Iranian Biomedical Journal 12 (4): 229-236 (October 2008)

Comparative Study of the Major Iranian Cereal Cultivars and some Selected Spices in relation to Support *Aspergillus parasiticus* Growth and Aflatoxin Production

Mansour Zare[1], Masoomeh Shams-Ghahfarokhi[2], Shahrokh Ranjbar-Bahadori[1], Abdolamir Allameh[2] and Mehdi Razzaghi-Abyaneh[*3]

[1] *Faculty of Veterinary Sciences, Azad University, Garmsar, Iran;* [2] *Faculty of Medical Sciences, Tarbiat Modares University, Tehran 14115-111, Iran;* [3]*Dept. of Mycology, Pasteur Institute of Iran, Tehran 13164, Iran*

Received 26 November 2007; revised 15 March 2008; accepted 14 April 2008

ABSTRACT

Background: Aflatoxins are toxic fungal metabolites enable to contaminate a wide range of natural substrates. This contamination can be host-specific for different plant species. In this study, the ability of a toxigenic *Aspergillus parasiticus* to produce various aflatoxins on major Iranian cereals was evaluated with special focus on plant susceptibility to toxin production at cultivar level. **Methods:** *Aspergillus parasiticus* cultured on major Iranian cereal cultivars and some selected spices was incubated in shaking condition at 28ºC for 6 days. The concentration of aflatoxins B_1 and total (B_1, B_2, G_1 and G_2) was measured by thin layer chromatography. **Results:** The amounts of aflatoxin B_1 produced on maize, wheat and rice cultivars were in the ranges of 1.0-33.9, 41.9-193.7, and 39.1-82.3 µg/g fungal weight, respectively. Interestingly, genetically modified *Bacillus thuringiensis* rice (GM rice) of Tarom Molaii cultivar examined for the first time in this study showed less susceptibility to aflatoxin production in comparison with its normal counterpart (P<0.05). The mean of aflatoxin production on maize cultivars was less than both wheat and rice cultivars that indicates considerable resistance of maize to aflatoxin compared with two other cereals. Unlike to *Cuminum cyminum*, both *Helianthus annuus* and *Carum carvi* seeds were highly resistant to aflatoxin production. **Conclusion:** These results indicate that inter- and intra-species differences exist in susceptibility of the major Iranian cereals as well as spices tested to *A. parasiticus* growth and aflatoxin production. Further studies are recommended to determine resistance markers of selected cultivars of Iranian cereals. *Iran. Biomed. J. 12 (4): 229-236, 2008*

Keywords: *Aspergillus parasiticus*, Aflatoxin (AF), Cereals, Spices, Genetically modified Bacillus thuringiensis rice (BT) (GM rice), Iran

INTRODUCTION

Aflatoxins (AF) are a group of closely related polyketide secondary metabolites produced mainly by some mycotoxigenic species of *Aspergillus* section *Flavi* especially *A. flavus*, *A. parasiticus* and *A. nomius* [1, 2]. The AFB_1, B_2, G_1 and G_2 are the major types among at least 16 structurally related toxins of this group. From these most economically relevant mycotoxins, AFB_1 has received major consideration because of its carcinogenic, mutagenic and teratogenic properties in human and animals. AF are capable of contaminating a wide range of natural substrates such as cereals, oilseeds and spices as well as different food and feeds in suitable conditions. This contamination is considered as an important agricultural, economic and health problem worldwide [3].

With respect to more than 40 years research by different scientists, the biosynthetic pathway of AF is thought to be the best known pathway among all secondary metabolites. This complex pathway is mediated by more than 25 clustered genes whit in a 75-kb DNA region on the fungal chromosome and involves at least 23 enzymatic steps in producing fungi [4-9].

Despite the large data exist now about different cellular and molecular aspects of AF from mycology to biochemistry and even prevention or control

*Corresponding Author; Tel. (+98-21) 6695 3311-20; Fax: (+ 98-21) 6646 5132; E-mail: mrab442@yahoo.com

[DOR: 20.1001.1.1028852.2008.12.4.8.7]

[DOI: -]

strategies, there is no common accepted procedure which be capable to completely resolve the AF contamination problem of crops and agricultural commodities. One of the major reasons is the complex plant host-mycotoxigenic fungus interactions which many of its aspects remain unclear. Different strategies have been used to prevent AF contamination of susceptible substrates [2-5]. These are mainly focused on host-mycotoxigenic fungus interaction i.e. fungal processes needed for plant invasion and contamination as well as various plant defense mechanisms against fungal invasion. Upon the observation of basic differences among various plant species and their selected cultivars to *Aspergillus*-AF contamination, special consideration has been made to screening for resistant strains and their potential chemical or genetic resistance biomarkers.

Data from several studies indicated that inter- or intraspecies variations exist among various plants from different geographic areas of the world which determine the susceptibility or resistance of a selected plant to *Aspergillus*-AF contamination [10-12]. Thus, the evaluation of new crop cultivars all over the world is important to find out resistant cultivars to select for cultivation as well as for further identification of their resistance biomarkers.

As in other parts of the world, rice, wheat and maize comprise the most economically cereal crops produced in amounts of thousand tons in different parts of Iran. Likewise, *Helianthus annuus* (sunflower), an annual plant in the family Asteraceae as well as two major spices of the genus Carum including *Carum carvi* (Caraway; Persian cumin) and *Cuminum cyminum* are routinely cultivated in Iran for using in food chain and in traditional medicine. Our recent study has shown that aflatoxigenic fungi are present in agricultural soil of Iran where they can contaminate various agricultural commodities either directly or indirectly in suitable conditions [13].

In this communication, the susceptibility of some important cultivars of the main Iranian cereals i.e. maize, wheat and rice as well as *H. annuus*, *C. carvi* and *C. cyminum* to AF production by *Aspergillus parasiticus* was studied for the first time in order to identify resistant cultivars for further research. Special consideration has been made to a genetically modified Bacillus thuringiensis rice (BT) (GM rice) of a local variety Tarom Molaii produced in a share project in Iran.

MATERIALS AND METHODS

Chemicals. AF standards (B_1, B_2, G_1 and G_2) were obtained from Sigma Chemical Co., St. Louis, MO, USA. All other solvents and reagents were of analytical grade purchased from E. Merck, Germany.

Seed samples. As indicated in Table 1, a number of 14 cultivars of cereals and spices of 250 g each were supplied by Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran. All cereals and spices were freshly prepared and transferred to the laboratory in the sterile nylon bags. They were kept in sterile conditions at 4ºC before they were examined. GM rice (Bt rice) has been approved by ABRII in collaboration with the Philippines-based International Rice Research Institute (IRRI) by inserting a bacterial gene that produces a toxin into a local variety of aromatic rice, Tarom Molaii. To ensure any previous contamination with AF, a portion of each cereal and spice sample was examined with thin layer chromatography (TLC) as describe in further steps.

Fungal strain and culture condition. *A. parasiticus* NRRL 2999, a toxigenic strain with capability of producing 4 major AF was used throughout the study. The fungus was cultured on different substrates according to the procedure of Shotwell *et al.* [14] with some modifications as follow: Uncracked seeds of each cereals and spices were surface sterilized by immersing in 1% sodium hypochlorite solution for 2 min. After thoroughly washing with distilled water, 50 g of each cereals and spices was separately transferred into 250-mL Erlenmeyer flasks containing 25 mL sterilized tap water. The flasks (test flasks) were allowed to stand in shaking condition for 2 h at room temperature and then inoculated with *A. parasiticus* spore suspension prepared from 7-day cultures on potato dextrose agar slants in amount of 10^6 spores/g substrate. Non-inoculated control flasks were also considered for each cultivar at the same conditions except that they were not inoculated with fungal spores. The cultures were maintained on a shaking incubator (JEIO TECH SI-900R, South Korea) at 28ºC with 150 rev/min for 6 days. Three flasks were considered for each cultivar and the experiment was done with two replicates.

[DOR: 20.1001.1.1028852.2008.12.4.8.7]
[DOI: -]

Table 1. The cultivar list of major Iranian cereals and some important spices. *A. parasiticus* growth rate on different cultivars of major Iranian cereals in comparison with those related to individual spices as *Helianthus annuus*, *Cuminum cyminum* and *Carum carvi* is shown.

Substrate	Cultivar	Fungal growth rate (g/50 g substrate)
Maize (*Zea mays*)	SC 301	17.3 ± 0.8
	SC 647	9.8 ± 0.4
	SC 700	16.2 ± 0.9
	SC 704	11.9 ± 1.0
Wheat (*Triticum aesativum*)	Foung	4.1 ± 0.3
	Gascogen	6.1 ± 0.7
	Light crass	7.4 ± 0.4
	Star	4.9 ± 0.3
Rice (*Oryza sativa*)	Tarom Molaii	16.9 ± 0.8
	Tarom Molaii (GM type)*	8.8 ± 0.4
	Astaneh	6.1 ± 0.3
Helianthus annuus	IRAN HA-1	16.6 ± 0.7
Cuminum cyminum (Cumin)	IRAN E-1	5.4 ± 0.2
Carum carvi (Percian cumin)	IRAN E-3	6.8 ± 0.2

*GM, genetically modified; as described in Materials and Methods section.

Fungal growth determination. Total weight of each flask was calculated at time zero (after inoculating with fungal spores) and after 6 days (at the end of growth period). The corrected weight of test flasks was calculated by considering the weight change of corresponding non-inoculated control flasks at the end of incubation period (the 6^{th} day). The fungal growth rate of 6-day cultures was estimated by reducing the corrected weight of inoculated test flasks from their initial weight at time zero.

Extraction of aflatoxins. Total cultures of each cereals or spices were separately blended in a waring blender for 5 min in presence of 250 mL water and then after adding 250 mL chloroform for an extra 5 min. The chloroformic phase (lower phase) was separated from the aqueous phase (upper phase) after centrifuging the mixture at 3000 ×g in 4ºC for 20 min. After passing the lower phase through anhydrous sodium sulfate for removing the remaining water, the clarified filtrate was concentrated using an EYELA rotary evaporator N-1000 (EYELA, Japan). The residue was dissolved in a known volume of chloroform for AF analysis.

Aflatoxin measurements. AF (B and G types) concentration was estimated by TLC method as described in our previous publication [15]. The sample extracts as well as AF standards (0.1, 0.5, 1 and 2 µg) were spotted on Silica gel 60 F_{254} pre-coated TLC plates and developed in a TLC chamber with chloroform:methanol (98:2, v/v) as mobile phase. The amount of AF was calculated at 365 nm using CAMAG TLC Scanner 3 (CAMAG, Switzerland) by comparing the area under curved of unknown samples with authentic AF standards.

Statistics. The results were analyzed by SPSS version 10 (SPSS Inc., Chicago, Illinois) programme for Windows. Analyses of variance (ANOVA) and Tukey's multiple comparison test at 5% significance level were used to compare the means of fungal growth and toxin production data. $P<0.05$ was considered significant.

Biosafety. All steps for culture of *A. parasiticus* as well as extraction and analysis of AF were carried out in an equipped laboratory with attention to biohazard cautions. The experiments were done under a biosafety cabinet using special gloves, eye wear and body dresses.

RESULTS

Fungal growth on different substrates. Growth rate of *A. parasiticus* NRRL 2999 on different substrates is shown in Table 1. The mean values of total *A. parasiticus* growth on cultivars of maize, wheat, and rice were recorded as 13.8, 5.6 and 10.6 g, respectively. The growth rate of fungus on *H. annuus*, *C. cyminum* and *C. carvi* were determined

[DOR: 20.1001.1.1028852.2008.12.4.8.7]
[DOI: -]

as 16.6, 5.4, and 6.8 g accordingly. Among maize samples, cultivar SC 301 was found to support the highest growth of *A. parasiticus* followed by SC 700, SC 704 and SC 647 cultivars. Fungal growth was supported in the range of 4.1 to 7.4 g by different wheat cultivars. Among these, only the differences between Light grass cultivars (7.4 g) were significant with two other cultivars including Fong (4.1 g) and Star (4.9 g) (P<0.05). For rice cultivars, the fungal growth was significantly higher in Tarom Molaii normal cultivar (16.9 g) compared with Astaneh cultivar (6.1 g) (P<0.05). Interestingly, GM type of Tarom Molaii cultivar was found to be less susceptible to *A. parasiticus* growth compared with its normal counterpart. The fungal growth on this cultivar was recorded as 8.8 g per total culture (50 g). *H. annuus* cultivar was found to be a suitable substrate in supporting *A. parasiticus* growth (16.6 g per total culture), while other spices tested i.e. *C. cyminum* and *C. carvi* with fungal growth rate of 5.4 and 6.8 g, respectively were considered as nearly resistant substrates in this regard.

Support of aflatoxin production. TLC analysis of AFB and G series produced on some selected cereal grains is shown in Figure 1. As shown in Figure 2, AF produced in different amounts on various cultivars of cereals within and among species. AFB_1 was reported to produce in the range of 1.0-33.9, 41.9-193.8 and 39.1-82.3 µg/g fungal weight on maize, wheat and rice cultivars, respectively.

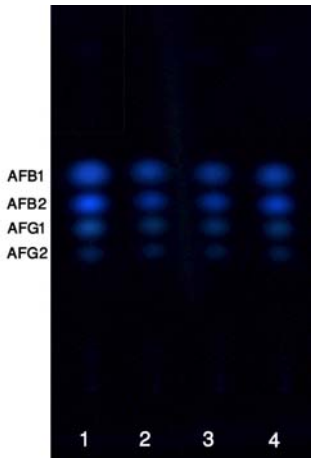

Fig. 1. Thin layer chromatography profile of aflatoxins B and G series produced on cereal grains tested. Line 1, Aflatoxins standard mixture (B and G series); Line 2, Wheat (Fong cultivar); Line 3, Maize (SC 647 cultivar) and Line 4, Rice (Astaneh cultivar).

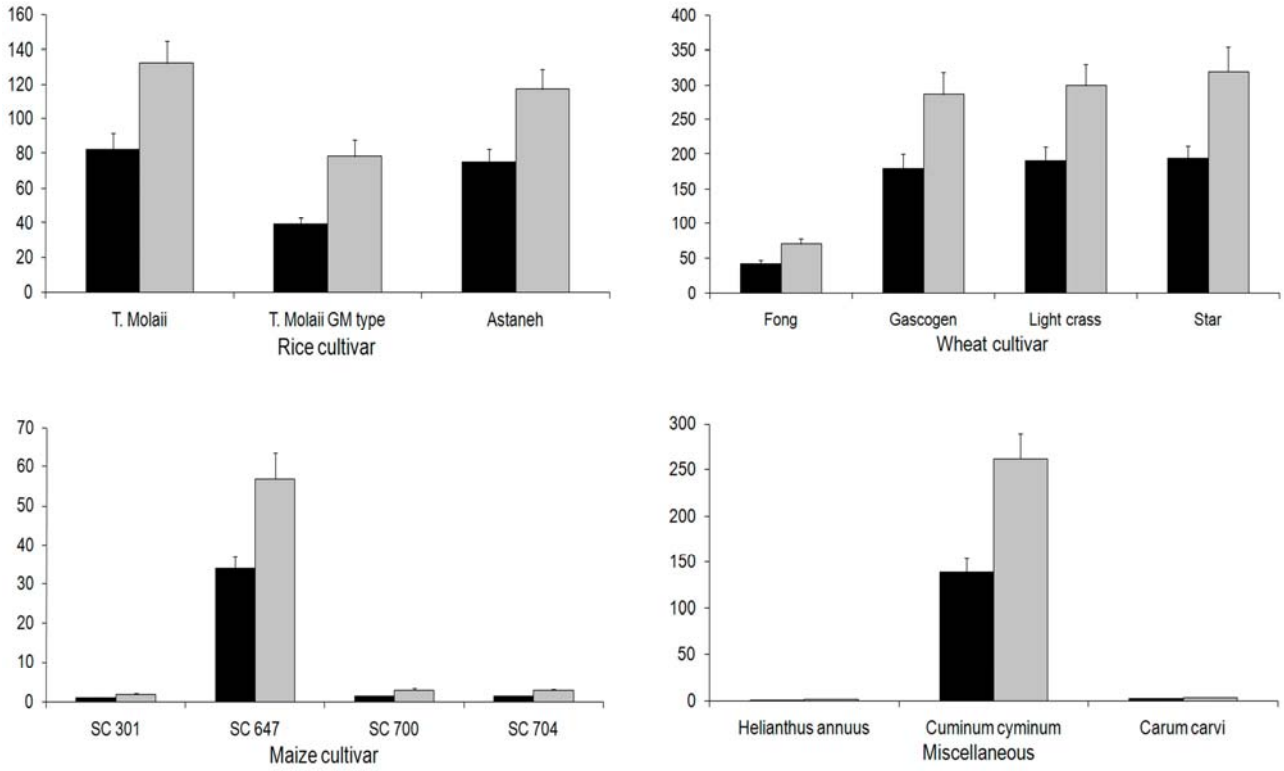

Fig. 2. Variability of different cultivars of major Iranian cereals and spices to support aflatoxin production by toxigenic *Aspergillus parasiticus*. Aflatoxin FB_1 and aflatoxin total (B_1, B_2, G_1 and G_2) amounts (µg/g fungal weight) are shown as black and gray columns, respectively. Data are the mean ± SEM obtained from 2 separate experiments in triplicate each. Error bars indicate SEM.

Fig. 3. Comparison of the mean values of aflatoxin (B_1 and total) produced on different cultivars of the major Iranian cereals. Data are the mean ± SEM obtained from 2 separate experiments in triplicate each. Error bars indicate SEM.

All maize samples except cultivar SC 647 supported very low concentrations of AFB_1 (less than 1.5 µg/g) and AF total (less than 3.0 µg/g) (P<0.05). These cultivars may be considered resistant to AF formation by *A. parasiticus*.

In contrast to maize, all wheat samples except Foung cultivar were capable of supporting high levels of AF production (Fig. 2, P<0.05). The amounts of AFB_1 and AF total were reported as 190.6 and 299.5, 179.8 and 286.9, 193.8 and 319.3, and 41.9 and 70.1 µg/g fungal weight for Light grass, Gascogen, Star and Foung cultivars, respectively. There was no significant difference in AF production (B_1 and total) among Gascogen, Light grass and Star cultivars at level of 0.05.

Both rice cultivars including Tarom Molaii and Astaneh were found to support AF production (Fig. 2). The amounts of AFB_1 and AF total production on Tarom Molaii (82.3 and 132.4 µg/g fungal weight) and Astaneh (74.9 and 117.5 µg/g fungal weight) cultivars showed significant difference at level of 0.05. Interestingly, GM type of Tarom Molaii was found to be less susceptible to AF formation (39.1 and 78.5 µg/g fungal weight for AFB_1 and AF total) as compared with its normal counterpart (P<0.05).

The amounts of AFB_1 and AF total production on *H. annuus* (1.0 and 1.9 µg/g fungal weight) and *C. carvi* (2.2 and 3.5 µg/g fungal weight) were very low, while for *C. cyminum*, relatively high levels of AF produced (139.5 and 261.8 µg/g fungal weight) (Fig. 2). These results show that unlike the *C. cyminum* and similar to the majority of maize cultivars, both *C. carvi* and *H. annuus* may be classified as resistant substrates to AF formation by *A. parasiticus*.

Mean values of AF (B_1 and total) production on different cultivars of Iranian cereals are compared in Figure 3. Based on these results, maize samples are thought to be resistant to AF formation, while wheat and rice cultivars should be considered as susceptible samples provide suitable conditions for AF production by *A. parasiticus*.

DISCUSSION

Plant contamination with polyketide mycotoxins specially AF produced by toxigenic *Aspergillus* species is a complicated process involving various genetic and biological factors [2-4]. Recent advances in genomics and proteomics era of *A. flavus* have been provided a unique opportunity for the development of effective strategies to prevent AF contamination and even elimination of these hazardous toxins from food chain of human and animals [4,10-12,16,17]. Despite that, limited information is now exist about an important aspect of AF contamination i.e. plant-toxigenic fungus interaction [4, 12].

A few pathogenic factors have been reported from *A. flavus* and *A. parasiticus* including proteases, lipases and various hydrolytic enzymes such as chitinases, cellulases and pectinases which produce during fungal invasion of crops [10,18]. On the other hand, several compounds have been isolated from host plants including fungal enzyme inhibitors, lipoxigenase-drived fatty acids, protein profiles and lipid and simple sugar contents [19-28].

At present, a definitive resistance mechanism as well as inter- and intraspecies variations contribute to susceptibility or resistance of a certain plant to *Aspergillus* colonization and subsequent AF contamination are lacking. This has been led to focus on necessity to examine more samples from different geographic regions of the world in order to identifying potential biochemical and genetic resistance markers [3, 29-33].

In this communication, the susceptibility of various Iranian cultivars of some important cereals and spices to AF production by *A. parasiticus* was studied under laboratory condition. Among all samples tested, various cultivars of maize, *H. annuus* and normal type of native rice cultivar, Tarom Molaii were found to capable of supporting *A. parasiticus* growth at different levels. However, all wheat cultivars as well as GM rice of Tarom Molaii, rice cultivar of Astaneh, *C. carvi* and *C. cyminum* showed low to moderate susceptibility to fungal growth. This may be contributed to resistance markers exist in some cultivars of certain plant species examined [10]. On the other hand, wheat and

[DOR: 20.1001.1.1028852.2008.12.4.8.7]
[DOI: -]

rice cultivars and also *C. cyminum* were able to support AF production in considerable amounts, while all maize cultivars, *H. annuus* and *C. carvi* showed high resistance to AF formation. Since fungal growth and AF production were not affected in a same manner by different plants tested at species or cultivar level, it is seems that various parameters may contribute to regulate AF synthesis by producing fungi.

Asis *et al.* [31] reported the resistance to *Aspergillus* infection and AF contamination of six peanut genotypes inoculated with 21 *Aspergillus* isolates obtained from the peanut production region of Cordoba, Argentina. They concluded that the resistance to fungal colonization and AF contamination was associated with seed coat integrity in the selected genotypes. Similarly, Brown *et al.* [32] showed that more than half the maize inbreds examined were resistant to AF accumulation with *Aspergillus flavus*.

Regarding to the resistance mechanisms to toxigenic fungal colonization of crops and subsequent AF contamination, the role of plant lipoxygenase (LOX) enzymes has been noticed in recent years. Based on the screening of maize LOX expressed sequence tags by Wilson *et al.* [29], they concluded that some maize cultivars possessing specific LOX genes were resistance to AF contamination. These data are in accordance to our data from present work which indicates a cultivar type in an important parameter in maize susceptibility to AF contamination.

GMBt such as Bt corn have received major considerations regarding to their resistance to pest damages in many parts of the world. Recently, Wu [34] reported an indirect benefit for Bt corn as the lower susceptibility to contamination with AF and another important mycotoxin named fumonisin. In the present study, GM rice (Bt rice) showed less susceptibility to AF formation as compared with its normal counterpart. GM rice is produced by inserting a synthetic *cry1Ab* gene from *Bacillus turingiensis* into a local variety of aromatic rice, Tarom Molaii. This genetically engineered rice is lethal to insects and nematodes and non-toxic for vertebrates. This property makes it safe from specific insect pests and results in substantial decreases in hazardous chemical pesticide use [33]. Higher resistance of GM rice to AF contamination by *A. parasiticus* compared to its normal counterpart is a novel promising finding reported here for the first time. It should be studied more regard to involved mechanism with special reference to the possible interactions of Bt gene (cry1Ab) with regulatory factors involve in growth and AF biosynthesis in producing fungi.

Among cumins tested, *C. cyminum* was found to support high levels of AF production comparable with 3 of 4 wheat cultivars, while *C. carvi* showed high resistance to AF formation by *A. parasiticus* in a similar manner with maize and *H. annuus*. These data clearly show that unlike *C. carvi* and *H. annuus*, *C. cyminum* can be used as a suitable substrate for laboratory production of AF.

In general, as shown in the present study, among major cereals cultivated worldwide i.e. maize, wheat and rice, as well as some spices tested, maize cultivars, GM rice, *H. annuus* and *C. carvi* were highly resistant to AF formation which indicate the possible involvement of AF inhibitory biomarkers exist in these plants. It is interesting to note that unlike the most wheat cultivars tested, maize cultivars and *H. annuus* considerably supported fungal growth while they showed very less susceptibility to AF formation by toxigenic fungus. This may be indicated that different plant factors are involved in complex host-toxigenic fungus interactions. Since there are not enough information about the structure and precise role of these interesting resistance factors, the results of this study further substantiate the need for examining more samples from different geographic areas to clarify the exact mechanism of plant-toxic fungus interactions.

Overall, selective substrate variability among cultivars of wheat, maize, and rice grains as well as cumins (*C. carvi* and *C. cyminum*) and *H. annuus* to support fungal growth and AF production by *A. parasiticus*, was reported in this study. So, comparative study of potential biochemical and genetic markers responsible for these differences in genomics and proteomics levels may led to identification of novel resistance markers useful for designing effective strategies to manage AF contamination of susceptible crops either in pre- or post-harvest conditions.

ACKNOWLEDGEMENTS

This work was financially supported by Pasteur Institute of Iran. The authors wish to thank Agricultural Biotechnology Research Institute of Iran (ABRII) for kind providing all cereal cultivars specially GM rice.

REFERENCES

1. Bennett, J.W. and Klich, M. (2003) Mycotoxins. *Clin. Microbiol. Rev. 16: 497-516.*
2. Keller, N.P., Turner, G. and Bennett, J.W. (2005) Fungal secondary metabolism: from biochemistry to genomics. *Natl. Rev. Microbiol. 3: 937-947.*
3. Cleveland, T.E., Dowd, P.F., Desjardins, A.E., Bhatnagar, D. and Cotty, P.J. (2003) United states department of agriculture-agriculture research service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest. Manag. Sci. 59: 629-642*.
4. Yu, J., Cleveland, T.E., Nierman, W.C. and Bennett, J.W. (2005) *Aspergillus flavus* genomics: gateway to human and animal health, food safety, and crop resistance to diseases. *Rev. Iberoam. Micol. 22: 194-202.*
5. Yu, J., Chang, P.K, Cary, J.W., Wright, M.S., Bhatnagar, D., Cleveland, T.E., Payne, G.A. and Linz, J.E. (1995) Comparative mapping of aflatoxin pathway gene clusters in *Aspergillus parasiticus* and *Aspergillus flavus*. *Appl. Environ. Microbiol. 61: 2365-2371.*
6. Minto, R.E. and Townsend, C.A. (1997) Enzymology and molecular biology of aflatoxin biosynthesis. *Chem. Rev. 97: 2537-2555.*
7. Yu, J., Chang, P.K., Ehrlich, K.C., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Payne, G.A., Linz, J.E., Woloshuk, C.P. and Bennett, J.W. (2004) Clustered pathway genes in aflatoxin biosynthesis. *Appl. Environ. Microbiol. 70: 1253-1262.*
8. Yu, J., Bhatnagar, D. and Cleveland, T.E. (2004) Completed sequence of aflatoxin pathway gene cluster. *FEBS Lett. 564: 126-130.*
9. Ehrlich, K.C., Yu, J. and Cotty, P.J. (2005) Aflatoxin biosynthesis gene clusters and flanking regions. *J. Appl. Microbiol. 99: 518-527.*
10. Cleveland, T.E., Yu, J., Bhatnagar, D., Chen, Z.Y., Brown, R., Chang, P.K. and Cary, J.W. (2005) Progress in elucidating the molecular basis of the host plant-*Aspergillus flavus* interaction: A basis for devising strategies to reduce aflatoxin contamination in crops. In: *Aflatoxin and Food Safety* (Abbas, H. eds.), CRC Press, Boca Raton, Florida, USA. pp. 167-193.
11. Kim, J.H., Campbell, B.C., Yu, J., Mahoney, N., Chan, K.L., Molyneux, R.J., Bhatnagar, D. and Cleveland, T.E. (2005) Examination of fungal stress response genes using *Saccharomyces cerevisiae* as a model system: targeting genes affecting aflatoxin biosynthesis by *Aspergillus flavus* Link. *Appl. Microbiol. Biotechnol. 67: 807-815.*
12. Luo, M., Liang, X.O., Dang, P., Holbrook, C.C., Bausher, M.G., Lee, R.D. and Guo, B.Z. (2005) Microarray-based screening of differentially expressed genes in peanut in response to *Aspergillus* infection and drought stress. *Plant Sci. 169: 695-703.*
13. Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Allameh, A., Kazeroon-Shiri, M., Ranjbar-Bahadori, S., Mirzahoseini, H. and Rezaee, M.B. (2006) A survey on distribution of *Aspergillus* section *Flavi* in corn field soils in Iran: population patterns based on aflatoxins, cyclopiazonic acid and sclerotia production. *Mycopathologia 161: 183-192.*
14. Shotwell, O.L., Hesseltine, C.W., Stubblefield, R.D. and Sorenson, W.G. (1966) Production of aflatoxin on rice. *Appl. Microbiol. 14: 425-428.*
15. Razzaghi-Abyaneh, M., Allameh, A., Tiraihi, T., Shams-Ghahfarokhi, M. and Ghorbanian, M. (2005) Morphological alterations in toxigenic *Aspergillus parasiticus* exposed to neem (*Azadirachta indica*) leaf and seed aqueous extracts. *Mycopathologia 159: 565-570.*
16. http://www.ncbi.nlm.nih.gov
17. http://www.aspergillusflavus.org/genomics
18. Brown, R.L., Chen, Z.Y., Cleveland, T.E., Cotty, P.J. and Cary, J.W. (2001) Variation in *in vitro* alpha-amylase and protease activity is related to the virulence of *Aspergillus flavus* isolates. *J. Food Prot. 64: 401-404.*
19. Chen, Z.Y., Brown, R.L., Lax, A.R., Cleveland, T.E. and Russin, J.S. (1999) Inhibition of plant pathogenic fungi by a corn trypsin inhibitor overexpressed in *Escherichia coli*. *Appl. Environ. Microbiol. 65: 1320-1324.*
20. Tsitsigiannis, D.I., Kunze, S., Willis, D.K., Feussner, I. and Keller, N.P. (2005) *Aspergillus* infection inhibits the expression of peanut 13S-HPODE-forming seed lipoxygenases. *Mol. Plant Microbe. Interact. 18: 1081-1089.*
21. Burow, G.B., Gardner, H.W. and Keller, N.P. (2000) A peanut seed lipoxygenase responsive to *Aspergillus* colonization. *Plant Mol. Biol. 42: 689-701.*
22. Mellon, J.K. and Cotty, P.J. (2002) No effect of soybean lipoxygenase on aflatoxin production in *Aspergillus flavus*-inoculated seeds. *J. Food Prot. 65: 1984-1987.*
23. Mellon, J.E., Dowd, M.K. and Cotty, P.J. (2002) Time course study of substrate utilization by *Aspergillus flavus* in medium stimulating corn (*Zea mays*) kernels. *J. Agric. Food Chem. 50: 648-652.*
24. Mellon, J.E., Cotty, P.J. and Dowd, M.K. (2000) Influence of lipids with and without other cottonseed reserve materials on aflatoxin B1 production by *Aspergillus flavus*. *J. Agric. Food Chem. 48: 3611-3615.*
25. Mellon, J.E., Dowd, M.K. and Cotty, P.J. (2005) Substrate utilization by *Aspergillus flavus* in inoculated whole corn kernels and isolated tissues. *J. Agric. Food Chem. 53: 2351-2357.*
26. Mahoney, N. and Molyneux, R.J. (2004) Phytochemical inhibition of aflatoxigenicity in

[DOR: 20.1001.1.1028852.2008.12.4.8.7]

[DOI: -]

Aspergillus flavus by constituents of walnut (*Juglans regia*). *J. Agric. Food Chem. 52: 1882-1889.*

27. Chen, Z.Y., Brown, R.L., Cleveland, T.E., Damann, K.F. and Russin, J.S. (2001) Comparison of constitutive and inducible maize kernel proteins of genotypes resistant or susceptible to aflatoxin production. *J. Food Prot. 64: 1785-1792.*
28. Guo, B.Z., Brown, R.L., Lax, A.R., Cleveland, T.E., Russin, J.S. and Widstrom, N.W. (1998) Protein profiles and antifungal activities of kernel extracts from corn genotypes resistant and susceptible to *Aspergillus flavus*. *J. Food Prot. 61: 98-102.*
29. Wilson, R.A., Gardner, H.W. and Keller, N.P. (2001) Cultivar dependent expression of a maize lipoxygenase responsive to seed infesting fungi. *Mol. Plant Microbe. Interact. 14: 980-987.*
30. El-Kady, I.A., Mohamed El-Maraghy, S.S. and Zohri, A.A. (1991) Mycotoxin production on different cultivars and lines of broad bean (*Vicia faba* L.) seeds in Egypt. *Mycopathologia 113: 165-169.*
31. Asis, R., Barrionuevo, D.L., Giorda, L.M., Nores, M.L. and Aldao, M.A. (2005) Aflatoxin production in six peanut (*Arachis hypogaea* L.) genotypes infected with *Aspergillus flavus* and *Aspergillus parasiticus*, isolated from peanut production areas of Cordoba, Argentina. *J. Agric. Food Chem. 53: 9274-9280.*
32. Brown, R.L., Chen, Z.Y., Menkir, A., Cleveland, T.E., Cardwell, K., Kling, J. and White, D.G. (2001) Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in West and Central Africa. *J. Food Prot. 64: 396-400.*
33. Ghareyazie, B., Alinia, F., Menguito, C.A., Rubia, L.G., Palma, J.M., Liwanag, E.A., Cohen, M.B., Khush, G.S. and Bennett, J. (1997) Enhanced resistance to two stem borers in an aromatic rice containing a synthetic *cry1Ab* gene. *Mol. Breed. 3: 401-414.*
34. Wu, F. (2006) Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. *Transgenic Res. 15: 277-289.*

[DOR: 20.1001.1.1028852.2008.12.4.8.7]

[DOI: -]