Barley Genetic Engineering Facility for FHB Research Community

Rong Di, Jun Qin and Michael Lawton

Department of Plant Biology
Rutgers University, NJ

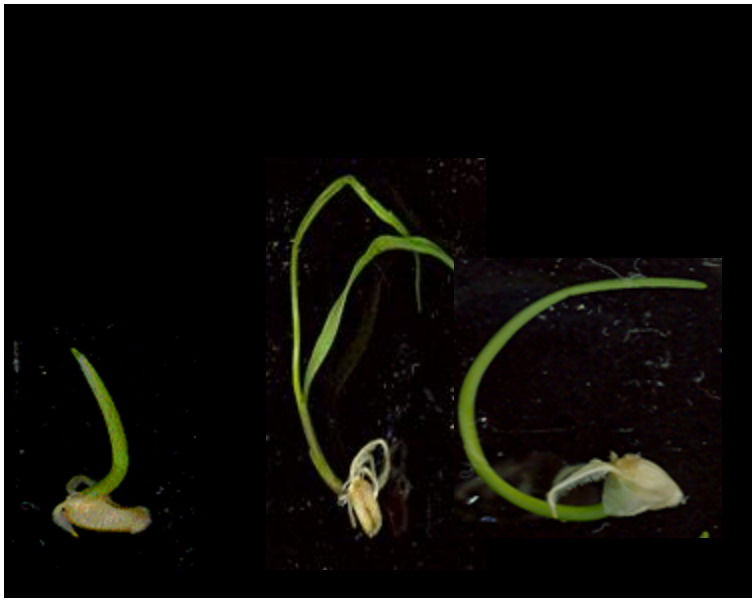
National FHB Forum
Dec. 8-10, 2024

Fusarium Head Blight and Deoxynivalenol (DON)

- ***Fusarium graminearum* (*Fg*) and *Fusarium culmorum* cause *Fusarium* Head Blight (FHB) in wheat and barley.**
- ***Fusarium* spp. produces trichothecene mycotoxin DON which causes severe diarrhea and death in human and livestock.**

Funding from USWBSI: We produced transgenic wheat plants overexpressing yeast RPL3Δ that resisted DON cytotoxicity in germination assay, *Fg* infection in the fields, and accumulated less DON in the grains. >>> GMO

U.S. Wheat & Barley Scab Initiative



wt RUT772 RUT8153

wt RUT772 RUT8153

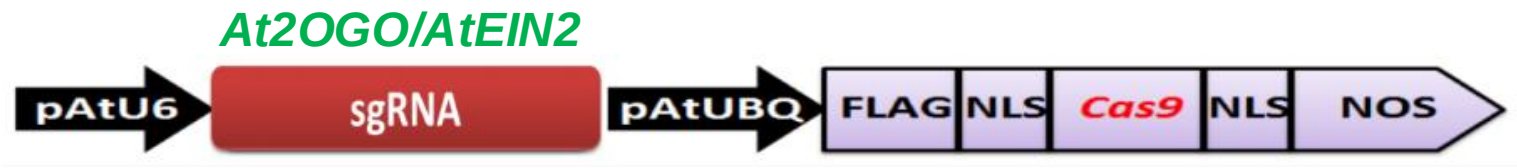
- Di, R., A. Blechl, R. Dill-Macky, A. Tortora, and N. E. Tumer. 2010. Plant Science 178:374-380.
- U.S. Patent #8,026,410 B2. Tumer, N.E. and R. Di. Sept. 27, 2011. Transgenic plants expressing L3 delta proteins are resistant to trichothecene fungal toxins.

Funding from NJAES and USWBSI:

RUTGERS
New Jersey Agricultural Experiment Station

U.S. Wheat & Barley Scab Initiative

- **We developed our own sgRNA-based CRISPR-editing vectors for dicots.**



- ***At2OGO* [*2-oxoglutarate Fe(II)-dependent oxygenase*] and *AtEIN2* (*ethylene insensitive 2*) are FHB susceptibility factors. *At2OGO*-KO and *AtEIN2*-KO Arabidopsis plants are resistant to *F. graminearum.***

- ***At2OGO*-KO and *AtEIN2*-KO Arabidopsis mutant plants over-expressing *Hv2OGO* and *HvEIN2* regained FHB susceptibility, indicating these two genes are true functional homologs and that their KO in barley might similarly improve FHB resistance.**

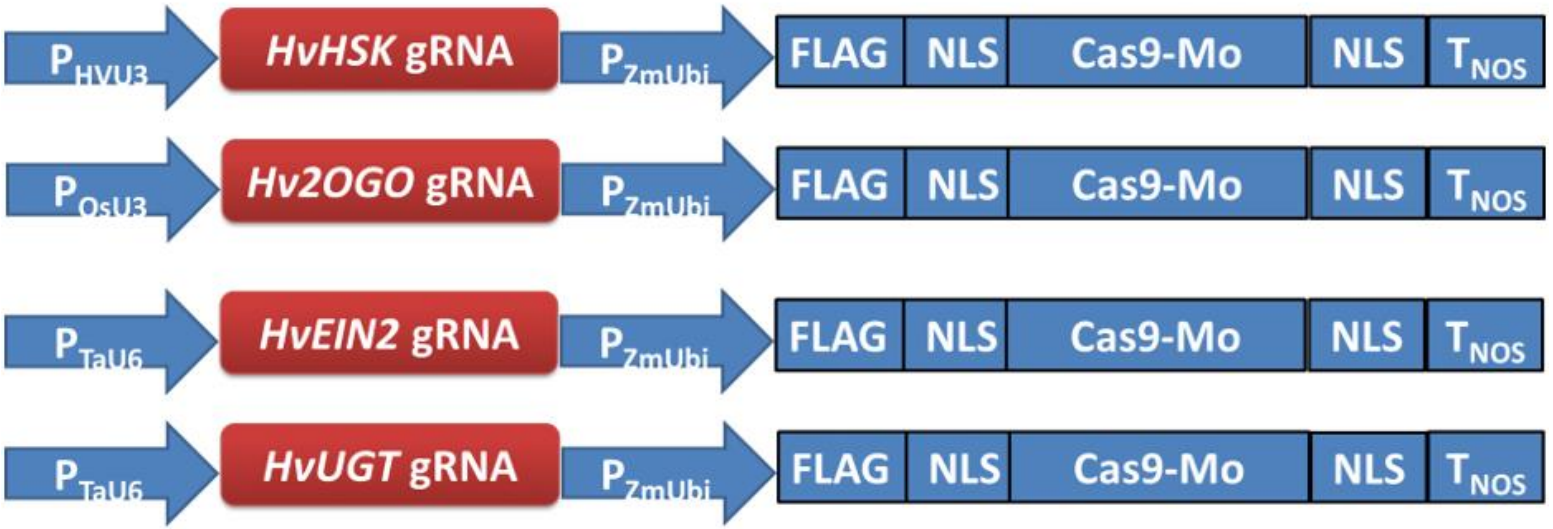
  - **Low, Y., M. A. Lawton and R. Di. 2020. Sci. Reports. 10:9935. DOI:10.1038/s41598-020-67006-5.**
  - **Low, Y. C., M. A. Lawton and R. Di. 2022 Plant Sci. 322:111361. DOI: 10.1016/j.plantsci.2022.111361**

Funding from NJAES and USWBSI:

RUTGERS
New Jersey Agricultural Experiment Station

U.S. Wheat & Barley Scab Initiative

- We developed our own sgRNA-based CRISPR-editing vectors for monocots.
- Several barley gene-editing vectors were constructed.



- Several barley mutants were produced.

New Funding from USWBSI:

Barley Genetic Engineering Facility for FHB Research Community

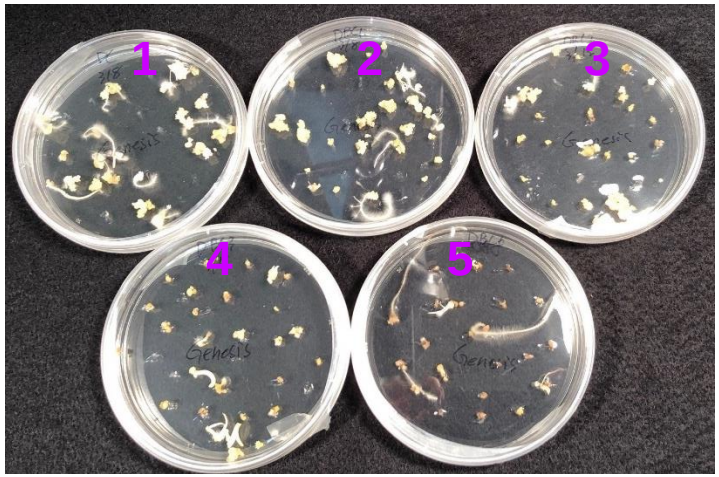
U.S. Wheat & Barley Scab Initiative

- **Similar to the Wheat Transformation Facility at Kansas State University, led by Dr. H. Trick**
- **Free of charge**
- **We need to test the regenerability of embryogenic calli induced from immature seeds of your chosen barley cultivars.**
- **Transformation by both gene gun and Agrobacterium**
- **Deliver T_1 seeds**

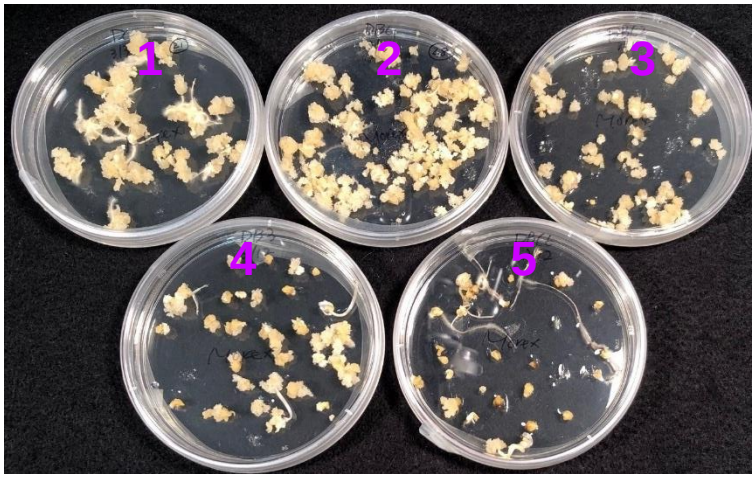
- **Contact: Rong Di at: rongdi@sebs.Rutgers.edu**
- **Fill out the transformation submission form**
- **Send the plasmid or the Agrobacterium**

We have optimized embryogenic callus induction for barley cv. ND Genesis (spring, 2-rowed), Morex (spring, 6-rowed) and Thunder (winter, 2-rowed).

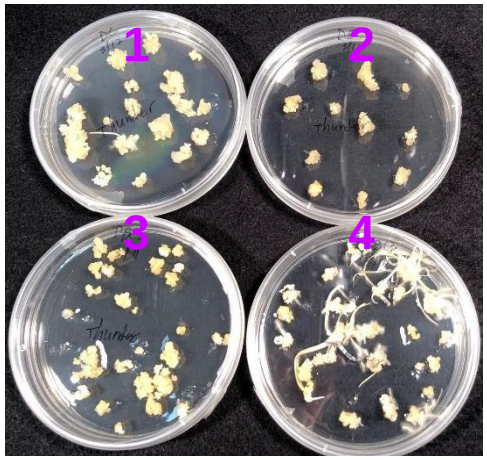
ND Genesis



Morex



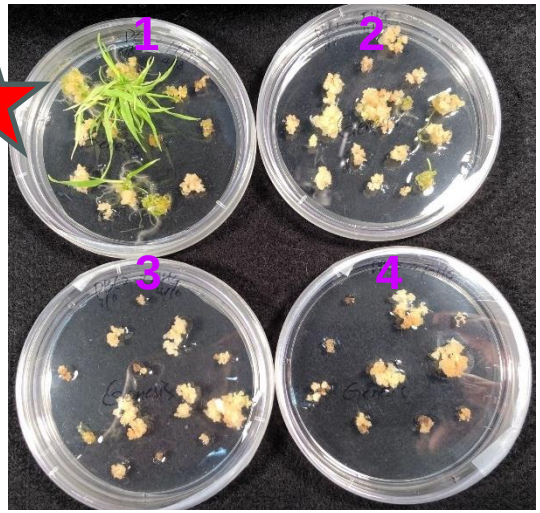
Thunder



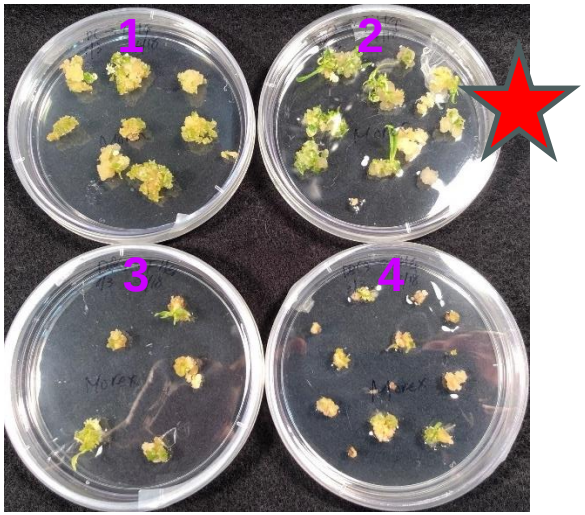
- Media #1,2,3,4,5 have different concentrations of 2,4-D and BAP.
- Seeds were grown in soil at 4 ^{o}C for 1 Mo. Seedlings were grown at 16 ^{o}C for 1 Mo. Plants were grown in G.H. for 3 weeks.
- Immature scutella as the explant were cultured on callus-induction media for 1 Mo.
- From seeds to calli: 3.5-4 Mo. Ready for transformation

We have optimized shoot induction for barley cv. ND Genesis, Morex and Thunder

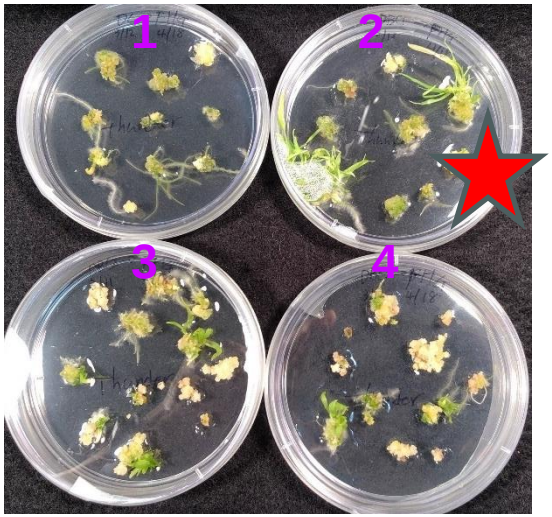
ND Genesis



Morex



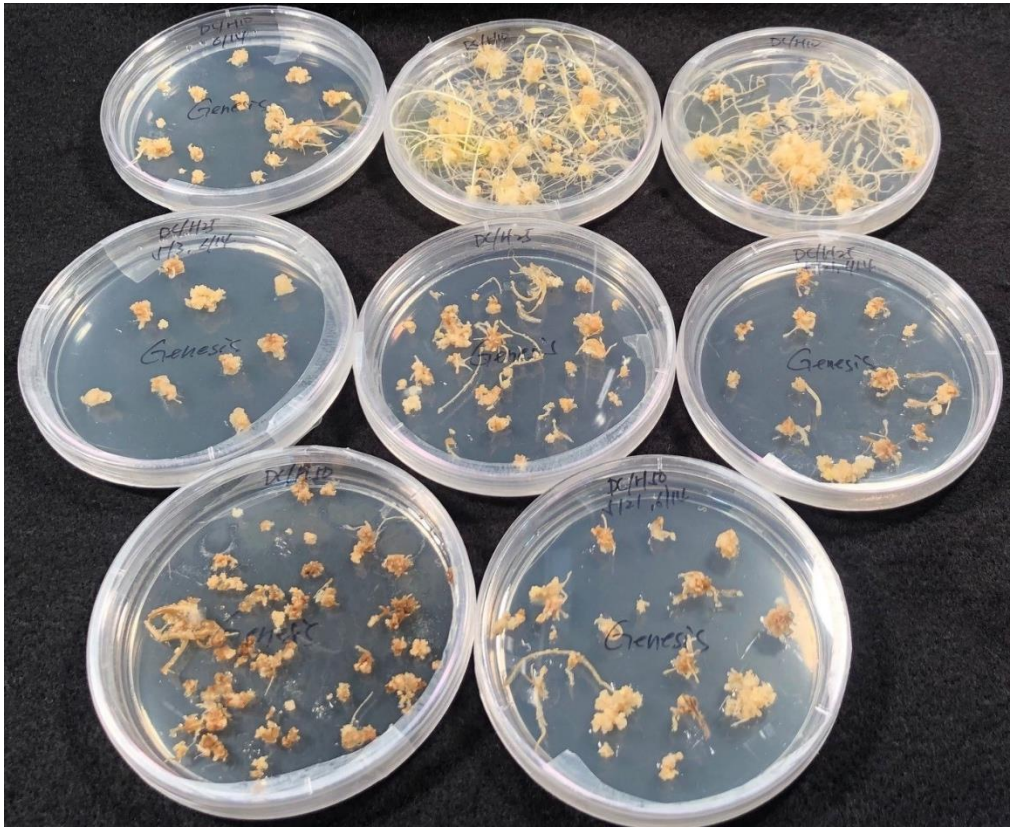
Thunder



- Calli induced on media #1,2,3,4 were transferred to FHG/0.1mg/L 2,4D/ 2mg/L BAP for 1 Mo.
- Shoots readily root in MS medium in 1-2 weeks.
- For transformation: add 1 Mo. for selection.

Totally from seeds to regenerated plants: ~ 6 months.

We have optimized hygromycin selection for barley



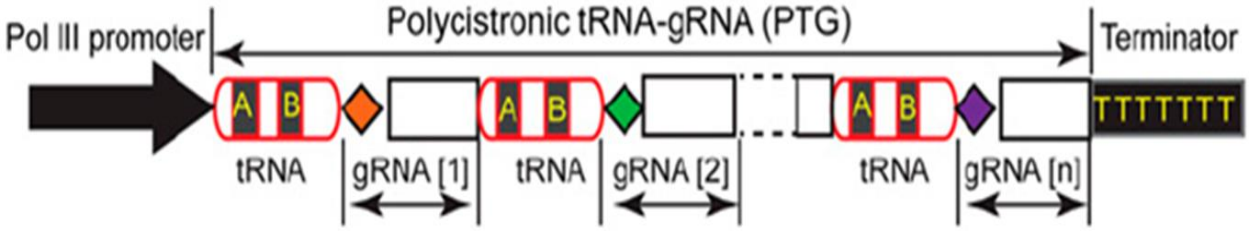
10 mg/L

25 mg/L

50 mg/L

ND Genesis

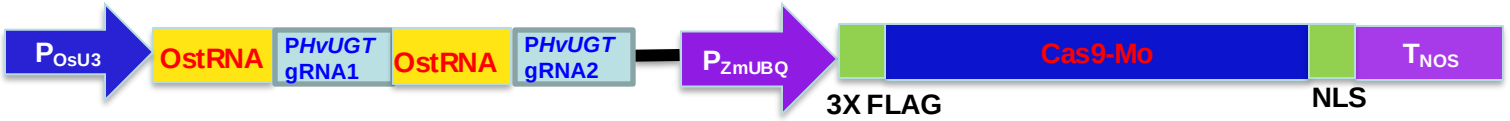
We have constructed dual tRNA (dtRNA)-based CRISPR vectors to enhance gene editing efficiency in barley



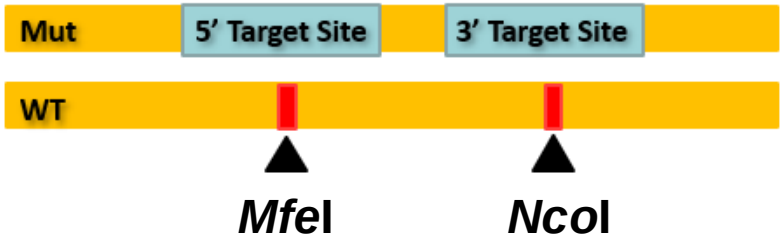
[Xie K, Minkenberg B, Yang Y. Proc Natl Acad Sci USA. 2015 112(11):3570-5]

Example: pRD549 (integrating vector)

To KO Morex *HvUGT* promoter to study the dynamics of *HvUGT* (*uridine diphosphate glycosyltranferase*) in response to *Fg* infection (in collaboration with Dr. Muehlbauer in UMN)

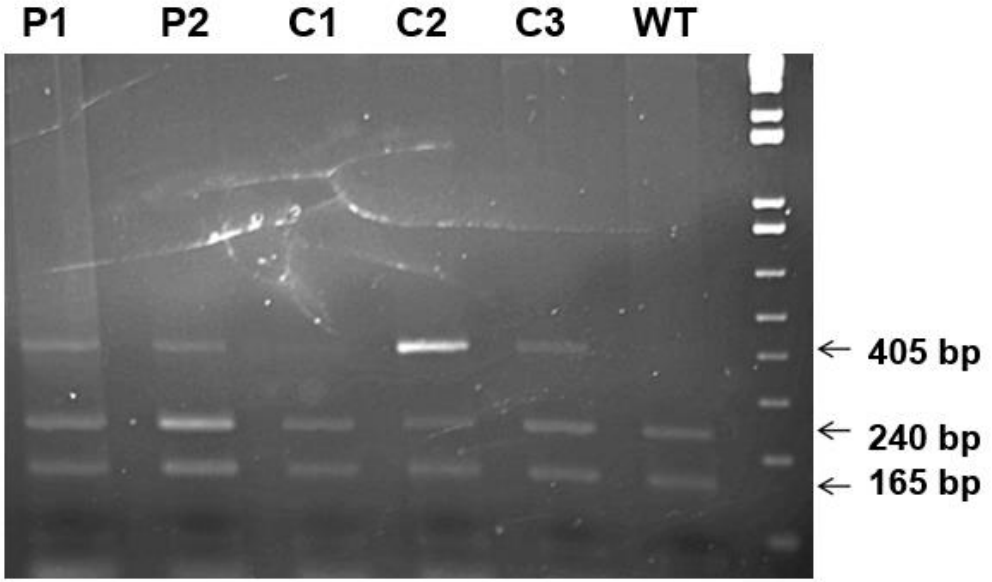


pRD549, targeting 2 sites in *HvUGT* promoter



- Transformed pRD549 into Morex protoplast by PEG, into Morex calli by gene gun
- PCR-amplified gDNA flanking *Nco*I target site
- RFLP by *Nco*I

- Monoallelic mutations in transformed samples

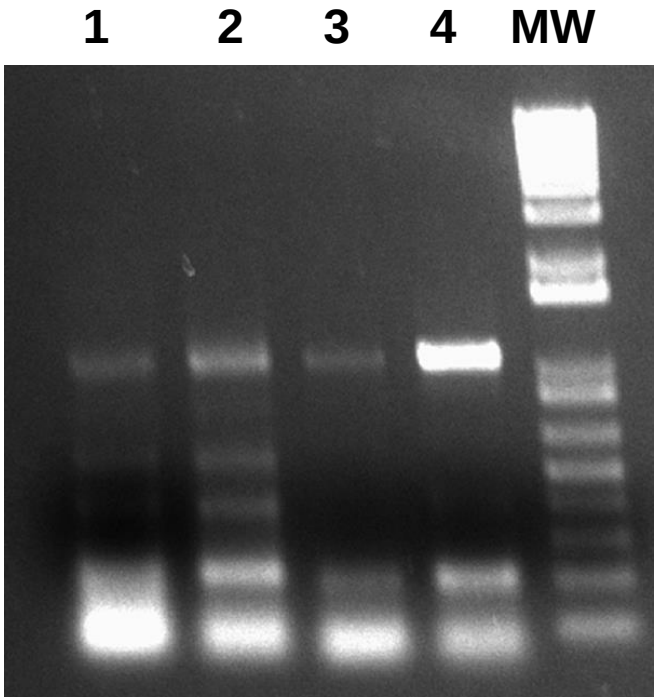


P1 and P2: Pooled, transformed protoplast gDNA
C1, C2 and C3: Pooled, transformed callus gDNA
WT: Pooled wild type protoplast gDNA

Both the transient (pRD543) and integrating (pRD549) *HvUGT* promoter CRISPR vectors induced mutations in PEG- transformed protoplasts

- Transformed pRD543 and pRD549 into Morex protoplasts by PEG
- PCR-amplified gDNA of *HvUGT* flanking both *Mfe*I and *Nco*I sites

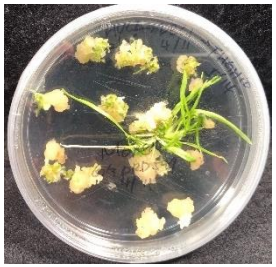
- Sequencing of smaller bands showed large deletions.



1. pRD543-transformed protoplasts
2. pRD549-transformed protoplasts
3. WT protoplast
4. WT protoplast

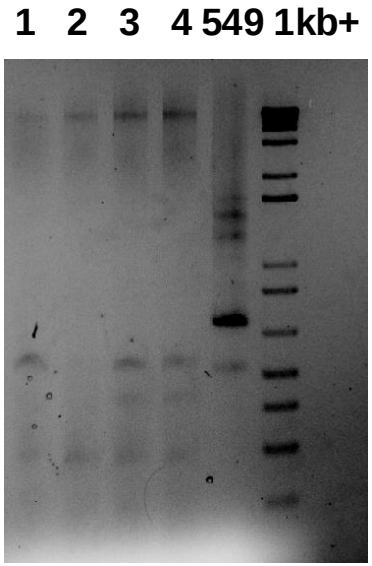
We have produced transgenic Morex plants transformed with pRD549 by gene gun

Selection of transgenic shoots



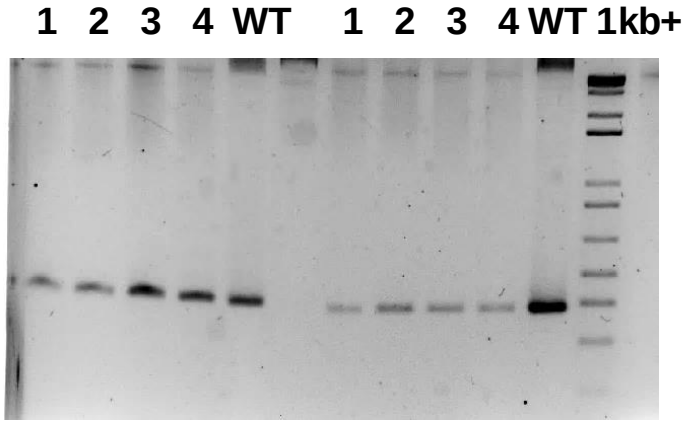
Root induction

PCR: presence of the dtRNA/gRNA cassette



549-1,2(?),3,4: transgenic
pRD549: 2 possible products, 543 bp, 721 bp

PCR: amplified the gDNAs flanking *Mfe*I and *Nco*I



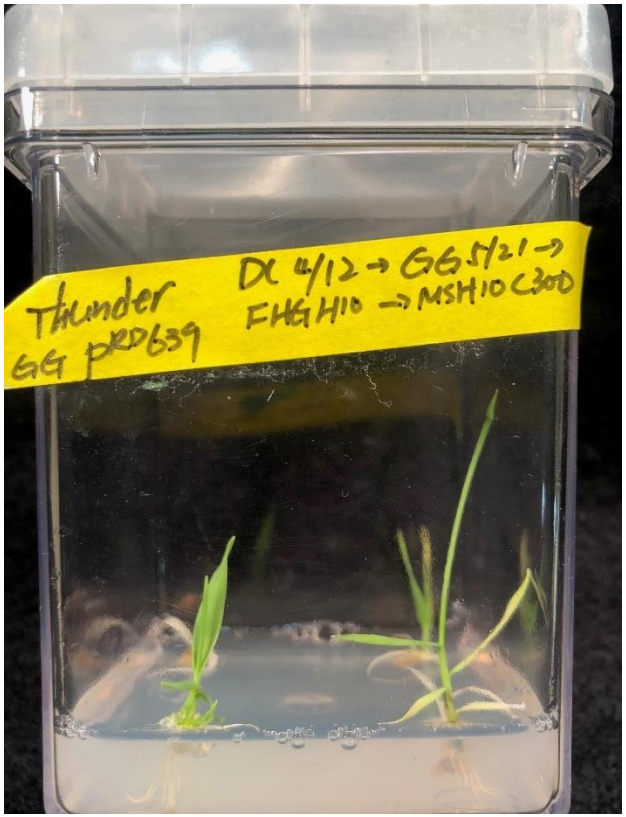
Being sequenced

We have transformed ND Genesis and Thunder using embryogenic calli as explant by gene gun

ND Genesis
pRD554
(*HvEIN2*-KO)

Transgenic shoot induction

Thunder
pRD639 (Cas9)



Root induction

Use of morphogenes to improve transformation and regeneration of recalcitrant plants: BBM, WUS, PLT etc. regulating cytokinin and auxin

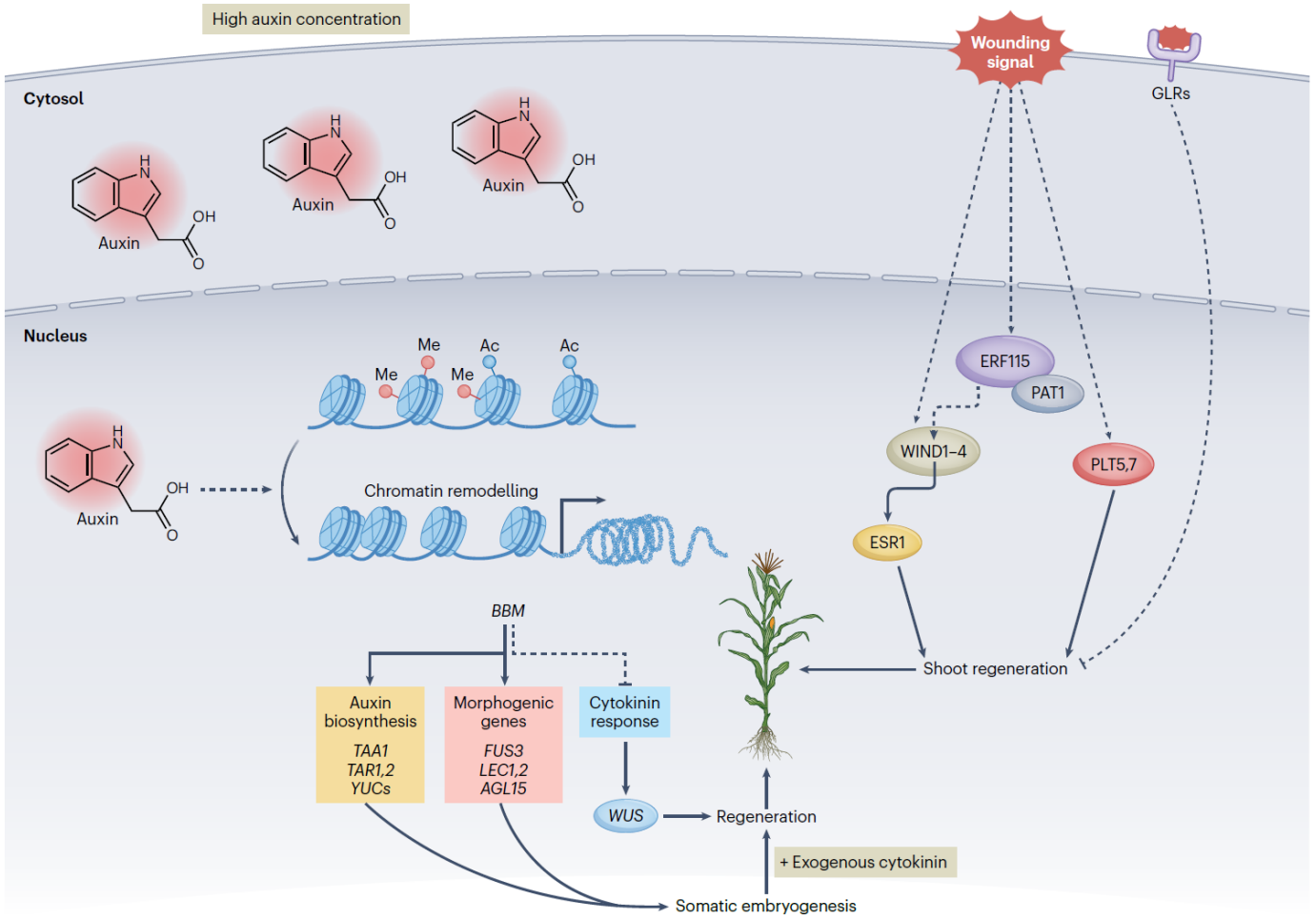
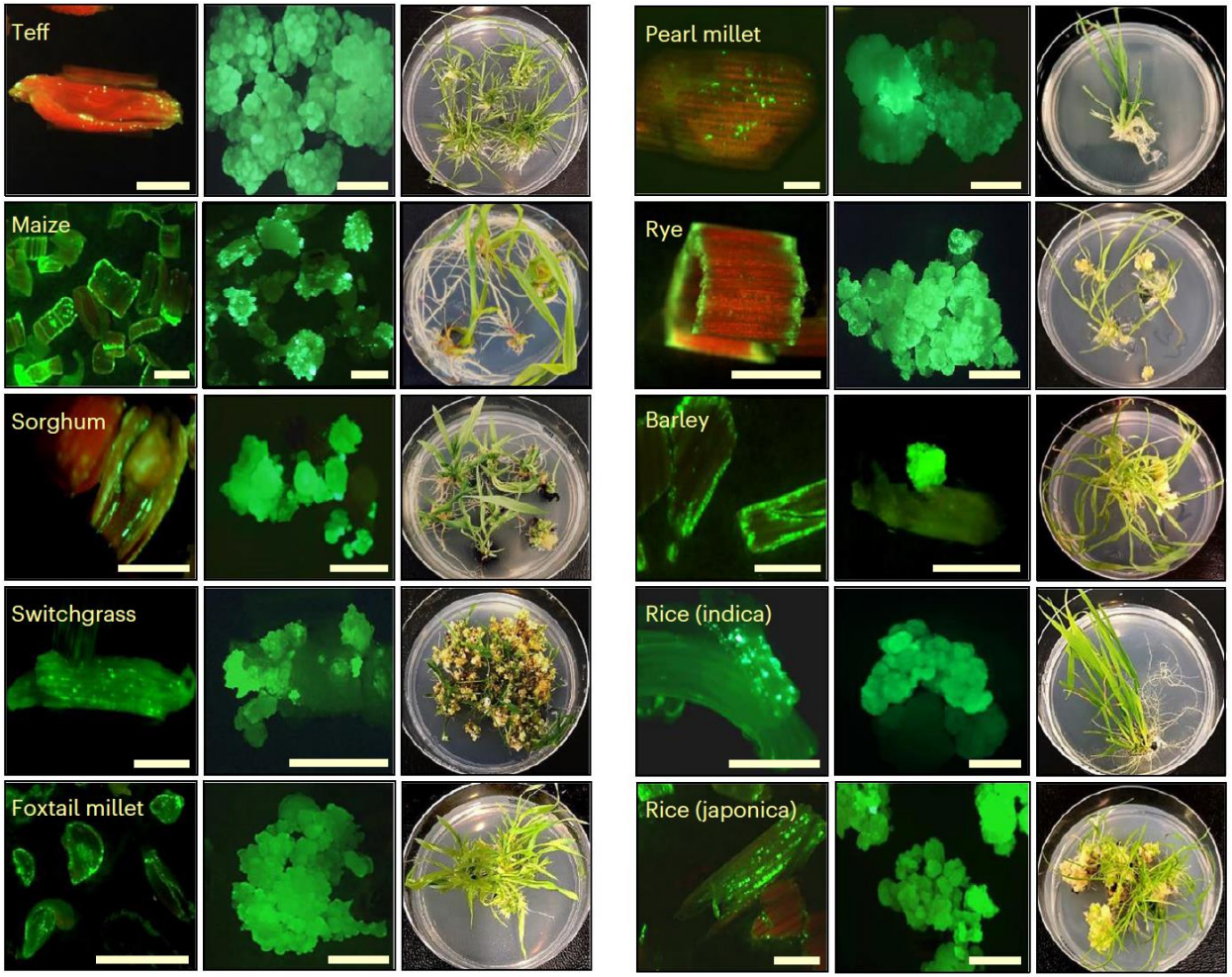


Fig. 2 | Plant regeneration pathways. Known molecular components of plant regeneration pathways. Solid lines indicate well-established relationships, while dashed lines indicate relationships where key molecular details are unknown; arrowhead ends indicate positive regulation and perpendicular ends indicate negative regulation. Created with BioRender.com.

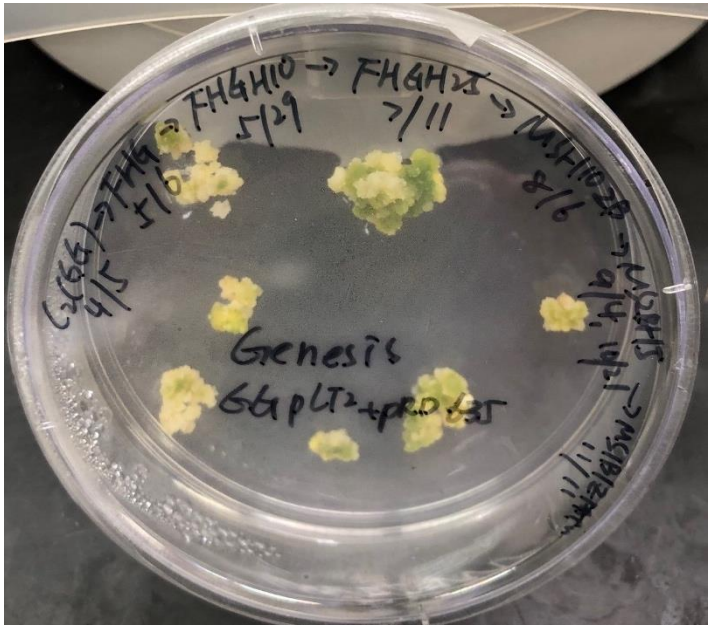
Chen/Gallavotti et al. 2022 Nature Plants

Use of WUS2/BBM to improve transformation and regeneration of monocot leaf tissues: avoiding immature embryos



Wang et al. 2022 Nature Plants

We have constructed our own transient and integrating vectors expressing *HvBBM, HvWUS*



- If the morphogenes are not removed, Genesis calli do not regenerate into shoots, even though they appear embryogenic.
- More testing is needed.

We have transformed Dr. John McLaughlin's (Rutgers) constructs into Genesis embryogenic calli

Arabidopsis lipid transfer protein4.4

Wheat lipid transfer protein3

Acknowledgement

Funding:

USDA/USWBSI: Barley FHB project
"Barley Genetic Engineering Facility for FHB Research Community" is funded by USWBSI at Rutgers (2022-2026).
USDA/Multistate, NJAES

Collaborators:

Rutgers: Dr. Jun Qin, Dr. Michael Lawton,
Alison Dineen Ying Chen and students
Rutgers: Dr. John McLaughlin
UMN: Dr. Gary Muehlbauer

Want to transform your barley cultivars with your constructs?

Contact: Dr. Rong Di, at

rongdi@sebs.rutgers.edu