Identification of *Aspergillus flavus* Isolates for Developing Biocontrol Agent Based on the Gene-Defects in the Aflatoxin Biosynthesis Gene-Cluster and Flanking-Regions

Sneha Meramanbhai Dodia, Gyan Prakash Mishra, Radhakrishnan Thankappan* and Jentilal Ramjibhai Dobraia

Directorate of Groundnut Research, PB 5, Junagadh - 362001, India.

(Received: 08 September 2014; accepted: 19 October 2014)

***Aspergillus flavus* is distributed throughout the world, but are more common in warm climate zones, thus the risk of infection is more in hot and dry weather conditions like that of Gujarat. We investigated defects in the aflatoxin gene cluster in 81 non-aflatoxigenic *A. flavus* isolates collected from different peanut growing fields of Gujarat state (India). PCR assays using aflatoxin-gene-specific primers grouped these isolates into 53 deletion patterns and three groups. It was revealed that 84% (group 2) and 11% (group 3) of the non-aflatoxigenic isolates had 1-7 and 19-27 gene-defects respectively. However 5% (group 1) of the non-aflatoxigenic isolates were found to have no gene-defects. No isolate was found defective for all the genes of aflatoxin biosynthesis gene cluster and flanking region. Thus, deletions in the gene-cluster among non-aflatoxigenic *A. flavus* isolates are not unusual, and the deletion patterns were quite diverse. Screening of isolates using gene specific PCR was found quite effective for the identification of gene-defects in *A. flavus*. Four most defective, group 3 isolates were identified, which can be used as bio-control agents in the form of "cocktails" for the Indian peanut growing areas.**

Key words: Aflatoxins, biocontrol strategy, gene-specific PCR, Pathway regulators, sugar utilization genes.

A. flavus is economically very important because it produces hepato-carcinogenic secondary metabolite aflatoxin[1]. Aflatoxins (AFs), a group of polyketide-derived furanocoumarins, were discovered in *A. flavus* about 40 years ago after an outbreak of Turkey X disease in England[2]. AFs contamination was more prevalent during times of high heat and drought, which may stress the host plant thereby facilitating *A. flavus* infection[3,4]. Currently, incidences of AF contamination of crops are limited to tropical and sub-tropical areas (between latitudes 40 °N and 40 °S) around the world[5] which also covers the major peanut growing area of the world[6].

AFs infestation continues to be a potential threat to food-, feed- and consumer-safety and to the world export markets[1,7]. Maximum levels of AFs have been set at levels below 20 ppb by most countries but allowable threshold levels may vary[8,9]. Although *A. flavus* infection does not affect the peanut yield significantly, but AF contamination leads to decrease in quality, thus incurs economic losses[1]. Development and maturation of peanut pods occurs inside the soil, therefore *A. flavus* might infect the pods or seeds at pre-harvest, during harvest or post-harvest stages[10].

AF biosynthesis involves complex inter-connecting network of pathway specific regulators,

* To whom all correspondence should be addressed. Tel.:+91-285-2673041; Fax: +91-285-2672550; E-mail: radhakrishnan.nrcg@gmail.com

Volume 8 No. 6 Page No. 4623-4635 Dec 2014