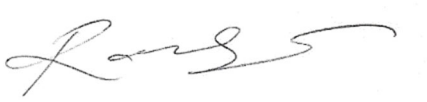
USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: July 29, 2021

Cover Page

Principle Investigator (PI):	Rong Di
Institution:	Rutgers University
E-mail:	rongdi@sebs.rutgers.edu
Phone:	848-932-6350
Fiscal Year:	2020
USDA-ARS Agreement ID:	59-0206-0-170
USDA-ARS Agreement Title:	Genetic Engineering Barley to Improve Fusarium Head Blight Resistance
FY20 USDA-ARS Award Amount:	$ 51,436
Recipient Organization:	Rutgers, The State University of New Jersey Division of Grant and Contract Accounting ASB 111, 3 Rutgers Plaza New Brunswick, NJ 08901·8559
DUNS Number:	00-191-2864
EIN:	22-6001086
Recipient Identifying Number or Account Number:	828846/127451
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
GDER	Genetic Engineering Barley to Improve Fusarium Head Blight Resistance	$ 51,436
	FY20 Total ARS Award Amount	**$ 51,436**



7/29/2021

Principal Investigator 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

Project 1: *Genetic Engineering Barley to Improve Fusarium Head Blight Resistance*

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

The goal of this project is to continue our effort in developing barley genetic engineering platform for the USWBSI barley community to employ transgene approach and the CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9 nuclease) technology to discover genes involved in *Fusarium* head blight (FHB) susceptibility and to engineer FHB resistance in barley.

Our specific objectives for this project are: (1) Production of *HvEIN2-, HvHSK*- and *Hv2OGO*-edited Conlon and ND Genesis plants and evaluation of mutant plants' resistance to FHB, (2) Production of *HvUGT* promoter-edited Morex mutant plants and evaluation of mutant plants' UGT level in relationship to FHB resistance, (3) Production of *HvNud*-edited Conlon and ND Genesis, *HvVrs1*-edited Morex plants and evaluation of the roles of hull and row types in FHB resistance, and (4) Development of barley anther culture for CRISPR-gene editing.

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?

With the previous cycle funding (2018-2020), we have constructed several CRISPR-editing vectors using barley (Hv), rice (Os) and wheat (Ta) U3 or U6 promoter. The gRNA target site is defined by the restriction enzyme immediately upstream of the PAM site for Cas9 nuclease. The monocot (mo) codon-optimized or humanized (h) Cas9 cassette is driven either by the maize ubiquitin/intron promoter (ZmUbi) or the rice ubiquitin promoter (OsUBQ). The transient vector is in the backbone of either pGEM3Zf(+) or pBS vector. The integrating vector is in the backbone of either pCAMBIA1300 (with hygromycin selection for plants) or pCAMBIA2300 (with kanymycin selection for plants). Here are the CRISPR-editing vectors to edit HvHSK, Hv2OGO, HvEIN2 and HvUGT promoter:

pRD330 [PHvU3::HvHSK-SacII::PZmUbi::Cas9-mo in pGEM3Zf(+)]
pRD388 (PHvU3::HvHSK-SacII::PZmUbi::Cas9-mo in pCAMBIA1300)
pRD380 (POsU3::Hv2OGO-NdeI::POsUBQ::Cas9-h in pBS)
pRD383 (POsU3::Hv2OGO-NdeI::POsUBQ::Cas9-h in pCAMBIA1300)
pRD279 [PHvU3::HvEIN2-SphI::PZmUbi::Cas9-mo in pGEM3Zf(+)]
pRD281 (PHvU3::HvEIN2-SphI::PZmUbi::Cas9-mo in pCAMBIA1300)
pRD282 (PHvU3::HvEIN2-SphI::PZmUbi::Cas9-mo in pCAMBIA2300)
pRD395 [PTaU6::HvEIN2-SphI::PZmUbi::Cas9-mo in pGEM3Zf(+)]
pRD403 (PTaU6::HvEIN2-SphI::PZmUbi::Cas9-mo in pCAMBIA1300)

pRD424 (PTaU6:HvUGTP-NcoI::PZmUbi::Cas9-Mo in pCAMBIA1300)
pRD438 (PTaU6:HvUGTP-MfeI::PZmUbi::Cas9-Mo in pCAMBIA1300).
The major activities for our specific objectives #1 to #4 within the reporting period 5/15/20 – 5/14/21 are:

(1) We have used both gene gun and Agrobacterium transformation methods to engineer Conlon, Genesis and Morex barley cultivars with pRD383, pRD388, pRD403, pRD438 and pRD424 targeting Hv2OGO, HvHSK, HvEIN2 and HvUGT promoter, using immature embryos as the explant for tissue culturing. We have produced many transgenic plants. The integration of the transgenes was verified by PCR, cloning and sequencing the PCR products.
(2) By cloning and sequencing the gDNAs spanning gene editing target sites, we have identified the following barley mutants: (a) RD383-C1: The Conlon Hv2OGO gene was mutated at the target site by gene gun, carrying L204Q H205R mutations. (b) RD383-C2: The Conlon Hv2OGO gene was mutated at the target site by gene gun, carrying S201P mutation. (c) RD383-CA2: The Conlon Hv2OGO gene was mutated at the target site by Agrobacterium, carrying S201P mutation. (d) RD388-C4, C5: The Conlon HvHSK gene was mutated off-target by gene gun, carrying E400D mutation. (e) RD388-C6: The Conlon HvHSK gene was mutated off-target by gene gun, carrying E400G mutation.
(3) The results above indicate that we can routinely produce transgenic barley plants with Conlon, Genesis and Morex cultivars.
(4) Our results also demonstrate that our CRISPR-gene editing platform is capable of inducing barley gene mutations.
(5) We are in the process of characterizing the other regenerated barley plants and the T1 generation of RD383-C1, C2, CA2 and RD388-C4, C5, C6 to investigate the heretibility of these mutations.
(6) We have cloned and sequenced the complete *HvNud* gDNA including an intron from ND Conlon and Genesis. An identical *Pvu*II target site has been selected. We are in the process of constructing the CRISPR-editing vector. We will collaborate with Dr. B. Steffenson on this objective.
(7) We have cloned and sequenced the complete *HvVrs1* gene including introns from Conlon, Genesis and Morex. Sequence alignment showed that the *HvVrs1* amino acid sequences are identical for Conlon and Genesis. However, there is a single nucleotide deletion at the 3' end of the Morex *HvVrs1*, leading to dysfunctional *HvVrs1*. It would be interesting to test if CRISPR-editing can be used to restore *HvVrs1* in Morex and change it to two-rowed and evaluate the row type's role in FHB susceptibility. We will collaborate with Dr. B. Steffenson on this objective. This test can pave the way for future gene replacement and gene analysis in barley.

(8) We have not produced barley mutant plants with mutations in the HvUGT promoter yet. But, we are actively transforming Morex calli with both pRD424 and pRD438 by gene gun.
(9) We have started the development of anther and microspore cultures for Conlon, Genesis and Morex. We have been able to induce calli from the anthers, but we have not been able to regenerate plantlets from these calli. Dr. Pat Hayes' group at OSU has been consulted to improve the tissue culturing of barley anthers and microspores.

b) What were the significant results?

(1) Most significantly, during the reporting period of 5/14/20 – 5/15/21, we have been working on improving the regeneration rate of Conlon, Genesis and Morex barley calli generated from immature embryos. Most of the transgenic or gene-edited barley plants reported in publications were from cultivar Golden Promise. We had been able to regenerate barley plantlets from our calli induced from immature embryos, which are the most suitable explant type for wheat, rice and barley. However, we had seen low regeneration rate with 1-2 plantlets regenerated from each half of the immature embryo from Conlon, Genesis and Morex. After revising our barley tissue culture regime, now, we are able to regenerate at least 4-5 plantlets from each half of the immature embryo from all three cultivars. This will greatly enhance the production rate of our transgenic barley plants. With the new tissue culture protocol, we have transformed Genesis and Morex with our barley CRISPR-gene editing vectors. We are in the process of regenerating plantlets from the transformed tissues.
(2) We have published our paper on the validation of the Hv2OGO gene involvement in conditioning barley FHB susceptibility in the CRISPR-edited At2OGO-knock out Arabidopsis plants, providing the molecular basis for targeting Hv2OGO to improve barley FHB resistance.
(3) We are in the process of submitting our paper on the validation of the HvEIN2 gene involvement in conditioning barley FHB susceptibility in the CRISPR-edited AtEIN2-knock out Arabidopsis plants. This work has also provided evidence that the HvEIN2 gene can be targeted to improve barley FHB resistance.

c) List key outcomes or other achievements.

(1) We have produced many transgenic barley plants in Conlon, Genesis and Morex cultivars with our integrating CRISPR-editing vectors by both gene gun and Agrobacterium.
(2) We have identified a few edited Conlon mutants with point mutations in Hv2OGOand HvHSK genes.
(3) We have greatly improved our barley tissue culture protocol using immature embryos as explants with enhanced callus regenerability.

(4) We have not been able to regenerate barley plantlets from anther and microspore cultures of Conlon, Genesis and Morex. Microspores are ideal explant materials for CRISPR-gene editing due to their sheer high number and amenability for gene gun transformation. Especially the high auto-doubling feature of the haploid microspores will greatly facilitate CRISPR-gene editing, allowing the production of homozygous mutant plants in one generation. We are currently in the process of modifying our tissue culture protocols for barley anther and microspore transformation and regeneration.

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.

Before the commencement of this project in May 2020, Rutgers, The State University of New Jersey ("Rutgers University") was mandated to shut down all research by the Gubernatorial "Shelter in Place" Executive Order on March 21, 2020, to slow the spread of COVID-19. My research programs, including my USWBSI project USDA-ARS-58-2050-8-012-824460 (FY2018-2020), was approved to continue during this difficult time but with a limited capacity and the presence of much reduced lab personnel. As a result, I requested the NCE for USDA-ARS-58-2050-8-012-824460 (FY2018-2020) to Jan. 31, 2021. With the approval of this NCE, I was able to support my former Ph.D student-turned-postdoc YCL to work on this project through the difficulties of the pandemic. We were able to publish one paper and are currently in the process of submitting the second one. With the departure of this postdoc early this year and the continual restriction of COVID-19 related regulations, it remains difficult to locate a capable researcher to participate in this project. Until only recently, I was able to hire a talented and hardworking lab technician AD to join this project. Since the addition of the new technician, we have been able to greatly improve our barley regeneration efficiency. Hopefully, the potential spread of the Delta variant will not force the university and the NJ government to impose new restrictions again.

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

This project has provided training for a postdoc YCL who was a Ph. D student of mine that worked on gene editing in *Arabidopsis* and the complementation assay with barley orthologous genes. This project has also provided training in molecular biology, CRISPR-gene editing and plant tissue culture for two other graduate students (DCS and JP), two undergraduate students (BF and JC) and an hourly laboratory technician (YC). This project now provides the majority of the funding for the employment and training of a full-time technicial AD, who is talented and hardworking with the background in Plant Science and Biochemistry.

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

Studies and results on the involvement of barley *Hv2OGO* and *HvEIN2* in FHB susceptibility and the feasibility of CRISPR-editing to improve FHB resistance have been presented in the form of YCL's Ph. D thesis defense seminar and as part of the PI's lectures to the students majored in Biotechnology and Plant Biology at Rutgers University. Our findings have also been presented to the participants at the annual meetings of our Multistate Project "NC1183: Mycotoxins: Biosecurity, Food Safety and Biofuels Byproducts" on May 17, 2021.

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. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☒Yes ☐No
   If yes, how many? 1

4. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY20 award period (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
N/A	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
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
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

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

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 published (submitted or accepted) or presentations presented 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.

NOTE: 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:

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

Journal publications.

Low, Y., M. A. Lawton and R. Di. 2020. Validation of barley *2OGO* gene as a functional orthologue of Arabidopsis *DMR6* gene in *Fusarium* head blight susceptibility. Sci. Reports. 10:9935. DOI:10.1038/s41598-020-67006-5.
Status: Published
Acknowledgement of Federal Support: YES

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

Nothing to report

Other publications, conference papers and presentations.

Di, R. and M. A. Lawton. 2021. CRISPR-editing barley to improve FHB resistance. NC1183 Multistate Project, Mycotoxins: Biosecurity, Food Safety and Biofuels Byproducts, May 17, 2021, Lincoln, NB (virtual).
Status: Published in the Multistate Project annual report
Acknowledgement of Federal Support: YES