PI: Ronald W. Skadsen **PI's E-mail: rskadsen@wisc.edu**

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

Project Title: Transformation of Barley with an Antifungal Gene Under Control of an Epicarp Promoter.

PROJECT 1 ABSTRACT
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The overal goal of the proposed research is to produce barley that is resistant to the Fusarium graminearum pathogen by expressing a barley seed antifungal hordothionin gene in the path of infection.

Goals: 1) Construct *Hth5* and *Hth6* vectors and perform transient expression testing on pericarps. 2) Stably transform Conlon with *Hth5* and *Hth6*. 3) Analyze transformants for HTH protein synthesis. 4) Perform preliminary anti-Fusarium testing on transformant epicarps.

In the proposed research, barley (Conlon cv.) will be genetically transformed with two improved vectors for the expression of modified hordothionin genes *Hth5* and *6*. In both vectors, a promoter that directs expression to the pericarp epithelium (epicarp) will be used. This promoter was cloned in this lab and shown to direct *gfp* expression to the epicarp in stable transformants. The *Hth5* modified gene contains no signal sequence and has two rare codons modified. This construct was proved to be translated in lemmas and leaves in a transient expression system, whereas an *Hth* gene previously used for stable transformation was not translated. *Hth6* is identical to *Hth5* but contains an *Ltp6* signal sequence, which should direct hordothionin secretion to the extracellular space. In the 5/04-4/05 grant period, transformants will be tested for Fusarium resistance by topical application of spore suspensions onto ovary epithelial hairs. Lines with apparent resistance will be tested in the field in the following year.

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.