Research Article

Polyphasic Detection of Atoxigenic *Aspergillus flavus* Isolates for the Biocontrol of Groundnut Aflatoxin Contamination

P Srilakshmi and H Sudini

International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502 324, Telangana, India. Email. pamireddysri@gmail.com

Abstract

Aflatoxin contamination by *Aspergillus* section *Flavi* is a major pre-and post-harvest problem in groundnut, causing significant trade losses globally. These secondary metabolites have carcinogenic, hepatotoxic and teratogenic effects on human and animal health. For sustainable management of pre-harvest contamination in groundnut, biological control using atoxigenic *A. flavus* is one of the viable options. However, selection of an appropriate atoxigenic isolate can be cumbersome, costly and also erroneous in view of the frequent false positives and negatives in detection methods. In our present studies, we have adopted a polyphasic approach for precise detection of an atoxigenic *A. flavus* isolate through cultural, analytical and molecular methods. Detection by growth of *A. flavus* (58 isolates of groundnut) and standard color reaction on coconut agar medium (CAM); yeast extract sucrose (YES) agar and exposure to ammonium hydroxide vapors was adopted in cultural methods. Further, the atoxigenic isolates obtained based on cultural methods were screened for aflatoxin production by enzyme linked immunosorbent assay (ELISA) and thin layer chromatography (TLC). Later, the atoxigenic isolates were screened for the absence of major structural and regulatory genes by amplifying them with gene specific primers using polymerase chain reaction (PCR). The obtained atoxigenic isolate was also screened for its α-amylase activity, gene for amylase production, and for production of anthraquinone pigments. Our results yielded a promising atoxigenic isolate, *A. flavus* (AF-334), that has shown negative reaction to the cultural and analytical screening methods. Further, the AF-334 has only five out of 23 structural genes and absence of both the regulatory genes responsible for aflatoxin biosynthesis. Besides, the AF-334 isolate has no α-amylase activity and the gene responsible for amylase production. Also, the major anthraquinone pigments that are intermediates in aflatoxin biosynthesis are also not produced by AF-334. Our results confirmed the atoxigenicity of AF-334 by using a polyphasic approach. Hence, the present investigation emphasize that prior screening of *A. flavus* (AF-334) is mandatory for its biocontrol potentialities, absence of toxigenic secondary metabolites other than aflatoxins before its recommendation as a candidate bioagent.

Key words: Aflatoxins, atoxigenic *Aspergillus flavus*, detection methods, groundnut

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