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PROJECT 1 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 goals of our proposed research are to produce transgenic barley which express potential antifungal proteins, but which do not contain selectable markers or plasmid DNA, or which express the AFPs in chloroplasts. Essential elements of the maize *Ac-Ds* transposable element system will provide the mechanism for separating the antifungal protein expression cassette from surrounding plasmid and marker gene sequences. Green tissue cultures systems will provide the basis for the ability to do chloroplast transformation. In an initial approach, we propose to: 1) Construct vectors designed for nuclear transformation which are *Ds*-bordered, ubiquitin-driven or actin driven AFP expression cassettes; or construct vectors for chloroplast transformation, which contain an AFP regulated by independent 5' and 3' regulatory regions and flanked by the sequences from the barley chloroplast genome to facilitate insertion, and introduce the resultant plasmids into *in vitro* cultured barley cells via biolistic bombardment. 2) Produce transformed plants that express AFPs 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 *Ds* AFP-containing plants with a 6-rowed *Ac*-transposase stock to mobilize the AFPs. 5) Select AFP-positive, plasmid- and transposase-free recombinant progeny. This research would meet the goals of the USWBSI in two ways. First, the production of transgenic barley containing AFPs 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, or which contain the gene of interest in the chloroplast genome and therefore not transmitted through pollen to nontransgenic plants, should facilitate public acceptance of transformation technology, and thus increase opportunities to use transgenically-encoded FHB resistance in commercial germplasm.