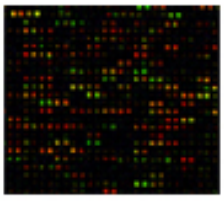

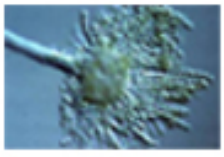

Proceedings of the 3rd Fungal Genomics, 4th Fumonisin, and 16th Aflatoxin Elimination Workshops

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Please join us in thanking the following peanut industry organizations for their generous sponsorship which has made this workshop possible:

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Improved Synthetic Peptide Technology for Expression in Plants

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Plants do not possess a complex immunoglobulin-based system such as that found in higher vertebrates to defend themselves against attacking microbial pathogens; however, they do have a wide variety of innate host defense mechanisms at their disposal. These include the production of antimicrobial reactive oxygen species (ROS), secondary metabolites, hydrolytic enzymes, and a wide array of antimicrobial proteins and peptides. Recombinant DNA technologies and plant transformation procedures have been used to introduce and express genes encoding these types of antimicrobial agents in plants in an effort to increase host resistance to plant pathogens. Of particular interest has been the identification and characterization of ribosomally-synthesized antimicrobial peptides. Antimicrobial peptides appear to be ubiquitous in nature being found in many organisms, from humans to bacteria. Various plants produce, either preformed or in response to microbial invasion, cysteine-rich antimicrobial peptides such as thionins, defensins, lipid-transfer proteins, and hevein- and knottin-type peptides. Examples of antimicrobial peptides of mammalian and insect origin include bovine or human defensins and protegrins, magainins from amphibians, and cecropins from the giant silk moth, *Hyalophora cecropia*. These peptides have been shown to be effective against a wide array of microorganisms including fungi. Antifungal peptides act either by lysing the fungal cell or by interfering with cell wall synthesis. Cecropin and cecropin analogs have been expressed in transgenic tobacco (*Nicotiana tabacum*) with mixed results regarding pathogen resistance. We were the first in the world to demonstrate enhanced disease resistance and reduced disease severity in transgenic tobacco expressing designed antimicrobial peptides upon infection with the bacterial pathogen, *Pseudomonas olanacearum*. However, tobacco plants expressing a native cecropin did not confer resistance to *Pseudomonas solanacearum*, presumably due to degradation of the peptide by host proteases. The advent of automated peptide synthesizers and combinatorial peptide chemistry has made it possible to rapidly synthesize and screen large numbers of peptides for their ability to inhibit the growth of target microbial pathogens. These linear peptides often can be less than half the size (10–20 amino acids) of their native counterparts and many times more potent without concomitant toxicity to host tissues. We recently reported on the antifungal activity of a 17 amino acid linear, synthetic peptide designated D4E1. This peptide was shown to interact with sterols present in the conidial cell walls and resist degradation by fungal and host proteases. *In vitro* assays with D4E1 demonstrated a minimal lethal concentration needed to kill 100% of germinating conidia of *Aspergillus flavus*, *A. fumigatus*, and *A. niger* of 12.5, 12.5, and 25 μM, respectively. We have been successful in the *Agrobacterium tumefaciens*-mediated transformation and expression of D4E1 in tobacco and subsequently, the demonstration of antifungal activity from crude extracts of the transformed leaves against *A. flavus* and *Verticillium dahliae*. Additionally, *in planta* assays demonstrated reduced disease severity upon inoculation of transgenic tobacco leaves with other fungal pathogens. In further experimentation, we have shown a number of herbaceous and woody species to possess enhanced resistance to bacterial and fungal pathogens when transformed with genes encoding our designed antimicrobial peptides. The use of further designed antimicrobial peptide-encoding genes for the production of plants with improved disease resistance characteristics is a reality and when applied to plants of agricultural import, significant increase in food and fiber would be realized.