Detection of Aflatoxin Producing *Aspergillus flavus* in Post-harvest Contaminated *Vigna ungulculata* Seeds

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Abstract: The present study was carried out with a specific objective to study post-harvest spoilage of Lobhiya (*Vigna unguiculata*) seeds contaminated with *Aspergillus flavus*. Infected seeds were collected and cultured on potato dextrose agar (PDA) media, at 25±2 °C. *Aspergillus flavus* isolates were primarily characterized by its morphological and microscopic characteristics. Collected fungal isolates were also screened for their afaltoxigenic nature on preliminary basis and at molecular level. For preliminary screening, 5 mm disc of fungal culture was soaked with few drops of liquid ammonia. Color change from yellow pigment to plum-red with different intensities showed the mycotoxic nature of the fungus. DNA from fungal isolates was isolated and amplified using PCR with aflatoxin specific primers, apa-2, ver-1 and omt-1. Amplicons of 1032 bp, 895 bp and 596 bp were obtained in most of the isolates regardless of primer set used which was useful to differentiate between mycotoxic and nontoxic isolates of *A. flavus*. The isolation of aflatoxigenic strains of *A. flavus* during post-harvest period of lobhiya seeds raise a serious concern over the quality of seeds and a threat to heath of consumers. It was concluded that *Aspergillus flavus* is responsible for postharvest spolilage of Lobhiya (*Vigna unguiculata*).

Keywords: *Vigna unguiculata* (L.) Walp.), post-harvest spoilage, *Aspergillus flavus*, aflatoxins.

Introduction

Lobhiya (*Vigna unguiculata* (L.) Walp.) is an edible legume plant and an important bean of family Fabaceae, cultivated globally for its nutritive value and edible importance. The seeds are pale-colored with prominent black spot, so known as black eyed pea or bean. Nutritionally, the lobhiya seeds are energy rich, having a very good concentration of carbohydrates, fats, proteins, vitamins and essential micro and macro elements (Davis *et al*., 1991).

In September - October 2013, local agricultural fields of Himachal Pradesh were surveyed for the post-harvest spoilage of Lobhiya seeds. A clear-cut yellowish green fungal infection was noticed on the seed surface. Therefore, the present study was conducted to ascertain the strains of fungi isolated from post harvested seeds of *V. unguiculata*. Collected fungal isolates were also screened for their mycotoxic nature.

Materials and Methods

The Lobhiya seeds showing typical symptoms of fungal association were collected from different location of the Himachal Pradesh, a North Indian state. About 30 samples were collected from all the locations. To clarify this fungus, collected seed samples were carried to laboratory and examined carefully. Infected seeds were observed under hand lenses and dissecting microscope for morphological characteristics of associated pathogen. With the help of sterilized needle or spatula, yellowish green fungal pathogen was cultured aseptically on Potato Dextrose Agar (PDA) media, and incubated at 25±2°C in darkness for 4-5 days. The fungus grown on culture media was isolated consistently and characterized primarily by its morphological and microscopic characteristics.

The mycotoxin producing potential of collected isolates of *Aspergillus flavus* is

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preliminarily carried out as per the method described by Saito and Machida (1999). A 5mm disc of fungal culture was soaked with few drops of liquid ammonia and observe disc for the color changed.

The aflatoxin producing nature *A. flavus* isolates was also studied at molecular level. DNA from fungal isolates was isolated following CTAB method (Shapira *et al.*, 1996; Konietzny and Geriner, 2003) and amplified using PCR with aflatoxin specific primers, apa-2, ver-1 and omt-1 (Shapira *et al*., 1996; Konietzny and Geriner, 2003).

Results and Discussion

Fungi namely *Aspergillus flavus* was consistently found associated to the described symptoms on Lobhiya seeds. Initially, the fungus appeared as yellowish-green on seeds surface that invaded healthy seeds later on (Fig.1 A-B). On PDA medium, colonies were yellowish-green, entire margin with umbonate elevations, exudate absent, reverse colorless to yellow and moderate to rapid in growth. Mycelium white, branched, septate; conidiphore 600 to 800 µm in length, 15 to 20 µm in diameter, vesicle globose to subglobose; conidia 20 to 45 µm in size, yellowish green, smooth; phialides covering nearly entire vesicle, biseriate primary and secondary phialides 7 to 10 µm in size, cleistothecia observed. On the basis of morphological and cultural characteristics the fungus was identified as *A. flavus* (Fig. 1 C).

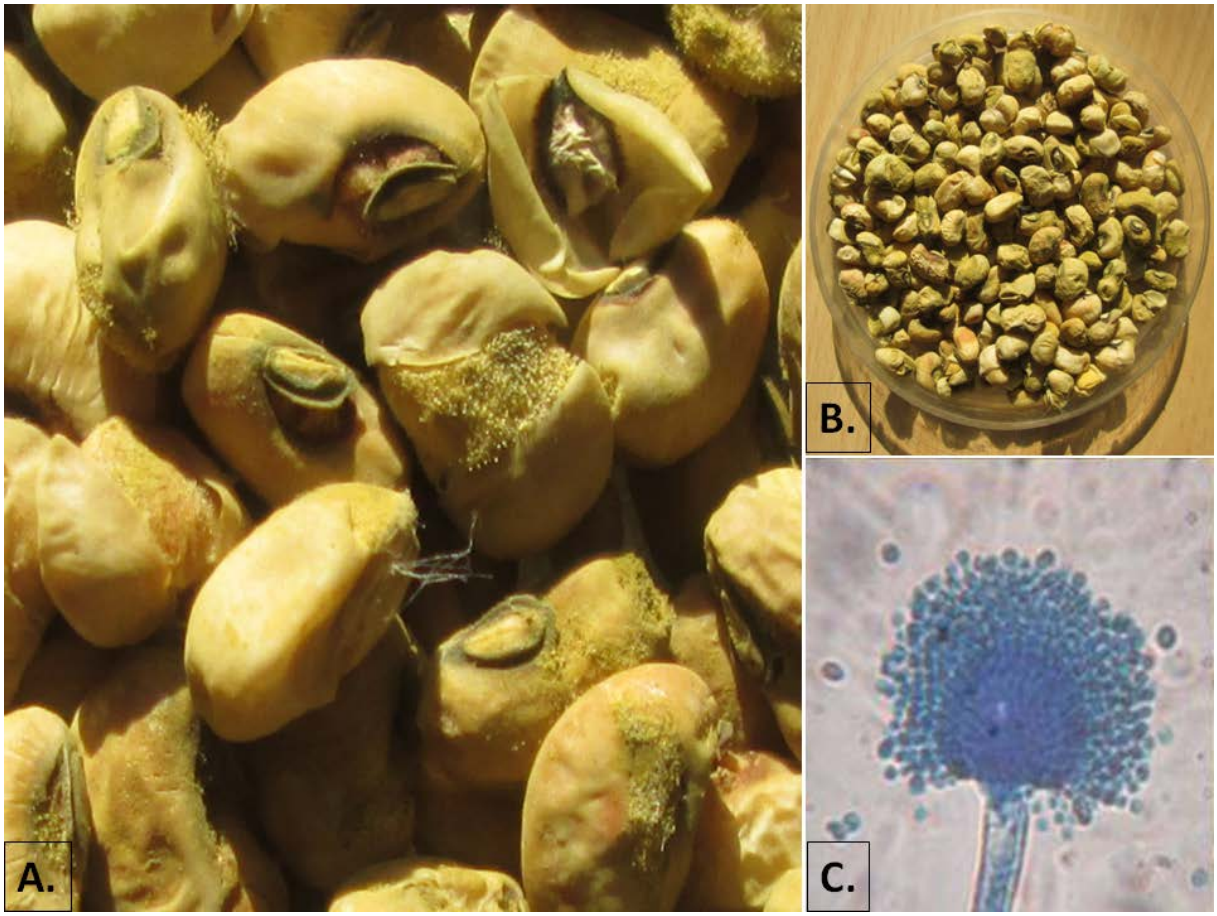

Figure 1. *Aspergillus flavus* associated with Lobhiya (*V. unguiculata*) seeds. **A-B.** Infected seeds; **C.** Microscopic view of *A. flavus*.

After exposure of *A. flavus* isolates to liquid ammonia, color changed from yellow pigment to plum-red with different intensities. This showed the mycotoxic nature of the fungus at preliminary level. In continuation, upon amplification of genomic DNA of *A. flavus* isolates with aflatoxin specific primers, apa-2, ver-1 and omt-1, amplicons of 1032 bp, 895 bp and 596 bp respectively, were obtained in most of the isolates regardless of primer set used. The results were useful in

determining the aflatoxigenic potential of *A. flavus* and also to differentiate between mycotoxic and nontoxic isolates of *A. flavus* (Figs. 2, 3).

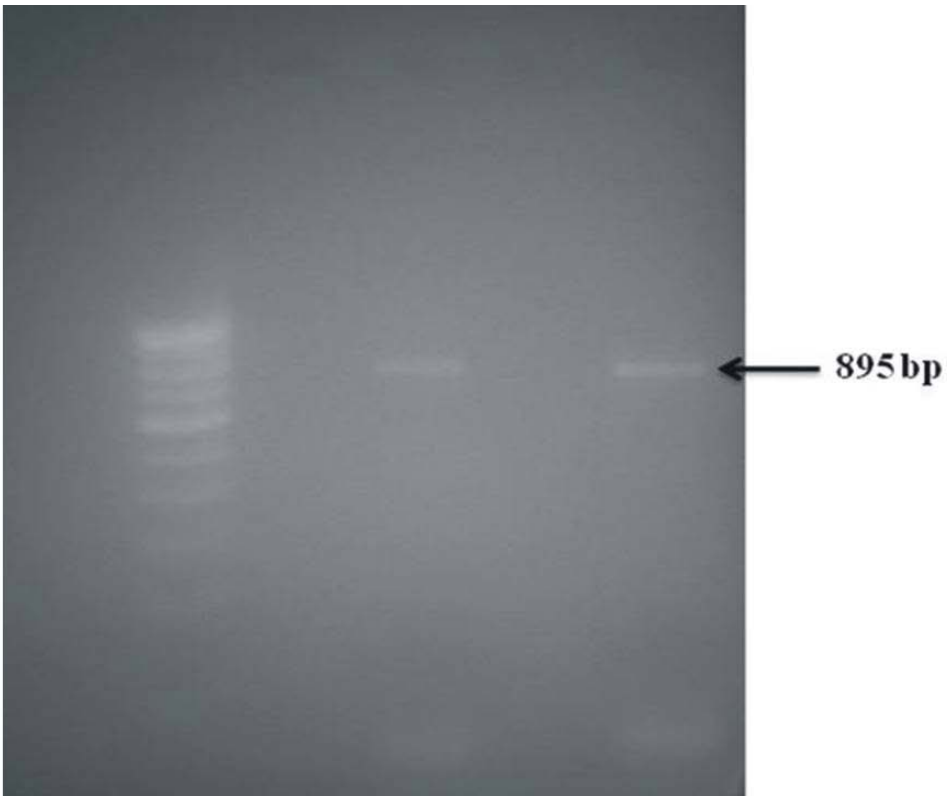

Fig. 2. PCR amplification of genomic DNA of *A. flavus* strains primed by ver-1, with 895 bp amplification.

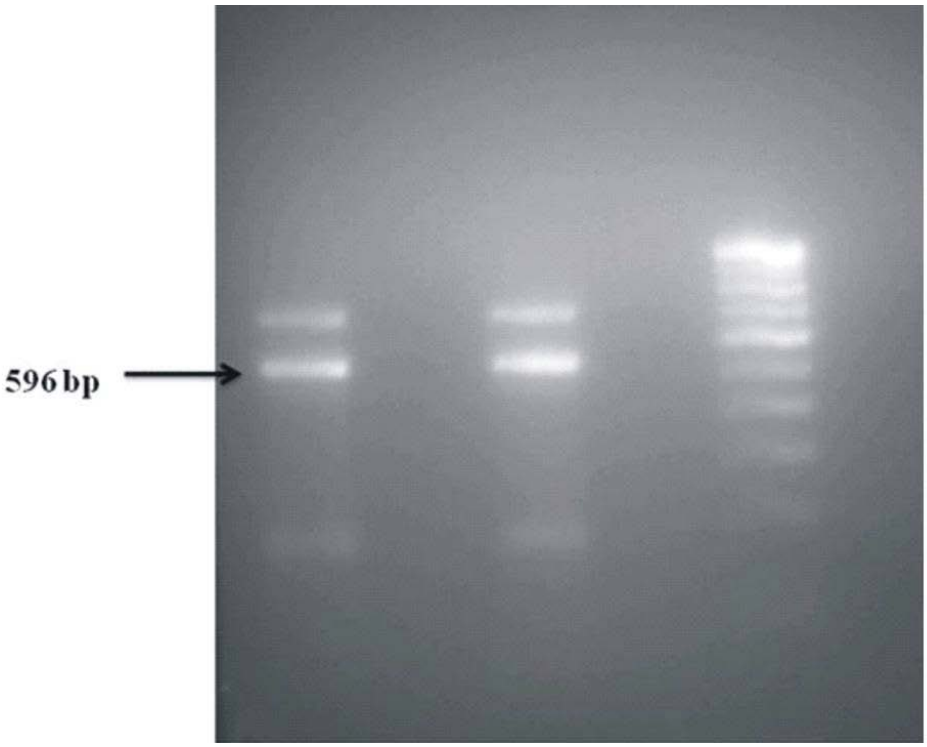

Figure 3. PCR amplification of genomic DNA of A. flavus strains primed by omt-1, with 596 bp amplification.

The findings of the present study showed that the *V. unguiculata* seeds were heavily contaminated by fungal species *A. flavus*, some of the isolates being highly toxigenic in nature. However, the association of *A. flavus* with *Vigna unguiculata* was reported earlier form South Africa (Kritzinger *et al*., 2003); West Africa (Houssou *et al.*, 2009), Nigeria (Fawole *et al.*, 2006), Egypt (Embaby *et al*., 2013) and even form India (Saad *et al*., 2008).

Since, aflatoxins are highly potent mycotoxins with immunosuppressive, carcinogenic and mutagenic nature. Therefore, isolation of aflatoxigenic strains of *A. flavus* during post-harvest period of lobhiya seeds raise a serious concern over the quality of seeds and a serious threat to heath of consumers. Such a contamination can be linked to harvest and storage conditions associated with the tropical climate.

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