USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-2-091
USDA-ARS Agreement Title:	Towards Development of Scab Resistant Barley Adapted for the State of South Dakota
Principle Investigator (PI):	Gazala Ameen
Institution:	South Dakota State University
Institution UEI:	DNZNC466DGR7
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$25,393
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Period of Performance:	May 1, 2022 – April 30, 2026
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Screening for Scab Resistance in Barley Lines Adapted for South Dakota	$25,393
	FY22 Total ARS Award Amount	$25,393

I am submitting this report as an: ☒ Annual Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

gazala 07-26-2023

Principal Investigator Signature Date Report Submitted

ǂ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring Wheat Region
SWW – Southern Soft Red Winter Wheat Region

Project 1: Screening for Scab Resistance in Barley Lines Adapted for South Dakota

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

Our goal is to identify the agronomically superior winter and spring barley lines with an improved FHB resistance. To fulfill the aim, scab severity evaluation was conducted at SDSU's Volga mist irrigated, inoculated FHB nurseries on 22 winter barley lines received from the breeding program at SDSU and 29 spring barley lines received from the breeding program at Montana State University, University of Minnesota and North Dakota State University. The winter Barley plots were evaluated for winter hardiness and the trait is being brought in the FHB moderately resistant lines for the state of South Dakota. The winter hardy lines and the 29 spring barley lines at the Volga plot were inoculated with *F. graminearum* using a grain spawn method and also macroconidial suspension spray at 50% anthesis and subjected to mist irrigated to promote the establishment of FHB disease [2]. Four replicates of each line were tested in randomized complete block design in the field plots. We used susceptible checks controls for the experimental evaluations.

Disease evaluation data were collected at 21 and 28 days after inoculation by severity scoring of FHB infection by visual estimation of 10 arbitrary spikes from each plot. The disease severity data was indicated in the percentage of infected kernels in each spike and is analyzed on the R software platform for tests of significance and to quantitatively visualize the disease ratings to establish the breeding value for the FHB resistance in testing progeny. The plots were hand harvested, threshed, and are being prepped for DON testing at the University of Minnesota DON testing Lab.

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

a) What were the major activities?

- A randomized complete block design with four replication was used as the layout. Misting units were present in each block.
- 22 winter barley lines tested for winter hardiness and FHB severity; however, data was obtained from 6 barley lines (Table1).
- 29 spring barley lines from neighboring states' breeding programs were evaluated for FHB disease severity (Table 2).
- A mix of *F. graminearum* isolates collected from SD was used to achieve high virulence, in both corn spawn and spray inoculation. For spray inoculation ~50000 macroconidia spores/ml at 50% anthesis stage were sprayed with a paint sprayer at ~5 cm above the canopy.
- FHB disease was scored at 21- and 28-days post inoculation on ten random heads from each block using 0-100% FHB rating scale for disease severity assessment.
- The entries were harvested individually and FDK were estimated by randomly 500 spikelets of each entry.
- The entries were grounded, and samples were submitted to the DON testing lab at the University of Minnesota DON testing Lab for the mycotoxin estimation.

b) What were the significant results?

- Out of 23 winter barley lines tested in the field, only six lines (Table 1) survived SD winters.
- The FHB severity of the 6 surviving lines was moderately susceptible to susceptible. However, they were not statistically significant from each other.

Table1. Winter barley lines evaluated for FHB severity.

S.No.	Winter Barley Lines	Origin
1	SD Barley	South Dakota State University
2	P-954	Nebraska Agricultural Experiment Station
3	NB 99845	Nebraska Agricultural Experiment Station
4	Weskan	Kansas State University Experiment Station
5	Dundy	Nebraska Agricultural Experiment Station
6	Hitchcock	Nebraska Agricultural Experiment Station

- The 29 spring barley lines had 100% incidence in the inoculated plots.
- Reading for FHB disease severity % was obtained from all 29 spring barley lines.
- We found a significant effect of genotype on the FHB disease severity % (ANOVA, p<.0001, 28 days) in spring barley lines. Tukey's Honest Significant Difference was performed to identify the significance of the difference between pairs of group means.
- Mean Disease severity% for each block was calculated from 21 and 28 days post inoculation severity data (Figure 4) from spring barley to represent a visual assessment.
- At 21 days the majority of lines were showing <40% of mean disease severity.
- Each spring barley sample was processed for FDK and mycotoxin analysis.

Table2. Spring barley lines evaluated for FHB severity.

S.No.	Spring Barley Lines	Origin
1	ND Genesis	North Dakota State University
2	AAC Connect	Agriculture and Agri-Food Canada
3	CDC Fraser	Crop Development Center, U of Saskatchewan, CA
4	AAC Synergy	Agriculture and Agri-Food Canada
5	ABI Cardinal	Anheuser-Busch at Global Barley Research
6	Conlon	North Dakota State University
7	Explorer	North Dakota State University
8	Pinnacle	North Dakota State University
9	Tradition	North Dakota State University
10	2ND32529	North Dakota State University
11	2ND36638	North Dakota State University
12	2ND36642	North Dakota State University
13	MT16M01801	Montana State University
14	MT16M02201	Montana State University
15	MT17M02507	Montana State University
16	MT17M01711	Montana State University
17	MT18M06011	Montana State University
18	Buzz	Montana State University
19	Robust	University of Minnesota
20	S2M184	University of Minnesota
21	S2M189	University of Minnesota
22	S2M190	University of Minnesota
23	S2M191	University of Minnesota
24	S2M192	University of Minnesota
[illegible]	25	S2M193	University of Minne[illegible]
26	S2M194	University of Minnesota
[illegible]ota	27	S2M195	University of Minne[illegible]
28	S2M196	University of Minnesota
[illegible]ota	29	S2M197	University of Minne[illegible]

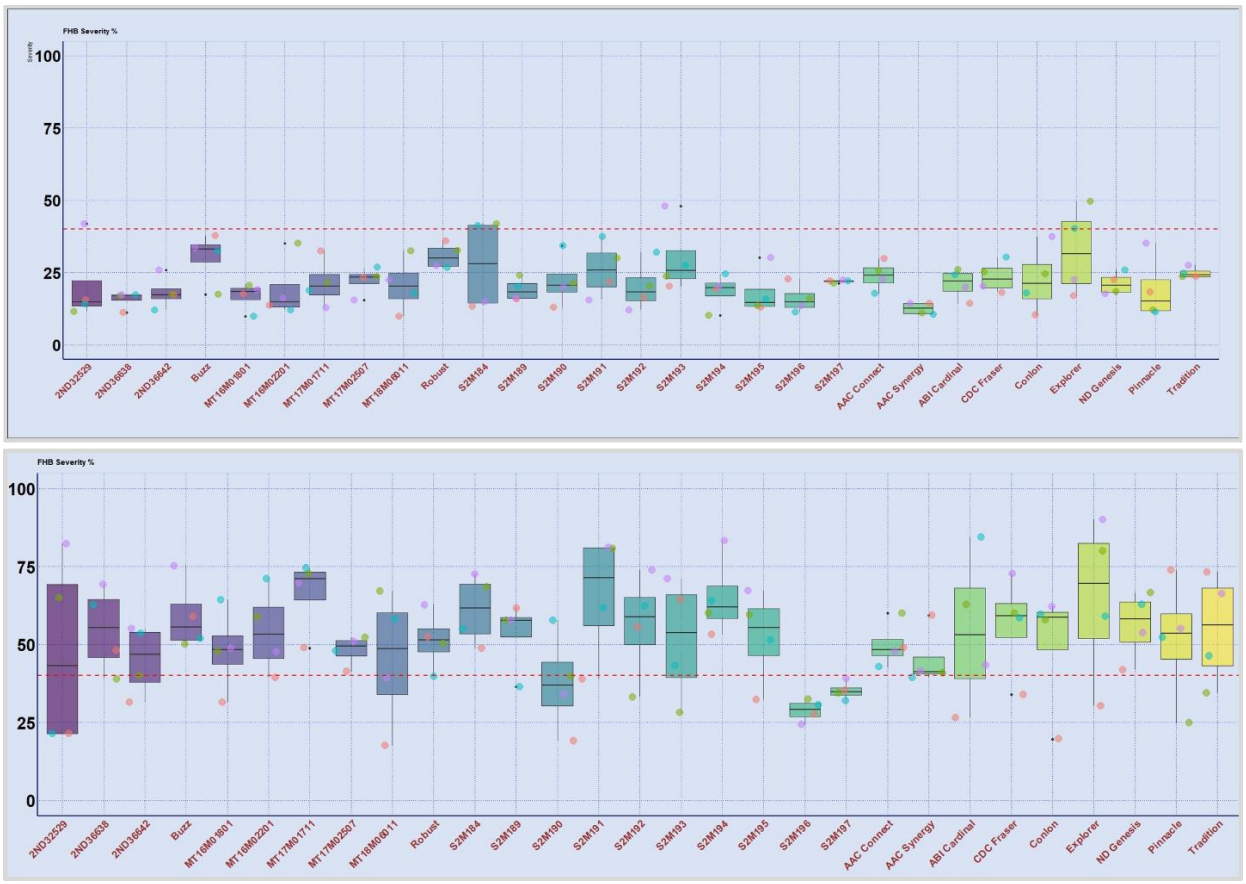

Figure 4. A jitter box plot representing the mean FHB severity % calculated for spring barley lines in four reps (orange, blue, magenta, and olive-green, circles) at 21 days (4a) and 28 days (4b). A horizontal red dotted line denotes the 40% FHB severity. X-axis represents spring barley genotypes and Y-axis represents the mean FHB severity % on 0-100 scale.

- We calculated the correlation coefficients between three variables: head_severity, DON, and NIV.
- The correlation coefficient between head_severity and DON is 0.040, which is a very weak positive correlation.
- The correlation coefficient between head_severity and NIV is 0.044, which is also a very weak positive correlation.
- The correlation coefficients between DON and NIV is 0.047, which is also a very weak positive correlation.
- In other words, there is a very weak positive relationship between all three variables. This means that as one variable increases, the other two variables are likely to increase slightly, but not significantly.
- Mycotoxin (DON and NIV) were detected significantly lower in Conlon (moderately resistant check) (figure 5) and the best-performing lines S2M196 and S2M197 for disease severity did not have significantly lower levels of mycotoxins.

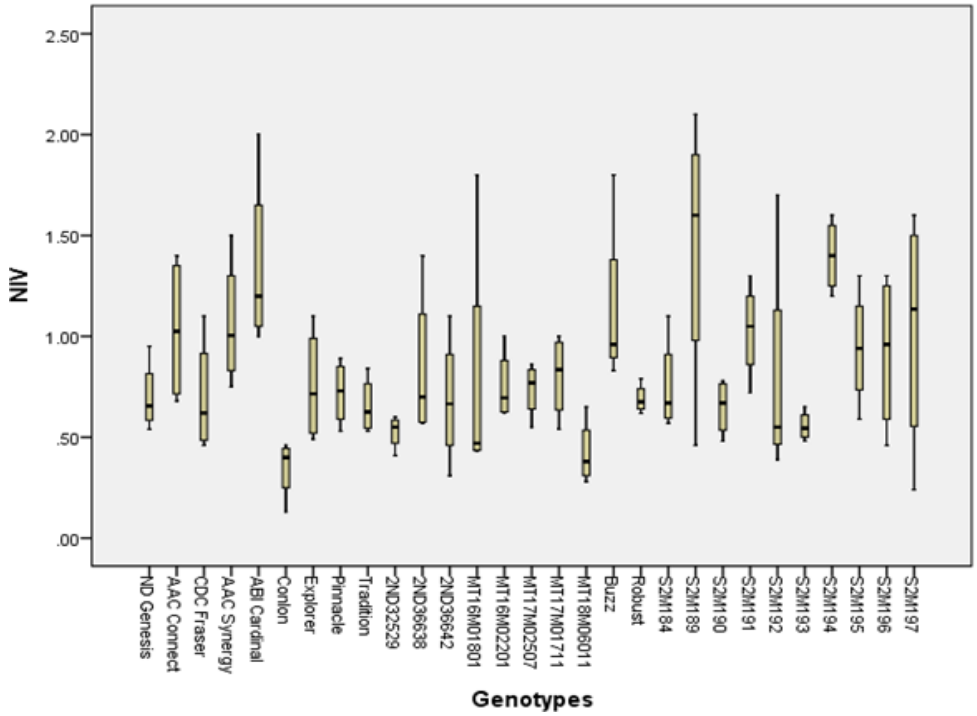

Figure 5a. A jitter box plot representing the mean mycotoxin Niv content in ppm calculated for spring barley lines in four reps at 28 days. X-axis represents spring barley genotypes and Y-axis represents the mean mycotoxin content.

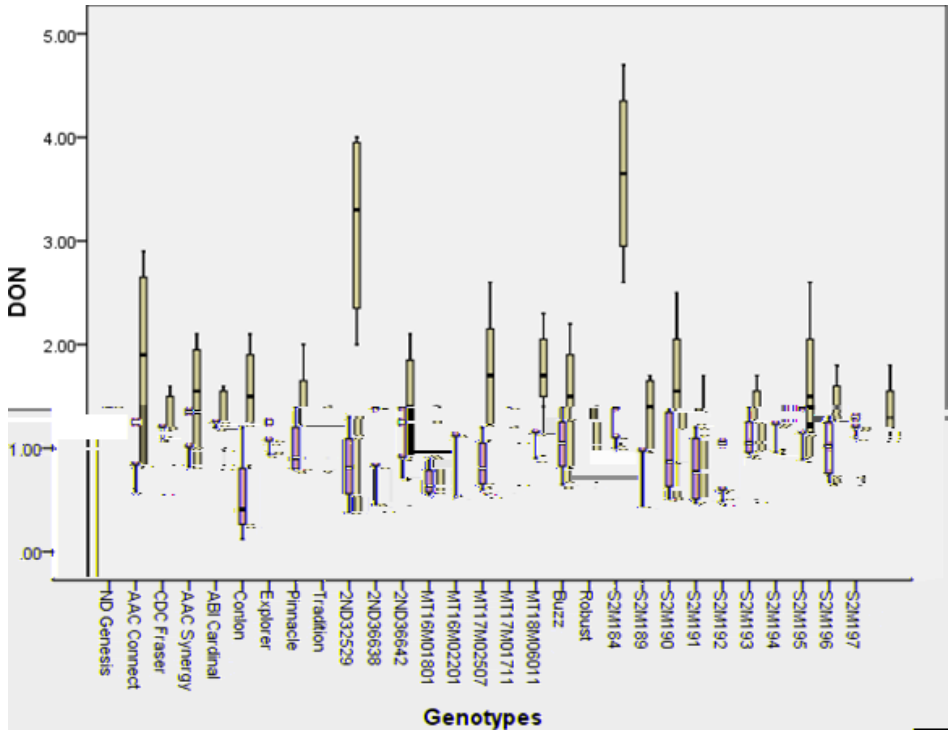

Figure 5b. A jitter box plot representing the mean mycotoxin DON content in ppm calculated for spring barley lines in four reps at 28 days. X-axis represents spring barley genotypes and Y-axis represents the mean mycotoxin DON content.

c) List key outcomes or other achievements.

- With this research funding, we were able to add South Dakota to a list of states conducting research on the FHB-barley pathosystem.
- We were also the first in the state to establish a barley disease nursery to evaluate germplasm performance for FHB.
- In this study, at 28 days S2M196 and S2M197 were found to have <40% mean FHB disease severity % in each block and we observed that the resistance was a decreased colonization of the *Fg* in the kernel as shown in Fig 4b.
- However, we did not detect a strong correlation between the mycotoxin and disease severity in these two lines.
- Overall, we also observed a weak correlation between the mycotoxin accumulated in the spikes at harvest with the disease severity observed.
- We went beyond the objectives and collected *F. graminearum* isolates from across states on many different hosts.
- We used the isolates to study their virulence on different hosts.
- We are uncovering the Fg isolates genome and mycotoxin encoding *Tri* gene cluster phylogeny to understand pathogen diversity and host speciation or cospeciation (**Figure 5)**.

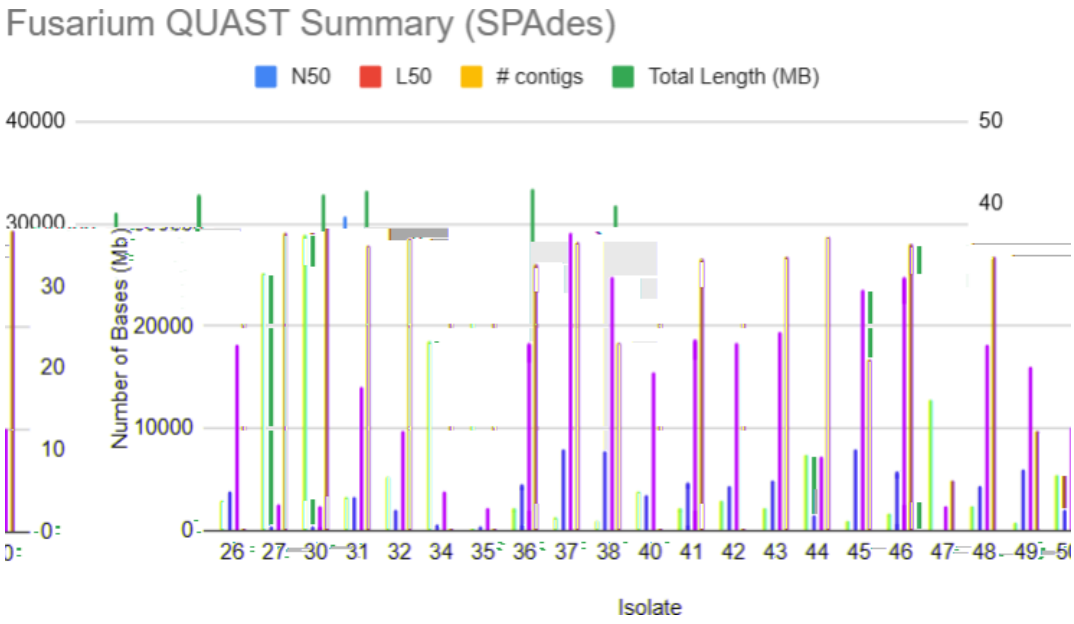

Figure5. Graphical representation of the 21 *Fusarium graminearum* isolates sequenced depicting their N50 (blue), L50 (red), and the number of contigs (yellow) and total length as green of genome assembly.

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

The research project gave opportunity to train three undergraduate students in our program. Two undergraduate student were recruited through the REU and REEU programs hosted at South Dakota State University and were mentored by one graduate student who is partially funded by the USWBSI. The four student have two female and two male students. One male student is an underrepresented minority in STEM.

The MS student (Tasneem Fathima, Plant Science) was trained effectively on barley germplasm collection, planting, nursery setup in the field, Fusarium fungal culturing, spore production, DNA extraction, library preparation for sequencing, spray inoculation, disease estimation, data collection, harvesting, data analysis, poster presentation skills and other related activities. The student also estimated Fusarium damaged kernel estimation, mycotoxin estimation, sequence analysis and Tri5 gene estimation of samples. The student presented the research at a national conference US Wheat Barley Scab Initiative 2022 in December. The graduate student is trained on soft skills and professional development, namely, maintaining lab notebooks, research planning and execution (experimental design, planning, data collection, data management, documentation of methods and results), data analysis, interpretation, and open science practices. She also presented her research at the USWBSI forum as a poster, and oral presentation at the Research Day at SDSU.

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

The USWBSI-funded project was presented by two undergraduate students at the EPSCoR symposium 2022 and 2023, we also disseminated the results at the Ag Horizon growers conference (largest in the state) and to the SD Wheat Commissioners.

The PI was also able to present the research at the 23rd North American Barley Researchers Workshop and 43rd Barley Improvement Conference and the USWBSI National Fusarium Head Blight Forum 2022.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY22 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period May 1, 2022 – April 30, 2023**?**

☒ Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY22 award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

N/A

Books or other non-periodical, one-time publications as a result of FY22 award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

N/A

Other publications, conference papers and presentations as a result of FY22 award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

1. Fathima, T., Sehgal, S.K., Graham, C., Gonzalez-Hernandez, J., Ali, S., Solanki, S., and Ameen, G. 2022. Development of scab resistance in barley lines adapted for the state of South Dakota. 23rd North American Barley Researchers Workshop and 43rd Barley Improvement Conference. UC Davis, CA. September 22-25.
2. Voirin, H., Fathima, T., Kaur, J., Zhou, E., Sehgal, S.K., Graham, C., Gonzalez-Hernandez, J., Ali, S., Ameen, G., and Solanki, S. 2022. Integrative genome analysis of Fusarium graminearum associated with the small grains in South Dakota. SD EPSCoR Undergraduate Research Symposium. Brookings, SD. July 29.
3. Fathima, T., Sehgal, S.K., Graham, C., Gonzalez-Hernandez, J., Ali, S., Solanki, S., and Ameen, G. (2022). Screening for scab resistance in barley lines adapted for the state of South Dakota. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, Florida. Dec 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf
4. Hugo Conde, Tasneem Fathima, Rachel C. Hall, Sahukat Ali, Jose Gonzalez, Gazala Ameen and Shyam Solanki. 2023. Integrative Genome Analysis of *Fusarium graminearum* and Associated Toxin Genes. SD EPSCoR Undergraduate Research Symposium. Brookings, SD. July 29.