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Molecular Characterization of Fumonisin Mycotoxin Genes of Fusarium sp Isolated from Corn and Rice Grains

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ABSTRACT: Fungi mycotoxins can be a serious risk to health and lead to substantial economic loss. The environmental conditions of Saudi Arabia, with its mostly warm temperatures, are conducive to the growth of toxigenic fungi resulting in mycotoxin production in different food items. The current study elucidates the natural occurrence of toxigenic fungi and mycotoxin production in grains in Saudi Arabia. Samples of white rice and corn (yellow, red) grains were collected from different local markets and houses. Three fungal isolates were obtained from the corn and rice grains and examined using Potato Dextrose Agar (PDA) and Carnation Leaf Agar (CLA) media. *Fusarium* spp. were the most prominent fungi in yellow corn, red corn and white rice grains. Three isolated *F. moniliforme* strains were identified using molecular characterization of the trichothecene 3-O acetyltransferase (*TRI101*) toxin gene. The DNA genome of the three *Fusarium moniliforme* isolates (namely, *F. moniliforme*_1, *F. moniliforme*_2 and *F. moniliforme*_3, which correspond to isolates from yellow corn, red corn and white rice, respectively) were used as a template for PCR to amplify trichothecene 3-O acetyltransferase (*TRI101*). Partially sequenced fragments amplified using a specific primer set were used to confirm the identification of, and to evaluate the phylogenetic relationships among the three isolates as well as to identify the corresponding antigenic determinants. The epitope prediction analysis demonstrated that there were four epitopes with scores equal to 1 in *F. moniliforme*_1, *F. moniliforme*_2 and *F. moniliforme*_3, respectively. Interestingly, there were great dissimilarities in the epitope sequences among the three isolates except in NSTPRACASEQEVS, STSSRADSSSLSTD and CTLCPRSLMASSVR. This indicates that the unique antigenic determinants predicted in the trichothecene 3-O acetyltransferase (*TRI101*) toxin gene could be used for designing a broad spectrum antibody for rapid detection of *Fusarium* spp. in foods.

Keywords: Fumonisin; PCR; Sequences; Phylogenetic tree; Antigenic determinants.

الوصف الجزيئى لجينات سموم فيومونيسين لفطرالفيوزاريوم المعزول من حبوب الارز و الذرة

لطيفة الحسينان، منيرة القحطانى و رانده محمد أحمد فرج

الملخص: مما لاشك فيه أن الاصابات الفطرية للمواد الغذائية تمثل عواقب وخيمة بسبب المخاطر على الصحة العامة والخسائر الاقتصادية.كما ان الظروف البيئية و ارتفاع درجات الحرارة بالمملكة العربية السعودية توفر البيئة المناسبة لنمو الفطريات مما يؤدي إلى إنتاج السموم الفطرية في المواد الغذائية المختلفة. توضح الدراسة الحالية التواجد الطبيعي للفطريات السمية والسموم الفطرية في الحبوب في المملكة العربية السعودية. تم جمع عينات من الأرز الأبيض والذرة (الصفراء والحمراء) من مختلف الأسواق المحلية والمنازل ,حيث تم عزل ثلاث عزلات فطرية من الحبوب المطحونة وحبوب الأرز باستخدام اوساط غذائية مناسبه لنمو الفطريات (Carnation Leaf Agar (CLA),(PDA Dextro . الفيوزاريوم كان الأكثرانتشار في الذرة الصفراء والذرة الحمراء وحبوب الأرز الأبيض. تم تحديد ثلاث سلالات معزولة من الفطر M. moniliforme باستخدام توصيف جزيئي لجين O- 3 acetyltransferase (TRI101). تم استخدام جينوم الحمض النووي لعزلات الفيوزاريوم الثلاثة وهي F. moniliforme (F. moniliforme_1 و F. moniliforme_2 و F. moniliforme_3 ، والتي تقابل العزلات من الذرة الصفراء والذرة الحمراء والأرز الأبيض ، تم استخدام أجزاء متسلسلة جزئياً تم تضخيمها باستخدام مجموعة برايمر محددة لتأكيد التحديد ، لتقييم العلاقات البينيه للاحماض الامينيه بين العزلات الثلاثة. أظهر التحليل الجزيئى للأحماض الامينية أن هناك أربع بروتينات ذات درجات تساوي 1 في F. moniliforme_1 و F. moniliforme_2 و F. moniliforme_3 على

التوالي. من المثير للاهتمام ، كان هناك اختلاف كبير في تسلسل الاحماض الامينية من بين ثلاث عزلات إلا في NSTPRACASEQEVS ، STSSRADSSSLSTD و .CTLCPRSLMASSVR هذا يشير إلى أن المحددات الفريدة لمولدات الاحماض الامينية المتنبأ بها في جينة 3 O- acetyltransferase (TRI101) يمكن استخدامها لتصميم جسم مضاد واسع النطاق للكشف السريع عن Fusarium ، .sp في الأطعمة لأغراض مراقبة الجودة.

الكلمات المفتاحية: الفيوموسين، تفاعل البلمرة المتسلسل، تتابع القواعد النيتروجينيه، شجرة العلاقات الجينية وتعين التغيرات الجينية للاحماض الامينية.

1. Introduction

Fungi cause major crop diseases during harvest and storage under higher temperature and humidity conditions [1]. While more than 25 different fungi species are known to invade stored grains and legumes [2], certain species such as *Aspergillus*, *Fusarium* and *Penicillum* are responsible for most spoilage and germ damage during storage [3,4]. They cause a reduction in baking quality and nutritive value, produce undesirable odors, color and change the appearance of stored food grade seeds [5]. Mycotoxins are secondary metabolites produced by fungi, which cause health hazards to animals and human beings [6, 7]. Moreover, fungal infestation of the seed coat may not only decrease seed viability, but also cause abnormal seedling development [1,7]. A large number of 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 of great significance worldwide [8], but knowledge regarding fungal seed decay and its importance for plant demographic and community processes is quite limited [9,10]. Fungal genera, such as *Aspergillus sp; Fusarium sp; Penicillium sp; Alternaria sp;* and *Epicoccum sp.* have been isolated from seeds of beans, cowpea, peas, and cocoa [3, 11, 12]. Regarding legumes in Saudi Arabia, very little information exists with respect to natural contamination with toxigenic fungi and mycotoxins. Aflatoxin(s) have been detected in some *Aspergillus* isolates while fumonisin has been found in some *Fusarium* isolates [13]. Among food contaminants, mycotoxins may cause substantial economic loss due to reducing availability of commodities with acceptable levels of mycotoxins present, and their possibly greater cost [14]. Mycotoxins continue to pose various health risks to consumers depending on the specific mycotoxin consumed and the level of exposure, and the health status of individuals in the population [15]. The majority of mycotoxins of greatest concern for human and animal health are produced by the genera *Aspergillus*, *Penicillium*, and *Fusarium*, the so-called field fungi, which frequently infect various food commodities [10, 15], and outbreaks of mycotoxicoses in humans and animals, caused by ingestion of products containing mycotoxins have been reported [4, 16]. However, further studies confirm that the toxic effects depend on intake dose, toxin type, duration of exposure, metabolism, mode of action, and defense mechanism [17,18]. Humans are exposed to mycotoxins throughout their lives due to consumption of fungus-contaminated food products, but sufficient quantities of mycotoxins in food and feedstuff can adversely affect human and animal health [14, 18]. Many human diseases, especially carcinogenic, teratogenic, hepatic, and gastrointestinal ones, have been found to be linked to the ingestion of mycotoxin-contaminated products [4, 19, 20].

This study was conducted to determine the bioinformatics characterization of Fumonisins isolated from corn and rice grain in Saudi Arabia. Fumonisins are produced by species of *Fusarium* genera, principally *F. proliferatum*, *F. verticillioides*, and *F. nygamai* [21]. Fumonisin B_1 (FB_1) is the most abundantly occurring and toxicologically most significant derivative [22]. Fumonisins are widely found as contaminants in corn, rice, figs, beer, and other commodities. Temperatures of 15 to 30 °C and 0.9 to 0.995 water activity have been reported as optimum for fumonisin production [23]. FB_1, due to its cancer-promoting activity, is designated as a possible human carcinogen [24], and fumonisins are probably linked to human esophageal cancer [4, 25]. It is also known that they are nephrotoxic and hepatotoxic [26] and cause neural tube defects in experimental animal species, and may also affect humans [27]. Several studies have revealed mycotoxin contamination worldwide in rice, for example, aflatoxins have been found in the United Arab Emirates [28], fumonisins in Iran and Argentina [25, 29], OTA in Morocco [30], ZEA in Nigeria [31], DON in Italy [32], nivalenol in Korea [33] and citrinin in Egypt [34]. In this study, we have hypothesized that mycotoxins affect human populations because the storage conditions in local markets and houses are conducive to mycotoxin production. As most of the corn and rice are grown during the wet season, they are 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* [35, 36]. Humidity, temperature, storage conditions, and transport time are the factors that influence mycotoxin production in rice. In the early 20th century, many human diseases occurring in Japan and other Asian countries were attributed to mycotoxins consumed in mold-damaged rice [4, 37]. Unfortunately, enactment of stringent rules for mycotoxin control in food is not always the best solution [4, 38]. A beneficial effect in Saudi Arabian mycotoxin-contaminated food is left for domestic population and developing grain producing countries [39].

The impact of mycotoxin standards is more drastic for the population of developing countries [1, 39]. Therefore, whilst in terms of quantity, availability of food for consumption might not be a problem, the availability of high-quality food which is free from mycotoxins, or at least, having toxin levels in permissible limits, is a matter of great concern in Saudi Arabia and other highly populated areas of the world [1, 39]. Thus, the regulatory authorities should aim to facilitate trade without compromising the protection of consumers' health [4, 39]. Therefore, the aim of this study was to determine the *Fusarium* species that are naturally occurring in contaminating corn and rice seeds (as the main crops imported in Saudi Arabia) by the molecular identification of toxigenic mycotoxin profiles of those species and protein structural analysis depicted from the gene(s) responsible for toxin biosynthesis. We have hypothesized that by studying

the molecular properties of Fumonisins, we could in future be able to produce vaccines for those species of *Fusarium* genera which have a higher prevalence in developing countries [4, 10, 39].

2. Materials and Methods

2.1 Grain samples: 150 samples of corn (yellow and red grains) and rice were collected from markets and houses from different areas of Saudi Arabia (Riyadh, Hail, Qasim, Asir,Tabuk, Jizan, Jouf, Jeddah and Dammam). About 0.5 - 1 kg of samples was taken randomly and collected in clean dry packaging.

2.2 Isolation of mycotoxigenic *Fusarium* species

Agar plate and blotter tests were used to isolate *Fusarium* spp. as described by Neergaard [40]. The 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. Half of each group was plated on potato dextrose agar (PDA). All dishes were incubated for 5 to 7 days at 25 °C.

2.3 Purification and identification of *Fusarium* species

Fusarium isolates were identified as species based on the morphological characteristics 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 light microscope [41]. When macroconidia, microconidia and mycelial characteristics from SNA were insufficient for identifying the species, further examination of the samples were done on different agar media. Potato Dextrose Agar (PDA) was used to identify colony pigment characteristics of aerial mycelium on the agar [40, 41] Carnation Leaf Agar (CLA) was used to identify macroconidia, chlamydospores and the presentation of aerial mycelium. A single colony was transferred and purified by the hypha tip technique onto a DA medium in the presence of streptomycin (50 mg /ml). Cultured fungi were processed for molecular identification using specific primers for the trichothecene 3-O acetyltransferase (*TRI101*) toxin gene. All conditions of isolation and purification of mycotoxins were performed under sterilization to prevent any external agent from polluting the seeds.

2.4 Molecular identification of trichothecene 3-O acetyltransferase (*TRI101*) toxin gene

2.4.1 Isolation of genomic DNA

The mycelium mass of *Fusarium* species isolates grown on a PDA broth medium was harvested by centrifugation at 6000 rpm for 10 min. The pellets were washed twice by PBS buffer and stored at 20 °C. Total DNA of three isolates was isolated using the lysozyme-dodecyl sulfate lysis method as described by Leach *et al*. [41].

2.4.2 Amplification and purification of trichothecene 3-O acetyltransferase (*TRI101*) gene

Specific PCR reactions were conducted to assess the presence of ***TRI101*** gene. The primers used were: FAD-U1 (5′-GATCTCGACATGGCCTTTGTCCCC-3′); FAD-D1 (5′-GAACAGGTGGTGAATGACGTGCTTC-3′) [40]. 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 (COT Thermocycler model 1105). Purification of PCR product was detected by electrophoresis using agarose 1.5% in 1x TAE buffer and stained with ethidium bromide [21, 41]. The trichothecene 3-O acetyltransferase (*TRI101*) gene fragment was excised from the gel and purified using a QIA quick gel extraction kit (Qiagen, Berlin, Germany).

DNA sequencing by purified PCR products were prepared for Sanger sequencing technology using the DNA sequencer technique (Sigma, central lab, PNU, KSA). DNA sequences of *Fusarium* isolates were aligned using Bio Edit software version 7 (www. Mbio-NCUs. Edu/bio. Edit) and were compared to the reference sequences accessions of *Fusarium* spp. available in the nucleotide database at NCBI using BIASTn-algorithm to identify closely related sequences (http/WWW.NCbI.Nih.Gov). Dendrograms were constructed using un-weighted pair group method with Arithmetic (UPGMA) on Genbank.

2.5 Epitope prediction and antigenicity

The primary amino acid sequence of the trichothecene 3-O acetyltransferase (*TRI101*) 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 the BCPREDS server with default parameters (http://ailab.cs.iastate.edu/bcpreds/), which implements a support vector machine (SVM) and the subsequence kernel method [42]. Flexible length linear B-cell epitopes were predicted using the FBCP red [43] 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, which makes use of the physicochemical properties of each amino acid and its frequency of occurrence in experimentally known segmental epitopes.

3. Results

The *Fusarium* isolates were selected for molecular identification using trichothecene 3-O acetyltransferase (*TRI101*) gene sequencing. Three *Fusarium* isolates represented grains from yellow corn, red corn and white rice and were designated as *F. moniliforme*_1, *F. moniliforme*_2 and *F. moniliforme*_3, respectively.

3.1 Molecular characters of *toxin* gene:

Total DNA was extracted from *Fusarium* isolates [*F. moniliforme*_1 (yellow corn isolate), *F. moniliforme*_2 (red corn isolate) and *F. moniliforme*_3 (white rice isolate)] from infected grains. The trichothecene 3-O acetyltransferase (*TRI101*) gene of three *F. moniliforme* isolates was amplified from isolated DNA of mycelium. The nucleotide partial sequence of the trichothecene 3-O acetyltransferase (*TRI101*) gene in the three isolates was compared with published isolates in the GenBank. The sequence homology revealed that the gene of interest was the trichothecene 3-O acetyltransferase (*TRI101*) gene and the test fungal isolates were *Fusarium moniliforme* isolates.

A multiple sequence alignment (MSA) was constructed using Clustal W software between the three studied isolates (Figure 1A). The alignment showed many conserved regions in all sequences and also distinguished the heterogeneity positions among the aligned sequences. Phylogenetic analysis was performed by construction of a phylogenetic tree using a neighbor-joining method to unravel the relationships among all *Fusarium moniliforme* isolates (Figure 1B). The phylogenetic tree resulted in two clades in which *Fusarium moniliforme*_1 (yellow corn isolate) and *Fusarium moniliforme*_2 (red corn isolate) were in the same cluster whilst *Fusarium moniliforme*_3 (white rice isolate) was separate in a different cluster (Figure 2A, B). Thus, the molecular identification based on sequence homology of the trichothecene 3-O acetyltransferase (*TRI101*) gene confirmed the identity and phylogeny of the studied three *Fusarium moniliforme* isolates.

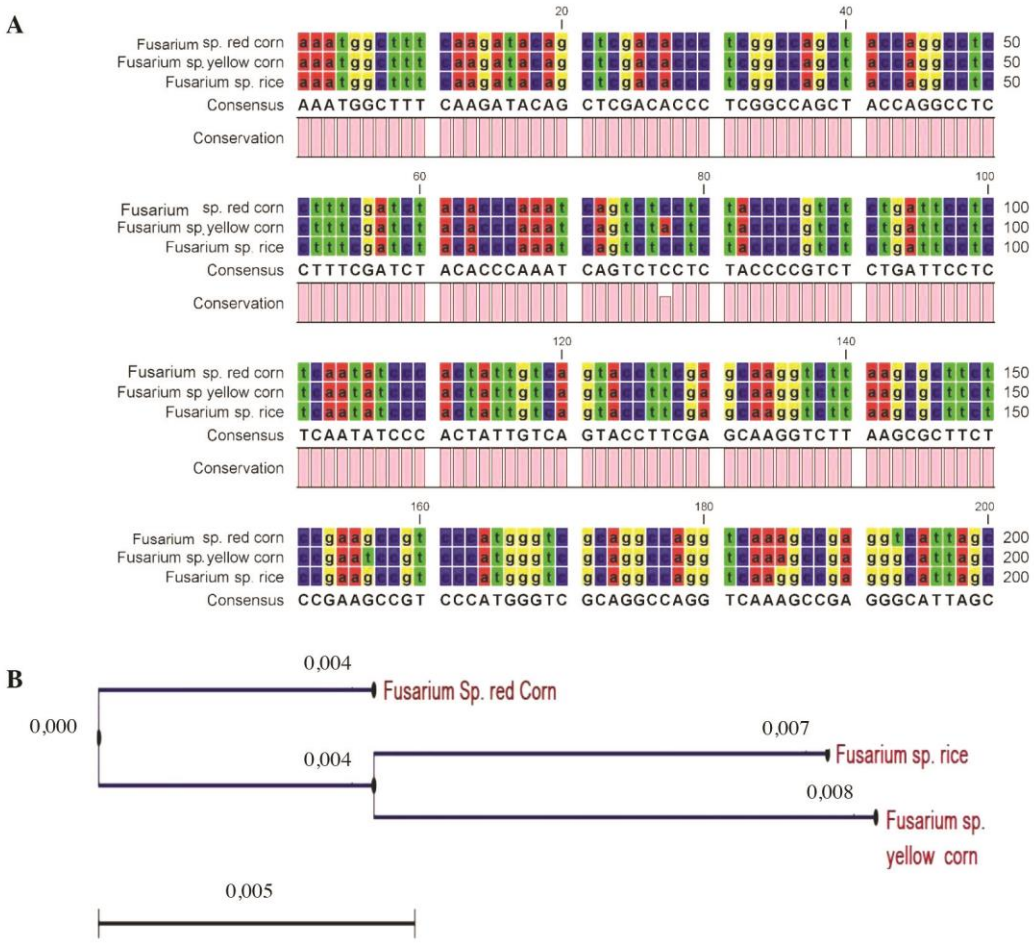

Figure 1. A and B are MSA for Phylogeny of the three studied *Fusarium* sp. isolates (*F. moniliforme*_1 (red corn isolate) and *F. moniliforme*_2 (white rice) were in the same cluster whilst *Fusarium F. moniliforme*_3 (yellow corn isolate) was separate in a different cluster.

A

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1         11        21        31        41        51        60
|         |         |         |         |         |         |
KWLSRYSSTPSASYQASFRSTPKSVYSTPSLIPLNIPLLSVPSSKVLSASPNPSHGSQAR 60
......EEEEEEEEEEEEE.........................EEEEEEEEEEEEE
SKPRALARETQELPLSSLSRTFLVLKTSAMILQRPRSRAERRHTLWRCLTRTSSRQGRRY 120
E.............................EEEEEEEEEEEEEE.............
LLDLVLVPTTQSLFYCSSTSSRADSSSLSTDITVLWIWAKMRSVYSPRRAVTTHSPKRKR 180
..EEEEEEEEEEEEE.EEEEEEEEEEEEEE.....EEEEEEEEEEEEE..........
PTSIARRFLTLKTTRLAPRIIRLSNLMLVVTLFSRRSVQAGRSSHSALRPCQSSRMLLPR 240
............................................................
LLTHQQSSCRLTMLFRRSSGSRPLACVSKESMALHLPSSAVLLMLDRQWVSRTTTQAFFK 300
..................EEEEEEEEEEEEE.......................E
TPTTTRPSAKSPTSHSAQQHHAFVQNSTPRACASEQEVSRRICTTTPTSPTYPRLMRTHL 360
EEEEEEEEEEEEE............EEEEEEEEEEEEE.EEEEEEEEEEEEE......
PASCVLGPRWDSGITTLDSDWLSPRLDGQSLSLLRACTLCPRSLMASSVRRLLGTRIWTD 420
.....EEEEEEEEEEEEE.................EEEEEEEEEEEEE..........
RRIRSGP 427
.......
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B

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1         11        21        31        41        51        60
|         |         |         |         |         |         |
KWLSRYSSTPSASYQASFRSTPKSVSSTPSLIPLNIPLLSVPSSKVLSASPKPSHGSQAR 60
.EEEEEEEEEEEEE.EEEEEEEEEEEEE.................EEEEEEEEEEEEE
SKPRSLARETQELPSSLLRTFLVLKTSAMILQRPRSRAERRDTLWRCLTRTSSRQGRRYL 120
E.EEEEEEEEEEEEE..............EEEEEEEEEEEEE...............
LDLVLVPTTQSLFYCSSTSSRADSSSLSTDITVLWIWAKMRSVYSPRRAVTTHSPKRRPT 180
.EEEEEEEEEEEEE.EEEEEEEEEEEEE.....EEEEEEEEEEEEE...........
SIARRFLTLKTTRLAPRIIRLSNLMLVVTLFSRRSVQAGRSSHSAARPCQSSRMLLPRLL 240
............................................................
THQQSSCRLTMLFRRSSGNRPLACVSKESMALHLPSSAVLLMLDRQWVSRTTTQAFFKSP 300
..................EEEEEEEEEEEEE.......................EEE
TTTRPSAKSPKSHSAQQHHAFVQNSTPRACASEQEVSRRICTTTPTSPTYPRLMRTHLPA 360
EEEEEEEEEEE............EEEEEEEEEEEEE.EEEEEEEEEEEEE........
SCVLGPRWDSGIKTLGSDWVSPRLDGQSLSLLRACTLCPRSLMASSVRRFLGTRIWTDRR 420
...EEEEEEEEEEEEE.................EEEEEEEEEEEEE............
IRSGP 425
.....
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C

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1         11        21        31        41        51        60
|         |         |         |         |         |         |
KWLSRYSSTPSASYQASFRSTPKSVSSTPSLIPLNIPLLSVPSSKVLSASPKPSHGSQAR 60
.EEEEEEEEEEEEE.EEEEEEEEEEEEE.................EEEEEEEEEEEEE
SRPRALAKETQELPLSSLLRTFLVLKTSAMILQRPRSRAERRDTLWRCLTRTSSRQGRRY 120
E.EEEEEEEEEEEEE...............EEEEEEEEEEEEE...............
LLDLLLVPTTQCLFYCSSTSSRADSSSLSTDITVLWIWAKMRPVYSPRRAVTTHSPKRKR 180
..EEEEEEEEEEEEE.EEEEEEEEEEEEE.....EEEEEEEEEEEEE...........
PTSIARRFLTLKTTRLAPRIIRLSNLMLVVTLFSRRSVQAGRSSHSAPRPCQSSRMLLPR 240
............................................................
LLTHQQSSCRLTMLFRRSSGIRPLACVSKESMALHLPSSAVLLMLDRQWVSRTTTQAFFK 300
...............EEEEEEEEEEEEE.............................E
TPTTTRPSAKSPTSHSAQQHHAFVQNSTPRACASEQEVSRRICTTTRTSPTYPRLMRTHL 360
EEEEEEEEEEEEE............EEEEEEEEEEEEE..EEEEEEEEEEEEE.....
PASCVLGPRWHSGITTLGSDWVSPRLDGQSLSLLRACTLCPRSLMASSVRRFLGTRIWTD 420
.....EEEEEEEEEEEEE.................EEEEEEEEEEEEE...RRIRSGP 427
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Figure 2. Amino acid residues of trichothecene 3-O acetyltransferase (TRI101) protein in **A:** *Fusarium* spp._1 (yellow corn), **B:** *Fusarium* spp._2 (red corn) and **C:** *Fusarium* spp._3 (white rice) showing predicted epitopes (Red) that are highlighted.

Table 1. Flexible length predictions of epitopes in the amino acids sequence of trichothecene 3-O acetyltransferase (TRI101) protein of the three studied *Fusarium* isolates

No.	Epitope/Fusarium sp._1 (yellow corn)	Score/	Epitope/ Fusarium sp._2 (red corn)	Score/	Epitope/ Fusarium sp._3(white rice)	Score/
1	RICTTTPTSPTYPR	1	RICTTTPTSPTYPR	1	ICTTTRTSPTYPRL	1
2	KTPTTTRPSAKSPT	1	KSPTTTRPSAKSPK	1	KTPTTTRPSAKSPT	1
3	NSTPRACASEQEVS	1	NSTPRACASEQEVS	1	NSTPRACASEQEVS	1
4	SSTPSASYQASFRS	0.998	SFRSTPKSVSSTPS	1	SFRSTPKSVSSTPS	1
5	SASPNPSHGSQARS	0.996	SASPKPSHGSQARS	0.998	SASPKPSHGSQARS	0.998
6	STSSRADSSSLSTD	0.993	STSSRADSSSLSTD	0.993	LGPRWHSGITTLGS	0.997
7	SRPLACVSKESMAL	0.974	QRPRSRAERRDTLW	0.984	STSSRADSSSLSTD	0.993
8	LGPRWDSGITTLDS	0.967	WLSRYSSTPSASYQ	0.974	QRPRSRAERRDTLW	0.984
9	QRPRSRAERRHTLW	0.94	NRPLACVSKESMAL	0.949	WLSRYSSTPSASYQ	0.974
10	CTLCPRSLMASSVR	0.892	LGPRWDSGIKTLGS	0.896	RSSGIRPLACVSKE	0.967
11	IWAKMRSVYSPRRA	0.871	CTLCPRSLMASSVR	0.892	PRALAKETQELPLS	0.953
12	DLVLVPTTQSLFYC	0.794	IWAKMRSVYSPRRA	0.871	CTLCPRSLMASSVR	0.892
13	-		DLVLVPTTQSLFYC	0.794	IWAKMRPVYSPRRA	0.883
14	-		PRSLARETQELPSS	0.704	DLLLVPTTQCLFYC	0.708

The epitope prediction analysis demonstrated that there were 1, 2, 3 and 4 epitopes with a score equal to 1 in *F. moniliforme* _1, *F. moniliforme* _2 and *F. moniliforme*_3, respectively. Also there were great variations in the epitope sequences among the three isolates except for NSTPRACASEQEVS, STSSRADSSSLSTD and CTLCPRSLMASSVR, which where common among all isolates. These residues with high frequencies of occurrence in antigenic determinants are highlighted (yellow) in the antigenicity profile (Figure 3). Figure 3 also shows the variability in the positions and types of amino acid residues with high antigenic frequency.

4. Discussion

Fungal infections not only cause considerable economic loss, there is no doubt that contamination of grains and foodstuffs with mycotoxins has become a danger that can't be ignored [40, 43]. Many species are well known mycotoxin producers with various toxicological properties which pose high risk to human and animal health [44, 45].

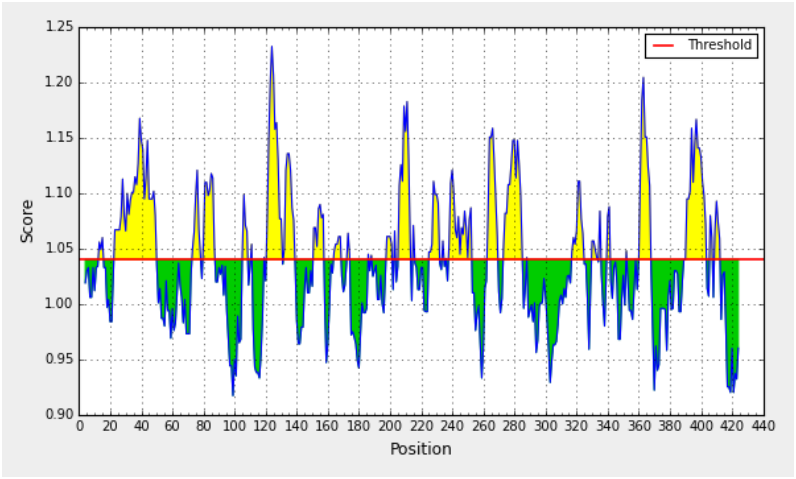
Environmental factors and host species have a strong impact on the occurrence of a specific chemotype and the incidence of *Fusarium* species [46]. The distribution of *Fusarium* species in maize is influenced by climatic conditions, pathogenicity and competition between other fungi [47]. The type of environmental factor identified in the incidence of *Fusarium* species was demonstrated in recent EU maize surveys [48]. In these studies, the prevalence of species varied year-to-year and is believed to be associated with the differences in climatic conditions between years [49, 50].

As was reported by [51], the presence of toxigenic fungi on small grains has a negative impact on the safety and quality of animal feed and human food [51]. The genus Fusarium includes cosmopolitan and ubiquitous mold fungi in which saprobes and plant pathogens are many. Fusarium species causes yield losses in processing and production [51, 52]. Being able to grow at low temperature, Fusarium spp. are responsible for spoilage of food through contamination during transport and storage [9, 17, 52]. In addition, reduction in nutritive value, insipidness and discoloration are other problems resulting from contamination of grains by *Fusarium* [53].

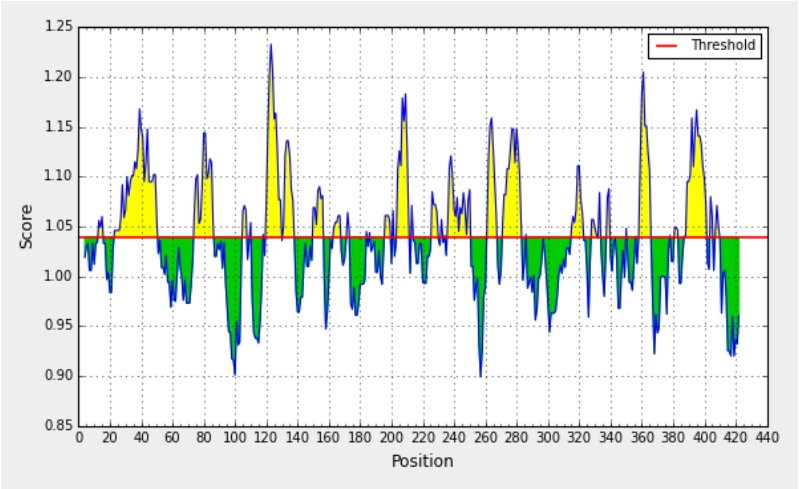
The advance of rapid and accurate identification of *Fusarium* and/or their metabolites are mandatory for the implementation of preventive measures in the whole food production system, as was reported by [54]. The molecular characterization of three *Fusarium spp.* isolated from small grains (yellow corn, white rice and red corn) using the mycotoxin gene, trichothecene 3-O acetyltransferase (*TRI101*), allowed for coupled identification and mycotoxin screening in the three *Fusarium* isolates [54, 55]. Following the molecular identification of *Fusarium* spp., B-cell epitopes in the trichothecene 3-O acetyltransferase (*TRI101*) 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 [56]. Epitope prediction saves labor and time for validation experiments. The identification of epitopes plays a crucial role in vaccine design, immunodiagnostic testing and antibody production [56]. In other study BCPREDS serves were used to predict epitopes found in the primary amino acids sequence of trichothecene 3-O acetyltransferase (TRI101) protein, where BCPREDS has proved highly efficient for predicting linear B-cell epitopes in SARS-CoV S protein [56, 57]. There was variability in the sequence and numbers of epitopes among the three toxin proteins analyzed [57].

In the present study, a fixed length of epitopes (14 residues) was observed. The epitope prediction analysis demonstrated that there were 1, 2, 3 and 4 epitopes with scores equal to 1 in F. moniliforme_1, *F. moniliforme*_2 and *F. moniliforme*_3, respectively. Interestingly, there were great dissimilarities in the epitope sequences among the three isolates except for NSTPRACASEQEVS, STSSRADSSSLSTD and CTLCPRSLMASSVR, which were common

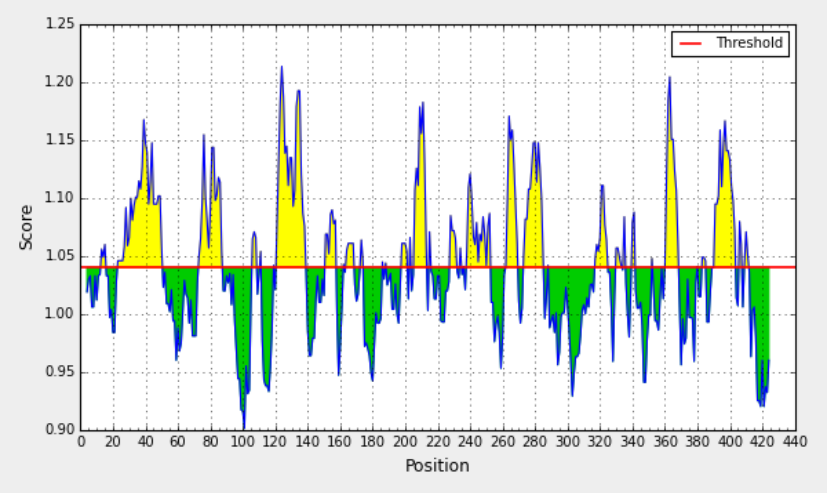
among all isolates. This result suggests its exploitation for the design of a specific antibody to be used for rapid detection of different *Fusarium* species in small grains. Epitope prediction has many implications in pathogen detection and differentiation applications. Consideration of the occurrence of *Fusarium* spp. on small grains is important in the risk assessment of mycotoxins and in proactively setting up preventive measures [57].



A



B



C

Figure 3. Kolaskar and Tongaonkar antigenicity scale for prediction of antigenic determinants in trichothecene 3-O acetyltransferase (TRI101) in A: *Fusarium* spp._1 (yellow corn), B: *Fusarium* spp._2 (red corn) and C: *Fusarium* spp._3 (white rice) Amino acid residues of high frequencies in epitopes are distinguished (yellow).

Conclusion

The identification of immunodiagnostic testing and antibody production of a fixed length of epitopes (14 residues) observed in the present study plays a crucial role in the vaccine design. This helps to control the incidence of mycotoxins in small grains (rice and corn). We also need to design bagged information about Epitope prediction for identification of mycotoxins in crops with economic value and high consumption rate.

Conflict of Interest

The authors declare no conflict of interest.

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