Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Paper

Molecular and pathogenic characterization of *Cochliobolus* anamorphs associated with common root rot of wheat in Azerbaijan

Göksel ÖZER[1,*], Mustafa IMREN[1], Mehtap ALKAN[1], Timothy C. PAULITZ[2], Harun BAYRAKTAR[3], Gülsüm PALACIOĞLU[3], Ibrahim MEHDIYEV[4], Hafiz MUMINJANOV[5], Abdelfattah A. DABABAT[6,*]

[1] *Department of Plant Protection, Faculty of Agriculture and Natural Sciences, Bolu Abant Izzet Baysal University, Bolu, 14030, Turkey*
[2] *United States Department of Agriculture, Agricultural Research Service, Wheat Health, Genetics and Quality Research Unit, Washington State University, Pullman, WA 99164-6430, USA*
[3] *Department of Plant Protection, Faculty of Agriculture, Ankara University, Ankara, 06110, Turkey*
[4] *Plant Health and Phytosanitary Control Sector of the Shaki Regional Division, Food Safety Agency of the Republic of Azerbaijan, Azerbaijan*
[5] *Food and Agriculture Organization of the United Nations, Plant Production and Protection Officer, Ankara, Turkey*
[6] *International Maize and Wheat Improvement Center (CIMMYT) P.O. Box. 39 Emek, Ankara, Turkey*
*Corresponding authors: gokozer@gmail.com, a.dababat@cgiar.org

Summary. Genetic variation among the Azerbaijani isolates of anamorphs of *Cochliobolus* spp., the causal agents of common root rot of wheat, was evaluated using pathogenicity assessments, sequence analyses of the internal transcribed spacer (ITS) region and glyceraldehyde-3-phosphate dehydrogenase (GPDH) gene, as well as inter-simple sequence repeat (ISSR) and inter-primer binding site (iPBS) markers. Twenty-eight isolates used in this study were obtained from diseased wheat plants in cereal growing regions of Azerbaijan in 2017. *Bipolaris sorokiniana*, *Curvularia spicifera*, and *Curvularia inaequalis* were identified. *Bipolaris sorokiniana* isolates were the most virulent on wheat seedlings, followed by isolates of *C. spicifera* and *C. inaequalis*. Phylogenetic analyses of a combined dataset of the ITS and GPDH regions grouped the isolates into three clusters, each of which contained isolates of one species. The dendrogram derived from the unweighted pair-grouped method by arithmetic average (UPGMA) cluster analyses based on the data of ISSR and iPBS markers divided the isolates into three clusters in concordance to their taxonomic grouping at species level, but without correlation to their geographic origins. Population structure of isolates was estimated based on Bayesian modelling, and this showed three populations (K = 3) supporting the separation of isolates in the dendrogram with the greatest mean value of Ln likelihood (-893,8). Utilization of the markers either separately or together produced a high level of polymorphism at interspecies level, which allowed for the separation of species. Although both marker systems had similar discrimination power to reveal genetic differences among the species, ISSR markers were more informative for eliciting intraspecies polymorphisms within *B. sorokiniana* and *C. spicifera* isolates.

OPEN ACCESS

Citation: G. Özer, M. Imren, M. Alkan, T.C. Paulitz, H. Bayraktar, G. Palacioğlu, I. Mehdiyev, H. Muminjanov, A.A. Dababat (2020) Molecular and pathogenic characterization of *Cochliobolus* anamorphs associated with common root rot of wheat in Azerbaijan. *Phytopathologia Mediterranea* 59(1): 147-158. doi: 10.14601/Phyto-10772

Accepted: February 18, 2020

Published: April 30, 2020

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Diego Rubiales, Institute for Sustainable Agriculture, (CSIC), Cordoba, Spain.

This is the first study on genetic diversity and population structure of anamorphic stages of *Cochliobolus* associated with common root rot of wheat using iPBS markers.

Keywords. ISSR, iPBS, phylogeny, aggressiveness.

INTRODUCTION

The species of *Cochliobolus* Drechsler are important pathogens associated with cereals. Their anamorphs are classified into *Bipolaris* and *Curvularia* genera based on morphological characteristics (Sivanesan, 1987). *Bipolaris sorokiniana* (Sacc. in Sorokin) Shoemaker [teleomorph: *Cochliobolus sativus* (S. Ito and Kuribayashi) Drechsler ex Dastur] is the most common species among *Cochliobolus* anamorphs, causing common root rot, spot blotch, seed black point, seedling blight, and head blight on wheat (Bakonyi *et al*., 1997). Common root rot (CRR) of wheat is a well-known and serious disease, which causes dark discolouration on lower leaf sheaths, crowns, subcrown internodes, and roots. CRR is responsible for annual yield losses of 6% in wheat production (Ledingham *et al*., 1973). Similar but less severe symptoms on cereals are also caused by different species of *Bipolaris* and *Curvularia*, including *B. bicolor*, *C. spicifera*, *C. inaequalis* (Gonzalez and Trevathan, 2000; Bach *et al*., 2003; Morejon *et al*., 2006; Safaee *et al*., 2008).

Using host genotypes/cultivars with natural genetic resistance and breeding new resistant cultivars to *Bipolaris* spp. and *Curvularia* spp. are likely to be the most economically and environmentally sound strategies for management of CRR. Differences both in host response and in pathogen virulence have been determined in previous studies to evaluate aggressiveness of CRR pathogens under controlled or field conditions on barley and wheat (Wildermuth *et al*., 1992; Valjavec-Gratian and Steffenson, 1997; Duveiller and Garcia, 2000; Gonzalez and Trevathan, 2000; Arabi and Jawhar, 2007, 2017). Accurate identification of isolates and analyses of the genetic and pathogenicity variabilities within pathogen populations provides key information to determine control strategies and breeding programmes. Due to their similarities, species identified as *Bipolaris* spp., *Curvularia* spp., and other anamorphs of *Cochliobolus* based on morphological characteristics pose difficulties (Sivanesan, 1987; Manamgoda *et al*., 2014, 2015). To overcome these, molecular analyses based on the internal transcribed spacer of ribosomal DNA (ITS), translation elongation factor 1-α (EF1-α), large subunit (LSU) ribosomal RNA and the glyceraldehyde 3-phosphate dehydrogenase (GPDH) locus have been successfully used for the identification of different species in these genera (Berbee *et al*., 1999; Manamgoda *et al*., 2012, 2014, 2015; Marin-Felix *et al*., 2017). Molecular marker techniques such as random amplified polymorphic DNAs (RAPD), retrotransposon microsatellite amplified polymorphism (REMAP) and amplified fragment length polymorphism (AFLP), have been most frequently and successfully utilized to reveal genetic variation among *B. sorokiniana* populations in Australia, the Czech Republic, Syria, Brazil, Poland, and the United States of America (Zhong and Steffenson, 2001; Müller *et al*., 2005; Arabi and Jawhar, 2007; Knight *et al*., 2010; Baturo-Ciesniewska, 2011; Ghazvini and Tekauz, 2012).

The inter-simple sequence repeat (ISSR) technique allows DNA amplification between identical SSR regions with a primer complementary to a target microsatellite region in PCR (Bornet and Branchard, 2001). The inter-primer binding site (iPBS) amplification technique is a universal method for DNA fingerprinting based on the universal presence of a tRNA complement as a reverse transcriptase PBS in long terminal repeats (LTR) retrotransposons (Kalendar *et al*., 2010). To date, both of these DNA marker techniques have been used in the analyses of population structures and differentiating fungal plant pathogens at intra- and interspecies levels (Özer *et al*., 2016; Altınok *et al*., 2018; Özer and Bayraktar, 2018; Skipars *et al*., 2018).

The present study was carried out to identify and characterize isolates of *Bipolaris* spp. and *Curvularia* spp. associated with crown and root rot of wheat in Azerbaijan, using sequence analysis of ITS and GPDH loci and pathogenicity tests. ISSR and iPBS molecular markers were also employed to assess genetic diversity within the isolates at intra- and interspecies levels.

MATERIAL AND METHODS

Fungus isolates

Samples of diseased plants were taken from 76 winter wheat fields located in the main wheat-growing regions of Azerbaijan, at the crop maturity stage and at harvesting time during June and July of 2017. Each sample consisted of at least 20 plants selected randomly from each field. Crown, root, sub-crown internodes, and

leaf sheaths of plants showing symptoms of CRR were washed thoroughly with running tap water to remove soil and organic particles for 3–5 min. Small sections from symptomatic tissues were surface-disinfested with 1% sodium hypochlorite solution for 1 min, rinsed with sterile distilled water, then dried between sterile tissue papers in a laminar flow cabinet. The tissue segments were then placed in 9 cm diam. Petri dishes containing 1/4 strength potato dextrose agar (PDA; Merck) supplemented with 100 mg L^{-1} streptomycin sulphate and 25 mg L^{-1} of chloramphenicol. After three-day-incubation at 22°C in constant darkness, the culture plates were examined under a dissecting microscope, and single conidia of 28 isolates similar to *Bipolaris* and *Curvularia* were picked off and transferred to Petri plates containing full strength PDA (Table 1). The plates were incubated under fluorescent lighting (12 h d^{-1}) at 22°C for 5 d. Morphological characteristics of the fungi were examined using a microscope (model DM1000, Leica Microsystems), and 30 conidia per isolate were measured at 400× magnification using the Leica LAS EZ software.

Pathogenicity assessments

The isolates were grown in 9 cm Petri dishes containing PDA and incubated at 22°C in the dark for 10 d to allow sporulation. For assessing pathogenicity, sterilized nursery soil was inoculated with a conidial suspension of each isolate to obtain an inoculum density of 250

Table 1. Isolates, their identities, locations, mean conidium dimensions, mean disease severity scores, and GenBank accession numbers related to sequences of their ITS and GPDH regions.

Isolate	Species	Location	Conidium length and width (µm)	Mean disease severity score[a]	GenBank accession numbers	
					(ITS)	(GPDH)
Co 01	*B. sorokiniana*	Qobustan	74.1 × 23.2	3.39 (ABCD)	MG661716	MH844812
Co 02	*B. sorokiniana*	İsmayıllı	75.8 × 22.2	2.85 (F)	MG654486	MK024315
Co 03	*B. sorokiniana*	İsmayıllı	79.4 × 23.0	3.36 (ABCDE)	MK022342	MH844813
Co 04	*B. sorokiniana*	İsmayıllı	84.7 × 20.6	2.88 (F)	MK022343	MH844814
Co 05	*B. sorokiniana*	İsmayıllı	81.7 × 18.9	3.05 (DEF)	MG654432	MH844815
Co 06	*B. sorokiniana*	Oğuz	71.1 × 18.9	3.53 (AB)	MG661717	MH844816
Co 07	*B. sorokiniana*	Oğuz	72.8 × 21.4	3.15 (BCDEF)	MK022344	MH844817
Co 08	*B. sorokiniana*	Oğuz	72.3 × 21.9	2.88 (F)	MK022345	MH844818
Co 09	*B. sorokiniana*	Şeki	71.3 × 21.2	3.48 (ABC)	MK022346	MK024316
Co 10	*B. sorokiniana*	Şeki	67.0 × 20.9	2.98 (EF)	MG661715	MK024317
Co 11	*B. sorokiniana*	Şeki	73.4 × 23.3	3.54 (AB)	MK022347	MK024318
Co 12	*B. sorokiniana*	Şeki	74.2 × 19.8	3.52 (ABC)	MG654433	MK024319
Co 13	*B. sorokiniana*	Şeki	73.0 × 21.9	3.33 (ABCDE)	MG654488	MK024320
Co 14	*B. sorokiniana*	Şeki	72.7 × 19.2	3.33 (ABCDE)	MG654487	MK024321
Co 15	*B. sorokiniana*	Ağdaş	83.0 × 20.9	3.44 (ABCD)	MG654434	MK024322
Co 16	*B. sorokiniana*	Kürdemir	73.0 × 18.9	2.97 (EF)	MK022348	MK024323
Co 17	*B. sorokiniana*	Kürdemir	81.2 × 18.9	3.60 (A)	MK022349	MH844819
Co 18	*B. sorokiniana*	Kürdemir	79.7 × 22.4	3.24 (ABCDEF)	MK022350	MH844820
Co 19	*B. sorokiniana*	Kürdemir	81.8 × 20.9	3.12 (CDEF)	MK022351	MH844821
Co 20	*C. spicifera*	Oğuz	27.4 × 9.7	2.01 (GHI)	MK022352	MH809683
Co 21	*C. spicifera*	Şeki	29.0 × 9.3	2.19 (GH)	MG654489	MH809678
Co 22	*C. spicifera*	Şeki	25.8 × 11.2	2.10 (GH)	MK022353	MH809679
Co 23	*C. spicifera*	Şeki	28.7 × 9.7	2.34 (G)	MK022354	MH809680
Co 24	*C. spicifera*	Şeki	30.2 × 9.5	2.31 (G)	MG661718	MH809681
Co 25	*C. spicifera*	Kürdemir	31.8 × 9.1	2.30 (G)	MK022355	MH809682
Co 26	*C. inaequalis*	Şeki	34.4 × 12.9	1.58 (J)	MG654437	MH844810
Co 27	*C. inaequalis*	Ağdaş	31.9 × 11.6	1.62 (IJ)	MK022356	MH844811
Co 28	*C. inaequalis*	Ağdaş	34.8 × 11.9	1.80 (HIJ)	MG654438	MK049127

[a]Means for each isolate within this column sharing the same letter are not significantly different ($P = 0.05$) according to the least significant difference test. LSD value for cultivar Seri-82 was 0.405.

conidia g^{-1} (Duczek *et al*., 1985). Seeds of wheat cultivar Seri-82 were sown (10 seeds per pot) into plastic pots (15 cm height, 9 cm diam.) filled with the inoculated soil. Sterilized soil was used as non-inoculated controls. Four replicates (ten seeds per replicate) were used for the experiment, which was repeated once. Resulting plants were maintained in a growth chamber with a 12 h photoperiod at 24°C for 7 weeks. Common root rot symptoms were assessed using the sub-crown internode index system (Ledingham *et al*., 1973); where 1 = no discolouration (clean), 2 = slight discolouration, 3 = extended linear lesions not surrounding the circumference (moderate), and 4 = discolouration of at least 50% and surrounding the circumference of the sub-crown internode (severe). Disease severity scores were analysed with analysis of variance, followed by Fisher's least significant difference test (LSD) at $P = 0.05$ using the Statistical Analysis System (SAS Version 9.0; SAS Institute Inc.).

DNA extraction from isolates

A modified version of the CTAB-based method, as described in the DArT protocol (http://www.diversityarrays.com), was used to extract genomic DNA from isolates. Approx. 100 mg of mycelia and conidia of each isolate were harvested by gently scraping the surface of respective cultures and was transferred into a 1.5 mL microfuge tube. Preheated (65°C) extraction–lysis buffer (750 µL; 125 mM Tris-HCl pH 8.0, 25 mM EDTA pH 8.0, 2% CTAB, 2% PVP-40, 0.8 M NaCl, 0.5% sodium disulfite, and 1% sarcosyl) was added to each tube. The sample was then uniformly homogenized by a micropestle and then incubated at 65°C for 60 min, shaken every 15 min. 750 µL of chloroform/isoamyl alcohol (24:1 v/v) was then added, mixed gently for 10 min and then centrifuged at 12,000 g for 15 min at 4°C. The supernatant was transferred to a clean centrifuge tube and 0.6 volume of isopropanol added to precipitate DNA. After centrifugation at 12,000 g for 5 min at room temperature, the supernatant was discarded. The pellet was washed with 70% ethanol (v/v) and then dried at room temperature. DNA was dissolved in 200 µL of sterile ultra-pure water. The concentration of DNA in each extraction sample was measured by spectrophotometer (DS-11 FX series, Denovix Inc.) and adjusted to 50 ng μL^{-1} for PCR assays.

ITS and GPDH amplification and sequencing

PCR amplifications were carried out in an Arktik Thermal Cycler (Thermo Scientific). PCR reactions were conducted in a 50 µL reaction mixture containing 5 µL of 10× PCR reaction buffer, 0.4 µM of each primer, 50 ng template DNA, 0.2 mM of each dNTPs, and 1.25 unit *Taq* DNA Polymerase (New England BioLabs). To amplify the ITS loci of genomic DNA, the primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White *et al*., 1990) were used. To amplify the GPDH loci, the gpd1 (5'-CAA CGG CTT CGG TCG CAT TG-3') and gpd2 (5'-GCC AAG CAG TTG GTT GTG-3') primers were used (Berbee *et al*., 1999). The amplification protocol consisted of a 3 min initial denaturation step at 94°C and a 10 min final extension at 72°C, followed by 35 cycles for 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C for the ITS, and with 1 min at 94°C, 1 min at 52°C and 45s at 72°C for the GPDH gene amplification. The amplified DNA fragments were purified with the Wizard SV Gel and PCR Clean-Up System (Promega) according to the manufacturer instructions and were subjected to bidirectional direct sequencing by a commercial company (Macrogen Inc.).

Genetic diversity evaluations using iPBS and ISSR markers

To evaluate genetic diversity among the isolates, genomic DNA was subjected to PCR amplification of 83 iPBS primers designed by Kalendar *et al*. (2010) and 20 ISSR markers including dinucleotide or trinucleotide repeats. Annealing temperatures for iPBS markers were performed as recommended, and ISSR markers were optimized depending on G+C contents of the primers. Five isolates with different morphological structures were screened with ISSR + iPBS markers to assess the ability of the markers to produce polymorphic and clear banding profiles. Five of ISSR markers and six of iPBS markers (Table 2) were selected to screen intra- and interspecific polymorphisms among all isolates. ISSR-PCR amplifications were carried out in a 25 µL reaction mixture containing 2.5 µL of 10× PCR reaction buffer, 0.24 µM of each primer, 50 ng template DNA, 200 µm of each dNTPs, and 0.8 unit *Taq* DNA Polymerase (New England BioLabs). The amplification was performed as follows: 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at an annealing temperature (between 52-54°C depending upon primers), and 2 min at 72°C, ending with one cycle of 10 min at 72°C. iPBS amplifications were conducted with Dream *Taq* DNA polymerase (Thermo Scientific) and *Pfu* DNA polymerase (Thermo Scientific) according to the protocols of Kalendar *et al*. (2010). Amplification products were subjected to electrophoresis in 1.4% (w/v) agarose gel over 1.5 h, and were visualized

Table 2. ISSR and iPBS markers used in this study and their characteristics.

Primers	Primer sequences (5'–3')[a]	T (°C)	GC (%)	TB[b]	PB[b]	PPB (%)[b]	PIC[b]	RP[b]
ISSR-SD2	ACACACACACACACACACRY	54	50	23	22	95.65	0.28	8.86
ISSR-SD3	GAGAGAGAGAGAGAGAGARY	54	50	21	21	100.00	0.27	7.93
ISSR-HR7	GAGAGAGAGAGAGAGAYT	52	47.2	16	16	100.00	0.32	4.36
ISSR-HR10	ACACACACACACACACYT	52	47.2	10	10	100.00	0.36	9.14
ISSR-HR13	BHBGAGAGAGAGAGAGA	52	51	21	21	100.00	0.29	8.50
Total				91	90			
	Average per primer			18.20	18.00	99.13	0.31	7.76
iPBS2395	TCCCCAGCGGAGTCGCCA	53	72.2	25	25	100.00	0.31	10.07
iPBS2077	CTCACGATGCCA	55	58.3	17	16	94.12	0.28	6.21
iPBS2219	GAACTTATGCCGATACCA	53	44.4	14	14	100.00	0.26	4.64
iPBS2393	TACGGTACGCCA	51	58.3	13	13	100.00	0.38	6.86
iPBS2389	ACATCCTTCCCA	50	50	13	13	100.00	0.31	5.50
iPBS2277	GGCGATGATACCA	52	53.8	13	13	100.00	0.29	5.07
Total				95	94			
	Average per primer			15.83	15.67	99.08	0.30	7.08

[a] Y, Pyrimidine; R, Purine; B, C, G or T and H, A, C, or T.
[b] T (°C) annealing temperature; TB, total band; PB, polymorphic band; PPB (%), percentage polymorphic band (%); PIC, polymorphism information content; RP, resolving power..

using a G:Box F3 Gel Documentation System (Syngene) after ethidium bromide staining.

Data analyses

The sequences were aligned with the ClustalW multiple sequence alignment method (Thompson *et al*., 1994). Phylogenetic analyses of the isolates, and reference isolates of *B. sorokiniana*, *C. spicifera*, and *C. inaequalis* available in the GenBank database, were performed with MEGA X software (Kumar *et al*., 2018) for combined datasets of the ITS region and GPDH gene. The neighbour-joining tree was constructed using the Tamura and Nei (1993) model, with 1,000 bootstrap replicates. The sequence of *Pyrenophora tritici-repentis* strain 200899 was included as an outgroup to root the phylogenetic tree.

All bands obtained by ISSR and iPBS analyses were scored as present (1) or absent (0) at positions to construct a binary data matrix. Each primer-sample combination was repeated in at least two different PCR amplifications, and only reproducible bands were evaluated. To evaluate the suitability of ISSR and iPBS markers to analyse genetic profiles of the isolates, the performance of the markers was measured using the polymorphic information content (PIC; Rolden-Ruiz *et al*., 2000) and resolving power (RP; Prevost and Wilkinson, 1999).

The data matrixes produced from each marker system were converted into a genetic similarity matrix, individually and in combination, using Jaccard's similarity coefficient with the NTSYS-pc numerical taxonomy package, version 2.02 (Rohlf, 2000). The unweighted pair-grouped method by arithmetic average (UPGMA) cluster analyses were used to construct a dendrogram. For each of the dendrograms obtained from the ISSR, iPBS and ISSR + iPBS combination data, a cophenetic matrix was generated. To check the goodness of fit of ISSR and iPBS markers, the Mantel significance test was used to calculate the correlation between the similarity coefficients matrix and the dendrogram, and compare each pair of the similarity matrices, using the MxComp Module of NTSYS 2.02 (Rohlf, 2000).

Polymorphisms among and within populations were analysed with Nei's gene diversity, Shannon's information index, degree of genetic differentiation and estimation of the amount of gene flow between populations, calculated using the Popgene statistical software ver. 1.32 (Yeh *et al*., 1999). All isolates belonging to one species were considered as one population. Further, evaluation of population genetic structure with data matrix was performed using the software Structure 2.3.4 with the Bayesian model-based clustering program (Pritchard *et al*., 2000). Structure Harvester (Earl and Vonholdt, 2012) was used to evaluate the level of genetic stratification for collating results generated by the program STRUCTURE.

RESULTS

Fungus isolates and pathogenicity assessments

A total of 106 *Cochliobolus* anamorph isolates were obtained from symptomatic wheat samples collected from 76 fields in the winter wheat growing regions of Azerbaijan in 2017. Species identification based on morphological and molecular tools showed that 96 isolates (from 19 fields) were *B. sorokiniana*, 14 isolates from six fields were *C. spicifera*, and six isolates were *C. inaequalis*. *Bipolaris sorokiniana* isolates were the most prevalent (19 of 28; 68%), followed by *C. spicifera* (six of 28; 21%) and *C. inaequalis* (3 of 28; 11%). One isolate from each of these species in the same field was selected for further studies.

The isolates were identified as *B. sorokiniana*, *C. spicifera*, and *C. inaequalis*. Mean conidium length and width dimensions (30 measurements) for these species were: *B. sorokiniana*, 76.1 (±7.2) µm × 21 (±2.4) µm; *C. spicifera,* 28.8 (±3) µm × 9.8 (±1) µm; and *C. inaequalis* 33.7 (±3.6) µm × 13.4 (±1.9) µm (Table 1).

All isolates of *B. sorokiniana* caused typical symptoms on wheat seedlings, including necrosis and discolouration of plant crowns and seminal roots, seedling blight and stunted seedlings. Discolouration of crowns and seminal roots was observed on the seedlings inoculated with *C. spicifera* isolates, but these symptoms were less severe than those induced by isolates of *B. sorokiniana*. Isolates of *C. inaequalis* caused slight discolouration of some crowns and seminal roots of the inoculated wheat seedlings. Mean disease severity scores ranged from 1.58 to 3.60 for all isolates, and differences (P = 0.05) were detected in aggressiveness among the isolates (Table 1). The mean disease severity scores were 3.2 for *B. sorokiniana* isolates, 2.2 for *C. spicifera* isolates and 1.7 observed for *C. inaequalis* isolates. No disease symptoms developed on control wheat seedlings.

Sequence alignment and phylogenetic analyses

PCR amplification of the ITS region of rDNA and GPDH region for all isolates produced DNA fragments ranging in size from 557-591 bp for the ITS region and 530-594 bp for the GPDH region. BLASTn queries based on the ITS and GPDH regions indicated that the sequences of all isolates were 99–100% identical to those of the species in the GenBank database. All obtained sequences were deposited in GenBank with the accession numbers listed in Table 1. Phylogenetic analyses based on a combined dataset of the ITS and GPDH sequences of isolates obtained from this study and reference sequences of the species available in the GenBank indicated that the isolates belonging to the different species were clearly separated from each other, and from *Pyrenophora tritici-repentis* strain 200899, with a bootstrap support of 100% (Figure 1).

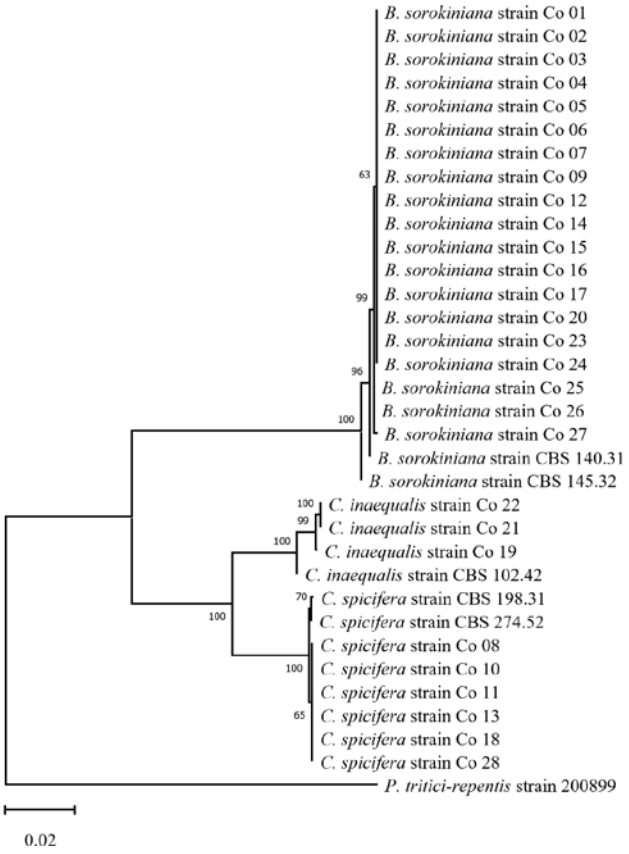

Figure 1. The neighbour-joining tree generated using Tamura and Nei (1993) for analyses of combined datasets of the ITS and GPDH sequences of 28 isolates from the present study, and for reference isolates. The phylogenetic tree was rooted with the *Pyrenophora tritici-repentis* reference strain. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to each branch.

iPBS and ISSR-PCR analyses

In total, 186 loci were obtained from PCR amplifications with five ISSR and six iPBS markers for 28 isolates. Both marker systems generated very distinct fragments, providing considerable variability between the isolates belonging to the different fungal species (Figure 2). A total of 91 fragments identified from ISSR-PCR were scored, out of which 90 (99%) were polymorphic (Table 2). Ninety-five reproducible fragments from iPBS PCR were scored, out of which 94 (99%) were polymorphic.

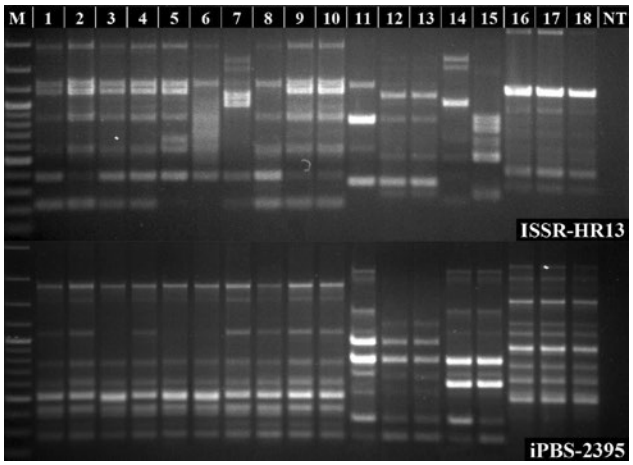

Figure 2. The representative of reproducible gel profiles with ISSR-HR13 and iPBS2395 primers. Lanes 1-10, *Bipolaris sorokiniana*; lanes 11-15, *Curvularia spicifera*; lanes 16-18, *Curvularia inaequalis*. Lane M is the DNA marker GeneRuler 100 bp plus (Thermo Scientific).

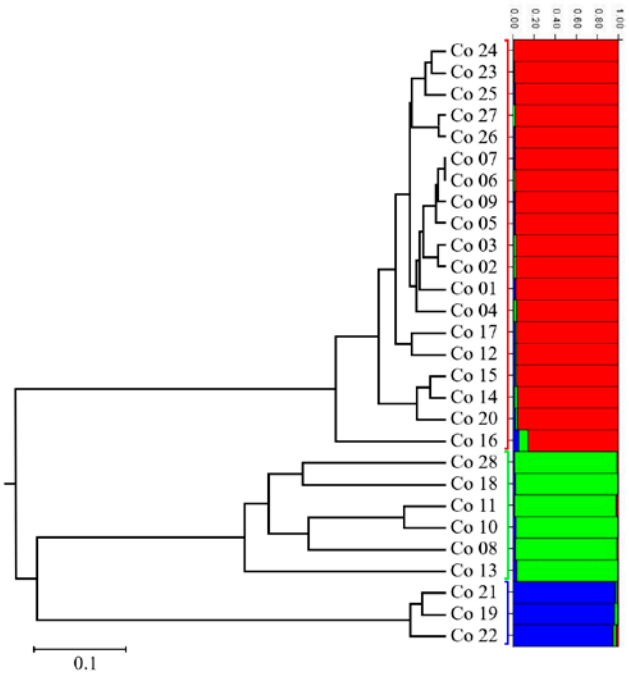

Figure 3. UPGMA tree based on the ISSR and iPBS combined marker data for 28 isolates, and STRUCTURE bar plots for individual assignment results for K = 3.

The number of amplified fragments with ISSR primers ranged from 10 (ISSR-HR7) to 23 (ISSR-SD2), giving a ratio of 18.2 bands per primer, while with iPBS primers ranged from 13 (iPBS2393; iPBS2389 and iPBS2277) to 25 (iPBS2395) with 15.83 fragments per primer.

PIC and RP indices were calculated for the two marker systems and are presented in Table 2. The greatest PIC index of 0.36 (ISSR-HR10) and the least PIC index 0.27 (ISSR-SD3), with an average PIC per primer of 0.31, were obtained from ISSR primers. For iPBS primers, PIC indices ranged from 0.26 (iPBS2219) to 0.38 (iPBS2393), with an average of 0.30. The means of RP indices, a parameter for the discriminatory potential of the primers, were 7.76 for ISSR primers and 7.08 for iPBS primers. Primer iPBS2395 produced the greatest RP value (10.07), while the ISSR-HR7 primer yielded the least RP index of 4.36.

The isolates on dendrograms from UPGMA cluster analyses of ISSR + iPBS combination data (Figure 3) grouped into three major clusters, which comprised the isolates of each particular species based on genetic similarity. Geographical-based clustering for the isolates did not occur in the phylogenetic tree. The cophenetic correlation values were 0.995 for ISSR data, 0.968 for iPBS data and 0.998 for ISSR + iPBS combination data. The correlation value between cophenetic matrix values obtained from these analyses was $r = 0.967$, suggesting very close similarity between ISSR and iPBS dendrograms.

The values of the genetic parameters were calculated with each dataset from ISSR, iPBS and ISSR + iPBS, including observed number of alleles (N_a), effective number of alleles (N_e), Nei's gene diversity (h) and Shannon's information index (I), to provide a detailed assessment of the distribution of genetic variation among populations that shared close similarities (Table 3). The value of h among the species was determined from the ISSR markers (0.30), which was similar to values detected by the iPBS and ISSR + iPBS data. The average values of G_{st} and N_m were 0.52 and 0.46 for ISSR, 0.67 and 0.25 for iPBS, and 0.60 and 0.34 for ISSR + iPBS. The value of genetic variation among the populations (G_{st}) was greater in iPBS (0.67) than ISSR (0.52), but the estimate

Table 3. Summary of genetic diversity indices analysed in populations of isolates.

Marker		Genetic diversity index[a]					
		N_a	N_e	h	I	G_{st}	N_m
ISSR	Mean	1.99	1.48	0.30	0.46	0.52	0.46
	St. Dev	0.10	0.30	0.14	0.17		
iPBS	Mean	1.99	1.48	0.30	0.47	0.67	0.25
	St. Dev	0.10	0.25	0.12	0.15		
iPBS + ISSR	Mean	1.99	1.48	0.30	0.47	0.60	0.34
	St. Dev	0.10	0.28	0.13	0.16		

[a] N_a, observed number of alleles; N_e, effective number of alleles; h, Nei's (1973) gene diversity; I, Shannon's Information index; G_{st}, degree of genetic differentiation; N_m, estimate of gene flow.

Table 4. Genetic variation values for three fungal species, from ISSR and iPBS markers.

Species	NI[a]	Marker	Value[a]				
			TB	PB	PPB (%)	h	I
Bipolaris sorokiniana	19	ISSR	47	30	63.83	0.147	0.240
		iPBS	37	2	5.40	0.015	0.024
Curvularia spicifera	6	ISSR	47	37	78.72	0.316	0.462
		iPBS	40	15	37.50	0.151	0.221
Curvularia inaequalis	3	ISSR	25	2	8.00	0.035	0.050
		iPBS	35	4	11.43	0.050	0.073

[a] NI, number of isolates; TB, total band; PB, polymorphic band; PPB (%), percentage polymorphic band; h, Nei's (1973) gene diversity; I, Shannon's Information index.

of gene flow among the populations (N_m) was more in ISSR (0.46) than iPBS (0.25). To reveal the genetic variability within populations, the ISSR markers provided a greater percentage of polymorphic bands, *h*, and *I* value, than those of the iPBS markers for *B. sorokiniana* and *C. spicifera*. In comparison, relatively high values of these genetic diversity parameters were obtained for *C. inaequalis* with the iPBS marker system (Table 4).

Analyses by STRUCTURE indicated that the isolates distinctly clustered according to their respective taxonomic groups, and the 28 isolates were in three clusters (Figure 3). The result of the harvester structure analyses showed that the best dataset number for the three populations was K = 3, providing the greatest mean value (-893,8) of Ln likelihood for the data.

DISCUSSION

Species of *Bipolaris* and *Curvularia*, which are the anamorphs of *Cochliobolus* species, are important plant pathogens of Poaceae host plants. Disease symptoms caused by these pathogens include leaf spots, blight, root rot, and crown rots (Sivanesan, 1987; Berbee *et al*., 1999; Manamgoda *et al*., 2014). The present study focused on isolates of *Bipolaris* and *Curvularia* species obtained from wheat afflicted with root and crown rot in Azerbaijan.

Twenty-eight isolates from diseased wheat plants were separated into three groups using morphological and molecular characterization methods. These were *B. sorokiniana* (19 isolates), *C. spicifera* (six isolates) and *C. inaequalis* (three isolates), as presented in Table 1. This is the first report of *C. spicifera* and *C. inaequalis* causing root and crown rot on wheat in Azerbaijan. *Bipolaris sorokiniana* was the predominant species occurring on crown and root tissues of wheat and was detected in 25% of the fields surveyed. This confirms previous observations, that this fungus has been the most frequently isolated from crown and root tissues of winter wheat (Fedel-Moen and Harris, 1987; Chen *et al*., 1996).

Comparison of the conidium sizes of the three species examined in this study was consistent with results from previous studies conducted by Sivanesan (1987), Koo *et al*. (2003), and Morejon *et al*. (2006). The conidium characteristics were useful criteria for the discrimination of *B. sorokiniana* isolates from other isolates, but could not be used for the differentiation between *C. spicifera* and *C. inaequalis* due to their morphological similarities. Previous studies have recommended using molecular tools to distinguish these species, due to inadequate differentiation using morphological characters (Berbee *et al*., 1999; Marin-Felix *et al*., 2017). The multi-sequence analyses of ITS and GPDH sequences have been suggested to identify and differentiate the species in *Bipolaris* and *Curvularia* (Manamgoda *et al*., 2012, 2014, 2015). The phylogenetic tree constructed in the present study, based on the ITS and GPDH gene of isolates, distinctly separated all the isolates into three clusters according to their taxonomic groups.

Pathogenicity tests demonstrated that all the isolates (all three species) were pathogenic to wheat but at a different level of pathogenicity. The study conducted by Ghazvini and Tekauz (2007) also reported no host specificity amongst similar fungi. However, pathogenicity comparisons, among these species showed that the *B. sorokiniana* isolates produced more severe symptoms on wheat seedlings than *C. spicifera* or *C. inaequalis* isolates. Similarly, Gonzalez and Trevathan (2000) showed that isolates of *C. spicifera* were virulent on wheat, causing discolouration of the seedling crowns and seminal roots, but these symptoms were less severe than those produced by *B. sorokiniana* isolates. Following identification and the pathogenicity test, *B. sorokiniana* was here shown to be the most aggressive of the three species associated with wheat, as well as the most widespread in Azerbaijan. This species has been reported as the most common fungus associated with root and crown rot of wheat in Turkey, the North China Plain, and Canada (Ledingham *et al*., 1973; Tunali *et al*., 2008; Xu *et al*., 2018). Statistically significant differences in virulence of the isolates belonging to each species were found in the present study. These could be explained by genetic differences between the isolates. Ghazvini and Tekauz (2012) found a close association between genetic diversity and virulence of *B. sorokiniana* on barley.

The possibility of genetic polymorphism among isolates was investigated with iPBS and ISSR markers. iPBS

markers have been successfully used to evaluate genetic diversity in fungi since this was discovered in 2010 by Kalendar *et al.* (Özer *et al.*, 2016; Özer and Bayraktar, 2018; Wu *et al.*, 2019; Ates *et al.*, 2019). Similarly, ISSR markers have been more extensively used for the analyses of different fungal populations (Bayraktar and Dolar, 2009; Altınok *et al.*, 2018). iPBS markers have not previously been employed for studying genetic diversity in these genera. In the present study, each of the UPGMA cluster analyses from the genetic dissimilarity matrices derived from ISSR, iPBS and combined ISSR + iPBS data provided clear differentiation among three anamorph species of *Cochliobolus* (Figure 3). The harvester structure analyses showed that the best dataset number for the three populations was K = 3, at which population of 28 individuals was divided into three clusters similar to the dendrogram. No apparent relationship, however, was obtained between the clustering and geographic origins of the isolates. This result is similar to those of Arabi and Jawhar (2014) and Knight *et al.* (2010). Although marker systems iPBS, based on retrotransposons, and ISSR, based on microsatellite repeat regions, differ in the nature of the respective evolutionary mechanisms underlying changes and distribution in the genome of the fungi, the highly significant correlation coefficient (r = 0.967) was determined between the co-phenetic matrices based on the genetic distance for ISSR and iPBS data. The high cophenetic correlation values indicate that both molecular markers were powerful tools for analysing interspecific variability among the isolates. The values of percentage polymorphic bands, polymorphism information content, and resolving power in these molecular marker systems were almost similar, which was in accordance with similar studies on *Lens* species (Baloch *et al.*, 2015). In all the isolates, the G_{st} and N_m values were, respectively, 0.67 and 0.25 for iPBS markers and 0.52 and 0.46 for ISSR markers. This showed that the majority of genetic variation obtained from these marker systems, especially the iPBS markers, resulted from differences among the species, not within the species.

To evaluate genetic variability among *B. sorokiniana* isolates from Azerbaijan, ISSR markers provided greater resolution, and were marginally more informative, than the iPBS marker system, with the values of 63.83, 0.147 and 0.24, respectively, for percentage polymorphic bands, *h* and *I* analyses. The mean of polymorphic bands (PB) produced by ISSR markers was 6.0 with the nineteen *B. sorokiniana* isolates, while Yadav *et al.* (2013) found 3.4 PB per RAPD marker with Indian isolates, and an average of 6.1 PB was produced by AFLP markers with Australian isolates (Mann *et al.*, 2014) and 11.9 PB with the isolates obtained from seven countries including the United States of America, Canada, Brazil, Poland, China, Uruguay, and Japan (Zhong and Steffenson, 2001). Similarly, the ISSR markers provided more detailed information to investigate polymorphisms among the isolates of *C. spicifera*, while iPBS markers were slightly better for isolates of *C. inaequalis*.

In the present study, high levels of interspecific variation among *Bipolaris* and *Curvularia* isolates was detected by the pathogenicity tests, sequencing of ITS and GPDH regions, and the ISSR and iPBS marker systems. These results confirmed that the sequencing of the ITS and GPDH regions could provide useful information for the identification and discrimination of the species used in this research. Comparisons of the information with marker systems revealed that ISSR markers showed highly informative markers for investigating genetic polymorphisms among the pathogen populations causing root rot disease on wheat. This study presented, for the first time, molecular data for *Cochliobolus* anamorphs of associated with common root rot of wheat using iPBS markers. The high polymorphism rate at the interspecies level, derived using ISSR and iPBS markers either separately or together, provided valuable information for species identification, and for elucidating evolutionary relationships among the pathogen species without any sequence data.

ACKNOWLEDGEMENTS

The authors thank the Food and Agriculture Organization of the United Nations, CIMMYT and Research and Development Unit (BAP) of Bolu Abant Izzet Baysal University (Project no: 2018.10.06.1392) for funding this study. The tremendous efforts are appreciated of Mr. Vugar Bashirov (National Project Management Consultant, FAO, Azerbaijan) during the project period. Colleagues are also thanked for their support during the project, especially Mr. Agil Gasimov, Mr. Jamal Guliyev, Mr. Taleh Shamiyev, Ms. Gular Ahmadova, and Ms. Sabina Khalilova. Colleagues in the survey regions provided invaluable support, particularly Mr. Talat Mehdiyev, Mr. Movsum Huseinov, Mr. Nazim Bakhshaliev, Mr. Asadov Akif, Mr. Javanshir Talai, Ms. Konul Aslanova, and Mr. Atabay Jahangirov.

LITERATURE CITED

Altinok H.H., Can C., Altinok M.A., 2018. Characterization of *Fusarium oxysporum* f. sp. *melongenae* isolates from Turkey with ISSR markers and DNA sequence

analyses. *European Journal of Plant Pathology* 150: 609–621.

Arabi M.I.E., Jawhar M., 2007. Molecular and pathogenic variation identified among isolates of *Cochliobolus sativus*. *Australasian Plant Pathology* 36: 17–21.

Arabi M.I.E., Jawhar M., 2014. Cultural and genetic evaluation of *Cochliobolus sativus* during successive passages through susceptible barley. *Advances in Horticultural Science* 28: 119–122

Ates D., Altinok H.H., Ozkuru E., Ferik F., Erdogmus S., Can C., Tanyolac M.B., 2019. Population structure and linkage disequilibrium in a large collection of *Fusarium oxysporum* strains analysed through iPBS markers. *Journal of Phytopathology* 167: 576–590.

Bach E.E., Barros B.C., Kimati H., 2003. Induced resistance against *Bipolaris bicolor*, *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in wheat leaves by xanthan gum and heat-inactivated conidial suspension. *Journal of Phytopathology* 151: 411–418.

Bakonyi J., Aponyi I., Fischl G., 1997. Diseases caused by *Bipolaris sorokiniana* and *Drechslera tritici repentis* in Hungary. In: *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot* (E. Duveiller, H.J. Dubin, J. Reeves, A. McNab, eds.) CIMMYT, DF, Mexico, 80–85.

Baloch F.S., Derya M., Andeden E.E., Alsaleh A., Cömertpay G., Kilian B., Özkan H., 2015. Inter-primer binding site retrotransposon and inter-simple sequence repeat diversity among wild *Lens* species. *Biochemical Systematics and Ecology* 58: 162–168.

Baturo-Ciesniewska A., 2011. Genetic variability and pathogenicity among Polish isolates of *Bipolaris sorokiniana* from spring barley. *Journal of Plant Pathology* 93: 291–302.

Bayraktar H., Dolar F.S., 2009. Genetic diversity of wilt and root rot pathogens of chickpea, as assessed by RAPD and ISSR. *Turkish Journal of Agriculture and Forestry* 33: 1-10.

Berbee M.L., Pirseyedi M., Hubbard S., 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia* 91: 964–977.

Bornet B., Branchard M., 2001. Nonanchored inter simple sequence repeat ISSR markers: reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter* 19: 209–215.

Chen C., Collins D. J., Morgan-Jones G., 1996. Fungi associated with root rot of winter wheat in Alabama. *Journal of Phytopathology* 144: 193–196.

Duczek L.J., Verma P.R., Spurr D.T., 1985. Effect of inoculum density of *Cochliobolus sativus* on common root rot of wheat and barley. *Canadian Journal of Plant Pathology* 7: 382–386.

Duveiller E., Garcia Altamirano I., 2000. Pathogenicity of *Bipolaris sorokiniana* isolates from wheat roots, leaves and grains in Mexico. *Plant Pathology* 49: 235–242.

Earl D.A., Vonholdt B.M., 2012. Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361.

Fedel-Moen R., Harris J.R., 1987. Stratified distribution of *Fusarium* and *Bipolaris* on wheat and barley with dryland root rot in South Australia. *Plant Pathology* 36: 447–454.

Ghazvini H., Tekauz A., 2007. Virulence diversity in the population of *Bipolaris sorokiniana*. *Plant Disease* 91: 814–821.

Ghazvini H., Tekauz A., 2012. Molecular diversity in the barley pathogen *Bipolaris sorokiniana* (*Cochliobolus sativus*). *Australasian Plant Pathology* 41: 283–293.

Gonzalez M.S., Trevathan L.E., 2000. Identity and pathogenicity of fungi associated with root and crown rot of soft red winter wheat grown on the upper coastal plain land resource area of Mississippi. *Journal of Phytopathology* 148: 77–85.

Kalendar, R. Antonius, K. Smýkal, P. Schulman, A.H. 2010. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. *Theoretical and Applied Genetics* 121: 1419–1430.

Knight N.L., Platz G.J., Lehmensiek A., Sutherland M.W., 2010. An investigation of genetic variation among Australian isolates of *Bipolaris sorokiniana* from different cereal tissues and comparison of their abilities to cause spot blotch on barley. *Australasian Plant Pathology* 39: 207–216.

Koo H.M., Lee S.H., Jung I.M., Chun S.C., 2003. A seedborne fungus *Bipolaris spicifera* detected from imported grass seeds. *The Plant Pathology Journal* 19: 133–137.

Kumar S., Stecher G., Li, M., Knyaz C., Tamura K., 2018. MEGA X: molecular evolutionary genetics analyses across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549.

Ledingham R.J., Atkinson T.G., Horricks J.S., Mills, J.T., Piening L.J., Tinline R.D., 1973. Wheat losses due to common root rot in the prairie provinces of Canada, 1969–71. *Canadian Plant Disease Survey* 53: 113–122.

Manamgoda D.S., Cai L., McKenzie E.H.C., Crous P.W., Madrid H., … Hyde K.D., 2012. A phylogenetic and taxonomic re-evaluation of the *Bipolaris - Cochliobolus - Curvularia* Complex. *Fungal Diversity* 56: 131–144.

Manamgoda D.S., Rossman A.Y., Castlebury L.A., Crous P.W., Madrid H., Chukeatirote E., Hyde K.D., 2014. The genus *Bipolaris*. *Studies in Mycology* 79: 221–288.

Manamgoda D.S., Rossman A.Y., Castlebury L.A., Chukeatirote E., Hyde K.D., 2015. A taxonomic and phylogenetic re-appraisal of the genus *Curvularia* Pleosporaceae: human and plant pathogens. *Phytotaxa* 212: 175–198.

Mann M.B., Minotto E., Feltrin T., Milagre L.P., Spadari C., Van Der Sand S.T., 2014 Genetic diversity among monoconidial and polyconidial isolates of *Bipolaris sorokiniana*. *Current Microbiology* 69: 874–879.

Marin-Felix Y., Senwanna C., Cheewangkoon R., Crous P.W., 2017. New species and records of *Bipolaris* and *Curvularia* from Thailand. *Mycosphere* 8: 1556–1574.

Morejon K.R., Moraes M.H.D., Bach E.E., 2006. Identification of *Bipolaris bicolor* and *Bipolaris sorokiniana* on wheat seeds *Triticum aestivum* L. in Brazil. *Brazilian Journal of Microbiology* 37: 247–250.

Müller M.V.G., Germani J.C., Van Der Sand S.T., 2005. The use of RAPD to characterize *Bipolaris sorokiniana* isolates. *Genetics and Molecular Research* 4: 642–652.

Nei M., 1973. Analysis of Gene Diversity in Subdivided Populations. *PNAS* 70: 3321–3323;

Özer G., Bayraktar H., Baloch F.S., 2016. iPBS retrotransposons 'A Universal Retrotransposons' now in molecular phylogeny of fungal pathogens. *Biochemical Systematics and Ecology* 68: 142–147.

Özer G., Bayraktar H., 2018. Genetic diversity of *Fusarium oxysporum* f. sp. *cumini* isolates analyzed by vegetative compatibility, sequences analyses of the rDNA IGS region and iPBS retrotransposon markers. *Journal of Plant Pathology* 100: 225–232.

Pritchard J.K., Stephens M., Rosenberg N.A., Donnelly P., 2000. Association mapping in structured populations. *The American Journal of Human Genetics* 67: 170–181.

Prevost A., Wilkinson M.J., 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics* 98: 107–112.

Rohlf F.J., 2000. NTSYS-pc, numerical taxonomy and multivariate analyses system, vol v. 2.1. Exceter Software, New York.

Roldàn-Ruiz I., Dendauw J., Van Bockstaele E., Depicker A., De Loose M.A.F.L.P., 2000. AFLP markers reveal high polymorphic rates in ryegrasses *Lolium* spp. *Molecular Breeding* 6: 125–134.

Safaee D., Okhovat S.M., Hedjaroude G.H., Younesi H., 2008. Diagnosis of *Bipolaris* form species involving root and crown rot of wheat, their distribution and disease severity in Kermanshah Province. *Journal of Science and Technology of Agriculture and Natural Resources* 12: 207–214.

Sivanesan A., 1987. Graminicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their teleomorphs. CAB International, Wallingford, UK, 261pp.

Šķipars V., Siaredzich M., Belevich V., Bruņeviča N., Brūna L., Ruņģis D.E., 2018. Genetic differentiation of *Phoma* sp. isolates using retrotransposon-based iPBS assays. *Environmental and Experimental Biology* 16: 307–314.

Tamura K., Nei M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526.

Thompson J.D., Higgins D.G., Gibson T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.

Tunali B., Nicol J.M., Hodson D., Uckun Z., Büyük O., Erdurmuş D., Bağci S.A., 2008 Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Disease* 92: 1299–1306.

Valjavec-Gratian M., Steffenson B.J., 1997. Genetics of virulence in *Cochliobolus sativus* and resistance in barley. *Phytopathology* 87: 1140–1143.

White T.J., Bruns T., Lee S.J.W.T., Taylor J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18: 315–322.

Wildermuth G.B., Tinline R.D., McNamara R.B., 1992. Assessment of yield loss caused by common root rot in wheat cultivars in Queensland. *Australian Journal of Agricultural Research* 43: 43–58.

Wu J., Xie X., Shi Y., Chai A., Wang Q., Li B., 2019. Analysis of pathogenic and genetic variability of *Corynespora cassiicola* based on iPBS retrotransposons. *Canadian Journal of Plant Pathology* 41: 76–86.

Xu F., Yang G., Wang J., Song Y., Liu L., Zhao K., Han Z., 2018. Spatial distribution of root and crown rot fungi associated with winter wheat in the North China Plain and its relationship with climate variables. *Frontiers in Microbiology* 9: 1054.

Yadav B., Singh R., Kumar A., 2013. Genetic variability and relationship analyses of *Bipolaris sorokiniana* isolates causing spot blotch disease in wheat using random amplified polymorphic DNA RAPD markers. *African Journal of Biotechnology* 12: 2526–2531.

Yeh F.C., Yang R.C., Boyle T., 1999. POPGENE version1. 32, Microsoft window-base software for population genetic analyses: a quick user's guide. University of

Alberta. Center for International Forestry Research, Alberta, Canada.
Zhong S., Steffenson B.J., 2001. Virulence and molecular diversity in *Cochliobolus sativus*. *Phytopathology* 91: 469–476.