Frequency of fungi associated with Creole corn seeds under different environmental conditions in Guerrero, Mexico

Guadalupe García-Solano[1], Francisco Palemón-Alberto[1], Santo Ángel Ortega-Acosta[1], Agustín Damián-Nava[1], Porfirio Juárez-López[2], Paul García-Escamilla[1], Edgar Villar-Luna[3], Blas Cruz-Lagunas[1]

[1]Departamento de Agronomía, Facultad de Ciencias Agropecuarias y Ambientales, Maestría en Ciencias Agropecuarias y Gestión Local, Universidad Autónoma de Guerrero, C. P. 40020, Iguala de la Independencia, Guerrero, México. [2]Facultad de Ciencias Agropecuarias. Universidad Autónoma del Estado de Morelos, Av. Universidad1001, Col., C.P. 62210, Cuernavaca, Morelos, México. [3]Instituto Politécnico Nacional, CIIDIR Unidad Michoacán, Justo Sierra 28, 59510, Jiquilpan de Juárez, Michoacán, México.
Corresponding author: Santo Ángel Ortega-Acosta (angelortega011185@hotmail.com)
Data de chegada: 28/01/2022. Aceito para publicação em: 08/09/2022

10.1590/0100-5405/260498

ABSTRACT

García-Solano, G.; Palemón-Alberto, F.; Ortega-Acosta, S.A.; Damián-Nava, A.; Juárez-López, P.; García-Escamilla, P.; Villar-Luna, E.; Cruz-Lagunas, B. Frequency of fungi associated with Creole corn seeds under different environmental conditions in Guerrero, Mexico. ***Summa Phytopathologica,*** v.48, n.4, p.158-162, 2022.

In Mexico, Creole corn has presented problems related to seed rot. Therefore, the objective of this study was to determine the frequency of fungi associated with Creole corn seeds. From January to February 2019, 13 Creole corn samples were collected from several localities in Guerrero, Mexico. The samples were processed in PDA culture medium and the frequency of each fungus was estimated based on cultural characteristics and taxonomic keys. Considering symptomatic corn seed samples, *Fusarium oxysporum* was recorded in eight samples at 97.2%, followed by *F. graminearum,* isolated from race "Olotillo 1" at 94.4% and from race "Cónico pepitilla" at 58.3%. *Phomopsis* sp. was isolated at 100% from race "Olotillo 2". *Gibberella moniliformis* was isolated from "Elotes Occidental 1" at 83.3%, while the least frequent isolate (19.4%) from "Pepitilla" was *Sarocladium strictum,* which was recorded in three samples at the low values of 11.1% and 2.8% for races "Arrocillo 2" and "Pepitilla", respectively; *Talaromyces variabilis* was found in two samples at 11.1% and 2.8% from "Arrocillo 2" and "Cónico pepitilla", respectively; *Stenocarpella macrospora* was isolated at 72.2% from "Pepitilla". *Aspergillus flavus* was obtained from "Cónico Pepitilla" at 16.7%, while *Setosphaeria turcica* and *Emericella* sp. were less frequently isolated. Considering asymptomatic seeds of race "Cónico pepitilla", *F. oxysporum* was isolated at 8.3%, *Fusarium acuminatum* at 8.3% and *Gibberella moniliformis* at 41.7%; from asymptomatic seeds of "Olotillo 1" and "Olotillo 2", *Phomopsis* sp. was isolated at 50% and *Rhizoctonia solani* at 50%, respectively. In the present study, the fungi *F. oxysporum*, *Phomopsis* sp., *G. moniliformis* and *F. graminearum* were most frequently associated with Creole corn seeds.

.**Keywords**: *Zea mays*, fungal disease, seed mycoflora

RESUMO

García-Solano, G.; Palemón-Alberto, F.; Ortega-Acosta, S.A.; Damián-Nava, A.; Juárez-López, P.; García-Escamilla, P.; Villar-Luna, E.; Cruz-Lagunas, B. Frequência de fungos associados a sementes de milho crioulo em diferentes condições ambientais em Guerrero, México. ***Summa Phytopathologica,*** v.48, n.4, p.158-162, 2022.

No México, o milho crioulo apresenta problemas de podridão das sementes. Portanto, o objetivo desta pesquisa foi determinar a frequência de fungos associados a sementes de milho crioulo. De janeiro e fevereiro de 2019, 13 amostras de milho crioulo foram coletadas em localidades de Guerrero, México. As amostras foram processadas em meio de cultura BDA e a frequência de cada fungo foi estimada com base nas características culturais e chaves taxonômicas. Nas amostras sintomáticas de sementes de milho, *Fusarium oxysporum* foi registrado em oito amostras com 97,2%, seguido por *F. graminearum* isolado na raça de milho "Olotillo 1" com 94,4% e na raça "Cónico pepitilla" com 58,3%. *Phomopsis* sp., foi isolado 100% na raça "Olotillo 2". *Gibberella moniliformis*, foran isolados de "Elotes Occidental 1" com 83,3% e o menos frequente (19,4%) em "Pepitilla" foi *Sarocladium strictum* registrado em três amostras com valores baixos de 11,1% e 2,8% na raça de milho "Arrocillo 2" e "Pepitilla", *Talaromyces variabilis* em duas amostras com 11,1% e 2,8% em "Arrocillo 2" e "Cónico pepitilla". *Stenocarpella macrospora* foi isolado em 72,2% da raça de milho "Pepitilla". *Aspergillus flavus* em "Cónico Pepitilla" com 16,7%, *Setosphaeria turcica* e *Emericella* sp., foi isolado com menor frequência. Em sementes assintomáticas da raça "Cónico pepitilla" foi isolado *F. oxysporum* com 8,3%, *Fusarium acuminatum* 8,3%, *Gibberella moniliformis* em 41,7%, em sementes assintomáticas de "Olotillo 1" e "Olotillo 2" *Phomopsis* sp., foi isolado em 50% e *Rhizoctonia solani* com 50%. Neste estudo, os fungos *F. oxysporum*, *Phomopsis* sp., *G. moniliformis* e *F. graminearum* foram os mais frequentemente associados às sementes de milho crioulo.

Palavras Chave: *Zea mays*, doença fúngica, micoflora de sementes.

In Mexico, native corn occupies around 70% to 80% of the cultivated area and is mostly used for self-consumption. Although it is a regional genetic material, native corn presents various problems that decrease the crop productivity, one of which is associated with seed rot induced by phytopathogenic fungi of different genera (27). The most frequent fungi causing economic losses throughout the world are *Fusarium* spp.; in such a large genus, the major species found in corn kernels and stalks are: *F. verticillioides*, *F. oxysporum*, *F. graminearum*, *F. equiseti*, *F. proliferatum* and *F. subglutinans* (3, 18), while in corn kernels for consumption and corn kernels for popcorn, *Aspergillus flavus*, *A. niger*, *A. calvatus*, *Rhizopus stolonifer* and *Fusarium proliferatum* were identified (28). The genus *Fusarium* commonly infects corn seeds and consequently reduces their ability to germinate; in some cases, the fungus remains colonizing plant debris for several periods, waiting for seedlings, roots and stalks (27). Therefore, the objective of the present study was to determine the frequency of fungi associated with Creole corn seeds in Guerrero, Mexico.

MATERIALS AND METHODS

Corn seed sampling

Corn samples were collected from January to February 2019. The sampling consisted of five symptomatic seeds and three asymptomatic seeds of the same race of Creole corn from the study locations (Table 1). The samples were placed in plastic bags, which were labeled and taken to the laboratory for processing.

Sample processing

Potato Dextrose Agar (PDA) culture medium (Bioxon®) was prepared according to the manufacturer›s instructions. In each Creole corn collection, 25 symptomatic seeds were selected for the isolation of fungi and 10 asymptomatic seeds were used as negative control. Six fragments of approximately 0.5 cm were obtained from each seed; they were disinfected with 1.5% NaClO for two minutes and rinsed in two changes of sterilized water for two minutes. The fragments were placed on previously sterilized absorbent paper and allowed to rest for 20 minutes to eliminate excess water. Then, the fragments of symptomatic and asymptomatic corn seeds were sown with a dissection needle; six fragments / Petri dish with PDA were deposited (90 x 15 mm diameter boxes) and incubated at 26°C for seven days under white light and alternating 12 h light / dark cycles.

Identification and frequency of fungi

Adopting the taxonomic keys proposed by Barnett & Hunter (5), Leslie & Summerell (17) and Crous et al. (9), the fungi associated with Creole corn seeds were morphologically identified, according to the methodology used in the present study.

After the incubation period, the presence of fungi and their growth were visually observed on Petri dishes with PDA. The frequency of fungi was calculated with the following formula:

$$\text{Frequency \%} = \frac{\text{Number of fragments per genus or species of fungus}}{\text{Total fragments per Petri dish}} \times 100$$

RESULTS AND DISCUSSION

In the present investigation, 10 species of fungi belonging to 8 genera were isolated. From symptomatic seeds, the following fungi were isolated: *Fusarium oxysporum*, *F. graminearum*, *Gibberella moniliformis*, *Sarocladium strictum*, *Talaromyces variabilis*, *Stenocarpella macrospora*, *Aspergillus flavus*, *Setosphaeria turcica*, *Phomopsis* sp., and *Emericella* sp.

Rhizoctonia solani, *Fusarium acuminatum*, *F. oxysporum*, *Gibberella moniliformis* and *Phomopsis* sp. were isolated from asymptomatic seeds (Table 2).

Fusarium oxysporum was isolated from eight samples of six races of Creole corn, showing an incidence of 97.2% in white seeds (Table 2). *Fusarium oxysporum* is considered the major cause of decreased corn production worldwide, particularly in Spain, Brazil and Mexico (1, 25). García-Aguirre & Martínez-Flores (14) reported that *F. oxysporum* was isolated from two samples of national white corn seeds and native white corn seeds at a frequency of 100%. Zhou et al. (29) isolated different *Fusarium* spp. from corn cobs and grains, detecting *F. verticillioides*, *F. proliferatum*, *F. meridionale* and *F. oxysporum* as the predominant species, and the latter was found at a frequency of 12.1%; those same species were considered pathogens that cause rot in corn ears and grains in China. Regarding the corn race "Pepitilla", *F. oxysporum* was less frequently isolated, reaching values of 2.8%. Considering asymptomatic fragments from race "Cónico pepitilla", that fungus was detected at 8.3%.

Fusarium graminearum was recorded in two samples of race "Olotillo 1" at a frequency of 94.4%. In Mexico, such a fungus was reported as the cause of ear rot, stem blight and rot in corn seedlings (19). In Argentina, it is also known as the cause of corn ear rot and stands out for its constant presence in corn grains (8). In Pakistan, Niaz & Dawar (21) reported the presence of *F. graminearum* in the microbiota

Table 1. Races of Creole corn (*Zea mays* L.) collected from five localities in Guerrero State, Mexico. Period: January – February 2019.

Region of Guerrero State	Municipality	Locality	Coordinates	Relative humidity (%)	Altitude (m.a.s.l)	Races of corn
Norte	Cocula	Tlanipatlán	18°9'34.05"N 99°44'52.51"W	58.80	1,067	Elotes Occidentales (morado 1), No identificado (blanco), Elotes Occidentales (morado 2) and Elotes Occidentales (rosado 3)
	Cuetzala del Progreso	Apetlanca	18°12'27.9"N 99°47'21.29"W	58.80	1,718	Pepitilla (blanco)
		San Francisco Lagunita	18°10'34.28"N 99°46'3.75"W	58.80	1,679	Cónico Pepitilla (blanco), Arrocillo delgado (blanco-crema), Arrocillo (blanco 1) and Arrocillo (blanco 2)
Centro	Chilapa de Álvarez	Cuamañotepec	17°33'0.07"N 99°3'9.68"W	62.69	2,096	Olotillo (blanco 1),
Costa Chica	San Luis Acatlán	Pascala del Oro	17°3'17.49"N 98°48'35.09"W	67.28	1,085	Olotillo (morado 2), Maíz Costeño (blanco 1 and blanco 2)

Table 2. Frequency of fungi in symptomatic and asymptomatic Creole corn seeds from five localities in Guerrero State, Mexico. Period: January – February 2019.

Race of corn	Fgra	Foxy	Facu	Sstr	Gmon	Smac	Phom	Tvar	Afla	Rsol	Stur	Emer	Sh
[a]Pepitilla	0[f]	2.8[f]	0[f]	2.8[f]	19.4[f]	72.2[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	2.8[f]
[b]Olotillo 1	94.4[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	5.6[f]
[b]Olotillo 1	0[g]	0[g]	0[g]	0[g]	0[g]	0[g]	50[g]	0[g]	0[g]	0[g]	0[g]	0[g]	50[g]
[c]Cónico pepitilla	58.3[f]	5.6[f]	0[f]	0[f]	0[f]	0[f]	16.7[f]	2.8[f]	16.7[f]	0[f]	0[f]	0[f]	0[f]
[c]Cónico pepitilla	0[g]	8.3[g]	8.3[g]	0[g]	41.7[g]	0[g]	0[g]	0[g]	0[g]	0[g]	0[g]	0[g]	41.7[g]
[c]Arrocillo delgado	0[f]	8.3[f]	0[f]	0[f]	0[f]	0[f]	75[f]	0[f]	0[f]	0[f]	16.7[f]	2.8[f]	0[f]
[c]Arrocillo 1	0[f]	13.9[f]	0[f]	8.3[f]	66.7[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	11.1[f]
[c]Arrocillo 2	0[f]	38.9[f]	0[f]	11.1[f]	0.0[f]	0[f]	0[f]	11.1[f]	0[f]	0[f]	0[f]	0[f]	38.9[f]
[d]Elotes occidentales1	0[f]	0[f]	0[f]	0[f]	83.3[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	16.7[f]
[d]Blanco no identificado	0[f]	97.2[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	2.8[f]
[d]Elotes occidentales2	0[f]	77.8[f]	0[f]	0[f]	0[f]	0[f]	22.2[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]
[d]Elotes occidentales3	0[f]	52.8[f]	0[f]	0[f]	44.4[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	2.8[f]
[e]Olotillo 2	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	100[f]	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]
[e]Olotillo 2	0[g]	0[g]	0[g]	0[g]	0[g]	0[g]	0[g]	0[g]	0[g]	50[g]	0[g]	0[g]	50[g]
[e]Maíz costeño 1	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	97.2[f]	0[f]	0[f]	0[f]	0[f]	0[f]	2.8[f]
[e]Maíz costeño 2	0[f]	0[f]	0[f]	0[f]	0[f]	0[f]	50[f]	0[f]	0[f]	0[f]	0[f]	0[f]	50[f]

a= Apetlanca, Municipality Cuetzala del Progreso; b= Cuamañotepec, Municipality Chilapa de Álvarez; c= San Francisco Lagunita, Municipality Cuetzala del Progreso; d= Tlanipatlan, Municipality Cocula; e= Pascala del Oro, Municipality San Luis Acatlan. Fgra= *Fusarium graminearum*, Foxy= *Fusarium oxysporum*, Facu= *Fusarium acuminatum*, Sstr= *Sarocladium strictum*, Gmon= *Gibberella moniliformis*, Smac= *Stenocarpella macrospora*, Phom= *Phomopsis* sp., Tvar= *Talaromyces variabilis*, Afla= *Aspergillus flavus*, Rsol= *Rizoctonia solani*, Stur= *Setosphaeria turcica*, Emer= *Emericella* sp., Sh= No fungal development. f= isolation of fungi from symptomatic seeds; g= isolation of fungi from asymptomatic seeds.

isolated from the seeds of corn crops. Broders et al. (7) pointed out that, in the United States, *F. graminearum* is considered an economically important pathogen, which causes the disease called “head blight” in wheat and is also responsible for stem and ear rot in wheat. On the other hand, in the present study, its presence was also reported in corn seeds of race “Cónico Pepitilla” at a frequency of 58.3%. Moreover, Ekwomadu et al. (11) mentioned that the genus *Fusarium* is the major cause of contamination of commercial corn seeds in South Africa.

The fungus *Phomopsis* sp. was isolated from six samples of symptomatic corn of races “Conico pepitilla”, “Arrocillo delgado”, “Elotes occidental 2”, “Olotillo 2”, “Maíz Costeño 1” and “Maíz Costeño 2”; it was also recorded in an asymptomatic sample of “Olotillo1”. The highest values were found for: “Olotillo 2” and “Maíz Costeño 1”; both were symptomatic samples and had frequency values oscillating between 100% and 97.2%, respectively. In addition, *Phomopsis* sp. was isolated from asymptomatic samples of “Olotillo 1” at a frequency of 50%. Lamprecht et al. (16) reported the presence of *Phomopsis viticola*, *P. asparagi*, *P. vaccinii* and *P. sclerotioides*, considered part of the fungal complex that significantly affects the survival of corn seedlings and the cause of ear rot in South African corn crops.

Gibberella moniliformis was isolated from four symptomatic seed samples; in this sense, the races “Elotes occidentales 1” and “Arrocillo 1” presented incidence values of 83.3% and 66.7%, respectively. That fungal species has been reported in Mexico as a pathogen of great importance, affecting different corn parts: cob, grains, root and stem (10). *Gibberella moniliformis* was also detected in a sample of asymptomatic seed fragments of race “Cónico Pepitilla”, showing incidence values of 41.7% (Table 2).

Sarocladium strictum was also isolated and, for symptomatic seeds, its incidence values were 11% in “Arrocillo 2”, 8.3% in “Arrocillo 1” and 2.8 in “Pepitilla”. That same species was recorded in South Africa at a higher incidence; as reported in the present study, its frequency was 18.4% isolation from corn grains and it was associated with corn ear rot (4). Differently, Gonçalves et al. (15) detected *S. strictum* in corn leaves.

In the present study, the fungus *Talaromyces variabilis* was isolated from two samples which showed a lower frequency: 11.1% in race “Arrocillo 2” and 2.8% in race “Cónico pepitilla”. Peterson & Jurjević (23) indicated that *Talaromyces* spp. were isolated from corn seeds in the United States.

On the other hand, *Stenocarpella macrospora* was detected in one single sample of symptomatic seeds at a frequency of 72.2% for corn of race “Pepitilla”. In Brazil, *S. macrospora* is the cause of corn stem, ear and leaf rot (26).

Aspergillus flavus was only detected in corn of race “Cónico Pepitilla” at a frequency of 16.7%. In Sonora, Mexico, research was carried out in several agroecological zones of the soils of corn crops, detecting values higher than 45% for *A. flavus*, which was considered a contaminant to corn crops; aflatoxin content of the said species was also analyzed (22).

Setosphaeria turcica exhibited colonial development in symptomatic seeds of race “Arrocillo delgado” collected from the town of San

Francisco Lagunita. That fungus is considered cosmopolitan and is of great economic importance for different crops worldwide; in East Africa and China, it has been reported as a severe pathogen, causing "leaf blight" that affects different races of sorghum (*Sorghum bicolor* L.) (24). Likewise, Félix-Gastélum et al. (12) confirmed the presence of *Exserohilum turcicum*, indicated as the anamorphic phase of *S. turcica* and the major cause of leaf blight affecting hybrid corn production in Sinaloa State, Mexico.

On the other hand, *Emericella* sp. was isolated from samples of race "Arrocillo delgado". *Emericella* sp. is considered to be present in food worldwide, both in its teleomorphic phase and in its anamorphic phase, which is *Aspergillus* sp. (2); in addition, both genera are regarded as potential producers of aflatoxins (13). So far, in reference to the current study, presence of *Emericella* sp. in corn seeds has not been reported in Mexico.

Fusarium acuminatum was isolated from asymptomatic seeds of race "Conico pepitilla", at 8.3% incidence. In Italy, 15 species of the genus *Fusarium,* including *F. acuminatum*, were identified as causing disease in corn stalks and ears (6).

Rhizoctonia solani was isolated from asymptomatic seeds of race "Olotillo 2", at a frequency of 50%. Globally, *R. solani* is considered a fungus of wide host range and capable of causing plant wilt and fruit rot, both in the field and in the greenhouse, thus generating economic losses (20).

The current results indicated the presence of several fungi associated with Creole corn seeds, leading to the conclusion that *F. oxysporum* was the most frequent organism, isolated from six races of Creole corn, followed by *Phomopsis* sp., *G. moniliformis* and *F. graminearum*. These were the most frequent fungi in the Creole corn seeds evaluated in the present study.

DISCLOSURE STATEMENT

There is no potential conflict of interest in the submitted paper.

ACKNOWLEDGMENTS

The first author thanks CONACyT for the scholarship granted to her Master's studies. The authors also thank "Maestría en Ciencias Agropecuarias y Gestión Local de la Universidad Autónoma de Guerrero" for supporting the present study.

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