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Research Area: **GET** **Duration of Award: 1 Year**

Project Title: Overexpression of Antifungal Genes with Tissue-Specific and *Fusarium*-Inducible Promoters.

PROJECT 1 ABSTRACT

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Our goal is to produce proteins that are toxic to *Fusarium graminearum* directly in the path of the infection, instead of producing the transgene product throughout barley plants. In addition, it would be advantageous to produce this protein only when the tissues are attacked. This requires the cloning of an inducible tissue-specific gene promoter that will direct the expression of any attached anti-*Fusarium* gene.

A germin-like protein gene (*GerF*), with preferential expression in the barley epicarp and lemma, was previously cloned. Studies showed that this promoter is strongly induced by infection of spikes with *Fusarium graminearum*. Thus, it appears to be an ideal tissue-specific inducible promoter. The *GerF* promoter will be linked to a green fluorescent protein (*gfp*) reporter gene, with and without an accompanying lemma thionin anti-*Fusarium* gene (Thio1841), and used to stably transform barley through the Agrobacterium system. Since germin is also an antifungal protein, the unaltered GerF gene will also be used to enhance germin levels. The peptide encoded by Thio1841 was shown to be lethal to *Fusarium*. Transformants will later be tested for resistance to *Fusarium*.

Transformants currently being produced in the current FY05 grant cycle will initially be tested for resistance in the FY06 cycle, when developing T_2 seeds become available. These transformants, expressing the Thio1841 gene under the control of the Lem2 lemma/epicarp promoter, will be propagated, and spikes of T_1 plants (T_2 seeeds) will be tested in vitro for *Fusarium* resistance. Seed will be amplified for later field testing.

Goals:

1. Insert cloned GerF promoter/coding gene constructs (GerF/gfp, GerF/Thio1841, and unaltered GerF nuclear gene) into Agrobacterium vector and transform.
   a. Analyze tissue-specificity of transformants.
   b. Test for thionin and germin expression and resistance to F.g. (in vitro tests with excised seeds).
2. Develop T_2 plants of current Lem2 promoter/Thio1841 Agrobacterium-mediated transformants and conduct initial (in vitro) *Fusarium* resistance studies.

This research corresponds to USWBSI Biotechnology goals of 1) developing methods for testing expression of antifungal genes in transgenic material, 2) transforming barley with anti-*Fusarium* genes and testing their effectiveness, 3) identification of promoter sequences to target transgene expression to specific spike tissues and targeting products to effective subcellular compartments.