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Unravelling the Severity Landscape of Brazilian *Magnaporthe oryzae* Triticum on Wheat Cultivars With and Without 2N^{V}S Translocation

Eduardo Alves da Silva[1] | Joao Leodato Nunes Maciel[1] | Carlos Augusto Pizolotto[2] | Luciano Consoli[1] | Eliseu Binneck[3] | Gisele Abigail Montan Torres[1] | Ana Lidia Variani Bonato[1] | Jose Mauricio Cunha Fernandes[1] | Walter Boller[2]

[1]Empresa Brasileira de Pesquisa Agropecuária (Embrapa Trigo), Passo Fundo, Rio Grande do Sul, Brazil | [2]Graduate Program in Agronomy of Universidade de Passo Fundo (PPGAgro/UPF), Passo Fundo, Rio Grande do Sul, Brazil | [3]Empresa Brasileira de Pesquisa Agropecuária (Embrapa Soja), Londrina, Paraná, Brazil

Correspondence: Joao Leodato Nunes Maciel (joao.nunes-maciel@embrapa.br)

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ABSTRACT

Wheat blast caused by *Magnaporthe oryzae* Triticum (MoT) threatens wheat production, affecting plants at various growth stages. Resistant cultivars, linked to the 2N^{V}S chromosomal translocation, offer potential control strategies, though questions persist about their effectiveness against diverse MoT isolates. This study aimed to (a) characterise and compare the virulence of 77 MoT isolates from wheat cultivated in Brazil based on the reaction of a set of 11 wheat cultivars to the pathogen infection; (b) characterise and compare the wheat genotypes based on their response to infection by MoT isolates, and correlate the presence of the 2N^{V}S translocation; and (c) identify effector genes and their variations among isolates through whole-genome sequencing of a subgroup of 25 representative Brazilian MoT isolates and analyse the effectors concerning the isolates' severity. A randomised design measured blast severity on detached wheat heads 7 days post-inoculation. Significant genotype-by-isolate interactions were observed, revealing that some isolates caused severe symptoms even in cultivars carrying 2N^{V}S. Resistance conferred by 2N^{V}S depended on the genetic background of the cultivars. Cultivar BRS 229, lacking 2N^{V}S, exhibited great resistance, comparable to Jagger and Santa Fe. Effector gene analysis identified 47 candidate genes, seven of which showed variations correlating with isolate aggressiveness. The findings highlight the complex interaction between MoT virulence and wheat genotypes, underscoring the need to broaden resistance strategies. These results provide essential insights into managing wheat blast, emphasising the importance of integrating genetic diversity in resistance breeding programmes, and the sequencing of 25 complete MoT genomes in this study provides a critical genomic foundation for future research in this important pathogen.

1 | Introduction

The genus *Magnaporthe* includes several species of pathogenic fungi that cause the disease called blast'. Among these fungal species, *Magnaporthe oryzae* (synonym to *Pyricularia oryzae*; Zhang et al. 2016) is the most important pathogen, due to its worldwide distribution in plants of the Poaceae family (Mehta and Baier 1998). The pathotype associated with the wheat crop is Triticum, a classification that has made the nomenclature *M. oryzae* Triticum (MoT) more commonly used.

Although it can occur at any stage of the development of wheat plants, the main damage caused by MoT in wheat crops is bleached spikes (Cruz and Valent 2017; Torres et al. 2022). The chemical control of the disease in these circumstances, which has been one of the practices used by wheat growers in South America, has been disappointing as it has many limitations (Cruz et al. 2019; Islam et al. 2019).

The use of resistant cultivars is a fundamental strategy for controlling wheat head blast (WHB), offering an effective and sustainable solution. Genetic resistance plays a crucial role in integrated disease management, reducing reliance on chemical control and enhancing long-term resilience in wheat production. Compared to the *Magnaporthe oryzae* Oryza–rice pathosystem, little is known about the molecular basis of the MoT–wheat interaction. Few genes have been reported as key effectors associated with resistance to WHB. Six resistance (R) genes have been reported in hexaploid and tetraploid wheat. The R genes *Rmg2* and *Rmg3* were identified in the bread wheat cultivar Thatcher (Zhan et al. 2008). *Rmg7*, found in the tetraploid wheat *Triticum dicoccum*, confers resistance to both leaf and spike infections (Tagle et al. 2015), and *Rmg11* was also found in tetraploid *T. dicoccum* (Islam et al. 2024); *Rmg8*, from the bread wheat cultivar S-615, offers partial resistance to leaf and spike infections caused by specific MoT strains (Anh et al. 2015). *RmgGR119*, identified in the wheat landrace GR119, works in combination with *Rmg8* to confer strong resistance to spike infections caused by Bangladeshi isolates (Horo et al. 2020; Wang et al. 2018), but they are less effective against recent MoT isolates (Navia-Urrutia et al. 2022).

Studies have also demonstrated the association between the presence of the $2N^VS$ translocation from *Aegilops ventricosa* in the wheat genotype and resistance to wheat blast; this finding has played an important role in opening new perspectives for disease control (Cruz et al. 2016; Hossain 2022; Singh et al. 2021; Vancini et al. 2023). The importance of sequence $2N^VS$ as a factor in the control of wheat blast has been demonstrated in evaluations of the reaction of cultivars to the disease under field conditions (Maciel et al. 2020). Furthermore, the interest in studying the $2N^VS$ sequence in relation to wheat blast has increased quite intensely (Ferreira et al. 2020; He et al. 2021), including the possibility of combining the effect of the sequence with other genes of resistance to the disease (Cruppe et al. 2020; Goddard et al. 2020).

A key question is whether the blast resistance conferred by $2N^VS$ could be overcome—a warning previously raised by Cruppe et al. (2021). Additionally, the resistance spectrum of $2N^VS$ against the genetic diversity of MoT isolates remains unclear. The interaction of the pathogen with the environment may favour the variability of MoT, generating more aggressive isolates and contributing effectively to this resistance breaking (Cruz and Valent 2017). Maciel et al. (2014) and Ceresini et al. (2018) evaluated populations of MoT from different Brazilian wheat-producing regions, finding significant variability in virulence among these populations. Recent studies have revealed substantial variability in genotype-by-isolate interaction (GII), indicating that the genetic background of the isolate or the wheat genotype may play a critical role in defining resistance (Cardozo Téllez et al. 2019; Ferreira et al. 2020; Inoue et al. 2020; Latorre et al. 2023; Martinez et al. 2019; Navia-Urrutia et al. 2022).

The *Rmg* avirulence group (*AVR*) of effectors plays a critical role in plant–pathogen interactions. These effectors are recognised by specific R genes in the host plant, triggering a hypersensitive response that leads to localised cell death and prevents the spread of the pathogen. This mechanism is a key aspect of the plant's immune system, helping to determine host species specificity and providing resistance against certain pathogens (Anh et al. 2018; Cumagun et al. 2014; Hossain 2022; Wang et al. 2018). So far, *AVR-Rmg8* is the most studied gene, which is recognised by two wheat resistance genes, *Rmg8* and *Rmg7* (Anh et al. 2018; Asuke et al. 2024). Three alleles of *AVR-Rmg8, eI*, *eII* and *eII′*, have been identified in isolates from South America and Bangladesh (Horo et al. 2020; Wang et al. 2018); two other alleles, *eII″* and *eII‴*, have been described by Latorre et al. (2023) and two other variants have been identified by Navia-Urrutia et al. (2022). Among these, the *eI* allele has shown greater effectiveness in triggering *Rmg8*-mediated resistance (Latorre et al. 2023).

In contrast to the limited understanding of the MoT–wheat interaction, the rice blast disease caused by *M. oryzae* Oryza has been extensively studied. Over 100 R genes have been identified in rice, of which at least 35 have been molecularly characterised (Kalia and Rathour 2019; Kou et al. 2024; Liu et al. 2024). In rice blast, resistance breakdown often results from the deletion of the corresponding *AVR* gene in the pathogen (Navia-Urrutia et al. 2022). Point mutations and transposon insertions can also inactivate *AVR* genes, enabling the pathogen to evade host resistance.

These circumstances contribute to the perception that fully successful generation of wheat cultivars with adequate blast resistance involves identifying and monitoring the virulence spectrum of MoT (Maciel et al. 2014). However, some aspects related to this issue still need to be considered for the phenotypic evaluation of wheat's reaction to pathogen inoculation. One of them refers to the definition of a set of wheat genotypes to differentiate MoT isolates (Cruppe et al. 2020). The other refers to the procedures to evaluate the virulence of the pathogen. In this respect, it is important to highlight that Maciel et al. (2014), Goddard et al. (2020) and Horo et al. (2020) have already demonstrated that the response of wheat detached heads is appropriate for evaluating the reaction of wheat genotypes to infections of MoT.

Understanding the dynamics of effector gene function in MoT is essential for effective disease resistance management, as these genes play a crucial role in the pathogen–host interaction, determining infection specificity and pathogen aggressiveness. Joint analysis of genomic sequencing data from isolates with disease severity data in different genetic backgrounds allows for indirect validation of the importance of genes in the fungal virulence process.

The objectives of this study were to (a) characterise and compare the virulence of MoT isolates from wheat cultivated in

Brazil based on the reaction of a set of 11 wheat cultivars to the pathogen infection; (b) characterise and compare the wheat genotypes based on their response to infection by MoT isolates, and correlate the presence of the $2N^VS$ chromosomal translocation from *A. ventricosa* with resistance to blast in these genotypes; and (c) identify effector genes and their variations among isolates through whole-genome sequencing of a subgroup of 25 representative Brazilian MoT isolates and analyse the effectors in relation to isolate severity.

2 | Materials and Methods

2.1 | Wheat Genotypes

The experiment was conducted at Embrapa Trigo, a Embrapa unit located in Passo Fundo, RS, Brazil. Eleven wheat cultivars were used in this experiment: five cultivars not carrying the $2N^VS$ translocation (Anahuac 75, BRS 229, BRS 234, BRS Buriti and Pampeano) and six cultivars carrying the $2N^VS$ translocation (Fuller, Jackpot, Jagger, Renan, Santa Fe and TBIO Mestre).

2.2 | Plant Material

Plants were grown in 8 L pots containing soil that was fertilised and pH-corrected according to its chemical analysis. Before being sown in the pots, seeds of the cultivars Fuller, JackPot, Jagger, Renan and Santa Fe were vernalised at 4°C for 45 days. After emergence, six plants per pot were maintained and grown in greenhouse conditions until the flowering stage, Z63 (anthesis) according to Zadoks et al. (1974).

Genomic DNA of the wheat cultivars was extracted from leaf tissues using a CTAB-based protocol (Bonato 2008; Saghai-Maroof et al. 1984). The quality of all DNA samples was evaluated on a 0.8% agarose gel stained with ethidium bromide, and the amount of total DNA was measured using Quant-iT PicoGreen ds reagent (ThermoFisher Scientific). The fluorescence was measured (Ex 480 nm/Em 520 nm) on a FLUOstar Omega plate reader (BMG LABTECH).

The presence of the $2N^VS$ translocation in wheat genotypes was validated using a molecular marker, according to Helguera et al. (2003), using primers VENTRIUP (5′-AGGGG CTACTGACCAAGGCT-3′) and LN2 (5′-TGCAGCTACAGC AGTATGTACACAAAA-3′), which generate a product of 259 base pairs.

2.3 | *Magnaporthe* Isolates and Inoculum Preparation

Seventy-seven monosporic isolates of MoT from the collection of the Phytopathology Laboratory of the Embrapa Trigo were used in the experiments (Pizolotto 2019). These isolates were obtained from segments of symptomatic wheat plants collected in seven Brazilian states from 2012 to 2017 (Figure 1; Table S1). The monosporic isolation and preservation were performed following the methodology described by Cruz et al. (2009) and Maciel et al. (2014).

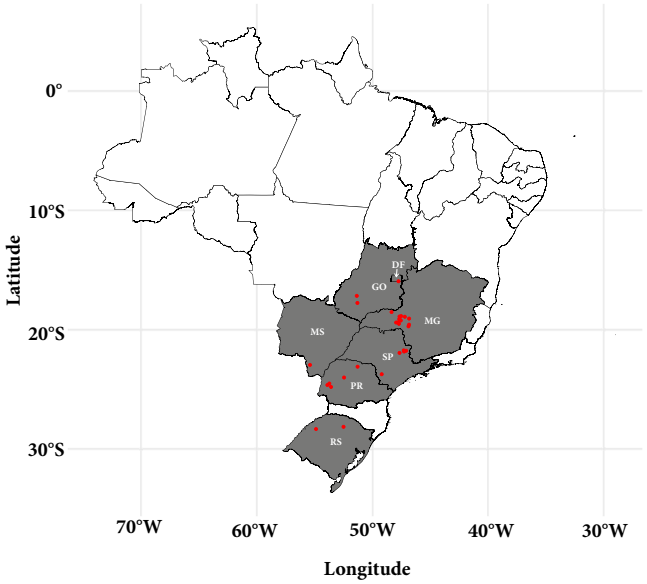

FIGURE 1 | Geographic distribution of pathogen isolate sampling sites across Brazil, highlighting the states of Rio Grande do Sul (RS), Paraná (PR), São Paulo (SP), Mato Grosso do Sul (MS), Minas Gerais (MG), Distrito Federal (DF) and Goiás (GO). Site of sampling points are marked in red. [Colour figure can be viewed at wileyonlinelibrary.com]

2.4 | Inoculation and Symptom Evaluation

Previously, the 77 isolates of MoT were preserved on filter paper. Subsequently, each isolate was transferred individually to Petri dishes containing oat agar medium. The Petri dishes were then incubated for 12–15 days under fluorescent lamps with a 12-h photoperiod at 25°C (Atkins et al. 1967; Ribeiro and Terres 1987).

Colonies of MoT were flooded with distilled water plus Tween 80 adhesive spreader (0.01%) and scraped with the aid of a brush to dislodge the conidia from the conidiophores. Then, the solution was filtered with four layers of sterile cheesecloth to eliminate the mycelia and any remaining medium. The conidia were counted in a Neubauer chamber under an optical microscope, and the concentration was adjusted to 10^5 conidia/mL.

Wheat heads were detached using scissors and fixed in phenolic foam cubes presaturated with water, and rachis were wrapped in plastic film. These cubes were then arranged in trays (40 × 20 × 10 cm), and the inoculation process was carried out with the aid of a 0.5 L plastic atomiser. After inoculation, wheat heads were kept in plastic bags for 24 h in a humid chamber. The photoperiod was then adjusted to 12 h, and the humidity was set to 70%. Seven days later, blast severity (BS) was evaluated through visual observation, ranging from 0% to 100%. Blast severity, expressed in percentage, refers to the diseased area of wheat heads and was used as a measure of pathogen severity.

2.5 | Statistical Analysis

The experimental design was a completely randomised design involving 11 cultivars inoculated with 77 isolates. The experiment was replicated once, where the detached heads fixed in each cube of phenolic foam were considered a repetition. Then, each cultivar was inoculated with 77 isolates with six repetitions, totalling 10,164 evaluated detached heads.

Statistical analyses were performed using the original data obtained from the evaluations on adult plants (detached heads). The adjusted means were estimated using the best linear unbiased estimator (BLUE) employing the analysis of mixed models:

$$Y_{ij} = \mu + I_i + G_j + GI_{ij} + Exp_k + t2NS_l + \varepsilon_{ijkl}$$

where Y_{ij} represents the observed data, μ denotes the fixed mean effect (intercept), with $E(\mu)=\mu$. I_i signifies the isolate effect (fixed effect), G_j represents the wheat cultivar effect (fixed effect), GI_{ij} represents the GII (fixed effect), Exp_k represents the experiment effect (fixed effect), $t2NS_l$ represents the presence of translocation effect (random effect) and ε_{ijkl} indicates the error deviation associated with Y_{ij}, with $E(\varepsilon_{ij})=0$ and $E(\varepsilon^2_{ij})=\sigma^2\varepsilon$. The linear mixed models were fitted using the 'lmer' function from the lme4 package (Bates et al. 2015) in the R software (R Development Core Team 2024).

The BLUEs were used to calculate Euclidean distances, and UPGMA clustering analysis was performed using the complete linkage method with R package pheatmap v. 1.0.12 (Kolde 2018) to identify how the isolates and cultivars are grouped based on the BS values. Our team decided to use a four-colour coding system to empirically classify the dataset values, solely to facilitate the visualisation of value variation. The response of wheat cultivars to infection by the isolate was categorised into four colour-coded classes as follows: dark green, BS $\leq$10%; light green, 10% < BS $\leq$30%; light yellow, 30% < BS $\leq$50%; and yellow, BS > 50%.

2.6 | Fungal Genomic DNA Extraction

Genomic DNA of the monosporic isolates of MoT was extracted using a CTAB-based protocol (Bonato 2008; Saghai-Maroof et al. 1984), with modifications. CTAB contained dithiothreitol at a working ratio of 0.16 g/100 mL and 10 μL of proteinase K at 10 mg/mL per sample. Before adding chloroform/isoamyl alcohol, the supernatant was transferred to a tube. Quantification was performed on a 1.5% agarose gel, stained with ethidium bromide, at 100 V for 2 h.

2.7 | Fungal Genomic Analyses

The genomic DNA samples of 25 monosporic MoT isolates were subjected to library construction using an Illumina DNA PCR-Free Preparation Kit. The genomic libraries were sequenced using Illumina NovaSeq6000 technology with the execution of 300 paired-end cycles (2 × 150 cycles) and production of at least 5 Gb of data per genome, which is equivalent to a minimum coverage of 100×, considering that each genome has an approximate size of 41 Mb.

The sequencing data were processed in paired-end mode using Trimmomatic v. 0.39 (Bolger et al. 2014) for quality trimming and primers/adapters clipping, with the parameters -phred33 ILLUMINACLIP:NexteraPE-PE.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:91 and de novo assembled using Spades v. 4.0.0 (Prjibelski et al. 2020) with default parameters. Potential misassemblies were corrected using RagTag v. 2.1.0 (Alonge et al. 2019) and the assembly GCA_004785725.2 (NCBI BioProject PRJNA355407) of MoT strain B71 (Peng et al. 2019) as a reference.

Gene prediction was performed with evidence-driven and ab initio approaches using GeneMark-ET v. 4.68 (Lomsadze et al. 2014), AUGUSTUS v. 3.5.0 (Stanke et al. 2008), SNAP (Korf 2004), CodingQuarry v. 2.0 (Testa et al. 2015), and Glimmerhmm v. 3.0.4 (Majoros et al. 2004). RNA-seq data of MoT strain B71 from NCBI Sequence Read Archive SRR9126640 and SRR9127597 (BioProject PRJNA355407; Peng et al. 2019) were used as transcript evidence. The consensus structures for gene annotations were computed using EVidenceModeler (Haas et al. 2008) and updated using PASA v. 2.5.3 (Haas et al. 2003). Signal peptide domains were detected using SignalP v. 4.1 (Nielsen 2017), and transfer RNA (tRNA) genes were predicted using tRNAscan-SE v. 2.0.12 (Chan and Lowe 2019).

The putative functions of genes were predicted by pattern matching with the InterPro (Blum et al. 2025), UniProtKB/Swiss-Prot (UniProt Consortium 2023), BUSCO/eudicotyledons_odb10 (Waterhouse et al. 2018), Pfam (Mistry et al. 2021), eggNOG (Huerta-Cepas et al. 2016), MEROPS (Rawlings et al. 2018) and CAZy (Drula et al. 2022) databases. Putative effectors were predicted from the annotation results and sequence comparison with the gene models annotated as known effectors or putative effectors in the genomes of *P. oryzae* from MycoCosm (Nordberg et al. 2014) and Uniprot (UniProt Consortium 2023).

The minimum and maximum numbers of predicted gene models in the 25 MoT sequenced genomes were 11,546 and 11,875, respectively. From all the predicted proteins from the protein-coding gene models, 500 BUSCO orthologues among the 25 genomes were randomly selected and concatenated for a phylogenomic analysis using IQ-TREE v. 2.3.6 (Minh et al. 2020).

3 | Results

3.1 | Assessment of Blast Severity Across Wheat Cultivars

Heads of all cultivars presented blast symptoms at 7 days after inoculation (dai), even those with the highest level of resistance. The present study showed significant variation in severity among MoT isolates in Brazil, with a 3- to 4-fold difference observed between the most aggressive and least aggressive isolates (17% versus 56% mean BS) at 7 dai.

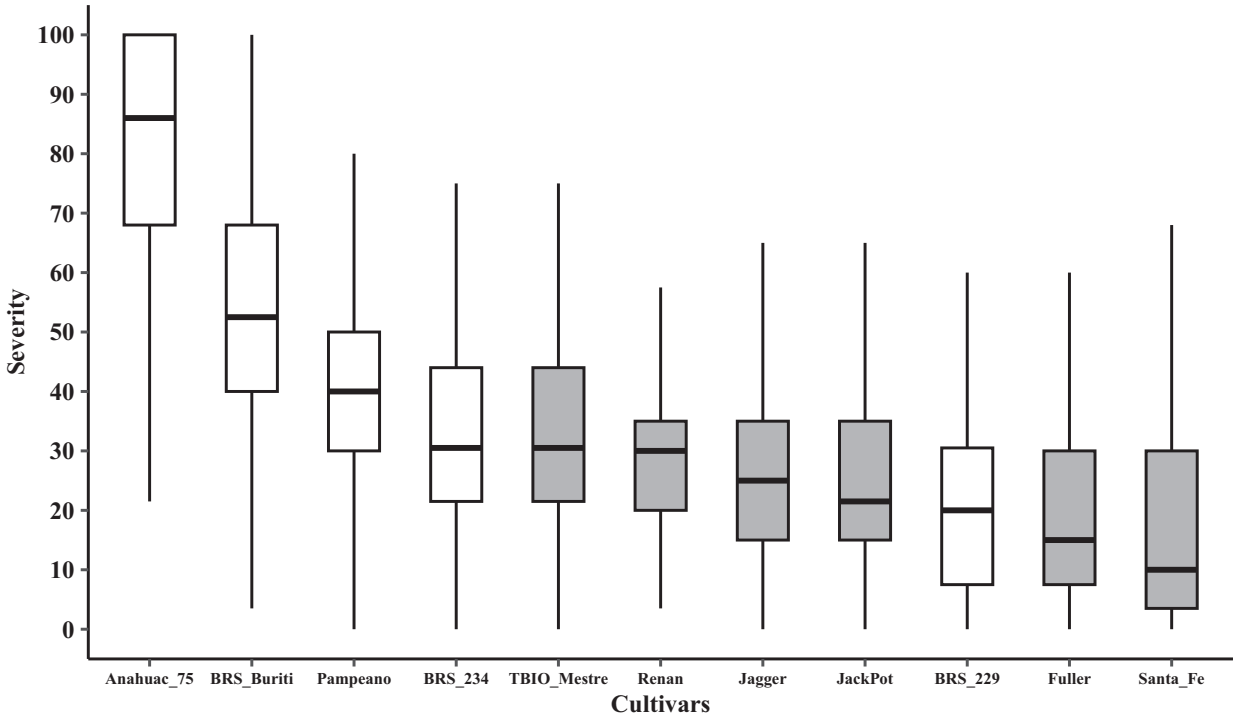

FIGURE 2 | Boxplot of blast severity percentage means on detached wheat heads subjected to inoculation with spore suspension of *Magnaporthe oryzae* Triticum (MoT) isolates. Grey and white boxes show wheat cultivars with and without the $2N^VS$ translocation, respectively.

TABLE 1 | Analysis of variance for fixed effects of the model for blast severity (BS) on detached wheat heads.

	df	SS	MS	*F*	*p*
Cultivar	10	217.140	27.140	2341.098	4×10^{-4}***
Isolate	76	80.425	1.058	114.093	2×10^{-16}***
Exp	1	1.034	1.034	111.523	2×10^{-16}***
Cultivar × Isolate	760	102.95	0.135	14.605	2×10^{-16}***
Residuals	9311	87.43	0.009		

***Significant at $\alpha = 0.0001$.

The mean value of BS for each inoculated wheat cultivar is shown in Figure 2. It was observed that cultivars BRS 229, Fuller, Jack Pot, Jagger, Renan and Santa Fe presented BS means below 30%. Cultivars BRS 234, Pampeano and TBIO Mestre presented BS means between 31% and 50%, whereas cultivars BRS Buriti and Anahuac 75 exhibited the highest BS means, reaching 53.6% and 81.7%, respectively.

3.2 | Analysis of Variance

The ANOVA results revealed a highly significant effect of wheat cultivars, MoT isolates, and their interaction ($p = 2\times10^{-16}$). Wheat cultivar × MoT isolate interaction was highly significant, indicating that the qualitative response has a highly relevant influence on the interaction between the isolates and the 11 cultivars (Table 1). Although the BS means of cultivars carrying the $2N^VS$ translocation were lower (24.71%) compared to the BS means of non-$2N^VS$ cultivars (45.8%), the effect of the translocation was not significant by likelihood ratio test (Table S2).

3.3 | Distribution of Head Blast Severity in Wheat Cultivars as a Result of Isolate × Cultivar Interaction

The wheat cultivars were not grouped according to the presence (black) or absence (white) of the $2N^VS$ translocation, as shown in Figure 3. Although several evaluations have demonstrated the positive effect of $2N^VS$ on blast resistance using a single fungal isolate (Cardozo Téllez et al. 2019; Cruz et al. 2016; Ferreira et al. 2020), our study reveals that the genetic background influences the observed effect on disease levels when a diverse set of MoT isolates is evaluated.

Furthermore, Figure 3 clearly illustrates the interaction between wheat cultivars and pathogen isolates. Among the cultivars analysed, only Anahuac 75 exhibited a reaction stability across the 77 isolates inoculated, with BS values consistently above 50% (yellow). In contrast, the other cultivars showed BS values distributed across at least three of the four BS value classes. Notably, even the TBIO Mestre cultivar, which carries the $2N^VS$, displayed BS values ranging from 7.5% to 74.8%, depending on the isolate used (Table S2). It is interesting to note the resistance of wheat

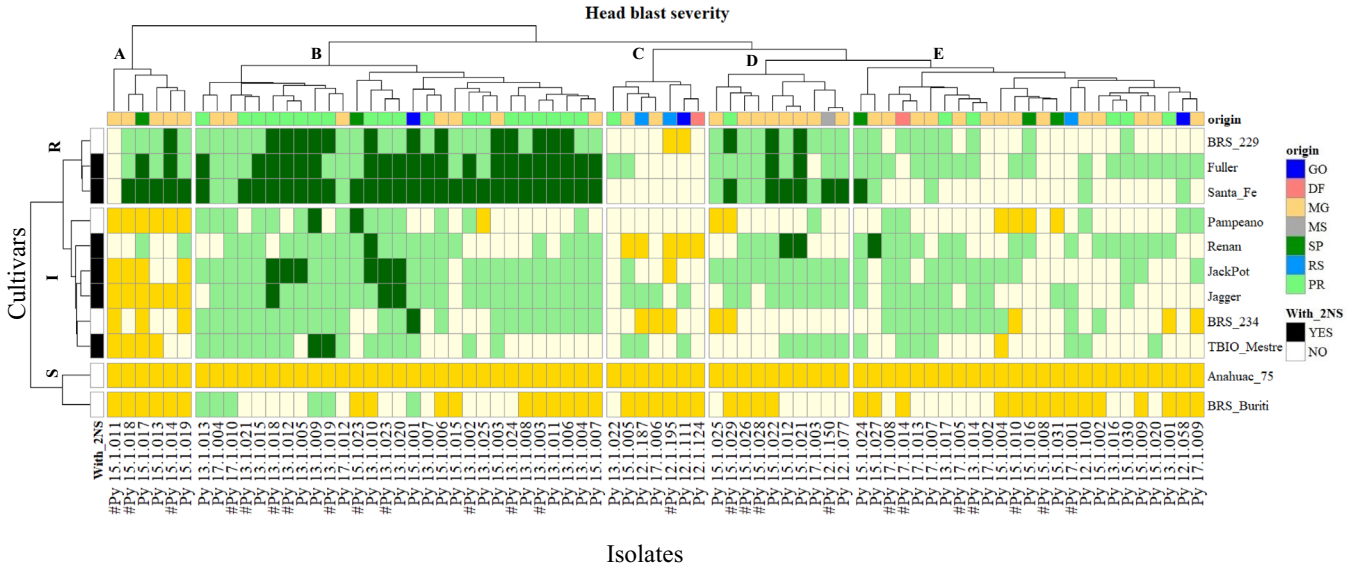

FIGURE 3 | Heatmap representing the hierarchical cluster of cultivars (lines) and isolates (columns) based on mean of blast severity (BS) on wheat heads inoculated with spore suspension of *Magnaporthe oryzae* Triticum (MoT) isolates. BS was categorised into four classes as follows: dark green, BS ≤10%; light green, 10% < BS ≤30%; light yellow, 30% < BS ≤50%; and yellow, BS > 50%. #, the 25 MoT isolates used for DNA sequencing. Clusters of isolates are labelled A to E for reference in the text. Isolates originated from the states of Rio Grande do Sul (RS), Paraná (PR), São Paulo (SP), Mato Grosso do Sul (MS), Minas Gerais (MG), Distrito Federal (DF) and Goiás (GO). Cultivar responses were classified as susceptible (S), intermediate (I) or resistant (R). [Colour figure can be viewed at wileyonlinelibrary.com]

cultivar BRS 229 to most MoT isolates, as this cultivar lacks the well-known source of blast resistance $2N^VS$ translocation.

Hierarchical cluster analysis revealed the presence of distinct groups of wheat cultivars sharing similar resistance or susceptibility patterns with the tested MoT isolates (Figure 3). Although there was a previous classification of the reaction of wheat cultivars to MoT into four groups (dark green, light green, light yellow and yellow), the analysis revealed a clear division of the 11 inoculated cultivars into three categories of reaction to the pathogen, namely resistant (R), intermediate (I) and susceptible (S).

The 'R' category consisted of cultivars BRS 229, Fuller and Santa Fe, presenting consistently low levels of BS, mainly within the ranges BS ≤10% (dark green) or 10% < BS ≤30% (light green). For the majority of the 77 MoT isolates inoculated, the mean of BS values was 18.44%. The intermediate (I) category comprised BRS 234, Jackpot, Jagger, Pampeano, Renan and TBIO Mestre with a BS mean of 31.13%. These cultivars showed increased susceptibility when faced with some specific isolates, with BS means above 50%, especially when inoculated with MoT isolates Py 15.1.011, Py 15.1.013, Py 15.1.017 or Py 15.1.018. Finally, the susceptible (S) wheat cultivars Anahuac 75 and BRS Buriti were grouped with higher BS means (67.60%).

The MoT isolates were grouped into five clusters (A–E) as shown in Figure 3. Cluster C stands out with seven isolates (Py 12.1.111, Py 12.1.124, Py 12.1.187, Py 12.1.195, Py 13.1.022, Py 15.1.005 and Py 17.1.006), inducing high BS values for all wheat cultivars inoculated. This group of isolates presented a BS average of 45.47% (30% higher than the mean). This cluster comprises the most aggressive isolates, which were collected in the Brazilian States PR, MG, RS and DF. From these regions, there is at least one MoT isolate able to infect wheat cultivars whose resistance is associated with the presence of the $2N^VS$ translocation.

Cluster B had the highest predominance of the dark green colour, indicating lower levels of BS. This cluster is mainly composed of isolates from PR (line of origin, green colour), which were revealed as less aggressive to inoculated wheat cultivars. The other isolates are distributed among the other branches of the dendrogram (A, D and E).

3.4 | Genomic Analysis of MoT Isolates

Using genomic data, maximum-likelihood (ML) phylogeny of the 25 isolates representative of the Brazilian MoT population showed a distinct regional pattern (Figure 4). These isolates, collected in several locations in Brazil, were phylogenomically analysed, revealing a significant correlation between genetic proximity and Brazilian morphoclimatic domains. The Brazilian morphoclimatic and phytogeographic domains are large territorial units characterised by their similarity in factors such as relief, climate, soil types, vegetation and hydrological conditions. These domains reflect the interaction and harmony of natural elements (Ab'Sáber 2003).

The isolates from Minas Gerais (MG), Mato Grosso do Sul (MS) and Distrito Federal (DF) formed a clade, indicating greater genetic similarity among them. These states have a predominance of the Cerrado morphoclimatic domain. Meanwhile, the isolates from Paraná (PR), São Paulo (SP) and Rio Grande do Sul (RS) also showed greater similarity, representing morphoclimatic domains Mares de Morros and Araucárias (Figure 4). This cluster grouped the isolates in such a way that the upper clade (G1) had a lower BS average (29%) compared to the isolates in the lower clade (G2; 39%).

To identify effector genes in the whole-genome sequencing data of 25 Brazilian MoT isolates, an in silico analysis was conducted using the annotations and functional predictions of the genomes.

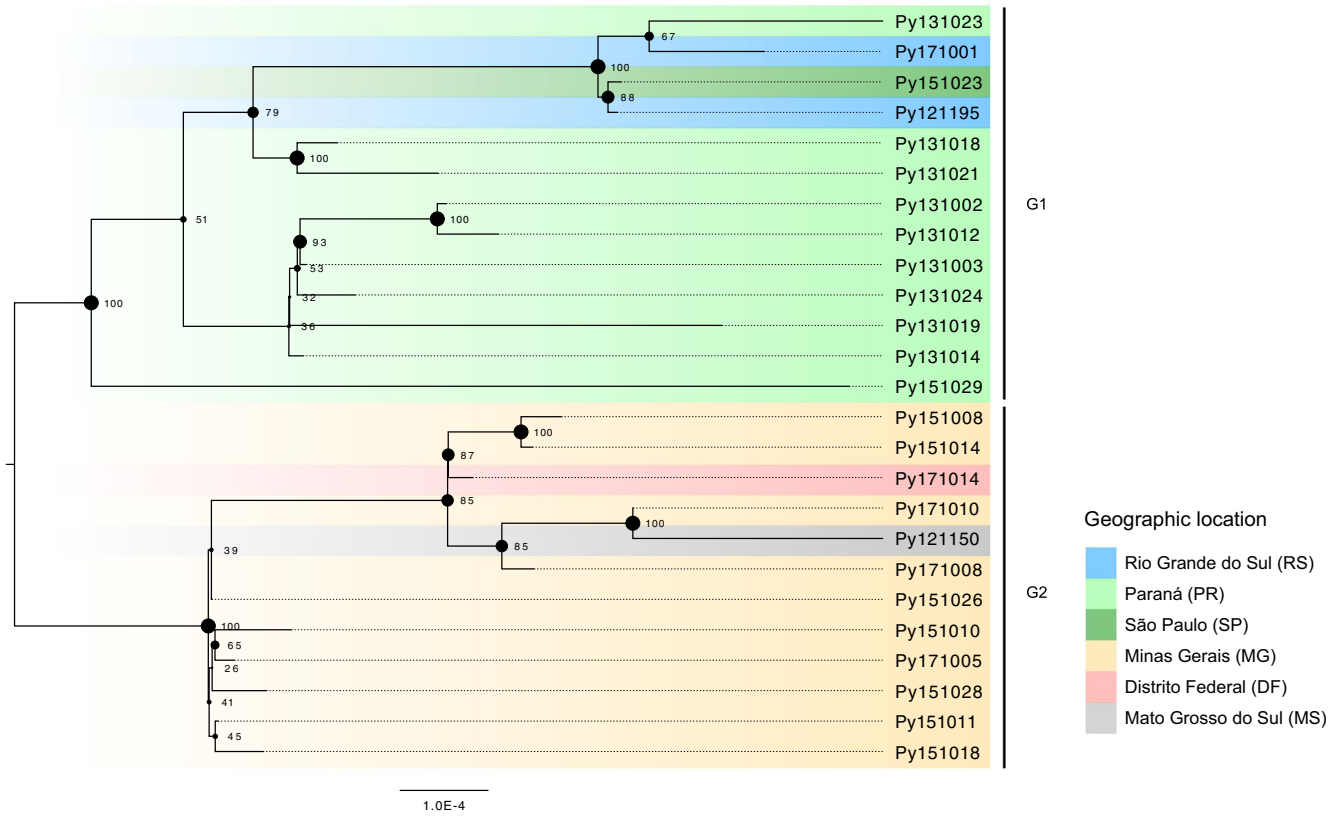

FIGURE 4 | Maximum-likelihood phylogenetic tree showing the relationships among 25 isolates of *Magnaporthe oryzae* Triticum collected from six regions of Brazil. Based on a concatenated alignment of 500 randomly selected BUSCO orthologues (final alignment length = 306,747 amino acid sites), the tree was inferred using IQ-TREE v. 2.3.6 with the VT+F+R3 model of substitution. Branch lengths are proportional to the scale bar representing the expected number of substitutions per amino acid site. Bootstrap support values (expressed as percentages of 1000 replications) are shown at branch points and represented by the circle shape size. [Colour figure can be viewed at wileyonlinelibrary.com]

This analysis was complemented by sequence comparisons with gene models annotated as known or putative effectors in the *P. oryzae* genomes available in MycoCosm (Nordberg et al. 2014) and UniProt (UniProt Consortium 2023). These analyses resulted in 47 possible effector genes, including seven genes exhibiting variations consistent with differences in severity among the isolates: *orth4636* (MGG_02784), *orth4981* (MGG_02114), *orth9974* (MGG_10556), *orth5618* (MGG_02648), *orth5400* (MGCH7_ch7g348), *orth8334* (MGG_00398) and *AVR-Rmg8*.

Phylogenetic analyses based on the ML model revealed distinct patterns of genetic clustering among MoT isolates, consistent with the Brazilian morphoclimatic regions described by Ab'Sáber (2003). These clusters, based on the putative AVR genes *AVR-Rmg8*, *orth4636*, *orth4981*, *orth5618*, *orth9974*, *orth5400* and *orth8334*, reflect both the genetic diversity and the relationship with the mean severity of the isolates analysed (Figure 5).

When studying the sequencing of the avirulence gene *AVR-Rmg8*, the occurrence of rearrangements in the promoter region associated with the proximity between the collection sites of the isolates was observed. The phylogenetic tree obtained from the alignment of the *AVR-Rmg8* sequences demonstrated a clear separation by morphoclimatic domain (Figure 5A), very similar to that presented with the 500 orthologous genes (Figure 4). The isolates were grouped into three main groups (G1, G2 and G3), with a clear distinction among BS levels caused by each group. Group 3 (G3) had more aggressive isolates, causing higher levels of disease severity, 39.4% (Figure 5A). Group G1 caused lower mean BS (29.7%), while G2, comprising only Py15.1.029, had 34.5% mean BS. Although *AVR-Rmg8* presents variants, it is still not enough to group only the isolates with the highest severity. Among the sequenced isolates, Py 17.1.005, with an average severity of 36.6%, had a mutation resulting from the loss of the start codon, while Py 15.1.023, with an average severity of 28.7%, presented an early stop codon, preventing the complete translation of the protein. These variations possibly attenuate the ability of these isolates to cause infection (Figure S1).

For the genes *AVR-Rmg8*, *orth4636* and *orth5400*, a strong regional grouping of the isolates with the Cerrado domain was observed, with a mean BS of 39.4% for these genes (Figure 5A,B,F). Two other genes, *orth4981* and *orth9974*, had isolates grouped according to the Cerrado domain with a single divergent isolate each, isolates Py 12.1.150 and Py 15.1.029, respectively. The first, although belonging to Cerrado regions, did not group with other isolates from this region but rather grouped with those from southern Brazil. The second isolate belonged to Mares e Morros regions and was grouped with Cerrado isolates (Figure 5C,E). For the other genes, there was a division of isolates belonging to the same morphoclimatic domain.

Considering the 500 orthologous genes (Figure 4), the Mares de Morros and Araucária domains are grouped (Figure 4).

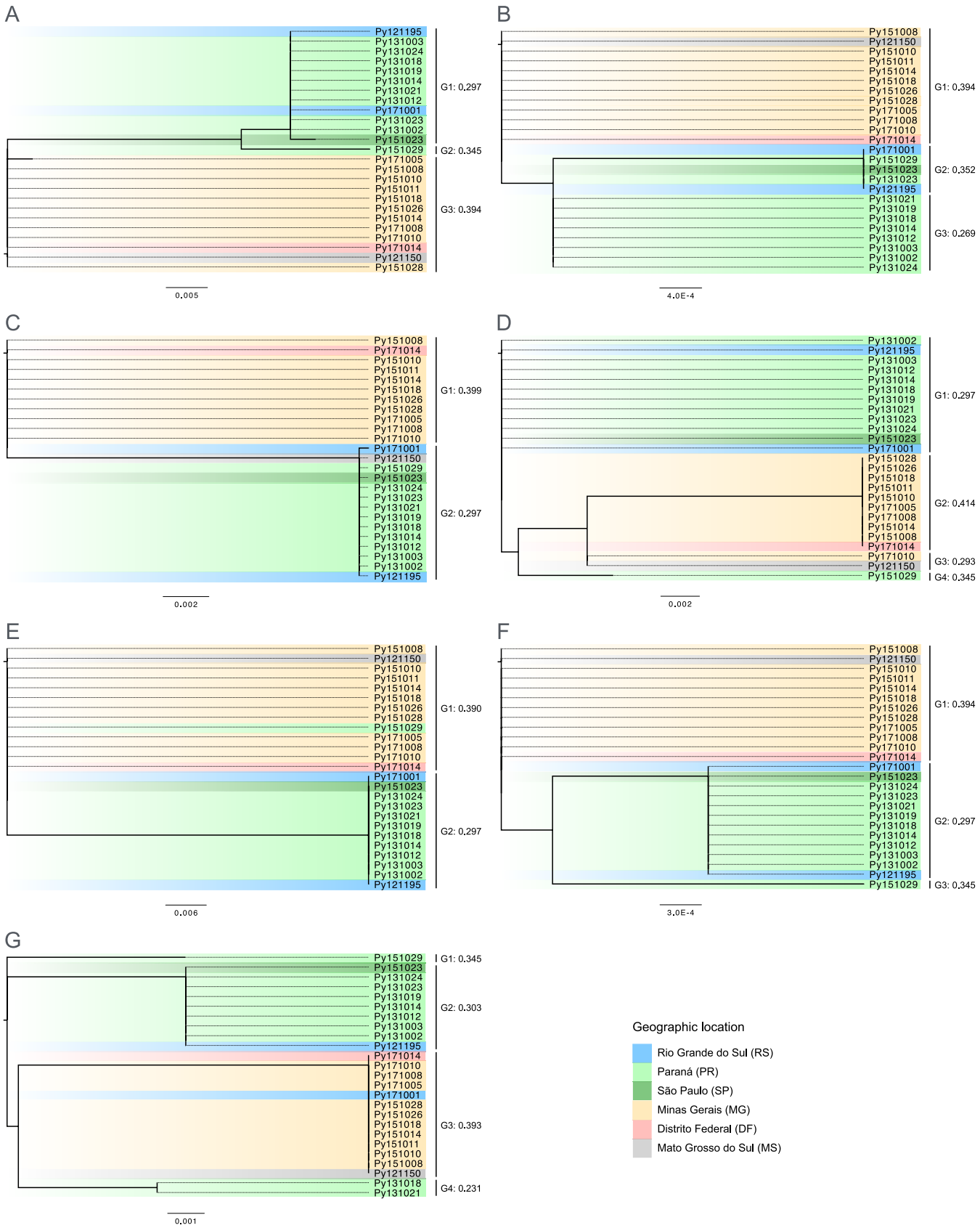

FIGURE 5 | Maximum-likelihood phylogenetic analyses based on nucleotide alignments of *AVR-Rmg8* (A), *orth4636* (B), *orth4981* (C), *orth5618* (D), *orth9974* (E), *orth5400* (F) and *orth8334* (G), showing the relationships among 25 isolates of *Magnaporthe oryzae* Triticum (MoT) collected from six States of Brazil and the mean blast severity (BS) caused by the identified groups (G1–G3). Branch lengths are proportional to the scale bar representing the expected number of substitutions per nucleotide site. For each phylogenetic tree, the mean levels of BS were calculated for each group. [Colour figure can be viewed at wileyonlinelibrary.com]

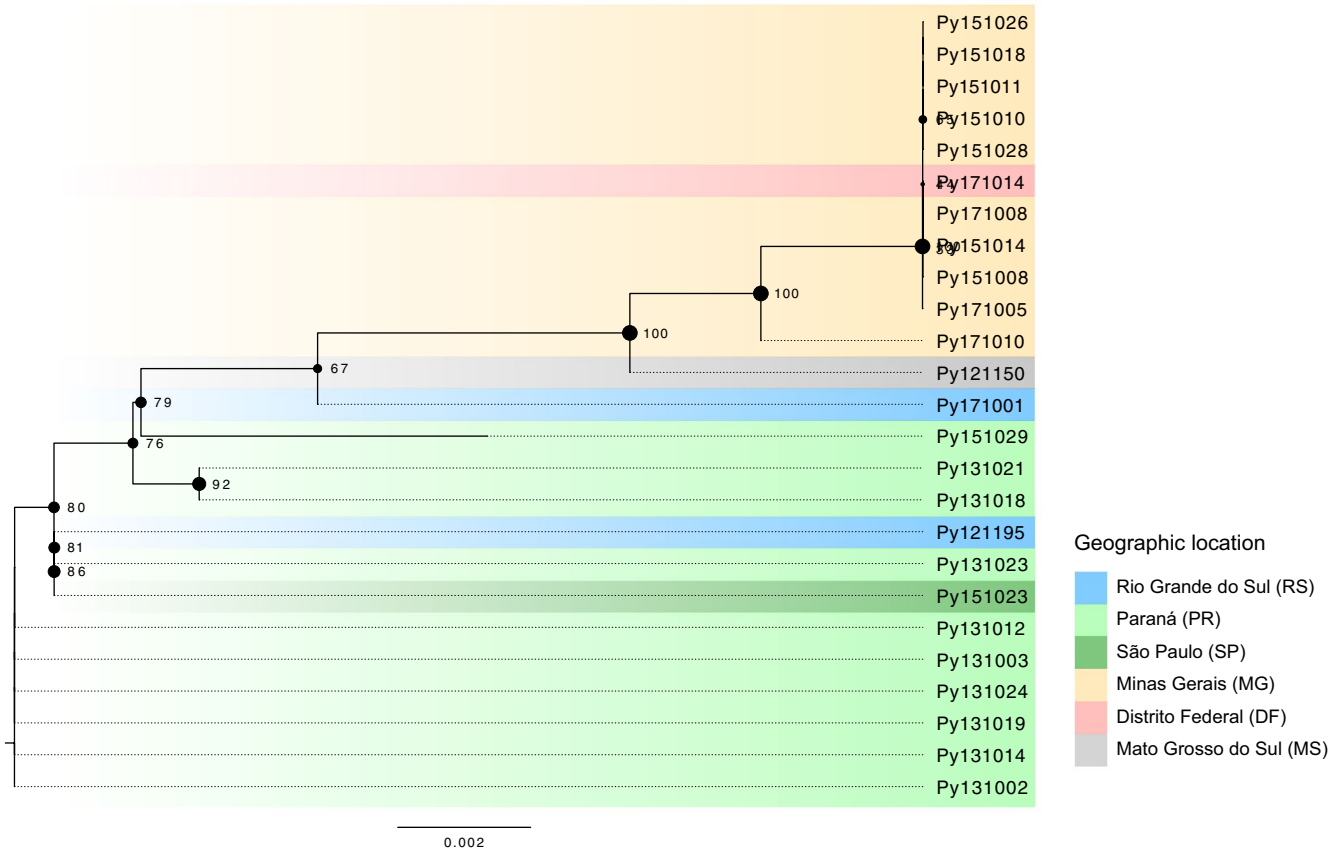

FIGURE 6 | Maximum-likelihood phylogenetic tree showing the relationships among 25 isolates of *Magnaporthe oryzae* Triticum collected from six States of Brazil. Based on a concatenated alignment of orth4636, orth4981, orth5400, orth5618, orth8334, orth9974 and AVR-Rmg8 protein sequences (final alignment length = 5396 amino acid sites), the tree was inferred using IQ-TREE v. 2.3.6 with the Q.bird+I model of substitution. Branch lengths are proportional to the scale bar representing the expected number of substitutions per amino acid site. Bootstrap support values (expressed as percentages of 1000 replications) are shown at branch points and represented by the size of the black circle. [Colour figure can be viewed at wileyonlinelibrary.com]

This trend was maintained only for the *orth5400* gene (Figure 5F). Segregation by polymorphism occurred in the other genes analysed, resulting in the division of the isolates. The isolate Py 15.1.029 presented a unique polymorphism and frequently alternated between the groupings of the different morphoclimatic domains. Furthermore, in four of the seven effector genes considered, this isolate exhibited unique polymorphisms.

The phylogenetic clustering was also performed using the concatenated sequences of the seven effectors (Figure 6). Similar to the clustering obtained from the 500 orthologous genes (Figure 4), this clustering showed the same tendency of grouping correlated with morphoclimatic domains. The isolates from the Cerrado tended to group within the same clade in the phylogenetic tree, while the others also clustered together.

4 | Discussion

The plant responses varied in terms of disease intensity levels, indicating different levels of resistance to pathogen infection. Under the conditions of this study, this variation can be predominantly attributed to genetic factors, either from the pathogen or the host genotype (Martinez et al. 2019).

A strong GII was observed in wheat cultivars infected by different MoT isolates. Similar variations in disease severity caused by the same isolate were also reported by Maciel et al. (2014) and Martinez et al. (2019). Significant differences in disease severity across cultivars, even for the same pathogen isolate, were evident from the colour variations within columns in Figure 3.

The GII poses a challenge for breeding disease-resistant crops. Breeding programmes must account for this variability to develop durable resistance against multiple isolates (Nelson et al. 2018). Under controlled conditions, using a mixture of isolates from different groups can help breeders evaluate and recommend resistant cultivars, as cultivar responses vary depending on the specific isolate.

Intragenotypic variation suggests that even wheat cultivars with the $2N^VS$ translocation may be susceptible to certain isolates. In this study, at least one isolate caused more than 30% BS in all tested genotypes at 7 dai. Other studies have also found MoT-susceptible genotypes carrying the $2N^VS$ translocation (Cardozo Téllez et al. 2019; Cruz et al. 2016; Ferreira 2019).

These findings reinforce the need to explore new genotypes and/or genes associated with lower pathogen severity, which can serve as a foundation for further studies. Identifying these novel

effector genes is crucial for the discovery of additional plant resistance sources beyond the $2N^VS$ translocation, emphasising the importance of diversifying resistance strategies.

Cruz et al. (2016) evaluated the resistance of 418 wheat genotypes to blast under controlled conditions and in field experiments conducted in Bolivia. They observed lower wheat blast intensity in cultivars carrying the $2N^VS$ translocation, although not all genotypes with the $2N^VS$ segment were resistant to the disease. This aligns with the notion that the presence of $2N^VS$ does not guarantee resistance to wheat blast, corroborating the findings of this study, where the isolate severity was, on average, only reduced in cultivars with the $2N^VS$ translocation.

This study confirms that the wheat cultivar BRS 229 is a significant source of resistance to wheat blast, despite lacking the $2N^VS$ translocation. This cultivar demonstrated remarkable resistance, as also reported by Ferreira (2019), who characterised BRS 229 as resistant under controlled environmental conditions. Similarly, de Campos Dianese et al. (2021) and Webber et al. (2023) identified BRS 229 among the top-performing genotypes without the $2N^VS$ translocation, achieving high grain yields under field WHB conditions. These results underscore the potential of BRS 229 as a resistance source independent of the $2N^VS$ segment.

Additionally, this study identified other promising genotypes for wheat breeding programmes aimed at blast resistance. Cultivars with the $2N^VS$ translocation, such as Fuller and Santa Fe, consistently exhibited resistance across most tested isolates and are viable candidates for use in breeding resistant cultivars. In contrast, Anahuac 75 and BRS Buriti were classified as susceptible due to their high head BS.

The grouping of isolates based on their regions of origin suggests a significant population structure influenced by factors such as geographic barriers, regional agricultural practices, climate, soil and topography. These regional similarities correspond to the concept of morphoclimatic domains (Ab'Sáber 2003). Although the primary goal of this study was not to characterise the population structure of *M. oryzae* isolates, the findings underscore the relevance of considering the pathogen's genetic diversity when selecting resistant genotypes. Multiple evolutionary mechanisms act in combination to shape this diversity (Ceresini et al. 2018; Gladieux et al. 2018; Maciel et al. 2014). These studies indicate a genetic structure compatible with a mixed reproductive system, involving sexual reproduction cycles followed by the dissemination of locally adapted clonal lineages (Castroagudín et al. 2017).

Clonal reproduction, which predominates in most *M. oryzae* populations, contributes to the maintenance of well-adapted lineages. Nonetheless, sporadic sexual recombination events, as evidenced by the presence of opposite mating types in some populations, may generate novel genotypes, albeit at low frequency (Bruno and Urashima 2001; Castroagudín et al. 2017; Galbieri and Urashima 2008; Urashima et al. 1993). Morphological evidence supporting the occurrence of sexual reproduction includes the formation of protoperithecia and perithecia (sexual fruiting bodies) during the saprotrophic stage on crop residues or senescent tissues of alternative hosts (Castroagudín et al. 2017; Moreira et al. 2015).

Moreover, point mutations, especially in avirulence genes, play a crucial role in overcoming resistance in previously effective cultivars, as shown by the indels observed in this study with the *AVR-Rmg8* gene. Beyond these intrinsic evolutionary factors, gene flow and population structure further modulate MoT's genetic landscape. The observed genetic similarities among geographically distant isolates could indicate long-distance dispersal through windborne spores or agricultural trade, facilitating the spread of favourable alleles. Conversely, the presence of well-defined clades among certain regions suggests that barriers to gene flow, such as ecological conditions and cropping systems, contribute to localised adaptation.

For instance, isolates from Paraná formed a well-defined clade with those from RS and São Paulo, indicating high genetic similarity and linking two domains: Mares de Morros and Araucárias. Similarly, isolates from MG grouped with those from MS, Goiás and the DF, reflecting genetic similarities potentially tied to adaptation within the Cerrado biome.

This regionalisation is crucial for developing targeted blast control strategies, as local adaptation of the pathogen can affect management effectiveness (Kou et al. 2024; Younas et al. 2024). Monitoring pathogen variability remains essential, especially given the ongoing evolution of the fungus through mutations. Characterising this variability helps clarify the dynamics of wheat blast resistance and gene-for-gene interactions.

When studying seven pathogen effector genes showing significant differences in their sequences: *AVR-Rmg8*, *orth4636*, *orth4981*, *orth9974*, *orth5618*, *orth5400* and *orth8334*, they displayed a phylogenetic clustering pattern that correlated with their morphoclimatic region of origin. Furthermore, they were able to group isolates into those causing higher and lower disease severity levels. These results suggest that these genes may play an important role in the plant–pathogen interaction process between MoT and *Triticum aestivum*.

Interestingly, studies with the *Rmg8* gene exhibit varying levels of wheat resistance to different MoT isolates (Anh et al. 2015). Latorre et al. (2023), when evaluating 71 *M. oryzae* strains, observed three distinct patterns of virulence in young leaves: virulent, intermediate and non-virulent, according to the polymorphism presented in the *AVR-Rmg8* gene sequences. This indicates that the efficacy of *Rmg8* to inhibit disease development may depend on the specific polymorphism of the pathogen genes. Furthermore, the ability of this resistance gene to recognise multiple pathogens, such as powdery mildew, highlights its adaptability (Asuke et al. 2024).

Although the influence of the *AVR-Rmg8* gene in the process of wheat resistance to leaf blast has been shown (Asuke et al. 2024; Horo et al. 2020; Latorre et al. 2023; Wang et al. 2018), the importance of this gene in spike resistance still generates inconclusive results. Some studies suggest a limited or variable contribution of *AVR-Rmg8* to host resistance at the spike stage, possibly due to tissue-specific expression patterns or differential activation of host defence mechanisms (Anh et al. 2018; Inoue et al. 2020; Wang et al. 2018). Further investigations are needed to clarify whether this gene plays a direct or indirect role in spike resistance and how its interaction with corresponding

R genes might vary depending on the wheat genotype or environmental conditions.

The alignment of the coding region of the *AVR-Rmg8* gene resulted in variations at the amino acid level between MoT isolates, revealing changes that may influence the resistance conferred by the *Rmg8* gene. Isolates such as Py 17.1.005 and Py 15.1.023 caused blast severities varying between 36.6% and 28.7%, respectively, and have alleles not yet described (Figure S1). Other MoT isolates presenting even higher disease severities reinforce the fact that, despite changes in *AVR-Rmg8*, there are other pathogen genes probably involved in the head wheat infection process by the pathogen.

The *AVR-Rmg8* gene groups MoT isolates into two major categories, *eI* and *eII*, which exhibit a 30% higher BS. However, it fails to exclusively cluster the most aggressive isolates, with grouping instead reflecting adaptations to morphoclimatic domains. The association of *AVR-Rmg8* with isolate severity remains unclear, requiring further analysis due to the complexity of genetic interactions. Studies indicate that wheat genotypes with the *Rmg8* allele for leaf blast resistance recognise the polymorphic *eI* allele in isolates Br 48 and BR 71 (Anh et al. 2018; Wang et al. 2018; Latorre et al. 2023). Interestingly, Wang et al. (2018) observed head blast symptoms in wheat cultivars deemed resistant, as their evaluation relied on discrete rather than continuous variables, with the lowest scores being classified as resistant. In the present study, the severity of the isolates carrying this *eI* polymorphism varied within the same cultivar. These results indicate that the *AVR-Rmg8* gene is not the main gene determining MoT isolate virulence, although it may play a role in overcoming wheat resistance to head blast.

The *orth4981* (MGG_*02114*) and *orth5618* (*MGG_02648*) genes, annotated as Interferon-induced GTP-binding protein Mx2 and Mx, belong to the GTPase protein superfamily associated with MoT infection in wheat. Zhong et al. (2016) linked GTPase proteins (Modnm1, Momdv1 and Mofis1) to the appressorium development of *M. oryzae* in rice, showing that their knockouts reduced virulence and vegetative spore growth. Knockouts of MoDnm2 (*MGG_02114*) and MoDnm3 (*MGG_02648*) did not significantly affect lesion formation in rice leaves; however, in the present study, they were able to separate MoT isolates into groups of higher and lower ability to cause disease in wheat spikes. Moreover, these pathogen effector genes were effective in grouping them according to the morphoclimatic domains from which they were collected.

The gene *orth9974*, annotated as HTR 9 (Host Transcription Reprogramming Factor 9), is part of the HTR superfamily. Effectors of this family, specifically MoHTR1, MoHTR2 and MoHTR3, bind to promoter regions of host target genes to influence the virulence process by acting as transcriptional repressors. This leads to interference with genes crucial for the plant immune response. Kim et al. (2020) demonstrated that such reprogramming significantly impacts rice defence against other pathogens. Lee et al. (2023) showed that MoHTR3 regulates defence-related genes in rice leaves. This suggests that HTR genes can also influence the overall susceptibility of the plant to infections. Our work suggests that HTR9 could specifically play a role in wheat head infection by MoT, as it was able to group the most aggressive isolate.

In summary, the $2N^VS$ translocation in wheat cultivars does not ensure resistance to wheat blast in spikes, emphasising the importance of combining it with other genetic backgrounds for durable resistance. The cultivar BRS 229 has shown resistance independent of the $2N^VS$ translocation. MoT virulence in Brazil varied based on cultivar genotypes ($2N^VS$ presence or absence) and the morphoclimatic domains. Additionally, DNA sequence analysis revealed associations between MoT isolate aggressiveness and new putative effector genes.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The genomic dataset of the 25 newly sequenced Brazilian *M. oryzae* Triticum isolates has been deposited at GenBank/ENA/DDBJ under the BioProject ID PRJNA1197378, in the WGS accessions. The phenotypic datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.