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Genetic diversity of *Pyrenophora tritici-repentis* in Algeria as revealed by amplified fragement length polymorphism (AFLP) analysis

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Tan spot caused by *Pyrenophora tritici-repentis* is a major wheat disease. DNA of 61 isolates of *P. tritici-repentis* from different cereal growing areas in Algeria were analyzed using amplified fragment length polymorphism (AFLP) in order to study the genetic diversity among this population. Initially, 78 primer combinations were tested, of which 12 were selected and applied to the 61 isolates. There was a high genetic diversity in this population of *P. tritici-repentis* with 61 different haplotypes among the 61 isolates selected. The Jaccard similarity index range was 1.43 to 68.37%. Cluster analysis showed that, clustering of isolates was independent of their race classification, geographic origin, or host plant. However, one isolate (Ptr24) that showed a new virulence pattern in our previous race analysis study was clearly distinguished from the rest of the population studied. This isolate had not only new virulence but also different genetic makeup to other *P. tritici-repentis* isolates and requires additional studies to decipher complete knowledge of host-pathogen interactions for tan spot of wheat.

Key words: *Pyrenophora tritici-repentis*, genetic diversity, AFLP, Algeria.

INTRODUCTION

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph *Drechslera tritici-repentis*) is the causal agent of tan spot disease of wheat, which is found in major wheat growing areas worldwide. This disease is very destructive and can cause high yield losses. In Australia, yield losses of 49% were reported under conditions that favor disease development (Rees et al., 1982). More recently, Loughman et al. (1998) reported, losses of 23 to 50% in cultivars affected by *P. tritici-repentis* and *Septoria nodurum*. Tan spot caused potential yield losses of 10 to 20% in Brazil, Paraguay, and Argentina (Annone, 1998 in Duveiller et al., 2007). In North America, yield loss of 15% was reported in Oklahoma (Evans et al., 1999), while in Europe, this disease damage, is estimated at 23

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Abbreviations: **RAPD**, Random amplified polymorphic **DNA**; **AFLP**, amplified fragment length polymorphism; **REP**, repetitive extragenic palindromic; **SSR**, simple sequence repeat; **ISSR,** inter simple sequence repeat; **ITS**, internal transcribed spacer; **PIC**, polymorphism information content.

to 53% of yield losses in Germany (Obst, 1988). In North Africa, Nsarallah and Mergoum (1997) reported yield losses by tan spot in the range 12 to18% in Morocco. The pathogen has been reported in Algeria and Tunisia, but the extent of economic losses is unknown. According to their ability to produce necrosis and/or chlorosis on a set of four differentials lines, the isolates of this fungus are currently grouped into eight races (Lamari and Strelkov, 2010). When three durum wheat genotypes were added to the differential set, a novel virulence pattern was identified (Benslimane et al., 2011).

Molecular biology techniques have been used to assess the genetic diversity in *P. tritici-repentis* populations throughout the world. These include the use of random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), repetitive extragenic palindromic (REP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), and internal transcribed spacer (ITS) (Mehta, 2001; Mehta et al., 2004; Dos Santos et al., 2002; Stenglein and Balatti, 2006; Moreno et al., 2008; Lepoint et al., 2010). A previous study in Algeria revealed that tan spot fungus was highly variable for virulence, and identified six races (1, 4, 5, 6, 7 and 8), also a new virulence pattern (Benslimane et al., 2011). Virulence markers may represent only a small portion of the total genetic variation present in the fungal population. However, in Algeria where tan spot has become one of the fastest growing disease problems (Benslimane et al., 2006), there has been no study on the genetic variability or relatedness of *P. tritici-repentis* races with neutral molecular markers. Knowledge of the structure of pathogen populations and their genetic diversity is crucial for the implementation of an efficient plant-breeding approach towards the control of this pathogen through the use of genetic resistance.

AFLP was chosen to study the genetic variation in the *P. tritici-repentis* population in Algeria, because it has been found to be very suitable for studies on the genetic structure of fungal populations where more specific markers system such as SSR markers are lacking (Majer et al., 1996; Campbell et al., 1999; Parrent et al., 2004; Leisová et al., 2008). This method has several advantages as follows: AFLP markers can be generated with no prior knowledge of the genomic makeup of the organism. It is highly polymorphic. Given relatively unlimited number of markers that can be generated, at least some AFLP markers will be located in variable regions and thus reveal even minor genetic differences within any given group of organisms. AFLP amplifications are performed under conditions of high selectivity thus eliminating any arti factual variation. AFLP amplifications show near perfect replicability and require minimal amounts of DNA (Mueller and Wolfenbarger, 1999).

This study's objective were to assess genetic diversity among the Algerian population of *P. tritici-repentis* using AFLP markers and to decipher the relationship between molecular markers and race classification on one hand, and between molecular markers and geographic origin and host plants (durum and bread wheat: *Triticum durum* and *T. aestivum* , respectively) on the other hand.

MATERIALS AND METHODS

Fungal isolates

Sixty one (61) single spore isolates of *P. tritici-repentis* were used in this study; obtained from several infected durum and bread wheat fields in 15 cereal growing areas in Algeria (Table 1 and Figure 1). These isolates were obtained as described by Benslimane et al. (2011).

Fungal cultures

To produce mycelia, isolates were grown in 250 ml Erlenmeyer flasks containing 100 ml of Fries liquid medium by adding 1 ml of spore solution. Cultures were grown for 10 days in darkness at 20°C. Mycelia mats were harvested by filtration through Whatman paper No. 3. The harvested mycelium was placed in liquid nitrogen and stored at -20°C, lyophilized and kept at -72°C until processed.

DNA extraction

Liquid nitrogen was added to 200 mg of fungal mycelium in the microtube. After the mycelium was ground to powder using Micro Pestele, 600 µl of nuclei lysis solution (50mM Tris HCl pH 7.4, 50mM EDTA, 3% SDS, 1% betamercaptoethanol) was added, the tubes were vortexed and incubated at 65°C for 15 min. Then, 10 µl (0.1 mg) of RNAase solution was added to the cell lysates and the samples mixed by inverting the tubes 2 to 5 times. The mixture was incubated at 37°C for 15 min, later the samples were allowed to cool to room temperature for 5 min before proceeding. For protein precipitation solution (3 M sodium acetate), 200 µl was added and the tubes vortexed vigorously at high speed for 20 s, and then centrifuged for 10 min at 13 000 *g*. The supernatant containing the DNA was transferred to a clean 2 ml micro-centrifiuge tube. One equal volume of phenol:chloroform was added to DNA solution, the tubes were mixed gently and centrifuged for 10 min at 13 000 *g*, then the aqueous layer was removed to a new tube. An equal volume of chloroform was mixed with the aqueous sample and the tubes were centrifuged as before. The supernatant containing the DNA was carefully removed and transferred to a clean 1.5 ml micro-centrifuge tube, 600 µl of isopropanol was added and the solution slowly mixed. The tubes were incubated for 30 min at 4°C to precipitate DNA, centrifuged again at 13 000 *g* for 10 min and supernatant was discarded. The DNA pellet was washed with 70% cold ethanol, dried at room temperature and re-suspended in 100 µl of TE (10 mM Tris, and 1 mM EDTA) by incubating at 65°C for 1 h.

AFLP analysis

DNA quality was checked before initiating the AFLP protocol. DNA was run in 0.8% agarose gels and the concentration of each sample adjusted to 25 ng/ml using Lambda DNA marker as a standard. Subsequently, DNA of each isolate was digested using the restriction enzymes *EcoR*I and *Mse*I. To carry out the reaction and for each DNA sample, an enzyme mixture was prepared containing 2 µl of OPA buffer, 0.3 µl of *EcoR*I enzyme (10 U/µl), 0.5 µl of *MseI* enzyme (10 U/µl), 0.5µl of 10mg/ml BSA, 6.7 µl of distilled H_2O (dH_2O) and 10 µl genomic DNA (250 ng), the mixture was centrifuged for a few seconds and then stored at 37°C for 3 h.

Table 1 . *Pyrenophora tritici-repentis* isolates used for the AFLP analysis.

Isolate	*Races classification	Location	Host plant
Ptr2	Race 1	Oued Abbas- Ain Defla	Bread wheat
Ptr4	Race 7	Berboucha-Tipaza	Durum wheat
Ptr6	/	Djendel-Ain Defla	Durum wheat
Ptr7	Race8	Bouira	Mixture
Ptr8	/	Didouche Mourad-Constantine	Durum wheat
Ptr9	Race 7	Cherchel-Tipaza	Durum wheat
Ptr10	Race 7	Oued Smar-Alger	Durum wheat
Ptr11	Race 7	Oued ElAlaig-Blida	Durum wheat
Ptr12	/	Ain-Sbara-Bouira	Bread Wheat
Ptr16	Race 1	Hamr El Ain-Tipaza	Bread wheat
Ptr17	Race 7	Mozaia-Blida	Bread wheat
Ptr18	Race 1	El-Harrach-Alger	Bread wheat
Ptr19	/	Oued Otmania	Bread whet
Ptr21	Race 7	El Kser-Bejaia	Durum wheat
Ptr 22	Race 5	Guelma	Durum wheat
Ptr23	Race 7	Iaazougen-TiziOuezou	Durum wheat
Ptr24	New virulence pattern	Laadjel Hela-Tipaza	Durum wheat
Ptr25	Race 6	Djendel-Ain Edefla	Durum wheat
Ptr26	Race7	Maskara	Durum wheat
Ptr30	/	Azazga-TiziOuzou	Durum wheat
Ptr31	/	Ain Tabahraot-Setif	Durum wheat
Ptr32	/	Ain Sbra-Medea	Bread wheat
Ptr34	/	Tagherourt-Bouira	Durum wheat
Ptr35	/	Bouira	Durum wheat
Ptr36	Race 1	El Khroub-Constantine	Durum wheat
Ptr37	Race	IbnZiad-Constantine	Durum wheat
Ptr38	Race 7	Berouaguia-Medea	Bread wheat
Ptr39	Race 4	Benihamiden-Constantine	Durum wheat
Ptr40	/	Hadjout-Tipaza	Durum wheat
Ptr42	Race 1	BeniSliman-Medea	Bread wheat
Ptr44	/	IghzerOuakar-Bejaia	Durum wheat
Ptr45	Race 5	Gramem Gouda-Mila	Durum wheat
Ptr46	Race 7	Ain Defla	Durum wheat
Ptr48	Race 1	Bouira	Bread wheat
Ptr49	/	Sidi Rached-Tipaza	Durum wheat
Ptr50	/	El Harrach-Alger	Durum wheat
Ptr51	/	Mhareza-Ain Defla	Durum wheat
Ptr52	/	Beni Mestina-Constantine	Bread wheat
Ptr53	Race1	Ain Sbaa- Bouira	Bread wheat
Ptr 55	Race1	Oued Smar-Alger	Bread wheat
Ptr 56	Race1	Oued Smar-Alger	Bread wheat
Ptr57	/	Oued Smar-Alger	Durum wheat
Ptr 61	Race1	Oued Elbared-Bouira	Bread wheat
Ptr 62	Race7	Ain Aloui-Bouira	Durum wheat
Ptr 63	Race1	Benselman-Bouira	Bread wheat
Ptr 64	Race7	El Hachimia-Bouira	Durum wheat
Ptr 65	New virulence pattern	Said Abid-Bouira	Durum wheat
Ptr66	/	AinBessam-Bouira	Bread wheat
Ptr 67	/	Tipaza	Durum wheat
Ptr 68	New virulence pattern	Tipaza	Durum wheat
Ptr69	Race1	Area1-Boumerdès	Durum wheat

Table 1. Contd.

Ptr72	Race1	Area4-Boumerdès	Durum wheat
Ptr73	/	Hmr El Alin-Boumerdès	Durum wheat
Ptr75	Race6	Hamr El Ain-Boumerdès	Durum wheat
Ptr76	New virulence pattern	Hamr El Ain-Boumerdès	Durum wheat
Ptr77	Race1	Oued Smar- Alger	Bread wheat
Ptr78	Race1	Oued Smar- Alger	Bread wheat
Ptr79	Race1	Oued Smar- Alger	Bread wheat
Ptr80	Race7	Ain Bessam-Bouira	Bread wheat
Ptr81	Race7	Ain Bessam-Bouira	Bread wheat
Ptr82	Race7	Ain Bessam-Bouira	Bread wheat

*According to Benslimane *et al*. (2011); /, Unknown races

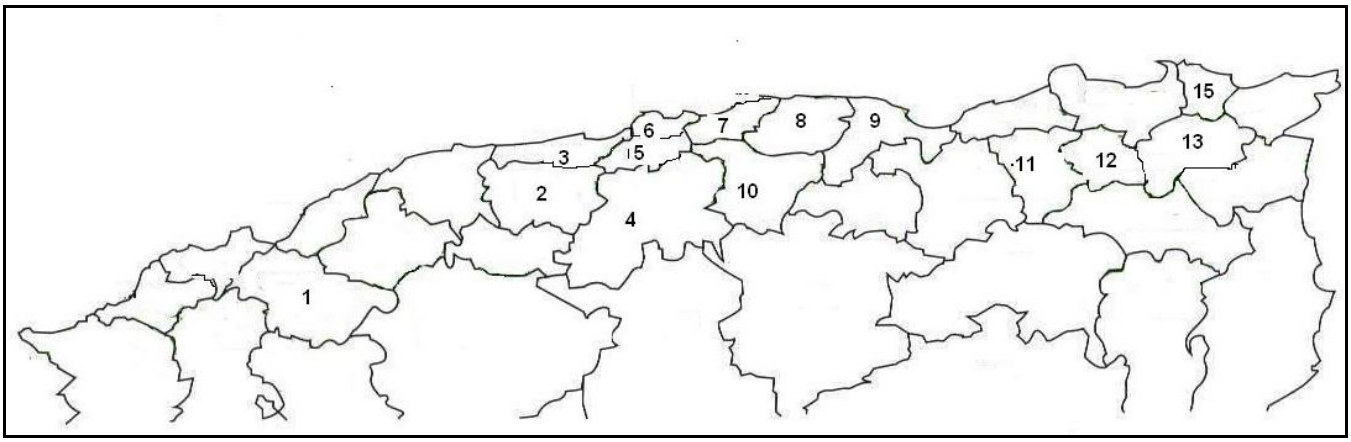

Figure 1. Map of wheat growing areas in Algeria showing the 15 localities where *P. tritici-repentis* isolates were collected. 1= Mascara; 2= Ain-Defla; 3=Tiaza; 4=Medea;; 5= Blida; 6=Alger; 7= Boumerdès; 8= Tizi-Ouzou; 9= Bejaia; 10=Bouira; 11= Mila; 12= Constantine; 13= Galma; ; 15= Anaba.

For adapter-ligation reactions for each sample, a mixture consisting of 0.25 µl of 10× OPA buffer, 0.1 µl of T4 DNA ligase (1 U), 0.25 µl *Eco*RI adaptor (5pmol), 0.25 µl *Mse*I adaptor (50 pmol), and 1.4 µl of dH_2O was added to 10 µl of digested DNA. The samples were incubated at 37°C over night.

The restriction-ligation reactions were diluted 1:5 then used in a pre-amplification step. The *Mse*I and the *Eco*RI complementary primers were used without base extension. The preselective mixture was prepared by adding 3 µl of diluted DNA from the restriction-ligation reaction, 2 µl of polymerase chain reaction (PCR) buffer (10×), 2.5 µl of dNTPs (0.25 mM), 1 µl of AFLP preselective primer (*Eco*RI+0), 1 µl of AFLP preselective primer (*Mse*I+0), 0.2 µl of *Taq* polymerase (1U; Promega, U K) and 10.3 µl of dH_2O. The pre-amplification was carried out in a thermal cycler programmed for 30 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 1 min.

The pre-amplification DNA was diluted five times with dH_2O and selective amplifications, with *Mse*I primers and fluorescently marked *Eco*RI primers, were performed. For the selective amplification, the reactions were set up as follows: 2 µl of diluted preselective amplification reaction product, 0.5 µl *Mse*1primers, 0.5 µl *Eco*RI primers, 1 µl of 10× PCR buffer, 1 µl of dNTPs (0.2 mM), 0.1 µl of *Taq* polymerase (1 U) and 4.9 H_2O. Selective amplification was carried out in a thermal cycler programmed at 94°C for 30 s, 65°C for 30 s and 72°C for 1 min, followed by 11 cycles over which the annealing temperature was decreased by 0.7°C per cycle. Finally, 23 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 1 min were used.

There were 78 combinations of primers tested and 12 pairs were selected (Table 2). These primer combinations were chosen because they generated a good number of fragments with sufficient fluorescence to be detected, and also a good level of polymorphism between the isolates tested. Amplification products were separated by capillary electrophoresis in an ABI PRISM 3100 (Applied Biosystems, USA) and analyzed using GeneMapper software (Applied Biosystems).

Data analysis

Data analysis was carried out using binary data generated on the basis of the presence or absence of AFLP amplification fragments. Genetic similarities between all isolates were computed using the Jaccard coefficient (Legendre and Legendre, 1998) while a dendrogram was generated from clustering with the unweighted pair-group method with arithmetic averages (UPGMA) using PAST software (Paleontological Statistics) version 2.03 (Hammer et al., 2001). Bootstrap support for the branches of the dendrogram was generated with 1000 replications using the same software. In order

Table 2. Primer combinations used in AFLP analysis.

Combinaison	RcoRI Primer		Mse Primer	
	Code	Nucleotide	Code	Nucleotide
C2	Eco32	AAC-3'	M13	AG-3'
C3	Eco32	AAC-3'	M29	
C11	Eco32	AAC-3'	M3	G-3'
C70	Eco32	AAC-3'	M55	TAT-3'
C21	Eco40	AGC-3'	M1	A-3'
C23	Eco40	AGC-3'	M3	G-3'
C45	Eco35	ACA-3'	M1	A-3'
C50	Eco35	ACA-3'	M48	CAC-3'
C51	Eco35	ACA-3'	M50	CAT-3'
C78	Eco35	ACA-3'	M55	CGA-3'
C60	Eco36	ACC-3'	M54	CCT-3'
C59	Eco36	ACC-3'	M50	CAT-3'

Table 3. Number of *loci* (bands) generated by each primer combination, and PIC values provided by each.

Primer combination code	Number of bands generated by each primer combination	PIC mean value	Maximum PIC value	Minimum PIC value
C2	83	0.11968884	0.37439459	0.03172938
C3	98	0.12940572	0.3749328	0.03172938
C11	104	0.14982498	0.37388642	0.03224059
C21	102	0.13807434	0.3716862	0.03172938
C23	129	0.1319415	0.3749328	0.03172938
C45	274	0.12022048	0.37439459	0.03172938
C50	150	0.13222671	0.3749328	0.03172938
C51	89	0.09916783	0.37472207	0.03224059
C59	98	0.12447819	0.3743528	0.03276852
C60	125	0.11367889	0.375	0.03224059
C70	81	0.15681037	0.37472207	0.03224059
C78	103	0.12177006	0.375	0.03224059

to represent the information value of a marker for detecting polymorphism within a population, the polymorphism information content (PIC) based on the formula of Botstein et al. (1980) was calculated using Power Marker software version 3.25 (Liu and Muse, 2005).

RESULTS

The 61 strains analyzed displayed 61 AFLP haplotypes; each one showed a different haplotype from the other. The 12 primer combinations used produced 1,251 DNA fragments which correspond to an average of 104.25 DNA fragment per primer combination. All of the bands obtained were polymorphic; no monomorphic band across all isolates was detected. The 12 primer combinations used where polymorphic across the 61 isolates tested and none of the primer combinations produced monomorphic DNA fragment. The maximum number of bands was generated when we used C45 primer combination with 274 bands, while the minimum was obtained with the C70 primer combination with 81 bands (Table 3).

There were 22 to 276 *loci* revealed for each isolate. Some *loci* were more frequent than others in the population. Indeed while some were found in almost all isolates, others were detected in only one. Primer combination C60 amplified a 154 bp DNA fragment that was found in 58 of the 61 isolates evaluated. Similarly, the same primer combination amplified 317 bp and 351 pb DNA fragments in 57 of the isolates. However, 478 markers were present in only one isolate. The PIC for each marker (*locus*) varied between 0.0317 and 0.375, the PIC mean values for each combination of primers used are compiled in Table 3. Our results show that the primer combination C70 amplified the markers which provided the highest PIC mean value, while C51 primer combination amplified markers with the lowest PIC mean

value. The C11 primer combination highlighted markers with PIC mean value close to the maximum PIC value found.

Jaccard's coefficient of similarity was used to assess the degree of similarity between each pair of isolates. Its value varied from 0.51% for isolates Ptr16 and Ptr44 to 73.50% for Ptr69 and Ptr72, indicating high diversity and an imbalance in the population (Figure 4). Isolates originating from the same host species had similarity coefficients ranging from 1.83% for isolates Ptr30 and Ptr24, to 73.5% for Ptr69 and Ptr72 for those from durum wheat. The bread wheat isolates showed a range between 8.7% for isolates Ptr18 and Ptr77, and 61.65 % for isolates Ptr55 and Ptr66. From the eastern geographic region the range was 12.34% (for Ptr22 and Ptr36) to 61.65% (for Ptr55 and Ptr66), while in the western region the strains showed a range of 10.77% (for Ptr26 and Ptr2) to 31.91% (for Ptr6 and Ptr2). In the central region, the variation was from 1.83 (for Ptr30 and Ptr24) to 73.50% (for Ptr72 and Ptr69). The isolate Ptr24, had a values of Jaccard similarity index that were relatively low compared to other isolates: its similarity ranged from 1.83% with isolate Ptr30 to 16.43% with Ptr2. Isolate Ptr24 from durum wheat was previously distinguished by a virulence pattern sufficiently novel to lead us to propose the existence of a new virulence pattern (Benslimane et al., 2011).

The cluster analysis based on UPGMA method using the Jaccard coefficient revealed two main groups at the level of linkage distance of 6%. The first group was formed by a single isolate Ptr30; isolated from durum wheat in the central area (Tizi-Ouzou). The second cluster included the rest of the isolates; with several identifiable groups at different levels of linkage, regardless of host species or geographical origin (Figure 2). Isolates from different locations were found in the same clusters. Isolates from durum and bread wheat were found together in the same cluster. We also noted that isolates from the same leaf spot were classified into different clusters: this was the case of isolates Ptr73, Ptr75 and Ptr76 which were linked only on at a level of 17% of similarity (Figure 2).

Cluster analysis based on the Jaccard similarity index, was performed a second time considering only isolates for which the race classification was known (Figure 3). Ptr24 differed greatly from the other isolates; forming separate cluster by itself on the level of linkage of 8%. This was well supported by bootstrap values of 100%. At the linkage distance level of 36%, there were 12 cluster and within some we found isolates belonging to different races. However at this level of similarity, one isolate of race 6 was distinguished in a single cluster. This was also true for Ptr76 and Ptr68, as each formed a separate cluster; these two isolates were also characterized by a new virulence pattern that indicated a new race (Benslimane et al., 2011). The last isolate of the new virulence pattern (Ptr65) was distinguished at a level of 45% of similarity. Our results also show that the single isolate of race 4 in this study comprised a separate cluster at a level of similarity of 48%.

When we analyzed all isolates, the bootstrap test generated values in the range 5 to 100%. Clearly some of these values were low; however, we noted that 52% had values ≥ 50%. The analysis for isolates for which race classification was known, showed bootstrap values in the range 12-100%, for this analysis 63% of the values were ≥ 50%. According to Moore and Frazer (2002) a bootstrap value ≥ 50% shows that the pseudo-replicated data strongly supports the branch, and < 50% indicates little support.

DISCUSSION

A high level of genetic variability was demonstrated between *P. tritici-repentis* isolates collected from different cereal growing regions in Algeria. Of the 61 isolates comprising the analyzed population, the AFLP markers have revealed the presence of 61 different haplotypes. Primer combination C70 and C11 showed the most informative values (0.156 and 0.149, respectively). Thus, for further analysis, it will be possible to choose a lower number of markers to make screening cheaper and quicker

Despite the absence of monomorphic bands, our results show that primer combination C60 revealed the presence of a 154 bp band on 58 isolates, representing ≥ 97% of the population. It also showed two fragments of 317 and 351 bp in the genome of 57 isolates accounting for 95% of the population. These three markers should be considered for development of specific primers. Obtaining such primers is very important because *P. trititci-repentis* causes symptoms very similar with to those produced by *Cochliobolus sativus*, rendering differentiation very difficult using only the symptoms (Luz and Bergston, 1987). The identification of pathogens through DNA may provide an important diagnostic tool.

Cluster analysis based on Jaccard's similarity index showed several clusters at relatively low similarity levels. This shows that isolates belonging to the new virulence pattern could be distinguished from other isolates with percentages of similarity ≤ 50% (a maximum of 45% was observed). One of them, Ptr24, was clearly distinguished from the rest of the population. The main characteristic of these strains is that they were isolated from durum wheat, and were able to attack only durum genotypes in the differential set (Benslimane et al., 2011). This new virulence pattern of isolates seems to involve production of one or more toxins yet identified; consequently they may have parts of their genome that differ to the eight known races. The AFLP technique is used to analyze the diversity of the entire genome, and so it is possible that some markers have highlighted DNA regions that are novel in this new virulence pattern. The single isolate of race 4 was found in a separate cluster at a level of 48%

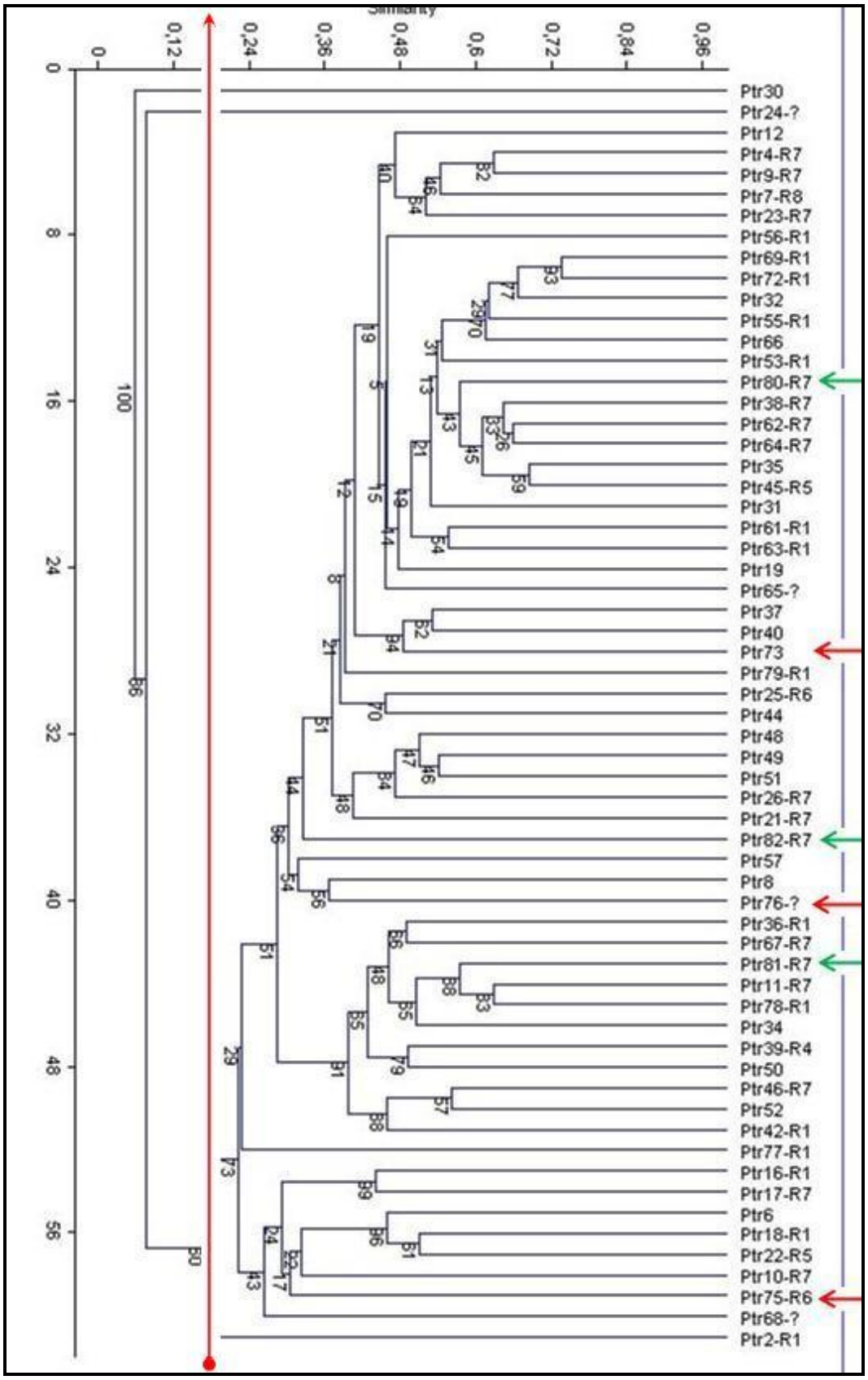

Figure 2. Dendogram produced using UPGMA cluster analysis based on Jaccard similarity coefficient calculated from AFLP DNA fragments from all the isolates (?=new virulence pattern).

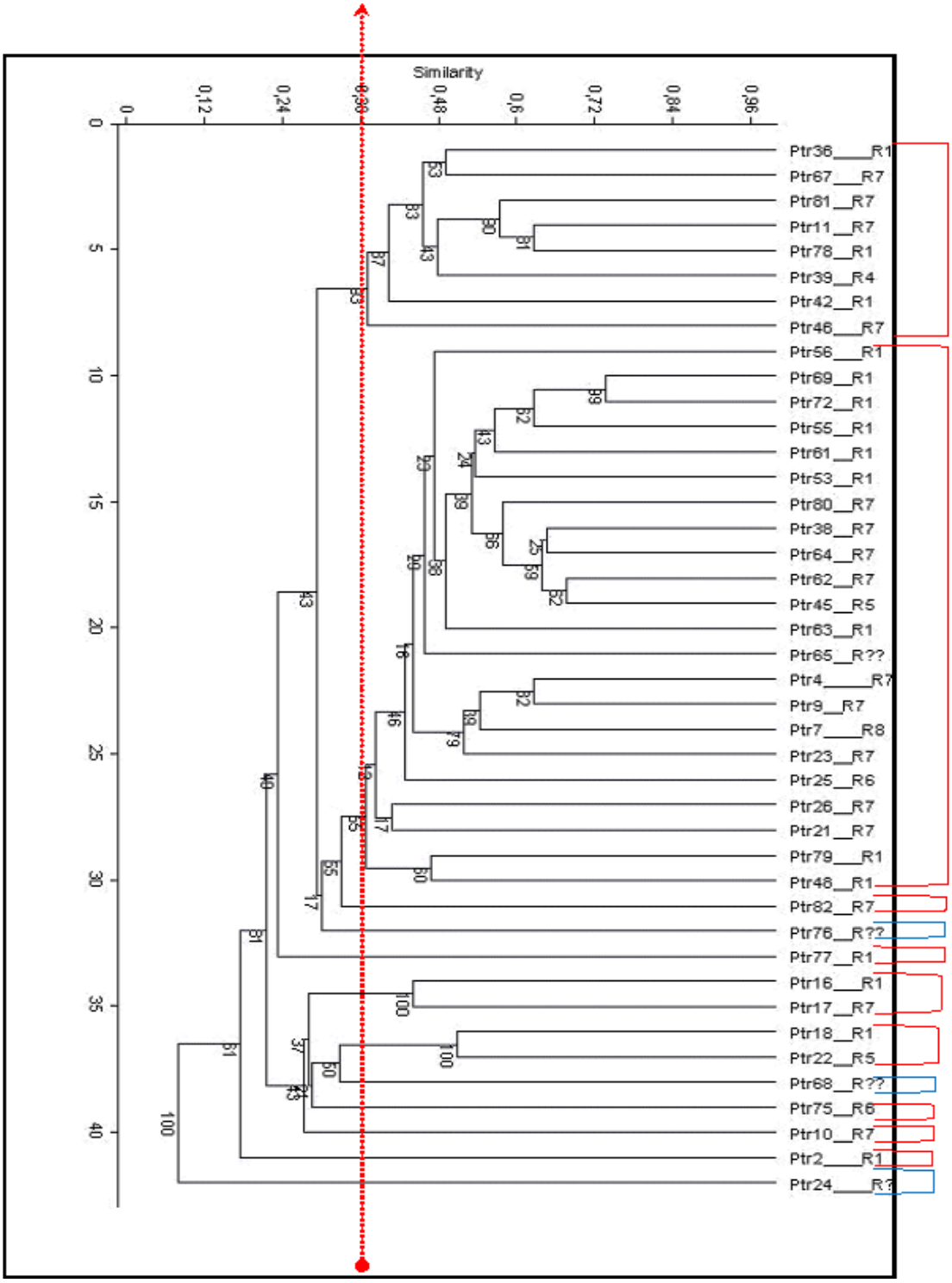

Figure 3. Dendogram produced using UPGMA cluster analysis based on Jaccard similarity coefficient calculated from AFLP DNA fragments from the isolates which the race classification is know.

of similarity. Race 4 is non-pathogenic on wheat, and previous studies (Lichter et al., 2002; Martinez et al., 2004) demonstrated its difference at the molecular level compared to other *P. tritici-repentis* races. Lichter et al. (2002) while comparing the karyotypes of virulent and avirulent strains, showed that part of a chromosome was completely absent in isolates of race 4. This part of the chromosome contains the ToxB gene, coding for PtrToxB

	Ptr2	Ptr12	Ptr24	Ptr36	Ptr46	Ptr67	Ptr56	Ptr79	Ptr4	Ptr1	Ptr25	Ptr37	Ptr48	Ptr69	Ptr57	Ptr80	Ptr6	Ptr17	Ptr26	Ptr38	Ptr49	Ptr72	Ptr61	Ptr81	Ptr7	Ptr18	Ptr30	Ptr39	Ptr50	Ptr73	Ptr62	Ptr82	Ptr8	Ptr19	Ptr31	Ptr40	Ptr51	Ptr75	Ptr63	Ptr68	Ptr9	Ptr21	Ptr32	Ptr42	Ptr52	Ptr76	Ptr64	Ptr10	Ptr22	Ptr34	Ptr44	Ptr53	Ptr77	Ptr65	Ptr11	Ptr23	Ptr35	Ptr45	Ptr55	Ptr78	Ptr66
Ptr2	1																																																											
Ptr12	23,22	1																																																										
Ptr24	16,44	8,416	1																																																									
Ptr36	7,509	32,45	6,615	1																																																								
Ptr46	14,16	22,29	5,556	32,63	1																																																							
Ptr67	7,442	20,46	7,955	48,94	38,67	1																																																						
Ptr56	24,07	35,63	5,96	20,37	20,37	20	1																																																					
Ptr79	17,49	35,47	8,065	25,76	19,61	15,97	32,41	1																																																				
Ptr4	22,69	46,69	7,177	23,71	19,69	19,36	37,1	34,13	1																																																			
Ptr1	11,5	23,22	14,87	21,62	14,16	17,86	12,29	13,19	15,22	1																																																		
Ptr25	15,66	32,52	3,425	16,87	21,07	13,36	38,34	30,21	37,92	3,226	1																																																	
Ptr37	12,75	35,82	3,279	23,6	17,61	14,73	33,19	34,09	33,21	11,11	35,78	1																																																
Ptr48	10,48	38,64	8,523	38,94	16,72	22,55	24,8	46,64	34,42	28,18	23,14	33,33	1																																															
Ptr69	18,8	52,03	6,757	36,95	24,07	19,69	43,5	50,16	42,32	24,11	40,41	44,7	52,38	1																																														
Ptr57	13,22	27,36	6,404	25,28	23,7	22,71	31,75	23,94	28,16	13,22	25,68	24,91	23,89	34,1	1																																													
Ptr80	15,39	37,5	6,989	25,81	23,39	19,79	42,53	36,01	40,37	15,94	45,02	40,41	34,63	47,93	30,18	1																																												
Ptr6	31,92	38,53	12,4	16,1	20,62	17,34	31,16	21,38	40,09	30,99	21,15	19,84	17,27	30,5	20,99	27,12	1																																											
Ptr17	10,39	20,16	10,26	20,47	12,64	18,03	12,39	11,36	17,05	44,07	4,425	13,22	24,32	19,7	13,59	23,48	26,37	1																																										
Ptr26	10,78	41,78	9,924	35,02	18,39	27,75	30,65	28,22	36,71	23,33	31,41	33,8	44,06	37,55	26,91	37,16	21,29	23,78	1																																									
Ptr38	18,42	42,46	5,747	28,97	25,09	23,13	51,52	34,21	45,6	18,42	45,5	44,93	31,35	47,27	34,34	54,8	33,8	18,5	50,27	1																																								
Ptr49	7,843	35,12	7,831	36,79	19,86	28,97	28,26	27,53	35,99	31,74	25,99	32,25	49,54	43,08	25,36	44,59	22,81	38,22	52,46	43,05	1																																							
Ptr72	19,81	55,2	6,5	35,89	25,58	21,28	46,22	50,17	45,56	23,3	43,5	49,17	45,16	73,5	34,62	48,99	36,52	19,68	39,73	52,81	43,21	1																																						
Ptr61	23,2	49,37	5,814	26,38	21,75	17,14	50,51	40,48	40,86	15,54	47,45	39,74	33,2	54,96	36,15	43,4	31,02	13,14	38,92	52,61	32,08	61,75	1																																					
Ptr81	14,89	35,09	6,122	44,12	35,78	41,96	32,86	30,11	32,93	18,5	31,54	31,92	29,62	41,56	30,91	53,7	26,67	25,46	30,44	42,35	39,09	42,05	33,56	1																																				
Ptr7	22,11	43,89	7,447	22,73	22,33	20,83	32,5	31,89	51,59	18,54	43,06	31,94	32,2	41,58	28,08	40,55	35,87	20,08	37,9	42,5	38,68	42,69	41,67	35,71	1																																			
Ptr18	24,28	31,7	7,407	17,1	15,7	11,5	18,85	20,97	24,65	34,38	11,95	17,28	20,68	28,77	12,34	23,22	41,75	31,47	14,64	20,31	25,1	29,43	23,81	25	27,69	1																																		
Ptr30	4,365	7,88	1,835	5,608	4,313	5,143	6,625	5,189	6,631	6,911	2,492	4,261	6,647	5,867	4,233	5,307	5,556	6,597	6,977	7,418	7,53	5,391	6,825	5,542	5,85	6,024	1																																	
Ptr39	12,09	30,18	6,855	43,48	34,8	37,46	25,74	31,45	26,78	11,27	27,4	37,12	26,77	37,95	23,8	27,66	17,69	9,718	24,57	30,65	23,84	38,66	30,42	42,73	27,41	16,18	5,226	1																																
Ptr50	12,27	27,87	6,719	38,89	29,85	35,51	27,39	24,75	28,94	13,09	23,2	30,5	21,93	33,05	29,62	28,4	23,67	12,7	23,75	31,83	26,56	35,91	30,35	40,06	25,15	15,94	4,918	49,24	1																															
Ptr73	13,99	37	4,425	23,28	17,6	16,46	39,13	39,39	32,91	14,94	28,95	44,87	34,86	46,92	22,89	32,54	28,14	15,88	26,32	37,73	27,49	50	41,67	29,14	30,9	21,19	6,701	36,42	30,51	1																														
Ptr62	21,81	45,2	6,818	29,01	27,9	24,63	52	39,19	47,79	23,12	46,04	40,51	36,18	55,51	37,41	53,81	38,28	24,66	44,72	64	46,15	62,27	55,98	47,45	45,99	27,71	6,706	31,94	36,18	43,4	1																													
Ptr82	11,92	33,22	4,717	20,53	24,04	15	30	25,28	29,68	16,89	29,02	27,88	24,24	35,18	20,49	47,84	27,67	23,29	23,37	36,09	30,77	39,15	32,84	39,49	29,25	28,94	5,541	20,38	21,41	26,99	37,08	1																												
Ptr8	16,6	36,68	5,668	23,99	20,68	20,77	26,09	27,63	38,68	12,27	25,6	31,82	24,77	30,46	27,73	31,65	27,92	13,07	29,86	34,56	28,16	33,96	29,97	29,62	37,38	23,34	7,073	28,88	31,27	24,45	34,55	25,07	1																											
Ptr19	17,41	45,67	7,735	25,74	25,87	24,91	41,36	34,3	44,23	19,8	43,13	34,92	31,54	43,17	30,61	40,56	33,94	18,64	41,83	50,9	40,43	47,97	49,33	36,09	43,5	23,11	7,184	28	28,35	33,91	51,34	33,69	34,64	1																										
Ptr31	16,59	44,77	6,103	31,79	26,54	21,57	40,16	41,07	45,52	20,81	42,31	41,07	37,13	51,81	31,79	45,42	33,47	21,48	44,89	56,41	47,95	54,9	46,77	39,43	47,13	24,04	6,316	31,47	31,01	40,74	56,78	33,33	33,84	51,41	1																									
Ptr40	17,19	43,6	6,977	26,99	21,6	17,44	41,51	39,25	34,82	16,58	34,1	53,02	34,55	48,43	26,69	37,25	28,38	14,47	36,54	47,27	30,74	56,2	48,39	32,45	32,05	21,71	6,785	41,61	35,31	51,61	47,09	31,64	32,78	41,95	43,53	1																								
Ptr51	7,653	33,21	8,974	36,05	16,32	22,01	23,81	30,46	31,58	24,85	24,22	38,77	52,4	42,91	25,74	35,98	19,91	27,5	44,39	34,2	54,04	42,44	32,76	30,85	31,2	20,97	5,136	31,21	31,13	39,23	39,56	25,18	27,72	32,1	39,68	38,39	1																							
Ptr75	21,17	25	12,61	15,64	17,41	13,87	24,23	19,93	22,98	29,69	15,42	23,29	17,75	27,71	19,28	21,21	37,81	23,53	17,19	26,79	20,93	33,03	27,67	19,65	21,98	29,44	4,483	20,14	21,45	27,82	31,86	18,82	19,16	35,29	26,34	31,19	24,26	1																						
Ptr63	21,61	38,49	8,333	22,93	21,59	16,6	37,37	34,28	33,33	17,96	38,42	33,78	26,78	42,45	31,2	44,65	30,46	20,5	28	44,39	28,19	51,87	55,56	33,81	31,38	21,7	5,363	23,61	29,93	34,36	56,25	30,98	28,18	40,18	39,1	40,28	29,95	30,94	1																					
Ptr68	16,58	20,88	10,18	14,05	13,68	9,635	18,11	24,77	17,8	19,68	11,92	20,07	16,96	24,83	12,66	27,44	26,67	22,83	9,231	16,55	19,48	24,74	18,38	25	19,58	33,05	5,848	20,54	19,19	23,68	21,93	26,13	18,99	18,38	19,61	24,14	21,57	27,4	20,82	1																				
Ptr9	22,3	51,69	2,985	24,8	18,93	16,52	39,65	36,73	62,65	4,511	53,57	44,38	40	48,17	25,55	56,41	34,44	7,519	46,58	53,01	44,83	54,65	49,43	37,62	57,14	21,26	4,383	35,92	28,14	36,19	54,09	33,66	38,7	44,57	45,88	44,69	41,89	17,28	40,39	16,77	1																			
Ptr21	12,06	42,35	9,559	28,91	24,45	21,4	26,35	36,74	38,74	32,87	28,39	27,14	43	45,46	22,22	33,16	29,53	24,47	40,68	38,2	40,59	45,06	37,65	28,63	38,33	29,01	9,302	22,47	23,68	27,23	41,62	30,19	28,69	44,31	43,01	31,75	37,5	26,39	31,33	18,09	40	1																		
Ptr32	23,6	50,64	7,831	33,66	27,88	25	42,51	46,55	42,46	25,71	45,18	44,64	44,89	66,07	30	51,13	33,97	20,55	42,35	53,37	44,04	67,31	56,16	43,64	45,26	29,83	6,25	34,23	33,22	41,07	59,9	36,26	34,24	50	52,97	48,37	40,55	29,35	50	24,13	51,85	49,08	1																	
Ptr42	18,83	23,53	5,66	40,12	36,49	35,04	25,46	20,76	19,17	18,83	16,55	19,81	13,46	28,31	22,98	20,32	27,45	15,3	13,68	27,72	13,61	30,13	27,92	38,37	18,63	22,92	4,416	39,06	35,54	23,44	29,12	19,29	19,51	21,75	23,78	24,66	14,01	27,08	26,32	18,57	14,92	16,26	28,21	1																
Ptr52	16,6	25,65	4,4	41,91	56,04	37,5	23,61	22,17	21,49	16,15	18,71	19,41	14,93	28,61	24,78	21,28	26,57	12,7	14,6	27,3	16,47	32,72	29,97	41,54	19,37	21,43	5,276	38,91	40,75	22,76	30,23	24,36	21,01	25,61	24,44	24,53	16,57	23,47	29,07	17,94	17,82	21,21	31,13	55,28	1															
Ptr76	11,88	30,3	4,196	27,54	22,49	22,65	30,19	27,32	29,54	9,355	27,76	33,03	21,59	35,64	35,84	30,93	22,02	12,65	22,46	32,33	22,32	38,58	32,12	33,51	26,32	14,13	4,158	35,86	36,82	37,26	34,76	22,37	36,61	30,15	32,96	35,19	26,19	19,94	30,16	15,57	29,71	21,6	34,89	27,13	29,4	1														
Ptr64	16,84	40,16	7,738	26,85	24,91	22,02	49,25	37,76	44,98	16,84	46,19	41,13	33,33	50,81	34,22	63,9	31,78	18,22	40,5	64,62	41,7	54,19	52,63	41,28	40,08	20,08	5,9	28,85	34,32	39,18	65,82	38,61	33,56	46,9	52,52	42,04	38,91	26,57	53,4	16,73	52,76	37,85	53,4	24,91	25,95	34,68	1													
Ptr10	17,75	30,59	8,219	20,87	17,88	21,12	25,69	24,03	35,74	26,75	21,01	20,72	30,9	31,46	22,68	25,2	33,51	25,91	27,72	25,74	35,65	31,86	25,42	23,03	49,76	28,57	4,348	19,94	18,15	26,35	30,74	21,74	22,95	32,05	34,39	25,21	28,51	26,46	20	21,14	38,67	27,37	31,98	16,15	15,39	16,71	23,43	1												
Ptr22	26,03	30,86	8,333	12,35	19,38	15,14	22,17	18,01	29,2	26,9	16,82	16,6	16	23,53	15,39	30,13	49,69	34,12	15,71	24,12	26,36	26,83	22,17	30,07	35,59	50,83	5,592	15,19	15,67	17,54	30,46	33,75	25	27,83	24,52	21,98	16,96	28	21,43	32,24	31,03	24,05	27,52	20,07	18,81	15,41	23,89	34,56	1											
Ptr34	13,04	23,89	6,522	43,07	40,41	42,7	26,67	24,73	25	8,745	28,47	23,81	18,43	29,59	25,6	33,78	20,56	9,635	20,21	33,22	20,64	31,85	27,24	49,68	23,24	13,64	4,444	44,27	41,44	23,45	33,68	24,41	24,19	28,99	28,61	27,91	18,91	16,43	26,6	12,9	28,96	20,08	32,87	44,56	47,13	30,97	35,46	13,25	17,41	1										
Ptr44	14,62	32	4,73	19,94	15,52	11,9	32,84	33,45	34,54	0,513	45,56	33,33	26,36	35,41	21,56	34,78	14,8	2,128	28,79	37,85	25,54	39,66	41,55	25,34	30,96	9,195	3,416	24,92	21,34	23,93	35,29	22,43	24,83	44,34	33,2	30,53	23,25	21,03	31,53	10,08	48,59	27,04	38,76	12,04	14,11	22,82	38,39	16,45	12,39	24,48	1									
Ptr53	22,56	41,95	6,667	25,81	25	16,79	41,54	38,13	39,92	22,56	36,22	39,91	30,09	49,58	25,76	46,51	38,1	16,11	33,33	54,64	32,88	56,67	49,75	36,23	33,05	27,75	5,625	29,15	33,56	35,91	52,76	33,07	31,36	42,01	46,15	46,34	33,65	31,69	47,83	21,46	45,91	41,45	56,92	31,78	33,69	30,19	54,69	18,1	28,22	36,74	33,5	1								
Ptr77	12,98	22,61	9,184	21,22	11,02	13,45	22,53	20,8	26,99	13,85	19,55	21,15	27,64	23,97	17,23	24,76	12,43	10,35	34,42	29,32	27,37	26,03	26,94	17,88	22,12	8,716	7,143	15,9	18,09	15,89	28,72	13,55	21,81	22,28	22,98	23,5	30,9	13,94	25,86	7,86	25,68	30,06	26,04	10,81	12,89	16,08	29,1	8,867	8,421	15,36	20,44	32,74	1							
Ptr65	19,05	34,46	8,876	25,84	21,18	21,32	44,93	35,55	36,33	23,63	31,67	33,74	33,47	46,69	28,52	50	33,18	28,1	33,33	45,29	41,78	45,87	37,61	41,84	37,9	29,22	5,848	28,17	26,94	34,29	53,27	33,58	30,62	40,17	45,82	35	34,2	29,27	36,41	28,57	38,42	34,25	45,66	25,09	22,63	28,45	51,42	33,63	31,63	25,41	26,07	35,14	19,9	1						
Ptr11	17,41	38,77	5,747	54,27	40	48,59	30,23	32,63	39,88	16,54	30,27	29,94	33,44	40,84	30,61	36,39	30	16,83	35,25	39,13	37,92	43,55	33,98	55,21	41,37	23,78	5,093	50,61	43,43	31,09	45,33	28,25	35,07	36,42	40,92	32,06	33,11	20,2	28,48	18,18	45,98	30,62	41,24	43,4	43,73	35,2	40	30,44	27,12	51,91	27,72	33,79	18,53	37,75	1					
Ptr23	22,51	46,03	9,04	28,18	22,6	21,07	36,12	39,93	51,01	16,42	36,36	39,67	39,92	46,21	26,3	46,84	34,25	19,31	38,86	42,92	45,13	51,46	40,85	39,59	47,48	34,58	6	31,85	28,14	30,95	47,81	36,4	39,93	40,41	46,48	41,1	36,91	20,71	32,61	25,66	57,06	41,62	48,42	18,04	19,19	27,71	44,74	34,06	41,06	25,08	41,4	40,46	26,73	43,53	41,67	1				
Ptr35	19,5	45,7	7,609	28,53	26,92	22,86	46,05	41,14	46,51	23,2	49,51	41,74	39,11	56,4	32,62	55,51	35,29	24,67	40,57	57,21	48,66	57,51	52,04	46,81	48,95	30,68	5,916	31,76	30,86	36,36	60,56	42,97	33,87	53,74	55,1	45,06	37,29	25,11	41,55	24,72	54,22	46,29	61,65	25,17	25,76	30,64	54,38	35,65	34,39	31,68	37,95	47,42	24,29	50,22	42,38	54,91	1			
Ptr45	20,56	44,44	7,976	28,08	29,66	24,81	53,68	37,93	49,58	21,23	53,8	41,41	34,58	51,85	36,47	58,37	39,9	20,83	44,5	62,89	42,66	59,72	53,92	45,19	49,11	23,89	5,357	33,22	34,9	38,35	67,54	39,92	35,99	54,98	56,33	46,3	37,9	31,8	48,45	21,07	57,79	46,25	61,14	27,6	29,28	35,65	63,02	34,26	32,21	37,09	37,32	56,15	29,19	53,4	41,67	49,77	68,37	1		
Ptr55	22,53	46,91	9,639	32,48	24,82	21,03	50,51	49,27	47,15	20,54	41,67	47,96	44,3	60,26	33,08	48,46	31,02	16,09	41	52,61	40,89	64,79	53,11	38,19	40,5	22,35	5,882	36,61	33,77	43,3	58,25	29,96	33	45,42	53,59	51,17	39,37	30,85	45,55	23,85	48,47	41,14	60,91	29,75	29,97	35,83	51,19	28,7	21,12	30,27	38,21	57,67	33,15	48,39	38,93	49,77	52,73	61,03	1	
Ptr78	17,31	32,23	6,024	47,9	39,74	45,39	31,94	34,44	32,93	15,53	30,63	30,67	29,65	42,81	30,93	35,6	25	16,72	30,47	38,83	34,8	42,43	35,35	59,47	35,69	21,61	4,492	50,16	44,54	30,66	45,2	25,93	30,71	36,96	41,59	32,46	30,87	21,48	34,29	19,17	33,61	29,48	44,04	45,1	48,46	33,16	38,28	28,14	21,81	50	24,59	36,2	18,91	37,42	62,62	34,97	42,66	43,48	42,05	1	
Ptr66	21,03	46,25	7,735	31,17	25	20,07	43,98	44,48	42,59	22,28	42,45	41,67	41,32	59,67	30,14	45,83	33,94	17,65	37,85	50,9	39,83	66,21	48,66	39,8	40,08	26,95	5,966	35,07	31,56	41,76	57,67	35,15	31,63	47,23	51,41	50,22	37,18	32,69	44,13	23,16	52,41	42,37	58,65	28,43	30,38	31,67	48,21	36,12	25,64	33,78	36,61	52,45	24,04	45,65	40,92	44,54	55,11	59,51	61,65	41,16	1

Figure 4. Similarity matrix in percentage for 61 isolates *of Pyrenophora tritici-repentis* using the similarity coefficient of Jaccard.

and some repeated sequences. In addition, race 4 is the only one whose genome contains the *toxb* gene which shows only 86% of similarity with ToxB (Martinez et al. 2004). In this case, it is also possible that AFLP markers were able to highlight parts of the genome that differ among non-virulent strains. Using AFLP markers, Leisová et al. (2008), reported the distinctness of race 4, but at a higher genetic distance. A bootstrap test generated some low values, with PIC ≤ 50%, indicating the branch had little support (Moore and Frazer, 2002). Leisová et al. (2008) also reported this fact with 39% of values ≥ 50%.

Furthermore, the AFLP analysis showed that the same leaf spot caused by *P. tritici-repentis* in wheat could be the result of genetically different strains. Not only were the haplotypes of isolates from the same leaf spot different, but the cluster analysis also showed that these isolates joined at a low similarity distance. Our results show that the genetic similarity could not be explained by race classification, with the exception of isolate Ptr24 which seems to belong to a new race. This lack of correlation is easily understood, in that the AFLP markers reveal random variation throughout the genome, while race classification is based on only three specific *loci*. It can also be explained by the fact that the acquisition of specific characters for pathogenicity evolution could be slower than specific genomic evolution. It has also been suggested the correlation between virulence and molecular markers is often low in populations with sexual reproduction (Burdon and Roelfs, 1985; Schilder and Bergstrom, 1992).

Our results also suggest that molecular similarity cannot be explained by geographical origin. However, the biology and epidemiology of this pathogen may provide some insights, according to De Wolf et al. (1998). In favorable conditions, conidiospores of *P. tritici-repentis* can move 10 to 200 km. It is also recognized that this pathogen is seed borne (Schilder and Bergstrom, 1995). Hence there is possible movement of inoculum over long distances. The long-distance dispersion of inoculum and the presence of sexual reproduction may contribute to increased genetic diversity independent of geographical origin, or race classification (Singh and Hughes, 2006). It was also not possible to establish any relationship between molecular similarity and host.

The lack of relationship between molecular similarity and geographical origin on one hand and race classification on the other in *P. tritici-repentis* has been reported previously. Friesen et al. (2005) showed similar results using AFLP markers to analyze 97 isolates from North America, South America and Europe. They explained that this indicates that, in wild populations, *P. tritici-repentis* had a tendency to out-cross. According to these authors, this situation is a result of the fact that pathogen introduction and its spread are recent or that there is a gene flow across different areas because of seed exchange. For our population, the second explanation seems more likely because of the high pathological variability that we noted in Algerian fields, which indicates a rather old introduction of the pathogen. Moreover, Singh and Hughes (2006) and Dos Sontos et al. (2002) who used RAPD markers and Moreno et al. (2008) who used ISSR markers also reported no correlation between genetic similarity and geographical origin and race classification. However, according to Leisova et al. (2008) the genetic similarity revealed by AFLP markers may be partially explained by race classification, the host plant and geographical origin. Each factor has an impact on the structure of the pathogen

An important factor in plant pathogenic fungi is the relative abundance of sexual and asexual reproduction within populations. *P. tritici-repentis* is a fungus with both types of reproduction; the occurrence of sexual recombination in nature is likely the reason for the high level of genetic variability among isolates. No cereals grass hosts, could also represent an important source of genetic variability, as they can be a large reservoir of new biotypes genetically different from those prevailing in cultivated wheat (Krupinsky, 1987; Ali and Lamari; 1997; De Wolf et al., 1998). Other factors affecting the variability of a fungus population should be not excluded; including mutations, gene flow, genetic drift, selection and heterokaryosis (McDonald, 1997; Agrios, 2005).

This work is the first study of genetic diversity of *P. tritici-repentis* in Algeria. It provides information about genetic structure of this population which should be used by plant breeders. Pathogen populations with large genetic variation gain advantage as they can rapidly respond to changing environments and overcome host resistance and fungicide treatments (Peltonen et al., 1996).

In Algeria until now, no resistance sources against *P. tritici-repentis* have been reported, the main cultivated genotypes of both durum and bread wheat seem to be susceptible to this pathogen. The present study showthat the pathogen population in Algeria was highly genetically diverse, as our previous study show that it was highly variable pathologically. Since this fungus possesses both types of reproduction spores, the risk that it overcomes more host resistance is increased. Sexual reproduction creates many new genotypes and allows new and existing virulence genes to be recombined into many different genetic backgrounds. For this purpose, McDonald and Linde (2002) hypothesized that pathogens undergoing regular recombination pose greater risks than pathogens that undergo no or little recombination.

In Algeria, resistance breeding combined with appropriate crop management approaches may minimize losses caused by *P. tritici-repentis*. Finding and using resistant genotypes is a high priority in the breeding program, in order to minimize selection pressure and to change the population structure. For this purpose, the future breeding program needs to focus on identifying resistance sources in presently grown wheat cultivars, especially in wheat landraces. Indeed, even ignoring the exact time of occurrence of this fungus in Algerian fields, it is not excluded

that it has been accompanied the introduction of wheat genotypes, qualified as higher yielding cultivars. Selection and incorporation of major genes is a very suitable approach, as resistance governed by major genes should be stable and it is unlikely to be broken down quickly, because the wheat *P. tritici-repentis* pathosystem follows the toxin model. Thus, to overcome resistance of cultivars, the fungus must produce a new toxin; the probability of occurrence of reverse mutation involving gain of function is very low, and the host plant has to have a specific receptor for the new toxin to develop susceptibility simultaneous occurrence of both these events very unlikely (Singh et al., 2010). Additionally, marker-assisted selection which has been used to improve resistance to pests and pathogens in various crops (Varshney et al., 2006), could be effectively used in the resistance breeding program, because of the known wheat resistance type against this pathogen and the opportunities to use toxins or cultures filtrates as screening tools.

Until resistance sources are identified, the following two actions appear urgent: removing the crops debris from wheat fields and practicing rotations with non-cereal crops. These are necessary, because the fungus survives as mycelium in stubble from infected crops, and under wet conditions, pseudothecia develop to produce the sexual stage of the fungus. Sexual reproduction is the main cause of genetic diversity and ascospores are the major sources of primary inoculum. In this context, previous research has showed that, continuous wheat cultivation and especially conservation or minimum tillage systems increased the incidence of this disease (Lamari and Bernier, 1994; Ciuffetti and Tuori, 1999).

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