Project FY22-SW-009: Winter Barley Mutant Resource to Increase FHB Resistance and to Reduce DON Content

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

1) Phenotypic Evaluation of M4 plants in head row design under misted scab nursery
2) Evaluation of selected mutant families in the greenhouse under point inoculation
3) Confirmation of the mutants and sharing the germplasm

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

What were the major activities?

We screened Nomini mutant population of 800 me derived M3 plants under misted and GH (point inoculation). In total in GH condition 3200 plants from Nomini mutant population were screened and data was recorded and contrasting mutant phenotypes were identified.

What were the significant results?

Since our screening at the misted nursery did not yield optimum results and the disease was not visible due to very low temperature, we screened the Nomini mutant population in the GH under point inoculation with *F. graminearum* culture. Our screening of 800 M2 derived M3 families (4 plants per family and 4 heads per plant) identified 9 contrasting mutant phenotypes as compared to the wild type Nomini (Fig. 1). These 9 mutants showed significantly improved response that Nomini wild type parental lines under three different replicated tests. The results of the analysis are presented in Fig. 2 and Fig. 3.

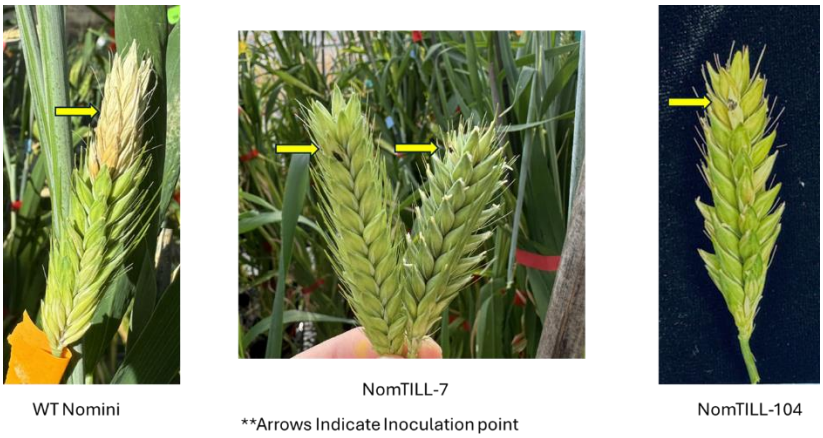

Fig. 1. Comparison of disease spread between wild type Nomini and two independent mutants (NomTILL-7 and NomTILL-104) with enhanced FHB resistance.

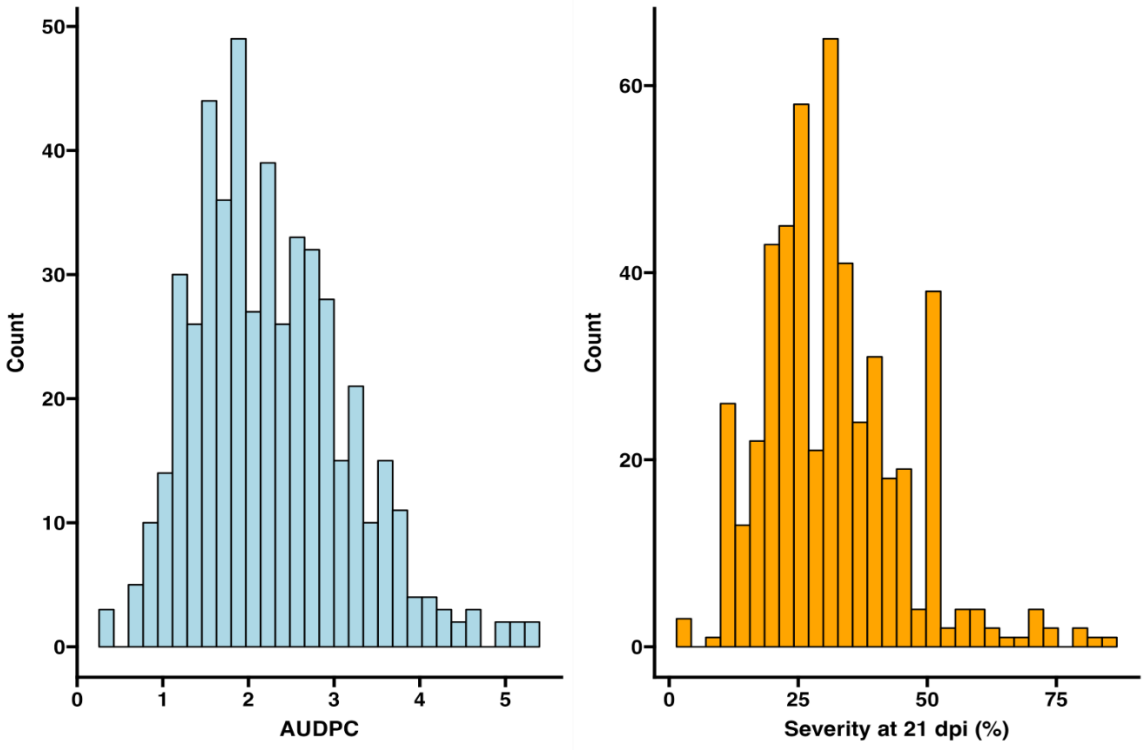

Fig. 2. Area under disease progression curve and severity index of barley mutant population under FHB inoculation.

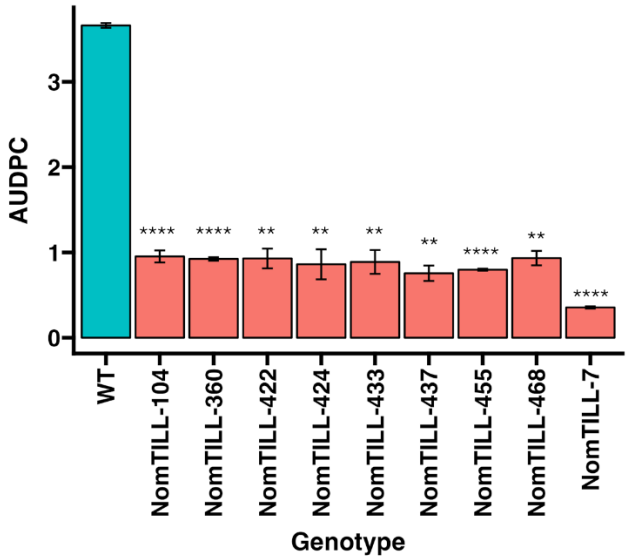

Fig. 3. Comparison of AUDPC of FHB progression in Nomini wild type and 9 independent mutants identified under at least two rounds of testing.

List key outcomes or other achievements.

Screening of Nomini EMS population for FHB resistance identified 9 independent mutants. A total of 3200 plants (from 800 M2 derived M3 families) were screened and a total of 1.1% of the population showed mutant phenotypes.

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

Three PhD students, one MS student, one postdoc, and three undergraduate students were trained under this project. All the trainees worked with the PI to conduct the nursery and collect and analyze data. These students also participated in conferences and commodity board meetings with their work.

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

An undergraduate student, Ms. Victoria Chang led the GH phenotyping part of the project, and she has presented the results at the MAS-ASPB meeting in her poster presentation. She also presented her work at a meeting at Cornell University. The PI presented the results in oral presentations and disseminated updates on FHB in wheat and barley through emails. PI presented the research updates in the FHB forum as well as online project update meetings. The graduate students in the team presented the results as posters and handouts to the stakeholders in commodity board meetings. The results were published as wheat trial Factsheets and was disseminated via emails and the UMD extension system to the broader grower community.

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

The project has expired but we are working on further validation of the mutants in multiple tests. We are also working on developing MutMap populations of two most promising mutants to identify the causal SNPs related with the resistant phenotype. We will be generating some concrete data and would then request funding support from USWBSCI to identify the causal susceptibility gene(s).

Project 2: A Double Haploid Initiative to Speed Development of FHB Resistant Soft Winter Wheat

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

This project strategically addresses each of the three priorities in the VDHR-SWW Action Plan.

(1) Increase the number of FHB resistant varieties available to farmers to reduce DON in the US grain supply. Utilizing doubled haploid (DH) technology decreases the breeding cycle from inception to pure line evaluation by a minimum of two years, which results in releasing FHB resistant varieties at a faster rate with improved efficiency. Saturating the number of FHB resistant varieties in the commercial marketplace for farmers is key to reducing DON presence within the national wheat supply chain. Approximately 1,400 DH lines will be generated from this project, with all of them potentially possessing multiple FHB resistance QTL in addition to high yield potential and quality.

(2) Increase efficiency of coordinated project breeding programs to develop and release FHB resistant varieties. Promising lines (from the 1,400 total DHs) selected by individual breeders will be shared among the VDHR-SWW group to evaluate within scab nurseries in additional locations for regional adaptation. This will practically ensure that no line goes unnoticed and robust, multilocation data for individual lines will provide appropriate information needed to justify release and licensing to companies for marketing to growers.

(3) Implement breeding technologies to enhance short term and long-term improvement of FHB resistance and to efficiently introgress effective resistance genes into breeding germplasm. Prior to selection of crosses for DH production, enrichment of FHB QTL and QTL for other agronomically important traits was completed for carefully chosen topcross populations using established markers and genotyping by Dr. Gina Brown-Guedira at the USDA ARS Eastern Regional Small Grains Genotyping Lab. This improves efficiency of the DH technology by eliminating undesirable lines and increasing the chance of stacking the appropriate genetics (FHB QTL + other QTL) in a DH line that will meet the requirements for release, or at a minimum, be used as a FHB donor parent in future crosses to effectively introgress resistance.

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

The grant period covers harvest (May-June) through preparation for planting for the programs of the VDHR-SWW. As per our group protocol we submitted F1 seed from crosses made in the spring of 2023 for creating new DHs for their planting in fall 2024. The parents of these F1s submitted for DH development were chosen to pyramid FHB resistance genes and have a high probability of combining yield, agronomic traits, other biotic pest resistance and resistance to FHB. About 50% of the DH lines that we received from HPI had less than 5 seeds, so we planted them in the GH and increased the seed volume for field testing. A total of 450 DH lines were generated in the performance period and tested in the headrows for the selection of high-yielding FHB resistant germplasm.

What were the major activities?

Selection of F1 lines based on genotypic data for making decision of DH creation.
Shipping of selected F1 seeds to Heartland Plant Innovation for DH production
Test and evaluation of selected DH lines for FHB resistance
Seed increases for plot testing for yield evaluations. In parallel we also started to optimize the DH protocol at the UMD.

What were the significant results?

Tested DH provided enhanced level of FHB resistance
Effectively reducing the generation time of cultivar development pipeline
We tested a highly selected set of 98 DH lines from previous DH cycles. These lines showed high yield and significant improvement on FHB resistance.

List key outcomes or other achievements.

Two high-yielding FHB tolerant DH lines are advanced for large scale seed production for their release and licensing process. A set of 42 selected DH headrows are advanced to statewide test for their critical evaluations.

Two high-yielding DH cultivars (MDW447-4 and MDW1-98) lines are being licensed to private seed companies.

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

Three PhD students, one MS student, one postdoc, and three undergraduate students were trained under this project. All the trainees worked with the PI to conduct the nursery and collect and analyze data. These students also participated in conferences and commodity board meetings with their work.

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

The PI presented the results in oral presentations and disseminated updates on FHB in wheat through emails. PI presented the research updates in the FHB forum as well as online project update meetings. The graduate students in the team presented the results as posters and handouts to the stakeholders in commodity board meetings. The results were published as wheat trial Factsheets and was disseminated via emails and the UMD extension system to the broader grower community.

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

We are increasing the seeds of 4 high-yielding DH lines with excellent FHB resistance in our MD germplasm and these lines will move forward for multilocation testing and advancement decisions. Update on these as well as new DHs will be presented.

Project 3: Developing FHB Resistant Soft Red Wheat Cultivars for Maryland

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

1) Breeding soft red winter wheat adapted to the Mid-Atlantic environment with resistance to scab and to increase the adaptation of FHB resistant lines by the wheat growers in Mid-Atlantic region.
2) Map and integrate new sources for FHB resistance in breeding germplasm and to enhance the collaborations with regional breeders.
3) Evaluation of advanced MD lines in Uniform Scab nurseries, Maryland State Test and Uniform Regional Nurseries and release of improved MD cultivars.

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

We made 300 hundred new crosses and ensured at least one prominent FHB resistant QTL was included in all the parental lines in the three-way crosses. New crosses were made to incorporate additional sources of FHB resistance in our breeding germplasm. Germplasm Jamestown, Bess/Truman, Neuse, and lines with major Fhb1, 2DL, 5AS have shown high resistance and increased grain yields. MAS, DH, and GS will be used to identify and incorporate resistant germplasm combining FHB with high yield, and resistance other biotic stresses. The resulting F1s from various FHB resistance sources will pyramid scab resistance in 3-way crosses. These crosses will include FHB-R parent, a rust-R parent, and a high–yielding adapted parent (powdery mildew-resistant) which will be used as female in the last cross. To accelerate the development of scab resistant germplasm, we have recently established the speed breeding protocol in our greenhouse that allowed us to advance 4 generations per year. Following this, marker-assisted selection was used in selected crosses to increase the number of scab resistant progenies by enriching breeding populations with markers. We used three independent approaches to combine FHB resistance with high yielding background to develop SRWW cultivars with improved FHB resistance: 1) Speed Breeding; 2) Double Haploids; 3) Genomic Selection. This year, a set of 50 advanced lines coming out of advanced yield and DH trials were tested in the statewide test along with highly competitive public and commercial cultivars.

What were the major activities?

Making FHB resistant germplasm using two-way and three-way crosses
Top 25 crosses were used for the speed breeding pipeline (100 lines per population)
Another top 12 crosses were used for DH generation (40 per population)
The rest of the germplasm was used for GS and marker assisted breeding pipeline.

What were the significant results?

Enhanced FHB resistance in the MD germplasm. In our advanced lines that we tested under 2023-2024 trial cycle about 75% of our germplasm contained Fhb1, about 65 percent of lines contained a combination of at least two Fhb resistance genes including Fhb1. Our nine MD lines (with excellent FHB resistance) were in the top 25 entries compared against several public and commercial wheat cultivars. Three of our lines ranked 1, 4, and 6th ranks.

List key outcomes or other achievements.

Based on three years of yield results and field performance, we are advancing to license two wheat cultivars to two seed companies. These prospective cultivars have excellent FHB resistance. MDW148 is an awnless, high yielding moderately resistant to FHB with two staked FHB R QTL and MDW23-2 is an awned with moderately susceptible to scab and has 6A and 3A QTL.

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

Three PhD students, one MS student, one postdoc, and three undergraduate students were trained under this project. All the trainees worked with the PI to conduct the nursery and collect and analyze data. These students also participated in conferences and commodity board meetings with their work.

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

The PI presented the results in oral presentations and disseminated updates on FHB in wheat and barley through emails. PI presented the research updates in the FHB forum as well as online project update meetings. The graduate students in the team presented the results as posters and handouts to the stakeholders in commodity board meetings. The results were published as wheat trial Factsheets and was disseminated via emails and the UMD extension system to the broader grower community.

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

PI plan to continue employing Speed Breeding to fast pace the integration of FHB resistant QTL and genes and to release FHB tolerant wheat cultivars.

Project 4: Mutant Population in Adapted SRW Wheat to Reduce FHB Susceptibility and DON Content

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

1) Development of an advanced generation mutant platform in adapted high-yielding FHB susceptible soft red winter wheat cultivar "Shirley"

2) Screening of mutant population to identify mutant lines showing moderate to resistant phenotypes and validate the phenotypes

3) Confirm the phenotypic mutants by characterizing them in greenhouse and field tests

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

Fig. 1. A snapshot of the GH showing the evaluation of Jagger mutant population for the FHB resistance. Jagger is highly susceptible so any phenotype showing moderate to resistance phenotype can be related to either disease escape or genetic resistance.

In initial screening we found Jagger mutant population exhibited much clearer phenotypic response than Shirley. So, we further evaluated a Jagger deletion mutant population of 854 individuals. This population has skim sequencing data so deletions can be quickly correlated with loss of a specific genomic region. Earlier we identified 12 Jagger and 8 Shirley mutant lines showing enhanced resistance under initial GH and field evaluations. Further evaluation confirmed 6 true Jagger deletion mutants. Further we screened another set of 625 lines (4 plants each) and it allowed us to identify another 21 mutants. So, this panel has allowed us to identify 27 total mutants from the screening of 854 from Jagger deletion panel. These

mutants are being tested again in replicated sets. Work is in progress in correlating the deletions with an increase in genetic resistance. Samples are already shipped for the DON analysis.

What were the major activities?
Advancing the generation of the mutant populations using single seed descend to avoid loss of undetected mutants due to segregation and mutant dilution.
Field testing of mutant lines in the inoculated misted nursery and tagging the possible mutant plants within the headrows. Harvesting the individual spike showing positive response.

What were the significant results?
We have identified 27 Jagger mutant lines showing enhanced resistance under initial GH and field evaluations. Since all the Jagger deletion lines are genotyped, we may be able to associate a specific genomic region with enhanced resistance.

List key outcomes or other achievements.
27 Jagger mutants with enhanced FHB resistance
6 mutants identified in previous set; we confirmed through a high-confidence rigorous phenotyping. These can be used to identify specific chromosomal regions harboring S genes.

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

Lines	Count
Lines screened	852
Resistant (score 2-4)	26
Moderately resistant (score 5-6)	10

Three PhD students, one MS student, one postdoc, and three undergraduate students were trained under this project. All the trainees worked with the PI to conduct the nursery and collect and analyze data. These students also participated in conferences and commodity board meetings with their work.

4. **How have the results been disseminated to communities of interest?**
The PI presented the results in oral presentations and disseminated updates on FHB in wheat and barley through emails. PI presented the research updates in the FHB forum as well as online project update meetings. The graduate students in the team presented the results as posters and handouts to the stakeholders in commodity board meetings. The results were published as wheat trial Factsheets and was disseminated via emails and the UMD extension system to the broader grower community.

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
To perform RH mapping to identify overlapping chromosomal bins to localize the physical location of FHB susceptibility gene (s).