PI: Nilgun Tumer **PI's E-mail: tumer@aesop.rutgers.edu**

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PROJECT 1 ABSTRACT

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To identify novel plant genes for resistance to trichothecenes, we screened an activation tagged Arabidopsis population and identified two novel lipid transfer protein (LTP) genes, designated as *AtLTP4.4* and *AtLTP4.5*, which were overexpressed in the resistant line *AtTRRF1*. Ltp4.4:GFP and Ltp4.5:GFP fusion proteins, which contain N-terminal signal peptides were localized to the cell wall/apoplast by confocal microscopy. In addition, Ltp4.4:GFP colocalized with chloroplasts and the endoplasmic reticulum (ER) in transgenic Arabidopsis plants. Overexpression of *AtLTP4.4* and *AtLTP4.5* in Arabidopsis demonstrated enhanced resistance to Tcin based on the ability to form roots on solid media containing 4 μM Tcin and reduction of chlorosis and cell death due to Tcin in transgenic Arabidopsis seedlings. We showed for the first time that trichothecenes cause oxidative stress by increasing reactive oxygen species (ROS) in Arabidopsis and in yeast and that *AtLTP4.4* overexpression protects plants against ROS damage. Furthermore, *AtLTP4.4* expression protected yeast against mitochondrial translation inhibition, disruption of mitochondrial membrane integrity and generation of mitochondrial ROS due to trichothecenes. We constructed a monocot expression vector with the *AtLTP4.4* and provided it to Dr. Harold Trick for transformation into wheat. Seven transgenic Bobwhite lines generated using this construct were confirmed to be positive for *AtLTP4.4* gene by PCR analysis. The transgenic wheat plants were phenotypically normal. We screened the entire collection of 5854 open reading frames (ORFs) in the yeast overexpression library and identified novel genes for trichothecene resistance.

The primary goal of this application is to determine if expression of *AtLTP4.4* will provide resistance to DON and FHB in transgenic wheat and barley and in an elite wheat cultivar. In addition, we will determine if overexpression of novel genes identified from the yeast overexpression library screen will protect transgenic plants against DON toxicity and FHB severity. Our specific objectives are:

1. Determine if transgenic wheat lines containing *At LTP4.4* show improved resistance to DON and FHB and protect wheat against ROS damage caused by trichothecene mycotoxins.
2. Determine if overexpression of wheat LTP and novel genes identified from the yeast overexpression screen will confer resistance to DON and FHB in transgenic Arabidopsis and wheat plants.

The novel genes we identified in combination with QTLs that drive FHB resistance may lead to pyramiding of suitable alleles to enhance resistance to FHB in elite wheat and barley cultivars. Furthermore, the novel approaches outlined in this proposal will continue to provide important insights into the mode of action of trichothecene mycotoxins. These studies fit very well with two FY14 research priorities of GDER: 2) Identify candidate genes for resistance against FHB and/or reduced DON accumulation; 3) Develop effective FHB resistance and/or reduced DON accumulation through transgenic strategies.