Chapter 8

Diseases affecting wheat: wheat blast

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- 1 Introduction
- 2 Symptoms and conditions for wheat blast development
- 3 Origin of wheat blast
- 4 Reproductive biology of Magnaporthe oryzae
- 5 The new causal agent of wheat blast: Pyricularia graminis-tritici
- 6 Alternatives for survival of the causal agent
- 7 Genetic control
- 8 Chemical control
- 9 Conclusion and future trends
- 10 References

1 Introduction

Pyricularia oryzae Cavara (syn. Magnaporthe oryzae B. C. Couch) (Couch and Kohn 2002) is a fungus that causes blast disease in more than 50 species of the Poaceae family (the grass family), including agricultural crops such as rice, wheat, barley, millet and oat (Urashima and Kato 1998; Couch and Kohn 2002; Takabayashi et al. 2002; Murakami et al. 2003; Couch et al. 2005; Prabhu and Filippi 2006; Castroagúdin et al. 2016). In Brazil, in addition to rice, the wheat crop is severely affected by outbreaks of the disease, especially in the states of Paraná, Minas Gerais, Mato Grosso do Sul, Mato Grosso, São Paulo and Goiás (Igarashi et al. 1986; Goulart and Paiva 1992; Anjos et al. 1996; Goulart and Paiva 2000). Wheat blast was first reported in Londrina, Paraná, Brazil, in 1985 (Igarashi et al. 1986). Reports of the rapid spread of the pathogen in other Brazilian states demonstrated the rapid dispersal of the pathogen from its original focus (Picinini and Fernandes 1990; Goulart et al. 1990; Goulart and Paiva 1992; Prabhu et al. 1992; Urashima and Kato 1994). Measures adopted for controlling wheat blast have not been effective, with no resistant wheat cultivars available and low efficiency of fungicides, conditions that are reflected in the losses caused by the disease, which may reach up to 100% (Goulart and Paiva 2000; Torres et al. 2009; Castroagúdin et al. 2015; Maciel 2011).

Although there have been reports of wheat blast occurrence in Argentina (Cabrera and Gutiérrez 2007), until the beginning of 2016 reports of outbreaks of wheat blast were restricted to three countries: Brazil, Bolivia and Paraguay (Duveiller et al. 2010; Maciel 2011). However,

in March 2016, a very severe wheat blast epidemic was reported in Bangladesh (Callaway 2016). More recently, in February 2017 there was a report about blast disease in Indian fields near the Bangladeshi border causing great damage to wheat grain production (Bhattacharya and Pal 2017). This situation has generated enormous concern within Bangladesh, and the concerned authorities have adopted extreme measures, such as burning affected wheat fields, in an attempt to eradicate the disease. The extreme severity and rapid spread demonstrated by wheat blast in Brazil since its occurrence in the country, in the mid-1980s, contrast with the condition that may have prevailed in India and Pakistan. Diekmann and Putter (1995) mentioned that in 1922 and 1943 there were reports of the natural occurrence of wheat blast in those two countries, respectively. However, the disease never reached epidemic levels. Based on phylogenetic analyses, Islam et al. (2016) proposed that wheat blast arrived in Bangladesh from wheat grain imported from South America.

2 Symptoms and conditions for wheat blast development

Wheat blast symptoms vary greatly in severity on leaves and heads according to weather conditions and wheat genotype (Urashima et al. 2009; Goulart 2005). The most favourable conditions for wheat blast development are a combination of temperature ranging from 23° to 28°C, excessive rain, long and frequent leaf wetness and poor fungicide efficacy (Goulart 2005). Danelli (2015) determined that the concentration of conidia of *Pyricularia oryzae* in the air in the municipality of Passo Fundo, southern Brazil, is enormous, although there may be some variation in the concentration (conidia/m³ of air) throughout the year.

Pyricularia oryzae affects both leaves and heads of wheat, but the most significant damage occurs on the heads (Cruz et al. 2015; Igarashi 1990; Urashima et al. 2009). The typical symptoms of wheat blast on the leaves, even though rare before the heading stage, are the presence of elliptical to elongate lesions with white to light brown centres and dark grey to reddish-brown borders. The main problem comes from the infection in the rachis, causing partial or total sterility of the heads. All the spikelets above the infection point in the rachis turn white. In general, symptoms on heads may vary from elliptical lesions with bleached centres on glumes to partial or total spike bleaching, sterility and empty grains (Goulart and Paiva 1992; Urashima et al. 2009). The infection in the rachis interrupts the translocation of photosynthates to upper parts of the spike. Blast-infected grains from highly susceptible cultivars are usually small, wrinkled, deformed and have low-test weight (Goulart 2005).

3 Origin of wheat blast

Several hypotheses were made with regard to the origin of wheat blast in Brazil after its first report in the mid-1980s. The most discussed hypothesis emphasized the relationship between rice and wheat crops, indicating that *Pyricularia oryzae* of wheat came from rice plants (Mehta et al. 1992). However, molecular and virulence assays detected important differences between isolates of *Pyricularia oryzae* coming from rice and wheat (Couch and Kohn et al. 2002; Maciel et al. 2014). New hypotheses emerged based on modern evolutionary theories. In this context, evidence showed that sympatric populations of *Pyricularia oryzae* adapted to

rice rarely migrated to wheat, suggesting that the population that infects wheat originated from a population infecting an unknown weed Poaceae, distinct from rice (Ceresini et al. 2011). Considering all these aspects, the most plausible hypothesis for the origin of wheat blast is from a clonal propagation of a well-adapted and epidemic lineage of *Pyricularia oryzae* belonging to populations of other host grasses. The bottleneck effect in the original *Pyricularia oryzae* population and intensification of wheat fields in southern Brazil are also part of this scenario (Ceresini et al. 2011; Maciel et al. 2014). Besides, it is likely that *Pyricularia oryzae* of wheat presents a mixed reproductive system in which sexual reproduction is followed by the dispersal of clones (Maciel et al. 2014), and that invasive plants of the Poaceae family influence the development of wheat blast (Maciel et al. 2013).

4 Reproductive biology of Magnaporthe oryzae

Pyricularia oryzae of rice is used as model fungal pathogen species (Talbot 2003). The teleomorph or sexual phase of *Pyricularia oryzae* is called *Magnaporthe oryzae* and, due to its self-incompatibility, it is formed only when there is a crossing between two sexually compatible and fertile individuals. This occurs when the female receptive structure called ascogonium is able to receive the compatible nucleus or nuclei of the male donor by means of conidia or receptor hyphae (Kang et al. 1994; Moreira et al. 2015).

What we know about reproductive biology of *Magnaporthe oryzae* in the literature was generated, mainly, with controlled crosses conducted in laboratories because the sexual phase of the pathogen is rarely found in the field (Saleh et al. 2012). Mating-type genes regulate the recognition of sexually compatible individuals (Turgeon 1998; Moreira et al. 2015). The gene that controls the compatibility in *M. oryzae* has two idiomorphs, *Mat1-1* and *Mat1-2* (Nottéghem and Sulué 1992; Peixoto 2014). The detection of gametic balance in populations of the pathogen is an indirect way of determining whether the reproductive mode of the pathogen in the field is mainly sexual or clonal (Maciel et al. 2014).

5 The new causal agent of wheat blast: *Pyricularia* graminis-tritici

Based on host specificity, mating capacity and genetic similarity, isolates of *Pyricularia* oryzae are characterized as belonging to several pathotypes (Urashima et al. 1993; Kato et al. 2000; Tosa et al. 2004; Tosa and Chuma 2014). The following is the list of the pathotypes of *Pyricularia* oryzae already described and the hosts in which it is pathogenic: Oryza, Oryza sativa (rice); Setaria, Setaria italica (foxtail millet); Panicum, Panicum miliaceum (millet); Eleusine, Eleusine coracana (finger millet); Triticum, Triticum aestivum (wheat); Oat, Avena sativa, (oat); and Lolium, Lolium perenne (ryegrass).

Pyricularia oryzae Triticum is the causal agent of wheat blast in South America, including Brazil, and is responsible for blast in barley, rye, triticale and *Brachiaria* (*Urochloa* spp., ex *Brachiaria* spp.) (Lima and Minella 2003; Verzignassi et al. 2012). As the wheat blast arose in an area in southern Brazil close to areas where rice fields were cultivated and occurrence of rice blast on those fields was common, the initial proposition was that *Pyricularia oryzae* Oryza had undergone some type of mutation and evolved virulence on

wheat (Igarashi et al. 1986). In southern Brazil, wheat and rice crops are grown in relatively close regions, although for reasons related to agricultural aptitude, the same areas are not used for cultivating the two crops. However, based on evaluations of pathogenicity, reproductive isolation and genetic analysis, Urashima et al. (1993) provided evidence that there were two distinct groups of *Pyricularia oryzae* that cause blast diseases in Brazil, one that infects wheat and another that infects rice. In these evaluations, it was verified that the isolates obtained from wheat plants were able to infect plants from six tribes of the family Poaceae. Urashima et al. (1993) also verified that *Pyricularia oryzae* Triticum isolates were able to recombine sexually, producing perithecia with viable ascospores, when crossed with isolates from *Eleusine coracana*, *Urochloa plantaginea* (ex *Brachiaria plantaginea*) and *Setaria italica*. However, when crosses were carried out between wheat and rice isolates, a complete absence of fertility was obtained, with no perithecia or ascospores.

Specific comparisons between isolates of *Pyricularia oryzae* belonging to the Oryza and Triticum pathotypes have already been made using different approaches. Using the RFLP technique, with the probes MGR563 and MGR586, Farman (2002) determined the existence of a substantial difference between the two pathotypes. According to analyses using 11 microsatellite loci, Maciel et al. (2014) showed a significant difference between *Pyricularia oryzae* Triticum and *Pyricularia oryzae* Oryza (FCT = 0.896, P \leq 0.001; FCT, variance between the two populations compared). Besides, Maciel et al. (2014) also determined that none of the 69 isolates of *Pyricularia oryzae* Triticum used in inoculation procedures were able to infect rice plants.

The genus *Pyricularia*, which has approximately 50 species, is quite complex, albeit it is believed to be monophyletic (Hirata et al. 2007; Choi et al. 2013; Klaubauf et al. 2014). Species of this genus have increasingly been subject to reclassifications and/or subdivisions, which have been mainly based on multilocus analyses of housekeeping genes. An important reclassification scheme divided *Pyricularia grisea* into two species, *Pyricularia grisea* and *Pyricularia oryzae* (Couch and Kohn 2002). Castroagúdin et al. (2016) proposed the existence of a new species *Pyricularia graminis-tritici*. According to the authors of the work, this species, together with the pathotype Triticum of *Pyricularia oryzae*, is the causative agent of wheat blast.

6 Alternatives for survival of the causal agent

Pyricularia oryzae is known to survive from one season to another on seeds and live plants or, as saprophytic mycelium, on straw (Ou 1987; Kimati et al. 2005). However, the relative extent and importance of these survival alternatives is unknown. Castroagúdin et al. (2016) indicated that plants of the genus *Urochloa* play an important role in the survival and generation of the pathogen variability. It is critical that we get more information about the characteristics of each one of the alternatives of survival of the causal agents of wheat blast in order to manage and control the disease.

Even though the proposal for the new species *Pyricularia graminis-tritici* is recent, this fungus also needs to be included in the discussion about its alternatives for the survival in the absence of wheat plants. However, this situation should be contextualized. Information on *Pyricularia graminis-tritici* is scarce and is restricted to the publication in which the proposal of this new species was presented (Castroagudin et al. 2016). In this sense, one of the most important factors reported by the authors is that both species *Pyricularia oryzae* and

Pyricularia graminis-tritici are widely distributed in the Brazilian wheat fields. In fact, there is little or no knowledge about the characteristics of *Pyricularia graminis-tritici*, especially in relation to phenotypic aspects, for example, its ability to survive in environments such as straw, seeds or alternative hosts. From now on, it seems most reasonable to imagine that the new studies on wheat blast, especially those conducted in Brazil, should consider the existence of the two species, *Pyricularia oryzae* and *Pyricularia graminis-tritici*, as the causal agent of wheat blast. Thus, the currently available knowledge on the alternatives of survival of the causative agent of wheat blast refers almost exclusively to the species *Pyricularia oryzae*, which represents the main content of this chapter. Currently the main source on *Pyricularia graminis-tritici* is the work of Castroagúdin et al. (2016) who isolated the pathogen in a number of grass species.

6.1 Infective activity in alternative hosts

The large number of plant species on which *Pyricularia oryzae* may survive represents a great difficulty in advancing the knowledge about the role that alternative hosts play in wheat blast outbreaks occurring in South America (Castroagúdin et al. 2016; Couch et al. 2005; Couch and Kohn 2002; Murakami et al. 2003; Prabhu and Filippi 2006; Takabayashi et al. 2002; Urashima and Kato 1998). The situation became even more complex with the new proposal that *Pyricularia graminis-tritici* was also a causal agent of wheat blast (Castroagúdin et al. 2016). This species has been reported as being capable of infecting *Urochloa* spp., *Echinochloa* spp., *Cenchrus echinatus*, *Rhynchelytrum repens*, *Eleusine indica*, *Avena sativa*, *Cynodon dactylon*, among others (Castroagúdin et al. 2016). Directly associated with this aspect is the problem of the lack of consistent information on the virulence of *Pyricularia graminis-tritici* on wheat and, much less, on wheat cultivars. One of the few pieces of information we have about the characteristics of *Pyricularia graminis-tritici* was demonstrated by Maciel et al. (2013) using isolates obtained from *Urochloa* spp. to inoculate leaves and heads of a set of wheat genotypes. They verified that *Pyricularia graminis-tritici* was as virulent as some isolates of *Pyricularia oryzae* Triticum used in the experiment.

6.2 Infection of wheat seeds

The first report of transmission of *Pyricularia oryzae* through seed was in rice seeds in Japan (Kuribayashi 1928). In relation to wheat, most of the studies on the detection of *Pyricularia oryzae* in wheat seeds were carried out in Brazil during the 1990s. The most important study is probably the one conducted by Goulart et al. (1995). In this work, carried out for 6 years (1987–92), 2238 samples of grain and wheat seeds, produced in 11 municipalities in the Brazilian state of Mato Grosso do Sul of several cultivars, were analysed. The presence of *Pyricularia oryzae* was detected in 16.5% of the samples and the average incidence of the fungus in the grains and/or seeds was 3% in 1990 and 0.2% in 1991 and 1992. The efficiency of the transmission of the pathogen by the seeds is high, capable of reaching 28% (Goulart and Paiva 1993).

Reis et al. (1995) determined that *Pyricularia oryzae* may survive for a relatively long period in wheat seeds. They tested seeds of two wheat cultivars, Anahuac and Tapejara, both harvested in the 1987 season, in the state of Paraná. Both samples had an average incidence of the fungal infection of around 45%, and it was possible to verify that *Pyricularia oryzae* survived in the seeds for 19 and 22 months, respectively, with a viability of 60% after 8 months of storage.

The presence of *Pyricularia oryzae* in seeds, besides damaging the plant that will emerge from this infected seed, may introduce the fungus in areas where it was not present. Considering the potential for dissemination of the pathogen through wheat seed, the use of healthy seeds, free from the pathogen, is an important measure in the management of blast. Currently, the seed cleanliness testing for *Pyricularia oryzae* is uncommon in South America, although this practice is becoming more prevalent in international seed trading (International Seed Testing Association 1996).

Detection of pathogenic fungi in cereal seeds may be difficult, especially when infection rates are very low. For a diagnostic test to be adequate, it should be sensitive and specific, avoiding cross-reactions with other fungal species (Konstantinova et al. 2002). The most commonly used seed tests for the detection of *Pyricularia oryzae* have been made on rice seeds using the Blotter test or growth on Petri dishes with culture medium (Neergaard et al. 1970). These procedures usually involve steps such as the isolation and cultivation of pathogens, as well as identification by the morphology of reproductive structures and biochemical tests. The test based on molecular methods, especially using PCR, is a viable alternative that has been shown to be very efficient for the detection of many fungi (Vandemark et al. 2000; Konstantinova et al. 2002).

The use of molecular techniques to detect *Pyricularia oryzae* in complex environments and under low concentrations has been demonstrated by Villari et al. (2017). In this study, a pathogen-specific quantitative-loop-mediated isothermal amplification (*qLAMP*) assay coupled with a spore trap system was developed for the rapid detection and quantification of airborne inoculums of *Pyricularia oryzae* from perennial ryegrass. In this sense, although the application of molecular strategies for the detection and differentiation of *Pyricularia oryzae* and *Pyricularia graminis-tritici* in seeds has not yet been demonstrated, it is likely that this procedure is achievable. The next step is the selection of specific primers and validation of the methodology.

6.3 Saprophytic activity in wheat straw

The great adaptability of the fungus *Pyricularia oryzae* is closely related to its nutritional requirements. The fungus is classified as a hemibiotroph, combining aspects of biotrophy and necrotrophy at different points during infection. *Pyricularia oryzae* can survive on dead plant tissue, but the timespan for viability of the pathogen in these circumstances is unknown. Soil type and tillage methods in addition to climatic conditions would affect viability. Most of the wheat fields in South America are in no-tillage areas, where plant residues remain on the surface of the soil (van Capelle et al. 2012). It is unknown if the slower degradation of wheat straw in no-tillage also means a longer survival period of *Pyricularia oryzae*. A great challenge is to relate the use of no-tillage with the survival of *Pyricularia oryzae* in straw and, more importantly, to establish the importance of this inoculum in the structuring of wheat blast outbreaks.

7 Genetic control

In Brazil, since the first report of the disease in the mid-1980s, the search for resistance to wheat blast has been intense, but the results are erratic and inconsistent (Goulart et al. 1995; Cruz et al. 2010). Wheat cultivars, apparently resistant in some localities,

160 -

are susceptible in other regions of the country, indicating interaction between cultivar and environment (Urashima et al. 2004). Considering that *Pyricularia oryzae* Triticum and *Pyricularia graminis-tritici* are present in all Brazilian wheat agroecosystems (Duveiller et al. 2010; Maciel et al. 2014; Castroagúdin et al. 2016), it is probable that both incidence and severity of blast are dependent on the fungal variant prevalent in that agroecosystem. Maciel et al. (2014) detected both complete and partial resistance in seven Brazilian wheat cultivars. Based on those results, it was possible to classify the wheat blast isolates used on the experiments in eight and fourteen virulence groups according to response in terms of complete and partial resistance, respectively. The high phenotypic variability of the pathogen will probably result in low durability of the resistance of wheat cultivars.

Selected wheat genotypes, which displayed high resistance to blast in early trials, have been evaluated in more detail, either under controlled conditions or in the field environment. The cultivar BR 18-Terena is widely used in wheat breeding programmes to obtain durable resistance to wheat blast. Results obtained in the field trials, under high incidence of the disease, determined that the bread wheat BR 18-Terena had head blast incidences varying from 8.2% to 24%, rates relatively low for wheat blast (Goulart et al. 1991; Goulart and Paiva 1992; Sousa 2002). Another wheat cultivar, classified as moderately resistant, that has demonstrated a good degree of resistance to wheat blast is BRS 229. In a field assay, conducted in Londrina, Paraná, Brunetta et al. (2006) evaluated BRS 229 for blast resistance and determined a mean score of 2 for severity on its heads, in a scale ranging from 0 to 9, with 9 as the highest intensity of the disease.

The winter wheat cultivar Renan was reported as having low disease rates (Danelli 2015; Tufan et al. 2009). In work conducted under controlled conditions, Danelli (2015) evaluated the reaction of Renan heads to individual inoculations of 144 isolates of *Pyricularia oryzae* obtained from wheat plants. There was considerable variability in the response of the cultivar, but overall the cultivar showed high resistance. In the evaluation of the cellular responses in Renan, the formation of papilla-like structures at the infection sites was observed, and the analysis of the transcripts associated with defence mechanisms (Tufan et al. 2009).

In Bolivia, many wheat growers use the cultivars Motacú, Urubo and San Pablo, which are classified as moderately resistant. More recently, cultivars, such as USDA 8 and CMT-50, with higher resistance levels to the disease have been available (Baldelomar et al. 2017).

Wheat cultivars currently available to South American growers are very susceptible to wheat blast with the exception of two or three cultivars, which at best may be classified as moderately resistant. This is thought to be due to the narrow genetic basis of wheat cultivars in Brazil. A solution to this situation is to use wide crosses to the progenitors of wheat (Cruz et al. 2010). It has already been shown that some *T. tauschii* accessions (synonyms *Aegilops squarrosa, Aegilops tauschii*) are resistant to *Pyricularia oryzae* (Urashima and Kato 1994). Bockus et al. (2012) evaluated 10 different genotypes of this species, with each one coming from a different Asian country, and showed a great deal of variability in blast responses in heads. The mean severity ranged from 5.1% to 99.9% depending on the genotype. Cruz et al. (2010), studying partial resistance to wheat blast, verified that synthetic wheat genotypes, developed by Moraes-Fernandes et al. (2000) from the crossing of *T. durum* and *T. tauschii*, showed lower blast severity than cultivars of wheat traditionally used in Brazil. This lower severity was observed in both leaf and head reactions.

7.1 Genetic resistance and molecular markers

The presence of Avr1-CO39 avirulence gene in Pyricularia oryzae Triticum and Pyricularia graminis-tritici prevents their infection on rice (Maciel et al. 2014) as this plant species carries the Pi-CO39 (t) resistance gene. The Pi-CO39 (t) gene is rare or absent in plant species belonging to the genera Avena, Urochloa, Eleusine, Eragrostis, Lolium, Panicum, Setaria, Triticum and also some species of the genus Oryza (Tosa et al. 2005). The corresponding Avr1-CO39 avirulence gene occurs generally in pathogen populations that do not infect rice, which include isolates of Pyricularia spp. of most of the above-mentioned hosts (Couch et al. 2005). These data indicate that the presence of Pi-CO39 in wheat genotypes, which is still unknown, appears as highly relevant scientific information to be obtained in the future, considering the possibility that this gene may be transferred to wheat genotypes using, for example, transgenic strategy since Pi-CO39 is cloned (Chauhan et al. 2002).

Approximately 100 rice blast-resistant genes have already been identified and mapped (Sharma et al. 2012; Wang et al. 2014). Of the genes mapped, 22 have already been cloned (Liu et al. 2010; Sharma et al. 2012; Singh et al. 2015; Wang et al. 2014; Xiao et al. 2015). Because it is a relatively recent disease, many aspects related to the molecular interaction between *Pyricularia oryzae* and wheat remain unknown. Eight resistance genes to wheat blast have already been identified, known as *Rmg1* to *Rmg8* (Anh et al. 2015; Nga et al. 2009; Tagle et al. 2015; Takabayashi et al. 2002; Vy et al. 2014; Zhan et al. 2008). In addition, transcriptome studies have also been conducted (Tufan et al. 2009; Ha et al. 2016). In a study published in 2016, evaluating differential expression in wheat in response to blast, the authors recommended pyramiding a large number of resistance genes to wheat blast (Ha et al. 2016).

Another study published in 2016 reported the association between the presence of the 2NS chromosomal translocation segment of Aegilops ventricosa and resistance to wheat blast (Cruz et al. 2016). The resistance of Aegilops ventricosa to wheat diseases including rust was first mentioned in the 1960s (Maia 1967). A long chromosomal segment of 25 to 38 cM located on the 2NS chromosome was transferred to chromosome 2AS of T. aestivum (McIntosh et al. 1995). The segment 2NS/AS carries the following genes: Rkn3, for gall nematode resistance (Meloidogyne spp.) (Williamson et al. 2013); Cre5, for resistance to the French pathotype Ha12 of the cereal cyst nematode (Heterodera avenae) (Jahier et al. 2001); and the genes Lr37, Sr38 and Yr17, which act against Puccinia spp., causative agents of leaf, stem and stripe rusts, respectively (Bariana and McIntosh 1993; McIntosh et al. 1995). The 2NS/AS fragment was initially transferred to the VPM1 wheat cultivar and then to other cultivars such as Madsen and Thatcher (Helguera et al. 2003). Helguera et al. (2003) developed molecular markers to confirm the presence of the 2NS/AS segment in wheat genotypes, and, based on them, Cruz et al. (2016) reported the association between the presence of this segment and the reduction of blast severity on heads of American cultivars of spring and winter wheat. According to Cruz et al. (2016), wheat genotypes with the 2NS/AS segment, when inoculated with wheat Pyricularia oryzae isolates, showed 64% to 80.5% less disease on the heads than those that did not have the segment. Nonetheless it is clear that other genes confer resistance in wheat against Pyricularia oryzae infection.

8 Chemical control

Chemical control is a major aspect part of wheat blast management and it is used in two situations. The first one is the seed treatment to prevent infection of seedlings

after germination. Tests show that fungicidal treatment of wheat seeds can satisfactorily control seedling infections caused by wheat blast. In Brazil, there are five commercial fungicides officially registered for wheat blast seed treatment (Anon. 2017). The active ingredients are iprodione, fluazinam, difeconazol, carboxin and thiram. The list of these products has not been subject to major changes since when the disease appeared and began to spread in the 1980s. Among them, the commercial mixture carboxin + thiram has been the most cost-effective product to control wheat blast via seed treatment (Goulart and Paiva 1991).

Leaves and heads can also be protected by applying fungicide one or more times, but results have been disappointing (Maciel 2011; Pagani et al. 2014; Rocha et al. 2014). This situation may be due to insensitivity of the pathogen to fungicides, use of too low a dose, poor coverage of the fungicide on the heads and inappropriate timing of the spray (Maciel 2011; Panisson et al. 2014; Castroagúdin et al. 2015; Oliveira et al. 2015). To remedy this situation, an important initiative started in Brazil in 2010, when a collaborative action was established between some Brazilian universities and wheat research companies (public and private). The 'Cooperative Assays Network' (CAN) conducts field trials using a standard protocol to evaluate the efficiency of fungicides to control wheat blast on heads. In 5 years, 39 field trials were held in the main wheat-producing regions of the country with a history of wheat blast. It was shown that there is no economic return for any fungicide sprayings if the incidence of head wheat blast in the field is more than 75%. Economic return was only guaranteed when the incidence of blast on the heads reached a maximum of 25%. When the incidence of the disease ranged from 25% to 75%, there was variation in relation to the economic viability, which was quite dependent on the vield of grains promoted by aerial application of fungicides. Another important result obtained in the scope of the CAN is that the products that contain mancozeb performed consistently well.

A question that still needs to be addressed is whether leaf infection of either Pyricularia oryzae Triticum or Pyricularia graminis-tritici leads directly to head blast. We think any analysis or speculations on the subject must take into account how South American growers, especially the Brazilian ones, cultivate their wheat crop. The occurrence of leaf blast is a rare event, and one reason that may contribute much to the difficulty in finding leaves with blast lesions is the increasing of the number of fungicide sprayings at early stages of development of the plants. In addition to mancozeb, the active ingredients of fungicide action most used in Brazil for the control of wheat blast belong to triazole or strobilurin chemical groups, which are registered and allowed to be sold in the country. Their performance to control the development of the causal agent of wheat blast in tests conducted in vitro has been considered satisfactory. Wheat growers use these products even before the heading stage with the aim of controlling foliar diseases such as mildew, leaf rust, tan spot, among others. In addition, Silva et al. (2015) showed that chemical control of blast on the wheat leaf is much more feasible than on the heads. In fact, the problem is that the applied fungicide does not fully cover the rachis adequately. The question of fungicide application technology is one of the factors that most needs to be investigated in relation to the chemical control of the blast. It is important to relate efficiency of the application with environmental aspects that occur during the crop season such as temperature and precipitation.

Evolved resistance to fungicides is a major concern. *In vitro* tests determined the sensitivity to the triazole tebuconazole of *Pyricularia oryzae* and *Pyricularia graminis-tritici* isolates obtained from the Federal District and from six Brazilian wheat-producing states.

Boaretto (2016) determined that 35% of the tested isolates had reduced sensitivity. In the case of trifloxystrobin (strobilurin chemical group), total insensitivity is widespread in the Brazilian population of *Pyricularia oryzae* Triticum and *Pyricularia graminis-tritici*, with 80% of the isolates demonstrating some kind of insensitivity to this fungicide (Castroagúdin et al. 2015). Examination of the cytochrome b (*Cyt-b*) gene, which is the target site, confirmed the results obtained *in vitro*. Of 234 tested isolates of *Pyricularia oryzae* Triticum and *Pyricularia graminis-tritici*, obtained from the major Brazilian wheat-producing regions in 2005 and 2012, 82% of them had the resistant G143A *Cyt-b* gene mutation. Castroagúdin et al. (2015) verified that the frequency of mutation had increased, from 36% in 2005 to 90% in 2012. The results indicate that, in Brazil, monitoring of populations of *Pyricularia oryzae* Triticum and *Pyricularia graminis-tritici* for sensitivity losses to triazole fungicides should be a priority and that alternatives to strobilurin fungicides are urgently needed.

9 Conclusion and future trends

The occurrence of wheat blast epidemics in Asia will bring a new perspective to this disease on the world stage. It is expected that there will be an increase in the number of people interested in developing research actions aimed at managing or controlling this disease, which has already demonstrated its harmful effects on the South American continent.

Regardless of who will do what and where it will be carried out, specific studies on wheat blast should be considered as priorities. Among these actions, we highlight the following that should be intensified: molecular and virulence characterization of the causative agents of wheat blast; molecular studies of the interaction of the pathogens *Pyricularia oryzae* Triticum and *Pyricularia graminis-tritici* in relation to the host *T. aestivum*; the search for new sources and resistance genes, considering the related species of *T. aestivum*, especially the species *Aegilops squarrosa* with a great potential to be exploited; development and evaluation of new molecules or alternative substances of fungicidal action to control wheat blast; evaluation of new application technologies that allow the fungicide to have a good covering action on the head, especially of the rachis; and better dimensioning of the role of alternative hosts, seeds and wheat straw as sources of inoculum for wheat blast.

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166 -

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