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Microbiota and Mycotoxins in Trilinear Hybrid Maize Produced in Natural Environments at Central Region in Mexico

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Abstract

Mycotoxigenic fungi and mycotoxins in 3 inbred lines (hybrids resistant to corn ear rot) were identified in twenty samples. The maize (*Zea mays*) accessions were collected in five plots of two municipalities in High Valley, state of Hidalgo. The fungal population was determined with a microbiological dilution method used two culture media (PDA and ELA), for the detection of mycotoxins with thin layer chromatography with visual inspection in UV light and a direct competitive enzyme-linked immunosorbent (ELISA). The results showed high moisture content in all hybrids evaluated on an average of 38.3% and a 1.8 × 10^3 UFC/g fungus, values within the permitted limits by the Mexican legislation; however the most prevalent fungi were *Fusarium* sp. (76%), *Alternaria* sp. (14%), *Penicillium* sp. (4%) and *Aspergillus* sp. (5%), and the species *Aspergillus nidulas*, *Aspergillus flavus*, *Fusarium verticillioides*, *Fusarium poae*, and *Penicillium ochraceum*. The aflatoxin concentration was observed in a range from 2 to 13 ng/g and 370 to 660 ng/g to fumonisins. It is concluded that trilinear corn hybrids have a variety of pathogenic potential fungi. The two genetic hybrids showed levels of aflatoxins and fumonisin safe for human consumption, contrary to one hybrid, with a content not suitable for human consumption. A better understanding of genetic hybrids corn will improve predictive mycotoxin contamination.

Keywords

***Zea mays* L., Mycotoxins, Fungi, Corn Hybrid**

1. Introduction

In Mexico, 19.7 million tons of corn annually occurs in an area of 7.4 million hectares, with enormous variations

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in production from one year to another [1], principally due to the attacks by fungus that can cause losses of up to 20% in harvest [2] [3]. The introduction of hybrid maize comes from three pure lines, with a resistance to fungus, which had improved productivity in some maize growing areas [4] [5]. In 2006, the central region from Hidalgo State, had losses in corn harvest, when appearing on their land plants falls, cobs smaller grain discolored dark and leaves with the presence of a pink powder.

Insects have been related to the transport of pathogenic fungi *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. [6]; among them, the Hymenoptera, Lepidoptera and Diptera; the most frequently funding in corn plant [6] [7]. Another factor that directly influences the development of fungi and mycotoxins is the weather, as intense rains during seed germination. Fungus uses their spores to reach the grain, causing physical and ear rot [8] deterioration and a high temperature can cause a decrease in grain yield per hectare [9]. The combined effect of oxygen and moisture environment promotes the synthesis of mycotoxins, which once installed cannot be deleted [10]; these mycotoxins constitute a risk to human and animal health [3]. The fungus *Aspergillus flavus* synthesizes aflatoxins [11]; they can cause hepatotoxicity in poultry, swine, cattle, sheep and laboratory animals, making morphological changes in liver cells as the change in the nucleolus, derangement and reduction in the number of ribosomes, proliferation of smooth endoplasmic reticulum and mitochondria degeneration and a decrease in protein synthesis; the toxicity is associated with low food intake, poor growth, low production, reduced fertility and immunosuppression. *Fusarium verticillioides* and *Fusarium oxysporum* have the same toxigenic effect as the fusariotoxin F-2, a mycotoxin with diverse estrogenic activity; in pigs, females and males, had shown an increase in the size of the mammary glands, include the human beings. *Fusarium verticillioides* synthesizes fumonisin, which has been involved with the development of esophageal cancer in humans [12] [13]. *Penicillium* sp. represents a large number of species of filamentous fungi, identified at least 300 species; *Penicillium ochraceus* produces Ochratoxin A, whose toxicity is associated with the isocumarine ring molecule that affects the kidney [14]; *Alternaria* sp. is an ubiquitous and saprophytic fungus, and *Alternaria* sp. synthesizes mycotoxins, called alternariol and alterotoxines, whose toxicity has not been sufficiently investigated in Mexico.

Toxic effects of mycotoxins on human health have been known since middle ages, recorded diseases in farm workers after harvest rye; intense documentation has been developed since then, the toxicity of mycotoxins has been intensively in target organs such as liver and lung, as well as the immune and nervous system [15].

Conventional techniques as microbiological isolation in raw and processed foods are still used, although the technique of polymerase chain reaction (PCR) currently identifies pathogens in less time [16]. The official mycotoxin monitoring technique is the thin layer chromatography (TLC). In some laboratories liquid chromatography coupled to mass spectroscopy-mass HPLC/MS/MS is used [17]. One of the quality concepts of maize seed is the germinating vigour, factors involved genetic constitution, maturity at harvest and pathogens [18]. Polyphenolic compounds commonly known as tannins, have been attributed natural insecticidal properties. Therefore the objective of this study was to isolate and identify the microbiota and mycotoxins in corn hybrids and probed the proprieties of hybrid maize.

2. Materials and Methods

The study was conducted during the spring-summer crop season, in the municipalities of Tlaxcoapan and Tlahuelilpan, located in the high valley at an altitude of 2,080 m, characterized by a temperate climate with summer rains. In the municipality of Tlaxcoapan threes locations, were sampled under a stratified system. In the municipality of Tlahuelilpan two locations with the same technique in cobs. The agronomic characteristics of corn hybrids are presented in **Table 1**. Tlaxcoapan: located at 20˚05'43"N coordinates 99˚13'12"S, at a height of 2,063 m. with a climate Cw, an average temperature of 17˚C and a rainfall of 850 mm per year; Tlahuelilpan is located in the coordinates 20˚07'47"N 99˚13'43"S, at a height of 2,067 m with the same climate, an average temperature of 12˚C and a rainfall of 620 mm per year. The cobs, were transported to the laboratory of toxicology where it was manually removed the grain, identified and stored.

Laboratory tests: moisture content was determined following the 44 - 40 method [18]; grains were dried at a temperature of 50˚C for 48 h.

For the total count of microorganisms, it were used randomly 100 grains of each commercial sample hybrid maize, disinfected with sodium hypochlorite (NaOCl 2%) and 20 grains were sown in Petri dish on a medium surface malt agar (MA), malt-salt agar (MSA) and Potato-dextrose-agar (PDA), and were incubated for 7 days at 25˚C. Colonies were counted and expressed as UFC/g. The identification of colonies after staining was per-

formed with Lactophenol blue light microscopy, based on the macroscopic and microscopic structures of each colony, [11] [19] microbiological density of each hybrid maize was expressed in colony forming units (CFU/g).

It was used thin layer chromatography under UV light by visual comparison to standards, for the screening analysis of aflatoxins and for fumonisins a commercial immunosorbent assay (ELISA). 500 g of each sample in duplicate were weighed and ground in electric mill, 50 g of ground corn was weighed and placed in a glass blender 500 mL with 5 g of NaCl; it were added 100 mL of methanol mixture and distilled water 80:20 (v/v) and mixed at high speed for 3 minutes. The extract was passed through a whatman No. 4 and it was collected 25 mL. Extract purification was performed under a C-18 column, eluting aflatoxins with 6 mL of chloroform and acetone at a ratio of 9:1 v/v. 50 µL of the purified extract and applied on silica gel plates 60 (Merck) reversed phase C-18 1 cm away, to be introduced into a development chamber 10 × 10 cm, in a solvent system to acetone and toluene base (1:1 v/v). The plates were exposed to ultraviolet light at a short wavelength (250 nm). Samples with a blue color and a distance equal to 0.50 indicated the presence of aflatoxin B type, having the same blue color and the front distance standards aflatoxin B_1 and B_2, and for fumonisin analyse a direct competitive enzyme-linked immunosorbent assay (ELISA) and the procedure provided by the manufacturer. 5 g of each sample were weighed and ground, a methanolic extraction was performed, an aliquot of 50 µL in each well of the microplate. A positive reaction is blue color. Finally the test was read to yield optical densities, and it was built a standard curve with controls and samples, to calculate the concentration of fumonisins.

3. Results

The contamination by microorganisms in maize hybrids is presented in **Table 2**, where it can be observed a high level of pollution in the commercial hybrid oso, with a total count of 2.5×10^3 CFU/g, followed by hybrid leopardo with 1.270×10^3 CFU/g, and hybrid 30V46 with 1.260×10^3 CFU/g. The identified fungus were *Fusarium* sp. and *Penicillium* sp. in the three varieties of hybrid maize; *Alternaria* sp. only hybrid maize oso. The species identified were *Ocraceus penicillium*, *Alternaria* sp., *Aspergillus flavus*, *Fusarium verticillioides*, *F. oxysporum* and *Fusarium moniliforme*.

Table 1. Agronomic characteristics of corn hybrids collected in the Mezquital Valley, State of Hidalgo, Mexico.

Genetic corn	Commercial identification	Genetics	Maturity
Hybrid	Oso	Three lines	Intermediate-early
Hybrid	Leopardo	Three lines	Early
Hybrid	30V46	Three lines	Intermediate

Table 2. Microbiota of commercial hybrid varieties of maize from the 2007-2008 harvest, Mezquital Valley, Hidalgo State.

Commercial hybrid	**Oso**	**Leopardo**	**30V46**
Municipality	**Tlaxcoapan**	**Tlaxcoapan**	**Tlahuelilpan**
Total microbiota*	2.5	1.3	1.2
***Fusarium* sp.**	1.4	0.99	0.89
***Penicillium* sp.**	4.0	5.0	1.0
***Alternaria* sp.**	14	NF	NF
Fusarium oxysporum	38	NF	NF
***Aspergillus* sp.**	10	NF	NF
Fusarium moniliforme	3	5	10
Aspergillus flavus	25	2	5

NF = not found.

In **Table 3**. Observed colony forming units in two culture media (potato dextrose agar and yeast extract agar) of samples taken from the grains and leaves before harvest and grain in the cellar.

In order to assess the percentage of fermentation of the varieties bear and leopard, germination test was performed, which is shown in **Table 4**. The leopardo variety had a higher germination percentage and higher average height germinated.

The presence and level of fumonisins, mycotoxins, tannic acid detected in corn hybrids is presented in **Table 5**, which shows that the hybrid leopardo had the most fumonisin contamination compared to the hybrid oso aflatoxin. The corn hybrid 30V46 was the least contamined, and tannin content with large variations between 1.4 mg/mL a 3.39 mg/mL.

4. Discussion

The high content of colony forming units detected in the commercial hybrid corn oso 2.5×10^3, indicates the susceptibility of genotype, which could be favored by excessive rain that occurred in the two locations in Tlaxcoapan, during the agricultural cycle. It should be noted that fungal colonies were identified in grains of corn hybrids, such as *Fusarium species*, *Alternaria* and *Aspergillus*, in agree with other authors. In this study it were identified *Fusarium verticillioides*, *F. oxysporum*, *Alternaria* and *Aspergillus flavus*, potentially produced fumonisins, aflatoxins and alternariol, highly dangerous chemicals to human and animal health [5]. Climate change affects all the agricultural field, the many pests that attack the corn plant or dryness, requires producers to care for and ideally manage their crop to achieve an economically and sustainable agricultural production in order to reduce the environmental impact of this activity [20]. The count of colony forming units (for bacteria and yeast) it was found within the national legislation, however is not desirable because the grain remain in storage for long periods sometimes. It is important to know the potential health risk [21]. The hybrid oso was less susceptible to fumonisin than aflatoxins. The 30V46 hybrid remained at low levels for both mycotoxins [22], being the less

Table 3. Colony forming units observed in two culture media (potato dextrose agar and yeast extract agar) of samples taken from the grains and leaves before harvest and grain post-harvest.

Corn	Pre-harvest grain		Grain store	
	PDA	ELA	PDA	ELA
Oso	987	995	1006	1026
Leopardo	635	854	987	995
30V46	1006	1026	635	854
Total	2628	2875	2628	2875

Table 4. Percentage of germination and size for three trilinear hybrid.

Genetic line	Seeds	Germination %
30V46	100	64
Oso	100	76
Leopard	100	87

Table 5. Identification and quantification of aflatoxin, fumonisin and tannins in improved maize.

Corn	DM (%)	Fumonisins content (ng/g)	Aflatoxins content (ng/g)	Tannic acid* (mg/mL)
30V46	85.99	370	2.0	3.38
Oso	85.74	250	13.0	1.40
Leopardo	86.00	660	7.5	3.40

DM = dry matter, *Millequivalents of tannic acid.

contaminated under the conditions of this study, it is important to consider the presence of any other metabolites in plants, such as tannins because they have qualities that can reach inhibit the growth of certain harmful microorganisms to the ground, men and animals [23].

5. Conclusions

The presence of fungus and mycotoxins in trilinear hybrids in maize showed a potential risk for human health.

This study showed that mycotoxin contamination depends not only from the genetic seed but also from several factors like the competition into microbiota and weather of each region.

It is important to choose a sensitive method for screening test, and establish the permitted levels of fumonisins in maize and derivatives in Mexico.

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