2002 BELTWIDE COTTON CONFERENCES, ATLANTA, GA – JANUARY 8-12

CONTROL OF PATHOGENIC AND MYCOTOXIGENIC FUNGI BY TRANSGENIC COTTON PLANTS EXPRESSING A SYNTHETIC PEPTIDE

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Abstract

Transgenic cotton plants expressing a gene coding for the synthetic peptide D4E1 have been identified through PCR and RT-PCR. The disease-resistant phenotype of transgenic plants expressing D4E1 is very promising. For example, *in vitro* assay results using crude leaf extracts from transformed cotton plants (R_0 and R_1) showed significant inhibition of the growth of pre-germinated spores of *Verticillium dahliae*, a cotton wilt pathogen that is very sensitive to D4E1. In a similar in vitro assay for the aflatoxin-producing fungus, *Aspergillus flavus*, leaf extracts from transgenic cottons reduced the number of colony forming units although the results were not highly significant compared to controls. This is possibly due to either sub-optimal level of gene expression or possible rapid degradation of the antifungal peptide in ground extracts by plant proteases. For example, we observed rapid degradation of D4E1 added to cotton leaf extracts within 30 minutes of incubation. *In situ* assays using immature cottonseeds, inoculated with Green Fluorescent Protein-expressing *A. flavus* strain, showed that the transgenic plants are capable of delaying and/or reducing the fungal advance in both seed coat and cotyledons. Detection and quantification of this small peptide (17 AA) in transgenic plant extracts poses a technical problem and availability of suitable antibodies is lacking thus far. In addition, synthetic peptides have a highly positive charge, tend to aggregate and as a result do not migrate into the gel properly making it difficult to perform western blots. Currently, we are increasing seed from R_1 progeny to assay for resistance to seedling diseases caused by any one of the following pathogens: *Pythium, Fusarium, Rhizoctonia solani* or *Thielaviopsis basicola*. We are also in the process of producing transgenic plants with stacked genes (providing insect and disease resistance) that will provide an additional level of protection from *A. flavus* by reducing bollworm damage in cotton bolls, a point of entry for the mycotoxigenic fungus.