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:	59-0206-0-170
USDA-ARS Agreement Title:	Genetic Engineering Barley to Improve Fusarium Head Blight Resistance
FY20 USDA-ARS Award Amount:	$51,436
Recipient Organization:	Rutgers University Department of Pant Biology 59 Dudley Rd., New Brunswick, NJ 8901
DUNS Number:	00-191-2864
EIN:	22-6001086
Recipient Identifying Number or Account Number, if any:	828846/127451
Project/Grant Period:	5/15/21 - 5/14/23
Reporting Period End Date:	5/14/2022

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 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

7/25/2022
Date Report Submitted

[*] 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 (gRNA) 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 may improve barley FHB resistance. Vectors pRD438 and pRD424 target two different sites of the *HvUGT* promoter. The disruption of *HvUGT* promoter may 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 FY21-22. 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 tRNA-based CRISPR vectors to target barley *HvEIN2* and the *HvUGT* promoter. We developed a protoplast system to test the gene editing efficiency of these tRNA-based CRISPR vectors. We also improved the barley transformation and regeneration protocol. We worked on the development of anther and microspore transformation and regeneration system for Genesis and Morex.

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, the gene editing efficiency is low; we have not recovered any Indel (insertion and deletion) mutant in

pRD383- and pRD388-transgenic Conlon plants. Even though pRD438 and pRD424 target two different sites of the *HvUGT* promoter, we have not recovered any transgenic Morex plant that carry the deletion intended to totally disrupt the *HvUGT* promoter.

Consequently, we constructed the tRNA-based CRISPR-editing vectors to target two different sites of the *HvEIN2* gene and the *HvUGT* promoter, anticipating a higher frequency of Indels and bigger deletions than the sgRNA-CRISPR vectors. The transient pRD543 and integrating pRD549 contain the dual barley glycine tRNA::*HvUGT* 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* 5' *Sph*I and 3' *Sph*I gRNAs under rice OsU3 promoter and the Cas9 cassette driven by ZmUbi promoter.

We developed a protocol to isolate protoplasts from young Genesis and Morex barley seedlings, and transform 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. Our results have shown that we are able to induce large deletions in both *HvEIN2* gene and *HvUGT* promoter. We are actively transforming the embryogenic calli of Genesis and Morex with the tRNA-CRISPR vectors in order to produce barley mutants at higher efficiency compared to our previous sgRNA-CRISPR platform.

We are 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 able to induce embryogenic calli from Genesis and Morex immature barley seeds and regenerate multiple shoots from calli induced from a single immature seed. This efficient regeneration system will aid the production of transgenic barley plants.

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 will continue to explore the feasibility of using these materials for barley transformation.

c) List key outcomes or other achievements.

During the course of FY21-22, 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 robust transformation and regeneration protocol using the immature scutellum explant and the protoplast system to evaluate the efficiency of gene-editing vectors.

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 a year ago with a B.S. degree in Plant Science and who is passionate about plant biotechnology, goal-oriented and hardworking. This project has also provided the training for several undergraduate (SG, DS), graduate students (LB, KA) and a part time technician (YC) in plant transformation and regeneration.

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

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

We have also presented our findings in 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 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).

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.

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).

None

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.

1. Oral presentation:

Dineen, A., **R. Di** and M. A. Lawton. 2022. CRISPR-gene editing to improve barley FHB resistance. NC1183 Multistate Project, Mycotoxins: Biosecurity, Food Safety and Biofuels Byproducts, May 17, 2022, Manhattan, KS.

2. Poster presentation:

Dineen, A., M. A. Lawton and **R. Di.** 2021. Genetic engineering of barley to improve *Fusarium* head blight resistance. In: *Proceedings of the 2021 National Fusarium Head Blight Forum* (pp.6). Virtual. U.S. Wheat & Barley Scab Initiative.