Genetic Transformation to Mitigate Drought and Aflatoxin-Related Losses in Peanut.

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The mitigation of aflatoxin and drought stress related losses and food safety risks are priorities for the peanut research community. The development of novel technologies in combination with recent advances in peanut genomics allow for the deployment of genetic transformation and genome editing to address this issue. Previously, we have found that drought tolerance and aflatoxin resistance are correlated with reduced accumulation of reactive oxygen species (ROS) in peanut and corn. It was hypothesized that modifying the accumulation of antioxidant enzymes in these plants may result in reduced aflatoxin accumulation and increased drought tolerance. To test this hypothesis, we have used biolistic transformation to independently overexpress three antioxidant genes, *AhAPX1*, *AhCAT1*, and *AhSOD1* in the cultivar Georgia Green. Conversely, we have engineered a novel polycistronic guide-RNA (gRNA) into the CRISPR-Cas9 cassette for genome editing to silence the expression of an isoform of *AhCAT1*. These approaches have resulted in the performance of six bombardments per construct/gene. Following regeneration and root induction, 27, 40, and 28 potentially transgenic T_0 plants have been generated representing 9, 11, and 6 independent transgenic events for overexpression of *AhAPX1*, *AhCAT1*, and *AhSOD1*, respectively. Regeneration of CRISPR-Cas9 plants is currently in progress. Genotypic and phenotypic evaluation is currently underway with both PCR and enhanced green fluorescent protein (eGFP) expression. Using eGFP expression as an initial screen in young root and foliar tissues, 37.0%, 47.5%, and 42.9% of regenerated plants (43.2% overall) for *AhAPX1*, *AhCAT1*, and *AhSOD1*, respectively, showed positive eGFP expression. This may indicate that these overexpression constructs are inserted into expressible regions of the peanut genome and warrant further evaluation. Effects of these genome modifications on antioxidant gene expression and ROS accumulation will be discussed along with the potential effects on aflatoxin contamination and drought tolerance.