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Research Article

Evaluation of wheat genotypes resistance to Fusarium head blight in Paraguay[1]

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ABSTRACT

Fusarium head blight (FHB), or scab, caused by a *Fusarium* spp. complex, is an important wheat disease in Paraguay. Among the strategies used to control it, the genetic resistance is considered highly efficient and cost effective. This study aimed to evaluate and compare the effects of *F. graminearum* on six wheat genotypes, including two comparison varieties, in two seeding dates. The genotypes were artificially inoculated in the spike, at the flowering stage, by injecting a pool of four pathogenic *F. graminearum* isolates. The FHB development was evaluated by scoring the disease incidence and severity, percentage of diseased spikelets and damaged kernels, as well as using the area under the disease progress curve. Besides the kernel infection, its impact on the development of mycotoxins (deoxynivalenol) and interactions with the genotypes were also evaluated. The results identified an advanced breeding line (Lin 84) with a resistance level to FHB comparable to that of the universally known resistance sources (Sumai 3 and Frontana). The other three genotypes (Caninde 11, Caninde 12 and Caninde 21), in spite of presenting a higher grain yield potential, were evaluated as moderately susceptible to susceptible. These results suggest that, although it is possible to transfer the FHB resistance to a higher agronomic type, combining such resistance with a higher grain yield potential remains an ongoing challenge.

KEYWORDS: *Fusarium graminearum*, *Triticum aestivum*, genetic resistance, scab.

RESUMO

Avaliação da resistência de genótipos de trigo à fusariose no Paraguai

A fusariose do trigo, ou giberela, causada por um complexo de *Fusarium* spp., é uma importante doença no Paraguai. Dentre as estratégias utilizadas para o seu controle, a resistência genética é considerada altamente eficiente e de baixo custo. Objetivou-se avaliar e comparar o efeito de *F. graminearum* em seis genótipos de trigo, incluindo duas variedades testemunha, em duas datas de semeadura. Os genótipos foram inoculados artificialmente na espiga, por meio da injeção de um pool de quatro isolados patogênicos de *F. graminearum*, na fase de floração. O desenvolvimento da fusariose foi avaliado pela pontuação da incidência e severidade da doença, porcentagem de espiguetas doentes e grãos danificados, bem como pela área sob a curva de progresso da doença. Além da infecção de grãos, foi determinado o seu impacto no desenvolvimento de micotoxinas (deoxinivalenol) e sua interação com os genótipos. Os resultados identificaram uma linhagem avançada (Lin 84), com nível de resistência à fusariose comparável ao das fontes universalmente conhecidas de resistência (Sumai 3 e Frontana). Os outros três genótipos (Caninde 11, Caninde 12 e Caninde 21), apesar de apresentarem maior potencial de rendimento de grãos, foram avaliados como moderadamente suscetíveis a suscetíveis. Esses resultados sugerem que, embora seja possível transferir a resistência da fusariose do trigo para um tipo agronômico melhorado, combinar a resistência com um maior potencial de rendimento de grãos continua sendo um desafio contínuo.

PALAVRAS-CHAVE: *Fusarium graminearum*, *Triticum aestivum*, resistência genética, giberela.

INTRODUCTION

The wheat production generates an immense economic impact worldwide, being one of the most important cereals. In South America, it constitutes the principal extensive winter crop in Argentina, Brazil, Bolivia, Chile, Paraguay and Uruguay (IICA 2010, FAO 2017).

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The wheat disease Fusarium head blight (FHB) is caused by a species complex of the *Fusarium* spp. genus and, in South America, *Fusarium graminearum* (teleomorph *Giberella zeae*) and *Fusarium culmorum* (teleomorph unknown) are the prevalent species (Arrúa et al. 2015). Fusarium head blight infection is largely dependent on environmental conditions, with optimal temperatures of 20-30 ºC and humidity of 80 %, for periods of 48-60 hours during the anthesis, which is the most vulnerable period of the crop (Siou et al. 2014, Reis et al. 2016).

Besides its severe impact on grain yield and quality, the fungus produces secondary metabolites (mycotoxins), including deoxynivalenol (FDA 2010, Kohli & Díaz de Ackermann 2013). The disease control must include multi-faceted strategies addressing genetic resistance, proper chemical application and efficient growing practices. Cultural practices primarily involve crop rotation and incorporating crop residues, in order to reduce the field inoculum load. While methods using preventive chemical control are prevalent, their efficacy has not been fully documented, and the use of resistant cultivars constitutes the most effective strategy to reduce the impact of the disease (Mazzilli et al. 2007, Díaz de Ackemann & Kohli 2013, Reis & Carmona 2013).

Schroeder & Christensen (1963) defined three types of genetic resistance against FHB. Type I is resistant to initial infection and evaluated based on disease incidence in the presence of natural inoculum or by forced-artifice spray infection (Miedaner et al. 2003). Type II refers to resistance against the spread of spike infection (e.g., test by point injections). The third type is related to the accumulation of mycotoxins, as proposed by Mesterházy (1995). It is well documented that all types of FHB resistance are complex, being based on multiple genes, with small effects.

In South America, the resistance sources used for wheat improvement include: Catbird, Pampeano, CEP 75203, Pel73101, Chum/Seri, Pel74142, Frontana, PF7815, Kvz/3/Tob/Cfen//Bb/4/Blo/F35.70/Mo/Nac/6/Bow, WRM/Ptm//Coc/Ning 68026 and Kvz-K 4500 L.A.4 OC 813 (Bainotti et al. 2013).

From China, the Sumai 3 cultivar has been reported as a source of Type I resistance to FHB. Catbird, another advanced breeding line, developed by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), and derived from a Chuan Mai#18/Bagula cross, is a source for Type II resistance, located on chromosome 7D (Cativelli et al. 2013). Catbird and Sumai 3 are considered to be stable sources of resistance to FHB, and have been widely used in breeding programs in South America and throughout the world (Kohli & Díaz de Ackermann 2013). Another variation frequently used in South America is the Frontana cultivar, from Brazil. It presents Type I resistance and is useful for comparisons with candidates for this type of resistance (Alves et al. 2013a).

Crop breeding programs must identify genotypes that produce higher yields and grain quality, while simultaneously reducing damages caused by diseases such as FHB (Pereyra & Lori 2013, Steiner et al. 2017). The incidence and severity of a disease may help to characterize its phenotypes (Engle et al. 2003, Sharan et al. 2004). The evaluation of relative severity over time allows calculating the area under the disease progress curve (AUDPC), and provides a better phenotyping (Siou et al. 2014). Considering its importance to the wheat production in Paraguay, this study aimed to evaluate the resistance to FHB in certain advanced wheat genotypes.

MATERIAL AND METHODS

Six wheat genotypes, presenting different reactions to Fusarium head blight (FHB), were obtained from the Instituto Paraguayo de Tecnología Agraria (IPTA) (Table 1). These were seeded on two seeding dates (May and June 2016), at the Centro Multidisciplinario de Investigaciones Tecnológicas - Universidad Nacional de Asunción (CEMIT-UNA), San Lorenzo, Paraguay. Seeding on two dates was done to expand the period of testing and observations.

Seeding was carried out using a randomized blocks design (plots of 1 x 0.5 m^2 each), with six replications per group (inoculated and a non-inoculated control), using the following varieties: Sumai 3, Frontana (resistant), Caninde 12 and Caninde 11 (susceptible). A substrate composed of a mixture of peat and gravel, at a ratio of 3:1, was used to plant the seeds, and urea (46 %) was used in two phases (80 kg ha^{-1} at tilling and 40 kg ha^{-1} at stem elongation). A standard chemical protection was applied in accordance with general recommendations for wheat crops before the anthesis (Kohli et al. 2012).

The Fusarium strains used for this study were previously isolated from wheat and identified microbiologically, using the Leslie & Summerell (2008) code, as well as a species specific marker,

Table 1. Wheat genotypes used to assess the resistance to Fusarium head blight (FHB).

Reference	Genotype	FHB reaction
Can 12	Caninde 12	Susceptible
Can 21	Caninde 21	Unknown
Lin 84	Ng8675/Cbrd//Sna5/Weaver/3/Itapua70	Unknown
Sum 3	Sumai 3	Resistant - Type II
Can11	Caninde 11	Moderately susceptible
Front	Frontana	Resistant - Type I

Fgram - *F. graminearum* (Kim et al. 2003, Gómez 2008). A partial sequencing of the elongation factor-1 alpha (EF-1 α) and beta-tubulin genes was performed. The sequences were deposited at GenBank (MK507898; MN416025; MN416024; MN416026).

The presence of trichothecene synthesis pathway genes, related to the production of deoxynivalenol, was determined by Tri5 marker (Doohan et al. 1999, Watson & Wang 2012). Upon verification of their aggressiveness, four mono-spore isolates were selected to evaluate the wheat genotypes (Table 2).

Fungal isolates were cultivated in a modified-CLA medium and incubated for 10 days, at a temperature of 22 ± 5 ºC, under continuous illumination (Cazal et al. 2014). Afterwards, the plates were scraped with 10 mL of sterile distilled water with 0.01 % Tween 20, and briefly shaken. Conidial suspension for each isolate was adjusted to 6×10^4 conidia mL^{-1}, and then mixed to obtain the isolate pool used for artificial inoculation (Mazzoni & Peixoto 2016).

Artificial spike inoculation with pathogenic isolates (Table 2) was performed by injecting a macro-conidial suspension (Engle et al. 2003) at flowering (61 to 65 anthesis) (Lancashire et al. 1991). The inoculation method involved micro-pipetting 1 mL of suspension onto the central spikelets, which were kept in a polythene bag for 24 h. The relative humidity of the chamber was maintained at around 80 %, with spray irrigation. Each spike was taken as an experimental unit and all spikes (inoculated and non-inoculated controls) were evaluated.

Table 2. *Fusarium graminearum* isolates selected for artificial infection.

Strain*	Local	Cultivar
c-395	Capitán Miranda - Itapúa	Catbird
c-150	Capitán Miranda - Itapúa	Caninde 12
c-51	Alto Paraná	Unknown
c-110	Capitán Miranda - Itapúa	Itapúa 40

* The isolates form part of the microorganism cultures collection of the Universidad Nacional de Asunción.

The FHB incidence was categorized by the presence or absence of the disease, and severity was calculated for three weeks (22 days) (Stack & McMullen 2011). The percentage of diseased spikelets was calculated as the number of *Fusarium*-diseased spikelets over the total number of spikelets, in each spike considered. The percentage of *Fusarium*-damaged kernels was calculated as the number of damaged kernels over the total number of kernels on each spike. The area under the disease progress curve (AUDPC) was used to combine multiple observations from four data points (8, 15, 22 and 30 days after infection) into a single value (Malbrán et al. 2012, Simko & Piepho 2012).

The empty grain percentage and 1,000-grain weight were considered yield components (Velazquez & Formento 2012). The mycotoxin content (deoxynivalenol) was evaluated using immunofluorescence, on a Vertu lateral flow reader (Vicam 2011). For the detection of deoxynivalenol, a sub-sample of 5 g was used, derived from the total sample of blocks per treatment, which were previously homogenized. The experiment was performed in triplicate.

Using multivariate analysis, the infection responses (FHB effects) of the Lin 84 and Caninde 21 genotypes were compared to both resistant and susceptible reference genotypes. The data set was generated from 78 Lin 84 observations and 131 Caninde 21 observations. For quantitative analysis, the FHB severity and AUDPC variables were used. Comparisons were made using non-parametric multivariate analysis of variance NP-Manova (Anderson 2001), with 9,999 permutations and Euclidean distance differences.

The normality tests used the Shapiro-Wilk analysis, and the variance homogeneity was verified using the Levene Test. Statistical conclusions were based on Kruskal-Wallis tests, when the normality assumption was not fulfilled. In case of normality, Anova supported by the Tukey test was conducted, and differences were considered statistically significant at a value of $p < 0.05$. All statistical analyses were performed with the Excel 2007 software (Microsoft Excel), Past 3 (Hammer et al. 2001), Infostat and R packages (e.g., ggplot2 and agricolae).

RESULTS AND DISCUSSION

Considering the wide adaptation of the genotypes under study to warmer growing conditions in Paraguay, differing numbers of spikes were evaluated for each cultivar. A total of 731 spikes were evaluated (112 for Caninde 11; 143 for Caninde 12; 138 for Caninde 21; 79 for Lin 84; 106 for Sumai 3; and 153 for Frontana).

Frontana and Sumai 3, known worldwide for their resistance, demonstrated the lowest FHB incidence rates [respectively 7.2 % and 2.9 % for the first seeding date (May), and respectively 39.8 % and 30.9 % for the second seeding date (June)]. The findings are consistent with those reported by other authors who observed that Frontana is considered a source of resistance to the initial infection progress (Bainotti et al. 2013), and thus slower the infection development (Alves et al. 2013b). In both cases, the FHB infection and its development were highly dependent on the local environmental conditions, which, in this case, were ideal for the second seeding date in June. However, Caninde 11 (moderately susceptible) and Caninde 12 (susceptible) demonstrated higher disease incidences for both seeding dates (Figure 1). In other words, while less than ideal environmental conditions are enough to promote higher FHB infection rates in susceptible germplasm, they are not sufficient to afford observational differences between moderately resistant and resistant genotypes.

Lin 84 and Caninde 21 were the new genotypes included in the study. Lin 84 presented disease incidences of 12.7 % and 33.3 %, respectively for the first and second seeding dates, and was classified as a moderately resistant genotype; while Caninde 21 presented 30.4 % and 71.5 %, respectively for the first and second seeding dates, and was considered a susceptible genotype.

The differences in the FHB incidence in the different genotypes and over the different seeding dates were supported by Bayes Factor tests. Generalized linear modeling, employing logistic regression, was applied to explain and predict the best model for incidence determinations. Taking into account the Akaike information criterion, the "genotype * climate" model explains the incidence for the genotypes in this study, where the incidence varies together with the tested genotype and climatic conditions (Table 3).

Table 3. Generalized linear model for factors affecting the Fusarium head blight incidence.

Generalized linear model	AIC	ΔAIC
Genotype + climate + genotype * climate	1,343.7	0.0
Genotype * climate	1,343.7	0.0
Genotype + climate	1,345.9	2.2
Genotype	1,490.7	147.0
Climate	1,498.8	155.1
1 (null model)	1,640.3	296.6

AIC: Akaike information criterion.

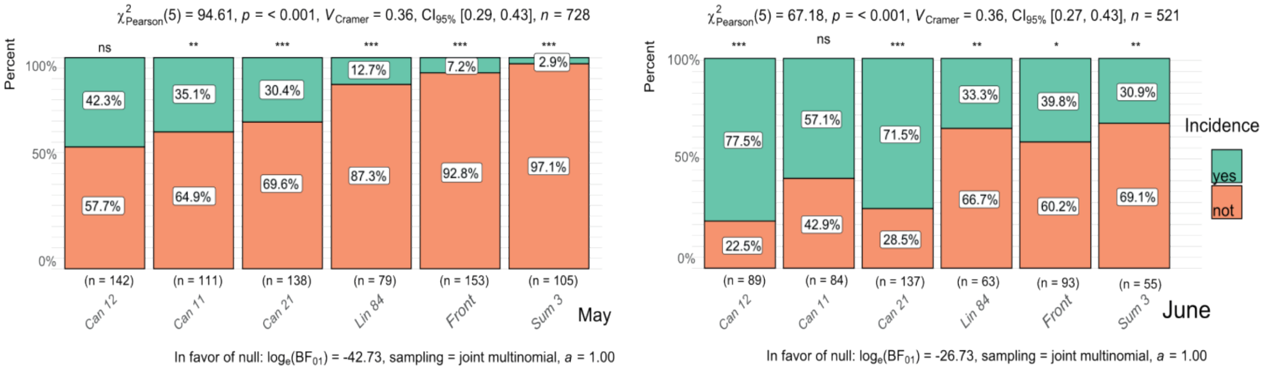

Figure 1. Fusarium head blight incidence on six wheat genotypes, for two seeding dates.

Our results also confirmed the lower FHB severity observed in the resistant cultivars Sumai 3 and Frontana (Table 4). The mycotoxin content analysis revealed cultivars below 1 ppm (Table 4) for deoxynivalenol. This low concentration of mycotoxin for these genotypes may be explained by the presence of Qfhs.ndsu-3BS, encoding a glucosyl transferase which is effective only against *Fusarium* strains that produce deoxynivalenol or structurally similar trichothecenes (Lemmens et al. 2005).

While Sumai 3 has been reported by some researchers as carrying a Type II resistance (Ittu et al. 2005, Niwa et al. 2014, Lahlali et al. 2016), its variable deoxynivalenol levels are attributed to its quantitative trait locus QTL aforementioned, which tends to accumulate different deoxynivalenol levels (Zhou et al. 2002, Dweba et al. 2017), thus confirming the general complexity of the FHB response. Our results revealed that Lin 84 also presents a Type II resistance, as described by Mesterházy (1995), and, in spite of the fact that it presented marginally infection levels higher than the resistant comparatives (Figure 1), it accumulated a lower deoxynivalenol concentration (Table 3).

On the other hand, the national varieties Caninde 11, Caninde 12 and Caninde 21 were all susceptible, revealing FHB severity values between 7.5 % and 12.7 %, and deoxynivalenol concentrations between 2.83 ppm and 4.14 ppm. In spite of the relatively low FHB infection rates under field conditions over the years, in this study, the new cultivar Caninde 21 was found to be moderately susceptible, as verified by the higher deoxynivalenol concentration (Table 3). Caninde 12 was identified as the most susceptible cultivar, presenting the highest severity for FHB and deoxynivalenol concentration.

It is also interesting to observe the progress of the disease over the 30-day period after infection on the seeding dates (Figure 2). The data shows that, for both the seeding dates, Frontana, Sumai 3 and Lin 84 presented similar median values for AUDPC, being well below those for Caninde 11, Caninde 12 and Caninde 21.

Table 4. Comparison of genotypes for Fusarium head blight (FHB) severity, percentage of *Fusarium*-diseased spikelets (FDS) and deoxynivalenol (DON) content.

Reference	FHB severity (%)	FDS (%)	DON (ppm)
Sum 3[r]	0.91 b*	1.19 a	0.65 b
Lin 84[u]	2.13 b	0.65 a	0.90 b
Front[r]	2.53 b	1.96 a	0.46 b
Can 21[u]	7.50 ab	2.04 a	3.09 a
Can 11[s]	8.24 ab	1.14 a	2.83 a
Can 12[s]	12.73 a	2.61 a	4.14 a

* Means compared by the Tukey test (α = 0.05). Means with a common letter are not significantly different (p > 0.05). [r] resistant; [s] susceptible; [u] unknown.

In both the seeding periods, Frontana presented low incidences, thus showing a difficulty for initial infection or Type I resistance (Figure 1). However, once the fungus overcomes the mechanical barrier of the spike, the disease progresses very quickly, as shown by the higher AUDPC values, in comparison to Sumai 3 and Lin 84 (Figure 2).

The disease severity, as evaluated by the incidence and progress of the disease, was low for Sumai 3 (Figures 1 and 2). This is attributed to its Type II resistance (ability to slow the infection progress) reported in other studies (Kubo et al. 2013, Niwa et al. 2014). Our study partially supports the results obtained by Mendes et al. (2018), who reported the lowest AUDPC in the resistant genotypes (BRS, Parrudo and Frontana), as compared to susceptible genotypes (Figure 2). In this study, the AUDPC for Frontana was lower than the susceptible genotypes for the first seeding date; but, for the second seeding date (ideal conditions for the disease development), its susceptibility was comparable to the already susceptible genotypes.

A significant finding of this study is the identification of Lin84, an advanced breeding line, which did not present a level of FHB severity lower than that for the susceptible comparisons (Table 3), but did present a significantly lower disease development over the period of 30 days post infection (Figure 2). Significantly, besides being comparable to the resistant genotypes Sumai 3 and Frontana, it also accumulates comparatively lower deoxynivalenol concentrations. Such a relationship was mentioned by Lemmens et al. (2005), who reported an association between disease progress (Type II resistance) and lower deoxynivalenol concentration. Our results confirmed a higher value of AUDPC in all the susceptible varieties (Caninde 11, Caninde 12 and Caninde 21); although the Caninde 11 values were markedly lower than the other two for both seeding dates.

The correlation analysis for the various infection parameters and deoxynivalenol concentration revealed some interesting results (Table 5). The susceptible genotypes (Caninde 11, Caninde 12 and Caninde 21) presented a high positive correlation with the deoxynivalenol concentration for all phenotypic

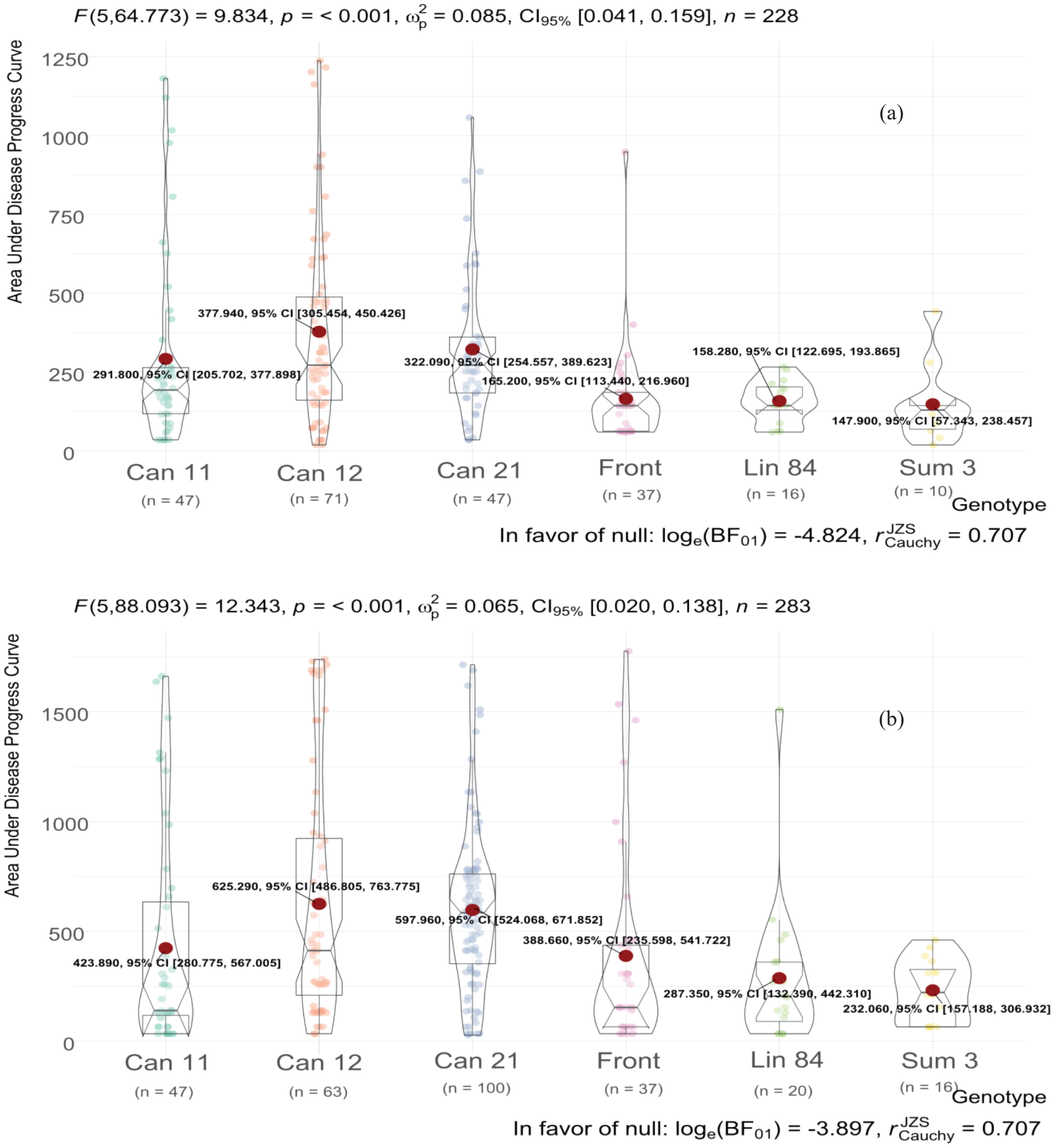

Figure 2. Area under the disease progress curve in wheat genotypes, for two seeding dates: May (a) and June (b).

Table 5. Correlation between Fusarium head blight infection variables and deoxynivalenol content, for resistant and susceptible genotypes.

Resistant\Susceptible	DON	AUDPC	FHB severity	FDK
DON	-	0.941**	0.880*	0.880*
AUDPC	0.698	-	0.935**	0.935**
FHB severity	0.759*	0.935**	-	1.000**
FDK	0.698	1.000**	0.935**	-

** Significant correlation at the 0.01 level. * Significant correlation at the 0.05 level. DON: deoxynivalenol; AUDPC: area under the disease progress curve; FHB: Fusarium head blight; FDK: *Fusarium*-damaged kernels.

variables (disease severity, AUDPC and *Fusarium*-infected kernels). The same was not true for the resistant genotypes (Frontana, Sumai 3 and Lin 84), with a positive correlation observed between the deoxynivalenol content and the FHB severity ($r = 0.759$; $p < 0.05$), but not for AUDPC or for the percentage of diseased kernels. In the susceptible genotypes, a high positive correlation between the deoxynivalenol concentration and the AUDPC ($r = 0.941$; $p < 0.01$) was observed for FHB severity and *Fusarium*-damaged kernels ($r = 0.880$; $p < 0.05$, for both cases) (Table 5).

These results support Spanic et al. (2019), who observed positive correlations between the AUDPC and deoxynivalenol concentration in inoculated wheat grains (r = 0.91; p < 0.01); and also Mesterházy et al. (2005), who observed highly significant correlations among FHB, *Fusarium*-damaged kernels, yield loss and deoxynivalenol contamination.

It should be mentioned that all genotypes under study presented positive and significant correlations between FHB severity and *Fusarium*-damaged kernels, for resistant (r = 0.935; p < 0.01) and susceptible (r = 1; p < 0.01) genotypes. This was also reported by Hernandez (2010), who observed similar correlations for winter wheat.

The FHB impact on grain yield (by genotype) was assessed by comparing the average number of spikes infected with *Fusarium* with each respective control group, for the percentage of empty grains and 1,000-grain weight (Figure 3).

In both cases, significant statistical differences were observed, confirming variable degrees of yield loss in the artificially inoculated genotypes. Sumai 3 presented a significant difference for 1,000-grain weight values, as compared to its control plot, while no differences were observed for Frontana and Lin 84 (Figure 3a). On the other hand, Caninde 12 and Caninde 21 presented significant differences between their inoculated spikes and control plots, for empty grain percentages. Similar effects were also observed for the resistant genotype Sumai 3 (Figure 3b). Although these results confirm the impact of FHB on grain yield and its components, the extent of variability present among the genotypes is an indicative of the difficulty in using a single parameter to identify resistant genotypes under development.

An additional consideration is that, under field conditions, genotypes behave differently. Caninde 11 and Caninde 12 demonstrated a better agronomic performance and higher grain yield potential, corroborated by systematic surveys conducted by the Instituto Paraguayo de Tecnologia Agraria (IPTA) (data not shown here). Similar results were reported in

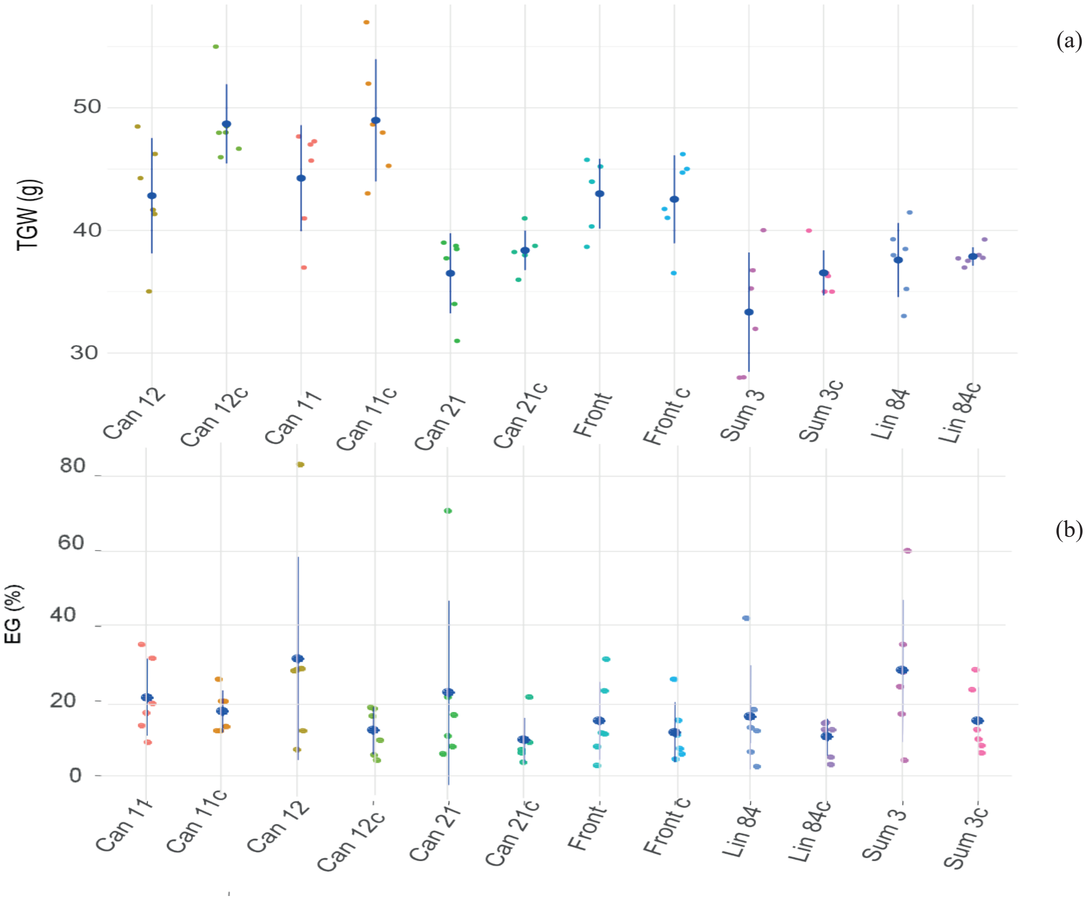

Figure 3. Results for 1,000-grain weight (TGW) of infected and non-infected wheat spikes, evaluated in six genotypes (a); and percentage of empty grains (EG) in inoculated and non-inoculated control wheat spike genotypes (b).

Table 6. Comparison between wheat genotypes with known and unknown Fusarium head blight reactions, using NP-Manova.

Comparison	F	p-value
All	23.25	0.0001
R *vs.* S	54.62	0.0006
R *vs.* Lin 84	0.02	1.0000
R *vs.* Can 21	30.24	0.0006
S *vs.* Lin 84	18.46	0.0012
S *vs.* Can 21	3.47	0.3702
Can 21 *vs.* Lin 84	11.75	0.0024

R: resistant genotype; S: susceptible genotype.

Argentina, where the Biointa-2004 cultivar presented high levels of FHB infection, diseased kernels and deoxynivalenol content, without depressed yields (Bainotti & Donaire 2015). The Bohemia variety, in spite of its lower expression of FHB and relatively higher grain yield, accumulated higher concentrations of deoxynivalenol (Chrpová et al. 2010).

Finally, to classify two new wheat lines (Lin 84 and Caninde 21) for their reactions to FHB, NP-Manova was used to compare their infections with the previously known resistant and susceptible genotypes in the study (Table 6). The analyses revealed that the FHB reaction of Lin 84 was not significantly different from the resistant genotype, while the performance of Caninde 21 was similar to the known susceptible genotype. Thus, they were respectively classified as resistant and susceptible to FHB.

CONCLUSIONS

1. It was possible to identify a new breeding line (Lin 84) as a source of Type II resistance against the Fusarium head blight (FHB), in a superiorly adapted agronomic plant type. The high yield potential Caninde 21 was classified as susceptible;
2. The controlled artificial inoculation of newly developed wheat genotypes is an effective tool for classifying the resistance or susceptibility to FHB. Yet, no single parameter, such as disease incidence or severity, may adequately guarantee the results. A combination of various characters, such as disease severity, rate of disease progress over time (area under the disease progress curve), infected or empty grain percentages, and, most importantly, mycotoxin (deoxynivalenol) buildup concentration are essential to categorize genotypes with unknown potential for FHB resistance.

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REFERENCES

ALVES, R.; NORA, T.; FRANCO, F.; COSTA, A.; STANGARLIN, J. Reação de resistência tipo I e tipo II a giberela em cultivares de trigo. *Summa Phytopathologica*, v. 39, n. 3, p. 167-171, 2013b.

ALVES, R.; NORA, T.; FRANCO, F.; COSTA, A.; STANGARLIN, J. Type-I resistance reaction to FHB in wheat cultivars. *Summa Phytopathologica*, v. 39, n. 2, p. 97-101, 2013a.

ANDERSON, M. J. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, v. 26, n. 1, p. 32-46, 2001.

ARRÚA, A. Avances en la investigación de la fusariosis de la espiga en Paraguay. *In*: SEMINARIO NACIONAL DE TRIGO "DEL GRANO AL PAN", 5., 2015, Asunción. *Anales*... Asunción: CAPECO/INBIO, 2015. p. 139-150.

BAINOTTI, C.; ALBERIONE, E.; LEWIS, S.; CATIVELLI, M.; NISI, M.; LOMBARDO, L.; VANZETTI, L.; HELGUERA, M. Genetic resistance to Fusarium head blight in wheat (*Triticum aestivum* L.): current status in Argentina. *In*: MAGLIANO, T.; CHULZE, S. (ed.). *Fusarium head blight in Latin America.* Dordrecht: Springer, 2013. p. 231-240.

BAINOTTI, C.; DONAIRE, G. Incidencia de fusariosis de la espiga en cultivares de trigo pan durante 2012-13 en Marcos Juárez (Córdoba - Argentina). *Revista FAVE-Ciencias Agrarias*, v. 14, n. 2, p. 1-13, 2015.

CATIVELLI, M.; LEWIS, S.; APPENDINO, M. Fusarium head blight resistance quantitative trait locus on chromosome 7D of the spring wheat cultivar Catbird. *Crop Science*, v. 53, n. 4, p. 1464-1471, 2013.

CAZAL, C.; ARRÚA, A.; MENDES, J.; DUJAK, C.; OVIEDO, R.; KOHLI, M. Comparación del efecto de dos medios de cultivos de origen natural sobre la esporulación de especies de *Fusarium graminearum*. *In*: CONGRESO

NACIONAL DE CIENCIAS AGRARIAS, 3., 2014, San Lorenzo. *Anales...* San Lorenzo: FCA-UNA, 2014. p. 498-499.

CHRPOVÁ, J.; ŠÍP, V.; ŠTOČKOVÁ, L.; MILEC, Z.; BOBKOVÁ, L. Resistance of winter wheat varieties registered in the Czech Republic to Fusarium head blight in relation to the presence of specific Rht alleles. *Czech Journal of Genetics and Plant Breeding*, v. 46, n. 3, p. 122-134, 2010.

DÍAZ de ACKERMANN, M.; KOHLI, M. Chemical control of Fusarium head blight of wheat. *In*: MAGLIANO, T.; CHULZE, S. (ed.). *Fusarium head blight in Latin America*. Dordrecht: Springer Science & Business Media, 2013. p. 175-190.

DOOHAN, F. M.; WESTON, G.; REZANOOR, H. N.; PARRY, D. W. Development and use of a reverse transcription-PCR assay to study expression of Tri5 by fusarium species *in vitro* and *in planta*. *Applied and Environmental Microbiology*, v. 65, n. 9, p. 3850-3854, 1999.

DWEBA, C. C.; FIGLAN, S.; SHIMELIS, H. A.; MOTAUNG, T. E.; SYDENHAM, S.; MWADZINGENI, L.; TSILO, T. J. Fusarium head blight of wheat: pathogenesis and control strategies. *Crop Protection*, v. 91, n. 1, p. 114-122, 2017.

ENGLE, J.; MADDEN, L.; LIPPS, P. Evaluation of inoculation methods to determine resistance reactions of wheat to *Fusarium graminearum*. *Plant Disease*, v. 87, n. 12, p. 1530-1535, 2003.

FOOD AND AGRICULTURE ORGANIZATION (FAO). *FAO STAT*: datos sobre alimentación y agricultura. 2017. Disponível em: http://www.fao.org/faostat/es/#compare. Acesso em: 3 jan. 2018.

FOOD AND DRUG ADMINISTRATION (FDA). *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. Rockville: U.S. Department of Health and Human Service, 2010.

GÓMEZ L. *Caracterización de cepas toxigénicas del género fusarium mediante técnicas de biología molecular*. 2008. Thesis (Doutorado em Ciencias Biotecnologicas) - Politécnica de Valencia, Valencia, 2008.

HAMMER, Ø.; HARPER, D. A. T.; RYAN, P. D. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, v. 4, n. 1, p. 1-9, 2001.

HERNANDEZ, J. *Fusarium head blight*: winter wheat cultivar responses and characterization of pathogen isolates. 2010. Thesis (PhD in Agronomy and Horticulture) - University of Nebraska, Lincoln, 2010.

INSTITUTO INTERAMERICANO DE COOPERACIÓN PARA LA AGRICULTURA (IICA). *Proyecto regional trigo*: principales logros y avances. Montevideo: Instituto Interamericano de Cooperación para la Agricultura, 2010.

ITTU, M.; SAULESCU, N.; CIUCA, M.; ITTU, G. Effect of single QTLs for wheat fhb resistance from Sumai 3 and F201R on phenotypic resistance traits and DON content. *Romanian Agricultural Research*, v. 23, n. 1, p. 13-20, 2005.

KIM, H.; LEE, T.; DAWLATANA, M.; YUN, S.; LEE, Y. Polymorphism of trichothecene biosynthesis genes in deoxynivalenol and nivalenol producing *Fusarium graminearum* isolates. *Mycological Research*, v. 107, n. 2, p. 190-197, 2003.

KOHLI, M.; DÍAZ de ACKERMANN, M. Resistance to Fusarium head blight in South American wheat germplasm. *In*: MAGLIANO, T.; CHULZE, S. (ed.). *Fusarium head blight in Latin America*. Dordrecht: Springer, 2013. p. 263-297.

KOHLI, M.; VIEDMA, L.; CUBILLA, L. *Guía para la producción de trigo*: fortalecimiento de la investigación y difusión del cultivo de trigo en Paraguay. Asunción: MAG/CAPECO/INBIO, 2012.

KUBO, K.; KAWADA, N.; FUJITA, M. Evaluation of Fusarium head blight resistance in wheat and the development of a new variety by integrating Type I and II resistance. *Japan Agricultural Research Quarterly*, v. 47, n. 1, p. 9-19, 2013.

LAHLALI, R.; KUMAR, S.; WANG, L.; FORSEILLE, L.; SYLVAIN, N.; FOBERT, P.; PENG, G.; KARUNAKARAN, C. Cell wall biomolecular composition plays a potential role in the host Type II resistance to Fusarium head blight in wheat. *Frontiers in Microbiology*, v. 7, e910, 2016.

LANCASHIRE, P.; BLEIHOLDER, H.; BOOM, T.; LANGELÜDDEKE, P.; STAUSS, R.; WEBER, E.; WITZENBERGER, A. A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*, v. 119, n. 3, p. 561-601, 1991.

LEMMENS, M.; SCHOLZ, U.; BERTHILLER, F.; ASTA, C.; KOUTNIK, A.; SCHUHMACHER, R.; ADAM, G.; BUERSTMAYR, H.; MESTERHÁZY, Á.; KRSKA, R.; RUCKENBAUER, P. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. *Molecular Plant-Microbe Interactions*, v. 18, n. 12, p. 1318-1324, 2005.

LESLIE, J.; SUMMERELL, B. *The fusarium laboratory manual*. Ames: John Wiley & Sons, 2008.

MALBRÁN, I.; MOURELOS, C. A.; GIROTTI, J. R.; AULICINO, M. B.; BALATTI, P. A.; LORI, G. A. Aggressiveness variation of *Fusarium graminearum* isolates from Argentina following point inoculation of field grown wheat spikes. *Crop Protection*, v. 42, n. 1, p. 234-243, 2012.

MAZZILLI, S.; PÉREZ, C.; ERNST, O. Fusariosis de la espiga en trigo: características de la enfermedad y posibilidades de uso de modelos de predicción para optimizar el control químico. *Agrociencia*, v. 11, n. 1, p. 11-21, 2007.

MAZZONI, E.; PEIXOTO, H. *Manual básico de técnicas fitopatológicas*. Brasilia, DF: Embrapa, 2016.

MENDES, G. da R. L.; PONTE, E. M. del; FELTRIN, A. C.; BADIALE-FURLONG, E.; OLIVEIRA, A. C. de. Common resistance to Fusarium head blight in Brazilian wheat cultivars. *Scientia Agricola*, v. 75, n. 5, p. 426-431, 2018.

MESTERHAZY, A. Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding*, v. 114, n. 5 , p. 377-386, 1995.

MESTERHÁZY, Á.; BARTÓK, T.; KÁSZONYI, G.; VARGA, M.; TÓTH, B.; VARGA, J. Common resistance to different *Fusarium* spp. causing Fusarium head blight in wheat. *European Journal of Plant Pathology*, v. 112, n. 3, p. 267-281, 2005.

MIEDANER, T.; MOLDOVAN, M.; ITTU, M. Comparison of spray and point inoculation to assess resistance to Fusarium head blight in a multienvironment wheat trial. *Phytopathology*, v. 93, n. 9, p. 1068-1072, 2003.

NIWA, S.; KUBO, K.; LEWIS, J.; KIKUCHI, R.; ALAGU, M.; BAN, T. Variations for Fusarium head blight resistance associated with genomic diversity in different sources of the resistant wheat cultivar "Sumai 3". *Breeding Science*, v. 64, n. 1, p. 90-96, 2014.

PEREYRA, S.; LORI, G. Crop residues and their management in the epidemiology of Fusarium head blight. *In*: MAGLIANO, T.; CHULZE, S. (ed.). *Fusarium head blight in Latin America*. Dordrecht: Springer, 2013. p. 143-156.

REIS, E. M.; BOARETO, C.; DANELLI, A. L. D.; ZOLDAN, S. M. Anthesis, the infectious process and disease progress curves for Fusarium head blight in wheat. *Summa Phytopathologica*, v. 42, n. 2, p. 134-139, 2016.

REIS, E.; CARMONA, M. Integrated disease management of Fusarium head blight. *In*: MAGLIANO, T.; CHULZE, S. N. (ed.). *Fusarium head blight in Latin America*. Dordrecht: Springer Science & Business Media, 2013. p. 159-173.

SCHROEDER, H. W.; CHRISTENSEN, J. J. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. *Phytopathology*, v. 53, n. 7, p. 831-838, 1963.

SHARAN, M. S.; KUMAR, A. K.; NAGARAJAN, S. Fusarium head blight (FHB) or head scab of wheat. *Proceedings of the Indian National Science Academy*, v. 70, n. 3, p. 255-268, 2004.

SIMKO, I.; PIEPHO, H.-P. The area under the disease progress stairs: calculation, advantage, and application. *Phytopathology*, v. 102, n. 4, p. 381-389, 2012.

SIOU, D.; GÉLISSE, S.; LAVAL, V.; REPINÇAY, C.; CANALÈS, R.; SUFFERT, F.; LANNOU, C. Effect of wheat spike infection timing on Fusarium head blight development and mycotoxin accumulation. *Plant Pathology*, v. 63, n. 2, p. 390-399, 2014.

SPANIC, V.; ZDUNIC, Z.; DREZNER, G.; SARKANJ, B. The pressure of Fusarium disease and its relation with mycotoxins in the wheat grain and malt. *Toxins*, v. 11, n. 198, p. 1-16, 2019.

STACK, R. W.; MCMULLEN, M. P. A *Visual scale to estimate severity of Fusarium head blight in wheat*. 2011. Disponível em: https://www.ag.ndsu.edu/ndipm/publications/wheat/documents/pp1095.pdf. Acesso em: 3 jan. 2018.

STEINER, B.; BUERSTMAYR, M.; MICHEL, S.; SCHWEIGER, W.; LEMMENS, M.; BUERSTMAYR, H. Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. *Tropical Plant Pathology*, v. 42 n. 3, p. 165-174, 2017.

VELAZQUEZ, P. D.; FORMENTO, A. N. Efecto de la fusariosis de la espiga (*Fusarium graminearum* y *Fusarium* spp.) sobre dos genotipos de trigo. *In*: JORNADAS FITOSANITARIAS ARGENTINAS, 14., 2012, Potrero de Los Funes. *Anales*... Potrero de Los Funes: INTA, 2012. 1 CD-ROM.

VICAM. *DON-V instruction guide*. Milford: Waters Corporation, 2011.

WATSON, R. J.; WANG, S. A method for making directed changes to the *Fusarium graminearum* genome without leaving markers or other extraneous DNA. *Fungal Genetics and Biology*, v. 49, n. 7, p. 556-566, 2012.

ZHOU, W.-C.; KOLB, F. L.; BAI, G.-H.; DOMIER, L. L.; YAO, J.-B. Effect of individual Sumai 3 chromosomes on resistance to scab spread within spikes and deoxynivalenol accumulation within kernels in wheat. *Hereditas*, v. 137, n. 2, p. 81-89, 2002.