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

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

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Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-0-136
USDA-ARS Agreement Title:	New Sources of Resistance to FHB and DON in Wheat
FY20 USDA-ARS Award Amount:	$33,763
Recipient Organization:	Kansas State University Department of Plant Pathology 4024 Throckmorton, Manhattan, KS 66506-5502
DUNS Number:	929773554
EIN:	48-0771751
Recipient Identifying Number or Account Number, if any:	AR9754 / GAPP006614
Project/Grant Period:	5/26/21 - 5/25/23
Reporting Period End Date:	5/25/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
HWW-CP	New Sources of Resistance to FHB and DON	$33,763
	FY21 Total ARS Award Amount	$33,763

I am submitting this report as an: ☒ Annual Report ☐ Final 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.

Principal Investigator Signature — Date Report Submitted: 07-25-2022

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: New Sources of Resistance to FHB and DON

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

This project is aimed at developing pre-breeding material resistance to FHB and reduced DON accumulation in wheat. The research activity involves in the identification of novel sources of FHB resistance in wild relatives of wheat and utilize them for developing wheat-alien translocations by directed chromosome engineering. The genomic region conferring FHB resistance will be transferred into adapted winter wheat cultivars. We have previously identified novel sources of FHB resistance derived from *Leymus racemosus, Fhb3, and Elymus tsukushiensis, Fhb6.* In addition, we are continuing the evaluation of wheat-alien introgression lines for the presence of novel sources of FHB resistance.

Objective 1: Transfer of *Fhb6* present in WGRC61 into adapted winter wheat cultivars Everest, Lyman, and Overland, with native FHB resistance and use molecular markers, genomic *in situ* hybridization (GISH) analysis, and field evaluations to recover the recurrent wheat genotype with the *Fhb6* gene.

Objective 2: New sources of FHB resistance are constantly being sought. In cooperation with Dr. Yanming Zhang from the Laboratory of molecular cytogenetics and genetic breeding, Harbin Normal University, China, who was a visiting scholar at the Wheat Genetics Resource Center, we have identified a potential new source of type-2 FHB resistance derived from *Thinopyrum intermedium,* designated as HSD2-32 (TA5117) and we are characterizing this new source of resistance using GISH and molecular markers.

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?

Objective 1: We have transferred *Fhb6* into adapted winter wheat cultivars Lyman and Overland. Homozygous *Fhb6* lines derived from *Fhb6*/Overland and *Fhb6*/Lyman were further analyzed for their FHB incidence and DON accumulation under field condition. The progenies of *Fhb6*/Overland had significantly reduced FHB incidence and DON content whereas, only minor effects were observed in the progenies of *Fhb6*/Lyman. Therefore, the progenies of *Fhb6*/Overland grown in the FHB nursery was harvested and stable homozyogous line for *Fhb6* were further selected for the seed increase. GISH was done to select the homozygous progenies of *Fhb6*/Overland. Twelve homozygous plants were identified and selfed for seeds increase. Around 200 grams of seeds were harvested. Screening of more homozygous *Fhb6*/Overland lines for seed increase are in progress.

Objective 2): We have further characterized the line HSD2-32 using molecular cytogenetic and molecular analyses but failed to detect any alien segment in this line. We also crossed this HSD2-32 with Chinese Spring and Everest wheat cultivar to produce

segregating populations, which can be used to map the FHB resistant loci. We continue screening the WGRC cytogenetic stocks carrying *Th. elongatum* chromosomes using point inoculation to identify the new source of FHB resistance.

b) What were the significant results?

Objective 1: The Overland containing *Fhb6* gene were harvested from scab nursery in Spring 2021 and checked for its homozygosity using genomic *in situ* hybridization. 12 homozygous plants were identified. Selfing was done to increase the seeds. About 200 grams of seed were harvested. We are screening more homozygous plants for seed increase.

Objective 2: Molecular cytogenetic analysis failed to detect any alien chromatin in germplasm HSD2-32, suggesting that the introgression may be either cryptic and smaller than the detectability of GISH (30 Mbp) or that the introgression was not derived from *Th. intermedium* or *Th. elongatum*. Skim-seq analysis was also done to identify putative *Th. intermedium* introgressions however, no *Thinopyrum* segment was detected in HSD2-32. The failure to identify any *Thinopyrum* introgressions in HSD2-32 line suggests that the source of FHB resistance in this line probably was not derived from *Th. intermedium* or *Th. elongatum*. However, skim-seq analysis revealed that HSD2-32 line had sizable regions in chromosome arms 2DL, 5BL, and 6AS (Figure 1), which were not able to be map to the reference genome suggesting that HSD2-32 line may have introgressions from other wheat relatives conferring FHB resistance. Mapping populations to map FHB resistant loci in HSD2-32 line were generated. The F2 population derived from the cross between Chinese Spring wheat and HSD2-32 was phenotyped for FHB resistance by point inoculation. GBS of F2 population is in progress. Phenotyping of the F2 mapping population showed that around 76 % of the plants were resistant having score less than 4. Genotyping is in progress.

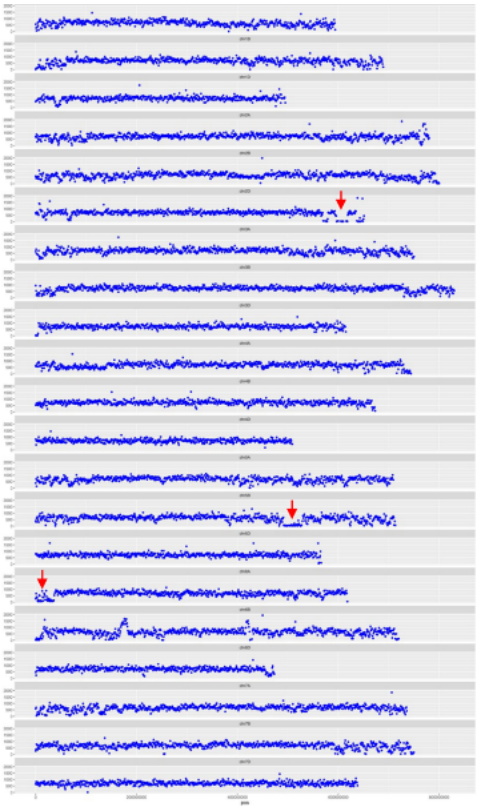

Figure 1. Skimseq-based digital karyotype of HSD2-32. Normalized read counts of a sample generated through skimseq (NEXTERA), where the reads were mapped on wheat reference genome. The reads were normalized to 10 million reads per sample. Some of the wheat genomes lack some segments (arrows) but there were no evidences of presence of *Thinopyrum* segment.

c) List key outcomes or other achievements.

Objective 1: HSD-32 was crossed with popular US wheat variety (Everest). The seeds of F2, F3 and BC1F1 of Everest x HSD2-32 were available. Around 150 F2 seeds of Everest x HSD2-32 were evaluated in Jessica Rupp's FHB nursery located at Rocky Ford, Manhattan, Kansas. It was observed that 70% of the F2 population were resistant for FHB. The detailed genetic analysis of FHB resistance in different wheat backgrounds is under way.
Fhb6/Overland selections with superior FHB resistance and reduced DON accumulation have been distributed to national wheat breeding programs together with molecular marker information to monitor the transfer into regional breeding programs.

Objective 2: The F2 mapping population derived from Chinese Spring and HSD2-32 was phenotyped. The progeny of F3 will also be proceeded with recombinant inbreed line generation using single seed descent method.

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

Dr. Yanming Zhang was visiting the Wheat Genetics Resource Center for one year and received training in state-of-the-art molecular cytogenetic techniques.

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

The results were presented at the 2020 National Fusarium Head Blight Forum and will be published in peer-reviewed international scientific journals. *Fhb6*/Overland and *Fhb6*/Lyman selections with superior FHB resistance and reduced DON accumulation have been distributed to national wheat breeding programs together with molecular marker information to monitor the transfer into regional breeding programs.

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 FY21 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?

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

☒ No, I have nothing to report.

Journal publications as a result of FY21 grant 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.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Books or other non-periodical, one-time publications as a result of FY21 grant 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.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers and presentations as a result of FY21 grant award

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