Breeding of Improved Non-GMO Cultivars and Germplasm (2024)
Principal Investigator: Carrie Miranda, PhD

a. Title: Breeding of Improved Non-GMO Cultivars and Germplasm (2024)

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b. Research Overview and Objectives

Research Overview: This project provides soybean farmers with improved non-GMO cultivars that have been developed by NDSU. The NDSU soybean breeding program has a long history of providing very competitive varieties, especially for conventional or non-GMO varieties. The project currently has improved non-GMO experimental lines that are close to being released as named cultivars or as specialty release food grade varieties. In addition, the non-GMO breeding effort is very important as a source of high-yielding parents for use in the development of glyphosate-resistant cultivars or other premium projects such as high oleic. Also, growers need information that enables them to select the best private company soybean cyst nematode (SCN) resistant varieties. This project tests private company varieties for yield in SCN infested soils. These data are then published online. Finally, as soybean sudden death syndrome (SDS) has been observed in several counties in North Dakota, genetic resistance is necessary to protect yields. It is possible to stack SDS resistance with SCN resistance to ensure a protection of yield potential in NDSU varieties. This project would allow for continued breeding efforts to stack SCN and SDS in NDSU germplasm.

Objectives:

This research project had three objectives which include:

- provide soybean growers in North Dakota with non-GMO cultivars which are genetically superior to cultivars that are currently grown
- to enable private companies and growers to compare yield of SCN-resistant cultivars and experimental lines at three North Dakota sites that are infested with SCN
- create germplasm with SCN and SDS stacked resistance

c. Materials and Methods:

Objective 1: Breeding methodology/pipeline: The conventional breeding project will now become the primary or core germplasm pipeline and herbicide traits will be introgressed by using a trait introgression backcrossing pipeline starting in fiscal year 2024/2025. This change has also caused reconsideration of the traditional breeding pipeline which is shown in Figure 1. This is still the currently used pipeline for majority of the program, however we are in the process of transitioning to an accelerated breeding pipeline that focuses on reducing time in the early generation advancement. This transition utilizes crossing in the growth chamber at NDSU during winter and then advancing lines from generations $F_1$ (or $F_2$) to $F_5$ at the winter nursery at Costa Rica Seeds in Upala, Costa Rica (Figure 2).

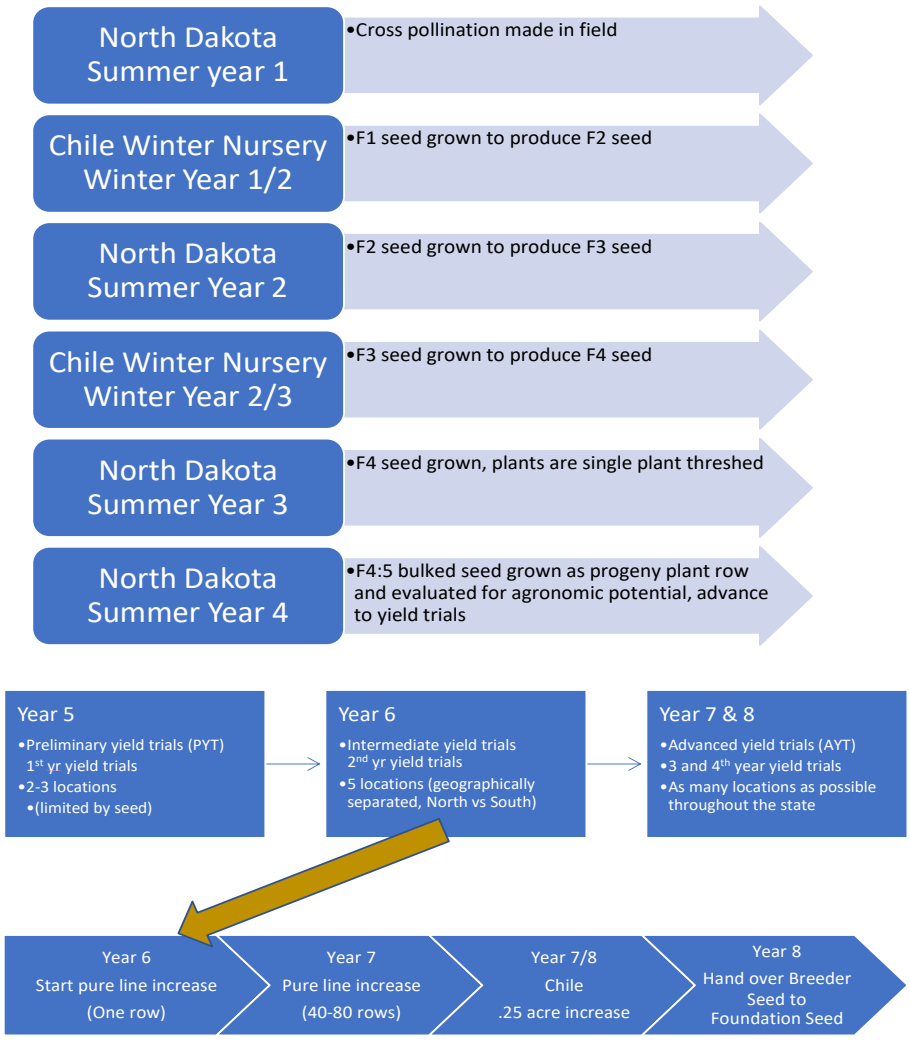


Figure 1: The traditional breeding pipeline. Crosses are initiated in the field during the summer growing season in North Dakota. Lines/populations are advanced over 4 years by shipping back and forth from ND to a winter nursery. By year 5 lines can be entered into yield trials where they will be tested for 4 additional years. During the time of yield testing, selected lines are also grown separately to create pure lines that will eventually become breeder seed.

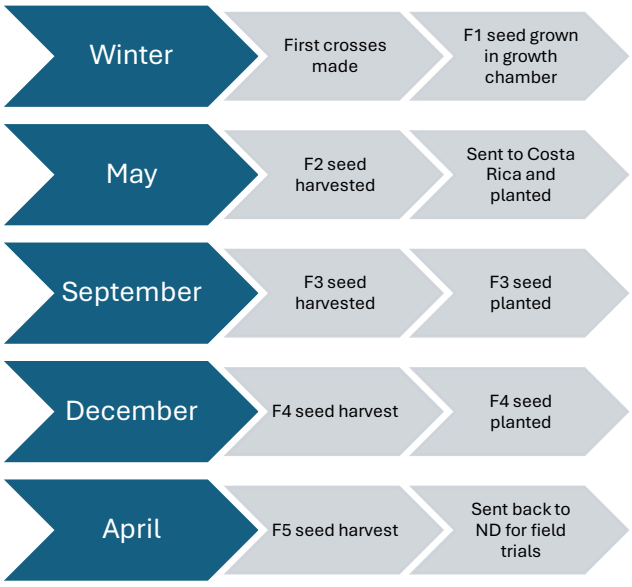


Figure 2. Accelerated breeding pipeline. Early generation advancement in months by utilizing growth chambers and an equatorial winter nursery.

Crossing: Was conducted in the field in 2023 and in growth chambers with high yielding lines from varying maturity groups. Three conventional populations were initiated in the field. These crosses were conducted with North Dakota materials only. A greater emphasis is on yield improvement utilizing high yielding materials from Illinois, Nebraska and Missouri. These crosses were conducted in growth chambers throughout the year. ~20 new populations were initiated with these materials to date in 2023. Illinois parents used were LD11-2170(III), LD15-5776793, LD17-10157, LD16-6787. Nebraska parents used are U14-910097 and U15-606207. Missouri parents are S16-12774 and S17-1980. All parents were selected based on superior performance in the USDA Northern Uniform Trials or personal recommendation from Dr. Brian Diers and Dr. George Graef. Missouri lines were recommended by Dr. Pengyin Chen for conventional Dicamba drift tolerance.

Early Generation advancement: A major accomplishment of this fiscal year was successful application of the accelerated breeding pipeline to 15 ND x MG II+ (IL, NB, MO) populations (eight conventional populations of the 15) that were initiated in 2022. These lines are the first trial of the new pipeline. Crosses were increased to $F_1$ seed in North Dakota in a growth chamber before being planted as $F_2$ seed in Costa Rica in May 2023. In April 2024, we received $F_5$ single plant thresh seed for 2111 lines. Lines were genotyped for maturity group prediction and planted in Casselton in June 2024.
We also received 429 $F_4$ seeds from 3 ND x MG II+ (IL, NB) populations that were sent to Costa Rica in 2023 as $F_1$ seed. These $F_4$ seeds were planted in Casselton in June 2024.

Yield trials: Yield trials were grown as follows: Preliminary yield trials- 2 rows/ 2 reps/ 2 location, Intermediate yield trials- 2 rows, 2 reps, 4 locations. These are planted in alpha lattice design. Advanced yield trials (3$^{rd}$ and 4$^{th}$ year)- 4 rows, 3 reps, 6 locations + 11 REC locations. Selected lines from advanced trials are also entered into USDA Uniform Yield trials for additional location data and mean comparison.

Genotyping:
All 15 ND x MG II+ (IL, NB, MO) populations (2111 $F_5$ lines) were genotyped for the *E1, E2,* and *E3* maturity genes per https://link.springer.com/content/pdf/10.1186/s12870-017-1040-4.pdf and allele combinations of these three genes were used to predict the maturity group of these lines. This was used to confirm true crosses as well.

Leveraging funds from the NCSRP SOYGEN project, 2023 and 2024 preliminary trial entries (~1600 lines) were genotyped with the Agriplex 1k SoySNP platform. This will be used for multiple purposes, primarily for future genomic prediction modeling.

Objective 2: Online applications were open in January 2023 for private company submissions for SCN testing. There was no fee charged as this project subsidized planting and field management expenses. Ten companies submitted 76 lines for testing. This was more than 3x the number of entries for 2022. Lines were planted as 4 rows, 3 reps, 3 locations. In 2024, a fee was imposed for the SCN trials and this objective was removed from the 2024/2025 core germplasm project. This year, one company submitted 7 lines for SCN testing. Dr. Wade Webster will oversee field maintenance and data analysis for this project and the NDSU Soybean Breeding Team will assist with planting and harvest. Lines were planted as 4 rows, 3 reps, 3 locations in June 2024.

Objective 3: In 2021, four populations were initiated to stack SDS resistance with NDSU lines with known PI 88788 SCN resistance. The SDS donor is MN1606SP from Dr. Aaron Lorenz at University of

Minnesota. The resulting ~1200 progeny were advanced with the traditional pipeline in Chile and Costa Rica. Genotyping for SCN resistance was done with known markers from the Dr. Dechun Wang lab submitted in the Agriplex 1k SoySNP trait marker panel. Dr. Febina Mathews was unable to phenotype for SDS resistance. Corteva markers for SDS resistance were considered unreliable. Due to this, in 2024, it was decided to grow preliminary trials of these $F_6$ progeny in one typical field (NDSU Agronomy Seed farm), and one heavily invested SCN field to apply selection pressure from disease. 384 experimental lines were planted in preliminary yield trials as 2 rows/ 2 reps/ 2 locations in June 2024.

d. Results:

Objective 1: The first plant/progeny rows of the ND x MG II+ (IL, NB, MO) will be grown in a North Dakota field in 2024 after crosses were initiated only 2 years ago. Lines were genotyped for accurate blocking based on maturity and to ensure true crosses. Due to the wide range of maturity possibilities from the broad cross, we applied Dr. Kristin Bilyeu's molecular maturity model for *E1, E2,* and *E3* genes to ensure proper experimental design and line evaluation. All lines will be planted regardless of anticipated maturity to ensure correct phenotyping and to leverage these data for future research projects. Of the 2111 $F_5$ lines received from the winter nursery, 1144 lines were considered to be derived from segregating populations. 202 lines are predicted to be MG 0 or MG 00, 271 lines to be MG I, and 671 to be late MG I or MG II. DNA was also extracted from all lines prior to planting for future whole genome marker analysis.

As this project transitions to the core germplasm project, emphasis is put on correct selection for both cultivar/germplasm development and also parental selection. Here is a table of selections for each yield trial

| Trial | Conventional entries (excluding GT, Objective 3 SDS lines and high oleic lines) | Yield BLUP and PM as days after Sept 1 |
|---|---|---|
| Preliminary yield trial | 158 | |
| Intermediate yield trial | 53 | |
| Advanced yield trial 3rd year | 6 | |
| Advanced yield trial 4th year | 1 | 44 bu/a and Sept 14 |

Lines were selected for SCN resistance potential based on pedigree and in advanced yield trials on HG type testing. Unfortunately yield gains may not be possible in the lines entered into intermediate yield trials and higher. These are the final lines from my predecessor and are heavily inbred resulting in yield stagnancy. However the backgrounds of these lines are useful parents due to IDC tolerance, *Phytophthora* race 4 resistance, and recently discovered Peking SCN resistance and of course, adapted maturity.

Objective 2: The new collaboration between Dr. Wade Webster and the Soybean Breeding Team is welcome help distribute workload for this project. The newly imposed fee laso reduced entry numbers. The 2023 trial overwhelmed our staff and was difficult to manage. With the work reduced and split between the Breeding Program and Webster lab, high quality data collection can continue for the companies that choose to participate.
Results are published online and in print the in NDSU A-843 publication in December every year.

Objective 3: We are grateful for the genotyping for SCN resistance available through the Agriplex trait marker panel (that I curated, so there's a little bragging there). With this help we were able to reduce the number of progeny rows without SCN resistance from 1200 lines down to 384 lines. It is unfortunate that the SDS phenotyping service was not provided because the original objective was to identify ~40 lines with stacked SCN and SDS resistance prior to field trials. 384 lines is an excessively large experiment for preliminary trials, however still manageable by our team. I feel confident that we can reduce the number of not useful lines by applying selection pressure from a heavily infected SCN field. We may lose lines with moderate resistance however.

e. Disclosure of variety release
None

f. Discussion: The first field tests with lines generated from the accelerated breeding pipeline is a highlight of my short tenure in this position. We went from cross to progeny row testing in only 2 years! That is a 2 year reduction in time from the traditional breeding pipeline. We produced ~2000 lines from this first trial which was completely manageable. There were also several important lessons learned before we scale up the entire program to this pipeline.
    1) Organization- At first we planned to send 4 shipments of crosses a year to Costa Rica, however we found that it was very hard to keep the seed inventory organized and also to coordinate with the yield trial pipeline. We have decided to only do two shipments a year, once in May and once in November. Seed inventory and prediction of $F_5$ line development still needs to be fine tuned but should become obvious with the reduction of seed shipments.
    2) Growth chamber space and crossing timelines- Growth chamber space is a consistent compromise of resources. We are concerned about bottlenecking our genetic diversity by using so few parents, however we do not have the space necessary to add more. I am interested in purchasing my own growth chambers for this purpose but have to get approval from the university. As much as we try to have a schedule for crossing, other breeding program tasks can take priority. Now that we know this is a successful operation that can potentially be scaled up, the priority needs to shift to creating new crosses. I believe that the reduction of shipments of Costa Rica will also reduce the time crunch for crossing. Instead of planting every week, we now plant every two weeks and crossing can occur with more time in between to accomplish other tasks.
    3) Winter nursery success with scaling up- The most challenging task for the winter nursery is completing the single plant threshes in time to ship for packing and planting in May. Our team typically takes 2-3 months to complete all the single plant threshing for 10,000 progeny rows with 1-2 people working. This year, the winter nursery successfully completed single plant threshing of 2,000 lines for us to receive the seed in April which allowed us time to do the three single marker maturity genotyping. However it is a concern that when we increase the lines to the full program, or 10,000 lines, they may not be able to handle that April deadline. This year we may send our greenhouse crosses in greater number (~5,000 $F_2$ lines) but in September to test if this is an improvement.
    4) Genotyping- I learned a very valuable lesson this year. With the traditional ND x ND crosses, we simply grow those progeny in the field as $F_2$ plants and discard rows that do not show segregation to ensure true crosses. However, it is not possible to do this with the accelerated breeding pipeline especially in the lines that are segregating for maturity. The simplest option is to genotype $F_1$ lines by seed chipping before sending to the winter nursery. This is can reduce payment to advance populations that are not true

crosses. We also have to genotype the ND x MG II populations for predicted maturity to block properly by maturity to facilitate note taking and sanity during harvest. This year, since we received the seed early, we sent tissue samples to a private company for DNA extraction and conducted the single marker analysis in our own lab as we do not have the capacity to do our own high throughput DNA extraction. I need to put thought into if that would be a worthwhile investment to our lab equipment. I want to thank the ND soybean council and Steve Schnebly for their advice and support of this project and the future core germplasm project. I am optimistic that we can expediate the creation of high yielding germplasm with this improved pipeline now. I look forward to sharing the results from this year's field season.

g. Benefit to North Dakota farmers
This project will become the main germplasm creation project for the NDSU Soybean Breeding Program. All potential variety releases will be generated with this project and have herbicide traits introgressed through the trait introgression pipeline. This year we successfully demonstrated that it is possible to save two years in germplasm development by utilizing the accelerated breeding pipeline which will allow creation of high yielding lines at a faster rate than previously possible. The next question is if we can scale up the entire program to this pipeline which be tested over the next two years. I feel optimistic that ND farmers will be growing primarily NDSU soybean varieties very soon.