0203-SK-102 Genetic transformation of barley with an altered hordothionin gene.

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PROJECT ABSTRACT
(1 Page Limit)

This research will focus on the transformation of barley with a hordothionin protein gene. The product of this gene, hordothionin (HTH), is highly toxic to *Fusarium graminearum*, the pathogenic fungus that causes head blight, or scab. Normally, HTH is produced in the starchy endosperm of the developing seed and is a safe dietary protein. We will attempt to constitutively express the gene so that HTH will be present in the path of Fusarium infection. For barley, this would involve the outer husk layers that enclose the developing seed. We have transformed barley with a full-length hordothionin gene (Hth-1), but this resulted in very low mRNA levels. In the current grant period, barley was transformed with an altered gene (Hth-2), achieving high expression at the mRNA level. We have also produced high titre specific antibodies to the HTH mature protein. Studies with these antibodies indicate that the leaves of our T_0 plants do not produce HTH protein. The proposed studies are designed to correct the likely cause of this problem and to produce basic knowledge and tools for improving gene expression in barley and wheat.

Studies will be conducted in three areas. 1) Since we have small numbers of seeds, we have not tested spike tissues. In the proposed research, we will test for HTH protein in other tissues of T_1 plants of Hth-2 transformants, including the lemma/palea and early developing seeds. In addition, oat that has previously been transformed with barley Hth, and producing mRNA in leaves (but no HTH protein), will be screened for HTH production in seeds. We will conduct translational control studies to determine why the *Hth-2* did not result in HTH protein accumulation. In preliminary studies, Hth-2 mRNA was found on leaf polyribosomes, indicating that the nascent HTH peptide may be degraded because it does not enter the endomembrane system. Studies will be conducted to determine whether the failed to attach to membrane-bound polyribosomes. In vitro translation will also be conducted to determine whether the polyribosome complex and mRNA are functional. These studies will provide insights for producing this protein and, possibly, thionins currently being utilized by other USWBSI researchers.

2) An expression vector will be designed from our current Gfp expression vector to allow preliminary screening of constructs through transient expression analysis in spike tissues. This will determine whether a specific transgene will function in the target tissue before stable transformation is conducted. The vector will be constructed so that parts or all of a candidate gene can be linked in frame with the downstream Gfp reporter gene.

3) Stable transformants will be produced in which the signal sequence of our seed Hth cDNA is exchanged with the signal sequences for two barley leaf-specific thionins.