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Research Category: **PBG** **Duration of Award: 1 Year**

Project Title: Development and Testing of Improved Enzymes for Transgenic Control of FHB.

PROJECT 1 ABSTRACT

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The primary goal of this proposal is to develop improved enzymes for the inactivation and degradation of fungal mycotoxins associated with Fusarium head blight and test their efficacy in barley. In the next year we plan to optimize the efficacy of trichothecene 3-O-acetylase from F. graminearum (Tri101) for inactivation of DON and nivalenol in barley. This will be accomplished by protein engineering starting from the structures and kinetic analyses of Tri101 from F. sporotrichioides and F. graminearum that were completed during the previous funding cycles. The prospect of success in this first phase is high because the kinetic analysis of this enzyme suggest that there are already significant differences in specificity between isozymes from different fungi. An important component of the investigation is the established program to test the improved enzymes against FHB in barley and as such this proposal represents an interdisciplinary collaboration. We also plan to continue to integrate the in vitro studies of Tri101 with the properties of the enzyme expressed in transgenic cereals to investigate whether the limited performance of the transgenic cereals is due to low expression, or inactive or posttranslationally modified protein. Thus the specific aims of the project are:

1. to apply protein engineering to the trichothecene 3-*O*-acetylase (Tri101) from *F. sporotrichioides* and *F. graminearum* to improve the function and stability of the enzyme and the expression level *in planta*. This is the first priority, since *Tri101* has been shown to provide partial protection against the spread of *F. graminearum* in transgenic wheat.
2. to test the efficacy of the new enzymes in providing resistance to FHB in barley. The improved genes will be inserted into plasmid pBract214 and transformed by Agrobacterium into barley to create transgenic strains. These will be tested for resistance to FHB once homozygous lines are identified.
3. to correlate the structure and function of the Tri101 protein produced in *E. coli* with that isolated directly from transgenic barley. This will ascertain the level of activity of the enzyme expressed in transgenic barley and establish a connection between the *in vitro* and *in vivo* studies of Tri101. This will establish a biochemical foundation for assessing the efficacy of Tri101 as a protective agent against FHB.

This proposal is consistent with the research priorities of both PBG and GDER.