REDUCTION OF FUNGAL INVASION AND GROWTH BY PUTATIVE TRANSGENIC COTTON PLANTS

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Abstract

Our objective is to develop transgenic cottons with innate resistance to invading phytopathogens. Utilizing two gene constructs, encoding either a bacterial haloperoxidase or a synthetic antimicrobial peptide, we have regenerated several cotton plants from multiple transformation experiments. We previously reported that crude leaf extracts from stably transformed tobacco plants, expressing either the haloperoxidase (from *Pseudomonas pyrrocinia*) or the synthetic antimicrobial peptide, D4E1, showed significant antifungal activity *in vitro* against *Aspergillus flavus*, *Verticillium dahliae* and *Fusarium moniliforme*. In addition, the transformed tobacco plants were shown to inhibit the growth *in planta* of a fungal pathogen, *Colletotrichum destructivum*, that causes anthracnose, and a bacterial phytopathogen, *Pseudomonas syringae* pv. *tabaci* that causes wildfire.

Using crude leaf extracts from putative transgenic cotton plants, we were unable to conduct antifungal assays similar to the tobacco model system due to the presence of phenolics and gossypol, which were, themselves, inhibitory to *Aspergillus flavus* growth. However, preliminary analyses using immature cottonseed (21 dpa) of the putative R_0 transgenic cotton plants (segregating populations of R_1) indicated that crude seed extracts from selected transgenic plants inhibited the growth of *Verticillium dahliae*. Immature cottonseeds from these plants were also infected with a green fluorescent protein (GFP)-expressing *A. flavus* strain; The GFP fluorescence, as a function of the fungal growth and spread after one week of incubation, was evaluated under a GFP microscope and quantified using a fluorometer (HTS 7000, Perkin-Elmer). Some of the putative transgenic plants showed reduction in GFP fluorescence indicating possible inhibition of *A. flavus* growth, as compared to the control seeds. Further characterization of the putative transgenic plants and their progenies by molecular and additional antifungal assays are in progress.

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