0203-DA-023 Spike-Specific Promoter Isolation from Bowman and Near-Isogenic Marker Lines.

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PROJECT ABSTRACT
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Current promoters used to control genes introduced by transformation give constitutive gene expression. Fusarium head blight mainly attacks the spikes of wheat and barley, so constitutive expression of potential resistance genes is an inefficient use of the plant's resources. The objective of the proposed research is to isolate spike-specific promoters for targeted transgene expression in wheat and barley. The first step to isolate such promoters is to identify genes that are only expressed in spike tissue. mRNA differential display techniques have been used to compare gene expression in Bowman spike tissues to genes expressed in the rest of the plant and to compare expression in normal and mutant tissues from near-isogenic morphological marker lines. Morphological markers used include: Blp1.b, black lemma and pericarp; Gle1.a, glossy lemma and pericarp; Pre2.b, red lemma and pericarp; gsh6.s and gsh2.f, glossy sheath and spike; alm1.a, albino lemma; yhd1.a, yellow head; glo-a.1003, globosum; cer-I.16, glossy spike; and rob1.a, orange lemma. So far, fifteen clones have been confirmed as being expressed in lemma, palea, and rachis tissue but not in leaf tissue, including two from Gle1.a comparisons, two from rob1.a, five from glo-a.1003, and six from cer-i.16. These will be sequenced, hybridized to restriction enzyme-digested genomic DNA, and those that are low copy number will be used to isolate BAC clones containing the full-length genes. Upstream controlling regions for each gene will be isolated and tested in a transient expression assay to determine the precise time and location of expression of each promoter. Potentially differentially expressed genes from additional comparisons will be cut out of the differential display gels, subcloned, and re-amplified. Candidate genes will be tested on reverse northern blots to identify those that show spike-specific expression, and confirmed differentially expressed genes will be treated as described above. Promoter sequences that drive gene expression in the appropriate tissues at the desired times will be used for barley transformation and made available for other transformation researchers.