Genetically Stable Atoxigenic Aspergillus Strains to Manage Pre- and Post-Harvest Aflatoxin Contamination (2024-052)

Development of novel Aspergillus strains using CRISPR-based gene editing to reduce aflatoxin contamination in the peanuts, corn, tree nut, and cotton industries.

Market Overview

Aflatoxins are toxic substances produced by certain fungi found on crops like corn, peanuts, cottonseed, and tree nuts. The contamination of peanuts by aflatoxins is a significant food safety concern, leading to restrictions on both export and domestic use of peanuts as food or animal feed. While some peanut genotypes resistant to aflatoxin have been identified in research, none demonstrate complete resistance and still accumulate aflatoxins that surpass U.S. food safety levels. The economic impact of aflatoxin contamination is significant. In the United States alone, peanut producers lose $25 to $58 million annually, while the corn industry faces losses of $280 million per year. Across all affected industries, including peanuts, corn, tree nuts and cotton, the total economic impact exceeds $1 billion. To address this problem, Clemson inventors have created genetically stable (non-reversible) atoxigenic Aspergillus strains for use as a biopesticide, ultimately reducing aflatoxin contamination in peanuts, corn, and cotton.

Technical Summary

The primary fungi that produce aflatoxins are *Aspergillus flavus* and *Aspergillus parasiticus*, which are abundant in warm and humid regions of the world. Inventors employed a CRISPR-based approach to develop genetically stable atoxigenic *Aspergillus* strains with an inherent capability to prevent toxigenic revertants. The strategy includes targeting *Aspergillus* polyketide synthase (pksA) *AflC* gene, disrupting *AflC* activity, and inducing mutations in the mating type gene upon sexual encounters. The process mitigates the risk of reverting to a toxigenic state by preventing subsequent mating events with compatible wild-type strains, thus ensuring continued mitigation of aflatoxin contamination.

Application

Agricultural industries that face aflatoxin contamination, including peanuts, corn, tree nuts, and cotton

Development Stage

TRL 2/3

Advantages

- Reduces aflatoxin contamination via genetically stable atoxigenic *Aspergillus* strains.
- Benefits growers of a wide range of crop species, including peanuts, corn, tree nuts, and cotton.
- Potential to reduce input costs and yield loss while establishing a population of nontoxigenic *Aspergillus* strains in soil.
- Significant (>$1 billion) economic opportunity.

About the Inventors

Sachin Rustgi

Associate Professor of Molecular Breeding and Faculty Scholar at Clemson University School of Health Research

Dr. Sachin Rustgi oversees the Molecular Breeding program at Clemson University Pee Dee Research and Education Center. Dr. Rustgi's research primarily focuses on developing dietary therapies for individuals with celiac disease or allergy to wheat or peanut, developing biomarkers for heat tolerance in soybean and peanut, and novel strategies to mitigate the major insect pests or fungal pathogens of the primary South Carolina crops.

Zachary Jones

PhD Student in Plant and Environmental Sciences at Clemson University

Zachary Jones graduated with a B.S. in Biological Sciences from Clemson University in December 2017. Following graduation, he worked as a Research Specialist in Dr. Rustgi's lab. In May of 2022, he began his PhD in Plant and Environmental Sciences and was selected to receive the MacDonald Graduate Fellowship Award. The goal of Zachary's research is to utilize plants to treat human diseases, focusing specifically on immunological conditions.

Ethan Matthews

Research Assistant at Clemson University

Ethan Matthews graduated with a B.S. in Biological Sciences from Francis Marion University in May 2023. His research at Clemson University focused on optimizing protoplast development and the genetic transformation of *Aspergillus* species.

Arpad Karsai

Research Associate at Clemson University

Dr. Arpad Karsai holds a Medical Degree and a Ph.D. in Biophysics. His past research has focused on protein biophysics, the mechanical properties of biomaterials, cell motility, and mechanical signal transduction in eukaryotic cells. At Clemson, Dr. Karsai has concentrated on developing genotyping methods for the characterization of *Aspergillus* transformants.

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