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BIOINFORMATICS ANALYSIS OF AFLATOXINS PRODUCED BY *ASPREGILLUS* SP. IN BASIC CONSUMER GRAIN (CORN AND RICE) IN SAUDI ARABIA

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ABSTRACT

The food contaminants by aflatoxins are inevitable even when all precautions and good agricultural practices are applied. Samples of white rice and corn (yellow, red) grains were collected from different local markets and houses. Three *Aspergillus flavus* strain isolated were identified using molecular characterization of *AFLR* (*aflR*) toxin gene. DNA genome of the three *A. flavus* isolates (namely *A. flavus* _ YC; *A. flavus* _ RC; *A. flavus* _ Rice) which corresponds to isolates from, yellow corn, red corn and white rice respectively were used as a template for PCR to amplify *Aspergillus flavus AFLR* (*aflR*) toxin gene. Partially sequenced was amplified using a specific primer set to confirm its identity, phylogenetic relationships between the three isolates as well as determination of the corresponding antigenic determinants. The epitope prediction analysis demonstrated that there were 1, 2, 3 and 4 epitopes whose score were equal 1 in *A. flavus* _ YC; *A. flavus* _ RC; *A. flavus* _ Rice, respectively. Interestingly, there were great dissimilarity in the epitope sequences among the three isolates except in RLQEGGDDAAGIPA, SPPPPVETQGLGGD, RPSESLPSARSEQG and PAHNTYSTPHAHTQ were found to be similar between all isolates. This work articulates that the molecular identification and characterization of three *A. flavus* using *Aspergillus flavus AFLR (aflR)* toxin gene and the unique antigenic determinants that could be used for design of a broad-spectrum antibody for rapid detection of *A. flavus* in foods and support quality system of food safety.

Keywords: PCR; sequences; phylogenetic tree; protein toxic gene; antigenic determinants

INTRODUCTION

Fungi caused major crops diseases during harvest and storage under higher temperature and humidity conditions **(Bhat et al., 2010; Pasquali et al., 2016)**. While more than 25 different fungi species known to invade stored grains and legumes **(Duan et al., 2007)** some species such as *Aspergillus, Fusarium, Penicillium* are responsible for most spoilage and germ damage during storage **(Boutigny et al., 2012; Aamot et al., 2015; Kachapulula et al., 2017)**. Crop transfers through international trade have made aflatoxins contaminated food a worldwide problem **(Passone et al., 2010)**. Mycotoxins are secondary metabolites produced by fungi, which cause health hazards to animals and human beings; the majority of mycotoxins of greatest concern to human and animal health are produced by the genera *Aspergillus, Penicillium,* and *Fusarium,* the so-called field fungi that frequently infect various food commodities **(Reddy et al., 2010)**. Toxicity of a mycotoxin will be manifested by its effect on the human and animal health and productivity of crops **(Abdel-Wahhab et al., 2006)**. The main routes of mycotoxins exposure are ingestion, inhalation or through skin contact. The toxicity of a mycotoxin is determined by metabolism involving the, transformation, administration, distribution, absorption, excretion and molecular interactions of the toxin and its metabolites. Nowadays the main mycotoxins of interest are aflatoxins (AFs), ochratoxins, **(Frisvad et al., 2019)**, trichothecenes, zearalenone, fumonisins, ergot alkaloids and deoxynivalenol **(Reddy et al., 2010; Cendoya et al., 2014; Covarelli et al., 2015; Singh and Cotty, 2019)**. Mycotoxin producing fungi which are associated with groundnuts, peanuts, cereals such as maize, rice, sorghum, wheat, barley and oats and spices such as black pepper, ginger, nutmeg, chilly, etc. are considered to be of greater significance for all over world **(Kumar et al., 2008; Brožková et al., 2015)**. Several studies have revealed mycotoxin contamination in rice worldwide: for example, aflatoxins in the United Arab Emirates **(Osman et al., 1999)** fumonisins in Iran, Argentina **(Alizadeh et al., 2012; Cendoya et al., 2014)** OTA in Morocco **(Juan et al., 2008)**, ZEA in Nigeria **(Makun et al., 2007)**, DON in Italy **(Lorè et al., 2011)** nivalenol in Korea **(Lee et al., 2011)** and citrinin in Egypt **(Abd Allah and Ezzat, 2005)**. As most of the corn and rice is grown during the wet season; it is

susceptible to mycotoxin contamination. Rice is shown to be a good substrate for toxigenic fungi like *A. flavus, A. ochraceus, Penicillium citrinum,* and *F. proliferatum* **(Aamot et al., 2015; Arino et al., 2007; Sánchez-Hervás et al., 2008)**. Humidity, temperature, storage conditions, and transportation period are the factors that influence mycotoxin production in rice **(Ariño et al., 2007)**. Regarding legumes in Saudi Arabia, very little information exists with respect to its natural contamination with toxigenic fungi and mycotoxins. Aflatoxins were detected in some *Aspergillus* isolates while fumonisin was detected in some *Fusarium* isolates **(Ibrahim et al., 1998; Abdel-Fatah et al., 2017)**. Among food contaminants, mycotoxins may cause substantial economic loss due to lower availability of commodities with acceptable levels of mycotoxins present and possibly greater cost of mycotoxin-safe and acceptable foods **(Mwanza et al., 2013; Samina, 2015)**. Mycotoxins continue to pose various health risks to consumers depending on specific mycotoxin consumed and level of exposure, and health status of individuals in the population **(Voss et al., 2014; Pasquali et al., 2016)**. Many human diseases, especially carcinogenic, teratogenic, hepatic, and gastrointestinal ones, have been found linked with the ingestion of mycotoxin-contaminated products **(Fung and Clark, 2004; Shephard, 2008; Samina, 2015)**. Outbreaks of mycotoxicoses in humans and animals, caused by ingestion of products containing mycotoxins, **(Peraica and Rašić, 2012)**. Risk assessments relating to food safety are frequently hampered by the lack of quantitative data **(Schmidt-Heydt et al., 2007)**. The sequencing fungal genomes and the studies of the molecular basis of fungal pathogenicity provide the study of risk factors associated with continuous exposer of mycotoxins **(Bilodeau, 2011; Taha et al., 2012)**. This paper was shown concentrated on aflatoxins produced by *Aspregillus* sp. and possibility for used the similarity between amino acid toxin gene of *Aspregillus* isolates strain for produced antibodies. Where the database of *Aspregillus* genomes provides a comprehensive resource of genomics data information's an important plant and human pathogenic fungal genus *Aspregillus*. It's given useful for discovery of genes encoding industrial enzymes, and antibiotics which may control in aflatoxin food contaminations **(Spröte et al., 2009; Taha et al., 2012)**. AFs are a group of polyketide-derived furanocoumarins, with at least 16 structurally related toxins that have been characterized. These toxins are produced by a number of different *Aspergillus* species are primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus* **(Geiser et al., 2007; Ito et al., 2001)**. There are four major AFs (AFB1, AFB2, AFG1, AFG2) all of which occur naturally **(Abbas et al., 2010)**. AFB1 is the most commonly occurring of the mold producing compounds **(Abdulkadar et al., 2004)**. AFB1 has been included in category 1A of active carcinogenic compound **(Abou-zeid et al., 1997; Abdel-Wahhab et al., 2006)**. Factors influencing the presence of mycotoxins in foods or feeds include environmental conditions related to storage that can be controlled **(Park et al., 2005)**. So, in this study, we have hypothesized that mycotoxins effects in human populations year to year because other factors as the fungal strain specificity, strain variation, and instability of toxigenic properties are more difficult to control **(El-Manzalawy et al., 2008a; El-Manzalawy et al., 2008b)**. However, by the articulate the molecular identification and characterization of mycotoxins, can be control in mycotoxins effects. In this study our aim advocate to using molecular detection and bioinformatics characterization for study the properties of protein toxin gene of aflatoxins in main consumer grains as rice and corn. Many human diseases occurring in Japan and other Asian countries were attributed to mycotoxins after consumption of mold-damaged rice **(Taligoola et al., 2011)**. Unfortunately, enactment of stringent rules for mycotoxin control in food is not always the best solution **(Samina, 2015)**. The impact of mycotoxin standards is more drastic for the population of developing countries **(Pasquali et al., 2016; Yassin et al., 2010)**.

Scientific hypothesis

Therefore, the aim of this study was to determine the *Aspergillus* species by the molecular identification of toxigenic mycotoxin profiles of those species that are naturally occurring in contaminating corn and rice seeds (as the main crops imported in Saudi Arabia) and protein structural analysis depicted from the gene(s) responsible for toxin biosynthesis. Which can be used as screening test for cost-effective control of mycotoxin and their products. We have hypothesized that by study the molecular characterizations and bioinformatics properties of Aflatoxins could in future be able to produce vaccine for species of *Aspergillus* genera which have higher prevalence rate in development countries **(Bhatnagar et al. 2003; Pasquali et al. 2016)**.

MATERIAL AND METHODOLOGY

Grains samples and isolation of mycotoxigenic *Aspergillus* species

One hundred fifty grains corn (yellow and red grains) and rice were collected from different area of Saudi Arabia (Riyadh, Hail, Qasim, Asir,Tabuk, Jizan, Jouf, Jeddah and Dammam), where collected from storage markets and houses. The collected grains were randomly and its weight between 0.5 – 1 kg of each grain in cleans and dries packaging. Agar plate and blotter tests were used to isolate *Aspergillus* sp. as described by **Neergaard (1977)**. Grains were divided into two groups, the first group was disinfected with sodium hypochlorite 1% for 2 min and the second group was non-disinfected. All grains were washed several times by sterilized water, and then dried between sterilized filter papers. The half of each group was plated on potato dextrose agar (PDA) (Sigma-Aldrich, USA). All dishes were incubated for 5 – 7 days at 25 °C. All isolation process under sterilized conditions to prevent any contamination of grain.

Purification and identification of *Aspergillus* species

Aspregillus species were initially identified to species based on the morphological characteristics **(Leslie, Summerell and Bullock, 2006)** of the macroconidia, microconidia and general mycelium presentation from a single spore isolate grown for 7 – 10 days on SNA with an Olympus BH-2 (Olympus America, New York) light microscope. Potato Dextrose Agar (PDA) was used to identify colony pigment characteristics of aerial mycelium on the agar **(Leslie, Summerell and Bullock, 2006)**.

Carnation Leaf Agar (CLA) (bio-WORLD, USA) was used to identify macroconidia, chlamydospores and the presentation of aerial mycelium single colony was transferred and purified by hypha tip technique onto PDA medium in the presence of streptomycin (50 mg.ml^{-1}). The developing fungi were prepared for molecular identification using primers specific for the *Aspergillus flavus AFLR (aflR)* toxin gene. All conditions of isolation and purification of mycotoxins were performed under sterilization for prevent any external agent of seeds pollutions.

Molecular identification of *Aspergillus flavus AFLR (aflR)* toxin gene

Isolation of DNA genome

The mycelium mass of *Aspergillus* species isolates grown on PDA broth medium was harvested by centrifugation at 6000 rpm for 10 min. The pellets were washed twice by PBS buffer and stored at 200 °C. Total DNA of the three isolates was isolated using lysozyme – dodecyl sulfate lysis method as described by **Leach et. al. (1990)**.

Amplification and purification of Aspergillus flavus AFLR (aflR) gene

Specific PCR reactions were conducted to assess the presence of *AFLR (aflR)* gene. The primers used as described by **Cary et al. (2000)** were: Omtl-F (*aflR*) (5′-GCCTTGCAAACACACTTTCA-3′); Omtl-R 5′-AGTTGTTGAACGCCCCAGT3′) and optimal annealing (Tm = 55 °C). The PCR amplification conditions included initial denaturation at 94 °C for 5 min then 35 cycles at 94 °C for 30 s, 55 °C for 60 s followed by extension step at 72 °C for 90 s. and a final extension at 72 °C for 7 min. The amplification reaction was performed by thermal cycler (Applied Biosystems, USA). Purification of PCR product was detected by electrophoresis (PNU, Faculty of science, Research center) using agarose 1.5% in 1x TAE buffer and staining with ethidium bromide (Qiagen, Berlin, Germany) **(Sambrook et al., 1989)**. The resultant fragment of *Aspergillus flavus AFLR (aflR)* toxin gene was excised from the gel and purified using a QIA quick gel extraction kit (Qiagen, Berlin, Germany).

DNA sequencing

The purified PCR products were prepared for Sanger sequencing technology using DNA sequencer technique (Sigma, central lab, PNU, KSA). DNA sequences of *Aspergillus flavus* isolates were aligned using Bio Edit software version 7 (www. Mbio-NCUs. Edu/bio. Edit) and were compared of the often accessions of *Aspergillys* sp. available in the NCBI data base using BIASI- algorithm to identify closely related sequences (http/WWW.NCbI.Nih.Gov). Dendrograms were constructed by using unweighted pair Group method with Arithmetic (UPGMA) on Gen bank.

Epitope prediction and antigenicity

The primary amino acids sequence of the *Aspergillus flavus AFLR (aflR)* toxin gene protein was evaluated from the corresponding nucleotide sequence using MEGA 6.0 software. The linear B-cell epitopes in the primary amino acid sequence of the coat protein was performed using BCPREDS server with default parameters (http://ailab.cs.iastate.edu/bcpreds/) which implements a support vector machine (SVM) and the subsequence kernel method **(El-Manzalawy et al., 2008a)**. Flexible length linear B-cell epitopes were predicted using FBCP red **(El-Manzalawy et al., 2008b)** method with a specificity cut-off; 75%. The antigenicity of each amino acid residue in the primary protein sequence was determined using a semi-empirical method **(Kolaskar and Tongaonkar, 1990)** which makes use of physicochemical properties of each amino acid and their frequencies of occurrence in experimentally known segmental epitopes.

RESULTS AND DISCUSSION

Three *Aspergillus* isolates from tested grains by PDA method was purified by single spore and hypha tip on PDA slant medium. The *Aspergillus* isolates were selected for molecular identification using *Aspergillus flavus AFLR (aflR)* toxin gene sequencing. Three *Aspergillus* isolates represented grains from yellow corn, red corn and white rice and designated as A. *flavus* _ YC; *A. flavus* _ RC; *A. flavus* _ Rice respectively.

Molecular characters of toxin gene

Total DNA was extracted from *A. flavus* _ YC; *A. flavus* _ RC; *A. flavus* _ Rice isolates infected grains. *Aspergillus flavus AFLR (aflR)* toxin gene was amplified from isolated DNA of mycelium using PCR reaction mixture and specific primer sets. PCR amplicons were allowed for sequencing reaction through cycle sequencing method. The DNA amplicons returned as electropherogram files. Electropherogram showed distinct peaks for each base cell as well as high *Q* values for each cell. Sequences obtained for each primer for each isolate had sufficient overlap between them and used to form one continuous sequence (Coting). The nucleotide partial sequence of *Aspergillus flavus AFLR (aflR)* toxin gene in the three isolates was compared with published isolates on Gen Bank. The sequence homology revealed that the gene of interest *Aspergillus flavus AFLR (aflR)* toxin gene and the test fungal isolates was *Aspergillus flavus* isolates. A multiple sequence alignment was constructed using ClustalW software (GNU Lesser GPL) between the three studied isolates. The alignment showed many conserved regions in all sequences as well as distinguished the heterogeneity positions among the aligned sequences (Figure 1a, 1b, 1c). Phylogenetic analysis was performed by construction of phylogenetic tree using a neighbor joining method to unravel the relationships among all *Aspergillus flavus* isolates (Figure 2). The phylogenetic tree resulted in two clades in which *A. flavus* _ Rice (white rice isolate) and *A. flavus* _ RC (red corn isolate) were in the same cluster whilst *A. flavus* _ YC (yellow corn isolate) was separate in a different cluster (Figure 2). Thus, the molecular identification based on sequence homology of the *Aspergillus flavus AFLR (aflR)* toxin gene confirmed the identity and phylogeny of the studied three *Aspergillus flavus* isolates.

The epitope prediction analysis demonstrated that there were 1, 2, 3 and 4 epitopes whose score were equal 1 in *A. flavus* _ YC (yellow corn), B: *A. flavus* _ RC (red corn) and C: *A. flavus* _ Rice (white rice). Also, there were great

variations in the epitope sequences among the three isolates except in RLQEGGDDAAGIPA, SPPPPVETQGLGGD, RPSESLPSARSEQG and PAHNTYSTPHAHTQ were found to be common between all isolates. These residues with high frequencies of occurrences in antigenic determinants were highlighted (yellow) in the antigenicity profile (Figure 3). Figure (3) also show the variability in the positions and types of amino acid residues with high antigenic frequency.

Mycotoxin detection is a major problem in developing countries where contaminated food commodities may readily reach food stores and homes **(Bilodeau, 2011; Boutigny et al., 2012; Taha et al., 2017)**. The risk of contamination by mycotoxins is an important food safety concern for grains and other field crops **(Abdulkadar et. al., 2004; Mwanza et al., 2013)**. Humans are exposed to mycotoxins throughout their life time due to consumption of fungus-contaminated food products and many human diseases, especially carcinogenic, teratogenic, hepatic, and gastrointestinal ones, have been found linked with the ingestion of mycotoxin-contaminated products **(Fung and Clark, 2004; Shephard, 2008; Mwanza et al., 2013)**. Sufficient quantities of mycotoxins in food and feedstuff can adversely affect human and animal health **(Qiu and Shi, 2014)**. Environmental factors and host species have a strong impact on the occurrence of a specific chemotype and the incidence of *Aspergillus* species **(Bhatnagar et al., 2003)**. The distribution of *Aspergillus* species in maize is influenced by optimal climatic conditions, pathogenicity and competition between other fungi **(Bhatnagar et al., 2006)**. The type of environmental factor identified in the incidence of *Aspergillus* species as demonstrated in recent EU maize surveys **(Creepy, 2002)**. In those studies, the prevalence of species varied year-to-year and was believed to be associated with the differences in climatic conditions between years **(Caldas et al., 2002; Scauflaire et al., 2011)**. As was reported by **Covarelli et al. (2015)** the emergence of toxigenic fungi on small grains has a negative impact on the safety and quality of feed and food. in this study We isolate mycotoxins strains of *Aspergillus* sp from contaminated corn and rice grains by a genomic library through amplication mycotoxins structure genes *(aflR)* by polymerase chain reaction (PCR) and sequencing of amplicans this result was agree with **(Chen et al., 2002)**. Data clearly reveal that the PCR technique is efficient in distinguishing mycotoxins **(Bilodeau, 2011; de Souza et al., 2005; Taha et al., 2012; Allam et al., 2015)** from commonly inhabiting stored grains. We isolate *Aspergillus* sp. from both zellow and red corn which used in food and feed in human and animal; also isolated from long and short rice **(Niessen, 2007; Fox and Howlett, 2008)**. *Aspergillus* species being able to grow at moderate to high temperature **(Ehrlich et al., 2003)** and its responsible for spoilage of food commodities during transport and storage. In addition, reduction in nutritive value, insipidness and discoloration are other problems resulted from contamination of grains by *Aspergillus* **(Dean et al., 2012)**. Rapid and accurate identification of *Aspergillus* and/or their metabolites are mandatory for the implementation of preventive measures in the whole food production system as was reported by **Bok and Keller, (2004); Bhatnagar et al. (2003)** and **Dean et al. (2012)**. The molecular characterization of three *Aspergillus* sp. isolated from small grains (yellow corn, white rice and red corn) using the mycotoxins gene, aflatoxin *AFLR (aflR)* allowed for coupled identification and mycotoxins screening in the three *Aspergillus* isolates **(Bhatnagar et al., 2006)**. Mycotoxins-producing fungi were isolated from sorghum grains from Saudia Arabia before **(Mahmoud et al., 2013; Yassin et al., 2010)**. Following the molecular identification of *Aspergillus* sp. B-cell epitopes in the aflatoxin *AFLR (aflR)* gene were predicted. The characterization of B-cell epitopes using computational tools is highly advantageous for the synthesis of specific antibodies for rapid detection of microbial pathogens in their environments. The epitopes prediction saves labor and time for validation experiments. The identification of epitopes plays a crucial role in the vaccine design, immunodiagnostic testing and antibody production **(Sette and Fikes, 2003)**. In this study, BCPREDS server was used to predict epitopes found in the primary amino acids sequence of aflatoxin *AFLR (aflR)* protein. BCPREDS proved high efficiency to predict linear B-cell epitopes in SARS-CoV S protein **(El-Manzalawy et al., 2008a; El-Manzalawy et al., 2008b)**. There was variability in the sequence and numbers of epitopes among the three toxin proteins analyzed. Here, a fixed length of epitopes (14 residues) was observed. The epitope prediction analysis demonstrated that there were 1, 2, 3 and 4 epitopes whose score were equal 1 in *A. flavus* _ YC; *A. flavus* _ RC; *A. flavus* _ Rice respectively. In this study, there were great dissimilarity in the epitope sequences among the three isolates. But we found that there some epitope sequences were common between all isolates in RLQEGGDDAAGIPA, SPPPPVETQGLGGD, RPSESLPSARSEQG and PAHNTYSTPHAHTQ. This result suggesting its exploitation for design of a specific antibody to be used for rapid detection of different *Aspergillus* species in small grains **(Ehrlich et al., 2003; Degola et al., 2007)**. The highly frequent residues with high antigenicity profiles such as valine, leucine, isoleucine, aspartic acid, glutamine and glutamic acid are mostly hydrophobic **(Duan et al., 2007)**. The occurrence of hydrophobic residues in epitopes is frequent and do have a hierarchy signature **(Zhang, 2002; Aftabuddin and Kundu, 2007; Suga and Galel, 2007; Scauflaire et al., 2011)**. Epitope prediction has many implications in pathogen detection and differentiation applications. The consideration of occurrence of *Aspergillus* sp. on small grains is important in risk assessment of mycotoxins and setting up preventive measures proactively. The main problems concentrated in high risk outbreaks of aflatoxicosis have been reported in different countries all over the world; that because the widespread of aflatoxin contamination especially in developing countries **(Pitt 2000a; Pitt 2000b; Kovacs, 2004)**. No doubt, that the safety control in food and feed processing should be by prevention of mycotoxin contamination in agriculture by used as simple and rapid screening tests for cost-effective control of food diseases by production of vaccines **(Sette and Fikes, 2003; Probst et al., 2014; Mwanza et al., 2013; Allam et al., 2015)**.

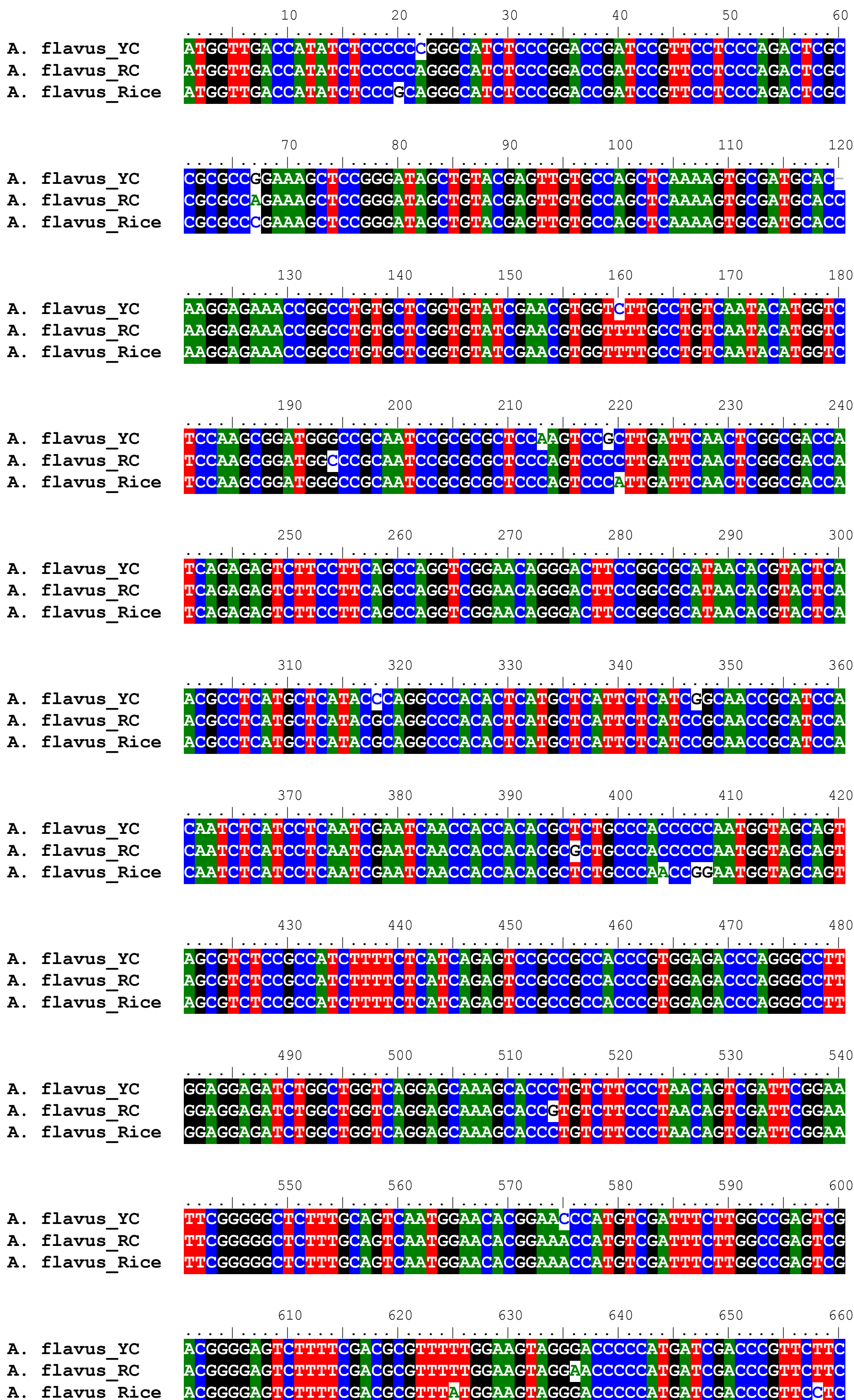

Figure 1a Multiple sequence alignment of the *aflr* gene partial sequence among three *Aspergillus flavus* isolated from yellow corn (YC), red Corn (RC) and rice.

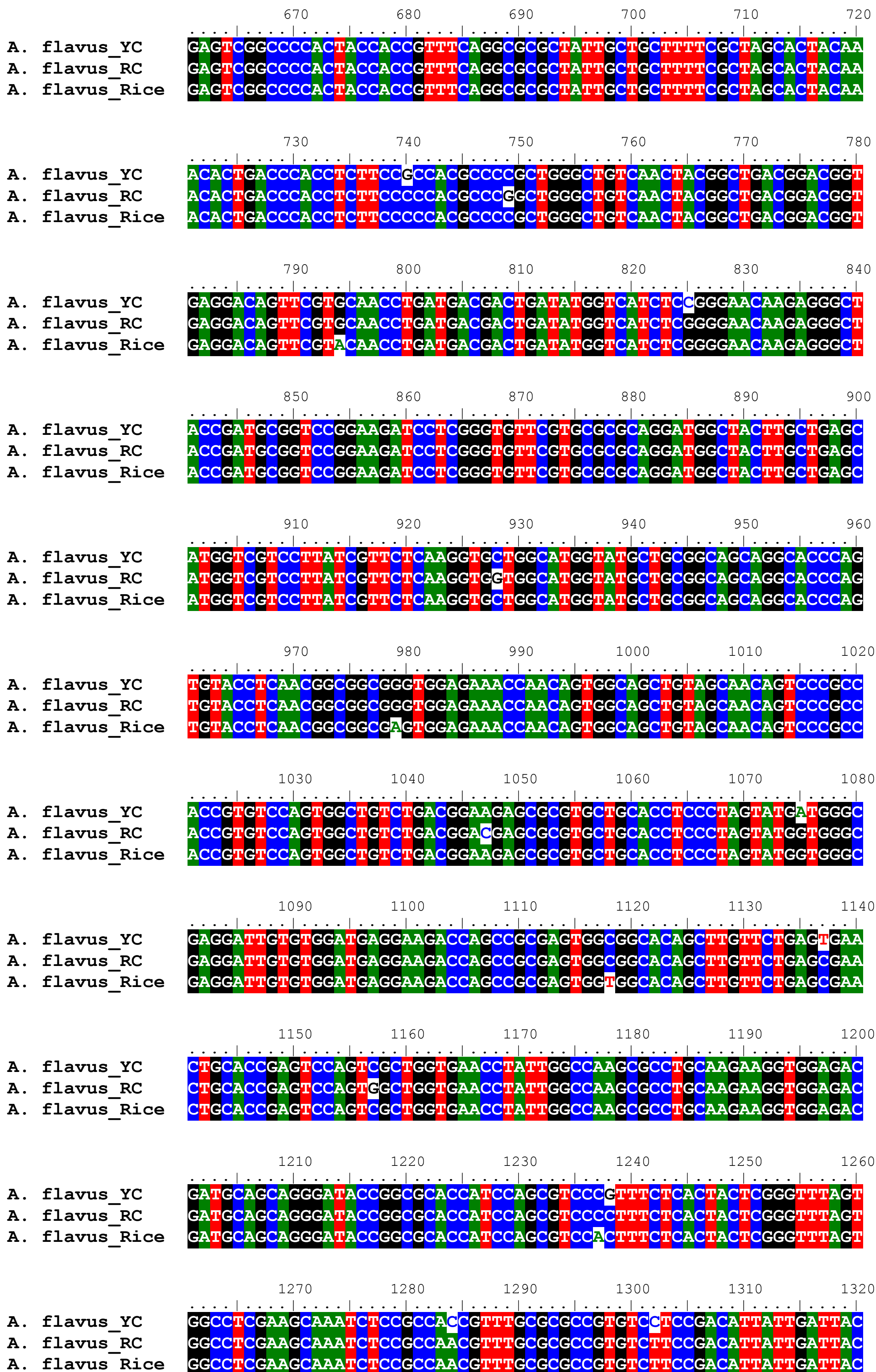

Figure 1b Multiple sequence alignment of the *aflr* gene partial sequence among three *Aspergillus flavus* isolated from yellow corn (YC), red corn (RC) and rice.

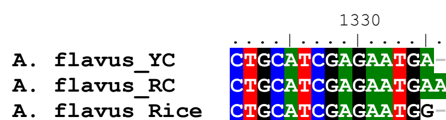

Figure 1c Multiple sequence alignment of the *aflr* gene partial sequence among three *Aspergillus flavus* isolated from yellow corn (YC), red corn (RC) and rice.

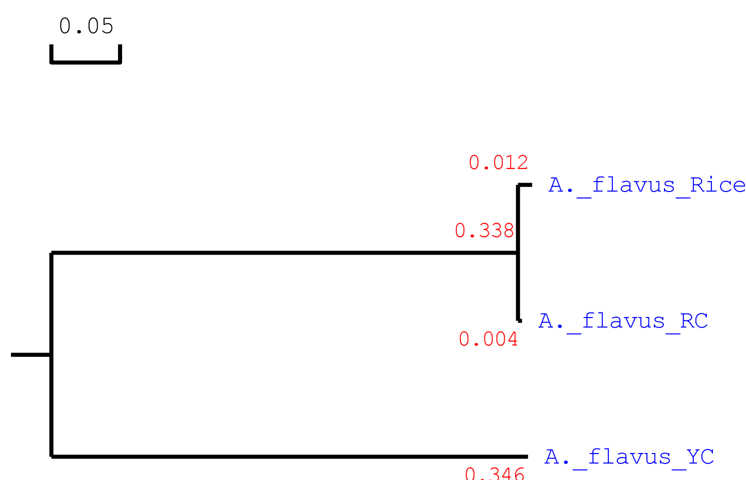

Figure 2 Phylogenetic tree using neighbor joining method among the three *Aspergillus* isolates.

Table 1 Flexible length predictions of epitopes in the amino acids sequence of *AFLR (aflR)* gene sequence protein of the three *Aspergillus flavus* isolates.

No.	Epitope/ A. flavus _ YC (yellow corn)	Score/	Epitope/ A. flavus _ RC (red corn)	Score/	Epitope/ A. flavus _ Rice (white rice)	Score/
1	RLQEGGDDAAGIPA	1	RLQEGGDDAAGIPA	1	RLQEGGDDAAGIPA	1
2	SPPPPVETQGLGGD	1	SPPPPVETQGLGGD	1	SPPPPVETQGLGGD	1
3	DHISPRASPGPIRS	1	DHISPRASPGPIRS	1	GETNSGSCSNSPAT	1
4	PPHALPTPNGSSSV	1	PPHALPTPNGSSSV	1	RRASPGPIRSSQTR	1
5	GETNSGSCSNSPAT	1	GETNSGSCSNSPAT	1	PHALPNRNGSSSVS	1
6	IDPFFESAPLPPFQ	0.997	IDPFFESAPLPPFQ	0.997	IDPFLESAPLPPFQ	0.996
7	MGRNPRAPSPLDST	0.994	MARNPRAPSPLDST	0.995	MGRNPRAPSPIDST	0.992
8	RPSESLPSARSEQG	0.99	RPSESLPSARSEQG	0.99	RPSESLPSARSEQG	0.99
9	PAHNTYSTPHAHTQ	0.936	PAHNTYSTPHAHTQ	0.936	PAHNTYSTPHAHTQ	0.936
10	MEHGTHVDFLAEST	0.847	VRCTKEKPACARCI	0.855	VRCTKEKPACARCI	0.855
11	SSGCLTEERVLHLP	0.842	TDGEDSSCNLMTTD	0.79	SSGCLTEERVLHLP	0.842
12	TDGEDSSCNLMTTD	0.79	VVLIVLKVVAWYAA	0.778	THLFPHAPLGCQLR	0.733
13	KVRCKEKPACARCI	0.773	VGEDCVDEEDQPRV	0.713	VGEDCVDEEDQPRV	0.713

CONCLUSION

A best strategy to control mycotoxins is only by prevention, because most mycotoxins are chemically stable, so they remain unaffected during storage and processing. For this reason, we believed that by using a highly sensitivity and selectivity methods as molecular identification and bioinformatics characterizations of protein toxic gene for mycotoxins gives chance for production of antibody against its toxicity. This paper was show that molecular identification in *Aspergillus flavus* in three isolates in small grains (rice and corn) with the epitope prediction analysis demonstrated that there were great differentiations in the epitope sequences among the three isolates except in four position (as described above) were found to be common between all isolates. This work articulates that the molecular identification and characterization of three *A. flavus* using *Aspergillus flavus AFLR (aflR)* toxin gene and the unique antigenic determinants that could be used for design of a broad-spectrum antibody for rapid detection of *A. flavus* in foods and feeds that conducive to control in aflatoxin levels and cover prevention of mycotoxins.

A

```
1         11        21        31        41        51        60
|         |         |         |         |         |         |
MVDHISPRASPGPIRSSQTRRAGKLRDSCTSCASSKVRCKEKPACARCIERGLACQYMVS 60
..EEEEEEEEEEEEEE...................EEEEEEEEEEEEEE...........
KRMGRNPRAPSPLDSTRRPSESLPSARSEQGLPAHNTYSTPHAHTQAHTHAHSHRQPHPQ 120
..EEEEEEEEEEEEEE.EEEEEEEEEEEEEE.EEEEEEEEEEEEEE..............
SHPQSNQPPHALPTPNGSSSVSAIFSHQSPPPPVETQGLGGDLAGQEQSTLSSLTVDSEF 180
.......EEEEEEEEEEEEEE.......EEEEEEEEEEEEEE..................
GGSLQSMEHGTHVDFLAESTGSLFDAFLEVGTPMIDPFFESAPLPPFQARYCCFSLALQT 240
......EEEEEEEEEEEEEE..............EEEEEEEEEEEEEE............
LTHLFRHAPLGCQLRLTDGEDSSCNLMTTDMVISGNKRATDAVRKILGCSCAQDGYLLSM 300
................EEEEEEEEEEEEEE..............................
VVLIVLKVLAWYAAAAGTQCTSTAAGGETNSGSCSNSPATVSSGCLTEERVLHLPSMMGE 360
..........................EEEEEEEEEEEEEE.EEEEEEEEEEEEEE.....
DCVDEEDQPRVAAQLVLSELHRVQSLVNLLAKRLQEGGDDAAGIPAHHPASRFSLLGFSG 420
................................EEEEEEEEEEEEEE..............
LEANLRHRLRAVSSDIIDYLHRE 443
.......................
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B

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1         11        21        31        41        51        60
|         |         |         |         |         |         |
MVDHISPRASPGPIRSSQTRRARKLRDSCTSCASSKVRCTKEKPACARCIERGFACQYMV 60
..EEEEEEEEEEEEEE....................EEEEEEEEEEEEEE..........
SKRMARNPRAPSPLDSTRRPSESLPSARSEQGLPAHNTYSTPHAHTQAHTHAHSHPQPHP 120
...EEEEEEEEEEEEEE.EEEEEEEEEEEEEE.EEEEEEEEEEEEEE.............
QSHPQSNQPPHALPTPNGSSSVSAIFSHQSPPPPVETQGLGGDLAGQEQSTVSSLTVDSE 180
........EEEEEEEEEEEEEE.......EEEEEEEEEEEEEE.................
FGGSLQSMEHGNHVDFLAESTGSLFDAFLEVGTPMIDPFFESAPLPPFQARYCCFSLALQ 240
...................................EEEEEEEEEEEEEE...........
TLTHLFPHARLGCQLRLTDGEDSSCNLMTTDMVISGNKRATDAVRKILGCSCAQDGYLLS 300
.................EEEEEEEEEEEEEE.............................
MVVLIVLKVVAWYAAAAGTQCTSTAAGGETNSGSCSNSPATVSSGCLTDERVLHLPSMVG 360
.EEEEEEEEEEEEEE............EEEEEEEEEEEEEE.................EE
EDCVDEEDQPRVAAQLVLSELHRVQWLVNLLAKRLQEGGDDAAGIPAHHPASPFSLLGFS 420
EEEEEEEEEEEE.....................EEEEEEEEEEEEEE.............
GLEANLRQRLRAVSSDIIDYLHRE 444
........................
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C

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1         11        21        31        41        51        60
|         |         |         |         |         |         |
MVDHISRRASPGPIRSSQTRRARKLRDSCTSCASSKVRCTKEKPACARCIERGFACQYMV 60
......EEEEEEEEEEEEEE................EEEEEEEEEEEEEE..........
SKRMGRNPRAPSPIDSTRRPSESLPSARSEQGLPAHNTYSTPHAHTQAHTHAHSHPQPHP 120
...EEEEEEEEEEEEEE.EEEEEEEEEEEEEE.EEEEEEEEEEEEEE.............
QSHPQSNQPPHALPNRNGSSSVSAIFSHQSPPPPVETQGLGGDLAGQEQSTLSSLTVDSE 180
.........EEEEEEEEEEEEEE......EEEEEEEEEEEEEE.................
FGGSLQSMEHGNHVDFLAESTGSLFDAFMEVGTPMIDPFLESAPLPPFQARYCCFSLALQ 240
...................................EEEEEEEEEEEEEE...........
TLTHLFPHAPLGCQLRLTDGEDSSYNLMTTDMVISGNKRATDAVRKILGCSCAQDGYLLS 300
..EEEEEEEEEEEEEE............................................
MVVLIVLKVLAWYAAAAGTQCTSTAASGETNSGSCSNSPATVSSGCLTEERVLHLPSMVG 360
...........................EEEEEEEEEEEEEE.EEEEEEEEEEEEEE..EE
EDCVDEEDQPRVVAQLVLSELHRVQSLVNLLAKRLQEGGDDAAGIPAHHPASTFSLLGFS 420
EEEEEEEEEEEE.....................EEEEEEEEEEEEEE.............
GLEANLRQRLRAVSSDIIDYLHREW 445
.........................
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Figure 3 Show the amino acids sequence of *AFLR (aflR)* gene sequence protein of the three *Aspergillus flavus* isolates.

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