ANALYSIS OF TRANSGENIC COTTON AND TOBACCO EXPRESSING LYTIC PEPTIDE GENES

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Abstract

Our goal is to transform commercial varieties of cotton with gene constructs encoding antifungal peptides/proteins to reduce aflatoxin contamination and the loss of lint yield and quality due to invasion by the fungus, *Aspergillus flavus*. We previously reported on the evaluation of a synthetic peptide for antifungal activity *in vitro.* The synthetic peptide, D4E1, at 1.5 mM and 12.5 mM has been shown to completely inhibit the growth of germinating conidia of *Fusarium moniliforme* and *A. flavus*, respectively, and is fairly resistant to degradation by both plant and fungal proteases. A vector construct utilizing a synthetic gene encoding D4E1 under control of the CaMV 35S promoter was introduced into cotton cells by *Agrobacterium*-mediated transformation. We have also transformed these constructs into tobacco cells as a means of quickly determining if the peptide is being expressed at efficacious levels. Previous bioassay of tobacco callus tissues transformed with the CaMV 35S-D4E1 construct demonstrated a 50-80% inhibition in the germination of *F. moniliforme* spores while transgenic cotton extracts demonstrated little if any inhibition. The level of inhibition demonstrated against *Fusarium* in the tobacco assays yielded an estimated concentration of 0.5-1.2 mM D4E1 which is approximately 10 fold less than required for inhibition of *A. flavus* spore germination (as determined by previous *in vitro* assays). These results indicated that the level of expression of the D4E1 gene under control of the CaMV 35S promoter was not great enough in either tobacco or cotton to effect inhibition of *A. flavus* spore germination (a conc. of 12.5 mM). Results of promoter strength experiments indicated that future constructs should utilize the CaMVd35S-TMV enhancer promoter in an attempt to increase expression of D4E1 to efficacious levels.

We have re-transformed both tobacco and cotton with the CaMV 35S-D4E1 construct and in addition have transformed both plants with a CaMV d35S-TMV enhancer-D4E1 construct capable of increased levels of expression of D4E1. Also transformed was a CaMV d35S-TMV enhancer -CSSP-D4E1 construct containing the cottonseed storage protein gene (CSSP) signal sequence. Inclusion of this DNA region in the construct should allow the D4E1 peptide to be secreted from the cell into the extracellular spaces. Tobacco leaf tissue obtained from *nptII* positive plantlets tested positive by PCR analysis for the presence of the promoter-D4E1 DNA. This was also true for a number of the cotton callus tissue DNA samples. Due to the small size of the D4E1 gene and lack of available specific antibody for D4E1, expression of D4E1 could not be measured at either the level of transcription or translation. However, *in vitro* and *in planta* antifungal and antibacterial bioassays were performed.

In vitro germinated spore assays performed from extracts of tobacco leaf tissue transformed with D4E1 demonstrated a high degree of efficacy against *Aspergillus flavus*, *Verticillium dahliae*, and *Fusarium moniliforme*. Slightly higher efficacy was noted for extracts of tobacco transformed with the CSSP-D4E1 construct. Preliminary analysis with transgenic cotton callus demonstrated lower levels of efficacy than tobacco against *A. flavus* and *V. dahliae*. *In planta* assays were performed on leaves of tobacco that had been transformed with either of the D4E1 constructs. Assay of *Colletotrichum destructivum* (anthracnose) lesion size showed inhibition of fungal destruction by both types of transgenes. Assay of *Pseudomonas syringae* pv. *tabaci* (fireblight) demonstrated reduced infection at lower inoculum levels for both transgenes tested. Results indicate that a higher level of efficacy may have been achieved with the improved CaMV d35S-TMV enhancer promoter controlling the expression of D4E1. This was noted in both tobacco and to a lesser extent in cotton callus. However, it should be noted that the cotton callus lines we assayed are chimeric and therefore optimal levels of D4E1 may not be present in these tissues. A better test of the efficacy of D4E1 in cotton will be determined once cotton plants are generated from somatic embryos we now have.

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