**Cover Page**

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| **Fiscal Year:** | 2015 |
| **USDA-ARS Agreement ID:** | 59-0206-1-121 |
| **USDA-ARS Agreement Title:** | A Genome-Wide Screen to Identify Novel Genes for FHB Resistance. |
| **FY15 USDA-ARS Award Amount:** | $ 65,112 |
| **Recipient Organization:** | Rutgers, The State University of New Jersey Division of Grant and Contract Accounting ASB 111, 3 Rutgers Plaza New Brunswick, NJ 08901-8559 |
| **DUNS Number:** | 00-191-2864 |
| **EIN:** | 22-6001086 |
| **Recipient Identifying Number or Account Number:** | 433617 |
| **Project/Grant Reporting Period:** | 04/24/15-04/23/16 |
| **Reporting Period End Date:** | 04/23/16 |

**USWBSI Individual Project(s)**

<table>
<thead>
<tr>
<th>USWBSI Research Category</th>
<th>Project Title</th>
<th>ARS Award Amount</th>
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<tbody>
<tr>
<td>GDER</td>
<td>Novel Plant Genes for FHB Resistance.</td>
<td>$ 65,112</td>
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**FY15 Total ARS Award Amount**

| FY15 Total ARS Award Amount | $ 65,112 |

* MGMT – FHB Management  
FST – Food Safety & Toxicology  
GDER – Gene Discovery & Engineering Resistance  
PBG – Pathogen Biology & Genetics  
EC-HQ – Executive Committee-Headquarters  
BAR-CP – Barley Coordinated Project  
DUR-CP – Durum Coordinated Project  
HWW-CP – Hard Winter Wheat Coordinated Project  
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:  
  SPR – Spring Wheat Region  
  NWWS – Northern Soft Winter Wheat Region  
  SWW – Southern Soft Red Winter Wheat Region
Project 1: Novel Plant Genes for FHB Resistance.

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

   Our goal is to identify novel genes for Fusarium head blight (FHB) resistance and develop wheat plants resistant to trichothecenes and FHB. We are also investigating trichothecene mechanism of action to understand how these virulence factors function in plants.

   In collaboration with Harold Trick we are generating transgenic wheat plants overexpressing Arabidopsis and wheat non-specific lipid transfer protein (nsLTP) genes, AtLTP4.4 and TaLTP3 (A wheat nsLTP gene: AY226580) in different wheat genetic backgrounds including Bobwhite, RB07, Rollag, and Forefront to determine if expression of these genes in transgenic wheat will confer FHB resistance.

2. What was accomplished under these goals?

   1) major activities

   We identified and Arabidopsis and a wheat non-specific lipid transfer protein (nsLTP) gene, AtLTP4.4 and TaLTP3 (A wheat nsLTP gene: AY226580), respectively.

   We showed that expression of a fusion protein containing EGFP fused to the C-terminus of AtLTP4.4 (AtLTP4.4:EGFP) in yeast and Arabidopsis provided enhanced resistance to trichothecin (Tcin) and other trichothecenes.

   We showed that expression of the wheat nsLTP gene, TaLTP3 in yeast conferred resistance to trichothecenes.

   In collaboration with Harold Trick we generated transgenic wheat plants overexpressing codon optimized AtLTP4.4 in the pAHC17 vector in four different wheat backgrounds, Bobwhite, RB07, Rollag, and Forefront. We were not able to detect protein expression in transgenic wheat lines, possibly because AtLTP4.4 is not stable in wheat when it is expressed by itself. To overcome this problem, we have made fusions with enhanced green fluorescent protein (EGFP) and showed that this fusion protein is expressed in transgenic Arabidopsis plants.

   To overexpress AtLTP4.4 and TaLTP3 as an EGFP fusion in transgenic wheat and barley, we constructed new transformation vectors. For wheat expression, both genes were cloned into B712p7o2x35s-UbiZmF-LGFP (DNA Cloning Services, Germany), which has the Bar gene driven by the CaMV35S promoter and the maize ubiquitin promoter driving expression of the transgene-GFP fusion.

   For barley transformation both genes were cloned into the B835p6o2x35s-UbiZm-LGFP overexpression vector, which has hygromycin (HPTII) selectable marker, and the maize ubiquitin promoter driving expression of the transgene:GFP fusion.
2) specific objectives: To determine if overexpression of \textit{AtLTP4.4} and \textit{TaLTP3} in wheat confers resistance to trichothecenes.

3) significant results: Four overexpression vectors (two for wheat and two for barley) containing the \textit{AtLTP4.4} and \textit{TaLTP3} genes were generated. Expression of the \textit{AtLTP4.4} and \textit{TaLTP3} genes was tested using transient expression assays in tobacco leaves. All four vectors containing the nsLTP genes were found to express the fusion proteins in tobacco leaves.

To generate transgenic wheat plants \textit{TALTP3} and \textit{AtLTP4.4} constructs were provided to Dr. Harold Trick. \textit{TALTP3} bombarded into wheat (Bobwhite) four times and \textit{ATLTP4} three times. \textit{TALTP3} was bombarded into Forefront and RBO7 callus. The plants are on selection media.

4) key outcomes or other achievements: We have identified the cause of the low \textit{AtLTP4.4} expression in transgenic wheat plants and constructed new vectors to overcome this problem.

We have generated a yeast (\textit{Pichia}) strain that overexpresses \textit{AtLTP4.4}, which will be used to isolate protein for the production of antibodies.

Analysis of \textit{Arabidopsis} protoplasts overexpressing \textit{AtLTP4.4} showed reduced reactive oxygen species (ROS) generation at the basal level and after Tcin exposure compared to the non-transgenic control. This result provides further evidence that \textit{AtLTP4.4} protects cells against oxidative stress due to trichothecenes.

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

The project has provided for the training of one MS student (Daniel Finn) and one undergraduate student (Mario Pinzas). Mr. Finn is using \textit{Arabidopsis} protoplasts to investigate the impact of trichothecenes on \textit{Arabidopsis}. He is also screening an \textit{Arabidopsis} activation tagging library to identify additional trichothecene resistant mutants.

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

Dr. John McLaughlin presented our results at the annual National Fusarium Head Blight Forum in December 2015.


Dr. Tumer was an invited speaker at the Corn Utilization and Technology Conference June 6-8, 2016 in St. Louis, MO.
Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 award period. The term “support” below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student’s stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY15 award period? No.
   If yes, how many?

2. Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY15 award period? No.
   If yes, how many?

3. Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant taken faculty positions with universities? No.
   If yes, how many?

4. Have any post docs who worked for you during the FY15 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies? No.
   If yes, how many?
**Release of Germplasm/Cultivars**

**Instructions:** In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY15 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.*

<table>
<thead>
<tr>
<th>Name of Germplasm/Cultivar</th>
<th>Grain Class</th>
<th>FHB Resistance (S, MS, MR, R, where R represents your most resistant check)</th>
<th>FHB Rating (0-9)</th>
<th>Year Released</th>
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Add rows if needed.

**NOTE:** List the associated release notice or publication under the appropriate sub-section in the ‘Publications’ section of the FPR.

**Abbreviations for Grain Classes**
- Barley - BAR
- Durum - DUR
- Hard Red Winter - HRW
- Hard White Winter - HWW
- Hard Red Spring - HRS
- Soft Red Winter - SRW
- Soft White Winter - SWW
Publications, Conference Papers, and Presentations
Refer to the FY15-FPR_Instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY15 grant. If you did not have any publications or presentations, state ‘Nothing to Report’ directly above the Journal publications section.

Journal publications.

Status: Published
Acknowledgement of Federal Support: YES

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

McLaughlin, J., Finn, D., Trick, H., McCormick, S. and Tumer, NE. Expression of a lipid transfer protein in wheat to alleviate oxidative stress induced by trichothecenes - A possible mechanism to increase resistance to FHB. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri, USA. December 6-8, 2015. Poster 21.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: YES

Status: Talk presented.
Acknowledgement of Federal Support: YES

Tumer, N. E. Expression of a lipid transfer protein alleviates oxidative stress induced by trichothecenes and confers resistance to Fusarium Head Blight. Corn utilization and Technology Conference June 6-8, 2016, St. Louis, MO. Invited talk.
Status: Abstract published and talk presented.
Acknowledgement of Federal Support: YES