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Phenotypic Reaction of Wheat Genotypes to Blast and Sporulation of *Pyricularia oryzae* Triticum

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ABSTRACT

Wheat blast, caused by *Pyricularia oryzae* Triticum (PoT), is one of the most destructive diseases of wheat. This study aimed to evaluate the phenotypic reaction to PoT in leaves and heads of plants of eight cultivars and 16 wheat lines and to evaluate the production of conidia in the rachis. The genotypes were inoculated at Zadoks stages 13 and 65 with PoT (10^5 conidia mL^{-1}), and the disease severity was evaluated at 5, 7 and 11 days after inoculation. The severity values were used to calculate the area under the disease progress curve (AUDPC). Rachis samples were collected to evaluate PoT sporulation. Symptoms of blast and PoT sporulation occurred in all genotypes. Seven genotypes showed increased resistance to blast in leaves: PF 190018, ORS Feroz, ORS Absoluto, TBIO Aton, UB 2016605, TBIO Duque and GD 150534. ORS Feroz and PF 190018 also showed increased head resistence, along with IPF 86775, PF 180135, IPF 86749 and PF 160660. Genotypes with decreased sporulation included PF 160660, IPF 86775, PF 180135, ORS Feroz, IPF 86749 and TBIO Duque. These findings will help breeders and farmers identify and select blast-resistant cultivars.

In tropical regions, global warming creates increasingly favourable conditions for the development of wheat blast (Pequeno et al. 2024), putting global food security at risk (Tembo et al. 2020). The causal agent of the blast is the fungus *Pyricularia oryzae* Triticum (PoT) (sexual phase *Magnaporthe oryzae* Triticum), which was first detected in 1985 in the state of Paraná, Southern Brazil (Igarashi et al. 1986). Since then, wheat blast has represented one of the main biotic challenges in the expansion of wheat farming in tropical regions (Rahman and Uddin 2017).

Blast manifests itself on both the leaves and the heads of wheat, with damage to the heads being the most detrimental to productivity (Valent et al. 2021). Given this, genetic improvement programmes have historically prioritized resistance to head blast. However, the leaf blast epidemic in Central Brazil in 2019 highlighted the need to include foliar resistance in young plants in selection protocols (Embrapa 2019), expanding the scope of research on this pathosystem.

Genetic resistance, achieved through the use of moderately resistant cultivars, has been one of the strategies for controlling blast (Valent et al. 2021). However, the effectiveness of genotypic resistance can be significantly influenced by a complex interaction of agronomic and environmental factors, such as plant development stage, planting date, climate and coexistence of other diseases (Naseri and Fareghi 2024). In this context, the results of investigations on the resistance of wheat genotypes at different

development stages are particularly relevant, considering the potential of this information to benefit countries with climatic conditions similar to Brazil.

In the evaluation of plant resistance, variables such as latency period and lesion size are important (Parlevliet 1979). However, there are relevant studies that have helped in the identification of genotypes resistant to blast using severity variables (Cruppe et al. 2020), area under the disease progress curve (AUDPC) (Fernández-Campos et al. 2020) and sporulation (Maciel et al. 2022). This study aimed to characterise the phenotypic response of wheat genotypes to PoT at the young plant (leaves) and adult (heads) stages, in addition to estimating the amount of PoT conidia produced in the wheat head rachis.

The trials were conducted in a controlled environment (greenhouse, inoculation chamber and laboratory) at Embrapa Trigo, Passo Fundo, RS, Brazil. Eight cultivars and 16 wheat genotypes indicated for cultivation in the Central Brazil region were used (Table 1). The genotypes that have the 2NS/2AS translocation, originally from a wild relative of wheat (*Aegilops ventricosa*), are reported as a source of resistance to blast (Roy et al. 2021). The cultivars present different reactions to blast, ranging from susceptible (BRS 264 and BR 18) to moderately susceptible (BRS 404) and moderately resistant (ORS Absoluto, ORS Feroz, ORS Soberano, TBIO Aton and TBIO Duque). The other genotypes are lines that are still in the selection process. The trials were conducted in a completely randomised design, containing 24 treatments, represented by the wheat genotypes, each evaluated in three replicates (pots). All trials were repeated.

To determine resistance to leaf blast, each replicate consisted of a pot (500 mL) containing peat-based substrate (Germina Plant), where 10 plants were grown and evaluated, subjected to inoculation 17 days after sowing (stage 13; Zadoks et al. 1974). To evaluate resistance in the heads, the genotypes were grown in 7.2 L plastic pots filled with soil. Ten plants were grown in each pot. Between 12 and 20 heads were evaluated in each pot. The heads were inoculated at the beginning of flowering (stage 65; Zadoks et al. 1974).

Seeds were not treated with any fungicide or insecticide. Plants used in the trials were grown in a greenhouse, where an automatic cooling system was activated when the temperature exceeded 26°C. Plants in both trials were watered daily and received nitrogen fertilisation with urea that was diluted in water (5 g L^{-1}) and applied weekly until the time of inoculation.

To prepare the inoculum, a proportional mixture of conidia from three PoT isolates available from the Embrapa Trigo PoT isolate collection was performed: two from the Central Brazil region (Py 15.1.010: Uberaba, Minas Gerais and Py 17.1.008: Sacramento, Minas Gerais) and one from Southern Brazil (Py 17.1.001: Passo Fundo, Rio Grande do Sul). The isolates used for the evaluations show high virulence in wheat leaves and heads (Pizolotto 2019). PoT inoculum multiplication procedures were performed according to Maciel et al. (2022).

TABLE 1 | Description of the wheat genotypes evaluated.

Number	Genotype[a]	2NS/2AS carrier	Genealogy
1	BRS 264		BUCK BUCK/ CHIROCA//TUI
2	BRS 404		WT 99172/ ALIANÇA
3	BR 18		Alondra Sel
4	ORS Absoluto	X	ORS Agile/ TBIO Audaz
5	ORS Feroz	X	ORS 1403/ IOR 351711
6	ORS Soberano	X	ORS 1403/ Desconhecido
7	TBIO Aton	X	MESTRE*2/ FUSTE
8	TBIO Duque	X	Toruk#3/ Celebra//Noble
9	IPF 86749	X	MRC/KAUZ// SKAUZ/3/ SUNSTATE/5/ VEE/LIRA// BOW/3/BCN/4/ KAUZ
10	IPF 86775	X	BOW/VEE/5/ ND/VG9144// KAL/BB/3/ YACO/4/CHIL/6/ CASKOR/3/ CROC_1/ AE.SQUARROSA (224)//OPATA/7/ PASTOR//MILAN/ KAUZ/3/BAV92
11	PF 190011		Seleção 38 dentro de BR 18
12	PF 160276		BR 18/IPR 144
13	PF 190018	X	TBIO Mestre/ BRS 229
14	UB 2009901		BRS Tangará// BR 18/IPR 85
15	GD 150534		PF 014366-B/ CD 113
16	PF 150505		PF 014366-B/ CD 113
17	UB 2016602		BR 18//PF 100164/ WT 07106
18	UB 2016604		BR 18//PF 100164/ WT 07106

(Continues)

TABLE 1 | (Continued)

Number	Genotype[a]	2NS/2AS carrier	Genealogy
19	UB 2016605		BR 18//PF 100164/ WT 07106
20	PF 200010		BR 18/QUARTZO
21	PF 200020		BRS Tangará// BR 18/IPR 85
22	PF 200031		BR 18//PF 100164/ WT 07106
23	PF 160660	X	Embrapa 21/ CPAC 07434
24	PF 180135	X	Embrapa 21/ CPAC 07434

[a]Genotypes 1 to 8 are cultivars and the others are lines from the Wheat Genetic Improvement Programme of Embrapa Trigo, Brazil.

For inoculation, a conidial suspension was prepared and applied homogeneously to the plants using a 0.5 L manual atomiser. Afterwards, the plants were placed in a plastic humidity chamber. They were then kept in the dark for 24 h, in a controlled environment (Menoncin), at a temperature of 25°C and a relative humidity of 90%–95%. After 24 h, the plastic humidity chamber was removed from the plants and the photoperiod was adjusted to 12 h, keeping them at a temperature of 25°C and a relative humidity of 70%–80% for 11 days.

The determination of PoT conidial production in the rachis of wheat heads of the genotypes was performed using heads evaluated in the wheat heads resistance studies. Heads were collected at 11 days, and spikelets were removed. Four rachises per wheat genotype were evaluated in each study, with two rachises randomly selected from each pot. The rachises of each genotype were disinfected with a sodium hypochlorite solution (1%) for 1 min. Afterwards, three rinses were performed with autoclaved distilled water (ADA). Using tweezers, the rachises were placed in a Gerbox plastic box (11 × 11 × 3.5 cm) labelled with two blotting papers moistened in a standardised manner with ADA. The Gerbox boxes containing the rachises were kept in a growth chamber with a 12-h photoperiod and a temperature of 25°C for 96 h.

After this period, the number of conidia produced was counted, evaluating each rachis individually. To release the conidia, each rachis was transferred, using forceps, to a 15 mL Falcon tube containing 2 mL of ADA. The tubes were then shaken in a MA 162 tube shaker (Marcon), performing standardised movements for 20 s in the upper part of the tube. Then, the tube was turned over and the movements were repeated for another 20 s in the lower part. The number of conidia produced in the rachis was estimated using a Neubauer chamber (Loptik Labor 0.0025 mm^2) and an optical microscope with 100x magnification. Afterwards, the wet weight of each rachis was determined using an analytical balance (precision 10^{-4}). The sporulation rate of PoT conidia in the rachis was obtained by dividing the mean conidia value by the wet mass, in grams, of the rachis.

The resistance of the genotypes was evaluated based on the severity of blast, attributed to each plant and head, at 5, 7 and 11 days after inoculation (dai). From the data obtained, the area under the disease progress curve (AUDPC) was calculated for each treatment (genotype), using the formula $AUDPC = \sum [(y1 + y2)/2] * (t2 - t1)$, according to Shaner and Finney (1977).

The original data on severity (%) and AUDPC, of the leaf and head, of each genotype, were transformed into $\sqrt{x + 10}$ and submitted to analysis of variance (ANOVA). Original data on PoT conidial production per gram of rachis were transformed into $\sqrt{x}$ and, due to their wide variation, were presented graphically with values on a logarithmic scale (Log_{10}). The means were grouped using the Scott- Knott test at a 5% probability of error level. The AUDPC and sporulation variables were submitted to Pearson correlation analysis ($\alpha = 0.05$). Data analysis was performed using the R Studio program (Allaire 2012).

All genotypes showed blast symptoms on leaves and heads, in addition to PoT sporulation on the rachis. On leaves, at 5 dai, the mean blast severity ranged from 0.11% to 52.87% (Figure 1A). The genotypes that showed the lowest mean severity were PF 190018 (0.11%), ORS Feroz (0.56%), UB 2016605 (1.40%), ORS Absoluto (1.43%), TBIO Aton (2.11%), and GD 150534 (2.88%). At 7 dai, the mean severity ranged from 0.23% to 64.65% (Figure 1B). The genotypes that presented the lowest severity means were (0.23%), ORS Feroz (1.24%), TBIO Aton (1.63%), ORS Absoluto (1.94%), UB 2016605 (2.30%), GD 150534 (4.48%), and TBIO Duque (4.55%). At 11 days, the average severity ranged from 0.80% to 89.58% (Figure 1C). The genotypes that presented the lowest severity means were PF 190018 (0.80%) ORS Feroz (1.97%), TBIO Duque (2.18%), ORS Absoluto (2.35%), TBIO Aton (3.81%), GD 150534 (4.05%), and UB 2016605 (5.43%).

In the heads, at 5 dai (Figure 2A), the average severity ranged from 0.76% to 33.76%. The lowest average severity was presented by ORS Feroz (0.76%), IPF 86775 (0.94%), BR 18 (1.82%), IPF 86749 (2.19%), PF 180135 (2.20%), PF 190018 (2.28%), ORS Absoluto (3.02%), PF 160660 (3.33), PF 150505 (3.98%), PF 200010 (4.08%) and TBIO Duque (6.92%). At 7 dai (Figure 2B), the average severity ranged from 1.64% to 79.08%. The genotypes that presented the lowest mean severity were ORS Feroz (1.64%), IPF 86775 (2.65%), IPF 86749 (4.06%), PF 180135 (5.55%), PF 160660 (6.66%) and PF 190018 (7.80%). At 11 dai (Figure 2C), the mean severity ranged from 4.29% to 99.19%. The genotypes ORS Feroz, PF 180135, and IPF 86775 exhibited the lowest mean disease severity, registering 4.29%, 8.81% and 9.40%, respectively.

In the analysis of AUDPC in the leaves (Figure 3A), the values ranged from 2.41 to 395.08. Seven genotypes presented the lowest values: PF 190018 (2.41), ORS Feroz (8.21), ORS Absoluto (11.95), TBIO Aton (14.63), UB 2016605 (19.15), TBIO Duque (22.35) and GD 150534 (24.41).

In the heads (Figure 3B), the AUCPD values ranged from 14.26 to 465.85. Six genotypes, ORS Feroz (14.26), IPF 86775 (27.10), PF 180135 (36.48), IPF 86749 (46.18), PF 160660 (48.60) and PF 190018 (66.87) presented the lowest AUCPD means.

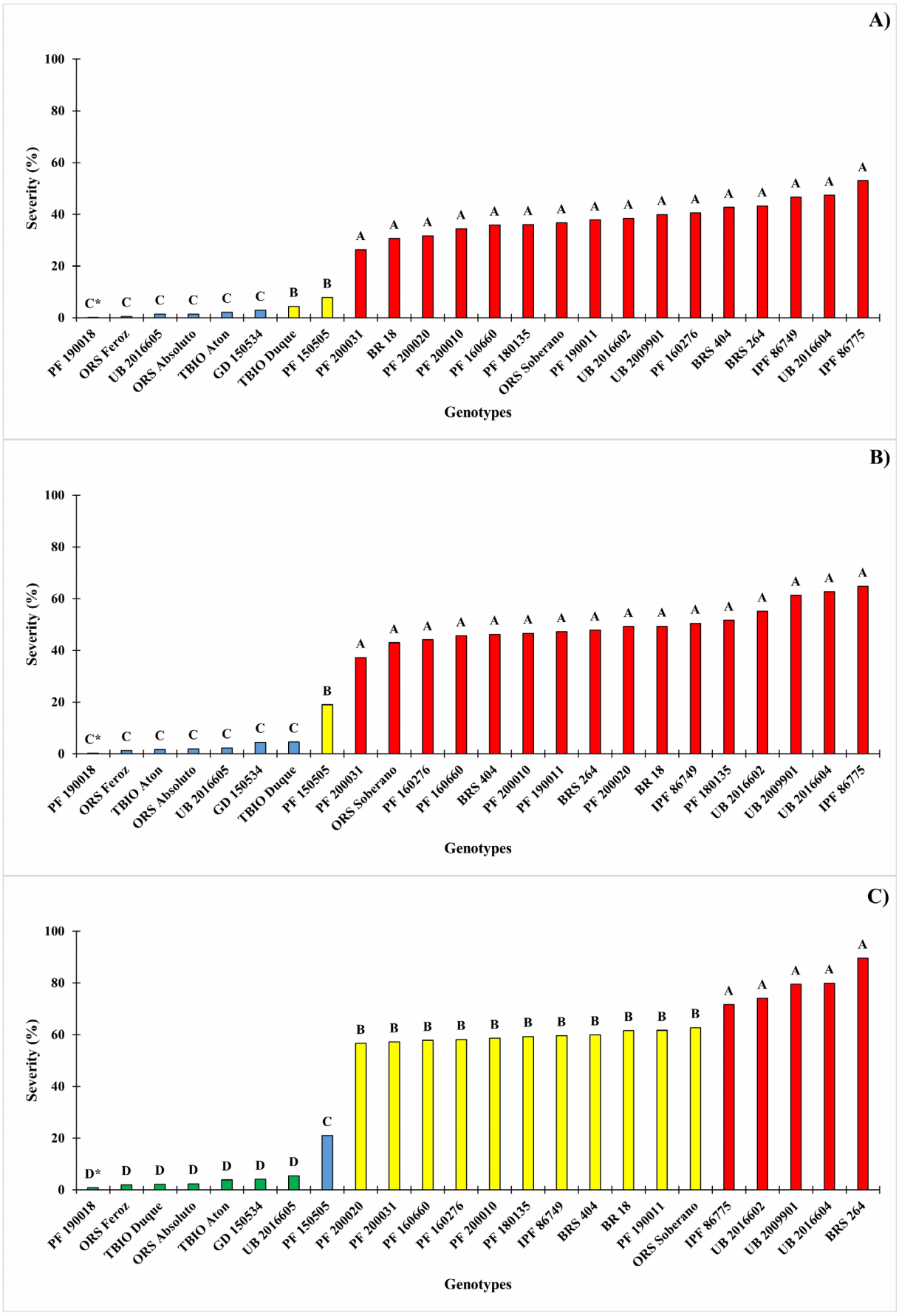

FIGURE 1 | Severity of blast in leaves of wheat genotypes at 5 (A), 7 (B), and 11 (C) days after inoculation. Means followed by the same letter do not differ from each other by the Scott- Knott test at 5% probability of error. Original data transformed to $\sqrt{x+10}$. CV (%): 5.60 (A); 4.76 (B); 4.54 (C).

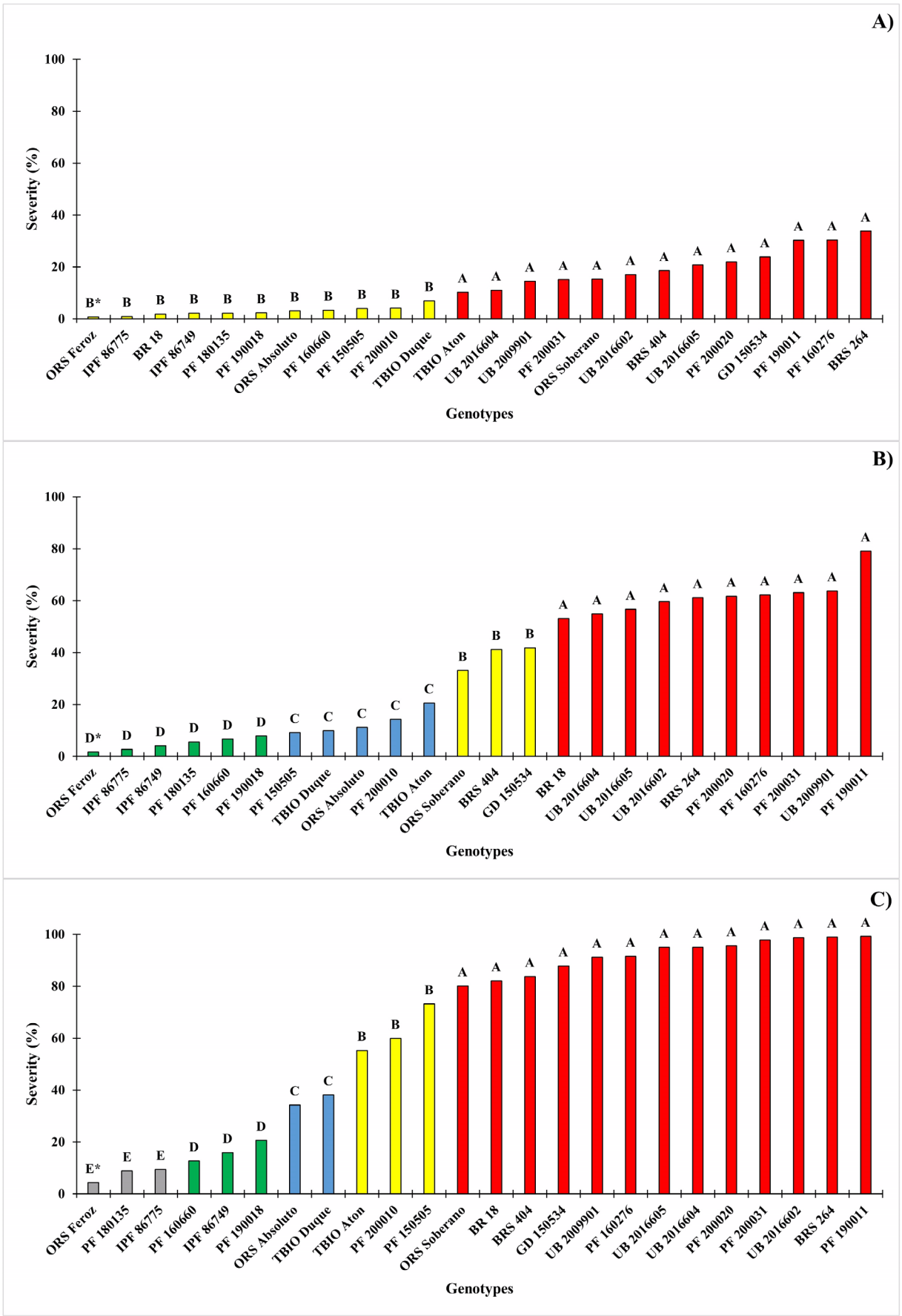

FIGURE 2 | Severity of blast in heads of wheat genotypes at 5 (A), 7 (B) and 11 (C) days after inoculation. Means followed by the same letter do not differ from each other by the Scott- Knott test at 5% probability of error. Original data transformed to $\sqrt{x+10}$. CV (%): 6.60 (A); 4.46 (B); 3.30 (C).

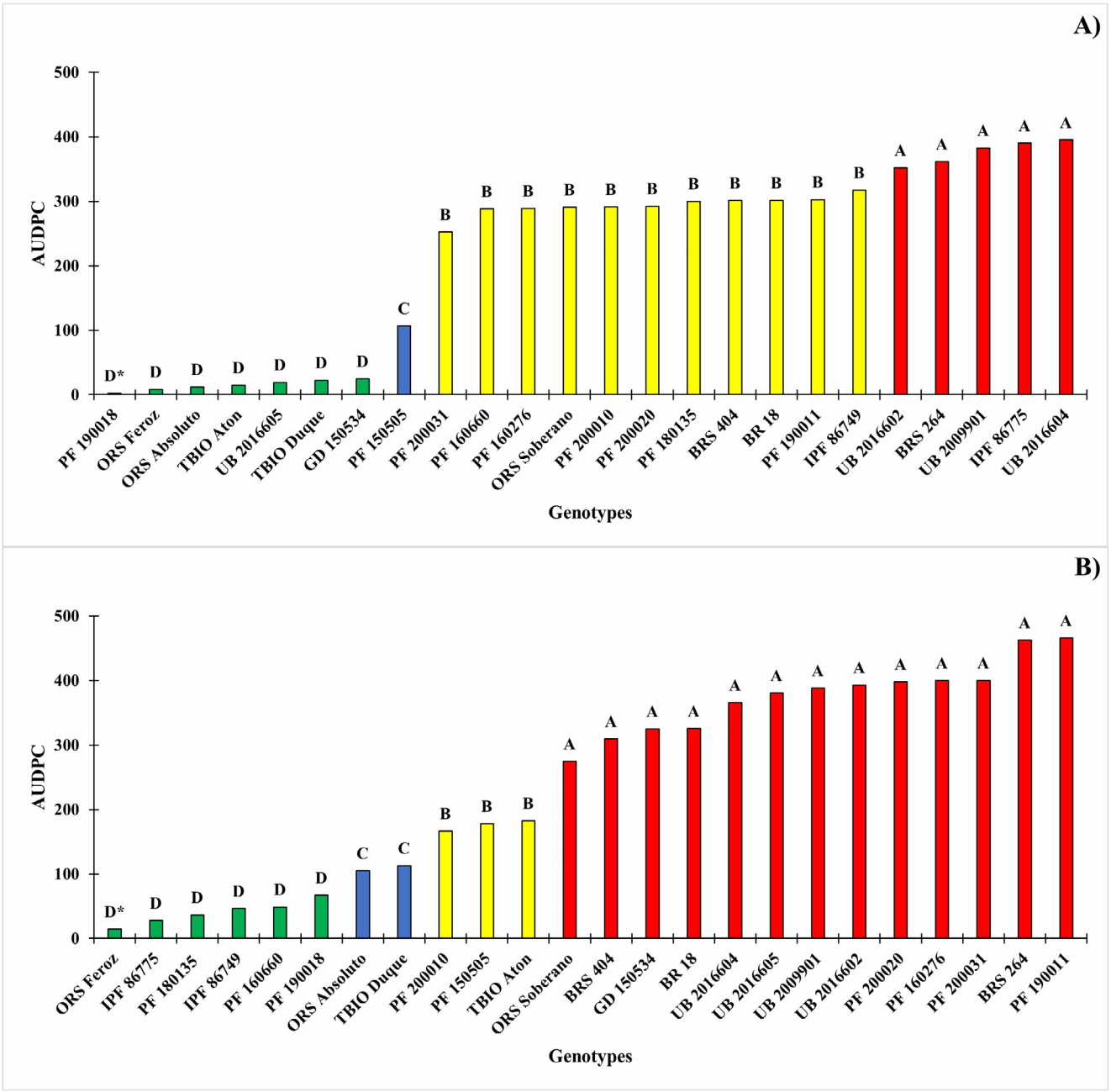

FIGURE 3 | Area Under the Disease Progress Curve (AUDPC) in Leaves (A) and Heads (B) of wheat genotypes. Means followed by the same letter do not differ from each other by the Scott- Knott Test at 5% probability of error. Original data transformed to $\sqrt{x+10}$. CV (%): 6.85 (A); 5.52 (B).

Regarding PoT sporulation, three genotype groups were formed, with Log_{10} values of conidia per gram of rachis ranging from 5.61 to 7.04 (Figure 4). The genotypes with the lowest sporulation rates were PF 160660 (5.61), IPF 86775 (5.63), PF 180135 (5.88), ORS Feroz (5.97), IPF 86749 (6.14) and TBIO Duque (6.30).

In this study, the only variables significantly correlated were the AUDPC of the head with sporulation in the rachis ($r = 0.59$; $p = 0.002$), indicating a tendency for PoT sporulation to increase as the severity of blast in the head increases. The classification of genotypes according to sporulation capacity has already been used to characterise partial resistance in other pathosystems such as soybean and the fungus *Phakopsora pachyrhizi* (Paul et al. 2011) and potato and oomycete *Phytophthora infestans* (Leclerc et al. 2019), based on the quantitative analysis of fungal sporulation (Parlevliet 1979). Likewise, for wheat blast, the classification of genotypes in relation to the quantification of the saprophytic sporulation potential of PoT in tissues, such as the rachis, can be a complementary variable in decision-making together with the severity/AUDPC analyses obtained from genotype phenotyping. In this sense, we highlight that 83.33% of the genotypes (5 of 6) classified in the group with the lowest conidial sporulation rate in the rachis, ORS Feroz, PF 160660, IPF 86775, PF 180135 and IPF 86749, were also classified in the group with the lowest AUDPC in the heads.

Despite the low frequency of resistance observed for wheat blast, the wide variation in blast severity, from 0.80% to 89.58% observed

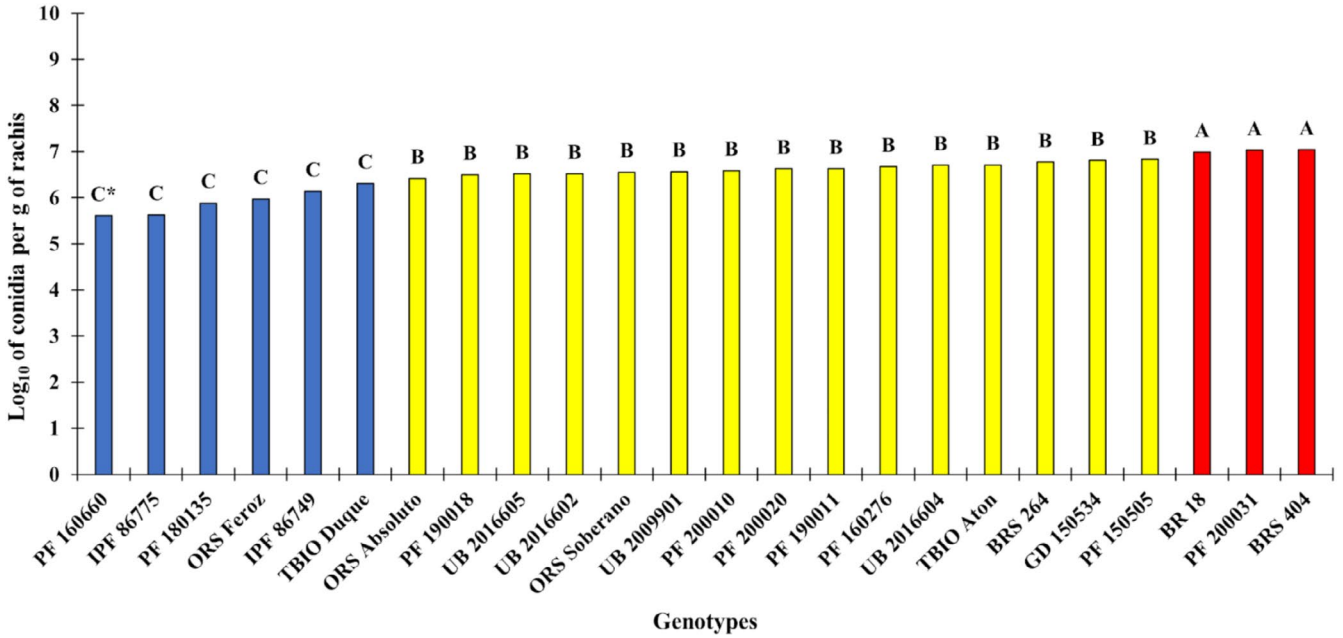

FIGURE 4 | *Pyricularia oryzae* Triticum sporulation in heads of wheat genotypes. Means followed by the same letter do not differ from each other by the Scott- Knott test at 5% probability of error. Original data transformed to $\sqrt{x}$. CV (%): 24.11.

in leaves and from 4.29% to 99.19% in heads, in addition to the significant amplitude of 10,555,453.69 in the number of conidia produced in the rachis (approximately 27.20 times more conidia between the genotype that produced the most and the one that produced the least), highlights the relevance of partial resistance for disease control. Its use was highlighted as an essential component to be integrated into blast management programmes, since, when combined with the use of fungicides, it can reduce disease severity in heads (Rios et al. 2016). In our study, we can mention the discrepancy in the results between genotypes such as IPF 86775 and PF 180135, both carriers of 2NS/AS (Table 1), as they were grouped among the genotypes most in the evaluation of resistance in leaves but which presented resistance to blast in the head, suggesting the existence of mechanisms that confer resistance only in the adult phase. In this sense, Cruz and Valent (2017) suggested that resistance to blast in heads conferred by the 2NS translocation does not guarantee resistance to blast in leaves.

On the other hand, genotypes UB 2016605 and GD 150534 showed resistance to leaf blast but were grouped among the most susceptible genotypes in the heads, together with the susceptible pattern BRS 264. A similar situation was observed in other studies in which genotypes considered resistant at the young plant stage did not confirm resistance in adult plants (Maciel et al. 2014). Although for other realities, such as Bangladesh, Asia, it is recommended to focus only on evaluations of resistance to head blast in the heads (Roy et al. 2021), our study highlights the relevance of including both stages of wheat development in evaluations for resistance to the disease, since evaluations in young plants evaluating only leaves, despite being faster and more economical, do not replace selection for resistance to head blast.

The identification of genotypes such as ORS Feroz and PF 190018, which exhibit resistance in both leaf and head, is extremely desirable to ensure greater safety in wheat cultivation, especially because infected leaves serve as a source of inoculum for head infection (Dorigan et al. 2024). Indeed, further investigation is needed to identify the molecular and physiological bases for the resistance observations.

In wheat, identifying the most appropriate sowing time contributes to reducing disease pressure, optimising control efficiency, increasing yield and prolonging the durability of genetic resistance (Naseri and Fareghi 2024). In this sense, we highlight that the management of blast using resistant genotypes needs to be complemented with sowing at appropriate times (Kohli et al. 2011), in order to reduce the exposure of the crop to hot and rainy conditions favourable to the pathogen that causes blast (Valent et al. 2021). We also recommend future studies during blast epidemics, in more than one growing season in the field, preferably combining the use of fungicides and evaluating productivity.

In this study, the resistance of wheat genotypes to blast was investigated. The data presented are useful (i) to guide the selection of genotypes for breeding, (ii) for decision-making regarding the release of commercial cultivars and (iii) for the positioning of wheat cultivars in different environments.

Author Contributions

João Leodato Nunes Maciel: conceptualisation; **Daniela da Silva, Mateus Mossolin and Marcos Kovaleski:** writing – original draft preparation; **João Leodato Nunes Maciel:** writing – review and editing; **João Leodato Nunes Maciel** and **Carolina Cardoso Deuner:** supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jph.70066.

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