Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i2.3502

Investigation note

Fusarium graminearum chemotype Don in malting barley grain grown in Mexico

Mirna Bobadilla-Meléndez[1]
Mauro R. Zamora-Díaz[2]
Ana María Hernández-Anguiano[3,§]

1 Campo Experimental Bajío-INIFAP. Carretera Celaya-San Miguel de Allende km 6.5, Colonia Roque, Celaya, Guanajuato, México. CP. 38110. (bobadilla.mirna@inifap.gob.mx).

2 Campo Experimental Valle de México-INIFAP. Carretera los Reyes-Texcoco km 13.5, Coatlinchán, Texcoco, Estado de México. CP. 56250. (zamora.mauro@inifap.gob.mx).

3 Posgrado de Fitosanidad-Fitopatología-Colegio de Postgraduados. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, Estado de México. CP. 56264. (ahernandez@colpos.mx).

Autora para correspondencia: ahernandez@colpos.mx

Abstract

Fusarium graminearum species cause Fusarium head blight, an important disease that affects grain yield and health quality in several barley-producing regions. Among the control measures is the use of resistant varieties, so this work aimed to isolate and morphologically and molecularly characterize isolates of *F. graminearum* from different malting barley-producing regions in Mexico and to identify those isolates with the greatest capacity to produce the Don toxin *in vitro* to recognize sources of genetic resistance. Thirty-nine isolates with morphological characteristics of *F. graminearum* were obtained, which were associated with malting barley grain from municipalities of High Valleys, El Bajío, and Tamaulipas. PCR reaction with species-specific primers Fg16N-F/Fg16N-R for *F. graminearum* confirmed the identity of 38 of the 39 isolates. The product sequences with Fg16N from 21 isolates annealed with the sequence of chromosome 1 of *Fusarium graminearum*, deposited in the GenBank-NCBI database. The PCR reaction with primers ToxP1/ToxP2 indicated that 17 of the 39 isolates correspond to the chemotype of *F. graminearum* that produces the Don toxin. Five of 33 isolates, analyzed by the Ridascreen® Fast Don test, registered the highest capacity to produce Don *in vitro* (3.4 and 17 ppm), so they can be considered to identify sources of resistance to Fusarium head blight in malting barley genetic improvement programs in the country.

Keywords:

Hordeum vulgare, deoxynivalenol, mycotoxin.

DOI: https://doi.org/10.29312/remexca.v16i2.3502

Fusarium head blight is a disease of great economic importance in small-grained cereals, such as barley (*Hordeum vulgare* L.). This disease is caused by a complex of *Fusarium* species, including *Fusarium graminearum* Schwabe [Teleomorph *Gibberella zeae* (Schweinitz)] (Mert-Türk *et al*., 2014). Gilchrist-Saavedra (2000) reported the presence of Fusarium head blight in Mexico in the regions of High Valleys and El Bajío. These regions include the states of Hidalgo, State of Mexico, Puebla, and Tlaxcala and those of Guanajuato, Jalisco, and Michoacán, where malting barley is grown under rainfed and irrigated conditions, respectively.

F. graminearum is a fungus that is characterized by producing toxins that harm the health of animals and people who consume contaminated barley grains or products (Bezerra *et al*., 2014; FDA, 2010), among which those of the trichothecene group stand out, such as deoxynivalenol (Don) and nivalenol (NIV) (Mert-Türk *et al*., 2014). These toxins are considered to be a virulence factor associated with the ability of the fungus to produce isolates with different degrees of pathogenicity (Mesterházy, 2002; Malihipour *et al*., 2012).

Among the recommended strategies for controlling Fusarium head blight was the use of resistant varieties to reduce the incidence of the fungus in the grain (McMullen *et al*., 2012). Therefore, it is necessary to have a collection of *F. graminearum* strains, isolated from the varieties and experimental lines of malting barley grown in the different geographical regions of Mexico, to identify sources of resistance that can be used in crosses carried out by the barley genetic improvement programs of national and international research institutions to control the disease (Bobadilla *et al*., 2019).

The main objective of this work was to isolate and morphologically and molecularly characterize *F. graminearum* isolates from different malting barley-producing regions in Mexico and to identify those strains with the greatest capacity to produce the Don toxin *in vitro*, for subsequent studies to search for sources of genetic resistance to Fusarium head blight.

During the 2021, 2022, and 2023 malting barley production cycles, 257 samples of spikes with and without symptoms of grain spot were collected, which were from the Adabella, Alina, Armida, Blanca, Esperanza, and Esmeralda varieties and from 131 experimental lines of the Barley Program of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP, for its acronym in Spanish). Sixty-eight samples were collected in the High Valleys region (19 in Hidalgo, 16 in Tlaxcala, 17 in the State of Mexico, and 16 in Puebla); 19 in Baja California; 156 in El Bajío (136 in Guanajuato, 12 in Jalisco, and 8 in Michoacán), and 14 in Tamaulipas. From each sample, 240 seeds were randomly taken for analysis by blotting paper and freezing (Warham *et al*., 1997).

Before the test, the seed was disinfested with 5% sodium hypochlorite (Reasol^{MR}) for 1 min in mechanical stirring and rinsed twice with sterile distilled water. Isolates with morphological characteristics of *F. graminearum* (Leslie and Summerell, 2006) were purified by monosporic culture in water-agar (BD Bioxon®), cultured in water-agar-carnation leaf and potato-dextrose agar (BD Bioxon®), and stored in mineral oil (Mier *et al*., 2002) and Whatman #1 filter paper (Fong *et al*., 2000).

DNA was extracted from each isolate from lyophilized mycelium using the cetyltrimethylammonium bromide (CTAB) method (Joint Research Centre of the European Commission, 2007). The quality and quantity of the extracted DNA was verified on a NanoDrop ND-1000. To confirm the identity and determine the chemotype by polymerase chain reaction (PCR), reactions were established with the species-specific primers Fg16N-Forward $^{5'}$ACAGATGACAAGATTCAGGCACA$^{3'}$ and Fg16N-reverse $^{5'}$TTCTTTGACATCTGTTCAACCCA$^{3'}$ (Nicholson *et al*., 1998) and with the primers ToxP1 5′GCCGTGGGGRTAAAAGTCAAA3′ and ToxP2 5′TGACAAGTCCGGTCGCACTAGCA3, which amplify an intergenic region between the Tri5 and Tri6 genes related to the synthesis of the Don and Niv toxins (Li et al., 2005).

Each reaction had a final volume of 12 µl with the same formulation, except for annealing. Initial denaturation 95 °C, 10 min; 40 cycles: denaturation 95 °C, 30 s; annealing 60 °C, 60 s (Fg16N) or 65 °C, 60 s (ToxP); elongation 72 °C, 72 s; final elongation 72 °C, 10 min. Reactions were established on an Eppendorf Mastercycler Gradient Thermal Cycler, products were analyzed by electrophoresis in 2% agarose and visualized on a PhotoDoc-ITTM UVP System, Fisher Scientific. As a reference, the marker ØX174 (PhiX174) /HaeIII (Fermentas and BioLabs, respectively) was included in the gel.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i2.3502

The PCR product with Fg16N of 21 strains was sent for sequencing to Macrogen CIA (http://dna.macrogen), Korea and the sequences were compared with Ncbi Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to verify their relationship with *Fusarium graminearum*. The ability of the isolates to produce Don *in vitro* was evaluated by the Ridascreen® Fast Don test (enzyme immunoassay (Elisa)) using flour samples (2 g) obtained from rice grains (Sos® Sushi Rice, super extra) colonized with the fungus, and with Ridascreen® Fast Don plates, reagents, and protocol.

The data were analyzed with the Statistical Analysis Software (SAS) Version 9.4, for Windows, under a completely randomized design, and the comparison of means by Tukey's method (α= 0.05). Of the total number of seeds (61 680 seeds) analyzed from High Valleys, El Bajío, and Tamaulipas, 39 isolates with morphological characteristics of *F. graminearum* were obtained (Leslie and Summerell, 2006) (Table 1).

Table 1. olecular identification of species and chemotype and production of Don toxin *in vitro*.

Locality	Isolate code	PCR		Annealed species	Don (ppm)[¥]
		Fg16N	ToxP		
Almoloya, Hidalgo	AL5R10	+	-		0.1c[*]
	AL6R32	+	-	*F. graminearum* [§]	0c
	AL9R32	+	-		0c
Cuautepec, Hidalgo	HGO1	+	+	*F. graminearum*	nd
	HGO2	+	+	*F. graminearum*	nd
Nanacamilpa, Tlaxcala	NA0R20	+	-		0c
	NA5R20	+	+	*F. graminearum*	1.6c
	NA8R20	+	+		2.6c
	NA9R10	+	+		2.9c
	NA11R30	+	+		0.1c
	NA16R10	+	-		0c
	NA16R20	+	-	*F. graminearum*	0.1c
Santa Lucía, Edo. de México	SL1	+	+		nd
	SL3R21	+	+		2.3c
	SL10R10	+	+	*F. graminearum*	3.4bc
	SL3R22	+	+		1c
	SL3R23	+	+	*F. graminearum*	9.2b
	SL3R24	+	+	*F. graminearum*	5bc
	SL6R10	+	-	*F. graminearum*	0c
	SL8R10	+	-	*F. graminearum*	0c
	SL4R10	+	-		0c
	SL5R30	+	-	*F. graminearum*	0.1c
Cuyuaco, Puebla	CU0R10	+	-	*F. graminearum*	0c
	CU1R30	+	+	*F. graminearum*	17a
	CU6R30	+	+	*F. graminearum*	3.6bc
	CU13R10	+	-		0.1c
	CU9R11E	+	-	*F. graminearum*	0c
	CU8R36E	+	-		0c
Celaya, Guanajuato	CEN1	+	-		0c
	CEN2	+	-	*F. graminearum*	0c

DOI: https://doi.org/10.29312/remexca.v16i2.3502

Locality	Isolate code	PCR		Annealed species	Don (ppm)[¥]
		Fg16N	ToxP		
	CEN3	+	-		0c
	CEN4	+	-		0.1c
	CEN8	+	-	*F. graminearum*	0c
	CEN9	+	-		0c
	CEE1	+	-	*F. graminearum*	0.1c
	CEFS31	+	-		0.1c
Tepatitlán, Jalisco	JA1	-	+	*F. graminearum*	nd
Río Bravo, Tamaulipas	TAMPS1	+	+	*F. graminearum*	nd
	TAMPS2	+	+	*F. graminearum*	nd

+/-= band amplification/non-amplification; nd= not determined; [*]= means by column with the same letter are not statistically different (Tukey α= 0.05); [¥]detection limit= 0.2 ppm kg; quantification limit= 0.36 ppm kg for oats; [§]= *Fusarium graminearum* chromosome 1.

The fungus was not isolated from the seeds from Baja California and Michoacán. Except for JA1, all isolates amplified the expected band of 280 base pairs (bp) with Fg16N-F/Fg16N-R (Nicholson *et al*., 1998). The sequences (approximately 250 bp) of the product with Fg16N of 21 strains (randomly taken by locality), including that of JA1, annealed with the sequence of chromosome 1 of *F. graminearum*, accession number HG970332, deposited in the GenBank-NCBI database (Table 1). The similarity rates were between 99 and 96%.

In the reaction with ToxP1/ToxP2, 17 isolates from High Valleys (14), Jalisco (1), and Tamaulipas (2) amplified a 300 bp band (Table 1). It is important to note that these primers simultaneously detect two fragments: a 300 bp fragment for the Don-producing chemotype of *F. graminearum* and a 360 bp fragment for the NIV-producing chemotype of *F. graminearum* (Li *et al*., 2005). No isolate of Guanajuato was amplified by these primers. Except for NA11R30, 10 of the isolates that amplified with ToxP1/ToxP2 recorded Don production *in vitro*, with differences (Tukey α= 0.05) in production levels.

Possibly, the isolates that amplified with Fg16N, but did not amplify with ToxP or record Don production, correspond to other chemotypes of *F. graminearum* (Walker *et al*., 2001).

In general, the results indicate that, in the malting barley-producing regions of High Valleys, El Bajío, Baja California, and Tamaulipas, the incidence of *F. graminearum* was low (less than 1%), and that the 39 isolates obtained show genetic variation (Tukey α= 0.05) not only among the isolates from different states, but also among the isolates in collections from the same municipality (Table 1). Cerón-Bustamante *et al*. (2018) research did not detect *F. graminearum* among the *Fusarium* species associated with wheat spikes with Fusarium head blight collected in the wheat-producing regions of High Valleys, El Bajío and Oaxaca.

Conclusions

Thirty-nine isolates with morphological characteristics of *F. graminearum* were obtained, which were associated with malting barley grain harvested in municipalities of High Valleys, El Bajío, and Tamaulipas. In 38 isolates, the identity was confirmed by PCR with the species-specific primers Fg16N-F/Fg16N-R. The sequences of the product with Fg16N from 21 isolates, including the one of JA1 that did not amplify, annealed with the sequence of chromosome 1 of *Fusarium graminearum*, deposited in the GenBank-NCBI database.

The PCR reaction with the primers ToxP1/ToxP2 indicated that 17 of the 39 isolates correspond to the *F. graminearum* chemotype that produces the Don toxin. The isolates SL10R10, SL3R23, SL3R24, CU1R30, and CU6R30, analyzed by the Ridascreen® Fast Don test, had a high capacity to produce Don *in vitro* (between 3.4 and 17 ppm), so they can be considered to identify sources of resistance to Fusarium head blight in malting barley improvement programs in the country.

DOI: https://doi.org/10.29312/remexca.v16i2.3502

Acknowledgements

To the National Council of Humanities, Sciences, and Technologies (CONAHCYT), for its acronym in Spanish for scholarship 1136066 awarded to the first author for doctoral studies. To Dr. Pawan K. Singh from the International Maize and Wheat Center (CIMMYT), for its acronym in Spanish for his support in conducting the analyses in his laboratory

Bibliography

1 Bezerra, R. M. E.; Oliveira, F. F. Ch.; Feitosa, M. F. E.; Florindo, G. M. I. and Rondina D. 2014. Mycotoxins and their effects on human and animal health. Food Control. 36(1):159-165. https://doi.org/10.1016/j.foodcont.2013.08.021.

2 Bobadilla, M. M.; Hernández-Anguiano, A. M.; Zamora-Díaz, M. R. y Vargas-Hernández, M. 2019. Evaluación de líneas de cebada maltera a fusariosis de la espiga y acumulación de deoxinivalenol. Revista Mexicana de Ciencias Agrícolas. 10(3):485-498. https://doi.org/10.29312/remexca.v10i3.916.

3 Cerón-Bustamante, M.; Ward, T. J.; Kelly, A.; Vaughan, M. M.; McCormick, S. P.; Cowger, C.; Leyva-Mir, S. G.; Villaseñor-Mir, H. E.; Ayala-Escobar, V. and Nava-Díaz, C. 2018. Regional differences in the composition of fusarium head blight pathogens and mycotoxins associated with wheat in Mexico. International Journal of Food Microbiology. 273:11-19 https://doi.org/10.1016/j.ijfoodmicro.2018.03.003.

4 FDA. 2010. Food and Drug Administration. Guidance for industry and FDA: advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed. https://www.fda.gov/Food/GuidanceRegulation/ucm120184.htm.

5 Fong, Y. K.; Anuar, S.; Lim, H. P.; Tham, F. Y. and Sanderson, F. R. 2000. A modified filter paper technique for long-term preservation of some fungal cultures. Mycologist. 14(3):127-130. https://doi.org/10.1016/S0269-915X(00)80090-7.

6 Gilchrist-Saavedra, L. I. 2000. Problemas fitosanitarios de los cereales de grano pequeño en los Valles Altos de México. Serie de Conferencias Magistrales. *In*: XXVII Congreso Nacional de la Sociedad Mexicana de Fitopatología, AC. Revista Mexicana de Fitopatología. 18(2):132-137. https://www.redalyc.org/articulo.oa?id=61218211.

7 Joint Research Centre of the European Commission. 2007. Maize seeds sampling and DNA extraction. Report on the validation of a DNA extraction method from maize seeds and grains. 1-10 pp.

8 Leslie, J. F. and Summerell, B. A. 2006. The *Fusarium* laboratory manual. First edition. Ed. Blackwell Publishing. USA. 387 p.

9 Li, H. P.; Wu, A. B.; Zhao, Ch. S.; Scholten, O.; Löffler, H. and Liao, Y. C. 2005. Development of a generic PCR detection of deoxynivalenol- and nivalenol-chemotypes of *Fusarium graminearum*. FEMS Microbiology Letters. 243(2):505-511. https://doi.org/10.1016/j.femsle.2005.01.015.

10 Malihipour, A.; Gilbert, J.; Piercey-Normore, M. and Cloutier, S. 2012. Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of *Fusarium* isolates causing head blight in wheat. Plant Disease. 96(7):1016-1025. https://doi.org/10.1094/PDIS-10-11-0866-RE.

11 Mert-Türk, F.; Gencer, R. and Kahriman, F. 2014. Chemotyping of the *Fusarium graminearum* isolates and variation in aggressiveness against wheat heads. The Journal of Animal & Plant Sciences. 24(6):1858-1862. http://www.thejaps.org.pk/docs/v-24-6/40.pdf. ISSN: 1018-7081.

DOI: https://doi.org/10.29312/remexca.v16i2.3502

12 Mesterházy, A. 2002. Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to Fusarium head blight. European Journal of Plant Pathology. 108(7):675-684. https://doi.org/10.1023/A:1020631114063.

13 McMullen, M.; Bergstrom, G.; De Wolf, E.; Dill-Macky, R.; Hershman, D.; Shaner, G. and Sanford, D. V. 2012. A unified effort to fight an enemy of wheat and barley: fusarium head blight. Plant Disease. 96(12):1712-1721. https://doi.org/10.1094/PDIS-03-12-0291-FE.

14 Mier, T.; Toriello, C. y Ulloa, M. 2002. Hongos microscópicos saprobios y parásitos: métodos de laboratorio. Universidad Autónoma Metropolitana Xochimilco (UAM)-X. México, DF. 54-57 pp. ISBN 970654609X.

15 Nicholson, P.; Simpson, D. R.; Weston, G.; Rezanoor, H. N.; Lees, A. K.; Parry, D. W. and Joyce, D. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiological and Molecular Plant Pathology. 53(1):17-37. https://doi.org/10.1006/pmpp.1998.0170.

16 Warham, E. J.; Butler, L. D. y Sutton, R. C. 1997. Ensayos para la semilla de maíz y de trigo. Manual de laboratorio. Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). El Batán, Texcoco, Estado de México. ISBN: 968-6923-71-3. 84 p.

17 Walker, L. S.; Leath, S.; Hagler, Jr. W. M. and Murphy, J. P. 2001. Variation among isolates of *Fusarium graminearum* associated with Fusarium head blight in North Carolina. Plant Disease. 85(4):404-410. https://doi.org/10.1094/PDIS.2001.85.4.404.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i2.3502

Fusarium graminearum chemotype Don in malting barley grain grown in Mexico

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 December 2024
Date accepted: 01 April 2025
Publication date: 16 April 2025
Publication date: Feb-Mar 2025
Volume: 16
Issue: 2
Electronic Location Identifier: e3502
DOI: 10.29312/remexca.v16i2.3502
Funded by: Consejo Nacional de Humanidades, Ciencias y Tecnologías
Funded by: Centro Internacional de Maíz y Trigo
Award ID: 1136066

Categories

Subject: Investigation note

Keywords:

Keywords:

Hordeum vulgare
deoxynivalenol
mycotoxin

Counts

Figures: 0
Tables: 1
Equations: 0
References: 17
Pages: 0