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

Correlação entre resistência à brusone em cultivares de trigo e taxa de esporulação de conídios de *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 objective of this study was to evaluate (a) the reaction to blast of Brazilian wheat cultivars and (b) to determine whether there is correlation between severity of symptoms on wheat spikes and “rate of sporulation of PoT conidia on 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 the log of Rscon. The cultivars ORS Feroz, ORS Destak, CD 116, ORS 1403, ORS 1401, TBIO Aton and TBIO Mestre stood out for being classified in the statistical groups with the highest resistance to blast for the four variables considered in the study. There was a correlation between blast severity on spikes at 5, 7 and 11 dai and Rscon.

Key words: Severity on wheat spikes, wheat spike rachis, 2NS chromosomal sequence.

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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 em 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 o log das Txcon. As cultivares ORS Feroz, ORS Destak, CD 116, ORS 1403, ORS 1401, TBIO Aton e TBIO Mestre se destacaram por terem sido classificadas nos grupos estatísticos de maior resistência à brusone para as quatro variáveis consideradas no estudo. Verificou-se a existência de correlação entre a severidade de brusone nas espigas aos 5, 7 e 11 dai e Txcon.

Palavras-chave: Severidade em espigas de trigo, ráquis da espiga de trigo, sequência cromossomal 2NS.

INTRODUCTION

Wheat blast is 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, 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 Bangladesh, in South Asia and in Zambia, in Africa (MALAKER, 2016; TEMBO et al., 2020).

In Brazil, blast is one of the main challenges to the expansion of wheat production to 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 the disease 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 for this disease 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 the disease have also not performed well when exposed to conditions quite favorable to the disease (MACIEL et al., 2014). More recently, wheat cultivars with greater resistance to blast have been made available to Brazilian wheat growers, among which we can highlight the following: ORS 1401, ORS 1403, TBIO Mestre, TBIO Sossego and CD 116 (MACIEL, 2020). What is possible to speculate is that such cultivars have the 2NS chromosomal sequence in their genome (CRUZ et al., 2016), a condition that has been associated with greater resistance to the disease.

A cooperative trial network, called Network of Cooperative Trials for Resistance to Wheat Blast in Spikes (RECORBE, acronym in Portuguese), has been conducted since 2018 in Brazil with the objective of evaluating the reaction of Brazilian cultivars to blast

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in 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 the disease in 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 disease resistance. 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) to determine whether there is correlation between severity of symptoms on wheat spikes and "rate of sporulation of PoT conidia on 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; MACIEL et al., 2022b).

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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 spores: *Py* 17.1.001, *Py* 17.1.008 and *Py* 15.1.010. These isolates belong to the collection of PoT isolates from Embrapa Wheat, are preserved at -18 °C using the filter paper technique and were classified by PIZOLOTTO (2019) into prevalent groups in Brazil and distinct from each other in relation to the demonstrated virulence during the infection of wheat and barley spikes (11 and one wheat and barley cultivars, respectively). The 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). 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 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× 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

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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 (Neubauer chamber). The conidia were counted in an optical microscope with a magnification of 100x. From each pot where the plants growed, the spore concentration was determined individually in four rachis. The number of conidia was converted to the number of conidia per g of wet rachis.

The experimental design was completely randomized and the analysis of variance of data was done with the values transformed and nontransformed. When transformed, severity and Rscon results were transformed to root x+10 and log x, respectively. Means were compared using the Scott-Knott test ($p>0.05$). Statistical analysis and the design of the boxplot graphs were performed using the R software (R Development Core Team, 2017). The mean obtained for each cultivar was used to determine the correlation coefficient (r) between the variable Rscon log transformed and the severity on spikes, without transformation, at 5, 7 and 11 dai 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.

RESULTS

The cultivars differed from each other regarding the variables severity on the spikes at the three evaluation moment, at 5, 7 and 11 dai, and in relation to the Rscon (Table 1). Based on Scott-Knott statistical tests, the 16 cultivars were separated into three groups according to the degree of blast severity on the spikes at 5 and 11 dai and the Rscon. On the severity assessment at 7 dai, four statistical groups were formed.

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 5.02 to 1.90, namely, ORS Feroz, ORS Destak, CD 116, TBIO Audaz, ORS 1403, ORS 1401, TBIO Aton and TBIO Mestre. At 7 dai, the mean blast severity on the spikes was 14.55, with the most resistant group presenting mean severity ranging from 6.61 to 2.60 and formed by the cultivars CD 116, ORS Destak, ORS Feroz, ORS 1403, TBIO Aton, TBIO Mestre and ORS 1401. At 11 dai, the mean blast severity on spikes was 30.29. The most resistant group presented mean severity ranging from 23.93 to 2.43 and was formed by the cultivars TBIO Duque, ORS Feroz, CD 116, TBIO Sossego, ORS Destak, TBIO Mestre, TBIO Aton, ORS 1403 and ORS 1401. 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 1.795×10^4 conidia per g of rachis, and eight of these cultivars were classified in the statistical group with the lowest sporulation

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rate. This group was formed by the cultivars ORS Feroz, ORS Destak, CD 116, TBIO Audaz, ORS 1403, ORS 1401, TBIO Aton and TBIO Mestre, with mean ranging from $7.411x10^3$ to $2.982x10^3$ conidia per g of rachis (Table 1; Figure 1A). The cultivars that presented the lowest and highest numerical value for Rscon were TBIO Mestre and BRS 264 with $2.982x10^3$ and $12.704x10^6$ conidia per g of rachis, respectively.

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 the disease 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 the disease, 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 means of cultivars in relation to Rscon (transformed to log x) and blast severity in spikes (without transformation) were used to determine the r between these two types of variables. The r obtained in the correlation analysis between Rscon and disease severity in the spikes at 5, 7 and 11 dai were 0.9938, 0.9655 and 0.9285, respectively. The ρ obtained for these same analyzes were 0.9985, 0.8850 and 0.8706 which, according to the scale described by Mukaka (MUKAKA, 2012), are of positive slope direction and are classified, respectively, as very high, high and high values, conditions that establish a strong association between the analyzed variables.

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DISCUSSION

The results obtained represent an updated analysis of blast reaction of 16 important Brazilian wheat cultivars. It is important to emphasize that 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 eight of the evaluated cultivars, ORS Feroz, ORS Destak, CD 116, ORS 1403, ORS 1401, TBIO Aton and TBIO Mestre, 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 eight cultivars is the fact that they presented Rscon lower than 10^4 conidia per gram of rachis. These are rates that must be considered very low if we compare, for example, with those obtained for the cultivar BRS 264 (1.27×10^7 conidia per gram of rachis), the most susceptible cultivar to blast among the 16 evaluated.

Most of the eight 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 1). 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 expectation placed on such cultivars is that they can form the differentiated genotypes that will serve as a basis, from which the expansion of wheat cultivation in Central Brazil will finally be consolidated. It is also important to mention that it is very likely that the better performance of these cultivars in terms of resistance to blast is associated with the presence of the 2NS sequence in their genome, although this association cannot go

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beyond the limits of speculation since, so far, of the 16 cultivars evaluated in the present study, this sequence was only described in TBIO Mestre (PIZOLOTTO, 2019) and TBIO Sossego (CRUPPE at al., 2020).

The confirmation by statistical tests (r and ρ) that there is a correlation between the severity of the disease on the spikes and the log-transformed data of Rscon supports the proposition that the variable Rscon is a reliable criterion to compare blast resistance in wheat genotypes. We understand, however, that its use will depend on the objectives of the evaluation in which there is interest in adopting it. Considering that the two types of variables (blast severity on spikes and Rscon) provide reliable data on the reaction of wheat genotypes, the most viable option to be adopted would be the evaluation of the severity, since it is much less laborious than the Rscon determination. According to our perception, the use of Rscon would be more recommended in specific studies. One example of this can be the identification of molecular markers associated with resistance to blast, which is an action that has greater chances in achieving its objectives if it is based on phenotyping carried out with great quantitative accuracy, such as the Rscon variable has the potential to offer, as demonstrated in the present study.

It is important to mention that the evaluation and sampling system used to determine the Rscon was significantly altered in relation to the one adopted by MACIEL et al. (2020a), which was characterized by the authors themselves as an “exploratory study” to assess the feasibility of this variable to compare wheat genotypes in terms of reaction to blast. 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 buckets carried out per cultivar were evaluated). In the evaluation made by MACIEL et al. (2020a), 6 observations were made per cultivar, each one representing a pot and the joint evaluation of 7 rachis. We understand that the changes adopted were efficient to provide greater reliability to the data collected in relation to the real condition of the experiments.

CONCLUSIONS

The variables used in the study were efficient to compare the 16 wheat cultivars in terms of resistance to blast on the spikes.

The cultivars ORS Feroz, ORS Destak, CD 116, ORS 1403, ORS 1401, TBIO Aton and TBIO Mestre 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.

There is a correlation between blast severity on spikes at 5, 7 and 11 dai and Rscon.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS' CONTRIBUTIONS

JLNM conceived and designed the experiments. MK, DS, JNC and CCC carried out the experimental procedures in the lab, greenhouse and incubation chamber. JLNM and MK did the statistical analyses and prepared the manuscript draft. All authors critically revised the manuscript and approved of the final version.

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Table 1. Blast severity in spikes of wheat cultivars and sporulation rate of *Pyricularia oryzae* Triticum on spike rachis.

Cultivar	Year of release	Severity on spikes			Conidia per g of rachis
		5 dai[1]	7 dai	11 dai	
BRS 264	2005	40.41 a[2]	83.12 a	95.06 a	12,704,863.40 a
TBIO Sonic	2017	14.26 b	26.00 b	60.14 a	85,480.10 b
ORS Senna	2020	13.04 b	19.16 b	36.92 b	62,443.60 b
BR 18 – Terena	1986	10.48 b	14.01 c	47.00 b	33,917.36 b
ORS Guardião	2020	9.80 b	10.48 c	38.65 b	26,506.05 b
TBIO Sossego	2015	8.49 b	10.25 c	18.36 c	20,029.86 b
BRS 404	2015	7.85 b	14.26 c	38.30 b	16,956.45 b
TBIO Duque	2019	7.02 b	12.80 c	23.93 c	13,362.55 b
ORS Feroz	2020	5.02 c	5.80 d	23.64 c	7,411.12 c
ORS Destak	2019	4.82 c	6.00 d	18.09 c	7,270.96 c
CD 116	2006	3.88 c	6.61 d	21.36 c	5,655.41 c
TBIO Audaz	2017	3.88 c	13.04 c	30.01 b	5,230.28 c
ORS 1403	2016	3.69 c	3.51 d	8.28 c	5,109.79 c
ORS 1401	2015	3.69 c	2.60 d	2.43 c	4,860.12 c
TBIO Aton	2019	2.60 c	2.60 d	8.92 c	3,642.68 c
TBIO Mestre	2012	1.90 c	2.60 d	13.52 c	2,982.97 c
Mean		8.80	14.55	30.29	17,947.34
CV (%)[3]		7.33	6.47	10.58	14.51

[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.

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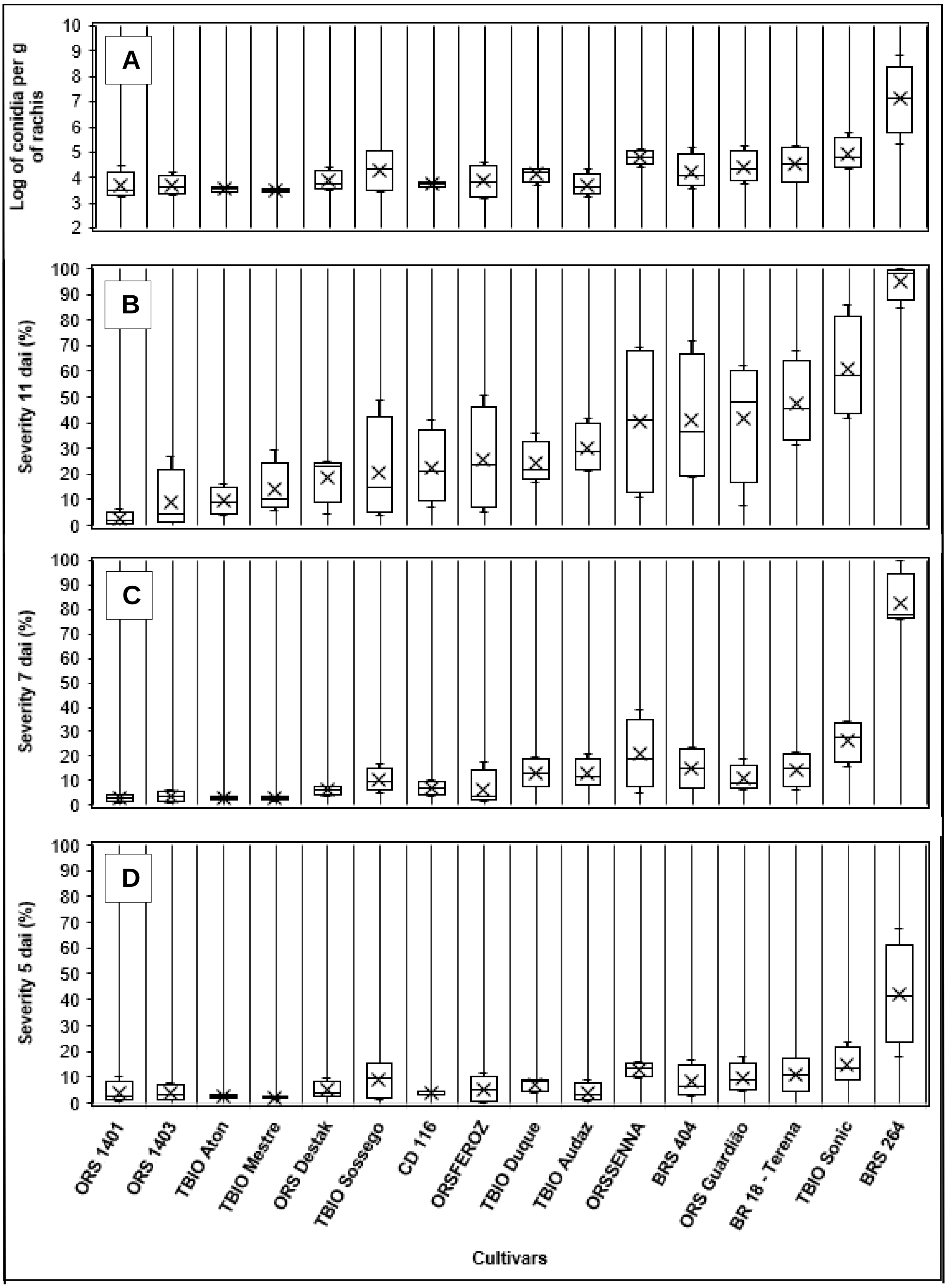

Figure 1. Distribution in boxplot graph of *Pyricularia oryzae* Triticum conidia production on wheat spike rachis (A) and blast severity on spikes of wheat cultivars 11 (B), 7 (C) and 5 (D) days after inoculation (dai) with conidial suspension of the pathogen.

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