Progress report for "A potential new method to control aflatoxin and other mycotoxins in corn."

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The main objective of this study, began in mid May 2009, is to express an α-amylase inhibitor (AI) from hyacinth bean in corn to effect a significant reduction in both fungal infection by *Aspergillus flavus* and aflatoxin accumulation in kernels. AI's inhibit the α-amylase activity of *A. flavus* and fungal growth as well, but don't inhibit the maize, porcine, or human α-amylases. Previous reports have demonstrated that the α-amylase inhibitors (AI) of hyacinth bean inhibited the break-down of starch necessary for both fungal growth and the subsequent accumulation of aflatoxins. By expressing an α-amylase inhibitor from this legume in corn, we expect to observe a significant reduction in both fungal infection by *A. flavus* and aflatoxin accumulation in kernels. Several of members of this gene family were previously cloned into the commercial vector pGEM-T easy and their sequence verified. Gene family member B01 has been cloned into the plant transformation vector 1300S, which is designed to expression B01 in all tissues. This plasmid was sent to the maize transformation facility at transformation facility at Iowa State University for biolistic insertion into corn variety Hi II. Nineteen transgenic lines with the B01 AI transgene have been regenerated and are being grown to maturity in the greenhouse at the University of Arkansas. Seed from each these lines will be tested for their ability to inhibit *Aspergillus flavus* infection and aflatoxin accumulation compared to the wild type control.

Concurrently, a second and third series of gene expression constructs are being made to express these amylase inhibitors in all tissues and in an embryo and kernel specific fashion. First, a ubiquitin (UBI) promoter is being fused to gene family members H07 and C12. The UBI promoter will not only expression the AI transgene in all tissues, it will express them at particularly high levels, compared to the 35S promoter above, in the kernel where aflatoxin accumulation occurs. Dr. Peter Quail, discoverer of the UBI promoter, has sent the PI plasmid pAHC17 that contains the UBI sequence along with a NOS terminator and this plasmid will facilitate a much simpler construction process. Upon completion, of these constructs, they will be sent to transformation facility at Iowa State University. The third promoter from the zein gene will be spliced onto the beginning of the open reading frame of the amylase inhibitor genes and a NOS terminator will be spliced onto the end of these gene construct. This promoter will express AI proteins only in the kernel. A yeast protein expression system for these AI family members will also be developed so that correctly folded AI proteins can be produced, purified, and used to develop AI specific antibodies. These antibodies will used to determine AI protein expression levels in leaf and kernel tissues. This data will be used in conjunction with fungal and toxin bioassays to determine the efficacy of AI's in inhibiting aflatoxin accumulation in corn.