Project FY22-HW-003: Transfer of FHB Resistance to NDSU Hard Red Winter Wheat Breeding Material

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1. **What are the major goals and objectives of the research project?**
   Objective 1. Systematically raise the frequencies of targeted FHB resistance genes in the HWW breeding program through convergent crosses, marker use and agronomic evaluation.
   Objective 2. Select for genetic background (native) FHB resistance among advanced lines and utilize it in the breeding program.
   Objective 3. Develop F_4 single seed descent (SSD) inbred lines from crosses that segregate for FHB resistance QTL (plus resistance to the wheat rusts) in each project year.
   Objective 4. Conduct an annual Winter Wheat x Fungicide field trial to evaluate genotype response to fungicide application for the reduction of DON content.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*
   What were the major activities?
   Objective 1. (a) The 2024 crossing block involved 37 parents which were crossed in 511 single cross combinations. Parents were chosen based on FHB resistance (14), wheat rust resistance (23), grain yield (27), height (33), plant phenotype (18) and winter-survival (30). (b) 506 new inbred lines were tested at the USDA Wheat Genotyping Laboratory, Fargo, ND using appropriate markers to predict *Fhb1*, *Qfhs.ifa-5A*, *Qfhb.rwg-5A.2*, and key rust resistance genes (*Sr2*, *Sr24*, *Lr34*, *Lr56*, etc.). (c) A third (modified) backcross was completed to transfer the *Fhb7* translocation (*Thinopyrum elongatum*) from the spring wheat germplasm XWC14-255-3-1 to winter wheat. 322 B_3F_2 from six backcrosses (involved eight winter wheat parents) were analyzed with markers to detect *Fhb7*, *Qfhb.rwg-5A.1*, *Qfhb.rwg-5A.2*, *Fhb1* and additional leaf and stem rust resistance genes. The same 322 plants were then evaluated for FHB Type II resistance and overall phenotype.
   Objective 2. (a) In a diallel study (11 parents and 55 F_1) to detect and use background FHB Type II resistance, F_2 of the six most resistant F_1 cross combinations were compared in a second greenhouse FHB trial to select possible transgressive F_2 segregates. F_3 of the selections were increased (greenhouse) and $F_{3:4}$ lines were then field planted (fall 2023). (b) A first Line (14) X Tester (4) analysis was done to detect background FHB resistance. The parents and 56 F_1 were evaluated (greenhouse) for FHB Type II resistance. F_2 from the most resistant F_1 were field planted for continued inbreeding and line selection. (c) A second Line (20) X Tester (3) analysis was done (greenhouse) to identify F_1 with multiple FHB resistance QTL. Tester #1 was an $F_{2:3}$ line, homozygous for *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* (markers) and carried background resistance from ND Noreen. Testers #2 and #3 were F_1 hybrids of ND Noreen with 18Nord-107 and 19Nord-129, respectively (each parent has background resistance (diallel)). The 20 lines were well-adapted but uncharacterized for FHB resistance.
   Objective 3. SSD inbreeding was initiated (annually) from promising crosses (high yield with resistance to FHB and other diseases) to achieve generation acceleration. ± 96 F_2 seedlings/cross were screened with mixed leaf and stem rust inoculum. During inbreeding the plants were also selected for height and fertility. The F_4 was field planted in the fall of year 2 and single plants were selected in year 3. F_5 pure lines were established in year 4.

Objective 4. A variety X fungicide evaluation trial with 22 entries was conducted at Casselton. The trial followed a split plot layout (three replicates) with half of the plots treated with Prosaro at 8.2 fl oz/acre at flowering (applied on three different dates to allow for variation in flowering dates among the varieties). Corn inoculum (FHB) was applied on two dates, the first application coincided with the onset of heading in the earliest varieties.

What were the significant results?

Objective 1. Of the 511 routine breeding program crosses that were made, 310 (61%) involved at least one FHB resistance source: (Native/none = 65 crosses; *Fhb1*/none = 164; *Fhb1*/native =26; *Fhb1*/*Fhb1* =16; *Fhb1*/*Qfhb.rwg-5A.1&5A.2* = 4; *Qfhb.rwg-5A.1&5A.2* /none = 14; *Fhb7*/none = 21). The progenies will be used for the selection of cold-tolerant, high yielding inbred lines with FHB plus multiple disease resistance.

In the *Fhb7* introgression attempt, (i) 22 robust B_3F_2 plants were selected for ongoing marker selection. The selected plants have either *Fhb7* on its own (four plants) or are pyramided genotypes with two to four of *Fhb7*, *Qfhb.rwg-5A.1*, *Qfhb.rwg-5A.2* and *Fhb1*. The pyramids are only partially homozygous and F_3 will be retested (markers) to increase homozygosity. (ii) Another 10 B_3F_2 plants had good agrotypes, strong type II FHB resistance and were homozygous for resistance QTL *Fhb7* (seven plants); *Fhb7* & *Qfhb.rwg-5A.2* (1 plant); *Fhb7* & *Qfhb.rwg-5A.1* (1 plant); and *Qfhb.rwg-5A.1* & *Qfhb.rwg-5A.2* (1 plant). The FHB resistance of the 10 selections will be confirmed in a replicated F_3 greenhouse trial.

Objective 2. (a) Diallel trial: The ratio of general combining ability (GCA) to specific combining ability (SCA) effects suggested that additive QTL effects were of primary importance. Overall, the resistance QTL showed incomplete dominance, an excess of dominant alleles, and a greater contribution of positive effect genes. Three genotypes exhibited useful (and different) levels of background resistance. F_2 of the six best crosses, that could include transgressive segregates, were retested in a 2nd FHB trial. Six F_3 seeds of each selection were replanted (greenhouse). Phenotypically better F_3 plants were harvested and 114 F_4 families were planted (fall 2023) for field evaluation (summer 2024). (b) First Line X Tester analysis: Significant differences in FHB disease severity were measured between lines, testers and F_1 hybrids as well as significant differences in GCA and SCA. Parents 21Jun318, Winner and Draper showed genetic background resistance comparable to that of ND Noreen. The six most resistant F_1 were identified and it appeared that additive gene effects contributed strongly to their superiority. (c) Second Line X Tester trial: F_1 data are being analyzed to calculate GCA and SCA and determine whether some of the tested inbred lines show significant background resistance. The most resistant individual crosses and F_1 plants from those crosses will be used to initiate field selection and pure line selection.

Objective 3. Three sets of SSD selections were handled during the report period: Two sets of SSD material were field planted at Casselton in September 2023, namely 1,680 F_5 head rows from 44 crosses made in 2021, and 332 F_4 rows from 31 crosses made in 2022. In addition, 672 F_3 lineages from 29 crosses made in 2023 were greenhouse planted in March 2024.

Objective 4. Dry, hot conditions prevailed at flowering resulting in very low FHB infection and development. As a result, no DON analyses were done. Fungicide treatment did not cause significant differential responses of varieties either. A new (2024) Winter Wheat Variety trial with 22 entries and the same statistical design was planted at Casselton in the fall of 2023.

List key outcomes or other achievements.

Objective 1. New inbred lines are being developed annually, a significant proportion of which possesses known FHB resistance QTL. These are evaluated in regional yield trials and provide next generation cross parents. Transfer of *Fhb7* resulted in additional segregating lineages that exhibited strong resistance (greenhouse) and some of these populations are now also being tested in the field. Resistant plants (greenhouse) showed no obvious phenotypic defects and some have already been utilized as parents in routine crosses.
Objective 2. (a) Three F_1 (diallel) crosses among the three parents with the best background resistance were also the most resistant of the 55 F_1 hybrids. Progeny of the three crosses are being used for field testing and pure line development. Also, remnant F_1 seed of the three crosses were included as cross parents (2023 routine program crossing block) and testers (2^{nd} Line X Tester trial). (b) 1^{st} Line X Tester analysis: F_2 of the six best performing F_1 hybrids (lowest disease severities) are being used for field testing and pure line development. Remnant F_1 seed from the four most resistant cross combinations were employed as cross parents in the 2024 crossing block. (c) 2^{nd} Line X Tester experiment: Since the background resistance and "larger"-effect resistance QTL are believed to be mostly dominant and complementing, the most resistant F_1 plants are likely to have combinations of several resistance QTL. Only the most resistant crosses will therefore be used for pure line breeding.
Objective 3. In 2024, the first group of 1,680 F_5 head rows will be evaluated and selected for agrotype at Casselton. The selections will advance to preliminary (Junior) yield trials and will be screened for resistance to wheat leaf diseases and marker analyses will be done (fall).
Objective 4. The results from the Variety X Fungicide trial were incorporated in the online publication: "North Dakota Hard Red Winter Wheat Trial Results for 2023 and Selection Guide" (A1196-23 September 2023).

3. **What opportunities for training and professional development has the project provided?**
Bhanu Dangi graduated in the spring of 2024 with MS thesis title: Diversification of FHB resistance QTL in winter wheat germplasm.
Kripa Rijal joined the project in August 2023. Her MS thesis title is: Improvement of Fusarium Head Blight resistance in winter wheat. Expected graduation: spring of 2025.

4. **How have the results been disseminated to communities of interest?**
Advanced lines were entered in regional nurseries [NRPN (4); RGON (30); Northern Scab (15); USDA stem rust (100); USDA KS Stripe rust (160); USDA WSU stripe rust (100)] and statewide variety trials [ND (4); SD (1); UMN (3)]. Data on the submitted material get listed in on-line reports of the respective nurseries. Variety X Fungicide trial results were incorporated in the NDSU producer's guide A1196-23 (September 2023)**.**

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
"Larger"-effect resistance QTL *Fhb1*, *Fhb7, Qfhb.rwg-5A.1* & *-5A.2* were introduced and six genotypes with significant, albeit uncharacterized, "smaller"-effect, background FHB resistance QTL were identified in our winter wheat germplasm. Marker-aided Pedigree breeding will be employed to widely disperse the variability within the breeding population and strive to combine the FHB resistance with winter survival, yield, broad adaptation, good processing quality and wide spectrum disease resistance (cereal rusts, tan spot, Stagonospora nodorum, bacterial leaf streak, etc.).