JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 77-85

Molecular Characterization of *Aspergillus flavus* Contamination of Barley Grains using RAPD and ISSR

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(Received: 10 September 2014; accepted: 29 October 2014)

Ten fungi belonging to five genera were isolated from barley (*Hordeum vulgare* L.) grain samples collected from different markets at three main regions of Saudi Arabia. The average of mycotoxigenic isolation frequencies were *Aspergillus* (29.5%), *Fusarium* (35%), *Alternaria* (13.2%) and *Penicillium* (10.9%). Thirteen isolates of *Aspergillus flavus* were screened by HPLC for their ability to produce aflatoxins (AFs). The percentage of aflatoxigenic *A. flavus* isolates were 61.6%, eight isolates were produced AFs. Random amplified polymorphic DNA (RAPD) and Inter simple sequence repeat (ISSR) DNA markers were used to genetically characterize strains of *A. flavus* and to discriminate between the aflatoxigenic and non-aflatoxigenic isolates. RAPD and ISSR analysis revealed a high level of genetic diversity in the *A. flavus* population, which was useful for genetic characterization. The RAPD and ISSR markers could not discriminate between aflatoxigenic and non-aflatoxigenic isolates, but the ISSR primers were somewhat better.

Key words: *Aspergillus flavus*; Barley; Aflatoxin; HPLC; RAPD PCR; ISSR PCR.

Barley grain is mostly used as feed for animals, malt, and food for human consumption. Approximately 25% of cereals consumed in the world are contaminated by Mycotoxins (Devegowda, *et al*. 1998). The most important groups of mycotoxins that occur quite often in food are: aflatoxins, ochratoxins, trichothecenes (deoxynivalenol, nivalenol), zearalenone and fumonisins (Kumar *et al*., 2008). Aflatoxins (AFs) are toxic secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus*. AFs can enter the food chain in the field, during storage, or later, under favorable conditions of temperature and humidity. The most potent of the four naturally occurring AFs (B1, B2, G1 and G2) (Kotsonis *et al*., 2001) B1 is, which is listed as a group I carcinogen by the International Agency for Research on Cancer (IARC, 1982). AFs are carcinogens, mutagens, and teratogens. Contamination of food and feed with AFs represents a high risk for human and animal health (Moss, 1996). The RAPD technique has been used to detect genetic variability between isolates of *A. flavus* and related species (Batista *et al*., 2008; Irshad and Nawab, 2012) and to discriminate between aflatoxigenic and non-aflatoxigenic isolates of *A. flavus* (Lourenço *et al*., 2007; Gashgari *et al*., 2010; Sepahvand *et al*., 2011). The ISSR technique has been employed to investigate the diversity and population structure of *A. flavus* (Batista *et al*., 2008; Tran-Dinh *et al*., 2009; Zhang *et al*., 2013) and to determine the similarities and dissimilarities between aflatoxigenic and non-aflatoxigenic isolates of this species (Tran-Dinh *et al*., 2009; Hatti *et al*., 2011).

The aims of the present study were: 1) to genetically characterize 13 isolates of the *A. flavus* group isolated from the barley crop found in three

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main regions in Saudi Arabia, 2) to determine the genetic relationship (comparing the genomic profile) between aflatoxigenic and nonaflatoxigenic isolates using PCR amplification of RAPD and ISSR molecular markers, and 3) to determine the AFs in these isolates using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Collection of samples

Fifteen samples (250 g each) of grain barley were collected from different markets located in Riyadh, Dammam, and Abha regions in Saudi Arabia during March 2011. The market survey of grain barley samples were divided Saudi Arabia into three regions, the first region Dammam, (eastern) where the weather was hot (30–45°C) and high humid, the second region Riyadh, (middle) where it was very hot (40-50°C) and dried and the third region Abha, (south western) where it was cool (15-30°C) and wet. Collected samples were transported immediately to laboratory for labelling, like date and place of collection and kept in cool place for further mycological analysis (Czerwiecki *et al*., 2002).

Isolation, purification and identification of pathogens

Fifteen samples were surface-sterilized with 5% sodium hypochlorite for 1 min, before they were rinsed three times with sterile distilled water. Five grains were placed randomly on potato dextrose agar (PDA) on three 9-cm Petri dishes. The Petri dishes were incubated at 25°C and observed daily for emergence of colonies for 5 days, after which the colonies were counted. Isolates were purified and identified up to the species level using keys and manuals (Raper and Fennell 19650. The isolation frequencies of fungal species were calculated according to the method of (Gonzalez *et al*., 1999). Identification of fungal isolates was carried out on the basis of morphological and microscopic characteristics at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Detection of aflatoxins based on fluorescence

All of the *A. flavus* isolates obtained were cultivated on PDA at 25°C for 7 days. A mycelium plug from each strain was placed in the center of a Petri dish containing test medium (PDA, Czapek agar (CZ) or malt extract agar (Davis *et al*., 1987).The plates were then incubated at 25°C for 4 days in the dark, and tested for the presence or absence of fluorescence in the agar surrounding the growing *Aspergillus* colonies was determined by exposing the Petri dishes to UV light (365 nm), which was expressed as positive or negative (Franco *et al*., 1998).

Determination of the toxigenic potential of *A. flavus* isolates

Isolates were grown in sterilized SMKY liquid medium (20 g sucrose, 0.5 g magnesium sulfate, 3 g potassium nitrate and 7 g yeast extract in 1000 mL distilled water) (Davis *et al*., 1987). Flasks were inoculated with 6-mm diameter discs of the toxigenic *Aspergillu*s spp. at 25 ± 2°C for 7 days (Paranagama *et al*., 2003). The experiments were performed in triplicate. After incubation, the contents of each flask were filtered through Whatman No. 1 filter paper. For AF extraction, filtration and separation using HPLC (PerkinElmer series 200 UV/VIS) were carried out according to (Christian, 1990).

DNA extraction of *A. flavus* isolates

A. flavus isolates were cultured in double layer media, consisting of a solid and a liquid layer, in 50-mm Petri dishes. The solid base medium was PDA, and the top liquid medium was peptone yeast glucose (PYG, 1200 ¼l). The fungi were incubated at 25°C for 2 days. DNA was extracted from 50 mg fresh mats according to (Amer *et al*., 2011).

RAPD PCR

We selected 3 primers from standard RAPD primer; sequences of primers (e-oligos, Gene link, USA) are listed in Table 1. Total DNA of *A. flavus* isolates were using a template. PCR amplifications were run using mixtures with a final volume of 25 ¼L containing reaction buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 3.4 mM $MgCl_2$, 0.25 mM dNTPs, 0.4 mM each primer, 2 U Taq DNA polymerase (BioLabs) and 25 ng genomic DNA.

ISSR PCR

PCR amplification of ISSRs was performed with the primers $(GTG)_5$, $(GACA)_4$ and $(AGAG)_4G$. Sequences of primers are listed in Table 1. The reaction mixtures had a final volume of 25 ¼L and contained reaction buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.25 mM primers, 1.25 U Taq DNA polymerase (BioLabs) and 25 ng genomic DNA.

DNA electrophoresis

For all samples, the amplified DNA (10 ¼L) was electrophoresed using an electrophoresis unit (Wide Mini-Sub-Cell GT, Bio-RAD) in 1.5% agarose containing ethidium bromide (0.5 ¼g/mL) at constant 75 V and 60 mA and was visualized with a UV trans-illuminator.

Gel analysis of DNA

The DNA gel was scanned for band Rf using a gel documentation system (AAB Advanced American Biotechnology, Fullerton, CA, USA). The different molecular weights of the bands were determined against a DNA standard (kb DNA ladder, Stratagene, Canada) with molecular weights of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, and 1500 bp. The similarity level was determined by unweighted pair group method with arithmetic mean (UPGMA).

RESULTS

Isolation frequencies of fungi recovered from barley grains from Riyadh, Dammam, and Abha regions

Ten fungi belonging to five genera were isolated from tested barley grains (Table 2). Isolation frequencies of isolated fungi were *Aspergillus* spp. (34.9%), *Alternaria* spp. (8.6%), *Fusarium* spp. (34.8%), *Penicillium* (13%) and *Rhizoups* stolonifer (8.7%) were isolated from Riyadh region. Ten fungi belonging to five genera were isolated from tested wheat grains. Isolation frequencies of isolated fungi were *Aspergillus* spp. (25%), *Alternaria* spp. (16.6%), *Fusarium* spp. (41.7%), *Penicillium* (12.5%) and *Rhizoups stolonifer* (4.2%) were isolated from Dammam region. Ten fungi belonging to six genera were isolated from tested wheat grains. Isolation frequencies of isolated fungi were *Aspergillus* spp. (28.6%), *Alternaria* spp. (14.3%), *Fusarium* spp. (28.5%), *Penicillium* (7.1%), *Eupenicillum alutaceum* (7.1%) and *Rhizoups stolonifer* (14.3%) were isolated from Abha region.

Table 1. Sequences of the nucleotide primers used in this study.

	RAPD primers		
Primer code	Sequence	Amplified region	References
RAPD primer 1	52 -GGTGCGGGAA-32	fragments from 1-10	Mahmoud et al., 2012
RAPD primer 2	52 -GTTTCG CTCC-32	genomic sites	
RAPD primer 5	52 -AACGCG CAAC-32	simultaneously	
	ISSR primers		
Primer code	Sequence	Amplified region	References
$(GTG)_5$	52 -GTGGTGGTGGTGGTG-32	Minisatellite-	Batista et al., 2008
$(GACA)_4$	52 -GACAGACAGACAGACA-32	region DNA	
$(AGAG)_4G$	52 -AGAGAGAGAGAGAGAGG-32		Hatti et al., 2010

Table 2. Isolation frequencies of isolated fungi from barley grains collected from different markets located in Riyadh, Dammam and Abha regions

Isolated fungi	Riyadh	Dammam	Abha
Aspergillus flavus	26.20	20.80	14.30
A. niger	08.70	04.20	14.30
A. parasiticus	00.00	00.00	0.00
Alternaria longipes	04.30	08.30	14.30
A. Chlamydospora	04.30	08.30	00.00
Fusarium monilinforme	08.70	12.50	07.10
F. oxysporum	17.40	16.70	14.30
F. solani	08.70	12.50	07.10
Penicillum funiculosum	08.70	08.30	00.00
P. implicatum	04.30	04.20	07.10
Eupenicillum alutaceum	00.00	00.00	07.10
Rhizoups stolonifer	08.70	04.20	14.30
Total	100.00	100.00	100.00

Detection of aflatoxins produced by *A. flavus* under UV irradiation (365 nm)

Thirteen *A.flavus* isolates were isolated from barley grains in all regions and detect of aflatoxins under 365 nm UV light (Table 3). All Isolates were expressed as positive for aflatoxin production expect, isolates B2 and B6 were expressed as negative for aflatoxin production, these isolates from Riyadh region. All isolates expressed as positive for aflatoxin production expect, isolate B9 was expressed as negative for

aflatoxin production, these isolates from Dammam region. All Isolates from Abha region were expressed as positive for aflatoxin production.

Determination of aflatoxins for *A. flavus* isolates from barley grains using HPLC

Eight isolates were capable of producing detectable levels of both B and G aflatoxins, although two isolates (B10 and B12) were failed to produce any detectable amount. The highest level (1.3 and 1.6 µg/ml) of B1 and B2 were obtained from isolates B7 and B1. Isolate B3 was the highest producer (2.1 µg/ml) of G1 while isolate B1 was the highest producer (2.5 µg/ml) of G2.

Genetic characterization of *A. flavus* isolates

Genetic characterization was evaluated by PCR amplification using a set of 6 primers (3 RAPD and 3 ISSR). The amplification products were analyzed for polymorphisms by gel electrophoresis to determine whether pathotypes could be distinguished at the molecular level.

RAPD profiles of 13 *A. flavus* isolates obtained with RAPD primer 1

Dendrogram analysis grouped the isolates into two main clusters at of 93.28% genetic similarity (GS) based on the banding pattern (Figure 1). GS between the isolates tested ranged from 93.28%-99.40%. The first main cluster included two isolates B1 and B6 from Riyadh region with 97.66% GS. Second main cluster included two groups at 94.01% GS. First group included two isolates B2 and B4 from Riyadh region with 99.12% GS. Second group divided into two sub-group at 96.30% GS. First sub-group divided into two subclusters at 97.13% GS. In these subclusters isolates B10, B8, B7 and B9 from Dammam region had more 97% GS. Second subgroup divided into two subclusters at 97.51% GS. First subcluster included one isolate B5. Second subcluster included isolates B12 and B13 from Abha region with 99.24% GS. With reference to this primer, there was very clear-cut relationship between clustering in the RAPD dendrogram and geographic origin of the tested isolates, especially with Riyadh and Abha regions.

Five subclusters included ten isolates; all subclusters included combination of aflatoxigenic and non-aflatoxigenic isolates for example, subcluster included two isolate B1 (producing AFB and AFG) and B6 (non-producing AFB or AFG) had 97.66% GS. No correlation between RAPD banding patterns and the ability of aflatoxin production or non-production of the tested isolates.

RAPD profile of 13 *A. flavus* isolates obtained with RAPD primer 3

Dendrogram analysis grouped the isolates into two main clusters 91.26% GS based

Table 3. Detection of aflatoxins produced by *A. flavus* isolated from barley grains collected from Riyadh, Dammam and Abha regions under UV light

A. flavus code	PDA+NaCl	Czapacks	YES
		Riyadh region	
B1	-	-	+
B2	-	-	-
B3	-	-	+
B4	+	-	+
B5	-	+	+
B6	-	-	-
		Dammam region	
B7	-	+	-
B8	-	-	+
B9	-	-	-
B10	-	+	+
B11	-	-	+
		Abha region	
B12	-	-	+
B13	+	+	+

Czapeks: Czapeks Dox agar, YES: yeast extract sucrose agar, PDA: potato dextrose agar (+) positive for aflatoxin production, (-) negative for aflatoxin production

Table 4. Aflatoxin production by *A. flavus* isolated from barley grains collected from Riyadh, Dammam and Abha regions in Saudi Arabia

A. flavus code	Aflatoxin (µg/ml)			
	B1	B2	G1	G2
		Riyadh region		
B1	0.9	1.6	2.0	2.5
B3	1.2	1.1	2.1	1.8
B4	0.7	1.0	1.9	2.1
B5	0.8	1.7	1.5	1.3
	Dammam region			
B7	1.3	1.1	1.2	1.9
B8	1.0	0.8	1.0	1.8
B10	0.0	0.0	0.0	0.0
B11	0.6	0.9	1.4	2.1
	Abha region			
B12	0.0	0.0	0.0	0.0
B13	0.5	0.8	1.2	1.6

on the banding pattern (Figure 2). GS between the isolates tested ranged from 91.26%-99.39%. The first main cluster included two isolates B6 and B10 with 97.66% GS. Second main cluster included two groups at 93.19% GS. First group included one isolate B1**,** second group included two sub-group at 94.72% GS. First sub-group divided into two subclusters at 95.57% GS. First subcluster included B2, B3 and B4 from Riyadh region with 98.72% GS. Second subcluster included B7, B8 and B9 from Dammam region with 98.02% GS. Second sub-group divided into two subclusters at 97.52% GS. With reference to this primer, there was clear-cut relationship between clustering in the RAPD dendrogram and geographic origin of the tested isolates, especially with Riyadh and Abha regions.

Five subclusters included ten isolates; two subclusters included combination of aflatoxigenic and non-aflatoxigenic isolates for example, subcluster included two isolate B5 (producing AFB and AFG) and B12 (non-producing AFB or AFG) had 99.39% GS.

Two subclusters included four aflatoxigenic isolates for example, subcluster included two isolate B3 and B12 had 99.48% GS. One subcluster included two non-aflatoxigenic isolates for example, subcluster included two isolate B6 and B10 had 97.66% GS. There was clear-cut relationship between clustering in the RAPD dendrogram and the ability of aflatoxin production or non-production of the tested isolates.

RAPD profile of 13 *A. flavus* isolates obtained with RAPD primer 5

Dendrogram analysis grouped the isolates into two main clusters at 84.96% GS based on the banding pattern (Figure 3). GS between the isolates tested ranged from 84.96%-99.33%. The first main cluster included one isolate B13 from Abha region. Second main cluster included two groups at 90.72% GS. First group divided into two subclusters at 96.82% GS. First subcluster included

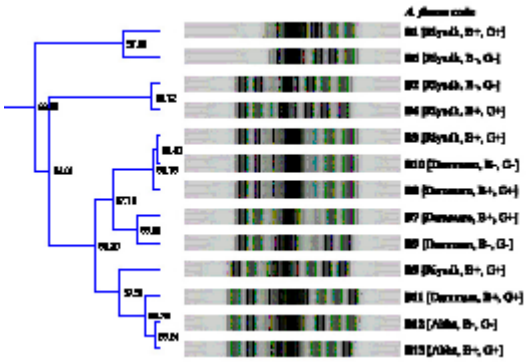

Fig 1. Dendrogram obtained by UPGMA method derived from PCR amplification banding of RAPD with RAPD primer 1 of 13 *A. flavus* isolates.

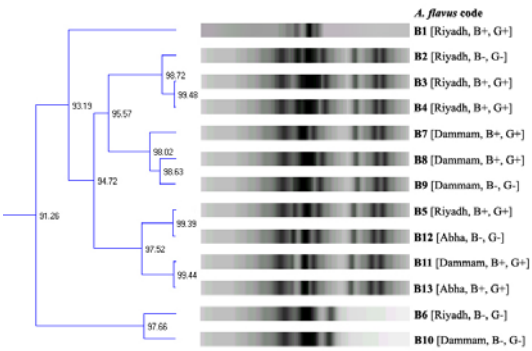

Fig 2. Dendrogram obtained by UPGMA method derived from PCR amplification banding of RAPD with RAPD primer 3 of 13 *A. flavus* isolates

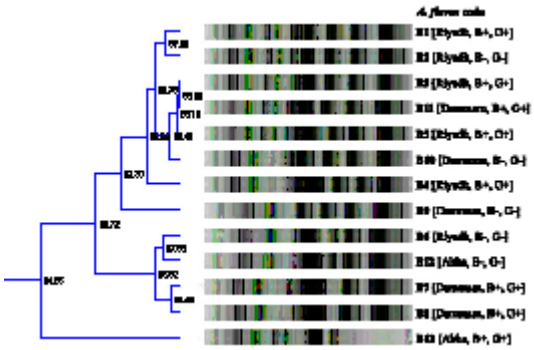

Fig 3. Dendrogram obtained by UPGMA method derived from PCR amplification banding of RAPD with RAPD primer 5 of 13 *A. flavus* isolates.

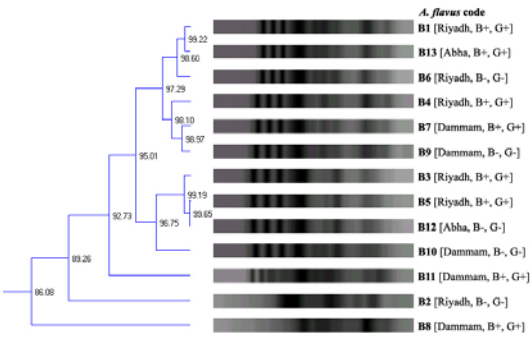

Fig 4. Dendrogram obtained by UPGMA method derived from PCR amplification banding of ISSR with primer $(GTG)_5$ of 13 *A. flavus* isolates.

B7 and B8 from Dammam region had 98.46% GS. Second subcluster included B6 and B12 from Riyadh and Dammam region respectively, had 98.46% GS. Second group divided into two subgroups at 93.30% GS. First subgroup included one isolate B9. Second subgroup included overlapping of many subclusters, in these subclusters B1 and B2 from Riyadh region with 97.98% GS. No correlation was found between RAPD data and geographic origin of the *A. flavus* isolates tested by using this primer.

Four subclusters included eight isolates; one subcluster included combination of aflatoxigenic and non-aflatoxigenic isolates for example, subcluster included two isolate B1 (producing AFB and AFG) and B12 (non-producing AFB or AFG) had 97.98% GS. Two subcluster included four aflatoxigenic isolates for example, subcluster included two isolate B3 and B11 had 99.33% GS. One subcluster included two non-aflatoxigenic isolates for example, subcluster included two isolate B6 and B12 had 97.66% GS. There was clear-cut relationship between clustering in the RAPD dendrogram and the ability of aflatoxin production or non-production of the tested isolates.

ISSR profiles of 13 *A. flavus* isolates obtained with primer $(GTG)_5$

Dendrogram analysis grouped the isolates into two main clusters at 86.08% GS based on the banding pattern (Figure 4). GS between the isolates tested ranged from 86.08%-99.65%. The first main cluster included one isolate B8 from Dammam region. Second main cluster included two groups at 89.76% GS. First group included one isolate B2 from Riyadh region. Second group included two subgroups at 92.37% GS. First subgroup included isolates B11 from Dammam. Second subgroup divided into two subcluster at 95.01% GS. First subcluster included isolates B3, B5, B12 and B10 with 96.75% GS. Second subcluster included isolates B1, B13, B6, B4, B7 and B9 and B10 with 97.29% GS. No correlation was found between ISSR data and geographic origin of the *A. flavus* isolates tested by using this primer.

Three subclusters included six isolates; two subclusters included combination of aflatoxigenic and non-aflatoxigenic isolates for example, subcluster included two isolate B7 (producing AFB and AFG) and B9 (non-producing AFB or AFG) had 97.66% GS. One subcluster included two aflatoxigenic isolates for example, subcluster included two B1 and B13 had 99.22% GS. No correlation between ISSR banding patterns and the ability of aflatoxin production or non-production of the tested isolates.

ISSR profiles of 13 *A. flavus* isolates obtained with primer $(GACA)_4$

Dendrogram analysis grouped the isolates into two main clusters at 87.39% GS based on the banding pattern (Figure 5). GS between the isolates tested ranged from 87.39%-99.19%. The first main cluster included two isolates B12 and B13 from Abha region with 95.24% GS. Second main cluster included two groups at 89.31% GS. First group included one isolate B8**.** Second group divided into two subgroups 93.48% GS. First subgroup included one isolate B1. Second subgroup divided into two subclusters at 94.76% GS. First subgroup included four isolates B3, B4, B2 and B6 from Riyadh region with 96.64% GS.

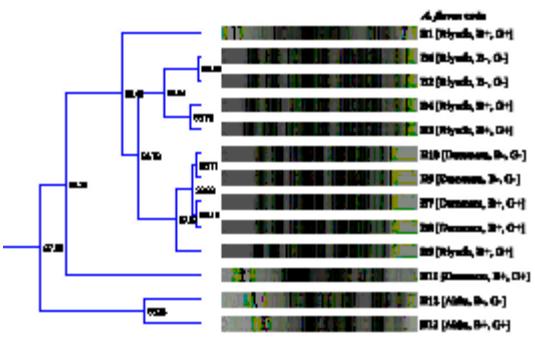

Fig 5. Dendrogram obtained by UPGMA method derived from PCR amplification banding of ISSR with primer $(GACA)_4$ of 13 *A. flavus* isolates.

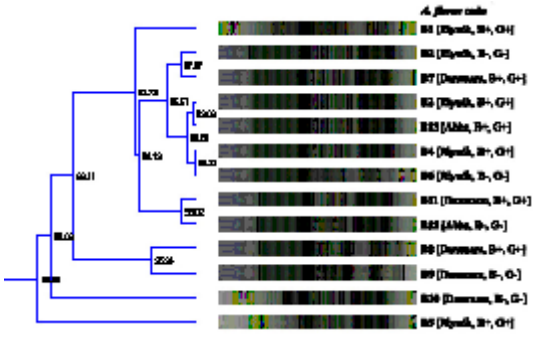

Fig 6. Dendrogram obtained by UPGMA method derived from PCR amplification banding of ISSR with primer $(AGAG)_4G$ of 13 *A. flavus* isolates.

Second subgroup included isolates B5 from Riyadh region and B8 and B7, B9 and B10 from Dammam region. With reference to this primer, there was clear-cut relationship between clustering in the RAPD dendrogram and geographic origin of the tested isolates, especially with Riyadh and Dammam regions.

Five subclusters included ten isolates; one subcluster included combination of aflatoxigenic and non-aflatoxigenic isolates for example, subcluster included two isolate B13 (producing AFB and AFG) and B12 (non-producing AFB or AFG) had 99.24% GS. Two subclusters included four aflatoxigenic isolates for example, subcluster included two isolate B4 and B3 had 98.73% GS. Two subclusters included two non-aflatoxigenic isolates for example, subcluster included two isolate B6 and B2 had 99.35% GS. There was clear-cut relationship between clustering in the ISSR dendrogram and the ability of aflatoxin production or non-production of the tested isolates.

ISSR profile of 13 *A. flavus* isolates obtained with primer $(AGAG)_4G$

Dendrogram analysis grouped the isolates into two main clusters at of 84.85% GS based on the banding pattern (Figure 6). GS between the isolates tested ranged from 84.85%-99.22%. The first main cluster included one isolates B5. Second main cluster included two groups at 86.08% GS. First group included one isolates B10. Second group included two subgroups at 88.11% GS. First subcluster included B8 and B9 with 95.12% GS. Second subgroup divided into two subclusters at 93.72% GS. First subcluster included many subclusters for nine isolates with 94.13% GS. No correlation was found between ISSR data and geographic origin of the *A. flavus* isolates tested by using this primer.

Five subclusters included ten isolates; four subclusters included combination of aflatoxigenic and non-aflatoxigenic isolates for example, subcluster included two isolate B7 (producing AFB and AFG) and B2 (non-producing AFB or AFG) had 97.87% GS. One subcluster included two aflatoxigenic isolates for example, subcluster included two B33 and B13 had 99.06% GS. No correlation between ISSR banding patterns and the ability of aflatoxin production or non-production of the tested isolates.

DISCUSSION

These results indicated that the type of fungal contamination of barley grains, at Saudi Arabia belongs to four genera (*Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria*) were qualitatively and quantitatively. Cereal grains contamination by fungi is a worldwide problem where several reports documented the presence of these fungi in samples of Argentina (Cavaglieri *et al*., 2007), Australia (Berghofer *et al*., 2003), India (Kumar *et al*., 2008), Italy (Covarelli *et al*., 2011), Saudi Arabia (Abd El-Aziz *et al*., 2013), Tunisia (Bensassi *et al*., 2011). Aflatoxigenic isolates produce blue fluorescence (B1 and B2) or green fluorescence (G and G2) on the reverse side of colonies under UV light, whereas non-aflatoxigenic isolates do not fluoresce (Davis *et al*., 1987). This method was improved by adding cyclodextrin (a methylated ²-cyclodextrin derivative) to common media used for testing AF production, to enhance the natural fluorescence of AFs (Fente *et al*., 2001).

HPLC determination of AF is sensitive and accurate in unprocessed cereals and cereal-based products (Pascale, 2009). Several authors have applied HPLC methods for low-ppb detection of AF (B1, B2, G1 and G2) and AF residues in sorghum grains (Yassin *et al*., 2010; Mahmoud *et al*., 2013).

RAPD was used for fourteen isolates of *A. flavus* from two geographically distinct sites in Brazil. Isolates were distributed in a non-random array, but one cannot assume that this is a fully discriminatory result, and thus, RAPD usefulness is debatable (Lourenço *et al*., 2007). Twenty-nine strains of *A. flavus* were used to study the genetic diversity among *A. flavus* population isolated from flour samples. RAPD-PCR technique was employed using two different primers ari1 and M13. All *A. flavus* isolates were repeatedly tested. The dendrogram produced by the combined cluster analysis of the two primers showed no correlation between DNA banding patterns and the ability of toxin production (Gashgari *et al*., 2010).

Microsatellite analysis of 84 Vietnamese *A. flavus* strains isolated from corn and peanut. High genetic diversity was observed in the 84 *A. flavus* strains, with no evident correlation being detected between strain toxigenicity and genotype (Tran-Dinh *et al*., 2009). *A. flavus* strains were isolated from different oil seeds (groundnut,

sunflower and soybean), and four of these isolates were found to be nontoxic, while eight were toxic. No correlation was found between AF production and an ISSR dendrogram (Hatti *et al*., 2010).

24 *A. flavus* isolates from peanut-cropped soils in China. ISSR PCR is effective molecular approaches for studying characterize the genetic diversity. The 24 *A. flavus* isolates were not clustered based on their produces aflatoxin. Some toxigenic and atoxigenic isolates appeared in one cluster.

ACKNOWLEDGEMENTS

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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