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Project Title: Tissue-Specific Overexpression of Antifungal Lemma Thionin Genes in Barley.

PROJECT 1 ABSTRACT
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Thionin antifungal genes normally expressed in the lemma and palea of barley will be cloned and used to transform barley to increase levels of antifungal thionin proteins. Expression will be targeted to the main routes of Fusarium infection, the lemma/palea and epicarp. Transformations include the vacuolar and cell wall thionin genes; the vacuolar form will also be recloned with a lipid transfer protein signal sequence to facilitate thionin secretion. Transformation will be by means of the Agrobacterium system to improve expression characteristics. An improved Agrobacterium vector will be developed with hygromycin selection resistance gene *HPT* under control of *Ubi1* and *gfp* selection, which should increase the efficiency of selection.

Goals:

1. Clone genes for lemma vacuolar and cell wall forms of thionin.
2. Synthesize mature peptides for vacuolar thionin and seed-specific thionin, and conduct in vitro anti-Fusarium testing.
3. Develop lemma thionin antibodies from vacuolar mature peptide.
4. Construct Agrobacterium transformation vectors with lemma thionin genes (cell wall-specific, vacuolar with native signal sequence, and vacuolar with *Ltp6* signal sequence) driven by *Lem2* (lemma/palea and epicarp) gene promoter.
5. Transform barley and test for thionin expression and resistance to F.g.
6. Cross transformants overexpressing vacuolar and cell wall thionins.

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.