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PROJECT 1 ABSTRACT

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To identify candidate plant genes for trichothecene resistance, we screened a collection of activation tagged *Arabidopsis* population and identified lines, which were able to grow and form roots on media containing trichothecin (Tcin) at doses, which completely inhibited germination of the wild type. Two of these mutants, *AtTRRF1*and *AtTRRF5*, were characterized by thermal asymmetric interlaced (TAIL) PCR and the genomic location of the insert was mapped. In *AtTRRF1* two lipid transfer protein (LTP) genes (designated *LTP4* and *LTP5*) were overexpressed relative to the control, and in *AtTRRF5* expression of the *TBL35* gene was disrupted due to a T-DNA insertion into this gene. To confirm that overexpression of the *LTP* genes conferred trichothecene resistance, *LTP5* was cloned, an LTP5:GFP fusion was constructed and transgenic *Arabidopsis* lines containing LTP5:GFP fusion were generated. To confirm that the loss of expression of *TBL35* conferred resistance, two independent homozygous T-DNA insertion lines in *TBL35* from the SALK collection were evaluated for resistance to trichothecenes. Both lines were able to germinate and form roots on Tcin, confirming that the resistance was due to the inactivation of *TBL35*. These results validated activation tagging as a valuable strategy to identify candidate plant genes for trichothecene resistance. The primary goal of this application is to determine if the novel genes identified from the activation tagging screen in *Arabidopsis* will confer resistance to DON and FHB in transgenic wheat and barley plants. Since my laboratory has pioneered the use of activation tagging to isolate candidate trichothecene resistance genes, we propose to use this functional genomics strategy to screen an activation tagged population in barley. Our specific objectives are:

- Determine if overexpression of *Arabidopsis* *LTP4* and *LTP5* genes in wheat and barley will confer resistance to DON and FHB.
- Determine if silencing *TBL35* homologs in barley will confer resistance to DON and FHB.
- Develop high throughput approaches to screen an activation tagged barley population to identify genes that can confer resistance to DON and FHB.

Knowledge gained from the proposed studies may lead to the development of FHB resistant wheat and barley plants. Furthermore, the novel approaches outlined in this proposal will provide important insights into the mode of action of trichothecene mycotoxins. These studies fit very well with both FY12 research priorities of GDER: 1) Increase efficiency of identification of candidate genes for resistance against FHB and reduced DON accumulation; 2) Develop effective FHB resistance through transgenic strategies.