0203-BR-038 Marker- and Plasmid-free Transgenic Barley Encoding Antifungal Proteins.

PI: Bregitzer, Phil; E-mail: pbregit@uidaho.edu
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PROJECT ABSTRACT
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Production of barley germplasm resistant to Fusarium head blight (FHB) may be accomplished by the non-sexual introduction of genes encoding antifungal proteins (AFPs). The goal of our proposed research is to produce transgenic barley which express potential antifungal proteins, but which do not contain selectable markers or plasmid DNA. Essential elements of the maize *Ac-Ds* transposable element system will provide the mechanism for separating the antifungal protein expression cassette from surrounding plasmid sequences. In an intial approach, we propose to: 1) Construct a *Ds*-bordered, ubiquitin-driven or actin driven *tlp1* (thaumatin-like protein) or *tlp4* expression cassette and introduce the resultant plasmid together with pAHC20 (ubiquitin-driven *bar-nos*) into *in vitro* cultured barley cells of elite 6-rowed germplasm via biolistic bombardment. 2) Produce transformed plants that express *tlp1* or *tlp4* and characterize them at the molecular level and for FHB resistance. 3) Move *Ac*-transposase activity into elite, 6-rowed germplasm by backcrossing and transformation. 4) Cross-hybridize *tlp1* or *tlp4*-containing plants with a 6-rowed *Ac*-transposase stock to mobilize *tlp*. 5) Select *tlp1*- or *tlp4*-positive, plasmid- and transposase-free recombinant progeny. Other potential AFP's, such as TR101 and TRI 12, will also be manipulated in a similar way. This research would meet the goals of the USWBSI in two ways. First, the production of transgenic barley containing *tlp1* or *tlp4* in an agronomically elite, 6-rowed malting background will enable FHB resistance assays that are more relevant to the germplasm in which resistance is needed (relative to Golden Promise-derived transformants). Such a system should enhance the rapidity with which candidate AFPs can be screened for efficacy. Second, the production of transgenics containing only the gene of interest, without plasmid and marker sequences, should facilitate public acceptance of transformation technology, and thus increase opportunities to use transgenically-encoded FHB resistance in commercial germplasm.