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Summary

Absence of 2NS/2AS in Wheat Resistance Sources to *Magnaporthe oryzae* in Brazil

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Abstract – Searching for resistance sources to *Magnaporthe oryzae* in wheat has been a major concern regarding wheat blast in Brazil, since its first report, in 1985. The aim of this work was to evaluate the presence of the translocation 2NS/2AS in wheat accessions identified as resistant, under Brazilian field conditions. The presence of this translocation was evaluated using public primers for amplification of one fragment of 262 base pairs in length. The line VPM1 and the cultivar Thatcher were employed as positive and negative controls for PCR reactions. In all, 17 genotypes (two susceptible and 15 resistant to *M. oryzae*) were characterized for the presence of the 2NS/2AS translocation. Both susceptible cultivars (Anahuac 75 and BRS 209) lack the 2NS/2AS translocation. Among the resistant ones, two (CBFusarium ENT014 and CPAC 07434) have the 2NS/2AS translocation. Among the resistant cultivars that do not have the translocation are BRS 229 and Trigo

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BR 18-Terena, which are examples of durable resistance to wheat blast in Brazil. This work offers new perspectives on the exploration of resistance sources to *M. oryzae* in addition to the 2NS/2AS translocation.

Index terms: wheat blast, *Pyricularia oryza*e, durable resistance.

Ausência da Translocação 2NS/2AS em Fontes de Resistência de Trigo a *Magnaporthe oryzae* no Brasil

Resumo – Em trigo, a busca por fontes de resistência a *Magnaporthe oryzae*, fungo causador da brusone, tem sido constante desde o primeiro relato mundial da doença feito no Brasil em 1985. O objetivo desse trabalho foi avaliar a presença da translocação 2NS/2AS, associada à resistência à brusone em condições de campo, em genótipos de trigo resistentes à doença no Brasil. A presença da translocação foi avaliada com o uso de primers de domínio público para amplificação de fragmento de 262 pares de bases. A linhagem VPM1 e a cultivar Thatcher foram utilizadas, respectivamente, como controles positivo e negativo das reações de PCR. Dezessete genótipos de trigo (dois suscetíveis e 15 resistentes a *M. oryzae*) foram caracterizados quanto à presença da translocação 2NS/2AS. As duas cultivares suscetíveis (Anahuac 75 e BRS 209) não possuem a translocação, e entre os 13 genótipos resistentes, dois (CBFusarium ENT014 e CPAC 07434) a possuem. Entre as cultivares resistentes que não possuem a translocação, estão BRS 229 e Trigo BR 18-Terena, que são exemplos de resistência durável à brusone no Brasil. Esse trabalho abre novas perspectivas de exploração de fontes de resistência a *M. oryzae*, além da translocação 2NS/2AS.

Termos para indexação: brusone, *Pyricularia oryzae*, resistência durável.

Introduction

Wheat blast is caused by *Magnaporthe oryzae* (B.C. Couch and L.M. Kohn, anamorphic synonym *Pyricularia oryzae*), also an important rice pathogen, and presents wide genetic variability. The first occurrence of blast on wheat spikes was described in Paraná state, Brazil (Igarashi et al., 1986). After 30 years of its report, the disease has already been registered in all regions with wheat fields in Brazil, and in Bolivia, Paraguay and Argentina (Kohli et al., 2011). In February 2016, wheat blast was registered in Bangladesh, Asia, reaching approximately 15% of the wheat cultivated area (Malaker et al., 2016) and in 2017 the disease was reported in fields in India, from the border with Bangladesh (Bhattacharya; Pal, 2017). Asia produces 135 million tonnes of wheat annually, and India is the second major wheat producer in the world with 90 million tonnes (Bhattacharya; Pal, 2017).

Searching for sources of resistance to the pathogen is a constant in Brazillian research. Most of the wheat cultivars analysed until the mid-2000s showed high susceptibility. However, several studies indicate the existence of genetic variability in the genotypes reaction (Urashima; Kato, 1994; Urashima et al., 2004; Prestes et al., 2007; Maciel et al., 2014; Coelho et al., 2016). Genetic studies indicate that wheat blast follows the gene-to-gene theory (Anh et al., 2015). Between 2008 and 2015, eight wheat resistance genes to *M. oryzae* isolates were identified: *Rmg1* (Takabayashi et al., 2002), *Rmg2* and *Rmg3* (Zhan et al., 2008), *Rmg4* and *Rmg5* (Nga et al., 2009), *Rmg6* (Vy et al., 2014), *Rmg7* (Tagle et al., 2015), and *Rmg8* (Anh et al., 2015). Four of them, *Rmg2*, *Rmg3*, *Rmg7*, and *Rmg8*, were effective against isolates obtained from wheat plants.

In addition to those genes which confer specific resistance to isolates, a positive correlation between the resistance of some wheat accessions to *M. oryzae* and the presence of a chromosomic translocation (2NS/2AS) from *Triticum ventricosum* (sin. *Aegilops ventricosa*) has been shown (Cruz et al., 2016). A total of 418 wheat cultivars was characterized by inoculation with *M. oryzae*, including winter and spring wheat and near isogenic lines. A subset of these materials was tested in field conditions in Bolivia. In both experiments, lower incidence of disease was observed in accessions with the 2NS/2AS translocation (Cruz et al., 2016).

In the same study, Cruz et al. (2016) observed that significant reduction of blast severity on the spikes was also present in genotypes without the 2NS/2AS translocation, suggesting that the genetic background and/or the environmental conditions could influence the resistance expression conferred by this translocation.

The 2NS/2AS translocation (Bariana; Mcintosh, 1993) was first introgressed from *A. ventricosa* (2n = 4x = 28) into the interspecific hybrid 'VPM1' (Maia, 1967) and possesses a gene cluster of wheat resistance to stripe rust (*Yr17*, to *Puccinia striiformis* f. sp. *tritici*), leaf rust (*Lr37*, to *Puccinia triticina*) and stem rust (*Sr38*, to *Puccinia graminis* f. sp. *tritici*). Thereafter, the same translocation presents resistance genes to the nematodes *Heterodera avenae* (Jahier et al., 2001) and *Meloidogyne* spp. (Williamson et al., 2013). Later on, Helguera et al. (2003) developed primers, of public domain,for efficient selection of this gene cluster (*Yr17-Lr37-Sr38*) to be used in the wheat breeding programs.

In Brazil, a collection of accessions with relevance to the wheat breeding program was characterized for blast reaction, aiming to identify resistance genes to *M. oryzae*, specifically at adult plant stage. This collection was named *Wheat BGIn* (Wheat Blast Genes Interaction). For three years (2010, 2011, and 2012), in three locations where blast is endemic (Planaltina, DF, Londrina, PR and Dourados, MS), 196 genotypes were evaluated in wheat blast nurseries, under natural conditions of infection. Thereafter, 15 of the accessions that had been identified as resistant, were selected for detailed phenotyping studies regarding the response to blast infection by two isolates of *M. oryzae*, followed by molecular characterization. The average of blast incidence in the resistant accessions selected in the field was 2,1%, differing statistically from the susceptible accessions Anahuac 75 and BRS 209, whose average incidence was 9,9%.

Anahuac 75 is a wheat cultivar from Cimmyt (Sousa; Caierão, 2014) and was widely grown in the 1980s and early 1990s in Brazil. Since the first wheat blast report (Igarashi et al., 1986), Anahuac 75 has been observed as highly susceptible to the causal agent of the disease. Inoculation studies have indicated its wide susceptibility to many *M. oryzae* isolates (Urashima et al., 2004). In experiments performed in controlled environmental conditions, Anahuac 75 showed high susceptibility in leaves inoculated with *M. oryzae* (Torres et al., 2015) in addition to the previously observed high susceptibility in the spikes.

BRS 209 is a wheat cultivar developed by Embrapa Soja in joint work with Embrapa Trigo (Sousa; Caierão, 2014). Since its release, it has been susceptible to blast (Brunetta et al., 2005). Thereafter, Prestes et al. (2007), evaluating the blast severity in spikes of 100 wheat genotypes under inoculation conditions, classified BRS 209 among those most susceptible ones, with disease severity average of 51%, at 10 days after inoculation.

The aim of this study was to evaluate the presence of the 2NS/2AS translocation in a set of 17 wheat genotypes previously phenotyped regarding the reaction to wheat blast in Brazil.

Material and Methods

The presence of the 2NS/2AS translocation was analysed in 17 wheat accessions previously characterized for reaction to blast (two susceptible and fifteen resistant) (Table 1).

Table 1. Wheat genotypes analysed for the presence of the 2NS/2AS translocation. Embrapa Trigo, Passo Fundo, RS, 2018.

Wheat genotype	BAG code Embrapa Trigo	Pedigree	Year of release	Country of origin
Anahuac 75	BGT00443	II-12300//Lerma-Rojo-64/II-8156/3/ Norteno-67	1975	Mexico
Bet Dagan 131	BGT01227	Pitic-62(SIB)/Florence-Aurore	1970	Iceland
BRS 209	BGT14405	Jupateco 73/Embrapa 16	2002	Brazil
BRS 229	BGT14408	Embrapa 27*3//BR 35/Buck Poncho	2004	Brazil
BRS Angico	BGT01454	PF 87107/2*IAC 13	2002	Brazil
CBFusarium ENT014	BGT15263	No information	-	Mexico
CPAC 07340	BGT20389	CPAC 96306/CPAC 9985	-	Brazil
CPAC 07434	BGT20388	Taurum/BRS 254	-	Brazil
Embrapa 27	BGT03372	PF 83743/5/PF 83182/4/CNT 10*4//Lagoa Vermelha*5/Agatha/3/ Londrina*4/Agent//Londrina*3/Nyu Bay	1994	Brazil
Huanca	BGT04275	Frocor/3/McMurachy/Kentana// Yaqui-50/4/Maria-Escobar/MN-2698/5/Maria-Escobar	1973	Peru

continua...

Tabela 1. Continuação.

Wheat genotype	BAG code Embrapa Trigo	Pedigree	Year of release	Country of origin
PF 020450	BGT16243	FL 72185A-A2-C1/Embrapa 40// CEP 24	-	Brazil
PF 909	BGT11109	PF 83743/PF 82252//PF 84433/ BR 35	-	Brazil
Safira	BGT15079	PF9099 /OR-1//Granito	2003	Brazil
Shanghai	BGT15211	(M)Yangmai-1	1978	China
Thatcher	BGT13324	Marquis/Iumillo(durum)//Marquis/ Kanred	1934	United States of America
Trigo BR 18-Terena	BGT13539	No information	1986	Brazil
Trigo Chapéu	BGT13588	No information	-	-

The primer names, sequences and PCR amplification conditions are presented in the Table 2. The expected length is 262 base pairs (bp).

The polymerase chain reactions (PCR) were performed with 100 ng of genomic DNA and final concentration of buffer 1X, 2.5 mM $MgCl_2$, 0.35 mM of each dNTP, 0.2 µM of each primer, 0.5 U of Taq polymerase (RBC Bioscience, #RT011C). The final volume of the reaction was 10 µL. The amplification products were analysed on 2% agarose gel. Molecular weight markers (50 bp DNA Ladder, Invitrogen, Cat. 10416-014) was used to estimate the length of the amplified fragments.

Table 2. Primer names, sequence and amplification conditions used to identify the marker associated to the 2NS/2AS translocation in wheat (adapted from Helguera et al., 2003). Embrapa Trigo, Passo Fundo, RS, 2017.

Primer name	Sequence (5´- 3´)	Amplification conditions
VENTRIUP LN2	AGG GGC TAC TGA CCA AGG CT TGC AGC TAC AGC AGT ATG TAC ACA AAA	Initial denaturation at 94 °C for 3 min, followed for 30 cycles of amplification. Each cycle includes a stage of denaturation at 94 °C for 45 s, annealing at 65 °C for 30 s and extension at 72 °C for 60 s. On last cycle 7 min of extension at 72 °C.

To analyse the presence of the 2NS/2AS translocation, materials known to carry the translocation were used as positive controls for the PCR. This preliminary study was performed with accessions from the Germoplasm Active Bank (BAG) from Embrapa Trigo, with genotypes that carry the 2NS/2AS translocation, according to the literature (Table 3). Thereafter, the 17 wheat genotypes were tested, using the genotype VPM1 (accessions VPM 1-1-1-2 R4; BAG identifier: BGT13964) as a positive control of the 2NS/2AS translocation (Helguera et al., 2003). The cultivar Thatcher, which is included in the group of genotypes identified as resistant to blast under field conditions in Brazil, is reported as not having the translocation (Bulos et al., 2006), and was used as a negative control of the amplification reactions.

Table 3. Wheat genotypes with or without the 2NS/2AS translocation and analysed as controls of the PCR test. Embrapa Trigo, Passo Fundo, RS, 2017.

Genotype	BAG Code Embrapa Trigo	Genealogy	Year of release	Country of origin	Presence of the 2NS/2AS translocation	References
Arche	BGT00484	Tribute, FRA/VS-73644-9-4-1	1989	France	positive	Robert et al., (1999)
Eureka	BGT03451	Mironovskaya-808/Maris-Huntsman/3/VPM-1/Moisson(R-1-5-2)//Courtot - 1991	1991	France	positive	Robert et al., (1999)
Hyak	BGT04292	VPM-1/Moisson-421//2*Tyee	1988	United States of America	positive	Helguera et al., (2003)
Madsen	BGT05725	VPM-1/Moisson-951//2*Hill-81	1988	United States of America	positive	Helguera et al., (2003)
Renan	BGT12239	Mironovskaya-808/Maris-Huntsman/3/VPM-1/Moisson//9*Courtot	1989	France	positive	Robert et al., (1999)
VPM 1-1-1-2 R4	BGT13964	Ae.ve./Tr.ca.//3*Marne	1967	France	positive	Helguera et al., (2003), Bulos et al., (2006)
Thatcher	BGT13324	Marquis/(Tr.dr.)lumillo//(hn-3001)Marquis/Kanred - 1934	1934	United States of America	negative	Bulos et al., (2006)

Seeds of wheat genotypes were germinated on filter paper for a period of seven days. The first leaves were collected and the DNA was extracted by the CTAB method (Doyle; Doyle, 1987). The quality and quantity of DNA from each sample were evaluated on 0.8% agarose gel stained with ethidium bromide. Electrophoresis was performed at 120 V, 0.19 A and 23 W for two hours.

Results and Discussion

All wheat genotypes reported in the literature as carriers of the 2NS/2AS translocation showed amplification of the 262 bp fragment (Figure 1): Arche, Eureka, Hyak, Madsen, Renan and VPM1. VPM1 and Thatcher (which does not have the translocation) proved to be good contrasting controls of the PCR.

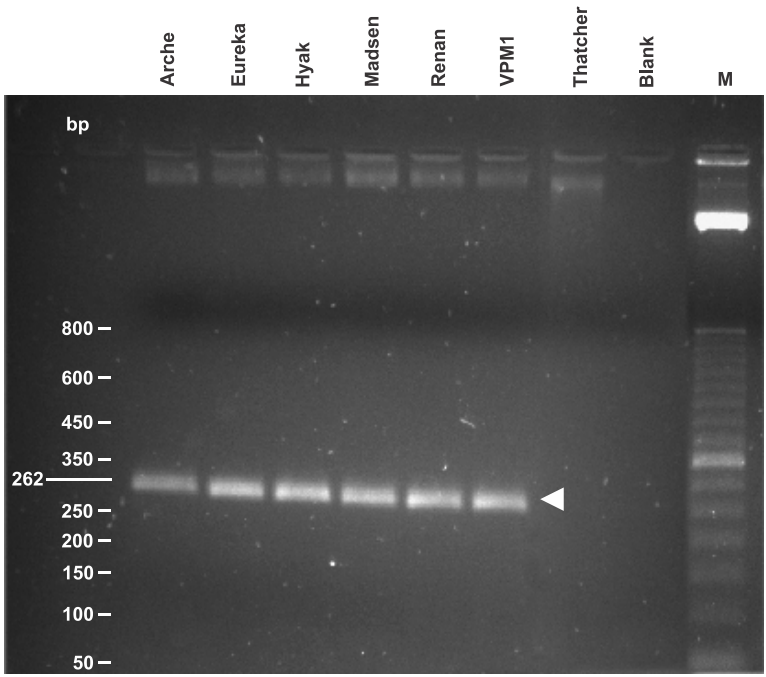

Figure 1. Amplification by PCR with the specific primers VENTRIUP and LN2 to the 2NS/2AS translocation in wheat, of genotypes reported in the literature to carry the translocation (Arche, Eureka, Hyak, Madsen, Renan and VPM1). White arrow indicates the PCR amplification product specific to 2NS with length of 262 bases pairs (bp). Thatcher, negative control; Blank, PCR without DNA; M, molecular weight markers (Ladder 50 bp, Invitrogen®).

The two cultivars susceptible to *M. oryzae*, Anahuac 75 and BRS 209, did not present the 2NS/2AS marker.

Among the 15 genotypes identified as resistant to the pathogen, in experiments carried out in Brazil (Table 4), only CBFusarium ENT014 and CPAC 07434 gave an amplification product with the use of the marker for the 2NS/2AS translocation (Figure 2). The remaining 13 genotypes did not amplify the marker for the translocation.

Table 4. Wheat genotypes characterized by the presence of 2NS/2AS translocation. Embrapa Trigo, Passo Fundo, RS, 2017.

Genotype	Blast reaction	2NS/2AS translocation
Anahuac 75	susceptible	absent
BRS 209	susceptible	absent
Bet Dagan 131	resistant	absent
BRS 229	resistant	absent
BRS Angico	resistant	absent
CBFusarium ENT014	resistant	present
CPAC 07340	resistant	absent
CPAC 07434	resistant	present
Embrapa 27	resistant	absent
Huanca	resistant	absent
PF 020450	resistant	absent
PF 909	resistant	absent
Safira	resistant	absent
Shanghai	resistant	absent
Thatcher	resistant	absent
Trigo BR 18-Terena	resistant	absent
Trigo Chapéu	resistant	absent

Among the 15 resistant accessions, six (40%) were obtained from other countries: Bet Dagan 131 (Iceland), CBFusarium ENT014 (Mexico), Huanca (Peru), Shanghai (China), Thatcher (United States of America) and Trigo Chapéu (no passport information). Trigo Chapéu was cultivated in Brazil and there is no reliable information about its origin or the institution that developed it (Sousa; Caierão, 2014).

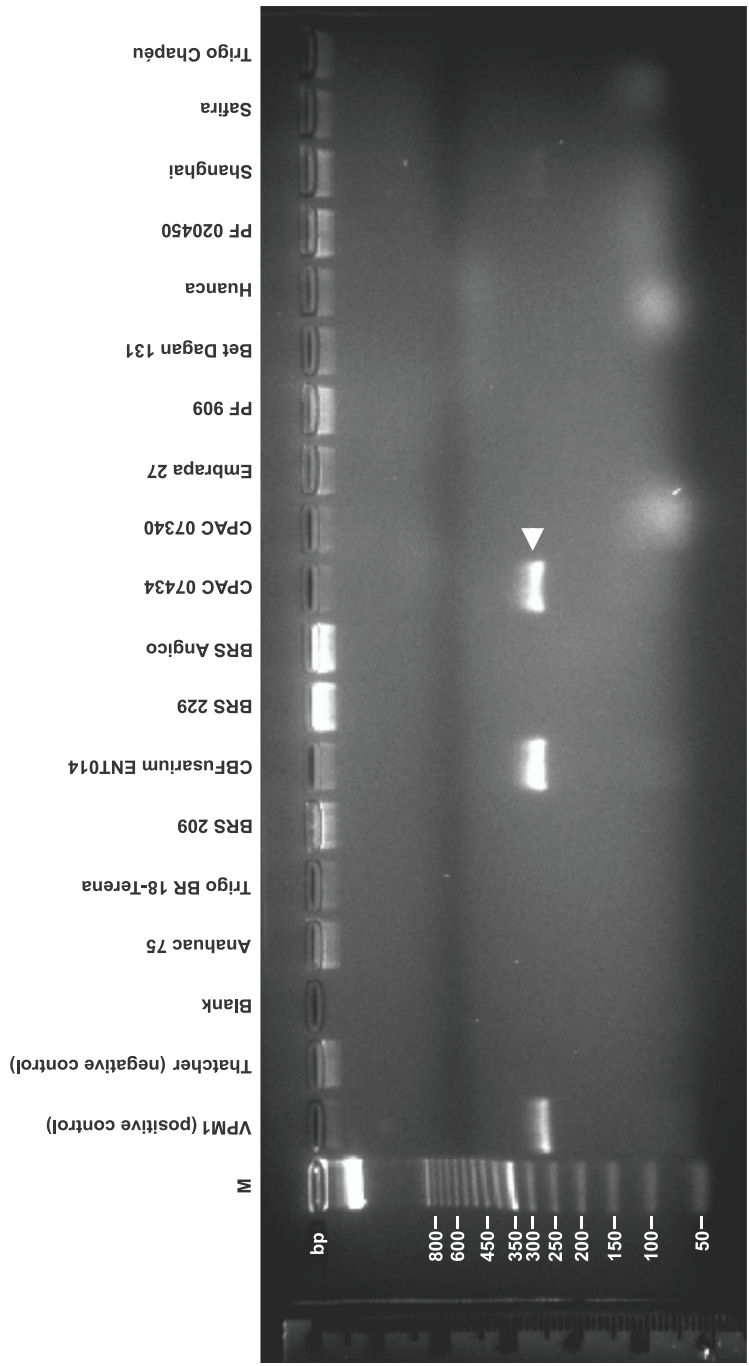

Figure 2. Amplification by PCR with the specific primers VENTRIUP and LN2 to the 2NS/2AS translocation in wheat. White arrow indicates the PCR amplification product specific to 2NS with length of 262 bases pairs (bp). VPM1 and Thatcher, respectively, positive and negative controls of the PCRs; Blank, PCR without DNA; M, molecular weight markers (Ladder 50 bp, Invitrogen®).

Thatcher was one of the first wheat cultivars specifically developed for stem rust resistance (Vanegas et al., 2008). In 2008, the first two blast resistance genes were identified in Thatcher, at seedling stage, to *M. oryzae* isolates obtained from wheat (Zhan et al., 2008).

From the six foreign accessions, only CBFusarium ENT014 has the 2NS/2AS translocation (Figure 2, Table 4), this is a synthetic wheat belonging to the Cimmyt crossing block (CB) (Van Ginkel et al., 2002). Crossing blocks are elite collections of breeding program that are used for assessments in multiple environments. Genotypes from each group carry specific genes for a given environment. In this case, it is the Fusarium head blight disease, caused by *Gibberella zeae*. CBFusarium ENT014 was used as a male parent of a doubled-haploid population, developed in the Biotechnology Laboratory at Embrapa Trigo.

There were four lines (CPAC 07340, CPAC 07434, PF 020450, and PF 909) and five cultivars (BRS 229, BRS Angico, Embrapa 27, Safira and Trigo BR 18-Terena) among the Brazilian wheat accessions evaluated for the presence of 2NS/2AS translocation. Among the wheat lines, only CPAC 07434 has the 2NS/2AS translocation and, among the cultivars, none of them has the translocation. Of these materials, BRS 229 was developed by Embrapa Soja, BRS Angico and Embrapa 27 were developed by Embrapa Trigo, Safira was developed by OR Sementes Ltda. and Trigo BR 18-Terena, whose crossing is unknown, was developed or introduced by Uepae-Dourados/ Embrapa Agropecuária Oeste (Sousa; Caierão, 2014). BRS 229 and Trigo BR 18-Terena are reported as moderately resistant (Torres et al., 2009) and/or resistant to *M. oryzae* (Reunião..., 2011, 2017). Trigo BR 18-Terena, from its release in 1986 to the 2017 harvest season, is part of the group of wheat cultivars recommended for cultivation in the central region of Brazil, and BRS 229 was indicated for cultivation in the state of Paraná from 2004 (Brunetta et al., 2006), due to resistance to major fungal diseases, among other characteristics.

BRS Angico (line PF 960198) was indicated for cultivation in Rio Grande do Sul in 2002 and to Santa Catarina (Del Duca et al., 2005) and Paraná (Só e Silva et al., 2005). Until then, BRS Angico had not been identified as a source of blast resistance (Reunião..., 2005, 2007, 2008).

Embrapa 27 was frequently employed in the 1990s for wheat crosses in Brazil (Sousa; Caierão, 2014), being important in the composition of cultivars from OR Melhoramento de Sementes Ltda. and Biotrigo Genética Ltda. (Caierão et al., 2014). That cultivar was characterized for reaction to inoculation by 18 isolates of *M. oryzae*, at the seedling stage, and was classified among the genotypes showing the lowest averages of disease severity in leaves (Cruz et al., 2010). In the present study, Embrapa 27 was identified as resistant at the adult plant stage, considering the disease in spikes.

Safira (line ORL 98204) is a cultivar developed in co-ownership of OR Melhoramento and Biotrigo. Since its first report, in Reunião... (2005), until Reunião... (2017), including the harvest season of 2007 to 2016, there was no information about the reaction of this cultivar to blast. As BRS Angico, this is the first report where Safira is identified as a source of blast resistance.

Trigo BR 18-Terena is an introduction in Embrapa Trigo from a line developed by Cimmyt, and was released to cultivation in Mato Grosso do Sul and Paraná, being extended to São Paulo and Rio Grande do Sul later (Sousa, 2002). Since released, it was classified as moderately resistant to *M. oryzae* in field conditions, although it does not have the translocation 2NS/2AS.

The results of this work agree with those obtained by Cruz et al. (2016), indicating that the relationship of 2NS/2AS translocation with resistance to *M. oryzae* is genotype dependent. These authors evaluated both accessions with and without the 2NS/2AS translocation to the reaction to wheat blast, observing that accesses carrying the translocation had lower rates of the disease.

Pizolotto et al. (2017), working with a restricted set of wheat genotypes, all with the 2NS/2AS translocation, observed a wide variability of reaction to the disease. These data indicated that the simple fact of the presence of the 2NS/2AS chromosomal segment in the genome does not necessarily mean spike resistance to the pathogen.

Further studies will be necessary to evaluate the contribution of this translocation to the resistance reaction observed in the two genotypes, CBFusarium ENT014 and CPAC 07434, under the conditions of wheat cultivation in Brazil.

Conclusions

This work opens new perspectives for the exploration of different sources of resistance to *M. oryzae*, besides the 2NS/2AS translocation, which are effective in the Brazilian field, from the 15 wheat genotypes considered resistant to M. oryzae, only two of them have the translocation. Among the other 13 resistant genotypes, without the 2NS/2AS translocation, are two of the most important sources of durable resistance to blast in Brazil: BRS 229 and Trigo BR 18-Terena.

The identification of resistant materials that do not possess the 2NS/2AS translocation opens up prospects for the study and the discovery of genes and/or QTLs and mechanisms of resistance not related to 2NS/2AS translocation and that are effective for the Brazilian conditions, where blast is endemic.

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