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RESEARCH ARTICLE

INTRASPECIFIC DIVERSITY OF WHEAT TAN SPOT CAUSING BY *DRECHSLERA TRITICI-REPENTIS* IN GEORGIA

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ABSTRACT

Using the random amplified polymorphic DNA (RAPD) method low molecular diversity was detected among *D. tritici-repentis* isolates recovered from wheat leaves collected in different locations in Georgia. The most of isolates were of highly similar. Similarity index varied between 0.333 -0.944. Maximum index was 0.944 for isolates A1a and A1b, low index (0.167-0.444) was seen for 23 pairs. High degree of genetic similarity was found among isolates obtained from Dusheti region and among Sachkhere isolates. The dendrogram of PARD data contains two groups. The grouping by DNA polymorphism showed no association with the geographic origin of the isolates.

INTRODUCTION

The fungus *Pyrenophora tritici-repentis* (Died.) Drechs, the anamorphic phase *Drechslera tritici-repentis* (Died) Shoem, cause of tan spot of wheat, is an important foliar pathogen worldwide. The disease was first identified in the United States in 1940s, and since then its incidence and severity have steadily increased (Wiese, 1991; Wolf *et al*., 1998). In Georgia tan spot was noticed and the disease has been identified in 2000 (Gorgiladze *et al*., 2007). Analyses of the intraspecific variability of plant pathogens have been revolutionized by molecular methods. Particularly, the polymerase chain reaction (PCR) assay has provided a framework to understand population structure of pathogens. Random amplified polymorphic DNA (RAPD) method has been extensively used to analyze virulence variability, to identify polymorphisms and to investigate the genetic similarity between different strains in phytopathogenic fungi (Chen *et al.,* 1999; Oliveira *et al*., 2002). It is recognized that *D. tritici-repentis* presents enormous variability in its morphology, genome and pathogenicity. In this study we used a RAPD-PCR to determine the intraspecific diversity of *Drechslera tritici-repentis* isolates collected from different regions of Georgia.

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MATERIALS AND METHODS

To determine the genetic variability of *Drechslera tritici-repentis* eighteen single-spore isolates collected in 2009 and 2010 crop surveys from different regions (Dusheti, Akhaltsikhe, Sachkhere) of Georgia a RAPD assay were used. Pathogen isolates were obtained from same wheat variety Bezostaya 1. The fungal strains used in this study are listed in Table 1. Samplings, isolation of single-spore cultures and RAPD – PCR of *Drechslera tritici-repentis* were carried out according to international methods (Bottcher *et al*., 1987, Jordah and Francl, 1992, Williams *et al*., 1990). RAPD-PCR was conducted accordance with the following steps: DNA extraction from fungal samples; amplification of DNA of separate isolates, data analysis. For optimization of the amplification conditions and selection of the primers, the genomic DNA extracted from original samples was used as a template for the amplification. Twenty grams of mycelium was used for DNA isolation. DNA was extracted from each isolates by modification of the method of Ashktorab and Cohen (1992). Approximately 0.2µm of DNA was obtained from 20mg mycelium.

In total 60 primers (OPA-1 - 20, OPB-1 - 20, OPC-1 - 20) were tested. Twenty four random primers were chosen due to the consistent reproducibility of *Drechslera tritici-repentis* isolates amplification products. These primers were OPA-1, OPA-2,

OPA-3, OPA-4, OPA-5, OPA-7, OPA-8, OPA-9, OPA-10, OPA-11, OPA-12, OPA-13, OPA-15, OPA-17, OPA-18, OPA-19, OPA-20, OPB-4, OPB-5, OPB-12, OPB-11, OPC-20, OPC-18, OPC-19. Amplification with selected primers was conducted according to standard methodology in thermal cycler "Techne TC 412"for 40 cycles.

RESULTS AND DISCUSSION

The results of gel electrophoresis showed that twenty four primers produced the most fragments; however number of fragments was different depending on used primers.

Table 1. Isolates of *Drechslera tritici-repentis* used in RAPD-PCR

Designation of isolate	Origin of isolate	Designation of isolate	Origin of isolate
D1a	Dusheti, commercial field , 2009	D1b	Dusheti field commercial, 2010
D2a	Dusheti ,commercial field, 2009	D2b	Dusheti field commercial, 2010
D3a	Dusheti, commercial field, 2009	D3b	Dusheti field commercial, 2010
S1a	Sakhere, commercial field, 2009	S1b	Sakhere field commercial, 2010
S2a	Sachkhere, commercial field, 2009	S2b	Sachkhere field commercial, 2010
S3a	Sachkhere, commercial field, 2009	S3b	Sachkhere field commercial, 2010
A1a	Akhaltsikhe, field trial, 2009	A1b	Akhaltsikhe field trial, 2010
A2a	Akhaltsikhe field trial, 2009	A2b	Akhaltsikhe field trial, 2010
A3a	Akhaltsikhe field trial, 2009	A3b	Akhaltsikhe, field trial, 2010

Table 2. Similarity coefficients of *Drechslera tritici-repentis* isolates based on RAPD analysis

Analysing 18 variables x 18 cases

Tolerance of eigenanalysis set at 1E-007

Gower General Similarity Coefficient

Similarity matrix

	D 1a	D 1b	D 2a	D 2b	D 3a	D 3b	S 1a	S 1b	S 2a	S 2b	S 3a	S 3b	A 1a	A 1b	A 2a	A 2b	A 3a	A3b
D 1a	1,000																	
D 1b	0,611	1,000																
D 2a	0,722	0,333	1,000															
D 2b	0,556	0,611	0,611	1,000														
D 3a	0,667	0,389	0,833	0,667	1,000													
D 3b	0,611	0,667	0,333	0,611	0,389	1,000												
S 1a	0,722	0,444	0,778	0,500	0,722	0,444	1,000											
S 1b	0,667	0,500	0,611	0,778	0,556	0,611	0,500	1,000										
S 2a	0,611	0,444	0,778	0,500	0,722	0,444	0,778	0,611	1,000									
S 2b	0,500	0,556	0,556	0,611	0,611	0,556	0,667	0,611	0,778	1,000								
S 3a	0,611	0,556	0,667	0,500	0,722	0,444	0,667	0,500	0,778	0,778	1,000							
S 3b	0,667	0,611	0,500	0,556	0,667	0,611	0,611	0,556	0,500	0,611	0,611	1,000						
A 1a	0,722	0,556	0,667	0,500	0,611	0,556	0,778	0,611	0,778	0,778	0,889	0,611	1,000					
A 1b	0,667	0,500	0,611	0,444	0,556	0,500	0,722	0,556	0,722	0,722	0,833	0,667	0,944	1,000				
A 2a	0,778	0,611	0,611	0,556	0,667	0,722	0,611	0,667	0,611	0,611	0,722	0,778	0,833	0,778	1,000			
A 2b	0,667	0,500	0,611	0,444	0,444	0,500	0,611	0,556	0,611	0,722	0,722	0,556	0,833	0,889	0,667	1,000		
A 3a	0,667	0,500	0,611	0,444	0,444	0,389	0,611	0,667	0,611	0,611	0,722	0,444	0,833	0,778	0,667	0,778	1,000	
A3b	0,556	0,278	0,722	0,333	0,556	0,167	0,611	0,444	0,611	0,389	0,611	0,333	0,611	0,667	0,444	0,667	0,778	1,000
	D 1a	D 1b	D 2a	D 2b	D 3a	D 3b	S 1a	S 1b	S 2a	S 2b	S 3a	S 3b	A 1a	A 1b	A 2a	A 2b	A 3a	A3b

Amplification conditions including cycle's number, DNA and $Mgcl_2$ concentration have been optimized. All reactions were carried out in a volume of 25 µ with 2 µl DNA, **2** mmol/ of each dNTps; 0.6 ml of primers; 5xBuffer (5 ml), 2mm $Mgcl_2$, 1.5ml Taq DNA polymerase. The following program was used in experiments: 96°C 2min, 1min at 30°C, 1 min at 94°C, 72°C 2min and final extension of 7 min at 72°C. Amplification products were electrophorized in 1% agarose gel, detected by staining with ethidium bromide and shot in UV light. Amplification with each primer was repeated at least twice to ensure the consistency of the banding patterns. Amplified bands were scored visually according to their presence or absence in each isolate. The binary matrix was built pair-wise, and presence or absence of a determined RAPD band scored 1 and 0, respectively. Similarity coefficients were calculated (Sneach&Sokal, 1973). Cluster analysis was performed using the unweighted pair set (UPGMA) method. Construction of dendrogram and clustering was done by the computer package MVSP 3.1.

The each isolate had specific RAPD specters with different size, number of amplicons and degree of expressiveness on electrophore-logramm. Together with common fragments other ones were recorded, those which were presented in one and absent in others. The best bands were obtained by primers - OPA-04, OPA-05, OPA-10 and OPA18. A gel showing products from amplification by selected primers is illustrated in Fig. 1,2,3,4.

Primer OPA4 amplified 8 fragments; primer OPA5 also produced 8 fragments, while primers OPA18 and OPA10 generated 11 and 16 fragments, respectively. In total 18 polymorphic fragments ranging from 300bp to 5,000bp were amplified by four primers. Majority of produced bands was common for all analyzed isolates. A 5,000bp fragment amplified only from isolate D1B. A 300bp fragment was generated from isolates D3B, S2B and A2B. Also, 400bp and 4,000bp fragments were amplified in D2B, D3B, S1B and D1B, D2B, D3B isolates,

respectively. Some isolates (S1A, A2B) did not amplify with primer OPA-04 after many repetitions.

The simple matching similarity coefficients of *Drechslera tritici-repentis* isolates based on computer analysis given in Table 2.

As Table 2 shows, considerable polymorphism was found with a similarity coefficient below 50%. The most of isolates were of highly similar. Similarity index varied between 0.333 - 0.944. Maximum index was 0.944 for isolates A1a and A1b, low index (0.167-0.444) was seen for 23 pairs.

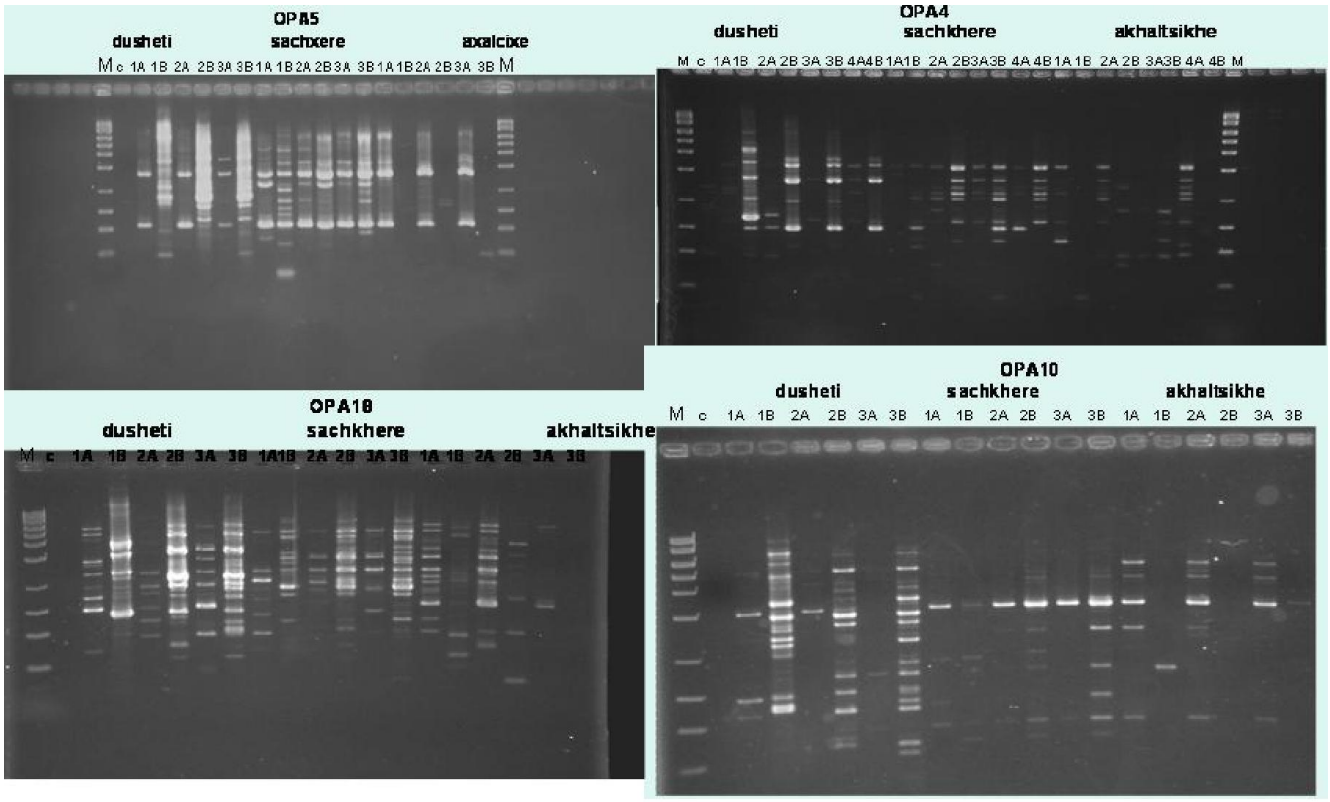

Fig. 1, 2, 3, 4. Amplification products of isolates of *Drechslera tritici-repentis* with primers OPA-04, OPA-05, OPA-10, OPA18. Line M is 1kb ladder DNA, c – negative control, lines 1A, 2A, 3A and 1B, 2B, 3B are isolates from Dusheti, Sachkhere and Akhaltsikhe regions collected in 2009 and 2010 growing seasons, respectively

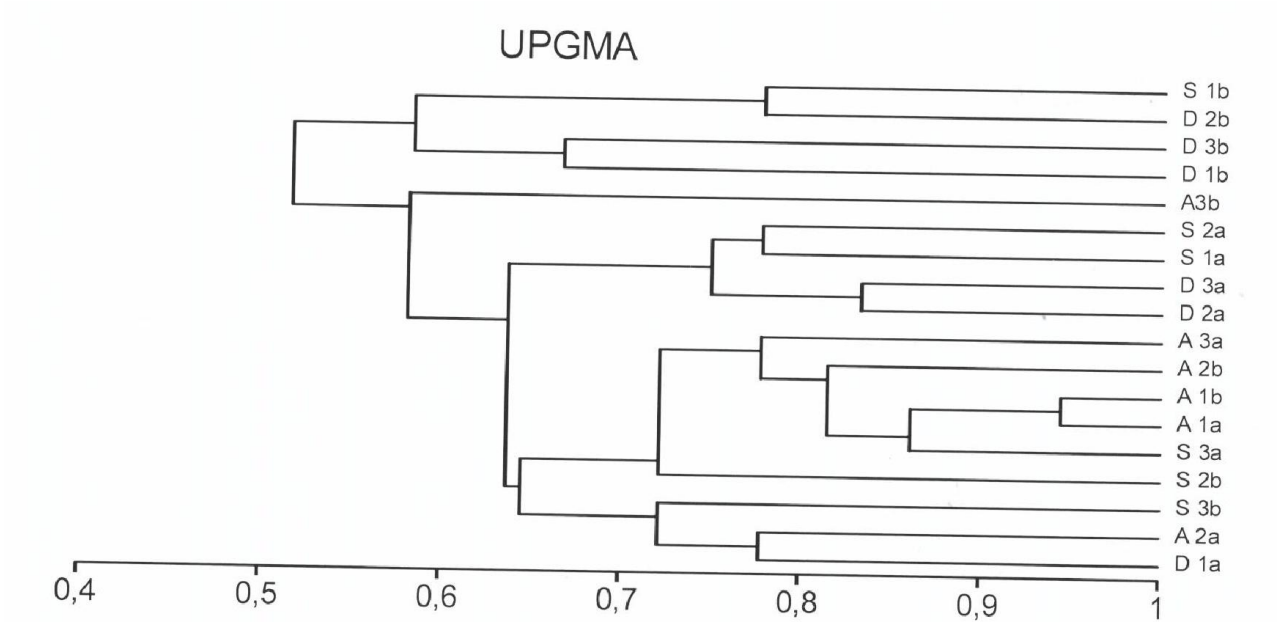

Fig.5. Dendrograms derived from banding patterns of RAPD analysis

High degree of genetic similarity was found among isolates obtained from Dusheti region and among Sachkhere isolates. Some difference was recorded among 2009 and 2010 collections. There was genetic variation among the isolates collected from Dusheti and Akhaltsikhe, Sachkhere and Akhaltsikhe populations. Low intraspecific variability was indicated among isolates from Dusheti and Sachkhere regions.

Figure 5 shows the dendrogram of PARD data and contains 2 groups. One group is formed by isolates A1a, A1b,A2a,D1a, S3a, A2b,A3a,D2a,D3a, S1a, S2a, S1b, D2b, D1b, D3b, D2b and S1b with isolates A1b and A1a showing the highest similarity coefficient (0.96). Isolate A3b stand alone as a separate group and has the lowest similarity coefficient (0.56). The grouping by DNA polymorphism showed no association with the geographic origin of the isolates. Our results are similar to research of Brazilian population of *Drechslera tritici-repentis* (Viera dos Santos *et al*., 2002).

As it is known the main source of the genetic variation in populations of plant fungal pathogens is mutation, gen recombination etc. A many environment factors (natural selection, plant host diversity, stable climatic conditions, sowing size) exert a strong selective pressure on plant pathogens (Burdon and Silk, 1997). Similarity of *p.tritici-repentis* isolates in different areas can explain that in Georgia wheat producing area is very small (~100 000ha) and wheat sowings is mainly occupied by old variety Bezostaya 1.

So, Using the RAPD technique we detected low genetic diversity among *D. tritici-repentis* isolates recovered from wheat leaves collected in different locations in Georgia. The results of the study are valuable for both theoretical understanding of the intraspecific structure and microevolution species *D. tritici-repentis* should be very useful benefit to specific breeding programs aimed at control of this pathogen.

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