RUTGERS

Enhanced resistance to trichothecenes and FHB by expression of *Arabidopsis* and wheat non-specific lipid transfer proteins (nsLTPs) in wheat

John McLaughlin

Tumer Laboratory

USWBSI 2017

Poster 25

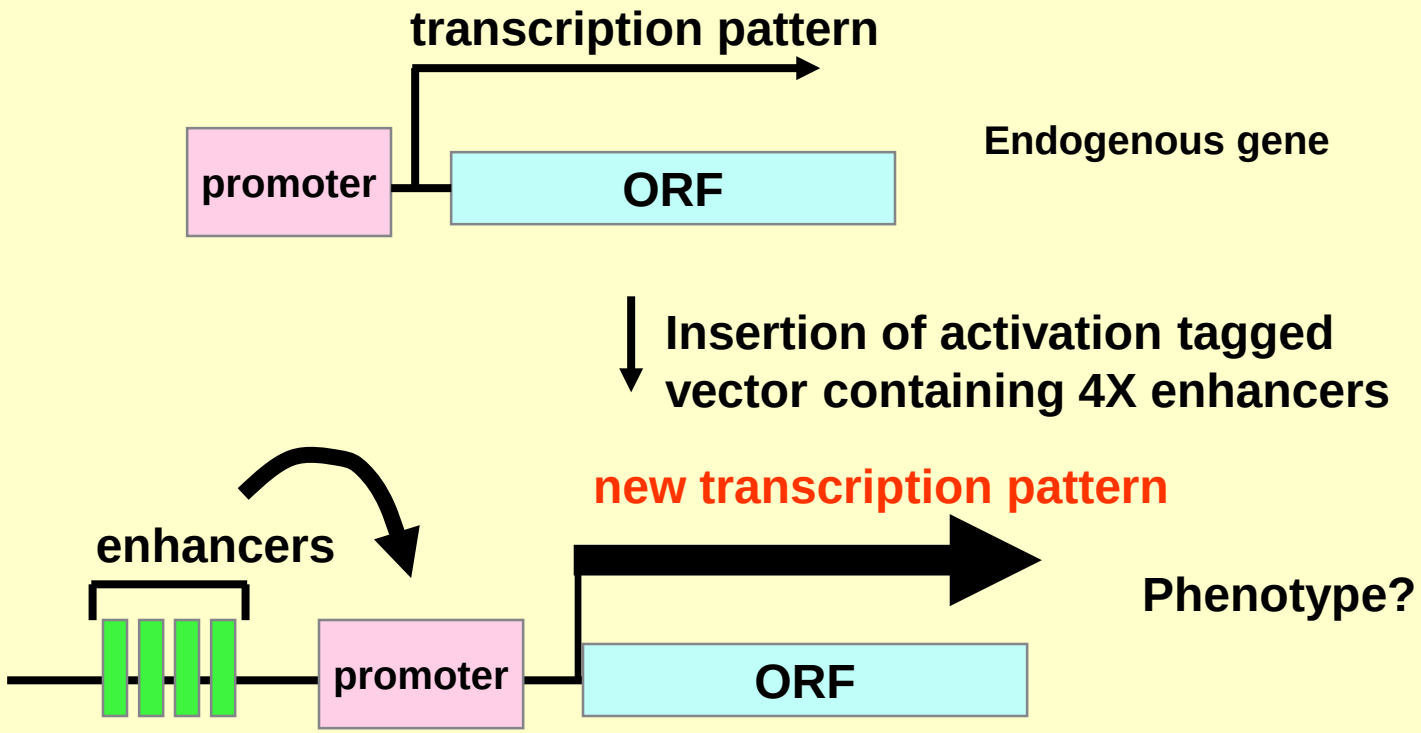
U.S. Wheat & Barley Scab Initiative

Goals

- **Identify plant genes that confer resistance to trichothecenes and Fusarium head blight (FHB)**

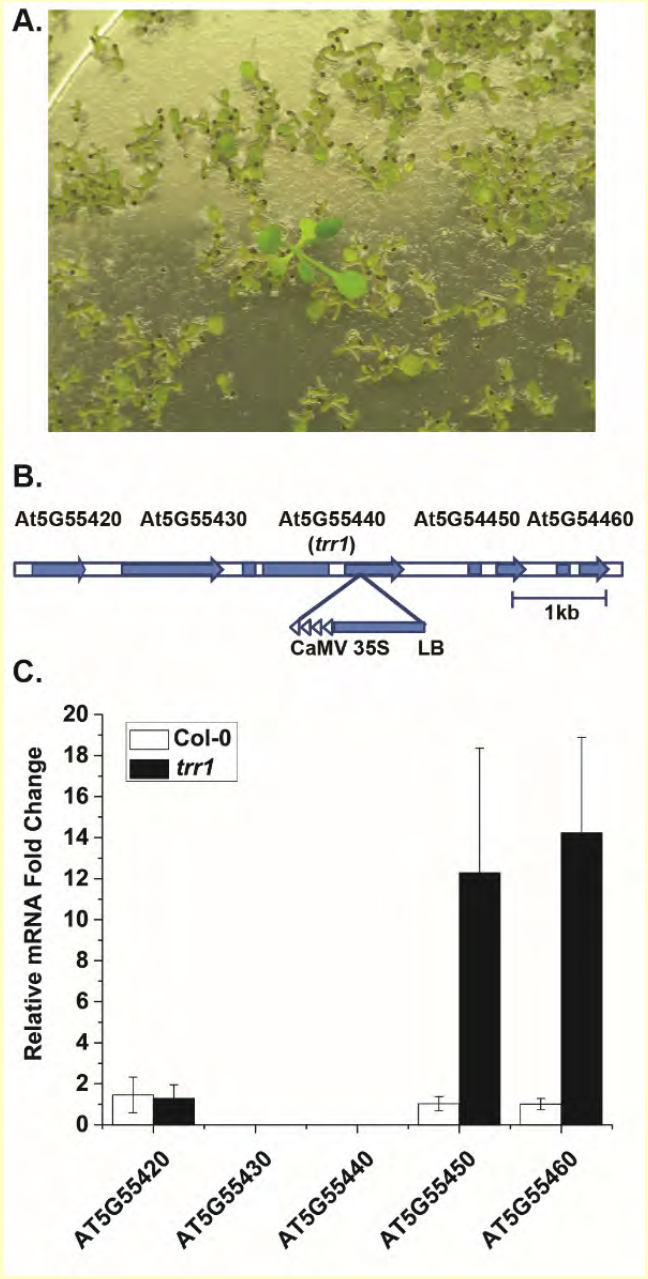
- **Test in yeast, Arabidopsis, wheat and barley**

Activation tagging: Dominant Gain-of-function



Perform a genome-wide screen in Arabidopsis for trichothecene resistant phenotypes

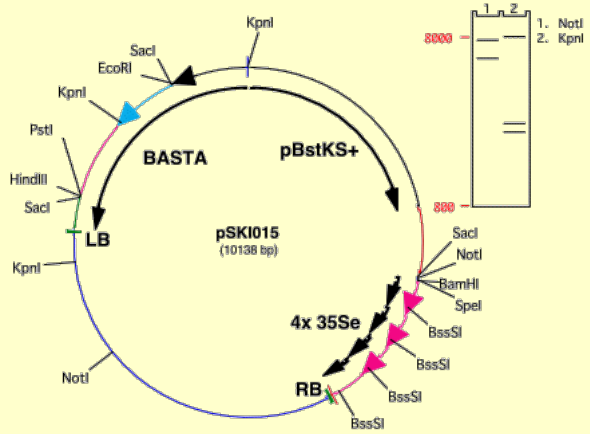
Arabidopsis Activation Tagging Screen for Resistance to Trichothecenes



Screen mutant population on 4 µM trichothecin (Tcin)

Identify tag on chr. 5

Two non-specific lipid transfer protein genes found to be upregulated AtLTP4.4 and AtLTP4.5 downstream from the activation tag.



Activation tagging vector contains 4 X CaMV 35S enhancers

McLaughlin, et al. "A lipid transfer protein increases the glutathione content and enhances Arabidopsis resistance to a trichothecene mycotoxin." *PloS one* 10.6 (2015)

RUTGERS

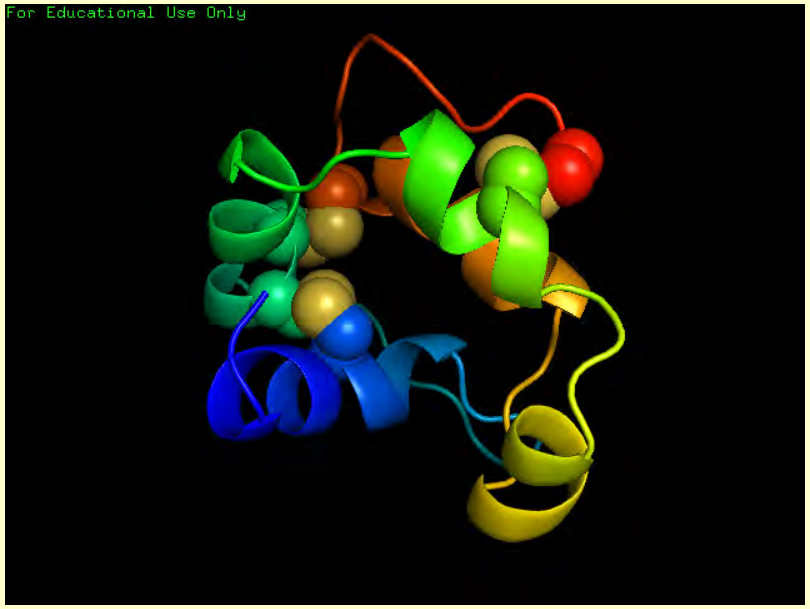
Plant nsLTP functions

- **Non-specific lipid transfer proteins (nsLTPs) bind to and transfer phospholipids between membranes *in vitro***
- **Proteins characterized by an eight cysteine motif (8 CM) backbone: C-Xn-C-Xn-CC-Xn-CXC-Xn-C-Xn-C**
- **The 4 disulfide bonds created by the 8 cysteine motif generate an internal hydrophobic pocket which can accommodate a lipid (or two)**
- **The high content of thiol cysteines of LTP1 (disulfide bonds broken during brewing process) in beer is the basis for its radical scavenging and antioxidant activities.**
- **Plant nsLTPs contain signal peptides, which target them to cell wall/apoplast**
- **Some nsLTPs are upregulated in response to infection and exhibit antimicrobial/antifungal activity**
- **The best studied plant nsLTP, DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1) in *Arabidopsis* plays a role in systemic acquired resistance (SAR). The lipid-derived chemical signals are believed to be C18 fatty acids (chloroplast lipids) and oxidized/cleaved by ROS.**

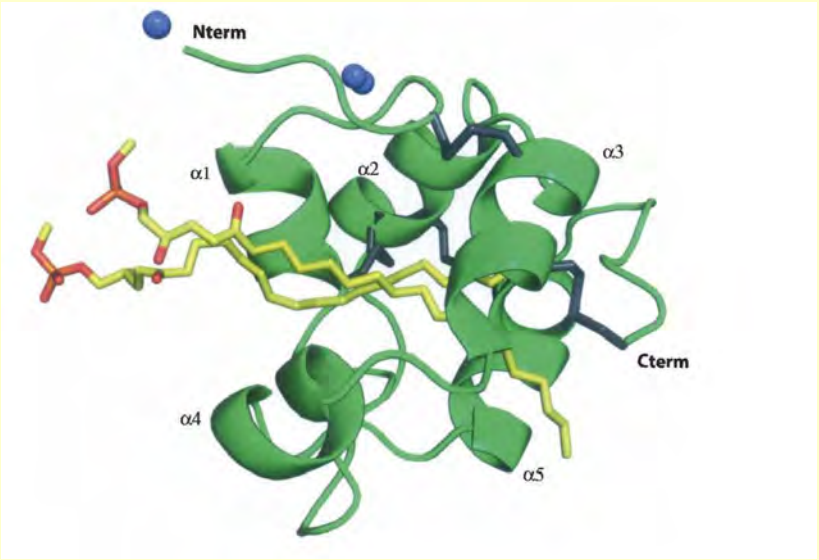
Plant nsLTP structures

AtLTP4.4

DIR1 (LTP4.1)



DIR1 (LTP4.1) with two lipids

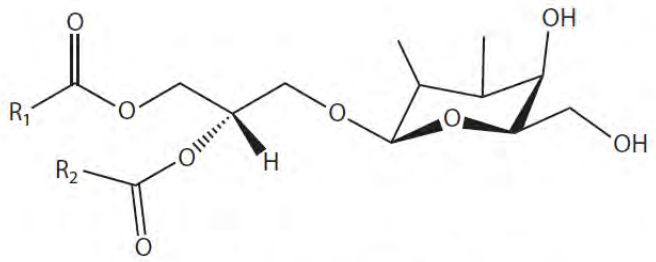


Predicted Structure for LTP4.4 compared to the known X-ray structure of the self-defense and signaling protein Defective in induced resistance (DIR1) from Arabidopsis. Crystallography of DIR1 shows a wide central channel, with two lipids (lysophosphatidyl choline) located inside the central cavity.

Lascombe et al 2008 (Protein Sci. 17: 1522-1530)

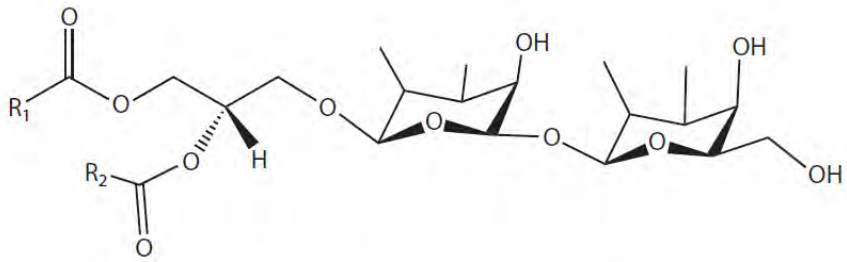
RUTGERS Chemical structure of two major chloroplastic lipids in plants/SAR

MGDG



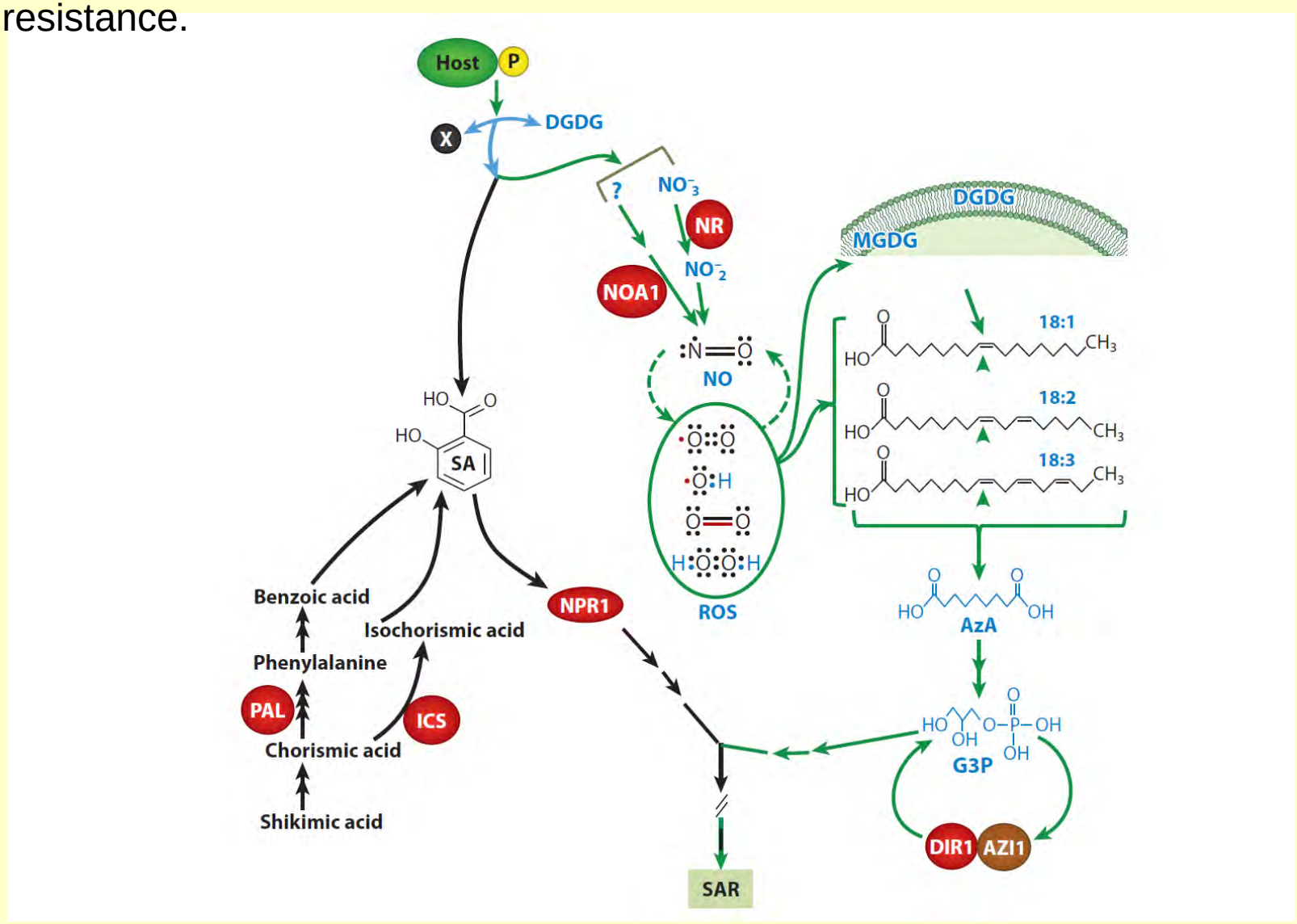
Monogalactosyldiacylglycerol

DGDG



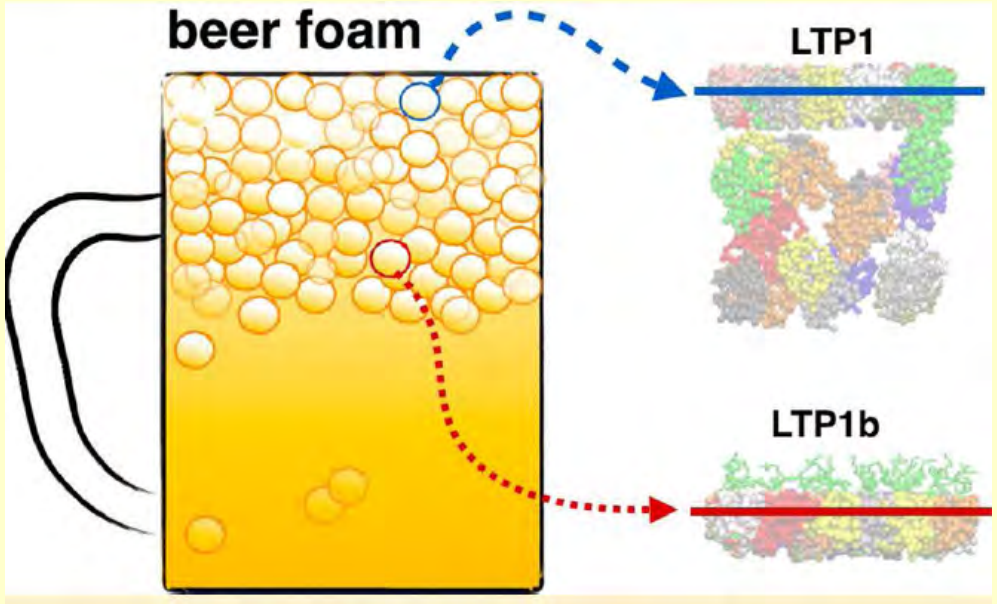
Digalactosyldiacylglycerol

Chemical signals and galactolipids (MGDG/DGDG) in systemic acquired resistance.



Annu. Rev. Phytopathol. 2017. 55:505–36

Barley LTPs play important role in beer



Langmuir 2017, 33, 4769−4780

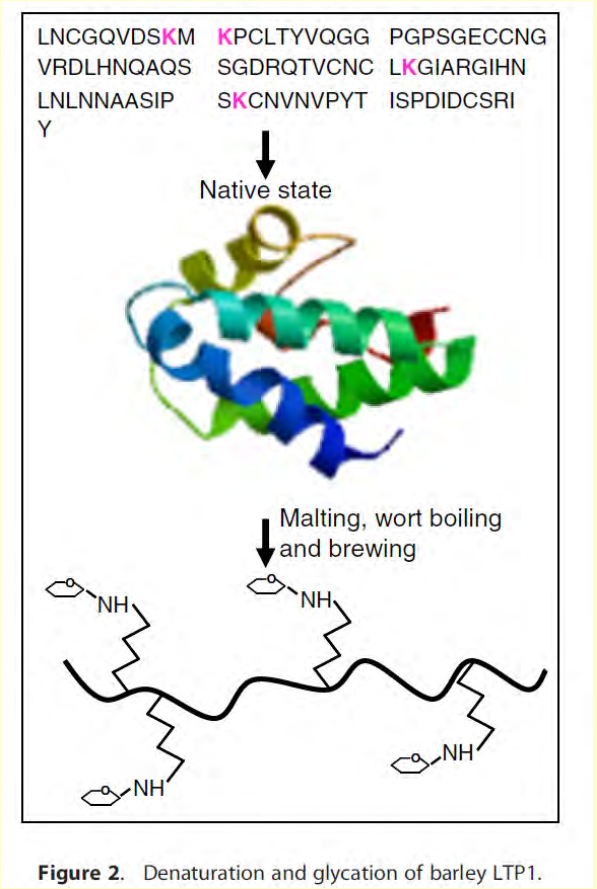


Figure 2. Denaturation and glycation of barley LTP1.

J. Inst. Brew. 2012; 118: 1–11

LTP1 plays important roles in beer: Foam, flavour stability, antioxidant.

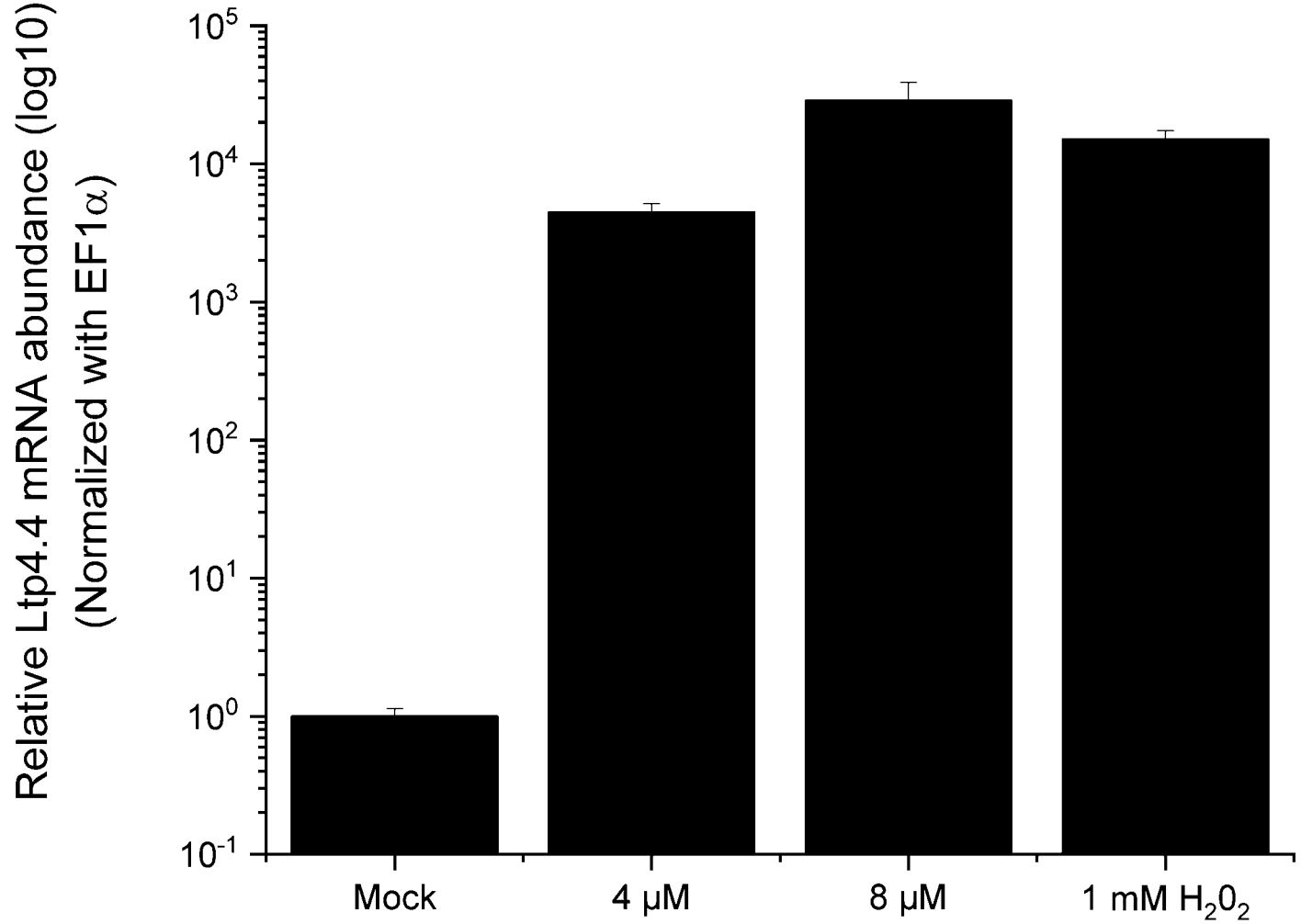
Antioxidant capacity rated based on free thiol content of LTP1.

LTP1 (free thiol groups) have ability to scavenge free radicals.

Identification of a Protein (LTP1) with Antioxidant Activity that is Important for the Protection against Beer Ageing. Int. J. Mol. Sci. 2011, 12, 6089-6103

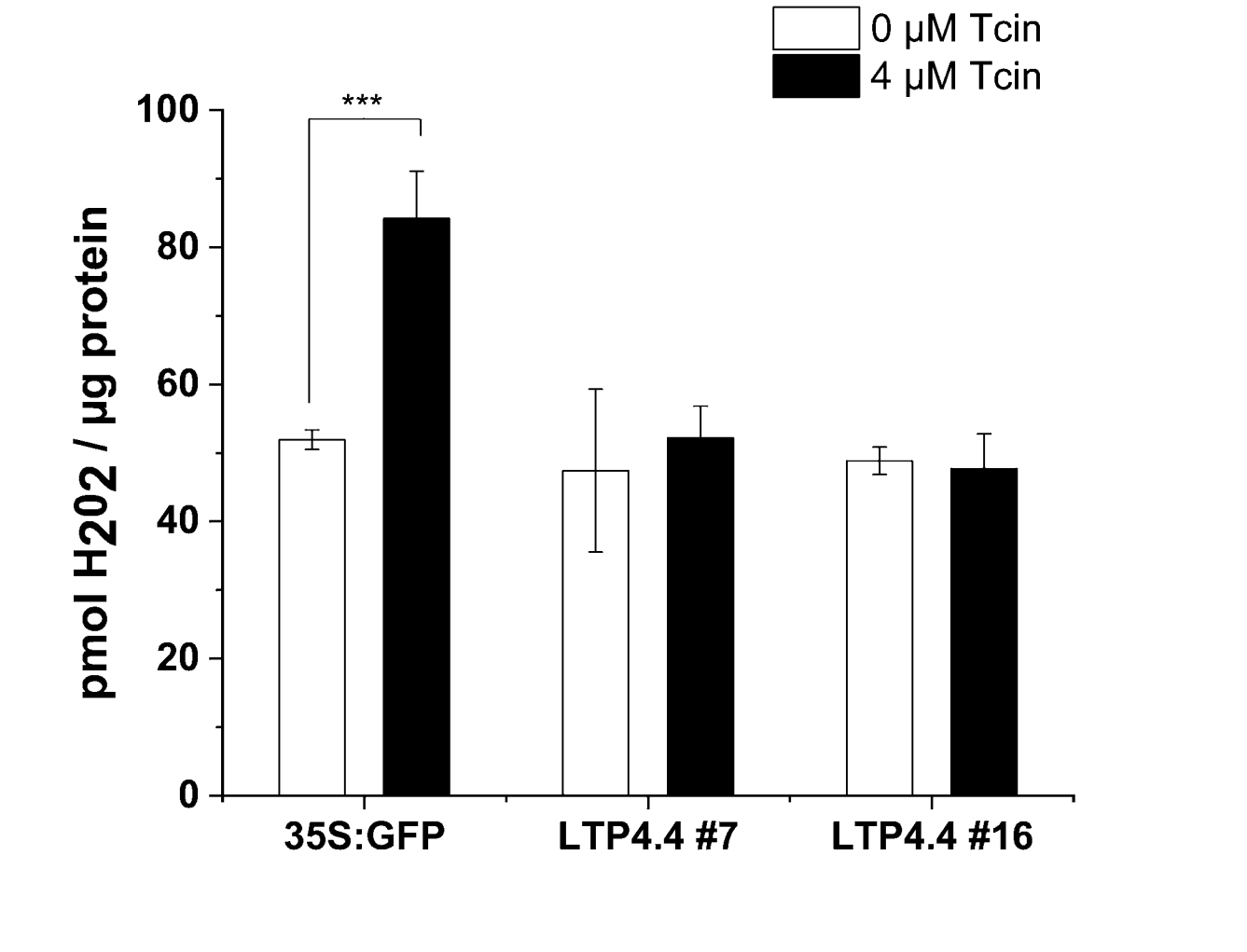
RUTGERS

Trichothecin and Hydrogen Peroxide induce LTP4.4 in *Arabidopsis* (Col-0)



RUTGERS

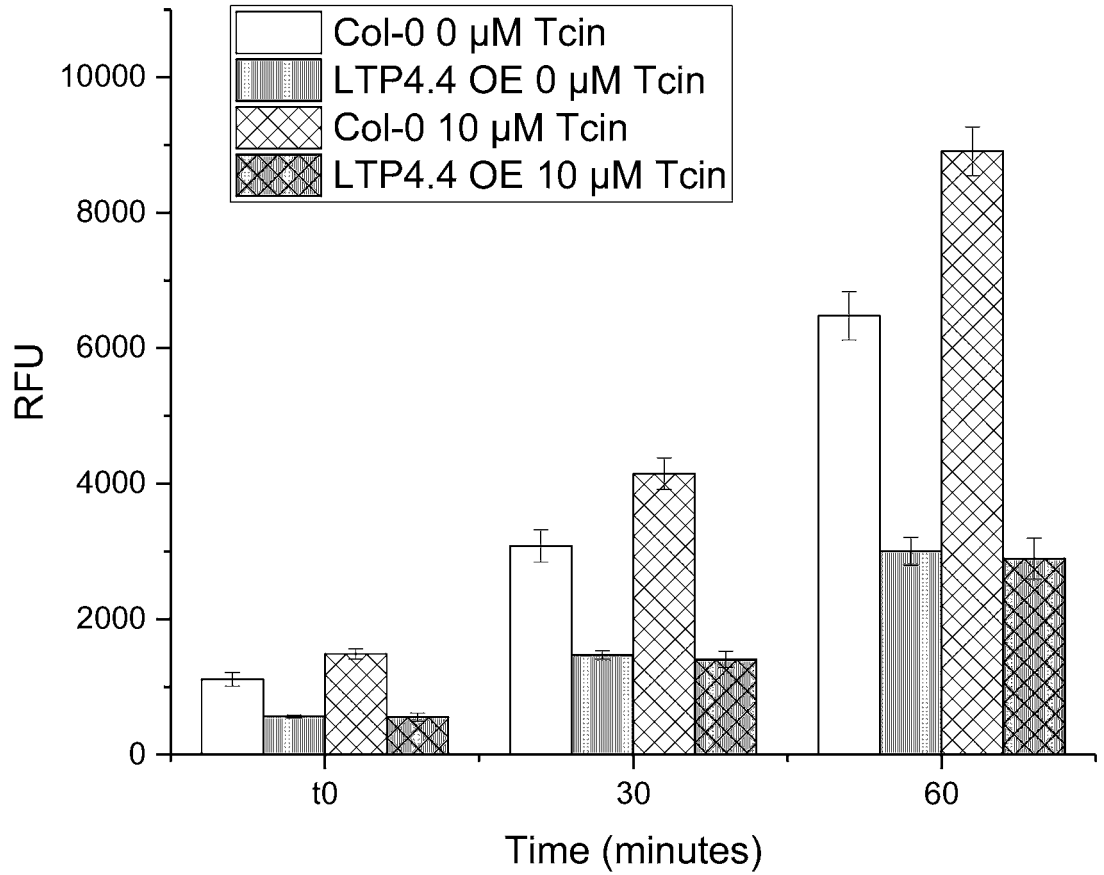
LTP overexpression prevents hydrogen peroxide increase upon Arabidopsis following Tcin exposure



Arabidopsis detached leaf assay to quantify H_2O_2 using Amplex Red. Tcin induces H_2O_2 accumulation in the vector control (35S:GFP) but that increase is prevented in the nsLTP overexpressing lines (24 hour treatment).

RUTGERS

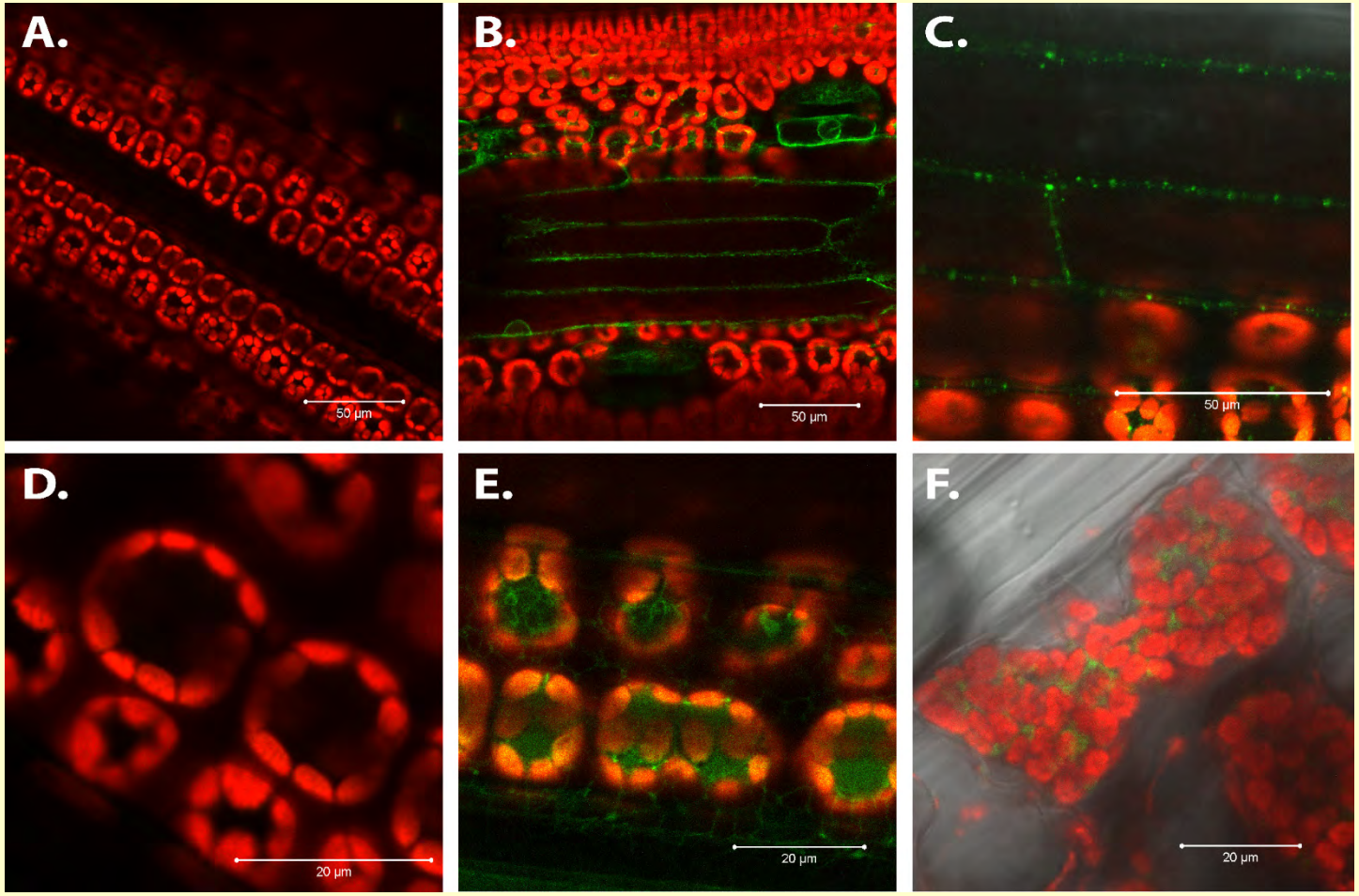
LTP overexpression lowers ROS levels In *Arabidopsis* Protoplasts



Arabidopsis protoplasts treated with 0 and 10 µM Tcin and ROS response measured using Dichloro-dihydro-fluorescein diacetate (DCFH-DA) and detected via flow cytometry. Note the basal level of LTP4.4 OE protoplasts is lower than the wildtype.

RUTGERS

Identification of transgenic Bobwhite OE AtLTP4.4 and TaLTP3- confocal imaging

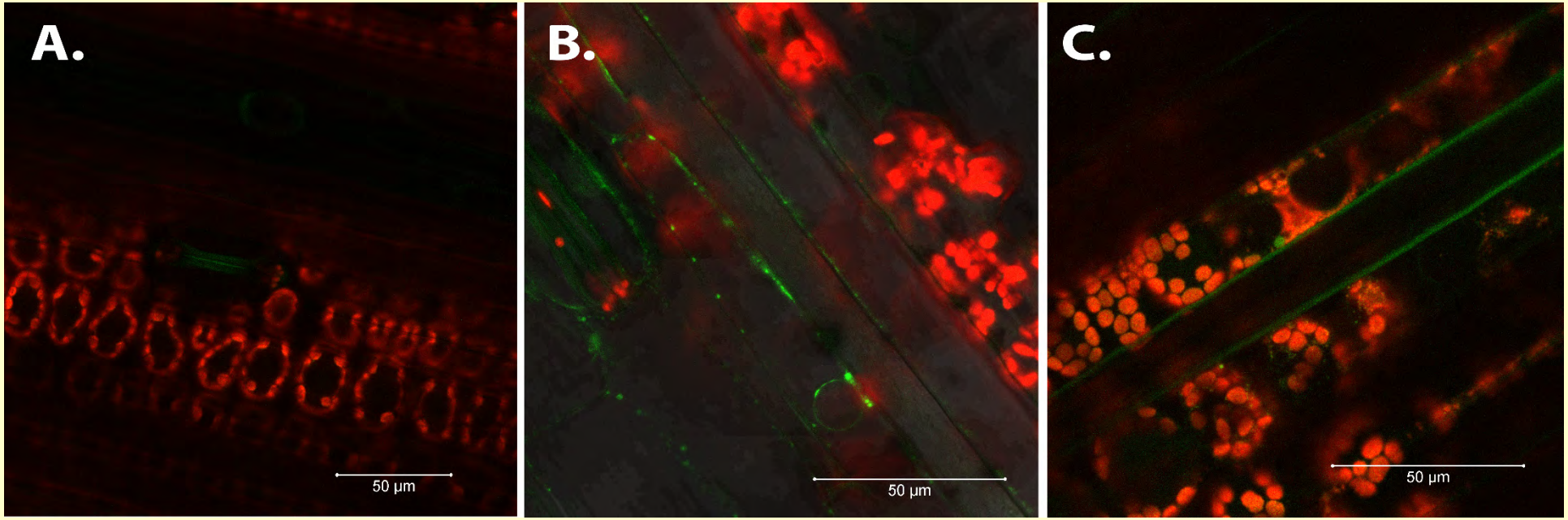


Bobwhite **9916(LTP4.4:GFP)** **9816 (TaLTP3:GFP)**

GFP-fused nsLTP protein (green) and autofluorescent chloroplasts (red) are shown.

RUTGERS

Identification of transgenic RB07 OE AtLTP4.4 and TaLTP3- Confocal imaging

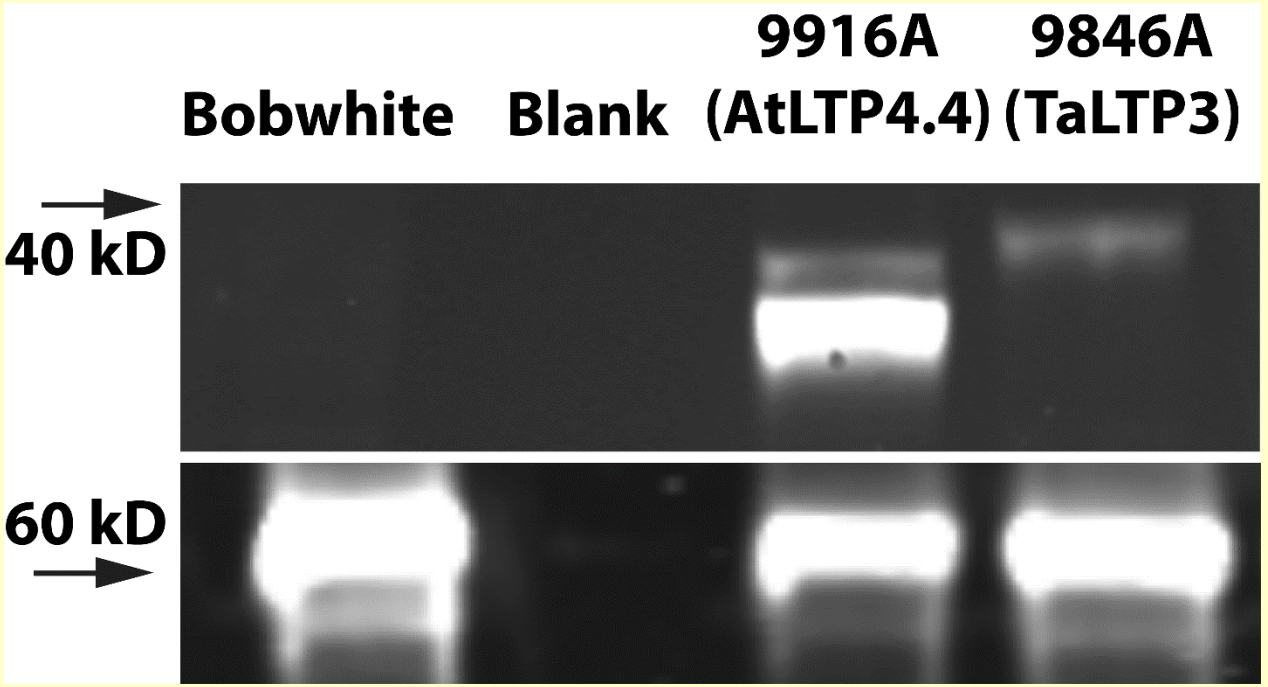


RB07 **1126 (AtLTP4.4:GFP)** **1088 (TaLTP3:GFP)**

GFP-fused nsLTP protein (green) and autofluorescent chloroplasts (red) are shown.

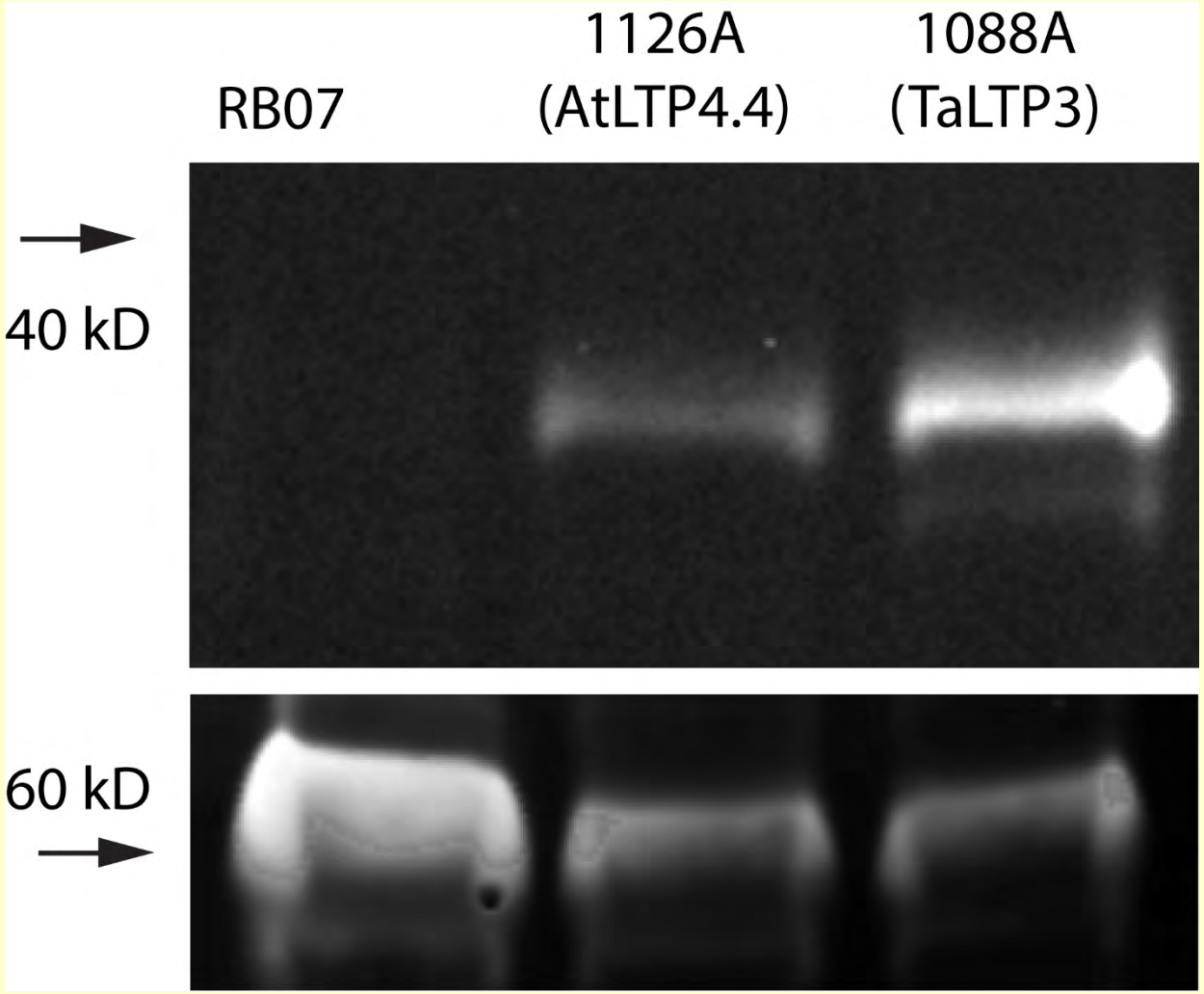
RUTGERS

Identification of transgenic Bobwhite expressing AtLTP4.4 and TaLTP3 (B712 vector)- Western Analysis



Loading Control

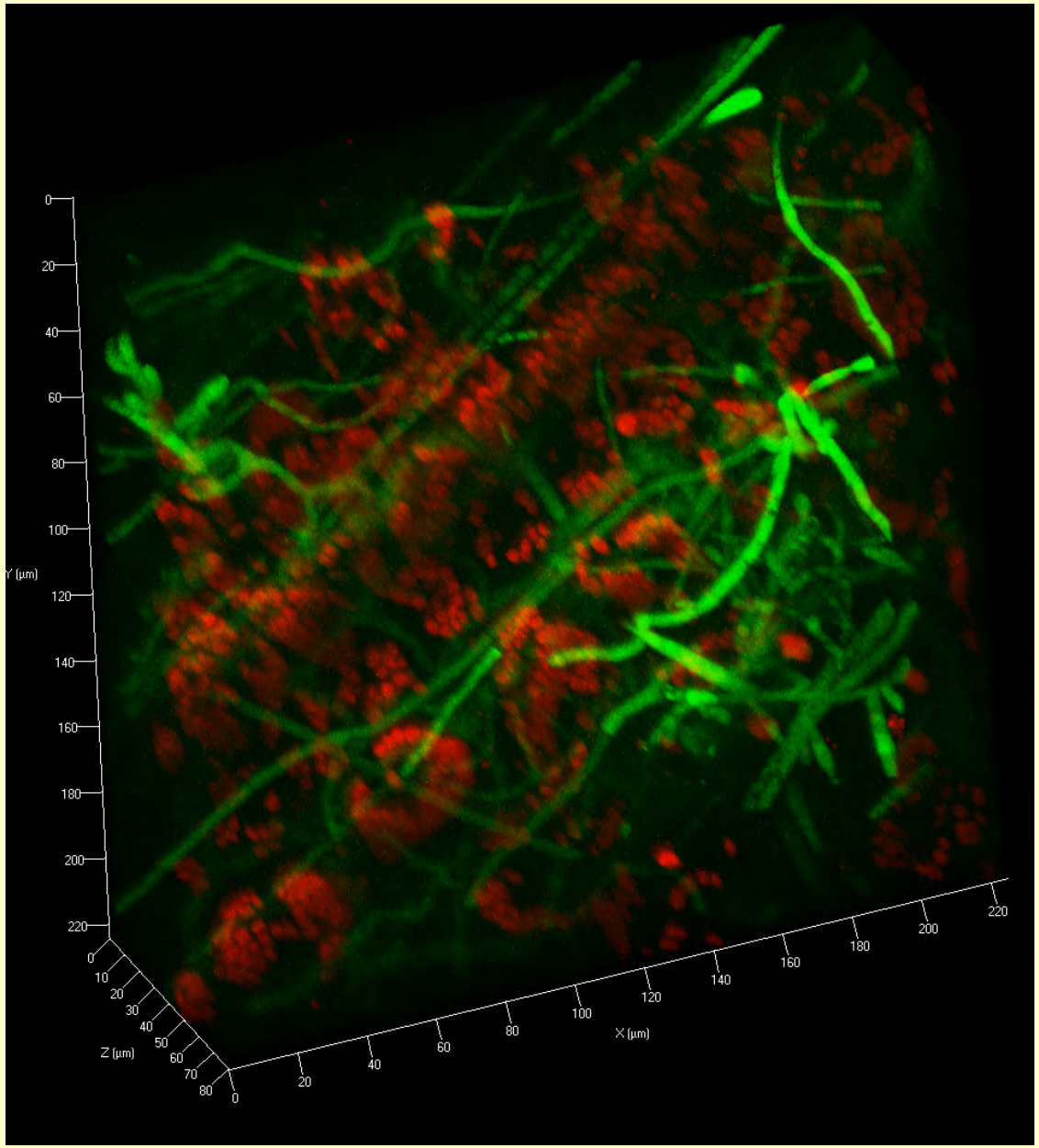
Identification of transgenic RB07 expressing AtLTP4.4 and TaLTP3 (B712 vector)- Western Analysis



Loading Control

RUTGERS

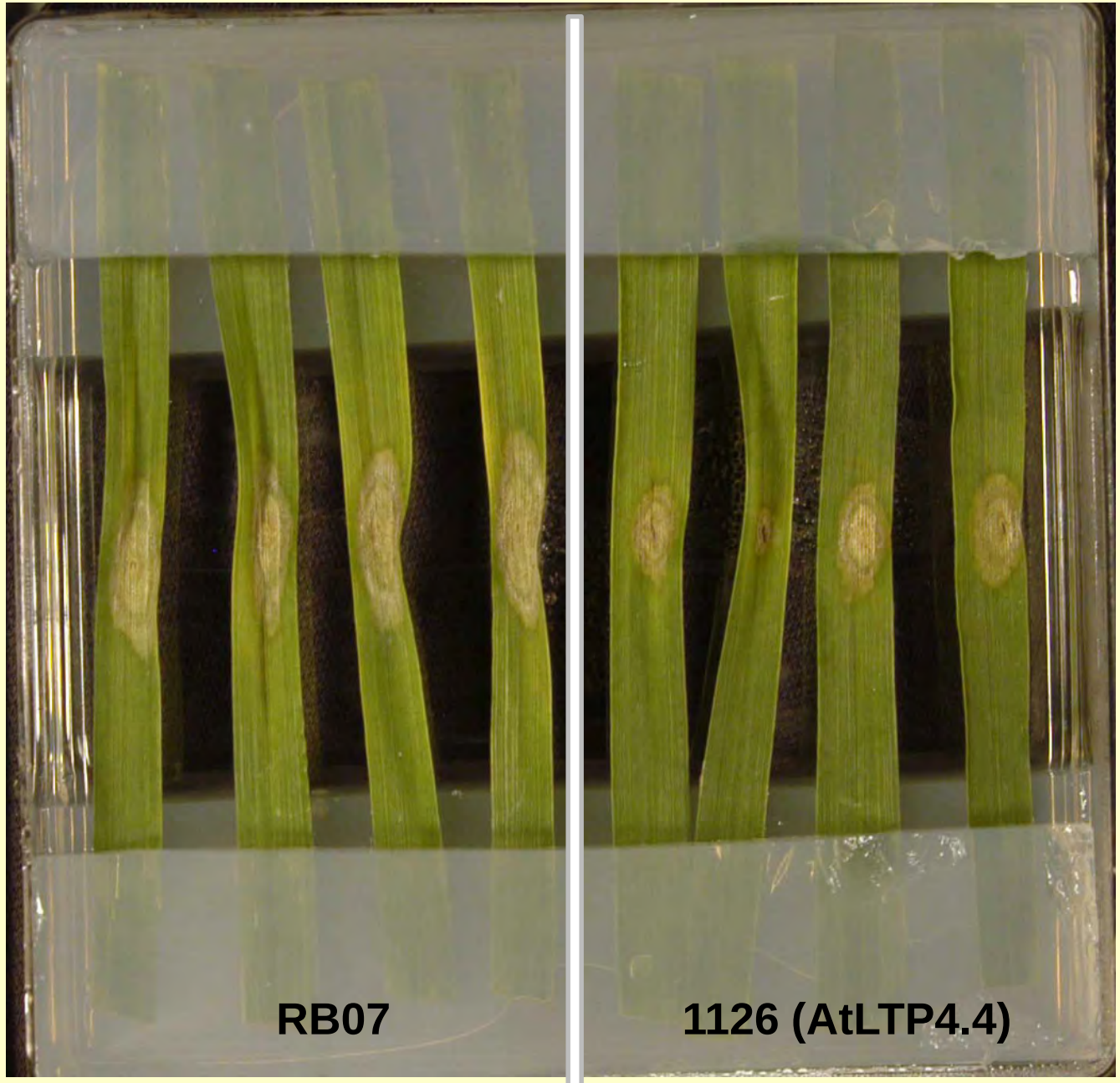
Test wheat OE nsLTP for Fusarium resistance



Fusarium graminearum **(GFP tagged, Fg8/1-GFP) obtained from Wilhelm Schäfer. Produces DON.**

Confocal Z-stack of wheat leaf tissue infected with the fungus.

Detached leaf bioassay to test for Fusarium resistance



Detached leaf assay:

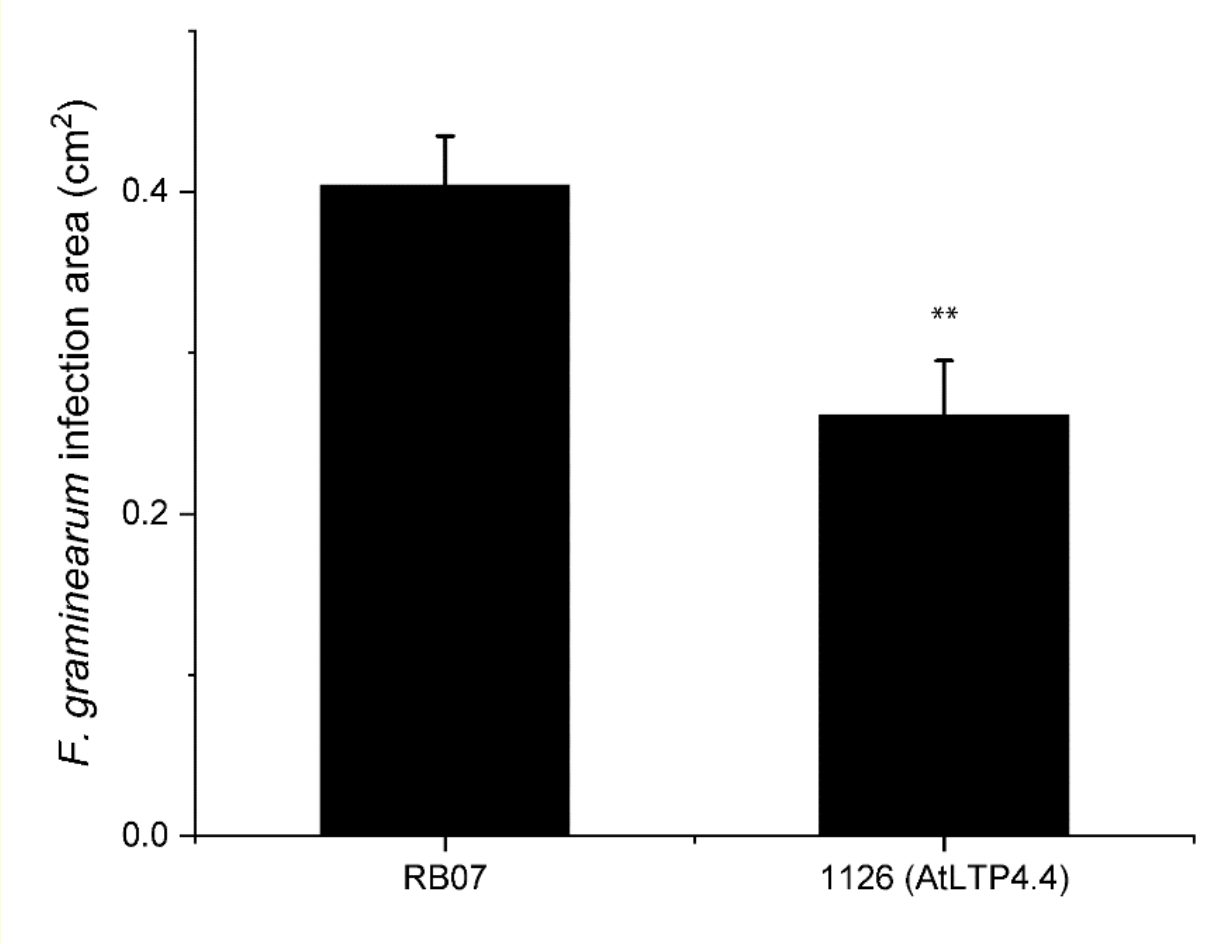
Wound with needle, apply 4,000 spores of Fusarium (Fg8/1-GFP).

Photograph at 3 DPI.

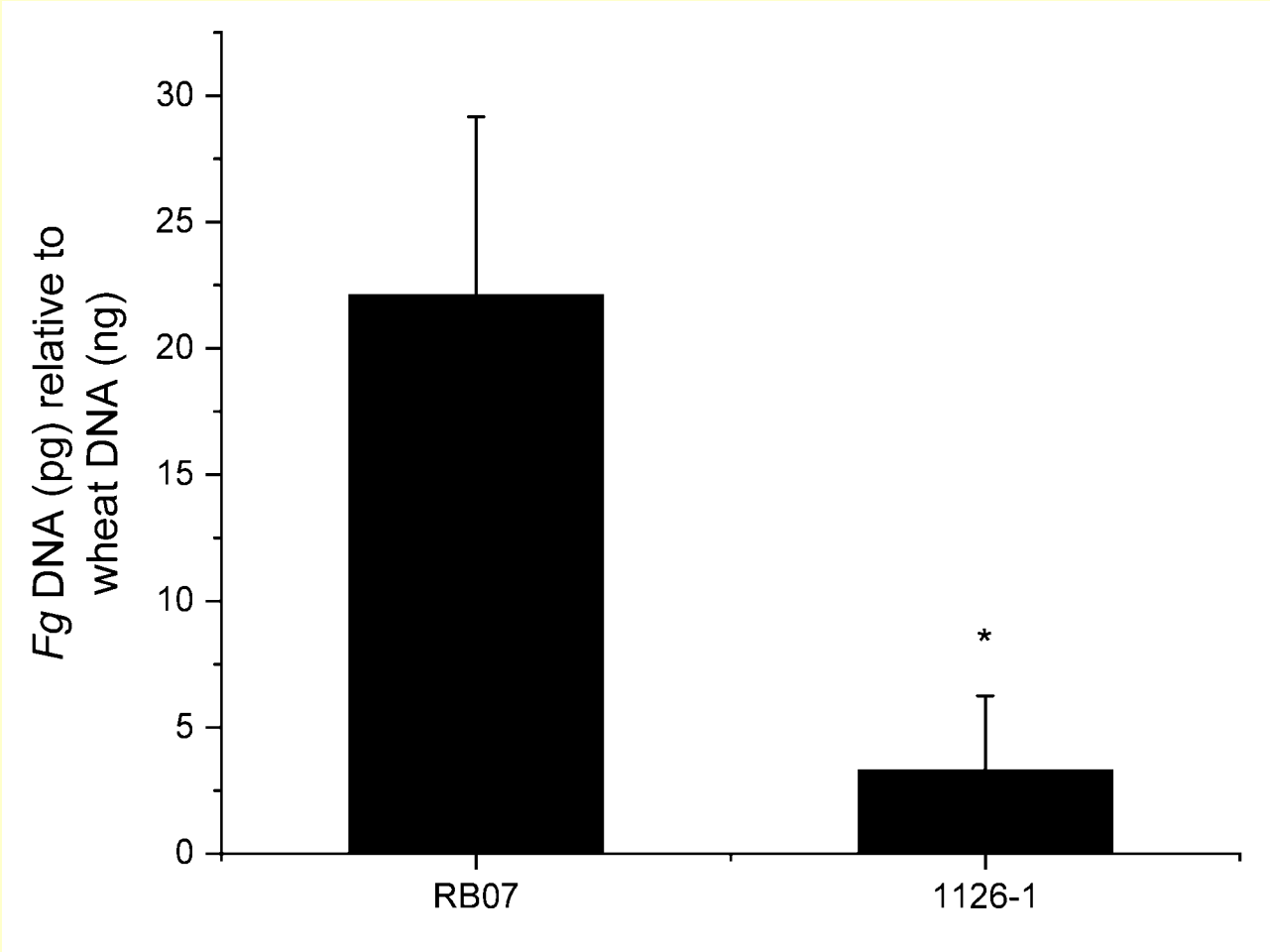
Method:Perochon and Doohan. 2016 Bio-protocol. Vol 6, No. 24.

Detached leaf bioassay to test for Fusarium resistance

Infection area quantification



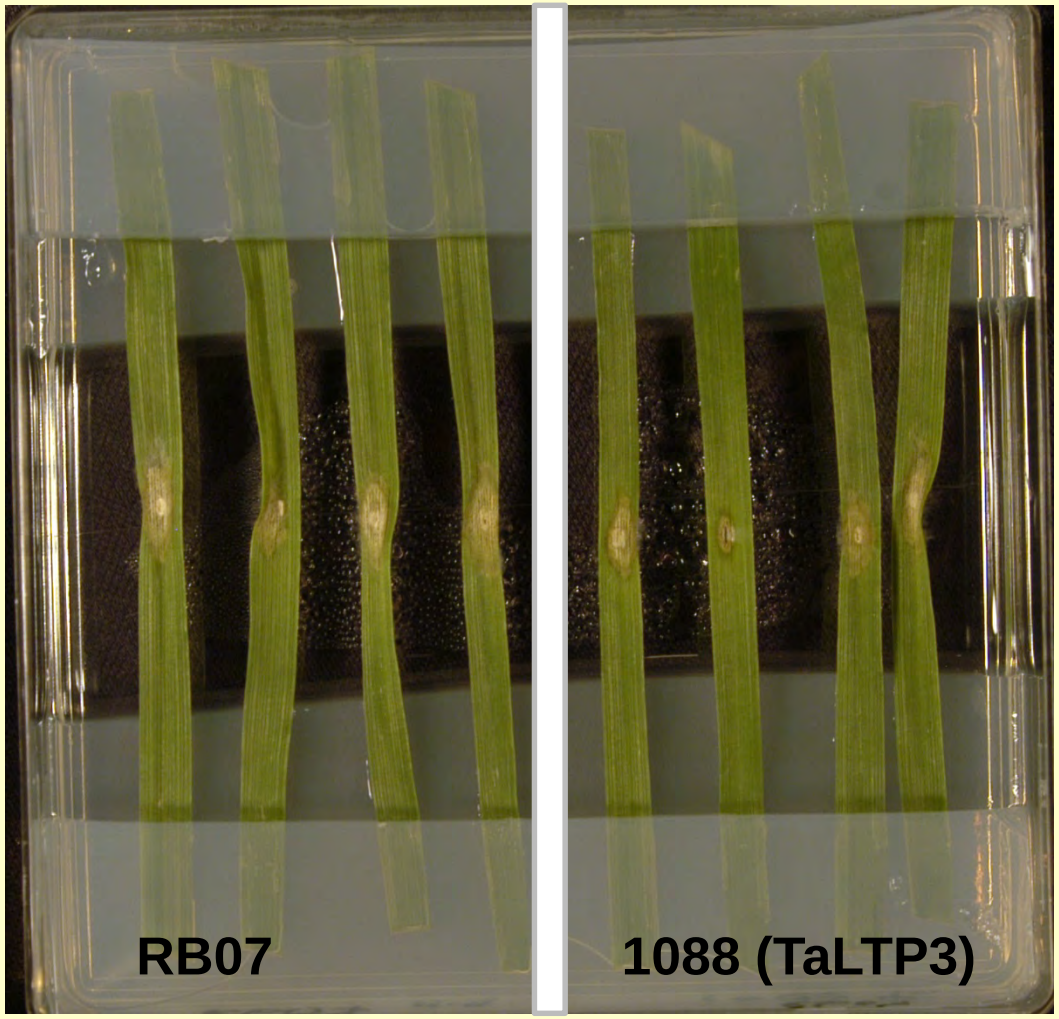
qPCR biomass quantification

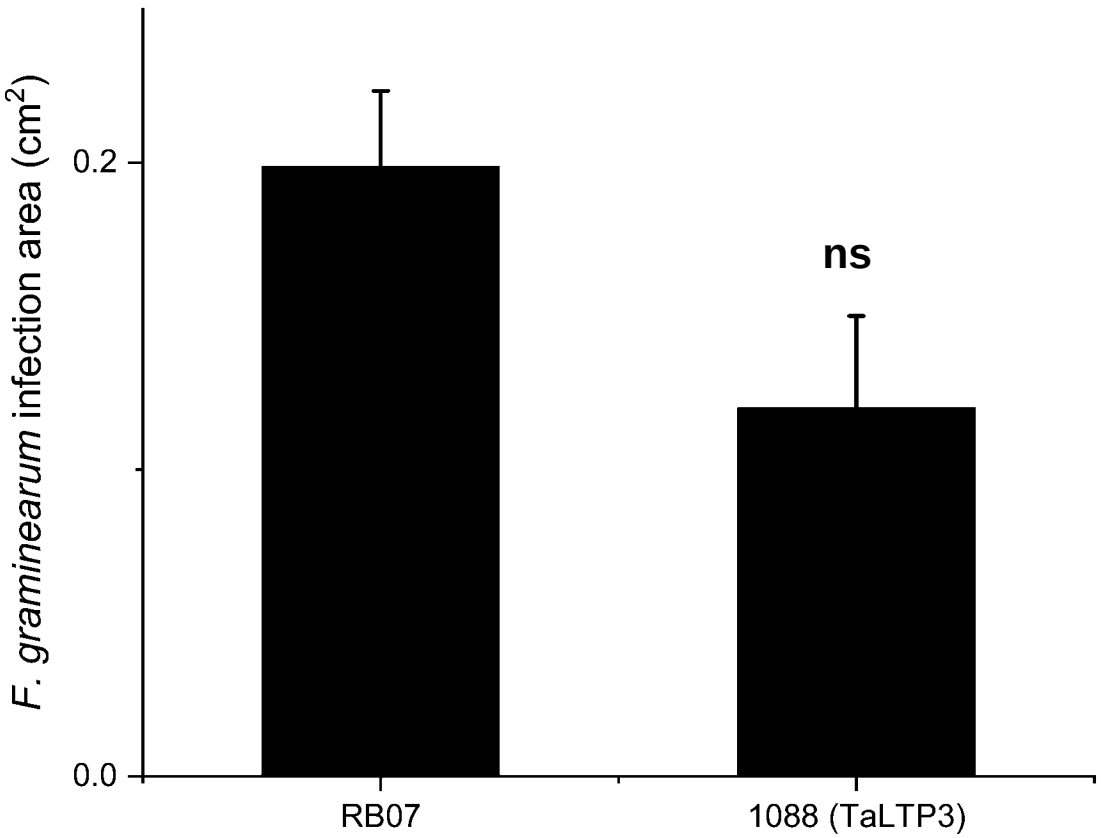


n=8, * (p<0.05), ** (p<0.01)

Primers:PR1 (Wheat), Tri6 (F.g)

Detached leaf bioassay (RB07 vs OE TaLTP3)





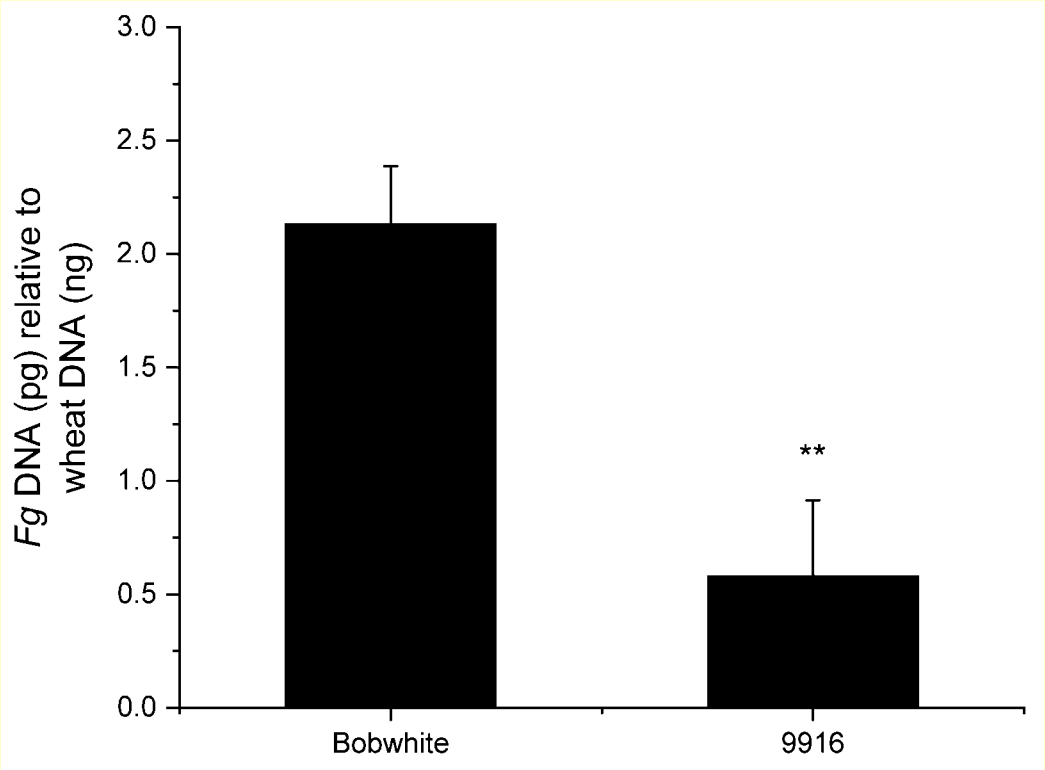
RUTGERS

Treat wheat heads with DON and inoculate with Fusarium in greenhouse

Overexpress *AtLTP4.4* in Bobwhite genetic background

FHB-infected spikes of T_3 plants from event #9916 in Bobwhite overexpressing *AtLTP4.4* 21 days post inoculation.

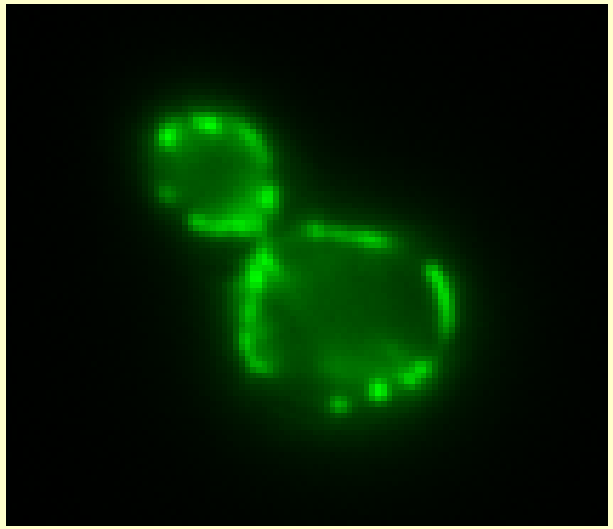
Bobwhite

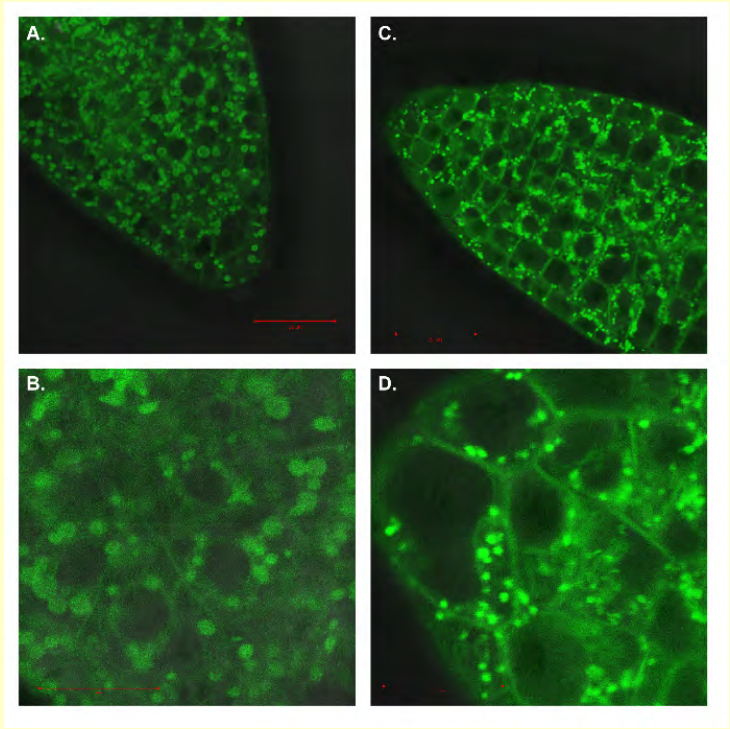


n=8, ** (p<0.01)

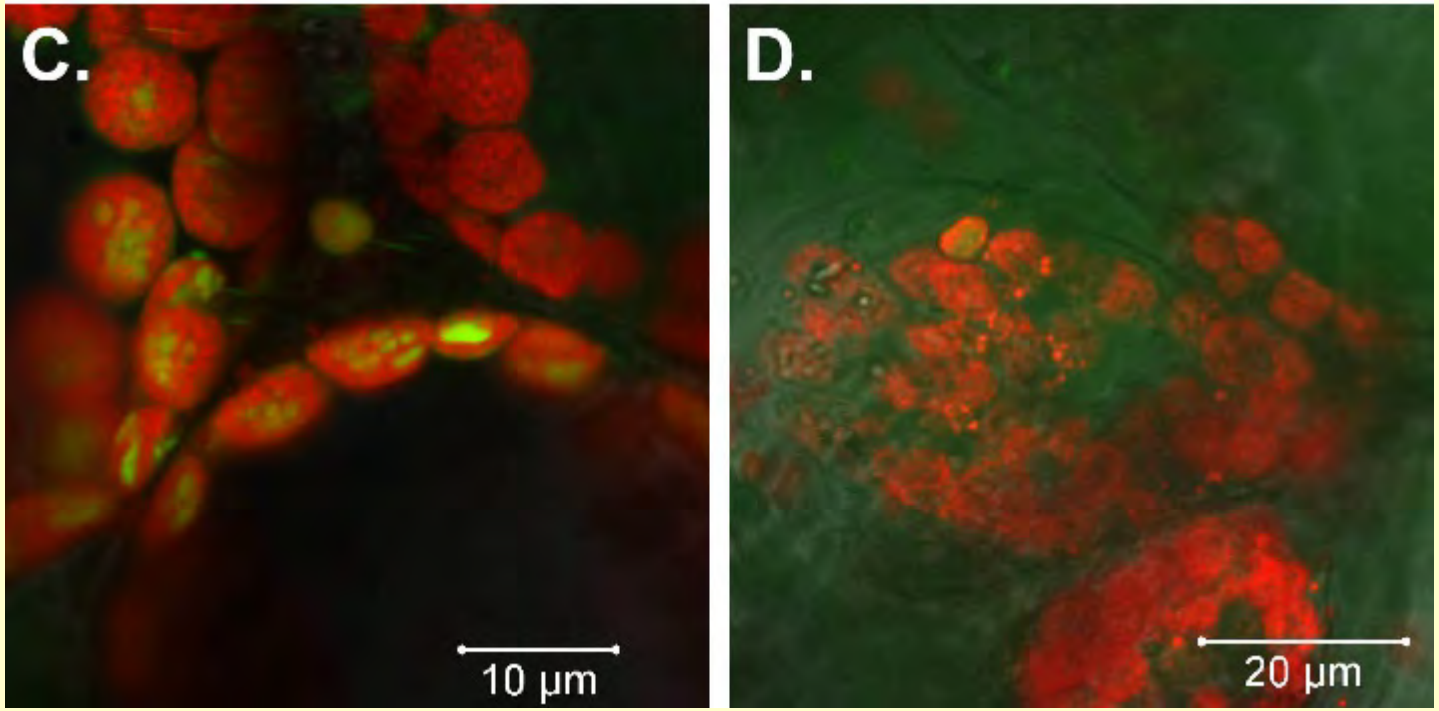
9916(AtLTP4.4:GFP)

Trichothecene impact on yeast mitochondrial and plant chloroplast membranes- lipid peroxidation





Apply DON and ROS sensitive dye to leaf tissue



Lipidomics to discover impact of trichothecenes on plants

- **Quantification of lipid changes in Arabidopsis seedlings exposed to 5 concentrations of Tcin sampled at 0, 24, 48, and 96 hours (triplicate samples prepared).**

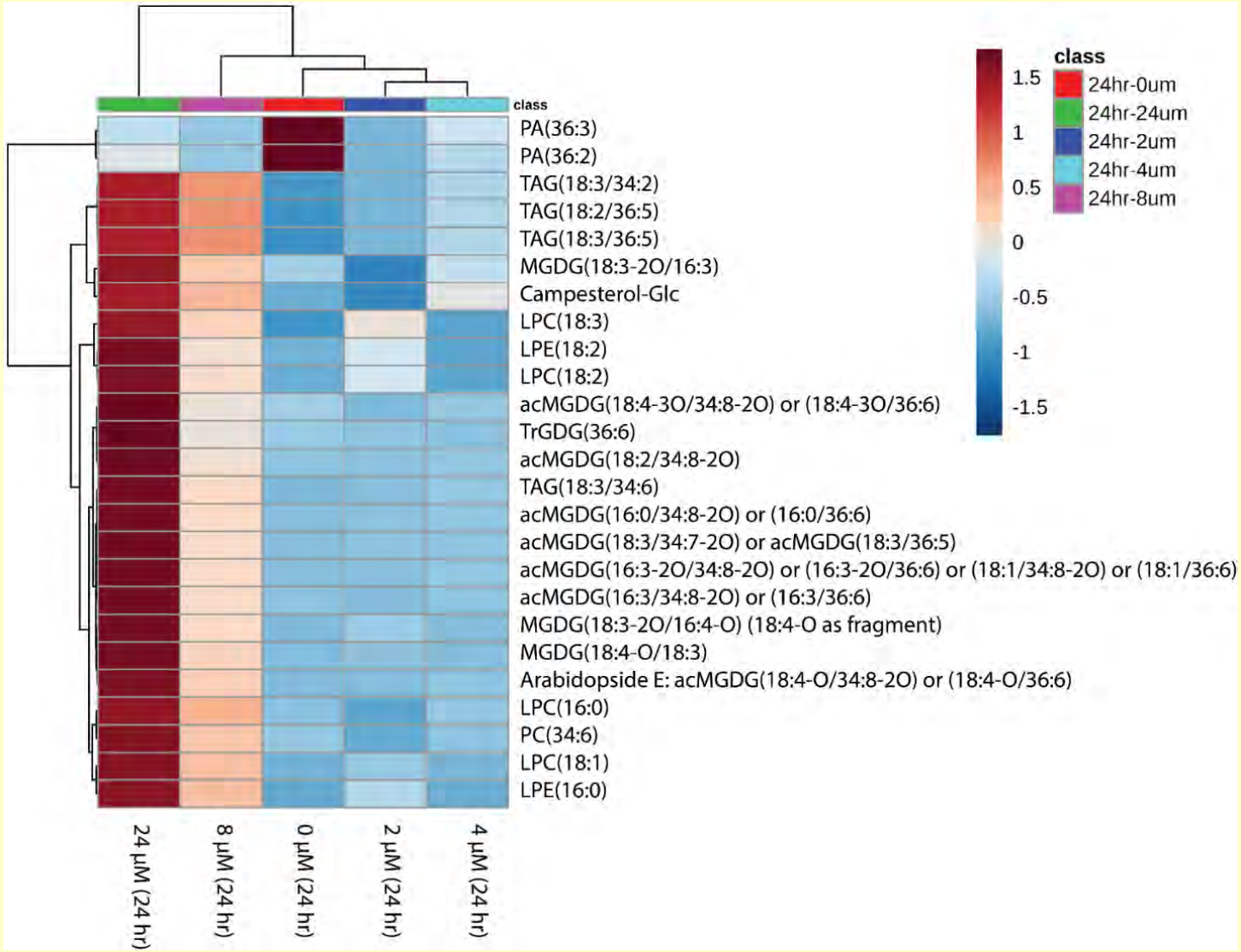
- **Kansas Lipidomics Research Center performed the lipidomics using a Xevo TQS mass spectrometer (Drs. Mary Roth and Ruth Welti, Kansas Lipidomics Research Center)).**

- **Top lipids changed include those found abundant in thylakoid membranes (MGDG and DGDG) indicating potential damage to the chloroplast.**

- **Observe increase in specific oxylipins upon Tcin exposure (ex. Arabidoside D,E).**

Lipidomics to discover impact of trichothecenes on plants

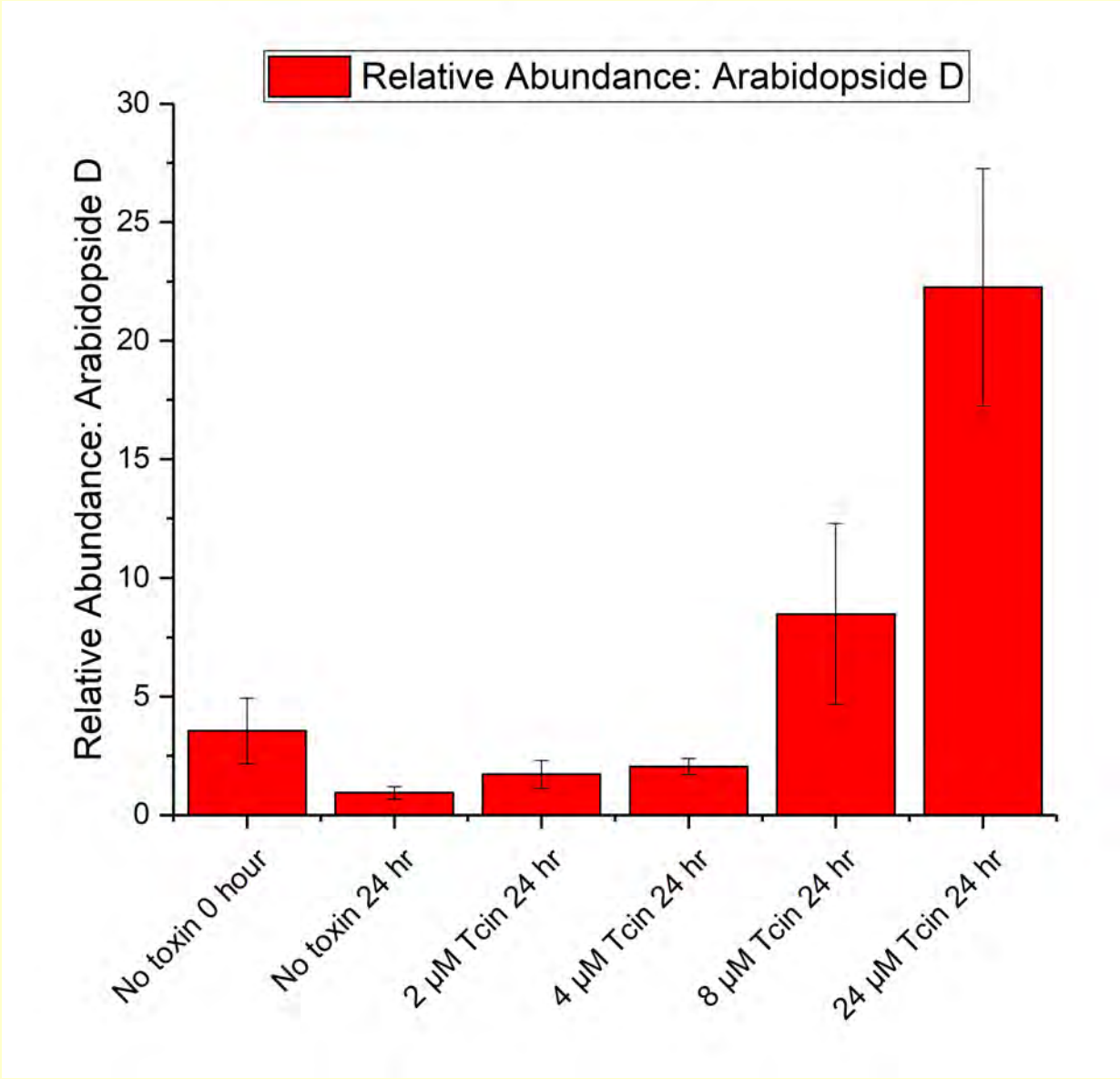
Top 25 lipid changes in Arabidopsis upon trichothecin exposure (24 hours)



See abundance of acylated galactolipids: monogalactosyldiacylglycerol (MGDG) in the top 25 of ~277 lipids analyzed by electrospray ionization (ESI) triple–quadrupole MS. Data analyzed at the Metabo-Analyst website (metabolanalyst.ca)

RUTGERS

Increase in oxylipin Arabidopsis D upon exposure to trichothecin



Arabidopsides are one class of oxylipins: Oxidation products of unsaturated fatty acids.

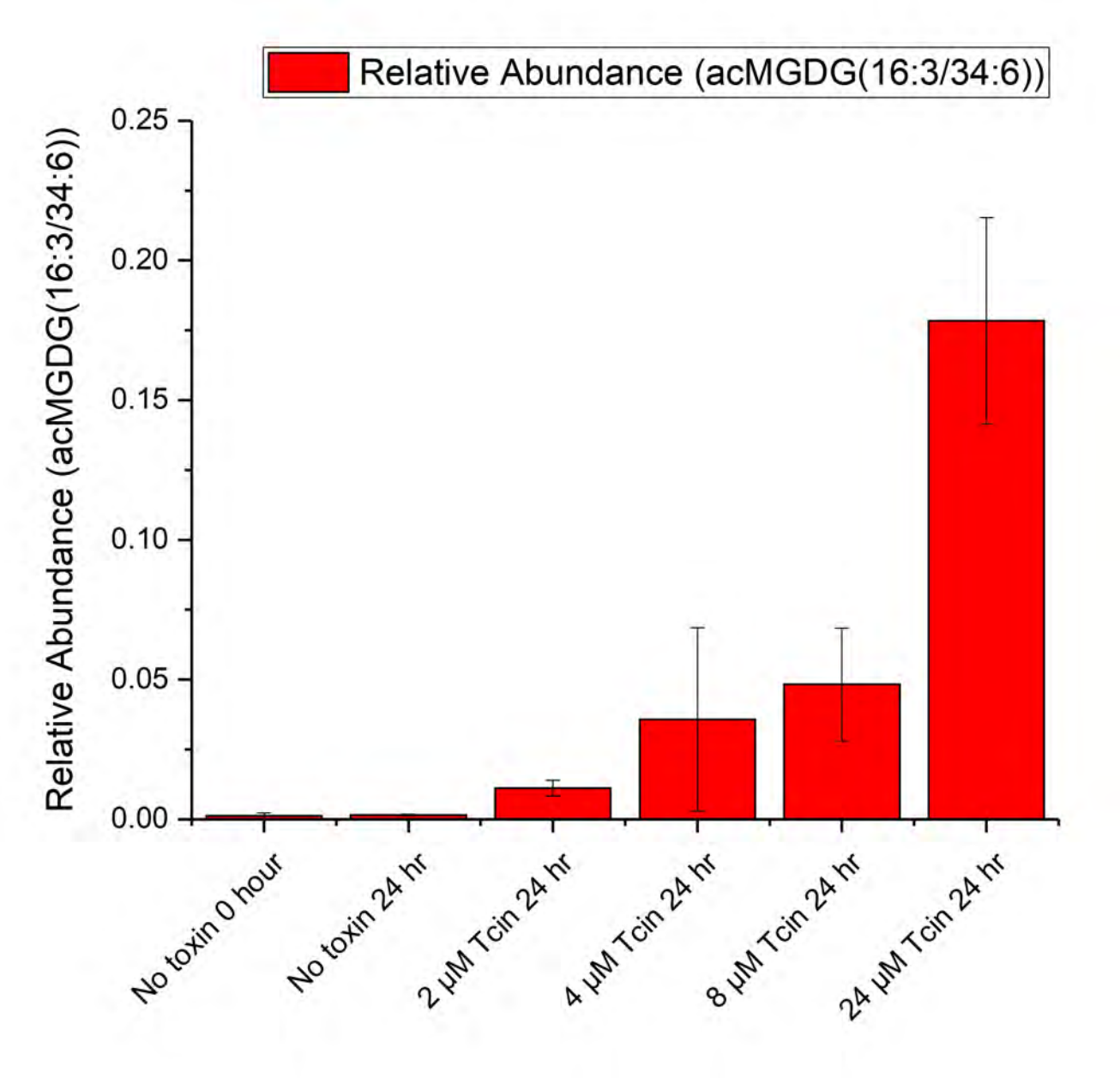
Function as signaling molecules in plants during development, wounding, and insect and pathogen attack.

Can have direct cytotoxic effects on pathogens.

Increases of Arabidopside D may be related defense reactions against pathogens

RUTGERS

Increase in acylated monogalactosyl diacylglycerol (acMGDG) upon exposure to trichothecin



Oxidized acylated MGDGs have been identified in Arabidopsis leaves following freezing stress, wounding, and bacterial infection (Vu et al. 2005). Here we show increases after treatment with Tcin.

Vu et al. 2005. Modifications of membrane lipids in response to wounding of Arabidopsis thaliana leaves, Plant Signaling & Behavior, 10:9.

Future Work

- Seed increase for field studies
- Test transgenic barley
- Characterizing the nsLTP protein using Pichia (lipid binding assays, Fusarium inhibition bioassays)
- Lipid analysis of Arabidopsis/wheat OE nsLTP relative to non-transgenic controls

RUTGERS

Summary

- We identified an nsLTP in Arabidopsis via activation tagging and showed that overexpression of this gene confers resistance to trichothecenes.

- Trichothecenes induce ROS and AtLTP4.4 overexpression reduces ROS levels.

- We have used a new expression vector (B712) and showed that both the Arabidopsis and wheat nsLTP proteins are overexpressed in transgenic Bobwhite and RB07.

- Using confocal microscopy we showed that nsLTPs are expressed in the cell wall/apoplast and in the ER in transgenic wheat.

- Transgenic wheat overexpressing nsLTPs showed improved resistance to DON and *Fusarium graminearum.*

- Lipidomics indicated that Tcin treatment caused major lipid alterations, increases in acylated membrane lipids and many chloroplast membrane lipids with oxidized acyl chains.

RUTGERS

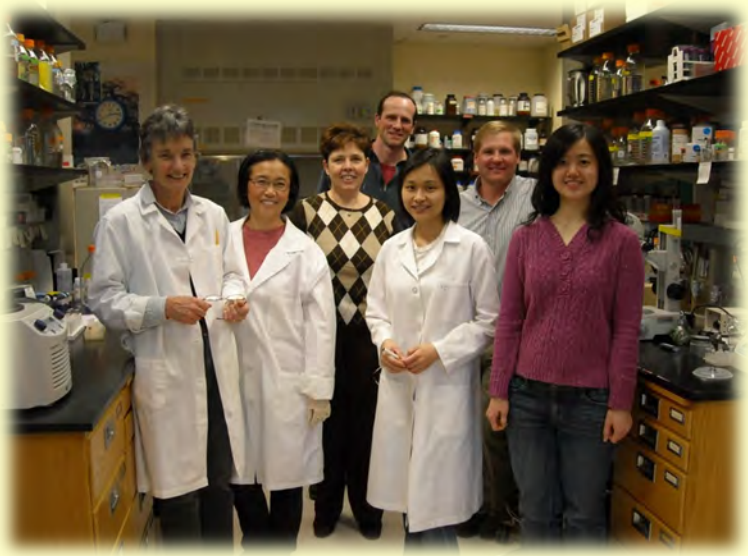
ACKNOWLEDGMENTS

Nilgun Tumer Lab

Dr. Susan McCormick

Dr. Harold Trick and Ms. Neerja Tyagi (Department of Plant Pathology, Kansas State University)

Drs. Ruth Welti and Mary Roth (Kansas Lipidomics Research Center)



Project Funding:

USDA NIFA (Lipidomics)

U.S. Wheat & Barley Scab Initiative