Molecular Detection, Gene Expression and Phylogenetic Analyses of Aflatoxin Producing of *Aspergillus flavus* Strains Isolated from Peanut Seeds in Georgia

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Aspergillus flavus infects peanuts and produces a mycotoxin called aflatoxin, a potent human carcinogen. In infected peanuts it can also affect peanut seed quality by causing seed rot and reducing seed viability, resulting in problems establishing plant stands. In 2020, peanut seeds in Georgia had lower than expected germination and a high frequency of *A. flavus* contamination. A total of 102 *Aspergillus* isolates were collected from seven seed lots and their identity, aflatoxin contamination and expression of the aflatoxin regulatory genes were studied. The isolates were confirmed as *A. flavus* by morphological characteristics and a PCR-based method using species-specific primers. Aflatoxin-producing strains identity and expression were assayed by targeting four genes involved in the aflatoxin biosynthetic pathway: *nor*1, *ord*1, *omt*A, and *afl*R. In all examined strains, 80 out of 102 (78%) were successfully amplified using all four genes, indicating aflatoxigenic activity. ELISA analysis further confirmed that the isolates produced high levels of aflatoxins (B1). A real-time PCR was used to analyze the level of expression of these regulatory genes in all collected *A. flavus* isolates. Our results showed a significant gene expression variation between aflatoxigenic and non-aflatoxigenic *A. flavus* isolate (Aflaguard). The variation of gene expression may be related to seed storage conditions as well as aflatoxin production. Overall, these studies presented that the 4 out of 25 biosynthesis-related genes may play an important role in the regulation of aflatoxin production in peanuts during storage. More detailed studies are needed to predict the potential risk of aflatoxin production and the functional importance of specific regulatory genes in stored commercial peanut seed.