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

FY21 FINAL Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-0-170
USDA-ARS Agreement Title:	Genetic Engineering Barley to Improve Fusarium Head Blight Resistance
Principle Investigator (PI):	Rong Di
Institution:	Rutgers University
Institution UEI:	M1LVPE5GLSD9
Fiscal Year:	2021
FY21 USDA-ARS Award Amount:	$51,436
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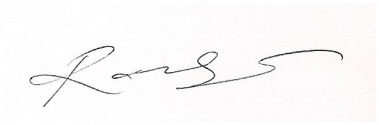
Period of Performance:	5/15/21 - 5/14/23
Reporting Period End Date:	5/14/2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Genetic Engineering Barley to Improve Fusarium Head Blight Resistance	$51,436
	FY21 Total ARS Award Amount	$51,436

I am submitting this report as a: ☒ 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.



7/21/2023

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: Genetic Engineering Barley to Improve Fusarium Head Blight Resistance

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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?

We have constructed several CRISPR-editing vectors using barley (Hv), rice (Os) and wheat (Ta) U3 or U6 promoter to drive the single guide RNA (sgRNA) expression and the maize ubiquitin/intro promore (ZmUbi) or the rice ubiquitin promoter (OsUBQ) to drive the expression of the monocot codon-optimized Cas9. Vector pRD383 targets *Hv2OGO*, pRD388 targets *HvHSK*, and pRD403 targets *HvEIN2*. Knocking out these genes would improve barley FHB resistance. Vectors pRD438 and pRD424 target two different sites of the *HvUGT* promoter. The disruption of *HvUGT* promoter would allow us to study the role that *HvUGT* plays in barley FHB resistance. We have used gene gun and *Agrobacterium*-mediated transformation methods to deliver the CRISPR-gene editing vectors into embryogenic calli from cultivars of Conlon, Genesis and Morex and produced several lines of transgenic barley plants.

We conducted the following major activities in FY22-23. We characterized the inheritance of *Hv2OGO* mutations in RD383-Conlon T_1 and *HvHSK* mutations in RD388-Conlon T_1 plants. We learned that the tRNA-based CRISPR vectors have several advantages over the conventional single-gRNA (sgRNA) vectors in that the gRNA can be multiplexed, the gRNAs can be expressed at higher level, and gene editing can be achieved at higher efficiency. Therefore, we constructed the dual tRNA-based CRISPR vectors to target two different sites of the Genesis *HvEIN2* gene and the Morex *HvUGT* promoter: the transient pRD543 and integrating pRD549 contain the dual barley glycine tRNA::*HvUGT* (Morex) promoter *Mfe*I and *Nco*I gRNAs under wheat TaU6 promoter and the Cas9 cassette driven by ZmUbi promoter. The transient pRD550 and integrating pRD554 contain the dual rice glycine tRNA::*HvEIN2* (Genesis) 5' *Sph*I and 3' *Sph*I gRNAs under rice OsU3 promoter and the Cas9 cassette driven by ZmUbi promoter.

We developed the protoplast and callus transient systems to test the gene editing efficiency of these tRNA-based CRISPR vectors. We optimized the transformation and regeneration protocol for Genesis and Morex barley. We worked on the development of anther and microspore transformation and regeneration system for Genesis and Morex.

We also improved the barley transformation and regeneration protocol for Genesis and Morex, with multiple shoots formed from a single immature scutellum versus single shoots formed as previously.

b) What were the significant results?

In analyzing the inheritance of *Hv2OGO* mutations in RD383-Conlon T_1 and *HvHSK* mutations in RD388-Conlon T_1 plants, we found that indeed the mutations induced in the T_0 plants were inherited into the T_1 generation. These results demonstrate that our sgRNA-based CRISPR vectors can lead to mutations in Conlon barley plants. However, we found that the gene editing efficiency by these sgRNA vectors is low, and that most of the mutations induced were single nucleotide changes and single amino acid changes. Therefore, it is not effective in creating Indels with insertions and deletions of nucleotides to result in gene disruptions. Hence, the dual tRNA-based vectors were constructed (see above).

We developed a protocol to isolate protoplasts from young Genesis and Morex barley seedlings and transformed protoplasts with the tRNA-CRISPR vectors by polyethylene glycol (PEG) to transiently assess the gene editing efficiency of these vectors. We then isolated the genomic DNA (gDNA) from the transformed protoplasts, PCR-amplified the gDNA regions spanning the target sites, and sequenced the PCR products or the clones of the PCR products. Similarly, we transformed calli by gene gun with our constructs and evaluated the gene-editing efficiency of our vectors. Our results showed that we were able to induce large deletions in both *HvEIN2* gene and *HvUGT* promoter. We then transformed the embryogenic calli of Genesis and Morex induced from immature scutella with the tRNA-CRISPR vectors by gene gun or Agrobacterium. Transformed calli are being selected with hygromycin and plants are being regenerated.

We are currently constructing the tRNA-based CRISPR vectors to target the *HvNud* and *HvVrs1* genes.

We worked on improving the barley transformation and regeneration protocol. With the explant of scutellum and modified media, we are now able to induce embryogenic calli from Genesis and Morex immature barley seeds and regenerate multiple shoots from calli induced from a single immature seed.

We tried various protocols for anther and microspore cultures of Genesis and Morex barley, however, we have not been able to regenerate any plant. The anther and microspore tissue culturing is cultivar dependent. Since the haploid microspore transformation will greatly facilitate the generation of gene-edited barley plants in one generation, we continue to explore the feasibility of using these materials for barley transformation.

c) List key outcomes or other achievements.

In 2022, we published our paper: Low, Y. C., M. A. Lawton and R. Di. 2022. *Ethylene insensitive 2* (*EIN2*) as a potential target gene to enhance *Fusarium* head blight disease resistance. Plant Sci. 322:111361. DOI: 10.1016/j.plantsci.2022.111361. This work is significant in that we proved *Fusarium* spp. exploits the ethylene signaling pathway to gain entry into plants which was demonstrated previously in Arabidopsis and wheat by knocking down the *EIN2* gene using the RNAi-mediated mechanism. We used CRISPR to specifically knock out the *AtEIN2* gene and showed that the FHB resistance was greatly enhanced in the mutant plants. We also showed the complemented *AtEIN2*-KO mutant Arabidopsis plants

with the barley *HvEIN2* cDNA regained their susceptibility to FHB, implying that *HvEIN2* is involved in *Fusarium* infection in barley.

Our other achievements include: the development of the more efficient gene editing CRISPR vectors utilizing the tRNA splicing mechanism; the protoplast and callus transient systems to evaluate the gene-editing efficiency of our CRISPR vectors; the robust transformation and regeneration protocol using the immature scutellum explant for both cv. Genesis and Morex.

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

This project provided the training and the fund for hiring of a talented technician, AD, who graduated from Rutgers in 2021 with a B.S. degree in Plant Science and who is passionate about plant biotechnology, goal-oriented and hardworking. AD joined the Plant Biology Graduate Program in the Fall of 2022 to study for an M.S. degree, while being a full-time technician supported by this project. This project has also provided the training for several undergraduate, graduate students and a technician in plant transformation and regeneration.

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

We have published our paper on *EIN2* in the journal of Plant Science in 2022. We have presented our progress in the National Fusarium Forum in December 2022 and the USDA Multistate NC1183 project annual meeting in May 2023. These are listed below.

We have also presented our findings in other meetings and the courses that Dr. Di teaches at Rutgers in the undergraduate and graduate programs in Biotechnology and Plant Science.

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 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).

Low, Y. C., M. A. Lawton and R. Di. 2022. *Ethylene insensitive 2* (*EIN2*) as a potential target gene to enhance *Fusarium* head blight disease resistance. Plant Sci. 322:111361. DOI: 10.1016/j.plantsci.2022.111361. (Yes, acknowledged federal support.)

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

None

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

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

1. Oral presentations:

Di, R. Aug. 16, 2022. "Genetic engineering of barley to improve *Fusarium* head blight resistance", Brewing Summit, Aug. 14-16, Providence, Rhode Island.

Di, R. 10/13/2022. "CRISPR-gene editing to improve plant disease resistance and stress tolerance". University of Massachusetts-Amherst.

Di, R. 5/16/2023. "CRISPR-gene editing for plant health and using *C. elegans* to study human health promoting compounds from plants". The International Conference and Workshop in conjunction with the 8th Indonesia Biotechnology Conference, Bali, Indonesia.

2. Poster presentation:

Dineen, A., M. Lawton and R. Di. 2022. Genetic engineering of barley to improve *Fusarium* head blight resistance. In: *Proceedings of the 2022 National Fusarium Head Blight Forum* (pp.13). Tampa, FL. U.S. Wheat & Barley Scab Initiative.

(Yes, acknowledged federal support.)