

Phytosanitary Quality of Genotypes of Wheat Seeds Used Northern Paraná State

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Abstract

Wheat seeds can be infested and/or infected by microorganisms that might cause deterioration of this propagation structure. The aim of this study was to evaluate the health quality of sixteen wheat genotypes grown in northern Paraná. Therefore, seeds of each genotype were submitted to the blotter test with 16 repetitions, 400 seeds per sample, for phytosanitary quality evaluation. The identification of the fungi was performed based on their morphological characteristics and quantified data. The results revealed variations in incidence, with 20 fungi genera in the analyzed samples. The fungi *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp. and *Bipolaris* sp. were found in 100% of the analyzed samples, while *Mucor* sp. and *Alternaria* sp. were in 89% and 78% of the samples, respectively. The main pathogens that cause diseases in the aerial part of wheat were not found, or were low incidence in all materials analyzed. The pathogens with the highest incidence associated with wheat seeds were groups of storage fungi and known to produce mycotoxins.

Keywords: *Triticum aestivum* L., seed pathology, blotter test

1. Introduction

Wheat (*Triticum aestivum* L.) is the cereal of the most economic importance worldwide, with over 600 million tons produced annually. In southern Brazil, it is most important chief winter crop, with annual production ranging from 5 to 6 million tons, and a consumption of around 10 million tons (Silva et al., 2011; CONAB, 2020).

During the development and maturation of plants in the field, these are attacked by fungi and other phytopathogenic organisms, resulting in diseased plants, seed quality is essential for the proper establishment of the plant population in the field, its full development and production (Dhingra, 1985; Souza et al., 2007). The vast majority of these fungi are agents of leaf stains and transmitted by seeds, from which they return to air organs through the transmission process (Reis & Casa, 1998; McMullen et al., 2012; Dweba et al., 2017).

The main difficulty encountered by wheat producers in southern Brazil is excess rainfall during the crop development cycle, covered days favorable to the occurrence and intensity of leaf diseases, directly interfering with the potential of phytosanitary quality of seeds (Reis, 2001). As leaf diseases in the Southern Region of Brazil that deserve to be highlighted are as caused by idiote (*Blumeria* (Sin. *Erysiphe*) *graminis* (DC) Speerf. sp. *tritici* Em. Marchal), a rust of the leaf, (*Puccinia triticina* Eriks), the yellow stain (*Drechslera tritici-repentis* (Died) Drechs.), and a septoriososis (*Septoria nodorum* (Berk. Berk.) (Reis & Casa, 2005).

The increase in the intensity of diseases caused by these fungi in a crop is directly related to the use of infected seeds, monoculture and no-tillage. The majority of the necrotrophic fungi survive in the seed, often in the form of dormant mycelium, and sometimes infesting them. The higher the incidence of the pathogen in the seeds, the higher the percentage of outbreaks in the field and the earlier the epidemic begins (Menten, 1995; Kobayashi & Pires, 2011).

The presence of phytopathogens in wheat seeds has been constantly reported, and this association has been responsible for the reduction of seed physiological quality, introduction, dissemination and early transmission of

pathogens to progeny. Therefore, this study aimed to investigate the phytosanitary quality of seeds of 16 wheat genotypes planted in the northern region of the state of Paraná, obtained in the 2018 crop.

2. Method

This study was performed at the Seed Pathology Laboratory, Agronomic Institute of Paraná (IAPAR), Londrina. The wheat genotypes used in this study are materials grown throughout the northern region of Paraná. These materials were collected in seed production field during the 2018 harvest in the northern region of Paraná. The genotypes used were, BRS Gaivota, BRS Graúna, BRS Gralha azul, BRS Tangará, BRS Pardela, BRS Sabiá, BRS Sanhaço, IPR Taquari TM, TBIO Mestre, TBIO Sintonia, FPS Virtude, LD 141103, LD 142114, LD 152109, WT 11167 and WT 13086. Field collected seed samples from each genotype were submitted to the sanity test by the Blotter test and vigor test. The seeds remained in paper packaging and were stores in cold room conditions at 7 to 10 °C and 30% relative humidity to decrease deterioration.

Each sample was partitioned into 16 repetitions, totaling 400 seeds per genotype, in which 25 seeds per gerbox were distributed equidistantly.

The seeds were incubated in a climate chamber with a temperature of 25 ± 2 °C and a photoperiod of 12 hours, for seven days. After incubation time, identification was performed, based on characteristics described by Barnett and Hunter (1998); Leslie and Summerell (2008); and Warham et al. (1996), and recording the number of phytopathogens present as well as the amount of infested seeds. The quantification of phytopathogens in wheat seeds was performed with the aid of a stereoscopic microscope (Stemi 2000-C, Zeiss), with a 40X magnification and optical microscope (Primostar, Zeiss). Seeds with presence of conidiophores and/or spores fungi were considered infested. Results were expressed as a percentage (MAPA, 2003).

Data were submitted to analysis of variance and averages were compared by the Scott-Knott test at 5% probability.

3. Results and Discussion

Twenty genera of phytopathogenic fungus were recorded, in addition to an unidentified fungus on the seeds of the 16 wheat genotypes analyzed, and the number of pathogens varied in diversity and their respective incidence varied according to genotype (Table 1).

Table 1. Incidence (%) of different pathogens on seeds of 16 wheat genotypes, detected by Blotter test, without asepsis, Londrina, 2018

| Pathogens | Wheat (<i>Triticum aestivum</i> L.) genotypes | | | | | | | | | | | | | | | RF ¹ (%) | I ² | |
|---------------------------------|--|-----------------|------------|-------------|-------------|-------------|-------------|-----------|-----------|-----------|----------|-----------|----------|---------------|-------------|------------------------|----------------|----------------|
| | BRS Sanhaço | BRS Gralha azul | BRS Graúna | BRS Gaivota | BRS Tangará | TBIO Mestre | BRS Pardéla | LD 142114 | BRS Sabiá | LD 152109 | WT 11167 | LD 141103 | WT 13086 | TBIO Sintonia | FPS Virtude | | | IPR Taquari TM |
| <i>Rhizopus</i> sp. | 31.25 | 2.75 | 1.75 | 1 | 28 | 62 | 14.25 | 11.75 | 17.75 | 8.25 | 50 | 63.75 | 21 | 24.25 | 11.5 | - | 349.25 | 15 |
| <i>Aspergillus</i> sp. | 4 | 2 | 10.75 | 4.25 | 15.25 | 2.75 | 9.5 | 6 | 6.25 | 11.25 | 24.25 | 7 | 3.5 | 24.75 | 2.75 | 0.75 | 135.00 | 16 |
| <i>Mucor</i> sp. | 1 | 4.25 | 28.5 | 32.25 | 1.25 | - | 8.25 | 3.75 | 3 | 17 | - | - | 5.5 | 12.5 | 5 | - | 122.25 | 12 |
| <i>Alternaria</i> sp. | 22 | 23 | 3.5 | - | 4.75 | - | 8 | 12.5 | 2 | - | - | - | - | - | - | 5.75 | 81.50 | 8 |
| <i>Penicillium</i> sp. | 0.5 | 3.25 | 5.25 | 2.75 | 7.25 | 5.75 | 0.25 | 0.75 | 6.25 | 8.25 | 11 | 3 | 2.25 | 3.25 | 6 | - | 65.75 | 15 |
| <i>Pyricularia grisea</i> | - ³ | 0.25 | - | - | - | - | - | - | - | - | - | - | - | - | - | 59 | 59.25 | 2 |
| <i>Bipolaris</i> sp. | 8 | 1.5 | 1 | 2.25 | 0.75 | 0.25 | 1.75 | 3.5 | 1.25 | 1 | 0.25 | 0.25 | 4 | 0.25 | 2 | - | 28.00 | 15 |
| <i>Fusarium verticillioides</i> | 2.25 | 1.75 | - | 0.25 | - | - | 0.75 | 8.25 | 0.25 | 2.5 | - | - | - | 2.5 | - | 6.5 | 25.00 | 9 |
| <i>Nicrospora</i> sp. | - | - | 0.25 | - | - | - | - | - | - | - | - | - | - | - | - | 11 | 11.25 | 2 |
| <i>Trichoderma</i> sp. | 0.75 | 0.75 | - | - | - | - | - | 6.25 | - | 2.75 | - | - | - | - | - | 0.5 | 11.00 | 5 |
| <i>Curvularia</i> sp. | - | - | 2 | 0.5 | 0.25 | - | - | - | 0.5 | - | 1.5 | - | - | 1 | 1 | - | 6.75 | 7 |
| <i>Cladosporium</i> sp. | - | 1.25 | - | 1.75 | - | - | - | - | - | - | - | - | - | - | - | - | 3.00 | 2 |
| <i>Chaetomium</i> sp. | - | - | - | - | - | - | - | - | 0.25 | 0.5 | 0.25 | - | 0.5 | - | - | - | 1.50 | 4 |
| <i>Fusarium poae</i> | - | 0.5 | - | - | - | - | 0.25 | - | - | - | - | - | - | - | - | 0.5 | 1.25 | 3 |
| <i>Phoma</i> sp. | - | - | - | - | 0.25 | - | - | 0.75 | - | - | - | - | - | - | - | - | 1.00 | 2 |
| <i>Bipolaris spicifera</i> | - | - | - | - | - | - | - | - | - | 0.25 | - | - | - | - | - | - | 0.25 | 1 |
| <i>Drechslera</i> sp. | - | - | - | - | - | - | - | - | 0.25 | - | - | - | - | - | - | - | 0.25 | 1 |
| <i>Fusarium</i> sp. | - | 0.25 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.25 | 1 |
| <i>Periconia</i> sp. | 0.25 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.25 | 1 |
| <i>Acremonium</i> sp. | - | - | - | - | 0.25 | - | - | - | - | - | - | - | - | - | - | - | 0.25 | 1 |
| Unidentified fungus | - | 1.25 | 0.25 | 0.5 | 0.75 | - | 9 | 0.25 | 0.25 | 0.25 | 0.5 | - | - | 0.75 | - | 13.75 | 27.50 | 11 |
| Diversity | 9 | 13 | 9 | 9 | 10 | 4 | 9 | 10 | 11 | 10 | 7 | 4 | 6 | 8 | 6 | 8 | 930.50 | - |

Note. ¹ Relative frequency (RF), ² Incidence in genotypes (I), ³ Absence of Phytopathogen (-).

The fungi found associated with the seeds were *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp., *Bipolaris* sp. were found in all wheat cultivars, followed by *Mucor* sp., *Fusarium verticillioides*, *Alternaria* sp. and *Curvularia* sp., which were recorded in 12 and 7 cultivars, respectively. These fungi together represented 87.4% of the phytopathogens recorded. Moreover, *Rhizopus* sp. and *Aspergillus* sp. were highly frequent, occurring in 37.5% and 14.5% of all seeds evaluated. In contrast, *Penicillium* sp. and *Bipolaris* sp., were found in only 7.07% and 3.01% of the seeds, respectively.

The fungus *Rhizopus* sp. had a higher incidence in the genotypes LD 141103, TBIO Mestre, WT 11167, BRS Sanhaço, BRS Tangará and TBIO Sintonia, which ranged from 63.7% to 24.2%. On the other hand, *Aspergillus* sp. had higher incidence in the TBIO Sintonia, WT 11167 and BRS Tangará genotypes (24.7% to 15.3%).

Regarding the genotypes BRS Gaivota, BRS Graúna and the lineage LD 152109, *Mucor* sp. was the fungus with higher incidence. Finally, *Alternaria* sp. were found in 23% and 22% of seeds of the genotypes BRS Gralha azul and BRS Sanhaço, respectively.

Trichoderma sp., *Cladosporium* sp., *Chaetomium* sp., *Fusarium poae*, *Phoma* sp., *Bipolaris spicifera*, *Drechslera* sp., *Fusarium* sp., *Periconia* sp. and *Acremonium* sp. were also found associated with wheat seeds, however, these fungi were present in few genotypes and with low incidence ($\leq 0.5\%$).

The highest diversity of pathogens were found in the genotype BRS Gralha azul, which had 13 different pathogens, followed by BRS Sabiá with 11 fungi. Twelve different pathogens were found in the genotypes BRS Tangará, LD 142114 and LD 152109. On the other hand, the cultivars LD 141103 and TBIO Mestre presented the lowest diversity (4), with predominance of the fungus *Rhizopus* sp. affecting 63.7% and 62% of the seeds, respectively.

Although the presence of fungi such as *Phoma* sp. and *Bipolaris* sp. was not constant, it indicates the transport and association of disease-causing pathogens in several crops, causing economic damage, whether in grains or

seeds. The materials that presented the highest incidence of *Phoma* sp. were LD 142114 (0.75%) and BRS Tangará (0.25%). Genotype LD 152109 was the only one to present the fungus *Bipolaris* sp. at 0.25% incidence.

Fungi of secondary importance such as *Curvularia* sp., *Cladosporium* sp., *Mucor* sp., *Nigrospora* sp., *Trichothecium* sp., *Periconia* sp., and *Rhizopus* sp. are part of the complex of grain-staining pathogens (Celano, Machado, Jaccoud, & Guimarães, 2012). The genotypes BRS Graúna (2%), WT 11167 (1.5%), TBIO Sintonia and FPS Virtude (1%), BRS Gaivota and BRS Sabiá (0.5%) and BRS Tangará (0.25%) incidence of the fungus *Curvularia* sp. Genotypes that presented incidence of the fungus *Cladosporium* sp. were BRS Gaivota (1.75%) and BRS Gralha azul (1.25%). The pathogen *Mucor* sp. showed a higher incidence in BRS Gaivota materials (32.25%) and BRS Graúna (28.5%). The pathogen *Nigrospora* sp. showed a higher incidence in the genotype IPR Taquari TM (11%). Genotypes LD 142114 (6.25%) and LD 152109 (2.75%) presented a higher incidence of *Trichothecium* sp., *Periconia* sp., presented incidence only in the genotype BRS Sanhaço (0.25%). *Rhizopus* sp. showed a higher incidence in genotypes LD 141103 (63.75%), TBIO Mestre (62%), WT 11167 (50%), BRS Sanhaço (31.25%) and BRS Tangará (28%).

The pathogen *Penicillium* sp., showed a higher incidence in genotypes WT 11167 (11%), LD 152109 (8.25%), BRS Tangará (7.25%) and BRS Sabiá (6.25%). *Fusarium* sp., presented a higher incidence in genotypes LD 142114 (8.25%) and IPR Taquari TM (6.5%).

The main group of fungi, known to produce mycotoxins, include species of *Alternaria* sp., *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp., however, the three latter are the dominant ones (Fisher et al., 2012). Additionally, *Alternaria* sp., *Cladosporium* sp. and *Fusarium* sp. are the most common field fungi (Vecchia & Castilhos-Fortes, 2007). The materials that presented the highest incidence of *Alternaria* sp. were BRS Gralha azul (23%), BRS Sanhaço (22%) and LD 142114 (12.5%). *Aspergillus* sp. presented greater focus on the materials of TBIO Sintonia (24.75%) WT 11167 (24.25%) and BRS Tangará (15.25%). *Penicillium* sp. in genotypes LD 142114 (8.25%) and IPR Taquari TM (6.5%). *Fusarium* sp., in genotypes LD 142114 (8.25%) and IPR Taquari TM (6.5%).

The presence of the fungi *Rhizopus* sp., *Mucor* sp. and *Alternaria* sp., which are typically decay fungi, stands out among as causative agents of root rot and leaf spots of various crops. Storage time under conditions where there is some seed-associated pathogen leads directly to loss of seed germination percentage (Casa et al., 2012).

The fungi *Aspergillus* sp. and *Penicillium* sp. are associated with seed deterioration, which is often related to inadequate storage conditions, however, contamination can still occur in the field soon after harvesting (Machado, 1982). In contrast, the fungus *Bipolaris* sp., may be associated with diseases such as yellow spot [*Bipolaris sorokiniana* (Sacc.) Shoemaker], which causes serious damage to wheat crop, with up to 20% crop losses (Brancão et al., 2008).

It can be evidenced the presence of fungi of the genera *Fusarium*, *Aspergillus* and *Penicillium* associated with seeds, these when present in seeds and grains, produce secondary metabolic, called mycotoxins, substances capable of damage cause on human and animal health (Freire et al., 2007).

Leaf stain caused by *B. sorokiniana* is considered a disease of great importance to wheat crop, with yield losses of 20-80%. According to Barros et al. (2006) in very favorable conditions, the disease occurs throughout the crop cycle, infected seeds is the main survival mechanism of the fungus. In stored seeds, the fungus has the ability to survive as mycelium, in the endosperm of the seed and colonize the root system until it reaches the shoot (Neergaard, 1979; Kobayasti & Pires, 2011).

The highest percentages of seeds with phytopathogens were found in the genotypes WT 11167 (88%) and IPR Taquari TM (86.3%), and were not statistically different by the Scott-Knott test, while the lowest percentage of seeds with phytopathogens occurred in BRS Gralha azul, WT 13086, BRS Sabiá, FPS Virtude with an incidence ranging between 41.5% and 28%, respectively (Figure 1).

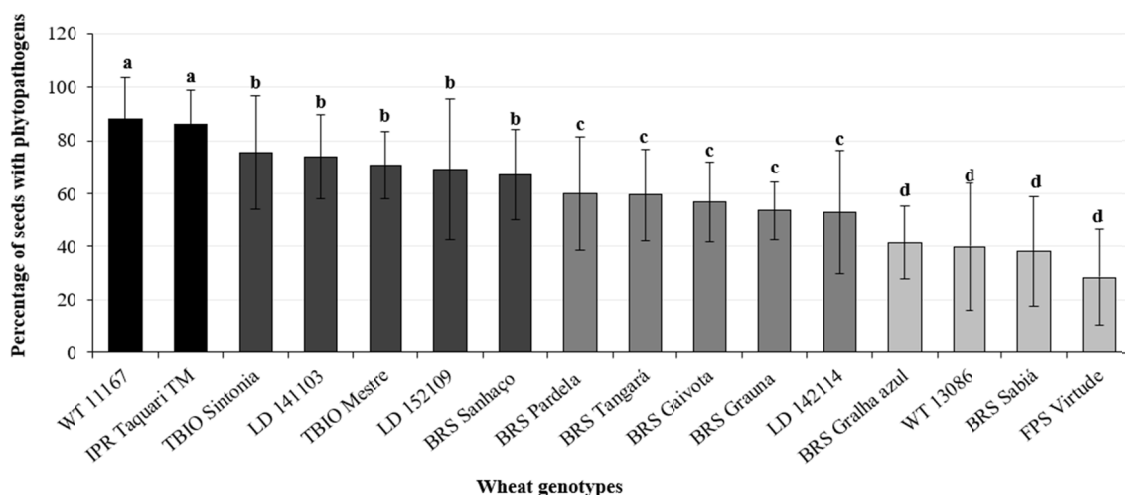


Figure 1. Percentage of phytopathogens detected in wheat seeds (unprocessed data). Averages followed by the same letter did not differ from each other by the Scott-Knott test at 5% probability. Coefficient of variation (CV) 30.6%; p-value < 0.01

The occurrence of fungi is detrimental to seed quality and can cause germination drop and reduce vigor, which can affect the establishment of plants under cultivation. The fungus *Rhizopus* sp. is considered of low economic importance in wheat seeds as a contaminant, however, as it is a fast-growing pathogen covering the seeds, it hinders usually the detection of other pathogens.

The incidence and severity of pathogens vary according to location, temperature and humidity (Brancão, 2008; Celano, Machado, Jaccoud, & Guimarães, 2012). Thus, it is important to monitor these factors in order to qualitatively and quantitatively observe the presence of pathogens associated with wheat seeds.

In addition to the control of plant pathogens under field conditions, technologies for monitoring the physical state of seeds can be implemented through health testing and sanitary treatment, which are fundamental to determine the causes of low germination, common in samples with high infection rates (Machado, 1982).

The fungus *Cladosporium* sp. is considered pathogenic to several plants and may reduce seed vigor (Rajput & Aslam, 2005). Moreover, this pathogen has been reported in association with wheat seeds (Brancão et al., 2002; Duan, Wang, Zhu, & Wu, 2007; Malaker, Mian, Bhuiyan, Akanda, & Reza, 2008).

One of the shoot diseases of major concern is the wheat blast, caused by *P. grisea*. Its importance is due to the impact on reducing grain yield and quality, which can reach up to 50% (Reis et al., 2005). Although there was no high incidence of *P. grisea* in the genotypes analyzed, the presence of this pathogen in seeds is of concern for the regions of the State of Mato Grosso, where conditions are favorable for its development. When infection occurs at the beginning of flowering and grain filling, they present deformed, small and with specific low weight (Embrapa, 2004). The materials analyzed in this study that presented the incidence of *P. grisea* in the seeds were BRS Galha azul with 0.25% and IPR Taquari TM with 59%. Both materials with the incidence of *P. grisea* are cultivars with high grain yield in the State of Paraná, wide adaptation, technological quality.

The genotypes BRS Gaivota, LD 152109, WT 13086, FDS Virtude, BRS Galha Azul, BRS Sabiá, BRS Grauna, TBIO Sintonia and BRS Pardela had the highest germination percentages between 98.3% and 87.3%, while the lowest germination index was recorded for Taquari TM (22%), followed by WT 11167 (37.3%), TBIO Mestre (44.3%). The other genotypes had intermediate level of germination, which indicates that the phytopathogens influenced the germination percentage for each wheat genotype evaluated (Figure 2).

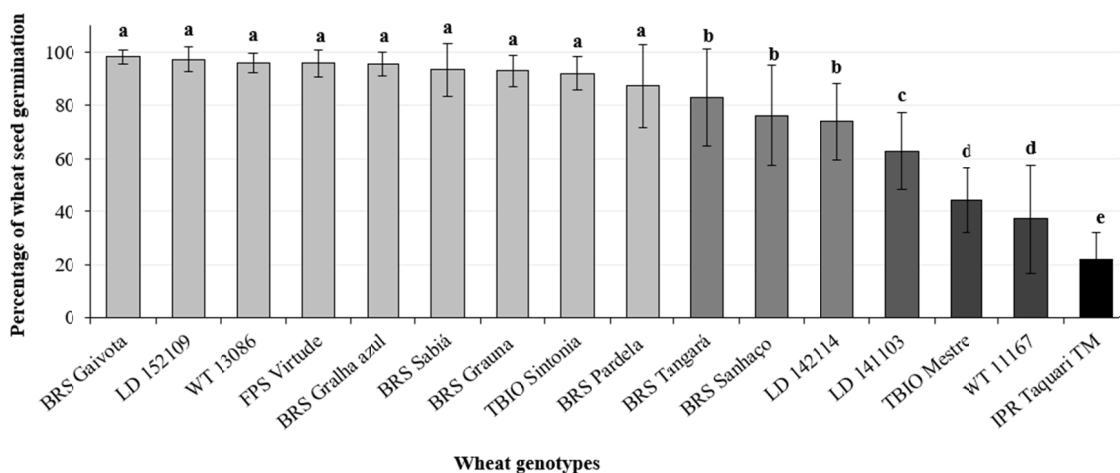


Figure 2. Percentage of wheat seed germination in 16 genotypes (unprocessed data). Averages followed by the same letter did not differ from each other by the Scott-Knott test at 5% probability. CV 15.3%; p-value < 0.01

Although the presence of fungi such as *Phoma* sp. and *Bipolaris* sp. was not constant, it indicates the transport and association of disease-causing pathogens in several crops, causing economic damage, whether in grains or seeds. The materials that presented the highest incidence of *Phoma* sp. were LD 142114 (0.75%) and BRS Tangará (0.25%). Genotype LD 152109 was the only one to present the fungus *Bipolaris* sp. at 0.25% incidence.

Fungi of secondary importance such as *Curvularia* sp., *Cladosporium* sp., *Mucor* sp., *Nigrospora* sp., *Trichothecium* sp., *Periconia* sp., and *Rhizopus* sp. are part of the complex of grain-staining pathogens (Celano, Machado, Jaccoud, & Guimarães, 2012). The genotypes BRS Graúna (2%), WT 11167 (1.5%), TBIO Sintonia and FPS Virtude (1%), BRS Gaivotá and BRS Sabiá (0.5%) and BRS Tangará (0.25%) incidence of the fungus *Curvularia* sp. Genotypes that presented incidence of the fungus *Cladosporium* sp. were BRS Gaivotá (1.75%) and BRS Gralha azul (1.25%). The pathogen *Mucor* sp. showed a higher incidence in BRS Gaivotá materials (32.25%) and BRS Graúna (28.5%). The pathogen *Nigrospora* sp. showed a higher incidence in the genotype IPR Taquari TM (11%). Genotypes LD 142114 (6.25%) and LD 152109 (2.75%) presented a higher incidence of *Trichothecium* sp., *Periconia* sp., presented incidence only in the genotype BRS Sanhaço (0.25%). *Rhizopus* sp. showed a higher incidence in genotypes LD 141103 (63.75%), TBIO Mestre (62%), WT 11167 (50%), BRS Sanhaço (31.25%) and BRS Tangará (28%).

Pathogens can be considered the most active due to their ability to penetrate plant tissues and lodge internally. The seeds are vehicle of transport of the inoculum of fungi, the transmission rate of the pathogen will depend, the place where it is located and the amount of the inoculum in the seeds. The most effective way for the seedling that will originate to present infection is through the presence of the pathogen in the embryo of the seeds (De Sá et al., 2011).

Other factors that can influence the infection of pathogens in seeds, such as genotype, practices used in crop management, severity of infection in the parent plant as well as the phase of infection in the plant, insect infestation and the way of management during seed processing (MACHADO, 2012).

Intermediate fungi attack the grains before harvest and continue to grow and cause damage during storage. This category includes some species of *Penicillium* sp. and *Fusarium* sp. (Vecchia & Castilhos-Fortes, 2007; Kobayasti et al., 2011). According to Medina et al. (2009), in the period after harvesting and during the transportation and storage of agricultural products, fungal growth can be influenced by many factors, mainly humidity level, temperature, aeration, insect damage, and storage time, among others. Moreover, the greatest effects of fungal development on stored grains and seeds are loss of germination power, loss of dry matter, mycotoxin production and change in nutritional value (Kobayasti et al., 2011).

4. Conclusion

The genera *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp., and *Bipolaris* sp. were the predominant phytopathogens in wheat seeds, with the first two presenting higher incidence percentages. Wheat seeds of the BRS Gralha azul and BRS Sabiá genotypes had higher incidence for different pathogens. On the other hand, WT 11167 and IPR Taquari TM genotypes presented the highest percentage of seeds with phytopathogens. Nine of

the sixteen genotypes investigated had the highest germination percentages, which were between 98.3% and 87.3%. The presence of fungi is detrimental to seed quality and may cause germination drop and reduce vigor, besides affecting the establishment of plants under cultivation.

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