Ciência Rural, Santa Maria, v.54:05, e20230086, 2024 http://doi.org/10.1590/0103-8478cr20230086

ISSNe 1678-4596
CROP PROTECTION

Correlation between blast resistance in wheat cultivars and conidia sporulation rate of *Pyricularia oryzae* Triticum

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ABSTRACT: The use of resistant wheat cultivars is a fundamental strategy to minimize the damages caused by blast, a disease caused by the fungus *Pyricularia oryzae* Triticum (PoT). The objetive of this stydy was to (a) evaluate the reaction to blast of Brazilian wheat cultivars and (b) determine whether there is correlation between severity of symptoms on wheat spikes and "sporulation rate of PoT conidia per gram of wheat spike rachis" (Rscon). Plants of 16 wheat cultivars were grown in greenhouse until flowering (Zadoks stage 65), when their spikes were inoculated with a suspension formed by mixing the conidia of three PoT isolates. The evaluated variables were blast severity on spikes at 5, 7 and 11 days after inoculation (dai) and Rscon. Rachis were evaluated individually to determine the Rscon. Correlation analyzes were carried out between blast severity means on spikes of cultivars at 5, 7 and 11 dai and Rscon. The cultivars TBIO Mestre, TBIO Aton, ORS 1401, ORS 1403, ORS Destak, ORS Feroz, and CD 116 stood out for being classified in the statistical groups with the highest resistance to blast for the four variables considered in the study. The correlation between blast severity on spikes at 5, 7 and 11 dai and Rscon is at most moderate.
Key words: genetic resistance, severity on wheat spikes, wheat spike rachis.

Correlação entre resistência à brusone em cultivares de trigo e taxa de esporulação de conídios de *Pyricularia oryzae* Triticum

RESUMO: A utilização de cultivares de trigo resistentes é estratégia fundamental para minimizar danos causados pela brusone, doença causada pelo fungo *Pyricularia oryzae* Triticum (PoT). O objetivo do estudo foi avaliar (a) a reação à brusone de cultivares brasileiras de trigo e (b) verificar a correlação entre a severidade dos sintomas na espiga e a "taxa de esporulação de conídios de PoT por g de ráquis de espigas de trigo" (Txcon). Plantas de 16 cultivares de trigo foram conduzidas em casa-de-vegetação até o florescimento (estádio 65 da escala de Zadoks), quando as espigas das mesmas foram submetidas à inoculação com uma suspensão formada pela mistura de conídios de três isolados de PoT. As variáveis avaliadas foram a severidade de brusone nas espigas aos cinco, sete e 11 dias após a inoculação (dai) e a Txcon. A Txcon foi determinada de forma individualizada para os ráquis. Foram realizadas análises de correlação entre as médias de severidade de brusone nas espigas das cultivares aos cinco, sete e 11 dai e a Txcon. As cultivares TBIO Mestre, TBIO Aton, ORS 1401, ORS 1403, ORS Destak, ORS Feroz e CD 116 se destacaram por terem sido classificadas nos grupos estatísticos de maior resistência à brusone para as quatro variáveis consideradas no estudo. A correlação entre a severidade de brusone nas espigas aos cinco, sete e 11 dai e Txcon é, no máximo, moderada.
Palavras-chave: resistência genética, severidade em espigas de trigo, ráquis da espiga de trigo.

INTRODUCTION

The world production of wheat grains in the season of 2022/2023 was 779.7 million tons (FAO, 2023). In Brazil, in this same season, the production was 10.55 million tons (AMIS, 2023), reaching the annual production record in the country. However, this amount produced still does not meet the country's consumption needs, which is around 12 million tons per year. Among the obstacles that limit wheat production in Brazil, one of the most important is wheat blast, which is a disease caused by the fungus *Pyricularia oryzae* Triticum (PoT) Cavara (teleomorphic form *Magnaporthe oryzae* (MoT) B.C. Couch) (COUCH& KOHN, 2002; VALENT et al., 2019). *Pyricularia oryzae* affects more than 50 species of grasses, with the first record in wheat occurring in 1985, in the north of the state of Paraná, Brazil (IGARASHI et al., 1986). In addition to being widespread in all wheat producing regions in Brazil, blast has already been reported in the main wheat producing countries in South America and, more recently, was diagnosed in South Asia and Africa, in Bangladesh and Zambia, respectively (MALAKER, 2016; TEMBO et al., 2020).

In Brazil, blast is one of the main challenges to the expansion of wheat production to

Received 02.13.23 Approved 08.08.23 Returned by the author 10.07.23
CR-2023-0086.R1
Editors: Leandro Souza da Silva Leonardo Araújo

the so-called Central Brazil (MACIEL et al., 2020), represented by states such as Goiás and Minas Gerais and the Federal District. A major disease management strategy in areas where wheat blast occurs involves timing the wheat planting date so that heading does not coincide with warm rainy weather (VALENT et al., 2021). In addition, one of the main difficulties faced by wheat producers, especially when climatic conditions are very favorable to wheat blast during heading time, is the low efficiency of its control based on the application of fungicides on the aerial part of the plants (CRUZ et al., 2019). These circumstances determine that the use of wheat cultivars resistant to blast represents a fundamental measure to be considered in the integrated management of the disease. However, there are limitations to adopting this strategy, as cultivars classified as having a higher level of resistance to wheat blast have also not performed well when exposed to conditions quite favorable to the disease (MACIEL et al., 2014).

A cooperative trial network, called Network of Cooperative Trials for Resistance to Wheat Blast on Spikes (RECORBE, acronym in Portuguese), has been conducted since 2018 in Brazil with the objective of evaluating the reaction of Brazilian cultivars to blast on their spikes under field conditions (MACIEL et al., 2020; 2022b). The initiative emerged at the 11th Meeting of the Brazilian Wheat and Triticale Research Commission, in 2017. The trials are standardized tests carried out in representative environments of commercial wheat cultivation in Brazil. Besides, the evaluation of wheat cultivars in terms of reaction to blast has also been carried out under controlled conditions (CRUZ et al., 2010; CRUZ et al., 2016; CRUPPE et al., 2020; GODDARD et al., 2020; MACIEL et al., 2014, 2022a). The main variable evaluated in such studies has been the severity and incidence of wheat blast on spikes. More recently, MACIEL et al (2022a) evaluated whether the variable "PoT sporulation rate on wheat spike rachis" could be an appropriate criterion to compare wheat genotypes in terms of resistance to wheat blast. Based on the results obtained in that study, MACIEL et al. (2022a) pointed out that this variable would be more efficient as a criterion for comparing genotypes in terms of resistance to blast if the evaluations were carried out individually for each rachis and the number of rachis that was evaluated per genotype had been higher than the number they used.

The objective of this study was to evaluate (a) the reaction to blast of Brazilian wheat cultivars and (b) determine whether there is correlation between severity of symptoms on wheat spikes and "sporulation rate of PoT conidia per gram of wheat spike rachis" (Rscon).

MATERIALS AND METHODS

The experiments were carried out between November 2021 and March 2022 in greenhouse, controlled environment chamber and phytopathology laboratory at Embrapa Trigo, Passo Fundo, RS, Brazil. Sixteen wheat cultivars were evaluated, the same ones that were used in the RECORBE trials conducted in 2020 (Table 1 and 2; MACIEL et al., 2022b). Among the cultivars used in the study, the cultivar BRS 264 was included due especially its great susceptibility to blast (MACIEL, 2020; 2022a and 2022b).

The wheat plants used in the experiments were grown under greenhouse environmental in plastic pots with a capacity of 8 L containing corrected pH and nutrition soil. For each cultivar, sowing was performed in 2 pots. Ten seeds were deposited in each pot but the number grown plants in each one of them was of 5 to 6. After 15 days, the experiment was repeated with new sowing of cultivars under the same conditions. When the plants reached flowering stage, they were inoculated with a suspension of PoT conidia. The number of spikes in each pot ranged from 6 to 12.

The pathogen inoculum was prepared by mixing conidia of the following PoT isolates with balanced concentrations of conidia: *Py* 15.1.010, *Py* 17.1.001, and *Py* 17.1.008. These three isolates are from the collection of PoT isolates from Embrapa Trigo and are preserved at -18 °C using the filter paper technique. They were classified in the virulence groups G3, G5, and G7, respectively, according to the classification established by PIZOLOTO (2019) based on the reaction on the spikes of a set of 11 wheat and one barley genotypes. This classification was done with individualized inoculation of 89 PoT Brazilian isolates on the plants, including the three isolates used in present the study.

The three PoT isolates were cultivated on Petri dishes containing oat-agar medium (60 g of oat, 12 g of agar, 1 L of water) and grown in incubation chambers for 10-12 days (25 °C and photoperiod of 12 h light/12 h dark, stimulatory conditions for sporulation of PoT). To prepare the inoculum, the Petri dishes were flooded with distilled water plus a Tween 80® adhesive spreader (0.01%). With the help of a brush or glass slide, the plates were scraped, in order to dislodge the conidia. The scraped material

Table 1 - Blast severity on spike rachis of wheat cultivars.

Cultivar	---------5 dai[1]-------		Cultivar	----------7 dai--------		Cultivar	----------11 dai-------	
BRS 264	40.41	a[2]	BRS 264	83.12	a	BRS 264	95.06	a
TBIO Sonic	14.26	b	TBIO Sonic	26.00	b	TBIO Sonic	60.14	a
ORS Senna	13.04	b	ORS Senna	19.16	b	BR 18 – Terena	47.00	b
BR 18 – Terena	10.48	b	BRS 404	14.26	c	ORS Guardião	38.65	b
ORS Guardião	9.80	b	BR 18 – Terena	14.01	c	BRS 404	38.30	b
TBIO Sossego	8.49	b	TBIO Audaz	13.04	c	ORS Senna	36.92	b
BRS 404	7.85	b	TBIO Duque	12.80	c	TBIO Audaz	30.01	b
TBIO Duque	7.02	b	ORS Guardião	10.48	c	TBIO Duque	23.93	c
ORS Feroz	5.02	c	TBIO Sossego	10.25	c	ORS Feroz	23.64	c
ORS Destak	4.82	c	CD 116	6.61	d	CD 116	21.36	c
CD 116	3.88	c	ORS Destak	6.00	d	TBIO Sossego	18.36	c
TBIO Audaz	3.88	c	ORS Feroz	5.80	d	ORS Destak	18.09	c
ORS 1403	3.69	c	ORS 1403	3.51	d	TBIO Mestre	13.52	c
ORS 1401	3.69	c	ORS 1401	2.60	d	TBIO Aton	8.92	c
TBIO Aton	2.60	c	TBIO Aton	2.60	d	ORS 1403	8.28	c
TBIO Mestre	1.90	c	TBIO Mestre	2.60	d	ORS 1401	2.43	c
Mean	8.80			14.55			30.29	
CV (%)[3]	7.33			6.47			10.58	

[1]dai = days after inoculation;
[2]Means followed by the same letter in the column do not differ from each other according to the Scott & Knott test at 0.05 probability;
[3]Coefficients of variation (CV) determined in analysis of variance performed with transformed data.

from the Petri dishes was filtered through a sieve with gauze inside. The spores count was done in a Neubauer Chamber (Loptik Labor 0.0025 mm^2) with the aid of a stereomicroscope, 400× of magnification, and the conidia concentration was adjusted to 10^5 conidia mL-1. The conidial suspension was sprayed with a manual atomizer directly onto the spikes when the plants were between phenological stages 58 to 68 on the Zadoks' scale (ZADOKS et al., 1974). The spikes were grouped, and three sprays were performed in front and three behind the spikes. Afterwards, the plants were protected with plastic bags and sent to a controlled environment chamber, being kept in the dark for 24 h at a temperature of 24 ± 2 °C and relative humidity greater than 90%. After 24 h, the photoperiod was adjusted to 12 h light/12 h dark. The plants remained in a controlled environment until 14 days after inoculation (dai).

The evaluations of blast severity on the all spikes subjected to inoculation were carried out at 5, 7 and 11 dai. At 14 dai, the spikes were harvested, separated according to the cultivar and pots, put inside paper bags, and kept at -20 °C. The spikelets were manually removed from each spike, in order to isolate the rachis. The rachis were disinfected in commercial sodium hypochlorite (2.5%) at a 1:1 (v/v) ratio for 1 minute, rinsed in distilled and sterilized water and arranged on previously moistened blotting paper in plastic Petri dishes. The plates were kept in an incubation chamber with a 12 h photoperiod and a temperature of 25 ± 2 °C for 96 h. After this period, each rachis was placed in a Falcon tube (15 mL) containing 2 mL of distilled and sterilized water. The tubes were shaken in a MA 162 tube shaker (Marcon®) for 40 s. An aliquot of liquid was removed and prepared in a hemacytometer in Neubauer chamber. The conidia were counted in an optical microscope with a magnification of 100x. From each pot where the plants grown, the spore concentration was determined individually in four rachis. This rachis were weighed wet, unlike MACIEL etal. (2022a) whose weighing was carried out before the incubation period.

The experimental design was completely randomized and data were previously analyzed to verify normality residue and variance homogeneity. After being transformed to root x+10 and log x, respectively, the data of severity and Rscon were subjected to analysis of variance. Means were compared using the Scott-Knott test with nontransformed data ($P > 0.05$).

The means of the cultivars in relation to the variables evaluated in the study were used to determine the correlation coefficient (r) between the severity of blast on spikes and the variable Rscon using the computer program Microsoft Excel (Microsoft Corporation, Seattle, USA). The Spearman correlation coefficient (ρ) between the variables was also determined using the Microsoft Excel program. Statistical analysis and the design of the boxplot graphs were performed using the R software (R DEVELOPMENT CORE TEAM, 2017).

RESULTS

The cultivars differed from each other regarding the variables severity on the spikes at the three evaluation stage, at 5, 7 and 11 dai, and in relation to the Rscon. Based on Scott-Knott statistical tests, the 16 cultivars were separated into three (a, b and c), four (a, b, c and d) and three (a, b and c) groups according to the severity assessment on the spikes at 5, 7 and 11 dai, respectively (Table 1). Two groups were formed according to the Rscon (a, and b; Table 2).

At 5 dai, the mean blast severity on spikes was 8.80 and the group with the most resistant cultivars was formed by cultivars with mean severity ranging from 1.90 to 5.02, namely, TBIO Mestre, TBIO Aton, ORS 1401, ORS 1403, TBIO Audaz, CD 116, ORS Destak, and ORS Feroz. At 7 dai, the mean blast severity on the spikes was 14.55, with the most resistant group presenting mean severity ranging from 2.60 to 6.61 and formed by the cultivars TBIO Mestre, TBIO Aton, ORS 1401, ORS 1403, ORS Feroz, ORS Destak, and CD 116. At 11 dai, the mean blast severity on spikes was 30.29 and the most resistant group presented mean severity ranging from 2.43 to 23.93, formed by the cultivars ORS 1401, ORS 1403, TBIO Aton, TBIO Mestre, ORS Destak, TBIO Sossego, CD 116, ORS Feroz, and TBIO Duque. The cultivar BRS 264 was the one that showed the highest severity value in the three evaluations carried out, 40.41%, 83.12% and 95.06%, at 5, 7 and 11 dai, respectively.

The mean Rscon for the 16 cultivars was 1.2 x 10^6, and 13 of them were classified in the statistical group with the lowest sporulation rate. With exception of the cultivars BRS 264, BRS 18 – Terena and BRS 404, all other cultivars were classified in the group with lower sporulation and in which the Rscon varied from 1.3 x 10^5 to 1.5 x 10^6 conidia per g of rachis (Table 2; Figure 1A). The cultivars that presented the lowest and highest numerical value for Rscon were ORS Destak and BR 18 - Terena with 1.3 x 10^5 and 4.4 x 10^6 conidia per g of rachis, respectively.

Table 2 - Sporulation rate of *Pyricularia oryzae* Triticum on spike rachis of wheat cultivars.

Cultivar	Year of release	------------Conidia per g of rachis-----------	
BR 18 – Terena	1986	4,447,788.5	a[1]
BRS 404	2015	3,965,990.5	a
BRS 264	2005	3,122,313.7	a
TBIO Audaz	2017	1,540,886.1	b
TBIO Duque	2019	1,211,152.7	b
CD 116	2006	1,191,501.8	b
ORS Guardião	2020	761,935.1	b
ORS Senna	2020	445,533.9	b
TBIO Aton	2019	429,270.8	b
TBIO Mestre	2012	298,270.8	b
ORS Feroz	2020	265,124.2	b
ORS 1401	2015	251,056.9	b
TBIO Sonic	2017	220,409.6	b
ORS 1403	2016	156,460.8	b
TBIO Sossego	2015	151,717.6	b
ORS Destak	2019	127,375.4	b
Mean		1,161,674.3	
CV (%)[2]		36.1	

[1]Means followed by the same letter in the column do not differ from each other according to the Scott & Knott test at 0.05 probability;
[2]Coefficients of variation (CV) determined in analysis of variance performed with transformed data.

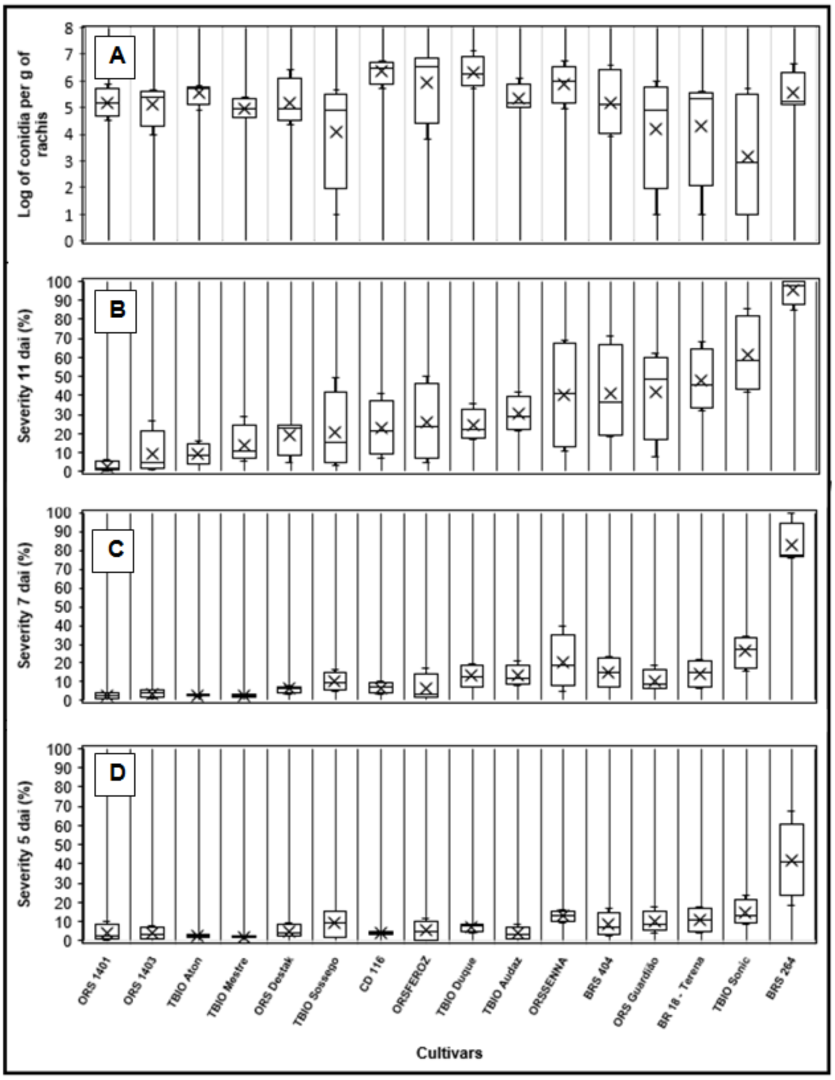

Figure 1 - Distribution in boxplot graph of *Pyricularia oryzae* Triticum conidia production on wheat spike rachis (A) and blast severity on spikes of wheat cultivars 11 (B), 7 (C) and 5 (D) days after inoculation (dai) with conidial suspension of the pathogen.

The boxplot graph shows the distribution of severity data and the evolution of symptoms over the three severity evaluations on spikes (5, 7 and 11 dai) on the cultivars (Figure 1B, C, D). There was an evolution in the development of wheat blast in the period between the three evaluations and an evident difference between the most susceptible cultivars to blast in relation to the most resistant ones. The outliers are more evident in the evaluation carried out at 11 dai and for the most susceptible cultivars. The cultivar BRS 264, due to its great susceptibility to wheat blast, plays a beacon role that helps the comparison of all cultivars. In the 1A graph, it is observed little occurrence of outliers for the variable Rscon, condition that was favored by the log-transformation of the data.

The r obtained in the correlation analysis between non-transformed data of Rscon and wheat blast severity on the spikes at 5, 7 and 11 dai were 0.23, 0.30, and 0.36, respectively (Figure 2A, C, and E). The ρ obtained for these same analyzes were 0.29, 0.52 and 0.60 which, according to the scale described by BABA et al. (2014), are of positive slope direction and are classified, respectively, as low, moderate, and moderate values. The r obtained in the correlation

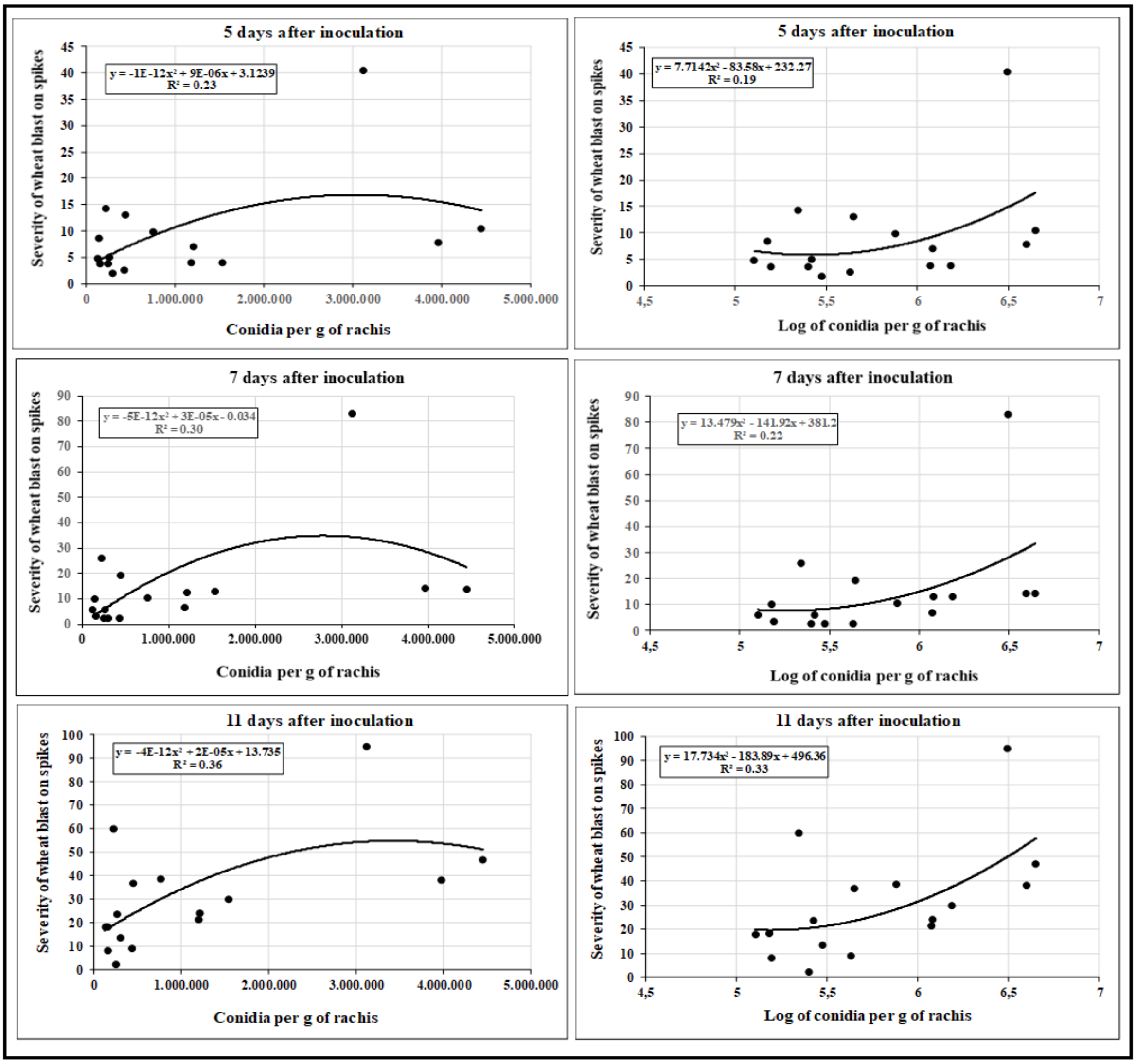

Figure 2 - Analysis of correlation between severity of symptoms on spikes of wheat cultivars and "sporulation rate of *Pyricularia oryzae* Triticum conidia per gram of wheat spike rachis" (Rscon). A, C, and E, analysis of correlation between wheat blast severity at 5, 7 and 11 dai*, respectively, and non transformed data of Rscon. B, D, and F, analysis of correlation between wheat blast severity at 5, 7 and 11 dai; respectively, and log transformed data of Rscon.

*dai = days after inoculation.

analysis between log transformed data of Rscon and wheat blast severity on the spikes at 5, 7 and 11 dai were 0.19, 0.22, and 0.33, respectively (Figure 2D, E, and F). The values of ρ were equals to those obtained when the nontransformed data were used in the analysis.

DISCUSSION

The results obtained represent an updated analysis of blast reaction of 16 important Brazilian wheat cultivars. This analysis reflects the reaction of these cultivars to a set of PoT isolates (*Py* 17.1.001, *Py* 17.1.008, and *Py* 15.1.010) previously characterized as representative of the prevalent virulence of the pathogen in Brazil (PIZOLOTTO, 2019). In addition, one of the most outstanding results obtained was the performance demonstrated by seven of the evaluated cultivars, TBIO Mestre, TBIO Aton, ORS 1401, ORS 1403, ORS Destak, ORS Feroz, and CD 116, which were classified in the statistical groups of greater resistance to blast for the four variables considered in the study. Another positive characteristic observed in these seven cultivars is the fact that they presented Rscon very lower than the cultivars BRS 264, BR 18 – Terena and BRS 404 (cultivars belonging to the statistical group with the highest Rscon values; Table 2). The exception to this condition was the cultivar CD 116, as for the other cultivars the Rscon was at least seven times lower than any of the three cultivars classified in the statistical group with the highest Rscon (for example, TBIO Aton in relation to BRS 264).

Most of the seven cultivars that stood out in terms of resistance to blast has the combination of two very relevant characteristics; are directed by their breeders for cultivation in Central Brazil and were launched commercially relatively recently, that is, from 2015 (Table 2). Many of these cultivars have already demonstrated a significant level of resistance to blast in previous evaluations, in experiments conducted both under controlled conditions and in the field (MACIEL et al., 2020a; 2020b; 2022).

The evaluation and sampling system used to determine the Rscon was significantly altered in relation to what was adopted by MACIEL et al. (2020a) in which six observations were made per cultivar, each one representing a pot and the joint evaluation of seven rachis. In that study, the correlation coefficients of the analysis established between the cultivar severity at 5 and 7 dai and the sporulation rate of PoT on spike rachis were relatively low (r=0.25 and r=0.20, respectively). Besides, the authors themselves characterized that study as being "exploratory" to assess the feasibility of this variable to compare wheat genotypes in terms of reaction to blast. The adopted alterations in the present study were efficient to give greater reliability to the collected data in relation to the real condition of the experiments. The main change adopted was the individual evaluation of the rachis of the infected spikes, plus the increase in the number of observations made per cultivar, i.e., 16 observations (four rachis of each of the four pots carried out per cultivar were evaluated). One of the effects of these changes was the increase in r values, which for the correlation between original data of Rscon and wheat blast severity on the spikes at 5, 7 and 11 dai, were 0.23, 0.30 and 0.36, respectively. We think that these circumstances were fundamental for the values of the correlation coefficients between the variables Rscon and wheat blast on the spikes to have been higher than those obtained by MACIEL et al. (2022). We also think that there is no specific number of rachis per genotype that have to be evaluated to generate more reliable and assertive results, but we consider that the procedures and number of rachis evaluated in the present study should be considered as a relevant reference for new evaluations with the same type of approach.

It is important to emphasize that, even if the correlation coefficients between the variables have increased in relation to the values obtained by MACIEL et al. (2022a), the strongest correlations between the assessed variables were classified in the present study at most as moderate (Rscon and wheat blast severity on the spikes at 7 and 11 dai). This classification is an important indicator that the sporulating capacity of PoT on rachis of wheat spikes is expressed in relatively independent of the severity of the disease produced by this pathogen on spikes. These circumstances help to compose the perception that the genetic control of PoT sporulation in wheat plants can be determined by a specific type of resistance; although, more studies on the subject should be encouraged with a view to obtaining more conclusive results on the theme.

The results obtained in the present study are very connected with the understanding that the greater production of spores of phytopathogenic fungi under the surface of plants installed in the field means greater potential production of new lesions and, consequently, an increase in new generations of the pathogen and of the damages caused by it (MONEY, 2016). In this sense, the present study also provides elements for analysis of how much and how PoT produces in terms of spores in the wheat crop, in addition to the strong and significant indication that the greatest damage will be in crops that use wheat cultivars with higher RScon.

Lastly, the option for using suspensions formed by mixing conidia of the three PoT isolates in the inoculation of wheat plants was adopted due to the understanding that this would be the most rational way, in terms of economics and practicality, to obtain data about the reaction of Brazilian cultivars to wheat blast caused by isolates of the fungus representative of the Brazilian variability in terms of virulence. Individualized assessment for each of the three isolates used would mean a tripling effort in terms of material and labor efforts, conditions that were not available or accessible within the scope of the present study.

CONCLUSION

The variables used in the study were efficient to compare the 16 wheat cultivars in terms of resistance to blast on the spikes. Besides that, the cultivars TBIO Mestre, TBIO Aton, ORS 1401, ORS 1403, ORS Destak, ORS Feroz, and CD 116 showed the best performance, as they were classified in the statistical groups with the highest resistance to blast for the four variables used in the study. Lastly, it was possible to verify that the correlation between blast severity on spikes and Rscon is moderate.

ACKNOWLEDGEMENTS

We are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing scholarships for the second (EncBolsasEMBRAPA2019- CNPq-380065/2022-3) and fourth (undergraduate-CNPq-PIBIC-145779/2022-0) authors and, to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 with PROSUC/CAPES, for providing scholarship for the third author (88887.667356/2022-00). We also thank EMBRAPA for the financial support, which was made available within the budget of project SEG 32.16.04.032.00.06.005.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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