PI: Xu, Steven | Agreement #:

Project FY22-SP-010: Development of Elite Spring Wheat Germplasm with Fusarium Head Blight Resistance

1. What are the major goals and objectives of the research project?

The major goal and objective of this project is to develop adapted hard red spring wheat (HRSW) germplasm by transferring FHB resistance from unadapted sources into the HRSW cultivars.

2. What was accomplished under these goals or objectives? (For each major goal/objective, address these three items below.)

What were the major activities?

- Evaluated a modified NAM (nested association mapping) population of 288 spring wheat genotypes, including 276 synthetic hexaploid wheat (SHW) derived HRSW lines and their 12 parents and checks. These lines were previously selected based on their reactions to FHB and bacteria leaf streak (BLS, caused by Xanthomonas translucens) from approximately 900 BC₁F₅ lines derived from 18 BC₁F₁ populations from backcrossing the synthetic hexaploid wheat (SHW) lines SW91, SW93, SW183, and SW187 to hard red spring wheat (HRSW) varieties 'Glenn', 'Barlow', 'Vitpro', 'Grandin', 'Linkert' and 'Bolles' and breeding lines ND828, NDHRS16-1436, and NDHRS16-13-89 in the FHB nurseries. The population were evaluated for FHB resistance using the randomized complete block design (RCBD) with three replications in greenhouse for two seasons in the fall 2023 and spring 2024 and in mistirrigated FHB nurseries in two locations (Prosper and Fargo, ND) during the summer seasons (May – August) in 2023 and 2024. Therefore, this population has been evaluated for FHB resistance in six environments. The BC₁-derived lines showed good segregation for FHB resistance, with 44 lines showing high FHB resistance with the mean FHB disease severities of \leq 30% across all experiments and 15 of the lines exhibiting FHB resistance (\leq 25%) comparable to Sumai3.
- Performed and completed genome-wide association studies (GWAS) using the modified NAM population above, FHB disease data collected from greenhouse and field nurseries, and the 90K SNP marker genotypic data set. A total of 19 unique SNPs were identified to be significantly associated with FHB resistance, These SNPs were distributed across chromosomes 1D, 2A, 2B, 3B, 3D, 4A, 4B, 5A, 6A, and 6B. Among these SNPs, three located on the short arms of chromosomes 1D, 2B, and 4A are likely associated with novel resistance loci. A manuscript from this study has been prepared and will be submitted to a journal for publication.
- Developed and evaluated approximately 170 elite hard red spring wheat lines that are homozygous for 5AL QTL and *Fhb1*, including six doubled haploid (DH) lines and 164 BC₁F₅ lines. These lines were previously selected by genotyping 700 BC₁F₂ plants (15FAR1157-1/2*ND Frohberg) and 15 DH lines (15FAR1157-1/ND Frohberg) using the STARP markers for PI 277012 derived 5AL QTL and *Fhb1*. A sub-set of 92 lines with adequate seeds were evaluated in the FHB nurseries in Fargo and Prosper, ND during the summer season in 2023. All 170 lines and their parents have been grown to BC₁F₅ generations in greenhouse, and they were evaluated in the FHB nurseries in two locations (Prosper and Frago, ND) during the summer seasons (May August) in 2024 and 2025. A sub-set of 141 lines and their parents were planted for seed increase and observation in the single-row plots in Prosper, ND. Approximately 40 lines showed higher levels of FHB resistance than ND Frohberg and

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22 of these lines also had lower DON. These lines also showed the same or similar agronomic traits and performance as the HRSW varieties used as the checks. A set of 24 lines were increased in greenhouse in spring 2025 and they are currently being grown in the yield trial in Prosper in the summer of 2025.

- Produced BC₁F₁ hybrids from backcrossing a wheat-*Th. ponticum* 7B/7el2 introgression line SN-26 carrying original *Fhb7* to ND Frohberg, ND Thresher, and ND Stampede.
- Produced BC₅F₁ hybrid seeds by backcrossing a wheat-*Th. elongatum* 7B/7E introgression line XWC14-255-13-1 (WGC002) carrying new *Fhb7* allele *Fhb7*^{The2} to ND Frohberg.
- Developed BC₃F₁ seeds from backcrossing Wangshuibai lines with ND Frohberg to simultaneously transfer major FHB resistance QTL *Fhb1*, *Fhb2*, *Fhb4*, and *Fhb5*.
- Six FHB resistance QTL were previously located on chromosomes 1D (*QFhb.rwg-1D*), 2D (*QFhb.rwg-2D*), 5B (*QFhb.rwg-5B*), and 7D (*QFhb.rwg-7D.1, -7D.2*, and *-7D.3*) using a population of 188 RILs from a cross between SHW line Largo and the susceptible wheat line ND495. In 2024, 10 KASP markers from the six QTL regions were developed and validated for marker-assisted selection.

What were the significant results?

- A set of 44 BC₁-derived lines derived from backcrossing SHW lines with HRSW varieties have high FHB resistance with the mean FHB disease severities of ≤ 30% across all experiments and 15 of the lines exhibiting FHB resistance (≤ 25%) comparable to Sumai3.
- Genome-wide association studies (GWAS)using the modified NAM population identified 19 unique SNPs significantly associated with FHB resistance. These SNPs were distributed across chromosomes 1D, 2A, 2B, 3B, 3D, 4A, 4B, 5A, 6A, and 6B. Among these SNPs, three located on the short arms of chromosomes 1D, 2B, and 4A are likely associated with novel resistance loci. A manuscript from this study has been prepared and will be submitted to a journal for publication.
- Among six DH lines (15FAR1157-1/ND Frohberg) and 164 BC₁F₅ lines (15FAR1157-1/2*ND Frohberg) that are homozygous for 5AL QTL and *Fhb1*, approximately 40 lines showed higher levels of FHB resistance than ND Frohberg and 22 of these lines also had lower DON. These lines also showed the same or similar agronomic traits and performance as the HRSW varieties used as the checks. A set of 24 lines were increased in greenhouse in spring 2025 and they are currently being grown in the yield trial in Prosper in the summer of 2025.
- Ten new KASP markers linked to the six FHB resistance QTL identified in SHW line Largo and HRSW line ND495 have been successfully developed and validated for marker-assisted selection.

List key outcomes or other achievements.

The elite hard red spring wheat lines that are homozygous for 5AL QTL from PI 277021 and Fhb1 with high levels of FHB resistance, including DH (15FAR1157-1/ND Frohberg) and BC₁F₅ lines (15FAR1157-1/2*ND Frohberg) will be useful germplasm for developing adapted HRSW germplasm and varieties for resistance to FHB and other diseases. Approximately 40 lines

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showed higher levels of FHB resistance than ND Frohberg and 22 of these lines also had lower DON. These lines also showed the same or similar agronomic traits and performance as the HRSW varieties used as the checks. A set of 24 lines were increased in greenhouse in spring 2025 and they are currently being grown in the yield trial in Prosper in the summer of 2025.

- A modified NAM population consisting of 276 SHW derived HRSW lines and their 12 parents and checks and their genotypic (90k SNP marker) data set is available for identifying and mapping the genes and QTL for resistance to FHB and other major wheat diseases. A number of QTL for resistance to FHB, BLS, tan spot, septoria nodorum blotch, and stripe rust have been identified using the modified NAM population.
- The SHW derived HRSW lines showing resistance to multiple diseases (FHB, BLS, tan spot, septoria nodorum blotch, and stripe rust) will be useful germplasm for developing adapted HRSW germplasm and varieties for resistance to FHB, BLS, and other diseases. Introgression.

3. What opportunities for training and professional development has the project provided?

This project provides training and professional development for a postdoctoral research associate.

4. How have the results been disseminated to communities of interest?

The results have been disseminated through publications and presentations at various workshops and seminars and communications with breeders and collaborators.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

- Conduct yield trials to evaluate elite HRSW lines (15FAR1157-1/2*ND Frohberg) and DH lines (15FAR1157-1/ND Frohberg) that are homozygous for Fhb1 and 5AS/5AL QTL.
- Perform final GWAS analysis using the modified NAM population above, FHB disease data collected from greenhouse and field nurseries so far, and the 90K SNP marker genotypic data set. Validate the QTL and their linked markers.
- Transfer the novel QTL identified from the modified NAM population by crossing and backcrossing two SHW-derived lines with ND Frohberg, ND Thresher, and ND Stampede.
- Develop BC₅F₁ seeds from backcrossing Wangshuibai and *Fhb7* introgression lines with ND Frohberg and newer variety or breeding lines carrying two PI277012-derived 5A QTL.