Article

Evaluation of malt barley lines to spindle fusariosis and accumulation of deoxynivalenol

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Abstract

The fusariosis of the spike by *Fusarium graminearum* is a disease that affects the yield and sanitary quality of the barley grain. Among the control measures is the use of resistant varieties, so this work had as objectives to evaluate the resistance to fusariosis and the accumulation of deoxynivalenol (DON) in 131 advanced malting barley lines selected for their tolerance to yellow rust, rust of the leaf, reticulated spot, leaf blight and scald of the leaf, of the barley program of the INIFAP-CEVAMEX. For three years, the advanced lines 30, 47, 65 and 123, as well as lines 73, 91 and 96, of six and two rows of grain, respectively, registered very low symptoms of fusariosis in response to inoculation with *F. graminearum*. Although the severity of the disease was not related (r= 0.5682) to the accumulation of DON, these lines registered very low or no toxin production. The information generated in this study is relevant because it indicates that these lines are resistant to the disease and have the potential to be used as sources of resistance in a crossbreeding program in malting barley that includes spike fusariosis in all diseases.

Keywords: *Fusarium graminearum*, *Hordeum vulgare*, DON mycotoxin, resistance.

Reception date: January 2019
Acceptance date: March 2019

Introduction

Fusarium head blight, also known as scab of the spike or fusariosis de la espiga (FHB), is an important disease in small grain cereals such as barley (*Hordeun vulgare* L.). This disease, caused by some *Fusarium* species, among which *F. graminearum* (Schwabe) stands out (Salas *et al*., 1999), attacks mainly in the stage of flowering to milky grains affecting the yield and sanitary quality of the grain. Ireta and Gilchrist (1994) reported losses of 17% in wheat yield in the production area of High Valley of Mexico; however, losses of up to 50% have been recorded in other parts of the world (Windels, 2000). The consumption of contaminated malted grain or barley products affects the health of people and animals due to the production of toxic compounds such as Deoxinivalenol (DON), from the group of trichothecenes by *Fusarium* (Bezerra *et al*., 2014).

Because of the implications to human and animal health of the DON toxin, also known as vomitoxin for causing vomiting in people and rejection of food in animals (Zain, 2011), the food industry has implemented limits of tolerance to the concentration of DON in the contaminated grain. In this regard, the US Food and Drug Administration (FDA) establishes the following limits on grains and by-products of wheat grains: 1 ppm for wheat products (flour, bran and germ) destined for human consumption, 5 ppm for food grain products for pigs, 10 ppm for grains and grain by-products for the preparation of feeds for cattle and poultry and 30 ppm for grains for distillery (FDA, 2010).

However, the brewing industry established a limit of 0.5 ppm for the production of malt (Windels, 2000). The foregoing, indicates that fusariosis is a disease that limits yield, affects the sanitary quality of the grain and has a negative impact on the agricultural, food and industrial sectors. On the other hand, Malihipour and Gilbert (2012) and De la Torre-Hernandez *et al*. (2014) report that the DON toxin is a virulence factor and associate it with the capacity of the *Fusarium* species that produce it to cause disease in its host. De Wolf *et al*. (2003) and Bondalapati *et al*. (2012) indicate that conditions of relative humidity around 90% and temperature of 15 to 30 °C during the anthesis period are favorable for the development of fusariosis and the accumulation of DON.

The Barley Program of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP) of the Valley of Mexico Experimental Field (CEVAMEX) has crossbreeding works for the improvement of malting barley, currently having 131 advanced lines with tolerance to yellow rust (*Puccinia striiformis* f. sp. *hordei* West), leaf rust (*P. hordei* Otth), reticulate spot (*Drechslera teres* Sacc), leaf blight (*Bipolaris sorokiniana*) and leaf scald (*Rhyncosporium secalis* (Oud) J. J. Davis), diseases considered as the most frequent in the producing areas of the High Valleys of Mexico (Gilchrist-Saavedra, 2000; Zamora *et al*., 2008).

It is important to mention that some advanced lines of barley have Gobernadora, Chevron and CI14064, as progenitors, which are used as sources of resistance for developing low levels of spindle fusariosis (Zhu *et al*., 1999; McCallum *et al*., 2004; Marchand *et al*., 2008). Considering that among the recommended control measures of spindle fusariosis is the use of resistant varieties to reduce the incidence of *Fusarium* in the grain (Horevaj *et al*., 2011; McMullen *et al*., 2012), the

objectives of this work were: to evaluate the resistance to fusariosis of the 131 advanced malting barley lines of the INIFAP-CEVAMEX Barley Program under greenhouse conditions and to quantify the production levels of DON toxin in malted barley seed harvested from spikes inoculated with *F. graminearum*.

The information generated in this work is relevant and can be used as a starting point in a malting barley breeding program that includes, in the set of diseases, the spindle fusariosis and the accumulation of DON toxin in grain.

Materials and methods

This study was carried out during the winter-spring cycle of 2015, 2016 and 2017, at the Postgraduate College, *Campus* Montecillo, State of Mexico. The *campus* is at an altitude of 2 240 meters above sea level, has a subhumid temperate climate, with an average annual rainfall of 700 mm, with a rainfall regime in summer. The plants were established in a greenhouse of 437.5 m^2 built with transparent plastic and dirt floor without controlled environmental conditions. During the period of inoculation of the plants, an average temperature of 20 °C was recorded in the greenhouse (with a minimum of 9 °C and maximum of 35 °C) and an HR of 46.6% (with a minimum of 26.5% and a maximum of 68.9%), with an HOBO H8 automatic sensor (Onset Computer corporation, USA).

Germplasm and establishment of plants

The seed of 131 advanced lines of malting barley, organized in sublots: 03 (30 lines), 02 (30 lines), 01 (25 lines) and Elite (16 lines), six rows of grain and sublot 02A (30 lines), of two rows, was provided by the Barley Program of the INIFAP-CEVAMEX. It is highlighted that the selection of the lines was from sublot 03 followed by 02A, 02, 01 and ending with Elite. That is, the sublots 03 and Elite represent the sublots of smaller and larger selection cycles, respectively. As control, seeds of the varieties were included: Seebe, CDC Copeland, Xena, AC Ranger, Kasota and AC Lacombe, provided by the International Center for Maize and Wheat Improvement (CIMMYT), El Batan, as well as the varieties: Governor, Adabella, Esmeralda and Shenmai 1, provided by the INIFAP-CEVAMEX (Table 1).

Table 1. List of varieties of barley used as a control and characteristics of number of rows of grain per spike and resistance to spindle fusariosis, according to the literature.

Variety	No. of rows of grain per spike	Resistance to disease
Resistant (R) or moderately resistant (MR)		
Gobernadora	2	Variety R, over time it has behaved like MR (McCallum *et al*., 2004; Marchand *et al*., 2008)
Seebe	2	Registered as MR, it sometimes behaves like R (Helm *et al*., 1996; He *et al*., 2015)
Moderately resistant (MR) or moderately susceptible (MS)		
Adabella	6	Variety MR with tolerance (Zamora *et al*., 2008)

Variety	No. of rows of grain per spike	Resistance to disease
Shenmai 1	2	Name used in China to refer to a segregating population of Gobernadora (F_4) (Vivar *et al*., 1997)
CDC Copeland	2	It behaves as MR and MS (He *et al*., 2015)
Xena	2	It behaves as MR and MS (He *et al*., 2015)
MS or susceptible (S)		
Esmeralda	6	Variety R but over time it has behaved like S (Zamora *et al*., 1997)
AC Lacombe	6	Variety considered between MS and S (Kibite, 1993; He *et al*., 2015)
AC Ranger	6	Variety considered between MS and S (Therrien, 2002)
Kasota	6	Variety S (Helm *et al*., 1996)

Seed was planted in duplicate in plastic bags (25 x 25 cm) with peat moss substrate (Promix®) sterilized with steam. 10 seeds per bag were deposited for each control line and variety. At the beginning of the crop, three irrigations by gravity with well water per week were given; after flowering, the plants were irrigated daily by sprinkling. For the fertilization of the plants, triple 16 (16-16-16), 18-46-00 and urea (Yara®) were used, applying the formula 100-46-30. Phosphorus and potassium were applied in full at eight days after sowing; nitrogen only 50% and the rest, at 60 days after sowing. During the 2017 cycle, a 75% shadow mesh was placed to generate cool conditions in the greenhouse.

Strains and inoculum preparation

Table 2 shows the relationship of strains of *F. graminearum* used for greenhouse inoculations during the three years of study. These strains are monosporic isolated from grains infected with *F. graminearum*. In 2015, the strain CIMFU1189 isolated from wheat grains collected in Toluca, State of Mexico, was used in 2016, the mixture of strains CIMFU1308, CIMFU1310, CIMFU1312 and CIMFU1313 isolated from wheat grains. All these strains were provided by the CIMMYT Plant Pathology Laboratory and were used for their high capacity to produce DON (Table 2).

Table 2. Relation of strains of *F. graminearum* used for greenhouse inoculations.

Year	Source Key strain	Origin Host	DON toxin production (ppm kg^{-1})	Inoculation† of spikes
2015	CIMMYT: CIMFU1189	Wheat	14.5	By sprinkling
2016	CIMMYT: CIMFU1313, CIMFU1310, CIMFU1308, CIMFU1312	Wheat	16.1, 17, 18.1 and 18.8, respectively (with an average of 17.5)	By puncture
2017	This studio: SL10R10, CU6R30, SL3R24, SL3R23 y CU1R30	Barley	3.4, 3.6, 5, 9.2 and 17, respectively (with an average of 7.6)	By sprinkling

†= in 2015 with 100 000 conidia mL^{-1}; in 2016 and 2017 with 70 000 conidia mL^{-1}

Meanwhile, in 2017 the mixture of strains CU1R30, SL3R23, SL3R24, SL10R10 and CU6R30 isolated from malted barley grains was applied in Cuyuaco (CU), Puebla and Santa Lucía (SL), State of Mexico. These last strains were isolated, identified and characterized for this study (data not shown) and were also selected for their high capacity to produce DON (Table 2). In 2016, the Plant Pathology Laboratory of CIMMYT provided the mixture with the four strains for the inoculation of the plants, while in 2015 and 2017 the mixtures were prepared in the Laboratory of Diseases in Post-harvest, Postgraduate Phytopathology, Graduate College.

For this a fragment of sterile Whatman™ 1 filter paper with fungal growth, stored for 5 months at 4 °C, was placed in a Petri dish with PDA and incubated at room temperature. After 10 days circles of medium with fungal growth (4 mm) were cut and placed in 250 mL flasks with 50 mL of liquid medium of Chinese bean (*Vigna radiata* (L.) Wilezek) sterile for the production of conidia (Bai, 1996). The inoculated flasks were kept shaking (Agitador Lab-Line®) with continuous white light (Fulgore® lamp, 250 V, 32 W) at room temperature for 7 days. The suspension obtained was filtered; through a sterile gauze and the concentration of conidia was estimated in a hematocytometer. All suspensions were maintained at -10 °C until use.

Spike inoculation

In 2015 the spikes were inoculated in the milky grain stage by sprinkling with an inoculum of 100 000 conidia mL^{-1}, whereas in 2016 by puncture with 70 000 conidia mL^{-1}, when the plants began to emit the spikes. For this, a gout of 10 µL was placed in the central spikelet of the spike with a hypodermic syringe (Engle *et al*., 2003). In 2017, the inoculation was at the beginning of the sprigging with an inoculum of 70 000 conidia mL^{-1} (Table 2).

Unlike the previous two cycles, in this cycle the spikes were inoculated again four days after the first inoculation by the same method and with the same amount of inoculum. Immediately after each inoculation, the ears were covered for 48 h with a transparent plastic bag, previously sprinkled with sterile water. The ground floor of the greenhouse was watered daily to maintain an environment of high relative humidity around the plants.

Evaluation of incidence, severity, and index of fusariosis

By line, 1 to 10 spikes were randomly selected at 21 days after spraying and at 7, 14, 21 and 28 days after inoculation by puncture. In the inoculation by aspersion type I resistance (resistance to initial infection) was evaluated while in the inoculation by puncture type II resistance (resistance to dispersion through the spine) (McMullen *et al*., 2012). The variables evaluated were.

Percentage of incidence (number of spikes with symptoms x 100/total of spikes inoculated); percentage of severity of the disease (number of grains with symptoms x 100/ total of grains per spike) with a scale of 1 to 9 where 1) <5%, 2) 5-17%, 3) 18-30%, 4) 31-43%, 5) 44-56%, 6) 57-69%, 7) 70-82%, 8) 83-95% and 9) >95% of pimples with fusariosis. The index of fusariosis (IFHB) was also evaluated by its acronym in English (% incidence x% severity/100 (Groth *et al*., 1999).

Statistical analysis

The values of the variables incidence percentage, severity percentage and IFHB index showed a non-symmetric distribution in a goodness-of-fit test, so the data was analyzed using a generalized linear model (Proc GLIMMIX) with Poisson distribution and link function (link) log with SAS software (Statistical Analysis Software) Version 9.4 for Windows.

DON toxin analysis

Seed of 56 lines of the Elite sublots (10 lines), 01 (8 lines), 02 (11 lines), 02A (14 lines) and 03 (13 lines), of the 2017 cycle, were analyzed with the reagents and protocol of RIDASCREEN®FAST DON (r-Biopharm) [enzyme immunoassay (ELISA)]. With the exception of lines 15, 33 and 131 of the Elite sublots, 01 and 03, respectively, all those lines (53 lines) behaved as resistant to fusariosis; the three remaining lines, as susceptible. Likewise, seeds of the Seebe, Shenmai 1 and Kasota controls were analyzed as reference of control R, MR and S, respectively. All the seed of the lines and that of the controls were harvested in physiological maturity from spikes inoculated with the mixture of strains SL10R10, CU6R30, SL3R24, SL3R23 and CU1R30, during said cycle.

The seed of each line and control was ground separately in an Iwatani 200W mill (AC 100 V, 50-60 HZ Pushon, Japan) for 40 s. The flour was recovered in 50 mL Eppendorf tubes with screw cap and mixed to homogenize. From each material, 2 g of flour was taken and placed separately in a 50 mL Eppendorf tube for analysis with some modifications proposed by CIMMYT. To this sample was added 40 mL of water and stirred at 400 rpm for 3 min (Lab-line agitator, model 4626). One milliliter of the suspension obtained was placed in a 2 mL Eppendorf tube to be centrifuged at 13 000 rpm (MIKRO 220R) for 15 min. From the supernatant obtained, 200 µL was taken and stored in refrigeration for further analysis.

The reactions were established in duplicate in 96-well plates, sensitized with capture antibodies against anti-deoxynivalenol antibodies. From each of the 59 samples and from each standard (0, 0.222, 0.666, 2 and 6 ppm of deoxynivalenol in water), 50 µL was taken and deposited on the plate according to a pre-established loading diagram. The reading of the plate was made in a reader of Elisa (Labomed Inc. EMR-500) at 450 nm and the visualization of the data with the program Ridawin.Exe Ver. 1.77.

Results and discussion

Response of the lines to the infection and to the spread of the disease

In this study, variation was found in the response between the 131 lines of malting barley as well as between the 10 control lines (varieties) and inoculation by puncture and aspersion with the *F. graminearum* strains. Therefore, to classify the response of resistant (R), moderately resistant (MR) and susceptible (S), the expected value of the control varieties (R, MR and S) was taken as a reference according to the cycle evaluated (Table 3).

Table 3. Index of fusariosis (IFHB) registered in the control varieties of barley in response to inoculation† with strains of *F. graminearum* during three years of evaluation in the greenhouse.

Variety	2015			2016			2017		
	R1	R2	Ẋ	R1	R2	Ẋ	R1	R2	Ẋ
Resistant or moderately resistant									
Gobernadora				4.5	1.4	3	3.2	2.5	2.8
Seebe	0	2.8	1.4	2.1	0.8	1.5	0.5	1.8	1.2
Moderately resistant or moderately susceptible									
Adabella	9.4	6.8	8.1						
Shenmai 1				3.5	7.3	5.4	12.1	3.6	7.8
CDC-Copeland	0	11.8	5.9	0.9	1.8	1.3	22.1	0.9	11.5
Xena	0	26.9	13.5	2.4	1	1.7	8	2.9	5.4
Moderately susceptible or susceptible									
Esmeralda	4	2.1	3.1						
AC Lacombe	8.9	8.8	8.8	4.4	2.3	3.3	14.9	0.9	7.9
AC Ranger	4.4	2.2	3.3	1.7	0.3	1	17.1	1.4	9.3
Kasota	6.7	13.3	10	2.8	5.9	4.4	12.4	24.9	18.6

†= 2015 by sprinkling with strain CIMFU1189. By puncture in 2016 with mixture of strains CIMFU1308, CIMFU1310, CIMFU1312 and CIMFU 1313 and sprinkling in 2017 with mixture of strains CU1R30, SL3R23, SL3R24, SL10R10 and CU6R30. R1= repetition 1; R2= repetition; 2. Ẋ= average.

The variation between repetitions (plants) of the same variety (Table 3) may be related to the phenological stage of the plant at the time of inoculation and to the microenvironment generated when the plant was covered with the plastic bag to favor the disease. It is possible that the difference in values between the replicates of a variety may be the result of an escape from the disease and not a reflection of the resistance of the plant. Thus, during the 2015 cycle, the value of IFHB registered in Seebe (1.4, R), Adabella (8.1, MR) and Xena (13.5, S) was taken as reference, leaving the 131 lines classified as: 82 lines (63%) R; 34 lines (26%) as MR and 15 lines (11%) as S, for registering a value from 0 to 5, from 6 to 10 and ≥11 from IFHB, respectively. All the sublots registered lines with the three types of response.

In this cycle the lines of sublot 02A (two-row lines) were very early and among them the line 94 stood out for having registered the highest IFHB index (29), followed by line 6 (23) of the Elite sublot and the line 33 (18) of sublot 01. These three lines registered level 3 (18-30%) on the severity scale (Chrpova *et al*., 2011) and incidence of 0 to 100%. Table 4 illustrates the classification of 56 lines by type of response R, MR or S for the 2015 cycle.

During the 2016 cycle, the IFHB index registered by the control Seebe (1.5, R), Gobernadora (3, MR) and Kasota (4.4, S) was taken as reference (Table 3). In general, there was an incidence of 25 to 100%, severity of level 2 (from 1 to 12%) and an IFHB index of 0 to 10. In most spikes, of all the lines, the necrosis developed only in the spikelet in which the inoculum was deposited; that is,

the spread of the disease through the rachis was low. However, in lines 84 and 92 of sublot 02A the dispersion in the rachis was beyond the point of inoculation, although the symptom of fusariosis was not observed in the nearby grains.

It is possible that the fungus has been confined to the spine of the spike of these lines. According to the results obtained: 18 lines (13.7%) behaved as R; 69 lines (52.7%) as MR and 44 lines (33.6%) as S for registering a value from 0 to 1, from 2 to 3 and ≥ 4 from IFHB, respectively. Of the resistant lines, lines 63 of sub-lot 02 and 73 of sub-lot 02A stood out, due to the absence of symptoms of disease. McCallum and Tekauz (2002) and Geddes *et al*. (2008) mention that barley naturally shows resistance to the spread of fusariosis through the rachis; whereas Jansen *et al*. (2005) indicate that the infection by *F. graminearum* is limited by the inhibition of hypha growth in the rachis of this crop.

The previous data indicate that the plants inoculated by spray (cycle 2015) registered greater severity (level 3) than those inoculated by puncture (level 2, cycle 2016). McCallum and Tekauz (2002) mention that when the dispersion in the spike is low, so is the severity of the disease. Table 4 illustrates the classification of 56 lines by type of response R, MR or S for the 2016 cycle. In 2017, the IFHB index was taken as reference, registered by the control Seebe (1.2, R), Shenmai 1 (7.8), MR) and Kasota (18.6, S) (Table 3) for the classification of the lines. In this cycle, the incidence of the disease varied from 0 to 100% and severity with a maximum level of 5 (0 to 43%), depending on the sublot and the line.

Considering the above, the lines were classified as: 103 lines (78.6%) R, 13 lines (9.9%) MR; and 15 lines (11.4%) S for having registered a value from 0 to 5, from 6 to 11 and ≥12 from IFHB, respectively. It is possible that the highest severity (level 5) recorded in this cycle of 2017 in relation to the one registered (level 3) in 2015, in the spikes inoculated by sprinkling, is due to several factors: the inoculum used (5 strains vs 1 strain), inoculum level (70 000 *vs* 100 000 conidia mL^{-1}), the origin of the strain (barley vs wheat), the number of inoculations (2 *vs* 1), the phenological stage (emission of spikes *vs* milky grain) and the environmental conditions of the greenhouse. Table 4 illustrates the classification of 56 lines by type of response R, MR or S for the 2017 cycle.

Table 4. Classification of barley lines into resistant (R), moderately resistant (MR) and susceptible (S) according to the response of IFHB† as well as to the accumulation of DON.

Sublot No. line	Response IFHB			DON (ppm kg^{-1})‡¥		
	2015	2016	2017	0-4 (R)	5-11 (MR)	≥12 (S)
Elite 1	S	MR	R			15.6 - 17.4
3	MR	MR	R	1.7 - 2		
4	R	MR	R	3 - 3.1		
5	MR	MR	R			13.4 - 20.3
7	MR	MR	R			10 - 14.8
8	R	MR	R		5.9 - 6	
9	MR	MR	R	3.5 - 4.4		
12	MR	MR	R	2.7 - 3.4		

Sublot No. line	Response IFHB			DON (ppm kg^{-1})‡¥		
	2015	2016	2017	0-4 (R)	5-11 (MR)	≥12 (S)
14	R	S	R			17.7 - 18
15	S	MR	S			17.1 - 19.1
01 18	R	MR	R		7.7 - 10.8	
21	MR	R	MR			11.3 - 16.2
23	S	MR	R			21.0 - 30.4
28	R	MR	R	0.11 - 0.13		
33	S	MR	S			16.7 - 19.7
30	R	R	R	0.7 - 0.8		
36	MR	MR	R			13.9 - 19.4
41	R	R	R		5.1 - 5.1	
02 42	R	S	R	2.1 - 2		
46	R	S	R			16.3 - 20
47	R	R	R	0.6 - 0.8		
48	R	MR	R	3.2 - 3.3		
53	MR	MR	R		6.4 - 6.9	
56	R	S	R	1.2 - 1.3		
58	R	S	R		5.1 - 5.2	
63	R	R	R		10.5 - 11.1	
65	R	R	R	0.01 - 0.04		
67	R	S	R			11.7 - 13.6
68	S	R	R			11.3 - 12.7
02A 72	R	MR	R		4.8 - 7.2	
73	R	R	R	0.01 - 0.02		
75	R	S	R		5.4 - 5	
79	R	MR	R		8.4 - 10.1	
81	R	S	R		6.8 - 8.9	
82	R	S	R			24 - 22.8
85	S	MR	R	3.6 - 4.2		
87	R	S	R	0.5 - 0.7		
90	MR	S	R	0 - 0		
91	R	R	R	0.1 - 0.1		
93	R	S	R	2.7 - 4.2		
96	R	MR	R	0 - 0		
98	R	S	R		5.4 - 6	
100	S	S	R	1.7 - 5.2		
03 103	R	S	R	0.8 - 1.3		
106	R	MR	R		7.7 - 9.3	
109	R	R	R		9 - 11.8	
112	R	MR	R	0.09 - 0.12		
114	MR	MR	R	0. 8 - 0.8		
116	R	MR	R	0.4 - 0.7		
119	MR	R	R			19.1 - 22.4

Sublot No. line	Response IFHB			DON (ppm kg^{-1})$^{‡¥}$		
	2015	2016	2017	0-4 (R)	5-11 (MR)	≥12 (S)
122	R	MR	R			14.5 - 14.6
123	R	R	R	1.5 - 1.7		
124	R	R	R		8.1 - 9.8	
125	R	S	R	1 - 1.5		
127	MR	S	R	2 - 4.8		
131	R	MR	S			12.4 - 13.9

†= inoculation by spray in 2015 and 2017 and by puncture in 2016. ‡reference = Seebe R, 3.4 - 4), Shenmai 1 (MR, 5.8-10.1) and Kasota (S, 22.0-22.8). $^{¥}$= detection limit= 0.2 ppm kg^{-1}; quantification limit= 0.36 ppm kg^{-1} for oats.

In the Figure 1 shows the symptoms of fusariosis in the spikelets and in the rachis of spikes inoculated with *F. graminearum*.

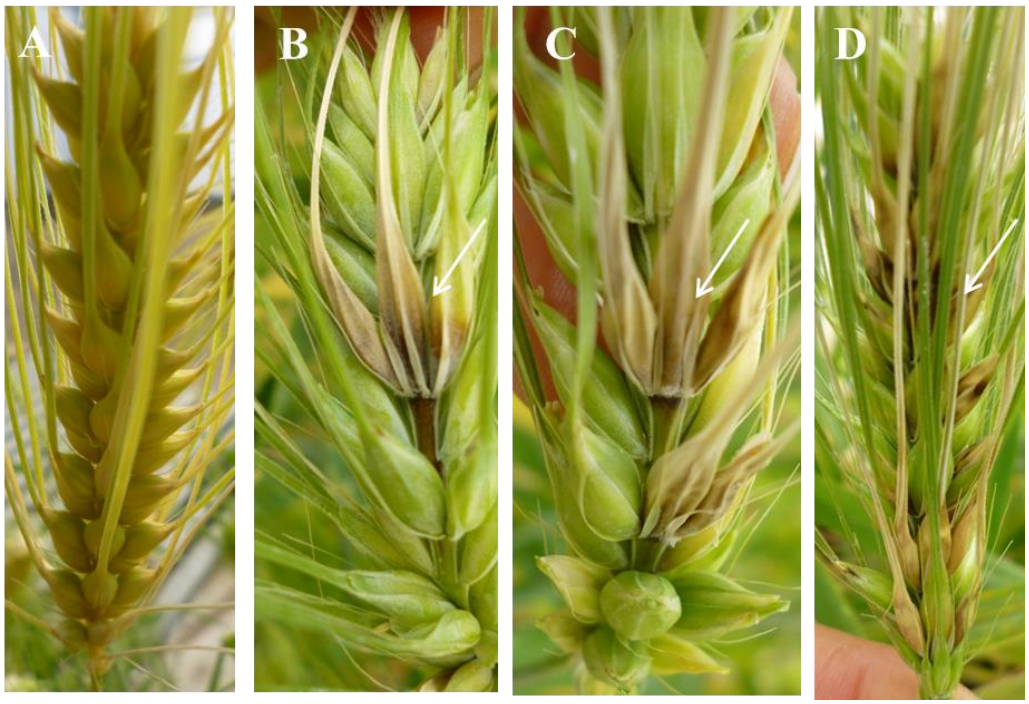

Figure 1. Spikes of malted barley lines after spraying with mixture of strains CU1R30, SL3R23, SL3R24, SL10R10 and CU6R30 of *F. graminearum* in greenhouse in 2017. Development of fusariosis. A) line 29 without symptoms; B) line 123 with low index; C) line 62 with moderate index; and D) line 33 with high index. Spikes in a state of doughy grain (A) and milky grains (B, C, and D). The arrow indicates the darkening and sucking of diseased spikelets.

The diseased grains were characterized by their wrinkled appearance, sucking and browning, characteristic symptoms of the disease. The statistical analysis of the values of incidence and severity of the disease showed difference ($p \leq 0.01$) between the lines and the sublots (Table 5).

This difference can be attributed to the aforementioned aspects or to a combination of both in addition to the genetic variability between lines by the number of selection cycles that exist in each sublot and the type of inoculation.

Table 5. Results of the analysis with the generalized linear model of the variables registered in the advanced lines of malting barley inoculated with *F. graminearum* for 3 years, in greenhouse.

Factor	GLN†	GLD‡	% incidence			% severity			IFHB		
			2015	2016	2017	2015	2016	2017	2015	2016	2017
Sublot	5	138	1	0.0001	0.0001	1	0.0001	0.0001	1	0.0001	0.0001
L (S)	133	138	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Generalized linear model, Poisson distribution, link (log); †GLN= degrees of freedom of the numerator; ‡GLD= degrees of freedom of the denominator; L(S)= lines (sublots).

Detection and quantification of DON in inoculated seed

The inoculation of 56 lines of the Elite sublots (10 lines), 01 (eight lines), 02 (11 lines), 02A (14 lines) and 03 (13 lines), by sprinkling with the mixture of strains CU1R30, SL3R23, SL3R24, SL10R10 and CU6R30 of *F. graminearum*, in the greenhouse in 2017, resulted in the accumulation of DON toxin in the seed of 49 malt lines analyzed (Table 4). Seed lines 28, 65, 73, 90, 91, 96 and 112 recorded values below the limit of detection (0.2 ppm kg^{-1}) and the limit of quantification (0.36 ppm kg^{-1}) for oats, according to the protocol of Ridascreen®Fast DON (r-Biopharm).

Taking as a reference the range of DON accumulation values in the controls Seebe (R, 3.4-4 ppm kg), Shenmai 1 (MR, 5.8-10.1 ppm kg^{-1}) and Kasota (S, 22.0-22.8 ppm kg^{-1}), the lines they were classified as: R, 26 lines, MR, 14 lines and S, 16 lines (Table 4). We found a low correlation coefficient (r= 0.5682) when relating the average values of DON content and disease severity of the 56 lines and the three control varieties (Figure 2).

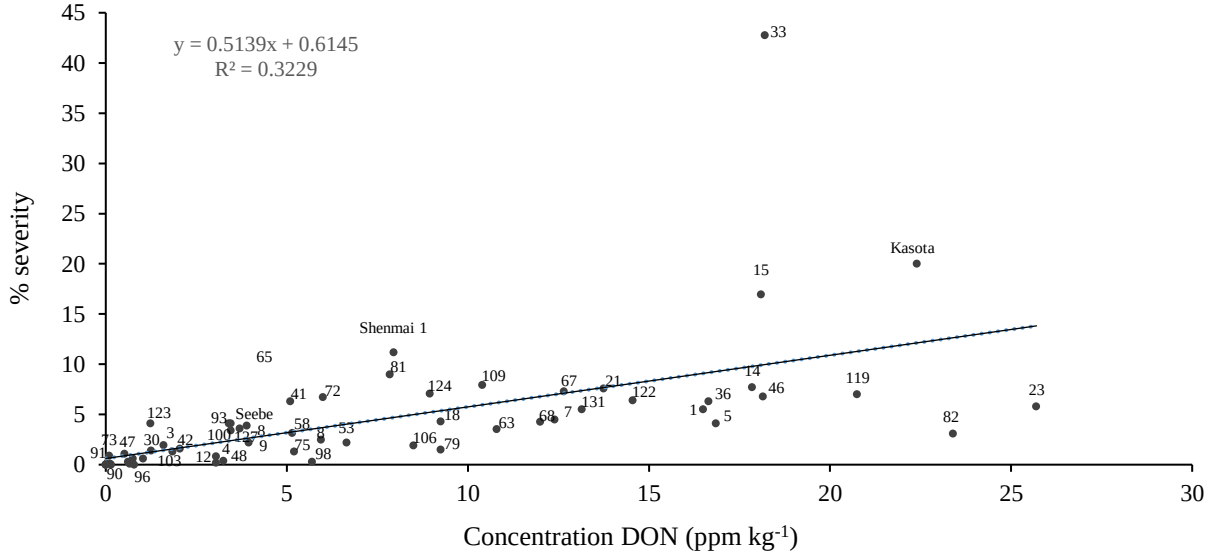

Figure 2. Correlation of severity of the disease and accumulation of DON in spike seed inoculated by spraying with mixture of *F. graminearum* strains, in greenhouse in 2017. The numbers inside the figure indicate the number of the line.

This low correlation is illustrated by the values obtained by lines 23, 82 and 119 (23.2 ppm kg on the average of DON) classified as R in that same year (Table 4). In contrast, lines 15, 33 and 131, classified as S to fusariosis in 2017, accumulated a high content of DON (16.4 ppm kg^{-1} on average). Therefore, lines 15 and 33 of the Elite and 01 sublots, respectively, could be considered as S control in future cross-breeding studies for resistance in barley.

In the literature, no consensus was found on the relationship between the DON content and the variables related to production. While Geddes *et al*. (2008) and Khatibi *et al*. (2012) reported low correlation (r= 0.36 and r= 0.44, respectively) between the DON content and the severity of fusariosis in barley, Chrpova *et al*. (2011) reported a high positive correlation between the values of visual symptoms of the disease and those of DON content (r= 0.76, $p< 0.01$) as well as those of DON content and grain weight per spike (r= 0.62; $p< 0.01$) and between those of the DON content and those of thousand grains (r= 0.71; $p< 0.01$) in that same crop.

Li *et al*. (2015) reported that transgenic wheat plants that synthesize the UDP-glucosyltransferase enzyme (HvUGT13248) from barley exhibited high resistance to spreading fusariosis in the rachis; that is, they showed type II resistance. The enzyme HvUGT13248 metabolizes the DON toxin to a less toxic compound (DON3-O-glucoside). In this study, the mechanism (s) involved in those lines classified in 2017 as R (26 lines) or MR (14 lines) to the accumulation of DON and to infection by *F. graminearum* (type I resistance) is unknown. When analyzing the responses for the IFHB index and DON accumulation of Table 4, it can be deduced that in general, as the selection cycle progressed, that is, from sublot 03 (lower selection cycle) to the Elite sublot (greater selection cycle) there were fewer lines resistant to fusariosis and more lines susceptible to the accumulation of DON.

This is because when transforming the total number of lines of each sublot in percentage, the sublots of lower selection cycle presented a higher percentage of lines classified as R. In contrast, the sublots with the highest selection cycle registered a higher percentage of lines classified as S.

Conclusions

For three years the advanced lines 30, 47, 65 and 123, and lines 73, 91 and 96, of six and two rows of grain, respectively, recorded very low symptoms of spindle fusariosis in response to inoculation with *F. graminearum*. Although the severity of the disease was not related (r= 0.5682) to the accumulation of DON, these lines registered very low or no toxin production. The information generated in this study is relevant because it indicates that these seven lines are resistant to the disease and have the potential to be used as sources of resistance in a crossbreeding program in malting barley that includes spike fusariosis in all diseases.

Acknowledgments

To CONACYT for the grant 1136066 granted to the first author during her doctoral studies. To CIMMYT; through Dr. Pawan Kumar Singh who generously shared his laboratory, materials and experience of Dr. Monica Mezzalama for her successful comments during the development of the

research and of MC Nérida Lozano Ramírez, for her support in the analysis of the DON toxin. To Dr. Aquiles Carballo Carballo of the College of Postgraduates for the facilities given in the greenhouse for the establishment of the tests of resistance evaluation.

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