing scheme we are using will result in BC3-F2 derived Rj1Rj1 (BNF+) and rj1rj1 (BNF-) NIL pairs in each of five differing MG 0, I, II, III, and IV high-yield varieties, as shown in the Appendix page. The backcross project was initiated in July of 2017 when the recurrent parent Rj1Rj1 was crossed to rj1rj1 donor parent. If the proposal is funded, on 1 Oct 2019, BC2F1 hybrid seed obtained from matings to be made in the 2019 Lincoln (LN) campus summer nursery would be ready to be shipped to the 2019-2020 Puerto Rico (PR) winter nursery. This would commence the 1st annual cycle of nursery advancement - LN mating that would involve using DNA markers flanking the cloned Chr 2 Rj1/rj1 locus (Lee et al., 2011) to: (1) confirm the hybridity of BC2F1 (Rj1rj1) seeds/plants generated by matings made in the 2019 LN summer nursery and grown in the 2019-20 PR winter nursery, and (2) identify, among the segregating BC2F2 seeds individuals with the genotype rj1rj1. These seeds will be planted 2020 LN summer nursery to perform matings and generate the BC3F1 hybrid seed to be planted in the 2020-2021 PR winter nursery to commence another cycle of the (1) and (2) marker analyses.
When the BC3F2 seeds are returned to Lincoln in the spring of 2021, however, the BC3F2 Rj1rj1 heterozygote segregates will be identified with markers. These Rj1rj1 seeds will also be genotyped with a set of "Recurrent Parent (RP) recovery" markers that will identify which of the many BC3F2 Rj1rj1 plants (for each of the five RPs) has the highest percentage of RP genome and seed will be planted in the 2021 summer nursery. The seed harvested from those 2021 LN summer nursery Rj1rj1 plants will be shipped to the PR winter nursery in Oct 2021 to produce BC3F2-derived, F3 seed that will segregate for the rj1 locus. When that seed is harvested and returned in spring of 2022 to LN, markers will be used to identify the Rj1Rj1 (BNF+) and rj1rj1 (BNF-) homozygote genotypes that will be grown in the LN summer nursery and will produce the seed that completes the creation of these two NILs.

4. Describe Deliverables & Significance Attained for Each Research Objective.

Six isoline pairs carrying contrasting alleles at the root-nodulating genetic locus rj1 in soybean that result in the expression of the mutant non root-nodule and the wild type (nodulated) phenotypes were developed to serve as a genetic tool for measuring the contributions of Biological Nitrogen Fixation and native nitrate availability (soil residual or fertilizer) in production environments of the North Central growing region. These genetic tools in the form of modern high-yielding isoline pairs across maturity groups 0, I, II, III and IV can be used by breeders, physiologists, and agronomists across the 12 states of the NCSRP region. These tools are expected to ultimately be beneficial to soybean producers who will improve their farm operation using the knowledge obtained from the study of the relationship between N derived from BNF and N derived from soil-applied urea (see attached word document for a summary of the final selected isoline pairs).

Project executed and reported by Dr. Luis Posadas, Research Assistant Professor.

4. Describe Deliverables & Significance Attained for Each Research Objective. (continued)

5. List where the Project Research Results/Findings were Publicized.

The manuscript involving the development these tools is currently being prepared and it is expected to be submitted for publication in 2023. Thank you very much for your continued support, particularly through the pandemic, challenging times. We learned a lot about root phenotyping and new ideas are coming forth to study the important dependence of seed yield with nitrogen. It's been a very fun project to work with.

Note: The Final Report comprised of the above listed items must be kept to THREE PAGES.

A Technical Report of no more than TEN PAGES (preferably fewer) can be appended to this report.

Submit the form with the following file name format: #XXX_FINAL_Project Title_LastName

Please submit this completed form with attached files to the Agriculture Research Division, jmcmahon10@unl.edu, based on the reporting schedule given to you.

If you have any questions, please call Jen McMahon at the Agricultural Research Division (402) 472-7082.

Please click to attach technical reports, etc. Attach files Please check your information before submitting the form. Submit by Email

Please note: Attach files button may not work in some versions of Acrobat Reader. You may need to save a copy of this form and then attach files to the copy.

Selected isoline pairs grouped together with their corresponding recurrent parent for comparison of some salient agronomic trait descriptors.

Recurrent Parent	BC3F3:5 Isoline	rj1 Status	Rhg1	Rhg4	ST	FC	PB	HC	MG	Estimated Date Of R8	MG Category	Seed Harvested (Lbs)
Sheyenne-1-03		**Rj1/Rj1**	**HET**	**Rhg4**	**I**	**P**	**G**	**Y**	**MG0**	**NA**	**0**	
	UX3933-1-028-003	rj1/rj1			I	P	G	Y	MG0	9/5/22	Early 0	0.7
	UX3933-1-028-015	Rj1/Rj1			I	P	G	Y	MG0	9/4/22	Early 0	1.3
	UX3933-1-040-015	rj1/rj1			I	P	G	Y	MG0	9/10/22	Late 0	0.9
	UX3933-1-040-006	Rj1/Rj1			I	P	G	Y	MG0	9/8/22	Late 0	1.3
U11-917032-1-20		**Rj1/Rj1**	**Rhg1-b**		**I**	**P**	**T**	**Bl**	**MGI**	**9/22/22**	**I**	
	UX3934-84-043-002	rj1/rj1	Rhg1-b		I	P	T	Y	MGI	9/15/22	Late I	1.2
	UX3934-84-043-012	Rj1/Rj1	Rhg1-b		I	P	T	Y	MGI	9/15/22	Late I	1.4
U11-920017-2-10		**Rj1/Rj1**			**I**	**P**	**T**	**Br**	**MGII**	**9/24/22**	**II**	
	UX3938-114-038-011	rj1/rj1			I	P	T	Br	MGII	9/28/22	Late II-ish?	1.4
	UX3938-114-038-001	Rj1/Rj1			I	P	T	Br	MGII	9/28/22	Late II-ish?	1.6
U11-614093-2-21		**Rj1/Rj1**			**I**	**P**	**T**	**Bl**	**MGIII**	**9/29/22**	**III**	
	UX3941-141-002-008	rj1/rj1			I	P	T	Bl	MGIII	10/3/22	III	0.8
	UX3941-141-002-014	Rj1/Rj1			I	P	T	Bl	MGIII	10/3/22	III	1.4
LD07-3395bf-1-11		**Rj1/Rj1**	**Rhg1-a**	**Rhg4**	**I**	**W**	**G**	**Bf**	**MGIV**	**10/6/22**	**IV**	
	UX4019-158-055-011	rj1/rj1		Rhg4	I	W	G	Bf	MGIV	10/3/22	Early IV	0.5
	UX4019-158-055-006	Rj1/Rj1		Rhg4	I	W	G	Bf	MGIV	10/7/22	Early IV	1.5

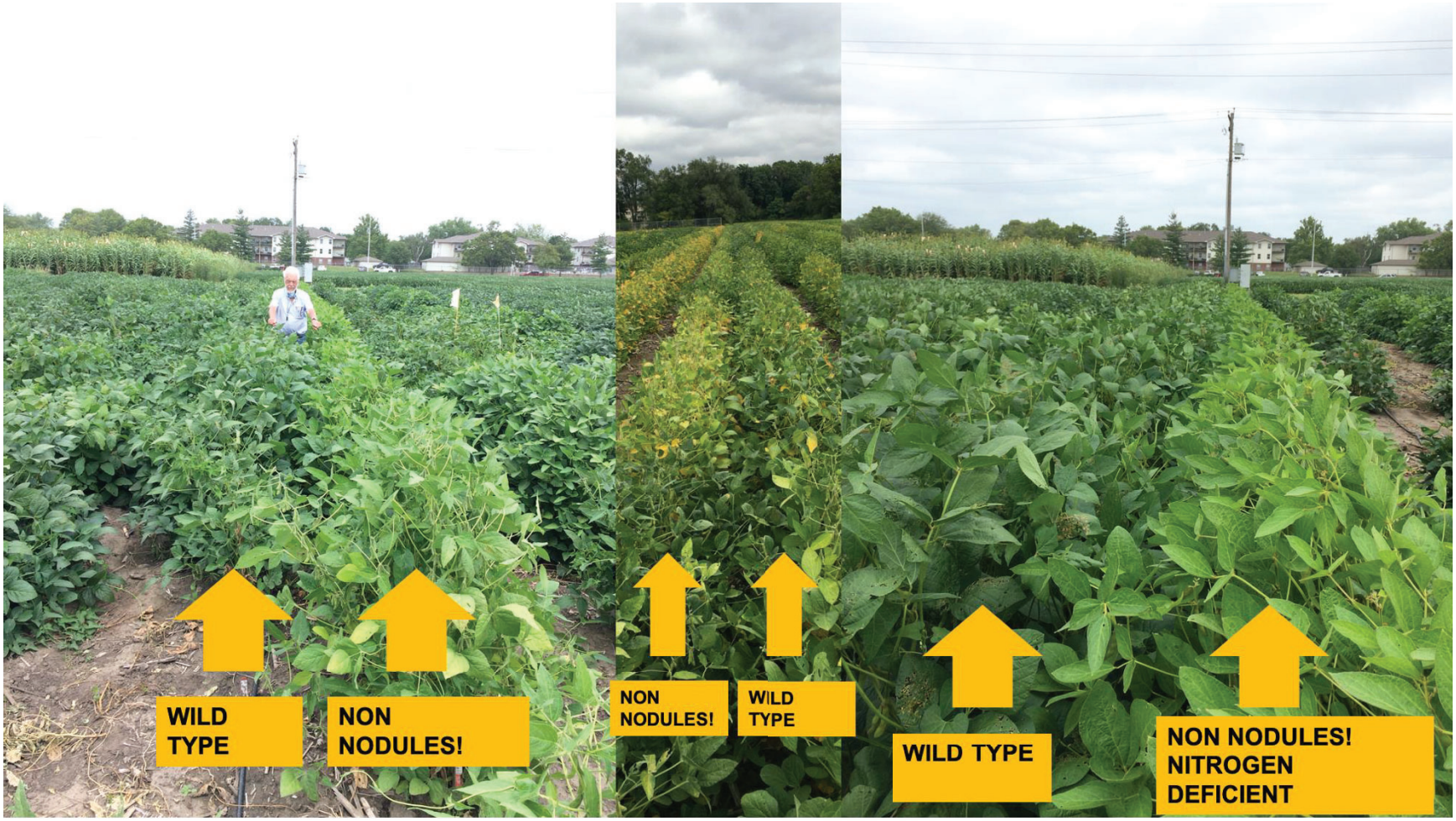
Non-destructive root phenotyping confirmation methods.

Root nodulation status confirmation methods for end-of-season harvest.

Plants with root nodules (wild type) capable of fixing atmospheric nitrogen through symbiosis with soil bacteria.

Plants with NO root nodules (mutant type) incapable of fixing atmospheric nitrogen through symbiosis with soil bacteria.

Side-to-side view of wild type rows next to mutant (*rj1/rj1*) nitrogen-deficient rows.



Molecular relationship of populations derived within each of the five different recurrent parent backgrounds (blocks), after three rounds of backcrossing.