PI: Ann Blechl
PI's E-mail: ablechl@pw.usda.gov

Project ID: 0506-BL-029
FY04 ARS Agreement #: NA

Research Area: BIO
Duration of Award: 1 Year

Project Title: Engineering Scab Resistance in Wheat by Combining Diverse Defense Mechanisms.

PROJECT 1 ABSTRACT
(1 Page Limit)

The goal of the work in this proposal is to develop wheat germplasm with host plant resistance to Fusarium head blight (FHB), the most cost-effective way to control this disease. Prominent among wheat's earliest responses to invasion by *Fusarium* and other pathogens is the generation of reactive oxygen species (ROS) that, in turn, activate defense genes including those encoding pathogenesis-related (PR) proteins such as glucanases, chitinases and peroxidases. Although research studies have shown that transgenic wheats engineered to constitutively express various PR genes often have improved Type II resistance, this strategy alone has been insufficient for engineering field resistance to FHB. Our strategy is to engineer ROS production in the outer tissues of the florets before anthesis, when wheat is most vulnerable to *Fusarium* infection. To accomplish this, we have introduced the *Aspergillus* glucose oxidase (*GO*) gene into wheat. Glucose oxidase is an apoplastic enzyme not naturally found in plants that catalyzes oxidation of β-D-glucose, generating H_2O_2, a ROS with multiple functions in plant defense. To act synergistically, we have also introduced barley genes for two peroxidases, *Prx7* and *Prx8*, whose expression is part of the defense response of leaves exposed to powdery mildew. All three of our anti-Fusarium (AF) genes are controlled by the barley *Lem1* gene promoter so that their expression is high in the outer floret organs at anthesis and is excluded from grain. Thus far, we have generated 100 transgenic lines carrying one or two of the candidate AF genes. We have shown that the transgene-encoded enzymes accumulate in the outer floral organs. Co-expression of these AF genes is expected to synergistically improve host resistance to initial fungal infection (Type I). We have shown that the recombinant GO and Prx8 proteins are targeted to the apoplast of the lemma and palea, where they would interact with the fungus during the initial biotrophic stage of infection. Hydrogen peroxide generated by GO could inhibit fungal growth directly. Intercellular Prx8 could mediate callose deposition and papilla formation, providing a physical barrier to infection. Intracellular Prx7 stored in the vacuole could encounter the fungus after the shift from the biotrophic to the necrotrophic stage of its interaction with the host. The transgenic plants are also expected to possess improved resistance to fungal spread (Type II) based on ROS-mediated activation of genes encoding PR proteins and accumulation of salicylic acid (SA). Our specific objectives for this funding period are to test singly and in combination these new AF genes for their abilities to 1) confer Type I, II and overall FHB resistance; 2) trigger accumulation of SA and the natural PR proteins of wheat; and 3) change the antioxidant status in the apoplast of outer floret tissues. This work addresses two objectives of the Biotechnology component of the scab Initiative: 1) "Transform wheat, barley, and durum to demonstrate the effectiveness of anti-*Fusarium* transgenes to limit damage resulting from *Fusarium* infection" and 2) "Develop methods to detect and measure levels of antifungal proteins encoded by transgenes."