academicJournals

Vol. 8(28), pp. 2697-2704, 9 July, 2014
DOI: 10.5897/AJMR2013.6575
Article Number: EB6DA8B45935
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Pathogenicity of monosporic and polysporic *Bipolaris sorokiniana* isolates to wheat seed and seedling under controlled conditions

Elisandra Minotto[1#], Michele Bertoni Mann[1#], Eduardo Vélez-Martin[2], Thaisa Feltrin[1], Luciana Pasqualini Milagre[1], Cristina Spadari[1], Sueli Teresinha Van Der Sand[1]*

[1]Post-graduation Program in Agricultural and Environmental Microbiology, Instituto de Ciências Básicas da Saúde (ICBS), Universidade Federal do Rio Grande do Sul (UFRGS), Av. Sarmento Leite 500, CEP 90050-170, Porto Alegre, Rio Grande do Sul, Brazil.
[2]Department of Ecology, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Bento Gonçalves, 9500, Porto Alegre, RS, 91540-000, Brazil.

Received 18 December, 2013; Accepted 14 June, 2014

***Bipolaris sorokiniana* may present considerable genetic diversity and highly variable pathogenicity and virulence. The pathogenicity of 99 *B. sorokiniana* isolates (27 polysporic and 72 monosporic isolates) from Brazil and other countries was assessed. Based on aborted germination, black point of seed, leaf spot, and coleoptile lesion, the principal component analysis (PCA) was used to evaluate the similarity patterns between isolates considering the variables of pathogenicity. Polysporic isolates presented higher virulence (over 60%), when compared with the monosporic isolates (43%) for all variables, except coleoptile injury. Of all isolates used to infect seeds, 8% were highly virulent, and the score obtained was over 75%, for all variables analyzed. The correlation of *B. sorokiniana* isolates with pathogenicity variables demonstrated that polysporic isolates were more virulent, especially upon seeds, as compared to aerial plant parts.**

Key words: Variability, virulence, *Triticum aestivum* L., spot blotch.

INTRODUCTION

Wheat (*Triticum aestivum* L.) has fundamental importance in humankind's food basis, and today it takes the first place in worldwide agricultural production figures (EMBRAPA, 2013). According to data published by the United Nations Food and Agriculture Organization, global wheat production is expected to reach a record number 708.5 million ton in the 2013 harvest (FAO, 2014). In Brazil, wheat production is about 5,000 to 6,000 ton. The largest cultivated areas, accounting for 90% of the country's production, are in southern (States of Rio Grande do Sul, Santa Catarina and Paraná) and midwest Brazil (States of Mato Grosso do Sul, Goiás and Distrito Federal) (EMBRAPA, 2013).

Wheat is subject to biotic and abiotic limitations, such

*Corresponding author. E-mail: svands@ufrgs.br.

#Authors contributed equally to this work.

as adverse climatic conditions, soil, pests and diseases. Among the limiting conditions, the phytopathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker,1995; (teleomorph, *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur) stands out as the causal agent of common root rot, leaf spot, seedling blight and black point in seeds of both wheat and barley cultures, apart from diseases in rye, oat, triticale, sorghum and fescue (Tinline, 1961). However, the most severe symptoms of these diseases are observed in wheat and barley cultures in hot and humid regions, with significant production losses (Kumar et al., 2002).

The pathogenic fungus *B. sorokiniana* uses all plant organs of winter cereals as substrate. For this reason, two distinct disease stages are discernible: the interference in photosynthesis, when the infection occurs in the aerial parts of the plant, and the interference in the search and absorption of water and nutrients, which is the stage that affects underground parts (Forcelini, 1991).

Due to this cosmopolitan nature, spot blotch caused by *B. sorokiniana* is estimated to affect 25 million hectares of wheat plantations worldwide to variable degrees, which accounts for 12% of the total area of the culture that is grown (Duveiller et al., 2005). Seeds infected with the pathogen are highly infectious and stand as the main survival mechanism of this fungus, representing the means by which hot spots of the disease remain active in the field (Forcelini, 1991). *B. sorokiniana* may also play a deleterious role in germination and in the establishment of cereal plantations, leading to aborted seed, seed rot, reducing seed viability, necrosis and discoloration of aerial parts of infected plants (Neergard, 1977).

Spot blotch has symptoms that usually appear on the aerial parts of plants and take the form of oval necrotic lesions surrounded by chlorotic halos. This infection may reduce photosynthetic area and eventually leads to premature plant senescence (Ghazvini and Tekauz, 2007).

Mehta (1978) has recommended the use of combined strategies to control *B. sorokiniana* in wheat and barley cultures. These strategies include the use of resistant varieties, chemical control of soils, waste management, and crop rotation. In spite of the fact that spot blotch is considered one of the most important diseases affecting wheat worldwide, control measures have not produced satisfactory results (Kumar et al., 2007).

In this scenario, the present study assesses the pathogenic potential of monosporic and polysporic *B. sorokiniana* isolates on wheat seeds and seedling under controlled conditions.

MATERIALS AND METHODS

Origin of microorganisms

The fungal polysporic isolates from different regions in Brazil were provided by Empresa Brasileira de Pesquisa Agropecuária- Trigo (EMBRAPA- Trigo, Passo Fundo, Brazil), while the other isolates used in this study were kindly provided by the International Maize and Wheat Improvement Center (CIMMYT, México). All isolates used were obtained from seeds and tissues of wheat plants. The biological material was deposited in the collection of the Environmental Mycology Laboratory, DMIP, ICBS, UFRGS. Ninety-nine *B. sorokiniana* isolates, characterized morphologically, physiologically and molecularly (Müller et al., 2005; Poloni et al., 2008; Nascimento and Van Der Sand, 2008; Mann, 2014; Mann et al., 2014) were used in the pathogenicity assay, of which 27 were polysporic and 72 were monosporic isolates (Table 1).

Table 1. Origin of *B. sorokiniana* isolates from different regions in Brazil and other countries.

Isolates code	Origin
98004 P, A, B, C	Cruz Alta, RS, Brazil
98007 P, A, C	Cruz Alta, RS, Brazil
98030 P, A, C	Cruz Alta, RS, Brazil
98032 P, A, B, C	EngenheiroBeltrão, PR, Brazil
CEV53 A, B	Guarapuava, PR, Brazil
98011 P, A, C	LagoaVermelha, RS, Brazil
98012 P, A, C	LagoaVermelha, RS, Brazil
98031 P, A, B, C	Nova Estância, PR, Brazil
98028 P	Pelotas, RS, Brazil
98025 P, A, C	Piratini, RS, Brazil
98026 P, B, C	Piratini, RS, Brazil
98042 P, A, B, C	Piratini, PR, Brazil
1992 B, C	Planaltina, GO, Brazil
98010 P, A, B, C	Santa Rosa, RS, Brazil
98041 P, A, B, C	União da Vitória, PR,Brazil
98023 P, A, B, C	União da Vitória, PR,Brazil
98013 P, A, B, C	União da Vitória, PR,Brazil
98017 A, B, C	Samambaia, PR, Brazil
CEV48 P, A, B, C	Tapera, RS, Brazil
98034 P, A, B, C	Unknown
NRRL5851 P, A, B, C	South Africa
CFO201 P, A, B, C	South Africa
A20 P, A, B, C	Canada
1965 P	Copenhagen, Denmark
BS15M2 P, A, B, C	Delicias, Chihuahua, Mexico
BS16M1 P, C	Delícias, Chihuahua, Mexico
BS18M2 P, A, B, C	Poza Rica,Vera Cruz, Mexico
CMO105 P, A, B, C	Mexico
BS52M1 P, A, B,	Monterrey, Nuevo Leon
CS1004 P, A, B, C	Hanoi, Vietnam

P: Polysporic *B. sorokiniana* isolate; A, B, and C: Monosporic *B. sorokiniana* isolate originated from the respective polysporic isolate; CEV: *B. sorokiniana* isolate from barley.

Monosporic and polysporic cultures

The monosporic cultures were obtained from the aerial mycelia of the polysporic cultures grown on plates with potato dextrose agar (PDA). A 0.85% saline solution was poured over the plated colonies, and the conidia were transferred to microcentrifuge tubes.

The contents of tubes were homogenized thoroughly to guarantee complete conidia release. The suspension was transferred to a Petri dish with PDA and incubated at room temperature for 2 h. Using a stereomicroscope with optical magnification of 40×, the conidia were transferred using plates with PDA. The plates were maintained at 24 ±2°C until the complete colonies developed, and then were stored at 4°C. Each spore culture was identified with a letter (A, B and C).

Fungal inoculum preparation

B. sorokiniana isolates were multiplied on a culture medium prepared with vegetable broth and carrot agar specific for sporulation and incubated in a BOD stove for 10 to 15 days at 25ºC in a 12-h photoperiod. To standardize the fungal inoculum, 5 mL sterile saline (0.85%) containing Tween 80 (0.1%) were added to colonies. Then, colonies were lightly streaked using a Drigalski spatula, spores were removed, and the suspension was transferred to sterile glass test tubes. Final spore concentration was adjusted to 10^6 spores/mL by counting conidia in a Neubauer chamber.

Pathogenicity assay

The pathogenicity assay was carried out using the 99 *B. sorokiniana* isolates. Samples of 100 wheat seeds, cultivar BRS Buriti, which is considered moderately susceptible to leaf spot, were disinfected using ethanol 70% for 2 min, sodium hypochlorite 2.5% for 2 min, and three wash runs with sterile distilled water. Samples were then placed in tubes containing the previously adjusted spore suspension and left at room temperature for 24 h. After, seeds were incubated according to a modified version of the Blotter test method. Each 100-seed sample was divided in groups of 25 seeds that were placed one by one on wet filter paper sheets, with four repeats. The sheets containing seeds were folded as a sachet, which was incubated in a seed germinator (JP-1000, J. Prolab) at 25°C in a 12-h photoperiod for 10 days. After incubation, wheat seeds and seedlings were individually assessed for aborted germination, black point of seed coleoptile lesion and leaf spot. The assay was carried out in10 blocks, each of which included a control group of seeds that were not challenged with *B. sorokiniana.* After the lesions were analyzed, all the tissues of the organs were submitted to re-isolation of the phytopathogen using culture conditions on PDA plates. Then, growth analysis of the structures under the microscope was carried out.

Statistical analysis

A descriptive statistics of the virulence of *B. sorokiniana,* was carried out for the four variables, expressed as percent values: for aborted germination, black point of seed, leaf spot, coleoptile lesion. This evaluation was visually determined by the presence or absence of symptoms.

The differences in pathogenicity between controls and groups of monosporic and polysporic isolates were assessed using the one-factor analysis of variance followed by an analysis of the differences between treatments using the randomization test, as described by Pillar and Orlóci (1996).

The differences in pathogenicity patterns between isolates based on the four variables assessed (aborted germination, black point of seed coleoptile lesion and leaf spot) was evaluated using the principal component analysis (PCA) (Person, 1901).

The statistical analyses were carried out using the application R (R Development Core Team, 2008) and the action interface for Excel (Estatcamp, 2013). Normality of variables was tested using the Shapiro-Wilk test. The analyses of variance and multivariate analyses were made in the MULTIV (Pillar, 1997).

RESULTS

Results of the four variables assessed indicated that the monosporic and polysporic isolates of *B. sorokiniana* strongly induced the diseases in wheat seed and seedlings, as compared to controls (Table 2).

The comparison between treatment groups (monosporic, polysporic and control isolates) revealed a significant difference between monosporic and polysporic isolates for aborted germination, black point of seed, leaf spot. The exception was observed for coleoptile lesion (Table 2).

The monosporic isolates virulence on germination, showed a median of 59.5 seeds with aborted germination, and the variation ranged from a minimum of 16 to a maximum of 100 seeds. The virulence of polysporic isolates had a higher median, of 75 seeds with aborted germination, and the variation ranged from a minimum of 43 to maximum of 110 seeds (Table 2 and Figure 1).

The highest virulence towards germination (values above the third quarter) was exerted by 18 monosporic isolates, with values over 73.25% for aborted germination, and by 6 polysporic isolates, with aborted germination values over 84% (Table 2 and Figure 1).

The virulence of *B. sorokiniana* isolates on wheat seeds led to high deterioration, with a median value of 100 seeds with black point, both for monosporic and polysporic cultures. The data analyses showed statistically significant differences between all treatments. Mean number of seeds affected by black point was higher after treatment with polysporic cultures (97.4% ±8.1), followed by seeds treated with monosporic cultures (83.9% ± 27.0) and controls (13.2%) (Table 2). On the other hand, 5.4% of monosporic isolates did not cause symptoms in seeds, and did not differ from controls. Allpolysporic isolates caused black point of seeds, with the lowest value of 61% of seeds.

Coleoptile lesion caused by polysporic and monosporic *B. sorokiniana* isolates presented medians of 82.0 ±34.1 and 67.9 ± 34.3, respectively (between zero and 100%). The analysis of variance, to compare groups, indicated a significant difference between the control and the treatment groups, though this difference was not observed between monosporic and polysporic isolates (Table 2 and Figure 1).

Incidence of lesions on leaf blades presented medians of 44.6 and 65.4 for monosporic and polysporic isolates, respectively (Table 2 and Figure 1). Among the most virulent isolates that caused leaf spot (above the third quarter), 25% were monosporic isolates, causing the effect in more than 69% of leaves, while 22.2% of polysporic isolates triggered the effect in more than 79.8% of leaves.

Analysis of the virulence of monosporic and polysporic isolates

The similarity pattern in the virulence of the isolates,

Table 2. Analysis of variance between the treatment groups of monosporic and polysporic isolates and control, for the variables: seed aborted germination, black point of seed, leaf spot and coleoptile lesion.

Variable	Treatment	N	Mean	median	Min	Max	SD	Lower limit (LL mean)	Upper limit (UL mean)
Aborted germination (1)	C[a]	11	25.7	24	16	41	7.7	20.6	30.9
	M[b]	74	60.7	59.5	16	100	19.9	56.1	65.3
	P[c]	27	72.4	75	43	100	17.5	65.5	79.3
Black point of seed (2)	C[a]	11	13.2	10	0	44	16.1	2.3	24
	M[b]	74	83.9	100	0	100	27.0	77.6	90.1
	P[c]	27	97.4	100	61	100	8.1	94.2	100
Coleoptile lesion (3)	C[a]	11	9.3	0	0	83.3	24.8	0	26
	M[b]	72	60.7	67.9	0	100	34.3	52.6	68.8
	P[b]	26	69	82	0	100	34.1	55.3	82.8
Leaf spot (4)	C[a]	11	5.6	0	0	54.5	16.3	0	16.6
	M[b]	72	43	44.6	0	100	33.7	35.1	51
	P[c]	26	59.9	65.4	0	100	31.3	47.3	72.6

C: Control, M: Monosporic isolate, P: Polysporic isolate, N: Number of treatments, Min: Minimum seeds, Max: Maximum seeds, SD: Standard deviation, CI: Confidence interval. Groups followed by different letters differ significantly from one another based on the probabilities obtained by the pairwise randomization test: (1) P= 0.001 for C-M. P= 0.001 for C-P and P= 0.01 for M-P; (2) P= 0.001 for C-M. P= 0.001 for C-P and P=0.009 for M-P; (3) P= 0.005 for C-M. P= 0.001 for C-P and P= 0.02 for M-P; (4) P= 0.001 for C-M. P= 0.001 for C-P and P= 0.296 for M-P.

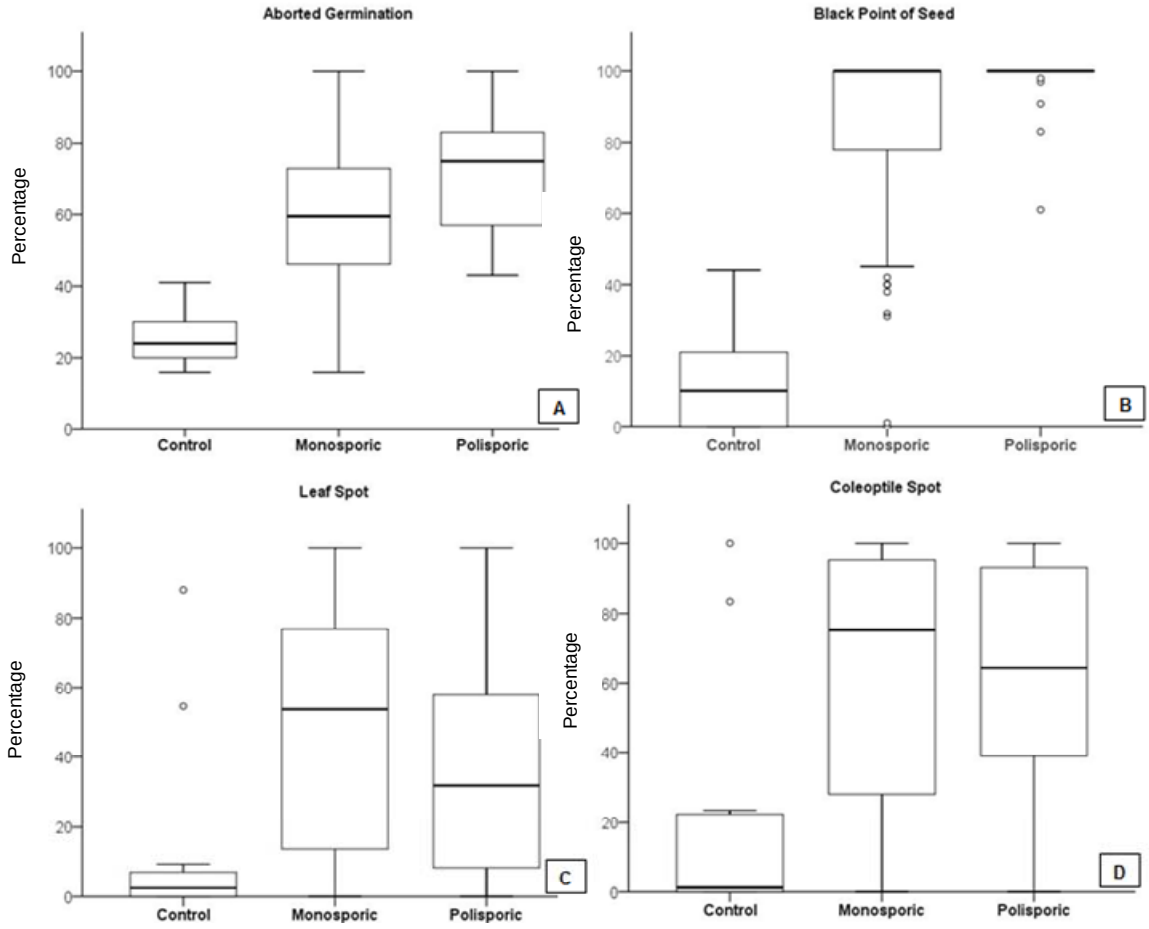

Figure 1. Boxplot of the variables: aborted germination, black point of seed, leaf spot, and coleoptile lesion in the pathogenicity test of the monosporic and polysporic isolates of *B. sorokiniana*.

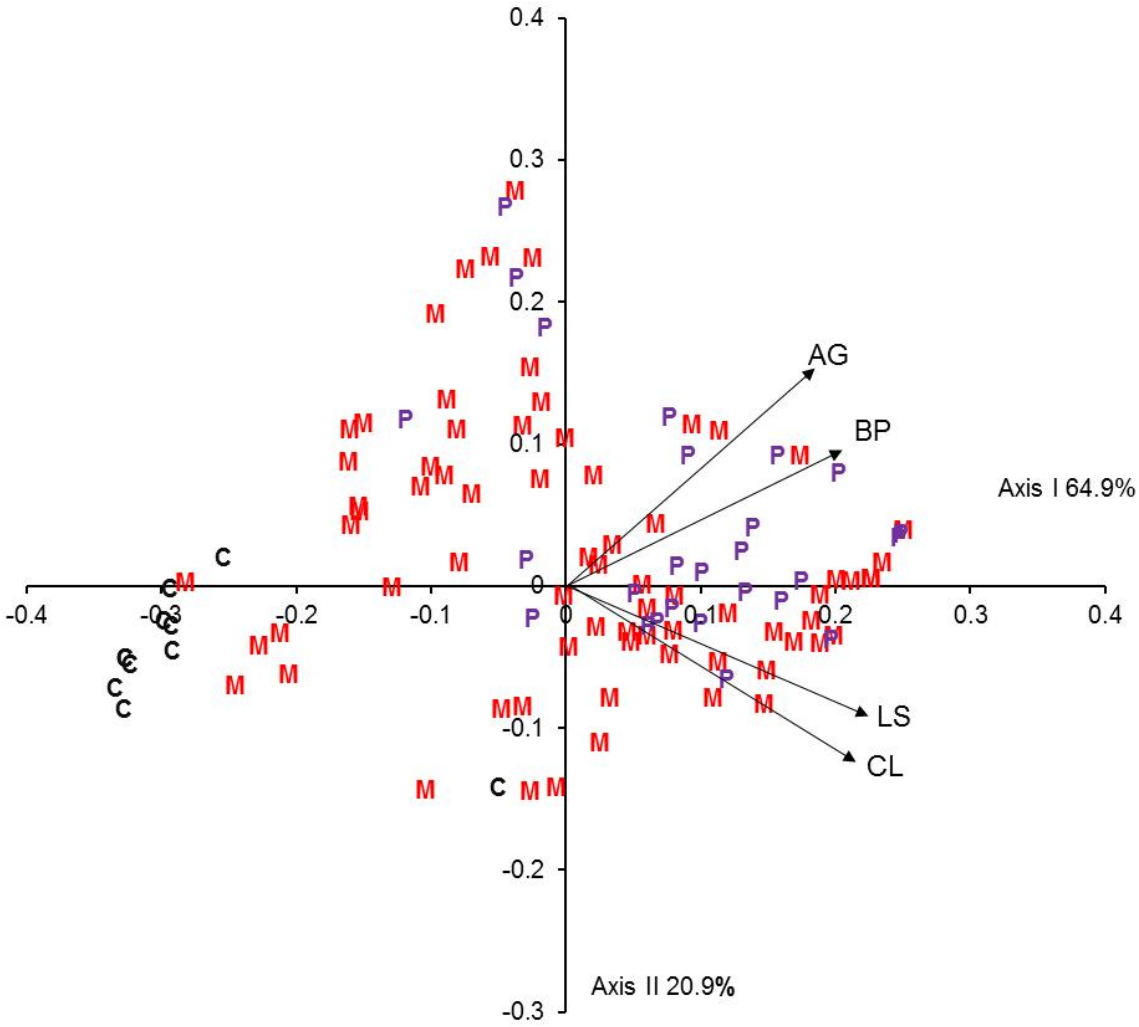

Figure 2. Ordination chart for *B. sorokiniana* isolates in terms of pathogenicity variables constructed based on the principal components analysis and correlation as a measure of similarity between the variables: (A) aborted germination (AG); (B) black point of seed (BP); (C) leaf spot (LS); (D) coleoptile lesion (CL). The percentage of variation in each axis and the variables that are correlated with at least one of the two axes is indicated. C: Control, M: Monosporic isolate, P: Polysporic isolate.

based on the four variables evaluated, simultaneously is shown in Figures 2 and 3. Ordination axis I contains 64.9% of the total variation of pathogenicity data, in which all variables exhibited high, positive correlation (aborted germination = 0.71, black point = 0.79, leaf spot = 0.87 and coleoptile lesion = 0.83) with this axis and the major contribution was that of leaf spot with 29.5% of the total variation in this axis. The position of isolates on axis I allows identifying the most virulent isolates, on the right, namely CEV48P, 98042P, 98042C and CFO201B. Low pathogenicity isolates are at the far end of the axis, especially 98012C, 98023B, 98026C, CFO201A and CS1004A.

Axis II contains 20.9% of the total variation in pathogenicity data and allows differentiating the most pathogenic isolates to wheat seeds from the most pathogenic to the aerial parts of the plant. Aborted germination and black point of seed had positive correlation with axis II (0.58 and 0.36, respectively), while leaf spot and coleoptiles lesion presented negative correlation (-0.35 and -0.48 respectively). The highest contribution was given by aborted germination, with 40.9%.

The ordination chart of isolates also reveals a clear distinction between control and monosporic and polysporic isolates (Figure 2). Also, monosporic isolates presented higher variation in virulence, as compared to polysporic isolates. However, these were a little more specialized in terms of pathogenicity, affecting more seeds than the aerial parts.

Of the isolates used to infect seeds, 8% presented the highest virulence, with virulence of over 75% for all

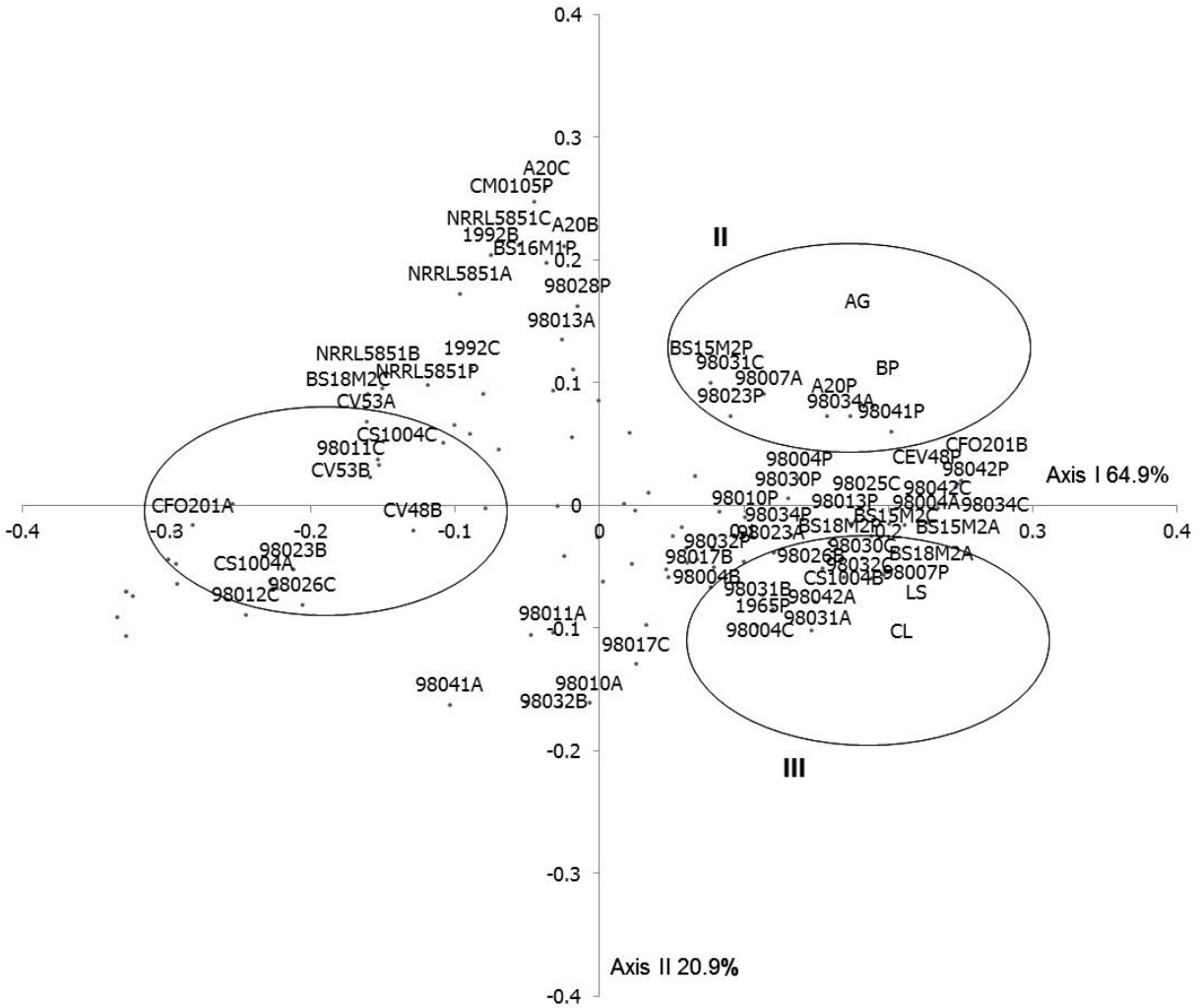

Figure 3. Ordination chart for *B. sorokiniana* isolates in terms of pathogenicity variables constructed based on the principal components analysis and correlation as a measure of similarity between the variables aborted germination (AG), black point of seed (BP), leaf spot (LS), and coleoptile lesion (CL). The identification codes of isolates with high and low values are shown in axes I and II. Isolates presenting intermediate values were not labeled, and are identified by dots. The ellipsis I indicates isolates with low pathogenicity, ellipsis II signals highly pathogenic isolates affecting mainly seeds, and ellipsis III indicates highly pathogenic isolates affecting mainly aerial parts.

variables assessed. The isolates with the highest and lowest scores in axis I and II were the most virulent when the four variables are considered as a set, 98004A, 98025C, 98034C, 98042P, 98042C, CEV48P and BS15M2A (Figure 3). Black point of seed was observed in all monosporic and polysporic isolates, except for isolates 98031P, 98012C, 98041A and CFO201A. On the other hand, monosporic isolates 98012C, 98041A and CFO201A presented low virulence, with symptoms observed in less than 1% of wheat seedlings and seeds.

DISCUSSION

In general, microorganisms present high genetic diversity, leading to differences in morphology, physiology and pathogenicity. Variations in the use of substrates, tolerance to determined temperature and pH ranges, production of toxins and other metabolites are among the manifestations of physiological distinctions in one population, which often result in variation of pathogenicity of biotypes (Machado, 1980).

The presence of symptoms and the wide variation in this pathogen's virulence patterns are reported based on the analysis of pathogenicity variables, which indicate that polysporic isolates are more virulent to leaves, with values over 60%, when compared with monosporic isolates, with values over 43% (Table 2). The monosporic cultures were used to reduce the effect of heterokaryosis, since one single conidium may be homokaryotic or present reduced variability, which makes it easier to identify pathotypes based on isolate virulence. This

characteristic may be linked with the different genes present in heterokaryotic cells of monosporic isolates, which in turn may manifest in different ways, depending on the quality and quantity of nuclei contained in cells and on the roles played by the environment and the host (Tinline, 1961). This may explain the wider spectrum of monosporic isolates on the pathogenicity variables shown in Figure 2.

Pathogenicity tests carried out by Christensen (1925) using 37 monosporic *H. sativum* isolates indicated that 18 formed zones in BDA medium, which differed from the parental colony as compared to morphology and pathogenicity. In a previous study, the virulence, morphology and growth rate in culture medium of 10 *B. sorokiniana* isolates from different regions in Brazil were analyzed in wheat. Wide variations in morphology and growth rates were observed between parental and re-isolated isolates. However, no relationship between morphological variability and virulence was detected between these two types of isolates (Oliveira et al., 1998) or origin of isolates (Valim-Labres et al., 1997).

The results obtained in the present study show that polysporic isolates exerted higher pathogenic action, predominantly in seeds, as compared to aerial parts. Often the pathogens that cause common root rot also cause different diseases in one single plant species; however, most specific symptoms in one plant are regulated by infection time and soil conditions, mainly temperature and humidity (Wheeler and Rush, 2001). Our results also reveal that polysporic isolates, which presented high pathogenicity levels in seed germination, did not show the same virulence indices in comparison with the respective monosporic isolates. For example, isolate 98041P inhibited germination in 97% of wheat seeds, while the monosporic isolates generated from the same polysporic strain reduced germination by approximately 50%. According to Mehta (1998), a likely source of variability may be inherent to the fungus itself, since its pathogenicity may vary with time.

Duveiller and García Altamirano (2000) showed that *B. sorokiniana* isolates from different parts of a plant did not cluster according to virulence, when they were reinoculated. In this sense, the authors discovered that the number of leaf spot varies with the isolate used for inoculation, and that this isolate does not depend on the organ from where it was isolated (Duvellier and García Altamirano, 2000). Fetch and Steffenson (1999) observed variation in virulence patterns of *Cochliobolus sativus* in relation to barley cultivars and to the development stage of plants.

In the present study, we observed that the most severe symptoms were associated with germination and black point of seed, with reduced germination and high levels of rot (Table 2). This condition is mainly due to the hemibiotrophic nature of *B. sorokiniana* and to the complex enzymatic apparatus it has, which is able to use any organ of a plant as nutritional substrate. The seed is considered one of the most efficient means of transmission and dissemination of phytopathogenic agents, especially overlong distances. In unaffected areas, seeds mediate the introduction of pathogens, which may be spread, selected and distributed by means of primary disease hotspots (Maffia et al., 1988).

The incidence of *B. sorokiniana* in wheat seeds is often observed negatively, affecting germination and triggering the occurrence of symptoms in plants and seeds, and even causing the death of plants (Lasca et al., 1983). The association of the pathogen to seeds is an efficient mechanism of survival and dissemination, and is the main reason behind the outbreaks of epidemics in wheat production regions in Brazil (Goulart et al., 1993). According to Kumar et al. (2002), infections may be so severe that the infected plants wither, without producing one single seed. Under conditions that favor the pathogen's life cycle, spikelets may be affected, causing seeds to dry out.

The pathogenicity assays were carried out using the wheat cultivar BRS Buriti, which is moderately susceptible to leaf spot and is recommended for the establishment of wheat plantations in winter, in southern Brazil. In this sense, the use of *B. sorokiniana* isolates from different regions of Brazil and the world may indicate that pathogenicity levels differ, which in fact was not observed. The results obtained did not afford to group *B. sorokiniana* isolates by geographic origin or the definition of similarity patterns in pathogenic action. Maraite et al. (1998) analyzed 360 wheat leaf samples from 10 countries presenting symptoms of the disease, and did not observe specific relationships between pathogenicity in terms of geographic origin and genotype.

Conclusion

The most interesting aspects observed in the present study are associated with the wide pathogenic variability of *B. sorokiniana* isolates. Virulence based on conidial origin were established using the correlation between monosporic and polysporic isolates for the variables, aborted germination, black point of seed, leaf spot and coleoptile lesion. Polysporic isolates presented higher virulence (over 60%), when compared with monosporic isolates (43%) for all variables, except coleoptile lesion, and increased aggressiveness was observed against the seed.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Christensen JJ (1925). Physiologic specialization and mutation in

Helminthosporiumsativum. Phytopathology 15:785-795.
Duveiller E, Garcia Altamirano I (2000). Pathogenicity of *Bipolaris sorokiniana* isolates from wheat roots, leaves and seeds in Mexico. Plant Pathol. 49:235-242.
Duveiller E, Kandel YR, Sharma RC, Shrestha SM (2005). Epidemiology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. Phytopathology 95:248-256
EMBRAPA (2013). Wheat. Available at: http://www.cnpt.embrapa.br/culturas/trigo/. Accessed 12/04/2013.
Estatcamp (2013). Action. Version 2.4. São Carlos: Estatcamp. Available at: http://www.portalaction.com.br/content/download-action. Accessed 15/04/2013.
Food and Agriculture Organization FAO (2014). Available at: https://www.fao.org.br/. Accessed March 2014.
Fetch TGJr, Steffenson BJ (1999). Rating scales for assessing infection responses of barley infected with *Cochliobolus sativus.* Plant Dis. 83: 213-217.
Forcelini CA (1991). Importância epidemiológica de fungos do gênero *Helminthosporium* em sementes de trigo e cevada. In: Menten JOM (ed) Patógenos em sementes detecção, Danos e Controle Químico. Escola Superior de Agricultura Luiz de Queiróz ESALQ, Piracicaba, Brazil.179-219.
Ghazvini H, Tekauz A (2007). Virulence Diversity in the Population of *Bipolaris sorokiniana.* Plant Dis. 91:814-821.
Goulart ACP, Paiva FA (1993). Eficiência do tratamento químico de sementes de trigo no controle de Pyriculariaoryzae e Cochliobolussativus (Heminthosporiumsativum). Summa Phytopathol. 19:199-202
Kumar D, Chand R, Prasad L, Joshi AK (2007). A new technique for monosporic culture of the most aggressive isolate in a given population of *Bipolaris sorokiniana*, cause of foliar spot blotch in wheat and barley. World J. Microbiol. Biotechnol. 23:1647-1651.
Kumar J, Schafer P, Huckelhoven R., Langen G, Baltruschat H, Stein E, Nagarajan S, Kogel KH (2002). *Bipolaris sorokiniana* a cereal pathogen of global concern: cytological and molecular approaches towards better control. Mol. Plant Pathol. 3(4):185-195.
Lasca CC, BrignaniNeto F, Chiba S (1983). Eficiência de fungicidas em tratamento de sementes de arroz para controle de *Pyriculariaoryzae* Cav. e *Phoma* sp. Summa Phytopatol. 9:93-94.
Machado AA (1980). Esporulação de*Macrophominaphaseolina (Tass.) Goid.* e variabilidade do método de inoculação de esporos em estudos de seleção de germoplasma resistente. MScdissertation, Escola Superior de Agricultura Luiz de Queiroz/USP, Piracicaba, Brazil.
Maffia LA, Muchovej JJ, Maffia AMC (1988). Fundamentos epidemiológicos no estudo da transmissão de patógenos por sementes. In: 111 Simpósio Brasileiro De Patologia De Sementes. Campinas, Brazil: Fundação Cargill. pp. 114-122.
Mann MB (2014). Molecular and isoenzymatic characterization of monosporic and polysporic Bipolaris sorokiniana isolates.PhD thesis. Universidade Federal do Rio Grade do Sul/UFRGS. Porto Alegre, Brazil.
Mann MB, Spadari CC, Feltrin T, Frazzon A PG, Germani JC, Sand ST (2014). Genetic variability of Bipolaris sorokiniana isolates using URP-PCR. Trop. Plant Pathol. 39(2): 163-171.
Maraite H, Di Zinno T, Longrée H, Daumerie V, Duveiller E (1998). Fungi associated with foliar blight of wheat in warmer areas. In: Duveiller E, Dubin HJ, Reeves J, McNab A (eds) Proceedings of the International Workshop on Helminthosporium Diseases of Wheat: Spot Blotch and Tan Spot, El Batán, Mexico: El Batán. 293-300.
Mehta YR (1978). Doenças do trigo e seu controle. Editora Agronômica Ceres. São Paulo. Summa Phytopatol. p. 190.
Mehta YR (1998). Constraints on the integrated management of spot blotch of wheat. In: Duveiller E, Dubin HJ, Reeves J, McNab A (eds) *Helminthosporium* Blights of Wheat: Spot Blotch and Tan Spot, CIMMYT, Mexico, D.F., Mexico. pp. 18-27.
Müller MVG, Germani JC, Van Der Sand ST (2005). The use of RAPD to characterize *Bipolarissorokiniana* isolates. Genet. Mol. Res. 4(4):642-652.
Nascimento EJM, Van Der Sand ST (2008). Restriction analysis of the amplified ribosomal DNA spacers ITS1 and ITS2 of *Bipolaris sorokiniana* isolates. World J. Microbiol. Biotechnol. 24(5):647-652.
Neergard P (1977). Seed Pathology. London: Macmillan 2:842-1187.
Oliveira AMR, Matsumura ATS, Prestes AM, Matos GS, Van Der Sand ST (1998). Morphological variability and pathogenicity in isolates of *Bipolaris sorokiniana*. Braz. Phytopathol. 23:349-353.
Pillar VD (1997). Multivariate exploratory analysis and randomization testing with Multiv. Coenoses 12(2):145-148.
Pillar VD, Orlóci L (1996). On randomization testing in vegetation science: multifactor comparisons of relevé groups. J. Veg. Sci. 7:585-592.
Poloni A, Muller MV, Pessi I, Van Der Sand ST (2008). Analysis of morphological and growth rate variability of polyconidial and monoconidial cultures of *Bipolaris sorokiniana*. Braz. J. Biociênc. (On-line) 16(1).
R Development Core Team (2008). R Foundation for Statistical Computing, Vienna, Austria.
Shoemaker RA (1955) Biology, cytology, and taxonomy of *Cochliobolussativus*. Can. J. Bot. 33:562-567.
Tinline RD (1961). *Cochliobolussativus* Drug resistant, color and nutritionally exacting mutants. Can. J. Bot. 40: 425-437.
Valim-Labres ME, Prestes A, Van Der Sand ST, Matsumura ATS (1997). Variação no aspecto cultural morfológico e virulência em isolados de Bipolaris sorokiniana. Fitopatol. Braz. Phytopathol. 22:483-487.
Wheeler T, Rush CM (2001). Soil inhabitant. In: Maloy OC, Murray TD (eds) Encyclopedia of Plant Pathol. John Wiley & Sons. New York. USA. 933-934.