



Scientific Article

Resistance of Triticale ($\times$ *Triticosecale*) varieties to *Fusarium* spp.

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ABSTRACT

Background/Objective. *Fusarium* head blight (FHB) is mainly caused by *Fusarium* spp. species, and is a disease that affects the yield and quality of cereal grain. The objective was to evaluate the resistance in adult plants of seven triticale varieties to *Fusarium boothii*, *F. camptoceras* and *F. graminearum*.

Materials and Methods. The experiment was established under greenhouse conditions at INIFAP-CEVAMEX. The pathogenicity tests were carried out during the flowering stage, by means of spot inoculation by the cotton method. The severity of the disease in the spikes was assessed at 4, 9, 14, 19 and 26 days after inoculation.

Results. The analysis of variance showed significant differences ($p \leq 0.01$) between varieties where variation in resistance was observed. The varieties Siglo TCL21 (3.1 to 6.4%) and Bicentenario TCL08 (0.8 to 6.8%) showed the lowest severities and were resistant to the three species of *Fusarium*, while Secano TCL96 was the most susceptible variety to *F. boothii*, presenting 47.6% severity; for *F. camptoceras* and *F. graminearum*, the most susceptible variety was Caborca TCL79, with severity percentages of 54.3 and 43.3%, respectively. Grain weight per spike was affected up to 90% in susceptible varieties.

Conclusion. It is important to consider in triticale breeding programs the incorporation of resistance to several *Fusarium* species, standing out in the present study Siglo TCL21 and Bicentenario TCL08.

Keywords: Severity, Blight, Spikes



INTRODUCTION

Triticale ($\times$ *Triticosecale*) is an intergeneric hybrid of wheat (*Triticum* spp.) and rye (*Secale cereale*). It was originally bred to combine the best qualities of both crops, offering an excellent alternative for regions unsuitable for wheat production (Dobrevá, *et al.*, 2016; Góral *et al.*, 2015; CIMMYT, 1976). In Mexico, triticale was planted on 24, 152 ha in 2019, producing 450, 518 t of green forage and 23, 039 t of grain. The main forage-producing states were Durango, Querétaro, and Chihuahua, while the State of Mexico led in grain production (SIAP, 2023). Although triticale is mainly grown for animal feed, recent genetic improvements have focused on enhancing grain quality for human consumption (Mcgooverin *et al.*, 2011; Mergoum *et al.*, 2009).

When first developed, triticale was considered resistant to various pests; however, the expansion of its cultivation area increased the selection pressure from harmful organisms, making this cereal more susceptible to insect pests and diseases common to rye and wheat. Among these, diseases have become one of the main constraints on triticale production, with *Fusarium* head blight (FHB) standing out as particularly significant (Dobrevá, *et al.*, 2016; Góral *et al.*, 2015).

Fusarium head blight is one of the most aggressive diseases affecting small grain cereals (Backhouse, 2014; Magliano and Chulze, 2013; Zillinsky, 1984). Severe FHB epidemics have been reported worldwide, reducing yields by up to 80% and significantly lowering grain quality (Alisaac and Mahlein, 2023). The disease decreases both grain weight and protein content, alters carbohydrate composition, and leads to contamination with trichothecenes mycotoxins mainly deoxynivalenol (DON) and nivalenol (NIV) which are harmful to human and animal health and pose a threat to food safety (Magliano and Chulze, 2013; Sobrova *et al.*, 2010; Veitch *et al.*, 2008).

Until recently, *Fusarium graminearum* (teleomorph: *Gibberella zeae* (Schwein)) was considered the main causal agent (McMullen *et al.*, 2012). However, other species may also be associated with the disease, depending on climatic conditions (temperature and relative humidity), geographic location, and the crop involved (Xu and Nicholson, 2009; Qu *et al.*, 2008). In Mexico, the predominant species are *F. graminearum* and *F. boothii*, as well as *F. culmorum*, *F. equiseti*, *F. avenaceum*, and *F. tricinctum* (Cerón-Bustamante *et al.*, 2018; Leyva-Mir *et al.*, 2017; Osman *et al.*, 2015; Ireta and Gilchrist, 1994). *Fusarium* head blight is a difficult disease to control due to its aggressive nature. Nevertheless, the use of resistant varieties is key in planning and implementing an integrated management strategy, as it is considered the most economical, effective, and sustainable control method (Gilbert and Fernando, 2004; McMullen *et al.*, 1997).

The few studies conducted on triticale report wide variation in resistance to *Fusarium* head blight. Góral and Arseniuk (2003) noted significant variation among triticale genotypes in resistance to *Fusarium*. According to Oettler and Wahle (2001) and Góral *et al.* (2021), triticale is less susceptible to *Fusarium* head blight than wheat. In a comparative study, Góral (2009) found that winter triticale lines infected with *F. culmorum* showed half the symptom severity observed in wheat lines.

Currently, there are no studies in Mexico on the levels of resistance that triticale has to *Fusarium* head blight. This underscores the need to conduct research to characterize the resistance of triticale varieties, which would also help identify new sources of resistance for use in breeding programs. The objective of this study was to evaluate, under

greenhouse conditions, the resistance levels of seven triticale varieties to infection by *F. graminearum*, *F. boothii*, and *F. camptoceras*.

MATERIALS AND METHODS

Experiment location. The experiment was conducted in 2018 and 2019 at the National Laboratory for Rusts and Other Cereal Diseases (LANAREC), part of the National Institute for Forestry, Agriculture and Livestock Research (INIFAP), at the Valle of México Experimental Station (CEVAMEX), located at 19° 29' N and 98° 54' W, at an altitude of 2,260 masl.

Varieties. Seven triticale varieties cultivated in Mexico were evaluated: Caborca TCL79, Eronga TCL83, Secano TCL96, and Impulso TCL2016, released by INIFAP; Pollmer and Siglo TCL21, developed by the International Maize and Wheat Improvement Center (CIMMYT); and Bicentenario TCL08, released by the Institute for Agricultural, Aquaculture and Forestry Research and Training (ICAMEX).

Inoculum. Three species were evaluated: *F. graminearum*, *F. boothii*, and *F. camptoceras*, provided by the Universidad Autónoma Chapingo, State of Mexico. These species were propagated through monospore cultures on carnation leaf agar (CLA) medium at the Mycology Laboratory of the Department of Agricultural Parasitology at the Universidad Autónoma Chapingo. The CLA medium consisted of 2% water agar (20 g of agar in 1 L of water) plus 10 to 12 sterile 5 mm carnation leaf pieces placed in Petri dishes. This method was used to promote the formation of *Fusarium* colonies producing macroconidia of uniform size and form (Leslie and Summerell, 2007).

Experimental design. A completely randomized design with a split-plot arrangement was used, where the main plots were the *Fusarium* species and the subplots were the triticale varieties. A total of 21 treatments were established (seven varieties × three *Fusarium* species), with three replications plus controls.

Planting and agronomic management. Under greenhouse conditions, the seven varieties were planted in seven-inch plastic pots filled with a sterilized substrate (60% soil and 40% peat moss). Five seeds were sown per pot and later thinned to retain the three most vigorous plants. A total of 12 pots were used per variety (three pots per replication). Fertilization was carried out using urea (46-00-00) and Tricel-20 (a foliar fertilizer), based on the crop's nutritional requirements.

Inoculation. Plants were inoculated at the anthesis stage (approximately 65 days after sowing). A spore suspension was prepared at a concentration of 1×10^6 conidia mL⁻¹, and the point inoculation method using the cotton technique was employed. Three previously marked spikes per pot were selected, and two 20 mg cotton tufts soaked in the spore solution were placed under the glumes at the midpoint of each spike. The inoculated spikes were then covered with glassine paper bags, following the methodology described

by Gilchrist-Saavedra *et al.* (2005) and Imathiu *et al.* (2014). The control for each genotype was inoculated with sterile distilled water using the same technique.

After the spikes were inoculated with *Fusarium* species, the plants were placed in an incubation chamber for 96 hours at an average temperature of 25 ± 10 °C, with a 12-hour photoperiod. Dew was produced for eight hours during the first 24 hours and for four hours over the following 72 hours to maintain relative humidity below 95%. The plants were then transferred to a greenhouse, where they were kept at an average temperature of 25 ± 10 °C until symptoms appeared.

Evaluations. Five evaluations of disease severity were carried out for each treatment at 4, 9, 14, 19, and 26 days after inoculation (DAI), using a visual scale for *Fusarium* head blight in triticale (Figure 1), adapted for assessing severity under greenhouse conditions. This scale was based on the visual scale for estimating *Fusarium* severity in wheat spikes proposed by Stack and McMullen (1998). Based on the average severity percentage obtained, triticale varieties were classified as: Resistant (0–10), Susceptible (20–40), and High Susceptible (41–100).

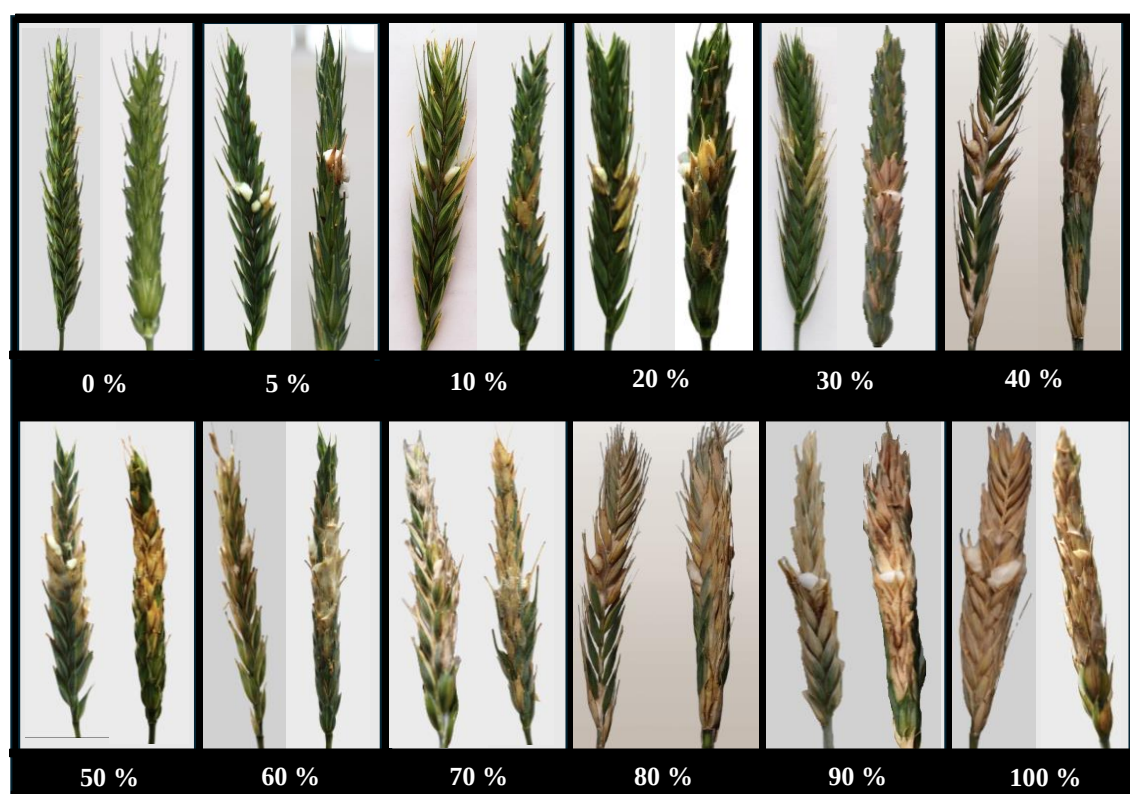


Figure 1. Visual scale for determining the percentage of *Fusarium* head blight severity in triticale spikes under greenhouse conditions.

Variables. The evaluated variables were: a) disease severity (DS), the percentage of the spike showing *Fusarium* infection; b) grain weight (GW), the weight of the grain obtained from each inoculated spike once it reached harvest maturity; and c) Area Under the

Disease Progress Curve (AUDPC), calculated from the five DS readings taken at set time intervals.

Statistical analysis. The data for the evaluated variables were statistically analyzed jointly using SAS 9.4 software (SAS, 2018), and mean comparisons for the studied varieties were performed using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Analysis of variance. The analysis of variance showed significant differences in the evaluations expressed in days after inoculation (DAI), among varieties, and in their interaction. This indicates that at least one variety exhibited a different pattern of severity across the five evaluations for each *Fusarium* species. For the variable Area Under the Disease Progress Curve (AUDPC), there were no significant differences among species; however, the sources of variation—varieties and the interaction between varieties $\times$ species—showed significant differences. This suggests that the varieties displayed different levels of disease development across the species. In contrast, for grain weight per spike (GW), significant differences were found among species, among varieties, and in the varieties $\times$ species interaction, indicating that the average GW varied both among species and among varieties.

Response of the varieties. Figure 2 shows the disease development pattern in the seven varieties over the five evaluations for the species *F. boothii*. Significant differences were found among varieties ($p < 0.0001$) in the final evaluation at 26 DAI. In other words, the severity percentage was significantly lower in the varieties Bicentenario TCL08 and Siglo TCL21, with values below 20%, compared to Secano TCL96, Impulso TCL2016, Caborca TCL79, and Eronga TCL83, which showed greater disease development.

For the species *F. camptoceras*, significant differences were found among varieties ($p < 0.0001$) (Figure 3). *Fusarium* head blight severity was significantly higher in the varieties Caborca TCL79 and Impulso TCL2016, with values exceeding 80% at 26 DAI, while Bicentenario TCL08 and Siglo TCL21 showed significantly lower severities of 13.6% and 16.7%, respectively.

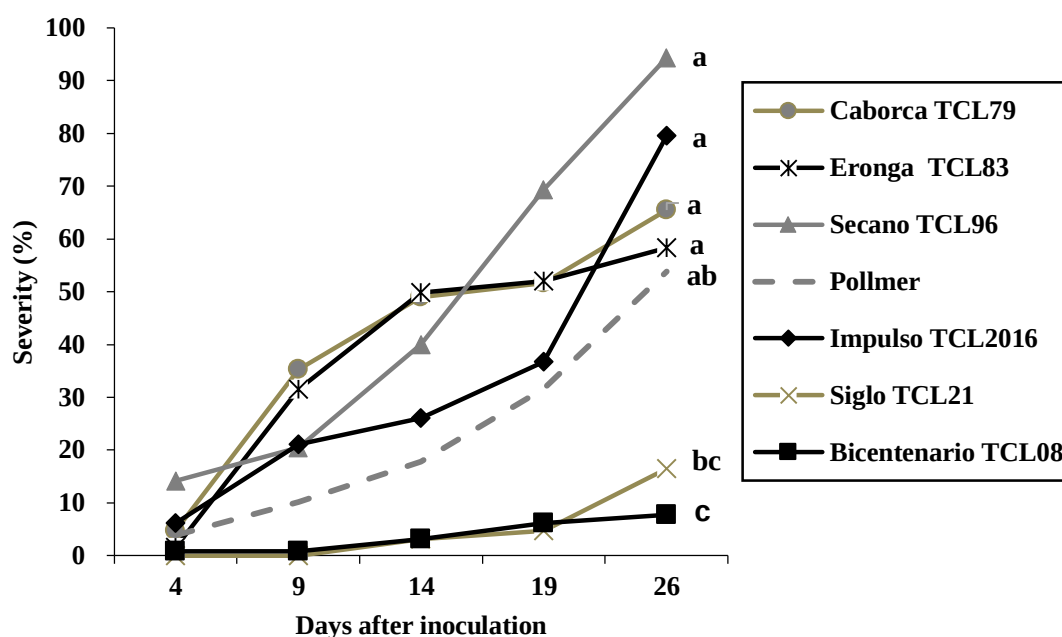


Figure 2. Performance of the varieties inoculated with *F. boothii* over the five evaluations.

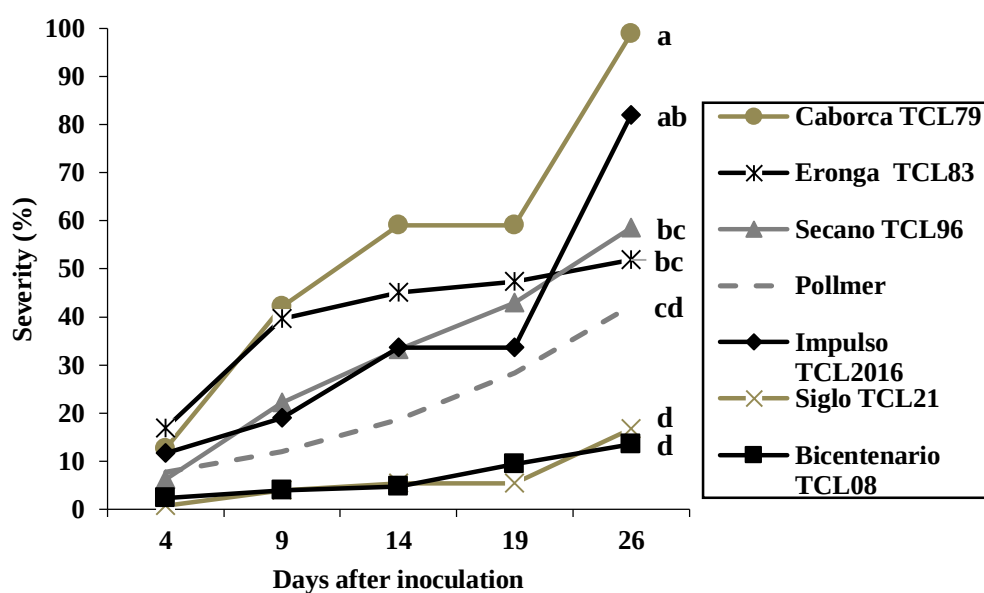


Figure 3. Performance of the varieties inoculated with *F. camptoceras* over the five evaluations.

Regarding disease severity caused by *F. graminearum*, the lowest final severity values were observed in Siglo TCL21 (1.56%) and Bicentenario TCL08 (7.78%), which were significantly different ($p < 0.0001$) from those of the other varieties. The highest severities were recorded in Pollmer (81.11%) and Caborca TCL79 (75.78%) (Figure 4).

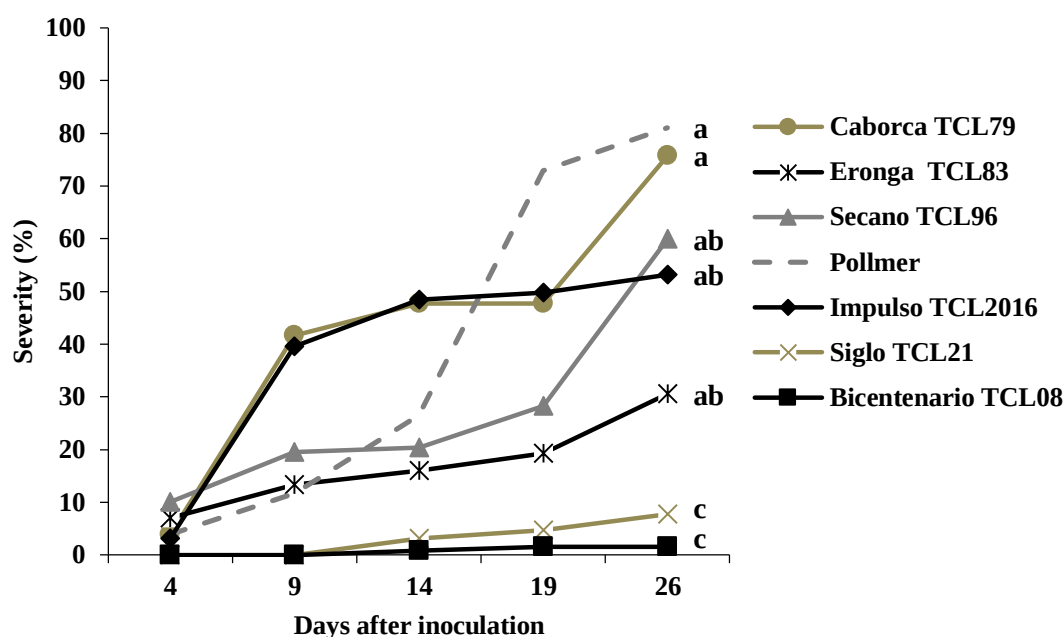


Figure 4. Performance of the varieties inoculated with *F. graminearum* over the five evaluations.

Most of the varieties began showing characteristic disease symptoms starting at four days after inoculation (DAI) with each *Fusarium* species. However, Bicentenario TCL08 began to show symptoms at 14 DAI for *F. graminearum*, and Siglo TCL21 showed delayed symptom development for both *F. boothii* and *F. graminearum*. Both varieties also exhibited severity percentages below 17% for all three *Fusarium* species. As suggested by Bai and Shaner (2004), Imathiu *et al.* (2014), and Schroeder and Christensen (1963), this behavior may be due to the fungus penetrating plant tissues without spreading through the spike, resulting in the absence of visible symptoms. In other words, *Fusarium* hyphae may have failed to invade the cells adjacent to the infection site, likely because these varieties possess genetic traits that prevented fungal progression or colonization.

Combined response of the varieties across the five evaluations. Table 1 presents the mean comparisons among varieties for the variable average severity percentage for each of the *Fusarium* species.

For *F. boothii*, significant differences were found among varieties ($p < 0.0001$). Bicentenario TCL08 (3.7%) and Siglo TCL21 (4.8%) were statistically different from Secano TCL96 (47.6%), Caborca TCL79 (41.2%), and Eronga TCL83 (38.7%), showing the lowest severity percentages. Similarly, for *F. camptoceras*, significant differences were observed among varieties ($p < 0.0001$). Siglo TCL21 and Bicentenario TCL08 had the lowest severity values, with 6.4% and 6.8%, respectively, while Caborca TCL79 showed the highest severity at 54.3%. Finally, for *F. graminearum*, significant differences were also found among varieties ($p < 0.0001$). Bicentenario TCL08 showed the lowest severity at 0.8%, and Caborca TCL79 the highest at 43.3%. The results indicated that average severity across varieties ranged from 3.7% to 47.6% for *F. boothii*, from 6.4% to 54.3% for *F. camptoceras*, and from 0.8% to 43.3% for *F. graminearum*.

Table 1. Mean comparisons of average severity (%) for each triticale variety in the combined analysis across the five evaluations for each *Fusarium* species.

Variety	<i>F. boothii</i>	<i>F. camptoceras</i>	<i>F. graminearum</i>	Reaction
Caborca TCL79	41.2 a ^z	54.3 a	43.3 a	High Susceptible
Eranga TCL83	38.7 a	40.2 b	17.2 cd	Susceptible
Secano TCL96	47.6 a	32.6 bc	27.7 bc	Susceptible
Pollmer	23.4 b	21.8 c	39.2 ab	Susceptible
Impulso TCL2016	34.0 ab	35.9 b	38.8 ab	Susceptible
Siglo TCL21	4.8 c	6.4 d	3.1 de	Resistance
Bicentenario TCL08	3.7 c	6.8 d	0.8 e	Resistance
HSD	14.04	12.17	14.42	

^zMeans within each column followed by the same letter are not significantly different (Tukey, $\alpha = 0.05$). HSD = Honestly Significant Difference.

Disease severity varied widely due to the different interactions established between the pathogen and the host. These variations were observed in the seven triticale varieties inoculated with the three *Fusarium* species, where significant differences were found in the average severity percentage ($p < 0.0001$). Notably, Siglo TCL21 and Bicentenario TCL08 consistently showed significantly lower severity values for all three fungal species, indicating resistance, while the remaining varieties were susceptible, with Caborca TCL79 being the most susceptible. This confirms the existence of a wide range of resistance levels among the evaluated varieties, in line with findings by Oettler and Wahle (2001), who reported variation in resistance to *F. culmorum* among 100 triticale genotypes despite infection pressure. Similarly, Kalih *et al.* (2014) observed broad variation in *Fusarium* head blight resistance in dwarf triticale lines, explained by the presence of QTLs that confer resistance despite the presence of the *Ddw1* gene, which reduces plant height. Góral and Arseniuk (2003) also found significant variation in resistance levels to *Fusarium* head blight among parental triticale genotypes.

Area Under the Disease Progress Curve (AUDPC). For the AUDPC variable, significant differences were found among varieties for all three species ($p < 0.0001$) (Table 2). The varieties Siglo TCL21 and Bicentenario TCL08 were statistically different from the others, showing the lowest disease development values for *F. boothii*, *F. camptoceras*, and *F. graminearum*. In contrast, Secano TCL96 showed the highest disease development for *F. boothii*, with an area of 1111.9 units (u), while for *F. camptoceras* and *F. graminearum*, the highest values were observed in Caborca TCL79, with 1262.2 u and 1015.4 u, respectively. Among the varieties, AUDPC ranged from 87.5 u to 1111.9 u for *F. boothii*, from 141.2 u to 1262.2 u for *F. camptoceras*, and from 18.7 u to 1015.4 u for *F. graminearum*.

Table 2. Mean comparisons of the Area Under the Disease Progress Curve (AUDPC) for each triticale variety in the combined analysis across the five evaluations for each *Fusarium* species.

Variety	<i>F. boothii</i>	<i>F. camptoceras</i>	<i>F. graminearum</i>
Caborca TCL79	981.7 a ^z	1262.2 a	1015.4 a
Eronga TCL83	930.4 a	964.9 ab	401.1 ab
Secano TCL96	1111.9 a	766.9 ab	624.9 ab
Pollmer	535.2 ab	506.5 bc	929.8 a
Impulso TCL2016	763.2 ab	803.2 ab	938.9 a
Siglo TCL21	101.1 b	141.2 c	70.8 b
Bicentenario TCL08	87.5 b	156.7 c	18.7 b
HSD	697.33	545.18	688.48

^zMeans within each column followed by the same letter are not significantly different (Tukey, $\alpha = 0.05$). HSD = Honestly Significant Difference.

Grain weight per spike. Table 3 shows the average grain weight per spike for the evaluated varieties under each fungal species and the control. A variability in grain weight per spike was observed among the triticale varieties in response to the different *Fusarium* species.

Table 3. Mean comparisons of grain weight (grams) per spike for each triticale variety across the five evaluations.

Variety	<i>F. boothii</i>	<i>F. camptoceras</i>	<i>F. graminearum</i>	Control
Caborca TCL79	0.22 bc ^z	0.23 a	0.19 b	0.65 c
Eronga TCL83	0.36 abc	0.50 a	0.58 ab	0.78 c
Secano TCL86	0.13 c	0.36 a	0.21 b	1.34 ab
Pollmer	0.42 abc	0.57 a	0.37 ab	0.75 c
Impulso TCL2016	0.59 ab	0.42 a	0.50 ab	1.36 ab
Siglo TCL21	0.73 a	0.55 a	0.56 ab	1.44 a
Bicentenario TCL08	0.61 ab	0.60 a	0.70 a	0.98 bc
HSD	0.41	0.39	0.44	0.44

^zMeans within each column followed by the same letter are not significantly different (Tukey, $\alpha = 0.05$). HSD = Honestly Significant Difference.

For *F. boothii*, significant differences were found among varieties ($p < 0.0002$), with Siglo TCL21 showing the highest grain weight per spike at 0.73 g. In contrast, for *F. camptoceras*, there were no statistically significant differences among varieties ($p < 0.0652$), indicating that the damage to grain was similar across varieties for this species. For *F. graminearum*, significant differences were observed ($p < 0.0046$), with Bicentenario TCL08 showing a significantly higher grain weight (0.7 g) compared to Caborca TCL79 and Secano TCL96.

All evaluated varieties showed lower grain weight per spike when inoculated with *F. boothii*, *F. camptoceras*, and *F. graminearum* compared to the control. This reduction was due to damage caused by *Fusarium*, which affected the pericarp, induced structural changes in the endosperm, and altered starch morphology, resulting in deformed and lightweight grains, as reported by Packa *et al.* (2008).

In this study, the variety Bicentenario TCL08 showed resistance and recorded the highest grain weight, while Secano TCL96 was the most susceptible, with grain weight reduced by up to 90% (Table 3). In contrast, Oettler and Wahle (2001) reported that infection by *F. culmorum* caused a 48% reduction in average grain weight per spike in triticale genotypes. Although grain can still be harvested from plants affected by *Fusarium* head blight, it is essential to assess the levels of mycotoxins present in the damaged grain. In a study by Góral *et al.* (2016), it was found that triticale grain can accumulate higher levels of trichothecene mycotoxins than wheat. For this reason, it is important to avoid planting genotypes that show high disease severity and high accumulation of these mycotoxins.

Although few studies have examined resistance to *Fusarium* head blight in triticale compared to other cereals, triticale may be as resistant as wheat and less resistant than rye, as noted by Gaikpa *et al.* (2019), Góral *et al.* (2016), Góral (2009), Miedaner *et al.* (2004), and Miedaner *et al.* (2001). However, since there is no research in Mexico on the levels of resistance that triticale varieties have to *Fusarium* head blight; this study identified moderate variation in resistance levels among varieties. This information can be useful for triticale breeding programs in Mexico aimed at developing new varieties with resistance to this disease.

CONCLUSIONS

The varieties Siglo TCL21 (3.1 to 6.4%) and Bicentenario TCL08 (0.8 to 6.8%) showed the lowest severities of *Fusarium* head blight caused by *Fusarium boothii*, *F. camptoceras*, and *F. graminearum*, and were considered resistant. The most susceptible variety to *F. boothii* was Secano TCL96 (47.6%), while for *F. camptoceras* (54.3%) and *F. graminearum* (43.3%) it was Caborca TCL79. Grain weight per spike was reduced by up to 90% in the case of Secano TCL96, which was considered susceptible to the disease. It is important for cereal breeding programs to incorporate resistance to different *Fusarium* species in triticale cultivation in Mexico.

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