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

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Transgenic maize development. The main objective of this study, which began in mid-May 2009, is to express an α-amylase inhibitor from hyacinth bean in corn in order to reduce both infection and aflatoxin accumulation by *Aspergillus flavus*. The α-amylase enzyme of *A. flavus* is necessary for the fungus to digest kernel starch and produce aflatoxins. Hyacinth bean contains α-amylase inhibitors that block the α-amylase activity of *A. flavus*, and inhibit spore germination and fungal growth as well. We have inserted the α-amylase inhibitor gene B01 from hyacinth bean into the corn line HiII, and have collected seed from 20 different transgenic lines (Table 1). This large number of lines will allow us to select those that have high levels of the α-amylase inhibitor, but that do not show any negative effects of transformation.

Analysis of transgenic corn lines. We have estimated expression of the α-amylase inhibitor by measuring RNA levels using Q RT-PCR. We have also developed an antibody that binds to the α-amylase inhibitor, allowing us to visualize the protein and accurately measure its abundance using an immunoblot assay (Figure 2). Because we expressed the α-amylase inhibitor in all tissues, we were able to extract proteins from leaves and use the antibody we developed to visualize the α-amylase inhibitor levels among different transgenic lines. Foliar tissue is much more abundant than in the early stage of development of the transgenic and can be easily tested to ensure expression of the transgene. Based on the measurements of transgene RNA levels, eleven lines with the highest transgene expression were analyzed for protein abundance by immunoblot analysis (Figure 2). Lines 115 and 146 express the greatest amount of α-amylase inhibitor. B73 is the non-transgenic pollen donor parent and expresses no α-amylase inhibitor (AILP). Lines 65, 68, 105, 156 and 168 express intermediate levels of the inhibitor while, line 19 expresses no visible α-amylase inhibitor protein.

Future Work: The PI will examine the protein of leaf extracts of the most promising transgenic lines to measure their inhibitor effects on *A. flavus* and aflatoxin accumulation. The PI will also produce this α-amylase inhibitor artificially in yeast and inject the yeast-made α-amylase inhibitor into corn ears and kernels to measure the amount of this protein necessary to inhibit ear rot and aflatoxin accumulation. This approach is much faster than taking these transgenic lines forward several generations and will be a valuable tool in optimizing the effectiveness of using α-amylase inhibitors in corn for aflatoxin control. We will also identify α-amylase inhibitors from soapberry using state of the art proteomics tools. These soapberry α-amylase inhibitors are completely novel and even more effective than those from hyacinth bean.

Table 1. Development of transgenic maize lines expressing AILP B01.

Materials	Number
Bialaphos-resistant callus lines (Hi II parent)	43
Callus lines regenerated to T0 plants	30
Callus lines with transgene confirmed by PCR	20
Total transformed T0 plants (≥3 plants per PCR-positive callus line)	74
Seeds of transgenic T0 maternal parent x B73 pollen	1101
Seeds of B73 maternal parent x pollen from transgenic line	512
Transgenic lines analyzed by RT Q-PCR	20
Transgenic lines analyzed by Immunoblots	11

Figure 2. Immunoblot to detect the α-amylase inhibitor (AILP) in transgenic maize lines (15-168). B73, which is not transgenic, is a negative control. The intensity of the band for AILP reflects how much protein is present.