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

Project Title: Engineering Scab-Resistant Wheat by Employing Oxidative Defense Responses.

PROJECT 1 ABSTRACT
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The goal of the proposed work is to use genetic transformation to create new sources of hexaploid and durum wheat germplasm with host plant resistance to scab. Transgene loci, by their very nature, carry molecular markers and thus can be readily introgressed into varieties adapted for growth in regions most severely affected by scab. In previous years, we tested several genes from *Fusaria* for their ability to protect against Fusarium head blight (FHB) when expressed in wheat. These have not proved effective in conferring useful levels of resistance, perhaps because they were poorly expressed in wheat cells. Thus far, sequence modifications we designed to increase mRNA accumulation of transgenes with *Fusarium*-derived coding sequences have been unsuccessful, although this work is still ongoing. In parallel with these experiments, we have characterized the expression specificity of the three promoters: maize *Ubiquitin1*, maize Glutamine Synthetase, and barley *Lem1*. Under the current funding, we showed that the *Lem1* promoter is active in the outer organs of the floret of transgenic wheat from anthesis to the soft dough stage, making it ideal for expression of anti-*Fusarium* genes. For 2004, we propose to utilize this specificity to implement a new strategy to engineer host plant resistance by expressing the *Aspergillus* Glucose Oxidase (GO) enzyme in wheat florets under control of *Lem1*. GO uses glucose to generate hydrogen peroxide, a type of Reactive Oxygen Intermediate (ROI) that has multiple anti-microbial effects, including direct growth inhibition and mobilization of plant defense signaling networks. Elevated H_2O_2 levels in wheat cells will be utilized by co-expressed barley peroxidase (PRX) genes that use hydrogen peroxide as a substrate in catalyzing cell wall lignification. Transgenic hexaploid wheat plants containing the *Lem1*:*GO* construct with and without the *Lem1:Prx* constructs will be characterized for enzyme activity, H_2O_2 content, cell damage, changes in cell walls, and inhibition of *Fusarium* growth *in vitro*.

In 2004, we will also continue to work towards making wheat cells resistant to the trichothecenes produced by *Fusaria* as virulence factors during infection. We will continue to evaluate the efficacy of the *TRI101* and *TRI12* genes in detoxifying DON in transgenic wheat. We will test whether or not increasing levels of ribosomal protein L3 will protect wheat cells from the mycotoxins. Making wheat resistant to trichothecenes and employing wheat's oxidative cell defenses are synergistic strategies. We will combine effective transgenes by genetic crosses. Our work addresses the needs of USWBSI Biotechnology Research Area, in particular by evaluating the efficacy of new anti-*Fusarium* proteins in enhancing host plant resistance, by developing methods to measure and localize such proteins, and by demonstrating the utility of a barley floret-specific promoter in wheat. By constructing plants that contain the proposed transgenes, and by evaluating the resultant changes in wheat's biochemistry and resistance, we advance the USWBSI goal of attaining cost-effective and environmentally friendly control of FHB.