Investigating the Role of Reactive Oxygen Species (ROS) in Host - *Aspergillus flavus* Interactions Under Drought Stress Using Genetic Engineering

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Aspergillus flavus is an opportunistic pathogen of plants such as peanut under conducive environments such as drought resulting in significant aflatoxin production. Drought-associated oxidative stress also exacerbates aflatoxin production by *A. flavus*. Our previous examination of host plant and pathogen responses to drought stress have shown that oxidative stress alleviation is central to these responses. In addition, drought sensitive lines accumulate higher levels of reactive oxygen species (ROS) in their leaf and kernel tissues compared to drought tolerant lines. These ROS levels are also correlated with aflatoxin accumulation in these lines when inoculated with *A. flavus* under drought. These ROS have also been found to stimulate aflatoxin production in *A. flavus in vitro*, and significantly regulate the expression of transcripts, proteins, and metabolites related to fungal secondary metabolism, pathogenicity, development, and reproduction. Therefore, it is hypothesized that ROS accumulation under drought stress in host plant tissues may stimulate aflatoxin production during *A. flavus* infection, and that increasing or decreasing ROS accumulation would increase or decrease aflatoxin contamination. To test this hypothesis, the expression of antioxidant genes in maize and peanut was manipulated using genetic engineering. In maize, *Agrobacterium tumefaciens* was used to introduce a DNA construct overexpressing the maize catalase III (*ZmCAT3*) gene using a kernel specific γ-zein promoter into the hybrid Hi-II. In peanut, using biolistic transformation, constructs constitutively overexpressing the peanut catalase I (*AhCAT1*), ascorbate peroxidase I (*AhAPX1*), or superoxide dismutase I (*AhSOD1*) were independently introduced into the variety Georgia Green. Using the same peanut system, a CRISPR-Cas9 genome editing construct was introduced with a customized polycistronic gRNA to introduce functional mutations in *AhCAT1* at multiple locations. This will allow for the examination of both increased and decreased antioxidant gene expression on ROS accumulation under drought and associated aflatoxin contamination. Effects on host plant agronomics, morphology, and biochemical composition will also be examined. Currently, regeneration and initial characterization of the primary transformants (T_0) is in progress. If successful, this will not only provide a novel approach to mitigating aflatoxin contamination and will also provide insight into the cross-talk between host plants and *A. flavus* during infection under drought, and the underlying mechanisms regulating drought-related aflatoxin production.