USDA-ARS

U.S. Wheat and Barley Scab Initiative FY20 Annual Performance Progress Report

Due date: July 29, 2021

Cover Page

Principle Investigator (PI):	Shaobin Zhong	
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Phone:	701-231-7427	
Fiscal Year:	2020	
USDA-ARS Agreement ID:	59-0206-0-162	
USDA-ARS Agreement Title:	Genetic and Molecular Characterization of New Sources of FHB	
	Resistance in Wheat	
FY20 USDA-ARS Award Amount:	\$ 183,830	
Recipient Organization:	North Dakota State University	
	Office of Grant & Contract Accouting	
	NDSU Dept 3130, PO Box 6050	
	Fargo, ND 58108-0650	
DUNS Number:	80-388-2299	
EIN:	45-6002439	
Recipient Identifying Number or	FAR0032880	
Account Number:		
Project/Grant Reporting Period:	5/15/20 - 5/14/21	
Reporting Period End Date:	5/14/2021	

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
DUR-CP	Identify and Map Novel QTL for FHB Resistance in Durum Wheat	\$ 45,071
DUR-CP	DUR-CP Expand FHB Screening Capacity for Durum Wheat VDHR-SPR Genetic Characterization and Integrated Deployment of FHB Resistance in Spring Wheat	
VDHR-SPR		
VDHR-SPR	Increase Capacity of Two Coordinated FHB Nurseries for Spring Wheat	\$ 33,915
	FY20 Total ARS Award Amount	\$ 183,830

07/29/2021
Date

* MGMT – FHB Management

FST - Food Safety & Toxicology

R- Research

S – Service (DON Testing Labs)

GDER – Gene Discovery & Engineering Resistance

PBG – Pathogen Biology & Genetics

EC-HQ – Executive Committee-Headquarters

BAR-CP – Barley Coordinated Project

DUR-CP - Durum Coordinated Project

HWW-CP – Hard Winter Wheat Coordinated Project

VDHR – Variety Development & Uniform Nurseries – Sub categories are below:

SPR - Spring Wheat Region

NWW – Northern Soft Winter Wheat Region

SWW - Southern Soft Red Winter Wheat Region

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Project 1: Identify and Map Novel QTL for FHB Resistance in Durum Wheat

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

The major goals of this research project are to identify, map, and deploy QTLs for FHB resistance in durum wheat. The specific objectives are:

- 1) Develop a mapping population with recombinant inbred lines (RILs) derived from the cross between Divide and PI 254188;
- 2) Phenotype FHB resistance and morphological traits of the mapping population from the Divide/PI 254188 cross in greenhouse and field;
- 3) Construct a genetic linkage map of the population using SNP markers;
- 4) Identify DNA markers linked to QTL for FHB resistance in the Divide/PI 254188 mapping population;
- 5) Transfer and pyramid FHB resistance QTL into adapted durum wheat cultivars.

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?

We evaluated ~200 RILs (F2:7) derived from the cross between Divide and PI 254188 for FHB resistance in both greenhouse and field experiments. We genotyped the RIL population using the genotyping by sequencing (GBS) approach and identified SNP markers. We constructed a genetic linkage map for the Divide/PI 254188 population with the SNP markers generated from the GBS genotyping. We conducted QTL analysis using the constructed linkage genetic map and the phenotype data from all greenhouse and field experiments. We also raised another RIL population from the cross between Joppa and one FHB resistant RIL derived from the cross between Divide and PI 254188. We backcrossed seven of the highly resistant RILs to Joppa and ND Riveland to improve the quality and yield of the transgression lines.

b) What were the significant results?

The RIL mapping population derived from the Divide/PI 254188 cross segregated for FHB resistance in the greenhouse evaluation; Some RILs had a high level of FHB resistance better than the resistant parent PI 254188 while some other RILs were very susceptible. GBS of the RIL mapping population and the parents generated over 8,000 SNP markers, and after filtration of low-quality ones, 4,192 SNP markers were used to construct a genetic linkage map, which consisted of 16 linkage groups corresponding to 14 chromosomes of the durum wheat genome. The total map size was 2653.47 cM. A major QTL on chromosome 2A and a minor QTL on chromosome 5A were detected

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in the Divide/PI 254188 RIL population. Interestingly, it appeared that Divide contains the 2A QTL contributing FHB resistance while PI 254188 has the 5A QTL conferring the FHB resistance. These QTL will be validated using the RIL population from the cross between Joppa and one FHB resistant RIL derived from the cross between Divide and PI 254188.

c) List key outcomes or other achievements.

- (1) A large number of SNP markers were generated for the RIL population derived from the Divide/PI 254188 cross.
- (2) A genetic linkage map with 4,192 SNP markers have been developed.
- (3) FHB phenotyping data were collected from both greenhouse and field inoculation experiments for multiple years and seasons.
- (4) One major QTL and one minor QTL for FHB resistance were detected in the RIL population based on phenotype data from both greenhouse and field experiments.
- (5) After several cycles of backcrosses with durum wheat cultivars, agronomic traits of the introgression lines with a high level of FHB resistance have been significantly improved. These lines will be used directly in durum wheat breeding programs.
- 3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Yes, the COVID-10 pandemic caused the university to partially close the campus and the research activities had to be reduced to the minimum. The FHB phenotyping in greenhouse was impacted because access to greenhouse is limited and number of people working in the same room is limited to one for keeping social distancing.

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

The project provided trainings to two research associate and two Ph.D. students on FHB phenotyping, QTL mapping and marker development. The PI and the participants of this project have attended three seminars and two conferences.

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

Nothing to report.

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Project 2: Expand FHB Screening Capacity for Durum Wheat

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

The major goal and objective of this research project is to expand the FHB nursery at Fargo location and establish a new FHB nursery at Langdon location to accommodate and screen more advanced breeding lines, mapping populations, and introgression germplasm from durum wheat researchers (Dr. Xiwen Cai, Dr. Steven Xu, Dr. Elias Elias, Dr. Xuehui Li, and Dr. Shaobin Zhong) working in the DUR-CP.

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?

In 2020 summer season, we planted approximately 4,200 hill plots of durum wheat materials in the FHB nursery at the Fargo location for FHB screening. The wheat materials included advanced breeding lines, mapping populations, and introgression germplasm from five Pls (Dr. Elias Elias, Dr. Xiwen Cai, Dr. Steven Xu, Dr. Xuehui Li, and Dr. Shaobin Zhong), who are working in the durum wheat CP. Fusarium-infested corn inoculum was applied in mid-June. We expanded water pipelines and upgraded overhead misting systems for disease development.

We also established a new FHB nursery at Langdon for FHB screening in collaboration with Dr. Venkata Chapara at the NDSU Langdon Research Station, but we didn't plant materials at that location for the 2020 summer season due to the pandemic.

We planted wheat materials at both Fargo and Langdon nurseries in the 2021 summer season.

b) What were the significant results?

Field plots has been increased, water pipelines expanded, and overhead misting systems upgraded at the Fargo location. The disease developed well and data collected from the FHB nursery facilitate QTL mapping, FHB resistance identification and cultivar development. FHB nursery was established at the Langdon location with the same inoculation method and misting system set up and 3000 hill plots were planted in the 2021 summer season.

c) List key outcomes or other achievements.

The FHB nursery at the Fargo location was expanded and facilities were updated, which provided larger capacity for screening of durum wheat materials from PIs involved in the

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USWBSI program. The FHB disease developed well under overhead misting conditions and high-quality FHB data were obtained from the 2020 summer season.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

We established a new FHB nursery at Langdon for FHB screening, but were not be able to plant materials at that location for the 2020 summer season due to traveling restrictions and lack of support personnel during the pandemic.

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

Nothing to report.

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

Nothing to report.

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Project 3: Genetic Characterization and Integrated Deployment of FHB Resistance in Spring Wheat

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

Our overall goal is to clone and characterize a major QTL for FHB resistance in PI 277012 and quickly deploy it along with another major FHB resistance QTL *Fhb1* in the four spring wheat breeding programs of the Spring Wheat Coordinated Project (SPR-CP). The specific objectives of this project are:

- 1) Clone and characterize the major FHB resistance QTL *Qfhb.rwg-5A.2* in the wheat line PI 277012.
- 2) Quickly introgress *Qfhb.rwg-5A.2* and *Fhb1* into elite spring wheat cultivars and breeding lines by backcrossing, marker-assisted selection and speed breeding approach.
- 3) Determine the expression of *Qfhb.rwg-5A.2* and its interaction with *Fhb1* in different genetic backgrounds.
- **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?

- (1) To clone the major QTL on 5AL for FHB resistance in the wheat line PI 277012, we collected 5A chromosomes from both Grandin and PI 277012 by chromosome sorting technology in collaboration with Dr. Jaroslav Dolezel at the Institute of Experimental Botany, Czeck Republic. We extracted DNA samples from the sorted 5A chromosomes from the two wheat genotypes and generated paired-end sequence reads from them by Illumina sequencing plateform. We assembled the sequence reads into a 5A scaffold using the Chinese Spring 5A pseudomolecule as reference. Due to the highly repetitive genome sequence, the quality of assemblies with the short-reads was not good. Therefore, we prepared high molecular wight DNA of PI 277012 and sent it to Mayo Clinic Genome Analysis Core Facility for HiFi whole genome sequencing. Eight SMRT cells were used, HiFi sequence reads with 12x genome coverage were generated, and whole genome assembly was conducted with the HiFi reads. We identified chromosome 5A contigs covering the whole region containing the target FHB resistance QTL and identified genes showing polymorphisms between Grandin and PI 277012. We also screened EMS mutants generated from PI 277012 in the greenhouse.
- (2) To develop user-friendly DNA markers for the novel QTLs for FHB resistance, we identified additional SNP markers for the 5AL QTL region. These SNP markers were converted into PCR-based markers for fine mapping and introgression of the 5AL QTL.

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(3) By backcrossing, marker-assisted selection and speed breeding approach, we have introgressed *Qfhb.rwg-5A.2* and *Fhb1* into 11 elite spring wheat cultivars or breeding lines developed by spring wheat breeders at NDSU, University of Minnesota, SDSU, and WSU, respectively. GP112, a wheat line derived from the cross between PI 277012 and Grandin, was used as the donor of *Qfhb.rwg-5A.2*. Alsen was used as the donor of *Fhb1*.

b) What were the significant results?

- (1) A total of 122.3 and 206.4 million paired-end reads (250 bp and 150 pb) were generated for the Grandin 5A chromosome, which were assembled into an 872.38 Mb scaffold. A total of 313.6 million paired-end reads (150 pb) were generated for the PI 277012 5A chromosome, which were assembled into a 933.66 Mb scaffold.
- (2) Whole genome sequencing of PI 277012 with the PacBio HiFi sequencing technology resulted in a high-quality genome assembly of 5827 contigs with N50=9.11 Mb. Analysis of genome sequences with DNA markers associated with the QTL detected two large contigs covering the target region. Comparative genomic analysis of the target region identified genes with polymorphism between Grandin and PI 277012. These genes will be further characterized for marker development and candidate gene identification for the QTL.
- (3) Screening approximately 400 M2 progenies derived from EMS M1 plants of PI 277012 in greenhouse identified several mutants that were much more susceptible to FHB compared to the parent PI 277012.
- (4) Fifteen additional PCR-based SNP markers were developed in the 5AL QTL region of PI 277012 and have been used in selection of the QTL in the process of introgression of the FHB resistance into adapted wheat cultivars
- (5) By backcrossing, marker-assisted selection and speed breeding approach, we have introgressed *Qfhb.rwg-5A.2* and *Fhb1* into eleven spring wheat cultivars. The backcross generations for each FHB resistance QTL are shown as follows:

Recipient cultivar or line	× GP112	× Alsen
Glenn	F1BC5	F1BC5
Alsen (with Fhb1)	F1BC5	-
ND VitPro	F1BC5	F1BC5
ND828	F1BC5	F1BC5
Linkert	F1BC5	F1BC5
Lang-MN (with Fhb1)	F1BC5	-
MN10201 (with <i>Fhb1</i>)	F1BC5	-
Surpass	F1BC5	F1BC4

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Recipient cultivar or line	× GP112	× Alsen
SD4539	F1BC5	F1BC4
WA8283	F1BC5	F1BC4
Dayn	F1BC5	F1BC4

c) List key outcomes or other achievements.

- 1) 5A chromosomes of both PI 277012 and Grandin were isolated by flow cytometer technology and sequenced with Illumina sequencing technology. High quality whole genome assembly of PI 277012 was also generated with HiFi sequence reads produced by the PacBio II sequencing plateform. These sequence resources will facilitate the isolation of the target gene on this chromosome.
- 2) Several FHB susceptible EMS mutants were identified from PI 277012. These EMS mutants will be useful for validation of the candidate gene(s) to be cloned.
- 3) The two major FHB resistance QTL (*Qfhb.rwg-5A.2* and *Fhb1*) were introduced into various spring wheat cultivars and breeding lines by backcrossing, marker-assisted selection and speed breeding approach, and near-isogenic lines at F1BC5 or F1BC4 generations have been developed.
- 3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Yes, the COVID-10 pandemic caused the university to partially close the campus and the research activities had to be reduced to the minimum. The FHB phenotyping in greenhouse was impacted because access to greenhouse is limited and number of people working in the same room is limited to one for keeping social distancing.

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

The project provided two research associate and three Ph.D. students with training on QTL mapping and marker development as well as map-based gene cloning. The PI and the participants of this project have attended three seminars and two conferences.

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

The FHB resistant wheat lines developed and DNA markers associated with the FHB resistance have been provided to and used by other wheat researchers and breeders.

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Project 4: Increase Capacity of Two Coordinated FHB Nurseries for Spring Wheat

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

The major goal and objective are to expand the FHB nursery at Fargo location and establish a new nursery at Langdon location to accommodate and screen more advanced breeding lines, mapping populations, and introgression germplasm from NDSU spring wheat breeding program (Dr. Andrew Green) and other researchers (Dr. Xiwen Cai, Dr. Steven Xu, and Dr. Shaobin Zhong) who are working in the spring wheat CP.

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?

In 2020 summer season, we planted approximately 5,000 hill plots of spring wheat materials in the FHB nursery at the Fargo location for FHB screening. The wheat materials included advanced breeding lines, mapping populations, and introgression germplasm from four PIs (Dr. Andrew Green, Dr. Xiwen Cai, Dr. Steven Xu, and Dr. Shaobin Zhong), who are working in the spring wheat CP. Fusarium-infested corn inoculum was applied in mid-June. Water pipelines were expanded to cover larger number of plots and overhead misting systems upgraded for better disease development.

We also established a new FHB nursery at Langdon location for FHB screening in collaboration with Dr. Venkata Chapara at the NDSU Langdon Research Station, but we were not able to plant materials at that location for the 2020 summer season due to the pandemic.

We planted wheat materials at both Fargo and Langdon locations in the 2021 summer season.

b) What were the significant results?

High quality disease data were collected from the Fargo nursery in 2020 summer season. These data have been used in FHB resistance identification, QTL mapping, and selection of breeding lines for wheat cultivar development.

c) List key outcomes or other achievements.

The FHB nursery at the Fargo location was expanded and facilities were updated, which provided larger capacity for FHB screening of spring wheat materials from PIs involved in the USWBSI program. The FHB disease developed well under overhead

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misted conditions and high-quality FHB data were obtained from 2020 summer season.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

We established a new FHB nursery at Langdon for FHB screening, but were not be able to plant materials at that location for the 2020 summer season due to traveling restrictions and lack of support personnel during the pandemic.

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

Nothing to report.

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

Nothing to report.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (5/15/20 - 5/14/21). The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1.		udents in your research program supported by funding from your their MS degree during the FY20 award period?
	If yes, how many?	Click to enter number here.
2.		udents in your research program supported by funding from your their Ph.D. degree during the FY20 award period?
	If yes, how many?	Click to enter number here.
3.		who worked for you during the FY20 award period and were ng from your USWBSI grant taken faculty positions with universities?
	If yes, how many?	Click to enter number here.
4.	supported by funding related companies ⊠Yes □ No	who worked for you during the FY20 award period and were ng from your USWBSI grant gone on to take positions with private agor federal agencies?
	If yes, how many?	One

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Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with <u>full or partial</u> support through the USWBSI during the <u>FY20 award period</u> (5/15/20 - 5/14/21). All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	Grain Class	FHB Resistance	FHB Rating (0-9)	Year Released
Nothing to report.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

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Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications <u>published</u> (submitted or accepted) or presentations <u>presented</u> during the **award period** (5/15/20 - 5/14/21) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

<u>NOTE:</u> Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/presentation. See example below for a poster presentation with an abstract:

Winn, Z.J., Acharya, R., Lyerly, J., Brown-Guedira, G., Cowger, C., Griffey, C., Fitzgerald, J., Mason R.E., and Murphy, J.P. (2020, Dec 7-11). Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat (p. 12). In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum*. https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.

<u>Status:</u> Abstract Published and Poster Presented <u>Acknowledgement of Federal Support:</u> YES (Abstract and Poster)

Journal publications.

Shrestha, S., Poudel, R. S., Zhong, S. 2021. Identification of fungal species associated with crown and root rots of wheat and evaluation of plant reactions to the pathogens in North Dakota. *Plant Dis.* (Accepted; first look)

Status: Published

Acknowledgement of Federal Support: YES

Wit, M., Leng, Y., Du, Y., Cegiełko, M., Jabłońska, E., Wakuliński, W., **Zhong, S.** 2021. Genome sequence resources for the maize pathogen *Fusarium temperatum* isolated in Poland. Mol. *Plant-Microbe Interact.* 34:214-217.

Status: Published

Acknowledgement of Federal Support: No

Wan, J., Jin, Z., **Zhong, S.,** Schwarz, P., Chen, B., and Rao, J. 2020. Clove oil-in-water nanoemulsion mitigates growth of *Fusarium graminearum* and trichothecene mycotoxin production during the malting of *Fusarium* infected barley. *Food Chemistry 312*:312120.

Status: Published

Acknowledgement of Federal Support: YES

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Books or other non-periodical, one-time publications.

None

Other publications, conference papers and presentations.

Leng, Y., Poudel, B., Bernardo, A., Bian, R., Karmacharya, A., Mullins, J., Bai, G., Xu, S.S., & Zhong, S. (2020, Dec. 7-11). Identification and molecular mapping of a major QTL on chromosome 2A conferring resistance to Fusarium head blight in emmer wheat (p. 19, Poster #7). In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum*.

https://scabusa.org/pdfs/NFHBF20 Proceedings.pdf.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)

Ren, S., Zhu, X., Leng, Y., Zhang, W., Talukder, Z., Zhong, S., Fiedler, J., Qi, L., & Cai, X. (2020, Dec. 7-11). Toward a better understanding of the hexaploid wheat-derived Fusarium head blight resistance in durum wheat (p. 20, Poster #8). In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum*. https://scabusa.org/pdfs/NFHBF20 Proceedings.pdf.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)

Wang, R., Hegstad, J., Xu, S., Elias, E., Zhong, S., & Li, X. (2020, Dec. 7-11). Developing durum wheat FHB resistant germplasm using interspecific crosses and phenotypic selection at early generations (p. 22, Poster #9). In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum*. https://scabusa.org/pdfs/NFHBF20 Proceedings.pdf.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)

Jiang, H., Wan, J., Zhong, S., Schwarz, P., Chen, B., & Rao, J. (2020, Dec. 7-11). Clove oil-in-water nanoemulsion mitigates growth of *Fusarium graminearum* and trichothecene mycotoxin production during the malting of *Fusarium* infected barley (p. 57, Poster #25). In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum*. https://scabusa.org/pdfs/NFHBF20 Proceedings.pdf.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)