Regional and varietal differences in prevalence and incidence levels of Bipolaris species in Brazilian rice seedlots

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ABSTRACT

A total of 722 rice seed lots were collected at six production regions of Rio Grande do Sul state during three consecutive seasons (2009/10 to 2011/12). For each seed lot, 200 seeds were assessed for the presence of Bipolaris spp. using a standard seed health blotter test. Results showed that B. oryzae and B. cynodontis were found in 62.5% and 10.4% of the seed lots, respectively. Overall mean incidence of B. oryzae and B. cynodontis were 0.5% and 0.06%, respectively. For the two most sampled varieties (75% of the seed lots), IRGA 424 and Puitá INTA CL, mean incidence levels were highest and lowest, respectively. Among regions, infection risk was highest in the southeastern and lowest in the western regions of the state, especially the Fronteira Oeste. The prevalence and the incidence levels of Bipolaris oryzae reported in this study were lower than previous reports in the same region - eighty percent of the seed lots showed incidence levels below the recommended 5% inoculum threshold. In conclusion, rice seeds produced by IRGA-certified growers showed an overall good health quality with regards to B. oryzae infection, which is the main Bipolaris species associated with rice seeds in southern Brazil.

Key words: Bipolaris oryzae, Oryza sativa, brown spot, seed pathology.

INTRODUCTION

Brown spot, caused by Bipolaris oryzae (Breda de Haan) Shoemaker, is among the most important diseases of rice (Oryza sativa L.) and is present in virtually all rice-producing areas worldwide (Ou, 1985; Lee, 1992; Savary et al., 2000). Brazil is the largest rice-producing country outside Asia and brown spot, together with leaf and panicle blast (Magnaporthe oryzae), is an economically significant disease that contributes to direct losses in both yield and grain quality and indirect losses due to seedborne inoculum (Aluko, 1975; Prabhu et al., 1980; Marchetti & Petersen, 1984; Datnoff et al., 1992).

When seedborne, B. oryzae mycelium becomes dormant and can be reactivated during seed germination and cause lesions on roots, coleoptile and primary leaves. The fungus eventually leads to seedling death depending on the inoculum density in the seeds (Lee, 1992; Nghiep & Gau, 2004). Seed-to-seedling transmission rate may reach up to 80% and lead to significant reduction in crop stand (Prabhu & Vieira, 1989; Toledo et al., 2006; Barnwal et al., 2013). In central Brazil, crop stand reduction ranged from 33% to 64.5% in field plots according to the incidence levels of B. oryzae in the seeds (Malavolta et al., 2002).

Information on rice brown spot in Brazil is scarce, and most recent research has focused on the efficacy of chemicals (Ottoni et al., 2000; Santos et al., 2005; Dallagnol et al., 2006), biological control agents (Ludwig et al., 2009), the effect of silicon on the increase of host resistance (Zanão-Junior et al., 2009; Dallagnol et al., 2009) or reducing seedling infection from B. oryzae seedborne inoculum (Dallagnol et al., 2013). Sporadic surveys have been conducted to identify Bipolaris associated with rice seeds produced in Brazil (Franco et al., 2001; Farias et al., 2007; Farias et al., 2011) but few attempted to identify Bipolaris at the species level (Malavolta et al., 2007).

Bipolaris comprises a large genus of fungi with more than 100 species, most of them being saprobes in soil and pathogens of plants, and some potentially able to infect humans and animals (Sivanesan, 1987; Cunha et al., 2012). While B. oryzae is the most commonly species infecting rice seeds, likely due to its association with foliar epidemics of brown spot, other Bipolaris species have been found during routine analysis of rice seedlots around the world, including B. bicolor, B. curvispora, B. cynodontis, B. halodes, B. hawaiensis, B. indica, B. spicifera and B. victoria (Sivanesan, 1987; Mathur & Neergard, 1973; Motlagh & Kaviani, 2008; Farias et al., 2011; Archana & Prakash, 2013).
In previous surveys conducted in southern Brazilian rice seedlots, two *Bipolaris* species possessing morphological features that were distinct from *B. oryzae* were found associated with rice seeds from all rice-growing regions of Rio Grande do Sul State in the 2004 season: *B. cynodontis* and *B. curvispora* with mean prevalence of 24.1% and 42.9%, respectively (Farias et al., 2011). Although critical, the information provided on that study was only the presence or absence of each species in a seed lot, and not the extension of the infection within a seed lot. The main objective of the present study was to identify and quantify both the prevalence and the incidence of *Bipolaris* species associated with commercial seeds of rice. Additionally, the infection risk associated with seasonal, regional and varietal differences across the seed lots was assessed.

**MATERIAL AND METHODS**

**Study area and sampling**

A total of 722 seed lots were collected from harvesting of commercial seed-production fields by certified-growers of the Instituto Riograndense de Arroz Irrigado (IRGA). Information available for each seed lot included growing season, municipality, production region and rice variety.

The seed lots were originated from fields located in all six rice-growing regions of the state of Rio Grande do Sul as defined by IRGA. Although the number of seed lots was more evenly distributed across the seasons (Figure 1), it varied widely among regions and varieties (Table 1). The most sampled region was Fronteira Oeste (FO) region, contributing with almost one third of the samples. The least sampled region was Depressão Central (DC) with only 57 seed lots (7.8%) (Table 1). Samples were not available for Campanha (CAM) and DC regions in 2009 and 2011, respectively.

The samples comprised ten varieties which were grouped, for some analyses, into four categories according to breeder and date of release: Embrapa (BRS series) and IRGA variety groups (400s series - 1980s and 1990s, and 420s series - 2000s), bred by Brazilian institutes, and an INTA variety, Puitá INTA CL, bred in Argentina. Both the IRGA 422 CL and Puitá INTA CL are imidazoline-resistant rice varieties carrying the proprietary Clearfield (CL) technology. Two varieties comprised 74% of the seed lots: Puitá INTA CL and IRGA 424, which contributed with 47% and 27% of the samples, respectively (Table 1).

**Detection and identification procedures**

In the laboratory, a sample of 200 seeds was randomly taken from each seed lot and subjected to a standard seed health blotter test for fungal detection (Brasil, 2009). Briefly, non-surface disinfested seeds were carefully placed on top of three layers of moist paper inside a plastic box (50 seeds/box), which was incubated at ±23°C and with a 12 h light-dark cycle during seven days. Afterwards, each seed was inspected visually by an experienced analyst with the aid of stereoscopic microscope and assessed for the presence of *Bipolaris* species. During the visual assessments, morphologically distinct groups of *Bipolaris* based on conidiophore and conidia features were further isolated to petri dishes containing potato-dextrose-agar media for vigorous growth. Slides were prepared to visualize the

![FIGURE 1 - Number of seedlots obtained from up to three harvesting years on each of six rice-growing regions in the Rio Grande do Sul state as defined by IRGA (Instituto Riograndense do Arroz): CAM, Campanha; DC, Depressão central; FO, Fronteira oeste; PCE, Planicie costa externa; PCI, Planicie costa interna; SUL, Zona sul.](image)
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pattern of conidia insertion in the conidiophore, which is a key marker that allows to segregate the most commonly species of this genus found in rice in the region (Farias et al., 2011). Results were expressed as percentage of seed lots (pooled or by each level of a factor) exhibiting at least one infected seed (prevalence), and percentage of infected seeds in the lot by each species (incidence).

**Data analysis**

Descriptive statistics summarized prevalence and incidence data pooled and stratified by levels of factors (season, region and variety). Incidence data in the form of count of infected seeds in 200 seeds (the original data which was transformed to percent incidence) was fitted to theoretical distributions of Poisson and negative-binomial to detect randomness or over dispersion in the data, respectively. The distributions were compared and the one that best described the data was defined based on appraisal of cumulative distribution function plots and AIC (Akaike Information Criterion), a measure of the relative quality of a statistical model; the smaller the AIC, the better the fit.

Because normality in the distribution of *B. oryzae* incidence data could not be assumed, Kruskal Wallis, a non-parametric version of a one-way ANOVA, was used to compare levels of factor independently. The varieties were combined into four groups as described previously. Factors for which their effect was significant, a post-hoc Mann-Whitney test with Bonferroni correction was used for pairwise comparison among levels of factors. Additionally, the association between prevalence and incidence was investigated. For such, datasets with pairs of prevalence-incidence data from regions where IRGA 424 and Puitá INTA CL varieties (the most sampled ones) had at least 10 seed lots were selected, totaling 24 datasets (13 from Puitá INTA CL and 11 from IRGA 424). Then, Kendall tau rank correlation coefficient, a non-parametric test, was used to measure the strength of relationship between prevalence and incidence because normality in the data could not be assumed. All statistical analyses and graphical work were conducted in R (The R Project for Statistical Computing).

**RESULTS**

**Species identification and prevalence levels**

Two species were accurately identified during the visual assessments of individual seeds: *B. oryzae*, the
most prevalent, detected in 62.5% of the seed lots and *B. cynodontis*, detected in 10.4% of the seed lots. The prevalence of these two species was affected by year, region and variety. The most noticeable differences in prevalence were found among regions and varieties, while being more similar among the years (54 to 58.3% for *B. oryzae* and 7.9 to 12.6% for *B. cynodontis*).

Among the regions, the lowest prevalence of *B. oryzae* (<41%) was found in the western regions of the state (FO and CAM), and highest prevalence levels (>75%) were found in the southeastern regions (PCI, PCE and SUL). An intermediate level of prevalence (63.2%) was found in the central region of the state (DC) (Table 2). *Bipolaris cynodontis* was more evenly distributed across the regions, with the lowest and the highest prevalence found in the FO region and at the SUL and DC regions, respectively (Table 2).

*Bipolaris oryzae* was detected in lots from all varieties and *B. cynodontis* was not found in the BRS Fronteira single lot and in the five lots of BRS7 Taim. The highest prevalence of *B. oryzae* among the group of varieties was found in the 420s series of the IRGA varieties (75 to 91%). For the IRGA 400s series of varieties (IRGA 409 and IRGA 417), prevalence of *B. oryzae* was much lower (10 to 15%) and *B. cynodontis* was found in 5 to 10% of the seed lots (Table 2).

For the group comprising the Embrapa varieties, only BRS Querência had a more representative number of seed lots analyzed. In this, *B. oryzae* was found in 35.7% and *B. cynodontis* in 9.5% of 42 seed lots. For the only INTA variety (Puitá INTA CL), *B. oryzae* was found in around 50% of the seed lots and *B. cynodontis* in 6.5% of the seed lots (Table 2).

**Incidence levels of Bipolaris species**

The relatively low prevalence levels for some varieties, especially Puitá INTA CL, which contributed with almost half of total number of seed lots, indicated that the fungus was not detected in a good portion of seed lots (many zeros). Overall mean and median incidence across the 722

### TABLE 2 - Summary statistics for prevalence (% of infected seed lots) and incidence (% of infected seeds in a seed lot) of species of the Bipolaris complex detected in rice seeds produced in Rio Grande do Sul state, Brazil. Mean values are shown for the data grouped by different factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th>N</th>
<th>Prevalence (%)</th>
<th>Incidence (%)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Bory</td>
<td>Bcy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean Med Max</td>
<td>Var</td>
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<td></td>
<td>2009/10</td>
<td>274</td>
<td>63.5 10.2</td>
</tr>
<tr>
<td></td>
<td>2010/11</td>
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<td>54.0 7.9</td>
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<tr>
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<td>40.8 10.7</td>
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<tr>
<td></td>
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<td>63.2 15.8</td>
</tr>
<tr>
<td></td>
<td>FO</td>
<td>201</td>
<td>37.8 7.5</td>
</tr>
<tr>
<td></td>
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<td>137</td>
<td>76.6 9.5</td>
</tr>
<tr>
<td></td>
<td>PCI</td>
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<td>90.2 8.9</td>
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<tr>
<td></td>
<td>SUL</td>
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<td></td>
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<td>42</td>
<td>35.7 9.5</td>
</tr>
<tr>
<td></td>
<td>BRS Sinuolo</td>
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<td>60.0 20.0</td>
</tr>
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<td></td>
<td>BRS7 Taim</td>
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<td>IRGA424</td>
<td>197</td>
<td>90.9 16.2</td>
</tr>
<tr>
<td></td>
<td>Puitá INTA CL</td>
<td>358</td>
<td>53.6 6.5</td>
</tr>
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</table>

1Percentage of infected seed in 200 seeds assayed using a standard seed-health blotter test. Data presented are the means, median (Med), maximum (Max) and variance (Var). The minimum value was zero for all situations.

²Rice-growing regions of Rio Grande do Sul state as defined by IRGA (Instituto Riograndense do Arroz): CAM, Campanha; DC, Depressão central; FO, Fronteira oeste; PCE, Planície costeira externa; PCI, Planície costeira interna; SUL, Zona sul.
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seed lots was 5% and 0.5% for B. oryzae and 0.06 and zero for B. cynodontis, respectively. Moreover, overdispersion was suggested based on variances much larger than the mean incidences for many situations (Table 2) and on the visual appraisal of distribution plots for pooled incidence data of each species (Figure 2). The negative binomial distribution had a better fit (AIC = 4055.04) to the count of infested seeds than the Poisson distribution (AIC = 17095.71), which confirmed overdispersion in the incidence data.

The maximum B. oryzae incidence (81.5%) was found for an IRGA 424 grown at the PCI region during the 2008/09 season. However, maximum B. cynodontis incidence was relatively very low, 1.5% found in two IRGA varieties (422CL and 424) and so significance of the effect of factors was assessed only for B. oryzae incidence data.

The non-parametric test for the effect of factors showed effect of season ($\chi^2 (2) = 13.01, P < 0.001$), group of varieties ($\chi^2 (3) = 277.42, P < 0.001$) and production region ($\chi^2 (5) = 213.74, P < 0.001$) in the incidence of B. oryzae. Significant differences by post-hoc tests among levels of factors are shown as different letters in Figure 2. Incidence of B. oryzae was higher in the 2009 year compared to the other two years (Figure 3A). Among the varieties, B. oryzae median incidence was highest (around 5%) for two 420 series IRGA varieties (422CL and 424) and lowest (<1.8%) for the other varieties (Figure 3B). Among the production regions, the highest incidence was found at PCI and SUL regions (Figure 3C), for which median incidence ranged from six to nine times higher than the pooled median (Table 2). The other four regions had median incidence values ranging from zero (FO and CAM) to 1.5% (PCE). Prevalence and incidence for the 24 datasets of the two most sampled varieties with at least 10 seed lots across the regions were highly associated based on the Kendall’s rank correlation coefficient ($r = 0.7, p < 0.01$).

DISCUSSION

This was the most extensive survey in relation to the number of seedlots assessed for identification of Bipolaris species conducted in commercial fields during three consecutive seasons in Brazil. Because the dynamics and size of pathogenic population may be largely influenced by climate and crop/disease management practices, which vary considerably over years or decades, continuing and systematic surveys may provide useful risk information for decision-making (Savary et al., 2000). In this study Bipolaris fungi currently associated with commercial rice seed production were identified at the species level and important factors associated with infection risk based on their incidence levels were provided. Bipolaris oryzae was the main species of the Bipolaris complex associated with rice seeds grown under irrigated areas of Rio Grande do Sul state. The occurrence of B. oryzae in levels similar to those reported in the present study has been documented elsewhere. For instance, in India, B. oryzae was found in 58% of 287 seed lots analyzed and, for 73% of those lots, fungal incidence ranged from 1 to 10%, with a maximum incidence of 60% (Gopalkrishnan et al., 2010). In Bangladesh, B. oryzae was found in 41% of 17 rice varieties with a mean incidence of 4.5% (Ora et al., 2011).

Likewise, B. cynodontis was detected in seed lots from all production regions, but at much lower prevalence than B. oryzae and only at trace incidence levels. This prevalence was lower compared to a previous report of B. cynodontis (one quarter of 165 seed lots) in seed samples originated from the same production regions of the country (Farias et al., 2011). Commonly associated with the wild grass Cynodon dactylon (L.) and previously reported in Oryza sp. (Sivanesan, 1987), B. cynodontis was first reported in Brazil infecting black-oat seeds (Farias et al., 2005), later causing leaf spots in Brachiaria brizantha (Macedo & Barreto, 2007) and, more recently, in rice seeds grown in Rio Grande do Sul state (Farias et al., 2011).

FIGURE 2 - Frequency distribution for incidence (percentage of infected seeds in 200 seeds) of Bipolaris species associated with rice seeds from a set of ten cultivars grown at the six rice-growing regions of Rio Grande do Sul State from 2008/09 to 2010/11. Note that length of x and y axis differs for each species. A. Bipolaris oryzae. B. Bipolaris cynodontis.
Intriguingly, although a considerably high number of seed lots were inspected, *B. curvispora*, which has been found in 43% of 165 rice seed lots from the same regions in a previous survey (Farias et al., 2011), was not detected in this survey. *Bipolaris curvispora* has a very unique conidia shape and it would be less likely to be misidentified by an experienced analyst. Thus, it is hypothesized that this may due to a relatively low mean incidence of the *Bipolaris* complex found in the present survey compared to the previous one (Farias et al., 2011).

Previous reports of *B. oryzae* mean incidence in the country were variable: 1.4% (Malavolta & Bedendo, 1999), 2.6% (Franco et al., 2001) and 9.3% (Farias et al., 2007). Such differences may be related to a combination of factors related to agronomic and environmental differences. In rice seeds grown in central Brazil, a two-year survey reported *B. oryzae* at incidence levels averaging 13.6% in the first year and only 1.6% in the second year across 22 rice varieties, suggesting a strong interannual variation (Malavolta et al., 2007).

Moreover, a considerably large number of seed lots analyzed in this study were originated from varieties exhibiting some level of resistance (e.g. Puitá INTA CL) and regions where environmental conditions may be less favorable for brown spot epidemics. Also, it cannot be disregarded the influence of best management practices adopted by IRGA-certified seed growers, which may have contributed to an improved overall seed health (C.R.J. Farias, *unpublished*), especially with the increase usage of fungicides known to suppress foliar epidemics of brown spot, compared to previous years (Ottoni et al., 2000). Information on the fungicides used in the fields from which the samples were originated was not available, but fungicide spray is a recommended practice for seed-growers in southern Brazil to protect rice seeds against a complex of diseases, including brown spot (Anonymous, 2012).

The rice variety and the production region affected the incidence levels at a greater extent than the season. IRGA 424 exhibited consistently higher incidence of *B. oryzae* than the other varieties regardless of the region and season. A previous study reported differences among ten rice varieties in a six-year survey (1993-1998) in the same regions, with the highest incidences reported for El Passo 144, IRGA 410 and IRGA 412 (5.8 to 7.1%) (Franco et al., 2001). On the other hand, the most sampled variety in this survey, Puitá INTA CL, exhibited consistently a low incidence of the fungus, suggesting host resistance effect. Interestingly, Puitá INTA CL is susceptible to dominant populations of *Magnaporthe oryzae* that causes rice blast in the region and IRGA 424 carry resistant genes that are still effective against rice blast pathogen populations based on recent survey (E.M. Del Ponte et al., *unpublished*). Hence, the trade-off for growing a variety that is less susceptible to brown spot is its susceptibility to rice blast, a disease that may be more devastating than brown spot and difficult to control.

It was shown that risk of *B. oryzae* seedborne infection was strongly affected by production region. The
western regions of the state should be preferred to grow seeds with lower risk of *Bipolaris* infection. This is in agreement with a previous survey that showed the rice seeds from western region showed lower incidence (10%) compared to the other regions (15 to 70%) (Farias et al., 2007). Climatic conditions differ across the six rice production regions of the state during the growing season, especially with regards to seasonal rainfall that increases from western to eastern regions of the state (Steinmetz, 2004). Moreover, soils from the eastern regions of the state are less fertile than those from western regions, which are known to favor the occurrence of rice brown spot (Mew & Gonzales, 2002).

The heterogeneity of the incidence data of *B. oryzae* for most datasets may be due to the differences in varieties, planting dates, weather conditions within a same region. The heterogeneity was depicted by variances higher than the mean incidences and the negative binomial model capturing the observed heterogeneity in the count data. This is in agreement with previous modeling that showed better fit of the negative binomial (for count data) or the beta-binomial (for incidence) models to fungal incidence datasets (Infantino et al., 2012). The significant relationship between prevalence and incidence is in agreement with a previous study with *Fusarium* spp. associated with wheat in Italy (Infantino et al., 2012). This suggests a hierarchical structure in which high number of infected fields (high prevalence) in a region is associated with high levels of incidence in the field.

The production of seeds with improved health quality is key for an integrated disease management and research has focused on the development of accurate and time-effective detection methods (Munkvold, 2009) as well as the establishment of inoculum thresholds. For *B. oryzae*, for example, a 5% incidence has been proposed as inoculum threshold for rice seeds produced in Brazil (Machado & Pozza, 2005). Data from the present study showed that 570 seed lots (79% of the total) had incidence of *B. oryzae* lower than the proposed 5% threshold. Thus, we can conclude that rice seeds produced in Rio Grande do Sul State by IRGA-certified growers showed an overall good health quality with regards to *B. oryzae*, which is the main species associated with rice seeds. Moreover, improved quality may be obtained with growing less susceptible rice varieties at low-risk regions combined with integrated management practices.

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