# INTRODUCTION OF PATHOGEN RESISTANCE FACTORS IN TO COTTON AND TOBACCO BY GENETIC TRANSFORMATION

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## Abstract

We are evaluating several antifungal factors for their activity in transgenic cells and callus cultures of cotton and tobacco. These factors include a linear, synthetic peptide (D4E1); a 14 kDa trypsin inhibitor gene identified from corn varieties resistant to *Aspergillus flavus* and a haloperoxidase gene construct of *Pseudomonas* origin.

*Agrobacterium* – mediated transformation of cotton has been accomplished with the antifungal peptide D4E1. Thus far, transformation of cotton has been carried out with *Agrobacterium* vectors containing the D4E1 gene driven by 35S:CaMV, ubiquitin 3 or ubiquitin 7 promoters. Several transformed callus cultures, identified by Southern hybridization of D4E1 – PCR products, have been induced to become embryogenic from which transgenic cotton plants have been developed. *In vitro* bioassays were conducted to test antifungal activity of transformed cotton callus cultures against germinated conidia of *Fusarium moliniforme*. Previous studies have indicated that the purified synthetic peptide D4E1 inhibited the further development of germinated conidia of *F. moniliforme* at concentrations as low as 1.5 µM. Preliminary bioassays using extracts from tobacco tissues transformed with the D4E1 constructs showed growth inhibition of *F. moniliforme* conidia by 50 to 80% compared to non-transformed tobacco controls. Similar bioassays with the transformed cotton cultures are in progress.

The potential of haloperoxidase (HPO) as an antifungal agent has been demonstrated using transgenic tobacco model system. Integration and expression of haloperoxidase in transgenic tobacco has been demonstrated by Southern and Western analyses. Halogenating activity due to the presence of haloperoxidase enzyme activity in transgenic tobacco has also been documented. The mode of action of haloperoxidase utilizes the fact that the plants generate $H_2O_2$ in response to invading pathogens. In the presence of haloperoxidase, enzymatic halogenation occurs which results in the formation of two potent microbicides – peracetic acid and hypochlorous acid. Bioassays of tobacco extracts for efficacy against fungal pathogens and transformation of cotton with haloperoxidase are in progress.

Tobacco plants transformed with the Trypsin inhibitor gene have been obtained. Expression of the protein in transgenic tobacco has also been documented by Western analysis. Antifungal activity of transgenic tissues has not been determined yet.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:489-489 (1998)
National Cotton Council, Memphis TN