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The use of RAPD to characterize *Bipolaris sorokiniana* isolates

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ABSTRACT. *Bipolaris sorokiniana* is a phytopathogenic fungus causing diseases of cereal crops such as common root rot, the leaf spot disease, seedling blight, and black point of the grain. Random-amplified polymorphic DNA (RAPD) assay was used to investigate the genetic diversity of 20 isolates collected from different cultivars in wheat-producing regions in Brazil. Seventy primers, with random nucleotide sequences, were tested. Reproducibility to amplify the genomic DNA of isolates was found for 30 of the 70 primers tested, generating between 1 and 17 fragments ranging from 0.35 to 2.0 kb (average size). The degree of similarity between samples was calculated through simple association and the dendrogram was assessed using the unweighted pair group method with arithmetical average. After the RAPD analyses 19 isolates were closely grouped, having a similarity coefficient of ≥ 78%. Isolate I017 showed very low similarity coefficients, ranging between 38 and 46%. The RAPD analyses provided important information as to the degree of genetic variability and the relationship between the iso-

lates investigated, revealing polymorphism and establishing electrophoretic profiles useful to characterize the phytopathogen.

Key words: *Bipolaris sorokiniana*, Phytopathogen, Variability, RAPD

INTRODUCTION

Wheat is one of the most economically important crops world-wide including Brazil. Nevertheless, considerable economic losses happen chiefly due to diseases caused by fungi. The phytopathogen *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker, 1959 (teleomorph): *Cochliobolus sativus* (Ito & Kuribayashi) Drechsl. ex Dastur causes diseases in wheat and other cereal grasses, and occurs widely in wheat-producing regions in Brazil. The diseases caused by the fungus are common root rot, leaf spot disease, seedling blight, and black point of the grain. The main source of inoculum is considered to be soil-borne conidia, which, under favorable conditions, may result in severe damage by common root rot (Duczek, 1984).

Bipolaris sorokiniana shows high morphological and physiological variability. The mechanisms involved in the variability of *B. sorokiniana* include heterokaryosis, which occurs chiefly through the anastomosis between adjacent hyphae, enables the parasexual cycle, which is the main source of genetic diversity in fungi with asexual reproduction (Day, 1974). This diversity is one cause of the infective success of phytopathogenic fungi in overcoming host resistance (Guseva et al., 1979).

The morphological, physiological and biochemical characterizations of *B. sorokiniana* have been the aim of many studies (Christensen, 1925; Mitra, 1931; Hrushovetz, 1956; Wilson and Murphy, 1964; Tinline, 1986). However, knowledge about the genetic structure of this fungus is less available (Zhong and Steffenson, 2001a,b; Zhong et al., 2002).

Fungal species that are pathogenic on plants or animals exhibit variation in many important traits. Variability in morphology can make pathogen identification difficult while physiological variability can affect the evaluation of the damage potential (virulence) of a particular pathogen. The correct species identification and recognition of physiological strains (pathotypes) is critical information to understand the pathogen-host relationship (Strongman and MacKay, 1993).

Molecular markers have been increasingly used to examine the dynamics of phytopathogen populations. The development and refinement of molecular techniques have provided tools to advance the study of genetic diversity, of the intra- and inter-specific phylogenetic relationships, and also the identification of races and pathotypes (Mullins and Faloona, 1987; Bruns et al., 1991; Batista, 1993; Gallego and Martinez, 1997).

Since its development, the random-amplified polymorphic DNA (RAPD) protocol has acquired a diversity of uses, such as: the establishment of the genetic similarity degree between individuals within a population (Anderson and Fairbanks, 1990), the construction of genetic maps as well as the localization of economically interesting genes (Williams et al., 1990), the production of a genomic fingerprint (Welsh and McClelland, 1990), and the study of genetic diversity along with the identification of fungi (Fani et al., 1993; Fegan et al., 1993; Henson and French, 1993; Jungehülsing and Tudzynski, 1997; McEwen et al., 2000).

The aim of the present study was to assess the genetic variability of *B. sorokiniana* isolates from different wheat cultivars, by means of the RAPD technique. The detection of genetic diversity and, if possible, the establishment of races and/or *formae specialis* are two important pre-requisites for the development of resistant wheat varieties and establishment of molecular markers to ascertain pathogen identification.

MATERIAL AND METHODS

Bipolaris sorokiniana isolates

The *B. sorokiniana* isolates examined in this experiment were obtained from wheat seeds of different cultivars grown in different wheat-producing regions in Brazil (Table 1). The seeds were provided by the Centro Nacional de Pesquisas do Trigo (EMBRAPA/CNPT), Passo Fundo, RS, Brazil.

Table 1. *Bipolaris sorokiniana* isolates used for molecular diversity.

Isolates	Host origin	Cultivar	Geographic origin
I003	Kernel/wheat	E120	Pelotas-RS/Brazil
I004	Kernel/wheat	E119	Cruz Alta-RS/Brazil
I006	Kernel/wheat	CEP27	Pelotas-RS/Brazil
I007	Kernel/wheat	RS8	Cruz Alta-RS/Brazil
I012	Kernel/wheat	BR35	Lagoa Vermelha-RS/Brazil
I014	Kernel/wheat	BR35	Lagoa Vermelha-RS/Brazil
I017	Kernel/wheat	E16	Samambaia-PR/Brazil
I018	Kernel/wheat	OR1	Vitória-PR/Brazil
I021	Kernel/wheat	E49	Vitória-PR/Brazil
I022	Kernel/wheat	BR35	Selbach-RS/Brazil
I023	Kernel/wheat	OR1	Vitória-PR/Brazil
I026	Kernel/wheat	BR35	Vitória-PR/Brazil
I029	Kernel/wheat	E40	Piratini-RS/Brazil
I030	Kernel/wheat	E120	Cruz Alta-RS/Brazil
I031	Kernel/wheat	OR1	Nova Estância-PR/Brazil
I032	Kernel/wheat	ANHAUAC	Eng. Beltrão-PR/Brazil
I033	Kernel/wheat	E16	Landoi-PR/Brazil
I040	Kernel/wheat	BR23	Vitória-PR/Brazil
I041	Kernel/wheat	OR1	Vitória-PR/Brazil
I043	Kernel/wheat	BR35	Pelotas-RS/Brazil

Isolation and maintenance of the polyconidial *Bipolaris sorokiniana* cultures

The wheat seeds were submersed in 2% sodium hypochlorite (w/v) for 2 min, rinsed three times in sterile distilled water, and subsequently transferred to Petri dishes containing potato dextrose agar. The samples were then incubated in a BOD at 24 ± 2°C for approximately 9 days with a 12-h photoperiod. After some replication of the isolates pure cultures were ob-

tained. Inoculum from the samples was transferred to slant test tubes containing PDA medium and incubated as before. When colonies developed and were identified, the test tubes were stored at 4°C.

DNA extraction

Genomic DNA was obtained from each isolate grown in liquid culture (potato dextrose broth) following a method already developed by Ashktorab and Cohen (1992), with modifications to suit the phytopathogen under study. Mycelium of each isolate was retrieved through filtration, rinsed, and macerated in liquid nitrogen until a fine powder was obtained. Wet mycelium extraction-lysis buffer (1 mL/g), which consisted of 200 mM Tris-HCl, pH 8.0, 250 mM NaCl, 25 mM EDTA, pH 8.0, 1% sodium dodecyl sulfate (SDS), 10 µL/mL β-mercaptoethanol (Sigma), and 50 µg/mL proteinase K, was added to this powder. The tubes were incubated at 64°C for 60 min, shaken occasionally and centrifuged at 5000 rpm for 20 min. One volume of phenol (equilibrated with Tris-HCl, pH 8.0) was added to the supernatant recovered from the centrifugation. Homogenization was followed by centrifugation at 5000 rpm for 20 min. The aqueous phase was removed, transferred to new tubes and the extraction routine took place twice with phenol:chloroform, and once with 1X volume chloroform:isoamyl alcohol. Pancreatic RNAse A (50 µg/mL) was added to the supernatant and then incubated at 37°C for 20 min. DNA sample was precipitated with 0.1 volume 3 M sodium acetate and 2.5 volume isopropanol at -20°C. DNA was then rinsed with cooled 70% alcohol (v/v), centrifuged at 5000 rpm for 10 min, dried at room temperature, and finally resuspended in milliQ water and stored at -20°C.

RAPD analysis

Genomic DNA from the *B. sorokiniana* samples was amplified using the RAPD protocol (Williams et al., 1990). The primers tested comprised the series W (W01-W20), E (E01-E20), P (P01-P20), and O (O01-O10) (Operon Technologies, Alameda, CA, USA). Thirty primers were selected for the analysis of genetic diversity observed on the basis of the polymorphic DNA standard obtained after the RAPD assays. The primers chosen, OPW02-W10, W18-W20; OPP01-P09, P14; OPE03, E07, E11, E12, E14, and E16-E18 (Table 2), were retested in RAPD assays to assess the reproducibility of banding patterns. Only strong and reproducible polymorphic RAPD profiles were analyzed. The amplification was tested in a final volume of 25 µL for each reaction containing 30 ng genomic DNA, a dNTP mix (2.5 mM of each dATP, dCTP, dGTP, dTTP), 30 ng oligonucleotide primer, 100 ng bovine serum albumin (BSA), 1X reaction buffer (10X), 1 U *Taq* polymerase (Cenbiot, RS, Brazil), and 0.001% mineral oil. The reactions took place in a thermal cycler Techne-Progene with 46 cycles as follows: 1 initial cycle (1 min at 94°C/5 min at 42°C/2 min at 72°C), 45 cycles (30 s at 94°C/30 s at 42°C/1 min at 72°C). Fragments were resolved by electrophoresis in 1.4% agarose gel, at 75 V for 2.5 h.

Statistical analysis

The RAPD data were assessed using the Statistical Package for the Social Sciences (SPSS) software, 2nd edition, through which similarity coefficients were calculated and the dendrogram for genetic distances was constructed. The similarity was evaluated through simple

Table 2. Primers chosen for the RAPD analysis and resulting number of amplified fragments.

Primers	Primer sequence 5'→ 3'	Fragment size (kb)
OPW-02	ACC CCG CCA A	0.53, 0.58, 0.72, 0.79, 0.82, 1.54
OPW-03	GTC CGG AGT G	0.50, 0.62, 0.68, 0.77, 1.44, 1.51, 1.86, 1.90, 2.00
OPW-04	CAG AAG CGG A	0.45, 0.50, 0.53, 0.64, 0.93, 0.96, 0.98, 0.99, 1.01, 1.03, 1.18, 1.19, 1.31
OPW-05	GGC GGA TAA G	0.40, 0.45, 0.58, 0.62, 0.63, 0.68, 0.78, 0.85, 0.87, 1.20, 1.28, 1.31, 1.75
OPW-06	AGG CCC GAT G	0.41, 0.47, 0.55, 0.66, 0.72, 0.84, 0.94, 1.10
OPW-07	CTG GAC GTC A	0.46, 0.71, 0.77, 0.78, 0.84, 0.86, 0.91, 0.95, 0.96, 0.99, 1.00, 1.04, 1.09, 1.17, 1.20, 1.37, 1.68
OPW-08	GAC TGC CTC T	0.54, 0.70, 0.93, 0.94, 1.21, 1.27, 1.28, 1.36, 1.42, 1.61, 1.67, 1.73, 1.78, 1.79
OPW-09	GTG ACC GAG T	0.59, 0.69, 0.74, 0.91, 1.05, 1.19
OPW-10	TCG CAT CCC T	0.39, 0.45, 0.60, 0.85, 0.93, 1.58
OPW-18	TTC AGG GCA C	0.61, 0.82, 0.95, 1.09, 1.20, 1.28, 1.98
OPW-19	CAA AGC GCT C	0.49, 0.54, 0.71, 0.78, 0.80, 0.83, 0.87, 1.02, 1.10, 1.58
OPW-20	TGT GGC AGC A	0.56, 0.60, 0.64, 0.77, 0.80, 0.94, 1.07, 1.19, 1.43, 1.50
OPE-03	CCA GAT GCA C	0.49, 0.69, 0.73, 0.74, 0.88, 1.02, 1.51
OPE-07	AGA TGC AGC C	0.55, 0.77, 0.74, 0.77, 1.03, 1.13, 1.30, 1.43, 1.47
OPE-11	GAG TCT CAG G	0.86, 0.91, 1.00, 1.15, 1.40
OPE-12	TTA TCG CCC C	0.43, 0.58, 0.70, 0.83, 1.17, 1.29, 1.30, 1.42, 1.63, 1.67, 1.90, 2.00
OPE-14	TGC GGC TGA G	0.52, 0.66, 0.72, 0.80, 0.87, 0.96, 1.23, 1.28, 1.74
OPE-16	GGT GACTGT G	0.66, 0.77, 0.94, 0.95, 1.05, 1.18, 1.69
OPE-17	CTA CTG CCG T	0.61, 0.74, 0.80, 1.11, 1.17
OPE-18	GGA CTG CAG A	0.54, 0.58, 0.64, 0.83, 1.13
OPP-01	GAT GCA CTC C	0.69, 0.70, 0.81, 0.85, 0.87, 0.91, 0.97, 1.12, 1.15, 1.22, 1.26, 1.58, 1.74, 1.85
OPP-02	TCG GCA CGC A	0.43, 0.47, 0.55, 0.62, 0.71, 0.74, 0.90, 1.00, 1.12
OPP-03	CTG ATA CGC C	0.40, 0.56, 0.66, 0.67, 1.94
OPP-04	GTG TCT CAG G	0.45, 0.50, 0.51, 0.52, 0.53, 0.54, 0.57, 0.63, 0.84, 0.91, 0.96, 1.51
OPP-05	CCC CGG TAA C	0.44, 0.54, 0.83, 0.86, 0.94, 1.04, 1.20, 1.41, 1.50, 1.81
OPP-06	GTG GGC TGA C	0.45, 0.48, 0.54, 0.56, 0.65, 0.67, 0.70, 0.83, 0.84, 0.96, 1.24, 1.90
OPP-07	GTC CAT GCC A	0.35, 0.41, 0.47, 0.51, 0.66, 0.70, 0.74, 0.79, 1.17, 1.38
OPP-08	ACA TCG CCC A	0.44, 0.46, 0.52, 0.56, 0.71, 0.76, 0.81, 1.05, 1.65
OPP-09	GTG GTC CGC A	0.50, 0.61, 0.65, 0.68, 0.78, 0.81, 0.85, 0.95, 0.99
OPP-14	CCA GCC GAA C	0.48, 0.56, 0.63, 0.65, 0.77, 0.79, 0.80, 0.84, 0.88, 1.07, 1.11, 1.23, 1.27
Total		282

association and the genetic distance as the Euclidean distance. The binary matrix was built pairwise, and the presence or absence of a determined RAPD band scored 1 and 0, respectively. The hierarchical groupings were based on the unweighted pair group using arithmetic averages - UPGMA (Sneath and Sokal, 1973).

RESULTS

A total of 282 fragments resulted from the primer amplifications with the DNA from the 20 isolates of *B. sorokinana* (Table 2). Fragments present in at least one and maximum 18 isolates (5-90% occurrence) were considered polymorphic, while fragments present in at least 19 isolates (95% occurrence) were monomorphic. This criterion established a total of 228 fragments (80.9%) as being polymorphic.

Figure 1A,B shows a monomorphic standard obtained with primer W06. The 1.10-, 0.94-, 0.84-, and 0.72-kb fragments were present in all samples, except in isolate I017, which did not amplify the 1.10- and 0.72-kb fragments. On the other hand, this was the only isolate to yield 0.41-, 0.47-, 0.55-, and 0.66-kb fragments (Table 2).

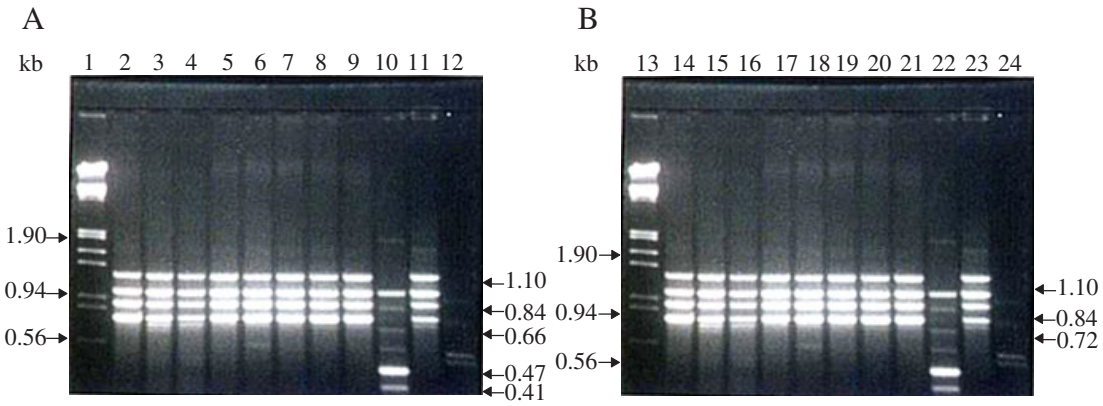

Figure 1. Amplification product for primer W06. *Lanes 1* and *13*: λ marker digested with *Eco*RI/*Hin*dIII. *Lanes 12* and *24*: negative control. **A.** Lanes 2 to 11, isolates I004, I003, I006, I033, I014, I022, I018, I012, I017, and I030. **B.** Lanes 14 to 23, isolates I031, I026, I032, I007, I023, I043, I041, I021, I029, and I040.

Figure 2A,B shows the amplification standard for primer P14. Fragments of 0.63 and 0.88 kb were monomorphic and did not amplify with isolate I017. In contrast isolate I017 was the only to amplify 0.56-, 0.65-, 0.79-, and 0.84-kb fragments. The 1.27-kb fragment was present in isolate I030 only. The 0.77-kb band amplified with isolates I032, I007, and I021 only, while the 0.80-kb band amplified with isolate I006. Isolates I004, I033, I014, I022, and I018 exhibited a 1.07-kb fragment. The 1.11-kb fragment was present in isolates I029 and I023 exclusively, and the 1.23-kb fragment was amplified with isolates I006, I033, and I022 (Table 2).

A dendrogram was constructed with 282 fragments generated from RAPD data as shown in Figure 3. The genetic similarity between the isolates assessed through simple association ranged between 0.3865 and 0.9681 (Table 3). The maximum coefficient (0.9681) was observed for pair I040-I029, while the lowest coefficient (0.3865) was observed for pair I006-I017. The remaining isolates had similarity coefficients between 0.9645 and 0.3936. The iso-

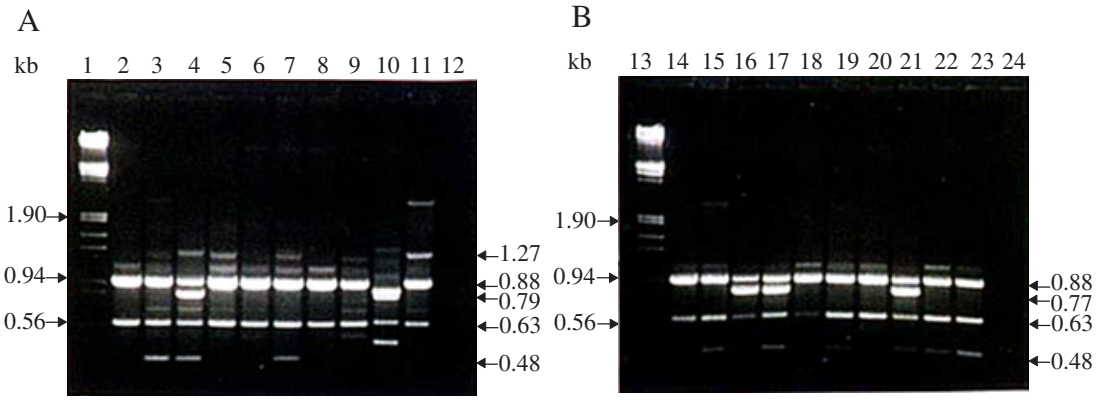

Figure 2. Amplification product for primer P14. *Lanes 1* and *13*: λ marker digested with *Eco*RI/*Hin*dIII. *Lanes 12* and *24*: negative control. **A.** Lanes 2 to 11, isolates I004, I003, I006, I033, I014, I022, I018, I012, I017, and I030. **B.** Lanes 14 to 23, isolates I031, I026, I032, I007, I023, I043, I041, I021, I029, and I040.

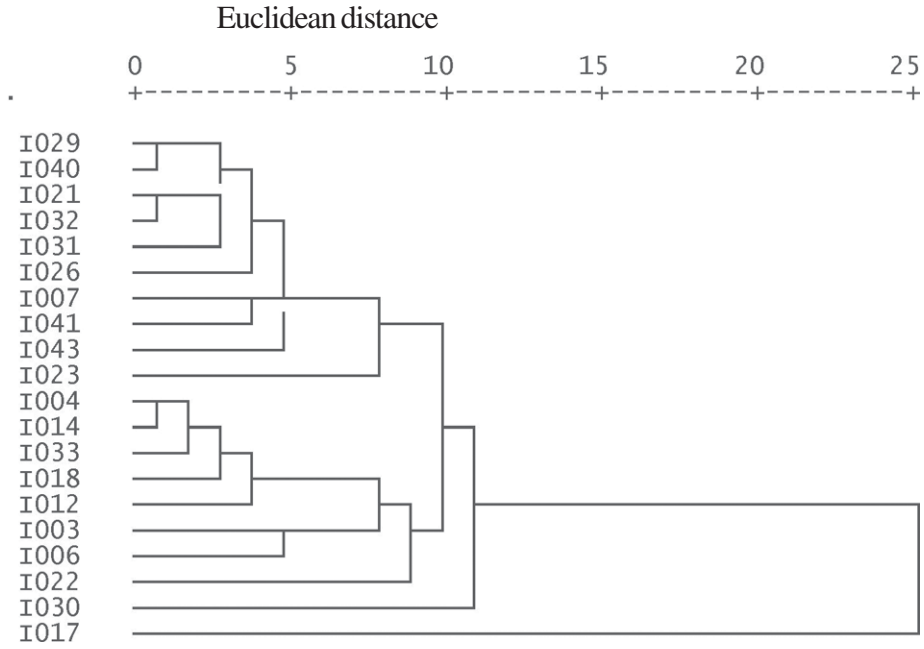

Figure 3. Dendrogram for the RAPD data obtained for the *Bipolaris sorokiniana* isolates, generated by the Euclidean distance, following UPGMA method.

lates were assigned to 3 groups: 2 closely related (groups I and II, with similarities ≥78%), and one group (group III), related to the other groups with similarity coefficients between 0.3865 and 0.4681.

Group I was established between pairs I029-I040 and I021-I032 joined by isolates I031, I026, I007, I041, I043, and I023. Group II comprised pair I004-I014, and isolates I033, I018, I012, I003, I006, I022, I030. Isolate I017, which forms group III, showed a banding pattern significantly different from the other isolates.

Table 3. Matrix for the similarity coefficient obtained between the *Bipolaris sorokiniana* isolates, after the RAPD assessment.

Isolates	1003	1004	1006	1007	1012	1014	1017	1018	1021	1022	1023	1026	1029	1030	1031	1032	1033	1040	1041
1004	0.9043																		
1006	0.9149	0.8972																	
1007	0.8227	0.8759	0.8156																
1012	0.8617	0.9362	0.8759	0.8404															
1014	0.8830	0.9645	0.8830	0.8688	0.9433														
1017	0.4078	0.4539	0.3865	0.4291	0.4681	0.4539													
1018	0.8546	0.9433	0.8617	0.8404	0.9220	0.9504	0.4397												
1021	0.8475	0.9078	0.8404	0.9255	0.8723	0.9078	0.4681	0.8936											
1022	0.8511	0.8830	0.8440	0.8014	0.8546	0.8830	0.3936	0.8688	0.8333										
1043	0.7943	0.8546	0.7801	0.8652	0.8333	0.8617	0.4645	0.8333	0.8972	0.7801									
1026	0.8298	0.8830	0.8156	0.9149	0.8546	0.8759	0.4362	0.8475	0.9184	0.8156	0.8582								
1029	0.8298	0.8901	0.8156	0.9220	0.8546	0.8830	0.4504	0.8617	0.9468	0.8085	0.8936	0.9291							
1030	0.8191	0.8511	0.8262	0.7908	0.8440	0.8369	0.4255	0.8227	0.8156	0.8050	0.7979	0.7979	0.7908						
1031	0.8546	0.9078	0.8404	0.9326	0.8652	0.9007	0.4468	0.8723	0.9362	0.8404	0.8830	0.9397	0.9326	0.8156					
1032	0.8475	0.8936	0.8333	0.9326	0.8652	0.8936	0.4610	0.8723	0.9645	0.8333	0.9043	0.9326	0.9397	0.8227	0.9574				
1033	0.8972	0.9504	0.9184	0.8475	0.9362	0.9504	0.4326	0.9291	0.8794	0.8759	0.8262	0.8546	0.8546	0.8582	0.8723	0.8652			
1040	0.8333	0.8936	0.8262	0.9397	0.8511	0.8865	0.4397	0.8582	0.9362	0.8191	0.8759	0.9326	0.9681	0.8014	0.9433	0.9362	0.8582		
1041	0.8227	0.8972	0.8227	0.9362	0.8759	0.8972	0.4645	0.8688	0.9326	0.8085	0.9149	0.9078	0.9291	0.8121	0.9255	0.9255	0.8759	0.9326	
1043	0.8404	0.8723	0.8050	0.9255	0.8298	0.8652	0.4255	0.8440	0.9078	0.8050	0.8688	0.9255	0.9043	0.7943	0.9362	0.9149	0.8369	0.9220	0.904

DISCUSSION

The results suggest that, notwithstanding the isolates originated from different wheat cultivars, for the sampling carried out, the genetic variability of *B. sorokiniana* isolates was low, with 19 of the 20 isolates showing a similarity coefficient of ≥78%. These results might be related to the geographic origin of the samples because all the isolates were obtained from wheat seeds from two states on the southern region of Brazil (Rio Grande do Sul and Paraná), the leading wheat-producing region in the country.

Zhong and Steffenson (2001b) assessed genetic diversity of *Cochliobolus sativus* based on virulence and AFLP markers. Ninety-five polymorphic AFLP markers were found with 8 primer pairs, and each isolate exhibited a unique AFLP marker. The cluster analysis did not reveal a close correlation between pathotypes and AFLP groups. In the same study, the authors also observed no close correlation between genetic similarity measured by AFLP markers and geographic origin, suggesting that these isolates were derived from the same founder source population and disseminated from one area to another in association with their hosts.

Chen et al. (1995) found a high degree of similarity between *Puccinia striiformis* individuals from the same *formae specialis*, and high degree of polymorphism was observed for individuals of different species. In the same study the RAPD groups formed generally were not associated with the geographic regions either. On the other hand, isolates of *Fusarium oxysporum* f. sp *vasinfectum* were differentiated into three main groups directly related to both virulence and geographic origin (Assigbetse et al., 1994).

The variation in intensity of some fragments in this study was observed, but this might be due to a more efficient amplification at some target sites, or to a greater complementarity between the primer used and the fungal DNA. Variation may appear in keeping with target sites present at highly repetitive sequences as well (Meunier and Grimont, 1993; Kelly et al., 1994; Tyler et al., 1997).

Nucleotide alterations, insertions and deletions at initiation sites may result in polymorphic DNA, which is detectable by the RAPD technique (Williams et al., 1990). In the present study, the multi-nucleated conditions of *B. sorokiniana* mycelial cells and conidia, with subsequent heterokaryosis - which in turn could lead to mitotic recombination and new haploidization arrangements - may account for a likely contribution to the DNA polymorphism detected for the phytopathogen. Even though, the amplification products of 33.3% of the primers W02 (1.54 kb), W05 (1.20 kb), W06 (0.84 and 0.94 kb), W07 (0.71 kb), W10 (0.93 kb), W19 (0.54 kb), P08 (1.05 kb), P09 (0.99 kb), P14 (0.63 and 0.88 kb), and E16 (0.66 kb) generated monomorphic fragments. These same primers are being used with monoconidial cultures of the isolates in order to verify the significance of the monomorphic fragments. If they repeat themselves it may be possible that these fragments can be used as specific molecular markers to identify *B. sorokiniana*.

Taxonomic identification of isolate I017 (Samambaia, PR, Brazil), was confirmed through conidial shape; hilum; germination; basal germ tube; direction of growth and position of emergence; septum ontogeny and conidiogenous nodes. This was the only isolate to show a very low similarity and the absence of significant monomorphic bands. Isolate I017 with all primers tested also showed a different banding pattern. This isolate had previously shown a differentiated pattern as to the degree of pathogenicity and established no infective interaction with wheat cultivars, and differed from other isolates analyzed (Santos AMPV, personal communication).The

isolate I017 was the only isolate that could be well distinguished from all the others in this study. More studies must be done with this isolate in order to classify it.

RAPD assays were used to determine *formae specialis* and physiological races in pathogenic and antagonistic species of *Fusarium oxysporum* (Migheli and Cavallarin, 1994). RAPD has been successfully used to distinguish pathogenic and non-pathogenic isolates of *Leptosphareia maculans* (Plummer et al., 1994) and to differentiate pathotypes in *Peronospora parasitica* (Tham et al.,1994).

In a work done with *Cochliobolus carbonum* (Jones and Dunkle, 1993) RAPDs were used to distinguish races at molecular level. In their studies they found that all pathogenic races were substantially different from nonpathogenic races. From the 20 primers tested on all five races, the banding pattern was similar for all pathogenic races with 19 of the primers. Only one primer showed race 3 specific differences.

A compilation of Tudzynski and Tudzynski (1996) lists 22 species of phytopathogenic fungi, where RAPD has been used. In most of these cases RAPD could be used to discriminate between physiologically or genetically well-defined species subgroups, or even between species, using RAPD as a molecular confirmation for the subgrouping obtained by other methods, and as a diagnostic tool for the grouping of new isolates (Jungehülsing and Tudzynski, 1997).

RAPD results obtained in the present study enabled a fast and efficient variability analysis for *B. sorokiniana*. These results also led to the generation of electrophoretic profiles, which discriminated intra-specific polymorphism in the isolates studied. Also, in order to find more variability among the isolates of *B. sorokiniana*, and maybe to separate them in races, a study with a higher number of isolates from the same, and different wheat cultivars from Brazil and other countries and also isolates from other cereal grasses will be necessary.

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REFERENCES

Anderson PW and Fairbanks DJ (1990). Molecular markers: Important tolls for plant genetics resource characterization. *Diversity* 6: 51-53.

Ashktorab H and Cohen RJ (1992). Facile isolation of genomic DNA from filamentous fungi. *Biotechniques* 13: 198-200.

Assigbetse KB, Fernandez D and Dubois MP (1994). Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. *Phytopathology* 84: 622-626.

Batista MF (1993). Métodos moleculares para identificação de patógenos de plantas. *Rev. Anu. Patol. Plantas* 1: 165-196.

Bruns TD, White TJ and Taylor JW (1991). Fungal molecular systematics. *Ann. Ver. Ecol. Syst.* 22: 525-564.

Chen X, Line RF and Leung H (1995). Virulence and polymorphic DNA relationships of *Puccinia striiformis* f. sp. *hordei* to other rusts. *Phytopathology* 85: 1335-1342.

Christensen JJ (1925). Physiologic specialization and mutation in *Helminthosporium sativum*. *Phytopathology* 15: 785-795.

Day PR (1974). Genetics and host-parasite interaction. W.H. Freeman and Company, San Francisco, CA, USA.

Duczek LJ (1984). Comparision of common root rot reaction of barley lines and cultivars in northwestern Alberta and central Saskatchewan. *Can. J. Plant Pathol.* 6: 81-89.

Fani R, Damiani G, Di SC, Gallori E et al. (1993). Use of random amplified polymorphic DNA (RAPD) for

generating specific DNA probes for microorganisms. *Mol. Ecol.* 2: 243-250.
Fegan M, Manners JM, MacLean DJ, Irwin JA et al. (1993). Random amplified polymorphic DNA markers reveal a high degree of genetic diversity in the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*. *J. Gen. Microbiol.* 139: 2075-2081.
Gallego FJ and Martinez I (1997). Method to improve reliability of random-amplified polymorphic DNA markers. *Biotechniques* 23: 663-664.
Guseva NN, Levitin MM and Mikhailova LA (1979). Genetic mechanisms of variability if phytopathogenic fungi. *Acta Phytopathol. Acad. Sci. Hung.* 14: 441-447.
Henson JM and French R (1993). The polymerase chain reaction and plant disease diagnosis. *Ann. Rev. Phytopathol.* 31: 81-110.
Hrushovetz SB (1956). Cytological studies of *Helminthosporium sativum*. *Can. J. Bot.* 34: 321-327.
Jones MJ and Dunkle LD (1993). Analysis of *Cochliobolus carbonum* races by PCR amplification with arbitrary and gene-specific primers. *Phytopathology* 83: 366-370.
Jungehülsing U and Tudzynski P (1997). Analysis of diversity in *Claviceps purpurea* by RAPD markers. *Mycol. Res.* 101: 1-6.
Kelly A, Alcala-Jimenez AR and Heale BW (1994). Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of *Fusarium oxysporium* f. sp. *ciceris* infecting chick-pea. *Phytopathology* 84: 1293-1298.
McEwen JG, Taylor JW, Carter D, Xu J et al. (2000). Molecular typing of pathogenic fungi. *Med. Mycol.* 38 (Suppl 1): 189-197.
Meunier JR and Grimont PA (1993). Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. *Res. Microbiol.* 144: 373-379.
Migheli Q and Cavallarin L (1994). Characterization of antagonist and pathogenic *Fusarium oxysporum* isolates by random amplification of polymorphic DNA. *Mol. Biotechnol.* 2: 197-200.
Mitra M (1931). Saltation in the genus *Helminthosporium*. *Trans. Brit. Mycol. Soc.* 16: 115-117.
Mullins KB and Faloona FA (1987). Specific synthesis of DNA *in vitro* via a polymerase-catalised chain reaction. *Methods Enzymol.* 155: 335-350.
Plummer KM, Dunse K and Howlett BJ (1994). Non-aggressive strains of the black leg fungus, *Leptosphaeria maculans*, are present in Australia and can be distinguished from aggressive strains by molecular analysis. *Aust. J. Bot.* 42: 1-8.
Sneath PHA and Sokal RR (1973). Numerical taxonomic. W.H. Freeman and Company, San Francisco, CA, USA.
Strongman DB and Mackay RM (1993). Discrimination between *Hirustella longicolla* var. *Cornuta* using random amplified polymorphic DNA fingerprinting. *Mycologia* 85: 65-70.
Tham FY, Lucas JA and Wilson ZA (1994). DNA fingerprinting of *Peronospora parasitica*, a biotrophic fungal pathogen of crucifers. *Theor. Appl. Genet.* 88: 490-496.
Tinline RD (1986). *Cochliobolus sativus*, a pathogen of wide host range. *Adv. Plant Pathol.* 6: 113-122.
Tudzynski P and Tudzynski B (1996). Genetics of phytopathogenic fungi. *Prog. Bot.* 57: 235-252.
Tyler KD, Wang G, Tyler SD and Johnson WM (1997). Factors affecting reliability and reproducibility of amplification-based DNA fingerprinting of representative bacterial pathogens. *J. Clin. Microbiol.* 35: 339-346.
Welsh RJ and McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7213-7218.
Williams JGK, Kuberlik AR and Kenneth JL (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6231-6235.
Wilson VE and Murphy HC (1964). Morphology and physiology of *Helminthosporium victoriae* and related species. *Phytopathology* 54: 147-150.
Zhong S and Steffenson BJ (2001a). Genetic and molecular characterization of mating type genes in *Cochliobolus sativus*. *Mycologia* 93: 852-863.
Zhong S and Steffenson BJ (2001b). Virulence and molecular diversity in *Cochliobolus sativus*. *Phytopathology* 91: 469-475.
Zhong S, Steffenson BJ, Martinez JP and Ciuffetti LM (2002). A molecular genetic map and electrophoretic karyotype of the plant pathogenic fungus *Cochliobolus sativus*. *Mol. Plant Microbe Interact.* 15: 481-492.