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

# FY22 Performance Progress Report

**Due date:** July 26, 2023

## Cover Page

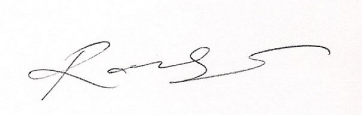
| | |
|---|---|
| **USDA-ARS Agreement ID:** | 59-0206-2-106 |
| **USDA-ARS Agreement Title:** | Fusarium Head Blight (FHB) Research Community Barley Genetic Engineering Facility |
| **Principle Investigator (PI):** | Rong Di |
| **Institution:** | Rutgers, The State University of New Jersey |
| **Institution UEI:** | M1LVPE5GLSD9 |
| **Fiscal Year:** | 2022 |
| **FY22 USDA-ARS Award Amount:** | $53,295 |
| **PI Mailing Address:** | Rutgers University, Department of Pant Biology<br>59 Dudley Rd.,<br>New Brunswick, NJ 8901 |
| **PI E-mail:** | rongdi@sebs.rutgers.edu |
| **PI Phone:** | 848-932-6350 |
| **Period of Performance:** | May 1, 2022 – April 30, 2026 |
| **Reporting Period End Date:** | April 30, 2023 |

## USWBSI Individual Project(s)

| USWBSI Research Category* | Project Title | ARS Award Amount |
|---|---|---|
| GDER | A Barley Genetic Engineering Facility for FHB Research Community | $53,295 |
| | **FY22 Total ARS Award Amount** | $53,295 |

I am submitting this report as an: ☒ Annual 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 _____ 7/24/2023 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:** A Barley Genetic Engineering Facility for FHB Research Community

---

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

The overall goals of this project are to: establish a barley genetic engineering facility to provide a no-cost transformation service for the *Fusarium* head blight (FHB) research community; continue to develop and apply CRISPR-gene editing technology to discover genes involved in FHB susceptibility; and engineer FHB resistance in barley cultivars grown in the U.S.

**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) We have worked with the USWBSI coordinators and established the "Barley Genetic Engineering Facility". A barley transformation request form has been uploaded to the USWBSI webpage. The description of the Facility has been published on the USWBSI Newsletter April 2023 Edition.

We have started working with other barley researchers to develop tissue culture protocols for their barley cultivars and to transform Genesis barley with several transgenes.

(2) We have developed the dual tRNA-based, multiplexing CRISPR platform to edit barley genes to improve FHB resistance.

We have also embarked on applying morphogenes and chemicals to enhance the regeneration of different barely tissues.

(3) We have transformed cultivars Genesis and Morex with our CRISPR-gene editing vectors and other transgenes to improve barley FHB resistance.

**b) What were the significant results?**

(1) We have worked with Dr. Patrick Hayes at Oregon State University since 2022, and developed greenhouse protocol to grow his spring barley cultivar Lightning and winter barley cultivar Thunder. We were able to induce embryogenic calli from the immature scutella of both cultivars and regenerate shoots from these calli.

We recently acquired the pUBQ:RUBY construct from Addgene, developed by Dr. Y. Zhao'group that can overexpress the "RUBY" reporter gene cassette that produces all of the enzymes required for betalain biosynthesis, leading to a vivid red color that serves as a marker for transformed cells that can be detected by the naked eye. We have bombarded cultivars Lightning and Thunder calli with pUBQ:RUBY by gene gun. The bombarded tissues are currently being screened on selective medium.

We have worked with Dr. John McLaughlin at Rutgers University since 2023 to produce transgenic barley cv. Genesis with the following overexpression constructs: (i) pB835, the base vector overexpressing GFP, (ii) pATLTP4.4, overexpressing Arabidopsis lipid transfer protein (LTP) (AT5G55450) and GFP fusion protein, (iii) pTaLTP3, overexpressing wheat LTP3 (AY226580) and GFP fusion protein. Dr.

McLaughlin's previous research has shown that LTP can confer Fusarium disease resistance. We have bombarded cultivar Genesis calli with these three constructs and the transformed calli are currently being selected.

(2) We have used our dual tRNA (dtRNA)-based CRISPR vectors, pRD545 ($P_{OsU3}$:OsdtRNA/*HvEIN2* gRNAs//$P_{ZmUbi}$:Cas9-Mo) to transform Genesis targeting two sites on the *HvEIN2* genome, and pRD549 ($P_{TaU6}$:HvdtRNA/*HvUGT P.* gRNAs//$P_{ZmUbi}$:Cas9-Mo) to transform cultivar Morex, targeting two sites on the *HvUGT* promoter. With our protoplast and callus transient assays, we have shown that these two integrating vectors and their equivalent transient vectors could induce "deletion" mutations at the target sites. The Genesis and Morex calli have been transformed with the vectors pRD545 and pRD549, respectively. Several putative transgenic shoots have been produced from the transformed Morex calli. We are in the process of characterizing these plantlets for DNA sequence changes within the target sites.

As we observed in our previous research on barley transformation and regeneration, the efficiency of barley mutation was low when we used our single guide RNA (sgRNA)-based CRISPR-gene editing vectors. We anticipate that the dual tRNA-CRISPR vectors will enhance the gene editing efficiency in barley.

It was recently shown that overespression of the proteinaceous morphogenic regulators BABY BOOM, GRF-GIF (Growth Regulating Factor - GRF-Interacting Factor) and others can greatly improve the regeneration of several important monocots such as maize, wheat and sorghum. We have acquired the construct pGGB from Dr. A. Gallavotti at Rutgers University, that overexpresses *ZmBBM* and *TaGRF4-GIF1*. We are currently evaluating the efficacy of this construct for its enhancement of barley transformation and regeneration.

Additionally, it was demonstrated in 2022 that perturbation of plant glutamate receptors improved regeneration in both monocots and dicots. We are currently evaluating one of such glutamate receptor-like protein inhibitors, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), on barley regeneration. Morex meristematic tissues pretreated with CNQX produced calli that seemed to have a greater incidence of embryogenic-like callus formation, compated to those that were not pretreated. This research is still at its very early stage but appears to be a promising approach, based on results in other systems. If this, and other chemicals, can indeed improve barley regeneration, it would provide a more accessible explant than the currently used immature scutella tissues and shorten the barley transformation and regeneration process. It also has the advantage of being applicable to any cultivar and does not require the expression of any additional transgenes.

(3) Genesis and Morex calli have been transformed with pRD545 and pRD549 respectively. Some putative transgenic shoots have been produced from the transformed Morex calli. We are in the process of characterizing these plantlets. If we obtain *HvEIN2* Genesis mutants, we will test their resistance to FHB using the detached leaf assay in which individual leaves are exposed to either GFB-tagged *F. graminearum* or DON toxin. If we successfully obtain *HvUGT* promoter Morex mutants, we will work with Dr. G. Muehlbauer to evaluate these mutants.

**c) List key outcomes or other achievements.**

(1) The barley genetic engineering facility is up and running. We have started collaboration with Dr. P. Haynes at Oregon State University, Dr. J. McLaughlin at Rutgers University and Dr. G. Muehlbauer at University of Minnesota.

(2) Our dual tRNA-CRISPR gene editing vectors induce deletion mutants in *HvEIN2* and *HvUGT* promoter in the transient assays.

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

This project provided the funding and training for AD, who is a full-time technician working on barley transformation and regeneration. Since Fall 2022, AD was accepted into the Plant Biology Graduate Program for an M.S. degree. AD is goal oriented, hardworking and dedicated to plant biotechnology. This project has also provided training for one undergraduate student DS and two graduate students DCS and DC in plant tissue culture and genetic engineering.

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

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 in the Publications section. 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 FY22 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 May 1, 2022 – April 30, 2023?**

☒ Yes, I've included the citation reference in listing(s) below.
☐ No, I have nothing to report.

## Journal publications as a result of FY22 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).

None

## Books or other non-periodical, one-time publications as a result of FY22 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 FY22 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.

2. Poster presentation:

Dineen, A., M. Lawton and R. Di. (2022). Genetic engineering of barley to improve *Fusarium* head blight resistance. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf
(Yes, acknowledged the USWBSI support.)