

**DEVELOPMENT OF PLASTID TRANSFORMATION VECTORS TO ENGINEER COTTON WITH
RESISTANCE TO ASPERGILLUS FLAVUS**

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Abstract

To effectively reduce aflatoxin levels in cottonseed to meet increasingly stringent regulatory levels, a variety of approaches will be necessary. We are pursuing a genetic engineering approach to develop cotton with enhanced resistance to *Aspergillus flavus*. We have identified several structural genes that confer enhanced resistance to *A. flavus* either *in vitro* or *in vivo*. Introduction of antifungal genes by *Agrobacterium*-mediated transformation has a number of disadvantages including transgene silencing, low level expression of the transgene, and transgene drift. To avoid these pitfalls we are pursuing plastid transformation as an alternative methodology to introduce and express genes in cotton. Our first goal is to identify plastid promoters that are appropriate for expression of antifungal traits during a variety of conditions and developmental stages. The ideal promoter would be active in cottonseed and at early stages of germination and development in both green and non-green tissues. We have tested 20 genes for expression in light and dark grown tissues, as well as tissues at various stages of development by dot blot analysis. From these results, 7 genes were identified for further expression studies using real time quantitative PCR. The promoters which best fit our criteria for expression will be engineered into expression cassettes that include selectable marker genes, sequences required for site-specific integration into the plastid genome, and cloning sites for introduction of the genes of interest.