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DNA MARKERS FOR IDENTIFICATION OF *PYRENOPHORA TRITICI-REPENTIS* AND DETECTION OF GENETIC DIVERSITY AMONG ITS ISOLATES

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ABSTRACT

In this study, specific primer pair DTR1-F and DTR1-R was designed for reliable PCR detection of *Pyrenophora tritici-repentis* in wheat leaf and seed samples and for its distinguishing from other pathogens, using standard agarose gel electrophoresis. Eight SSR primer pairs were also designed for assessment of genetic diversity in a group of 13 Slovak, 1 Czech and 10 Finnish isolates of *P. tritici-repentis*. Only five SSR primers showed polymorphism with an average of 4.2 bands and average diversity index 0.454 per primer. By use of 5 published RAPD primers, we found much higher polymorphism, with an average of 19 bands and an average diversity index 0.912 per primer. Dendrograms with Principal Component analysis based on SSR and RAPD data did not show association between genetic diversity of the isolates and their geographic origin.

Key words: tan spot, *Pyrenophora tritici-repentis*, PCR detection marker, SSR, RAPD.

INTRODUCTION

The fungus *Pyrenophora tritici-repentis* (PTR) (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (DTR) (Died) Shoem., Shoemaker, 1962) is one of the main wheat pathogens, which causes tan spot of wheat and can cause yield losses from 3% to 53%. This disease is mostly spread in USA, Canada, Argentina, but in recent years, it has become important component of the leaf spot complex also in European countries, including Slovakia. Increased incidence and severity of tan spot is connected with shifts from conventional tillage and stubble burning to conservation tillage, shorter crop rotations and continuous wheat cultivation (De Wolf et al., 1998). *P. tritici-repentis* is not easy to study because of the high level of variation in disease symptoms, significant interactions among isolates, genotype, environment and physiological variation in virulence (Strelkov et al., 2002). This pathogen shows two distinct symptoms: tan necrosis and extensive chlorosis (Lamari and Bernier, 1989) and 11 pathotypes have been identified based on their virulence on a set of differential host genotypes (Gamba and Lamari, 1998; Strelkov et al., 2002; Lamari et al., 2003). Besides this, pathogen reduces quantity and quality of wheat grain (total yield, kernel weight, number of grains per head, total biomass, red-smudge symptoms); it also produces 4 known host specific toxins (Ptr Tox A, B, C, D), which can be important from the point of healthy food production.

Taking into account the high morphological and physiological variability of this pathogen, as well as its difficult differentiation from another important wheat pathogen – *Parastagonospora nodorum* – based on visual symptoms, correct, fast and precise diagnostic test for *P. tritici-repentis* in various plant life stages is demanded. Such diagnostic tests for pathogens are based on detection of pathogen DNA in plant material

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by DNA markers using PCR (e.g. Taylor, 1993; Reeves, 1995; Parry and Nicholson, 1996; Smith et al., 1996; Doohan et al., 1998; Matusinsky et al., 2010, 2011). In case of *P. tritici-repentis*, DNA markers (RAPD, AFLP, ISSRs, SSAP, IRAP) were used for detection of intraspecific genetic diversity among isolates of this pathogen (Di Zinno et al., 1998; Santos et al., 2002; Friesen et al., 2005; Singh and Hughes, 2006; Iram and Ahmad, 2007; Leisova et al., 2008; Moreno et al., 2008; Leisova-Svobodova et al., 2010).

The main objective of this work was the development of specific primers for PCR detection of *P. tritici-repentis* in wheat leaf and seed samples and its differentiation from *P. nodorum*. The aim of this study was also to develop microsatellite (SSR) primers and use them together with some published RAPD primers for study of genetic diversity in a group of mainly Slovak and Finnish isolates, to describe the relationship between this variability and geographic origin of pathogen isolates.

MATERIAL AND METHODS

Biological material

In years 2004-2006 thirteen *P. tritici-repentis* (PTR) isolates were obtained from primary wheat leaves collected in different regions of Slovakia, one isolate from Czech Republic and 10 isolates collected from Finland (Table 1).

The isolates of *Zymoseptoria tritici* and *Parastagonospora nodorum* used in this study were provided by Prof. W. Bockus (Kansas State University, USA), two isolates of *Rhynchosporium secalis* were provided by Dr. S. Salamati (Kvithamar Research Center, Stjørdal, Norway), isolate of *Pyrenophora teres* f. *maculata* was provided by Dr. V. Minarikova (Agricultural Research Institute Kromeriz, Ltd., Czech Republic), isolate of *P. teres* f. *teres* and isolate of *P. teres* f. *maculata* were provided by Prof. K.J. Williams (Cooperative Research Center for Molecular Plant Breeding, South Australian Research and Development Institute, Urbane, Australia) and isolates of *Fusarium culmorum*, *F. graminearum*, *F. poe* and *F. avenaceum* were provided by Dr. Šliková (National Agricultural and Food Centre, Research Institute of Plant Production Piestany, Slovakia). All isolates have been maintained on potato dextrose agar at room temperature (20 C ± 2°C) in dark.

Table 1. The list of *P. tritici-repentis* isolates used in this study; first letter in the names of the samples corresponds to *Drechslera* (D, anamorph) and second letter corresponds to the country of origin, i.e. Slovakia (S), Finland (F) and the Czech Republic (C)

Code	Country	Town	Collection year
DS1	Slovakia	Maly Saris	2005
DS2	Slovakia	Maly Saris	2005
DS4	Slovakia	Trencianske Teplice	2006
DS5	Slovakia	Kalna nad Hronom	2006
DS6	Slovakia	Vidina	2006
DS7	Slovakia	Spisska Bela	2006
DS8	Slovakia	Martin Valca	2006
DS9	Slovakia	Velke Ripnany	2006
DS10	Slovakia	Dolne Plachtince	2006
DS11	Slovakia	Bodorova	2006
DS12	Slovakia	Roznava	2005
DS13	Slovakia	Bzince pod Javorinou	2005
DS14	Slovakia	Turcianske Teplice	2006
DC	Czech Republic	Kromeriz	2006
DF2	Finland	Mietoinen	2004
DF3	Finland	Ylistaro	2004
DF15	Finland	Inkoo	2004
DF23	Finland	Inkoo	2004
DF31	Finland	Mietoinen	2004
DF32	Finland	Mietoinen	2004
DF35	Finland	Mietoinen	2004
DF42	Finland	Jokioinen	2004
DF45	Finland	Päikäne	2004
DF65	Finland	Inkoo	2004

Pathogenicity tests were conducted in greenhouse experiments by inoculating the leaves of four wheat cultivars (Salamouni, Glenlea, Katerwa and Coulter) provided by Dr. L. Lamari (Department of Plant Science, University of Manitoba, Winnipeg, Canada) at two leaf stage with conidial suspensions of 3×10^3 spores/ml of the *P. tritici-repentis* isolates by using pressurized sprayer. Samples of leaves were taken for testing eleven days after inoculation.

DNA extraction

The total fungal genomic DNA was extracted from single-spore-derived pathogen cultures grown on agar plates. Fungal mycelium was scraped off, homogenized in liquid nitrogen and DNA was extracted using the Adgen DNA Extraction System (Adgen Ltd.) and Wizard DNA Clean-Up system (Promega).

These extraction systems were also used for extraction of mixed DNA (wheat and pathogen DNA) from wheat leaves (1g of leaves from 1 plant) artificially infected by *P. tritici-repentis* and wheat seed (1 g from milled seeds) naturally infected by this pathogen.

Specific detection of *P. tritici-repentis*

PCR primer pair DTR1-F (5'-ACCAATATGAAGCCGGACTG-3') and DTR1-R(5'-CTCGGGAGAGAGACAAGACG-3') was designed for specific PCR detection of *Pyrenophora tritici repentis* from AF163060 sequence (GenBank database; www.ncbi.nlm.nih.gov). These primers amplify 382 bp long amplicon of the target nucleotide sequence from ITS1 - ITS2 regions of ribosomal RNA gene (Figure 1).

Figure 1. The structure of *P. tritici-repentis* ribosomal RNA operon and localization of primers DTR1-F, DTR1-R

Primers were designed using Primer3 online program (http://frodo.wi.mit.edu/, Rozen and Skaletsky, 2000) and specificity of primers was tested using BLAST (www.ncbi.nlm.nih.gov/BLAST). The PCR with DTR1 primer pair had the following optimal reaction mixture: 1 x PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$), 0.5 μM of each primer, 0.2 mM dNTP, 1 U of proofreading *Taq* DNA polymerase, recombinant (Invitrogen™), and 25 ng of DNA. Amplifications were performed in total volume of 25 μl using the GeneAmp® PCR System 9700 (Applied Biosystems®) with the following amplification conditions: initial denaturation at 94°C for 1 min, 35 cycles at 94°C for 30 s, 60°C for 30 s, 72°C for 1 min and extra extension at 72°C for 5 min. Electrophoretic detection of PCR products was performed in 1.4% agarose gel stained with ethidium bromide. PCR products after amplification of samples DC, DS1 and DF2 with primer pair DTR1 were purified by 3M sodium acetate and ethanol and then commercially sequenced (Laboratory of DNA sequencing, Faculty of Science, Charles University in Prague, Czech Republic). The resultant sequences originating from DC, DS1 and DF2 samples were subsequently deposited into the GenBank database (accessions KF447151 - KF447153).

PCR conditions for SSR

GenBank database and Primer3 programme were also used to design eight microsatellite primer pairs (Table 2) for assessment of genetic diversity in group of *P. tritici-repentis* isolates according to the following criteria: primer length 18-27 bp with optimum 20 bp, annealing temperature 57-63°C with optimum 60°C, GC content 20-80%, PCR product size 100-200 bp, dimers avoided as much as possible. PCR amplification was carried out in 20 μl reaction mixture containing 1 × PCR buffer (Invitrogen™), 1.5 mM $MgCl_2$ (Invitrogen™), 0.2 μM of each forward and reverse primer, 0.2 mM dNTP (Invitrogen™), 0.8 U of *Taq* DNA polymerase (Invitrogen™), and 25 ng of template DNA. Amplifications were run in the GeneAmp® PCR System 9700 (Applied Biosystems®) with the following conditions: initial denaturation at 94°C for 3 min, 35 cycles at 94°C for 30 s, annealing temperature for 30 s, 72°C for 40 s, and final extension at 72°C for 10 min. Five microlitres

of the reaction mixture were loaded into 6% denatured polyacrylamide gel and gel was stained by silver staining method (Bassam et al., 1991).

PCR conditions for RAPD

Five the most polymorphic RAPD primers published by Singh and Hughes (2006) were used for this analysis (Table 2). The composition of PCR mixture with RAPD primers and amplification conditions were according to Singh and Hughes (2006). Amplifications were performed in total volume of 15 μl using the GeneAmp® PCR System 9700 (Applied Biosystems). Electrophoretic detection of PCR products was performed in 1.5% agarose gel stained with ethidium bromide.

Statistical analyses

Polymorphic DNA segments amplified with all microsatellite and RAPD primers were considered as different bands, assigned a size (letter) and each band was scored as present (1) or absent (0). Based on the frequencies of bands, index of diversity (DI) $1-\sum P_{ij}^2$ (P_{ij} = frequency of the j-th band of the i-th primer), the probability of identity (PI) $\sum p^4_i+\sum\sum(2p_ip_j)^2$ and polymorphic information context (PIC) $1-(\sum p^2_i)-\sum\sum(2p^2_ip_j^2)$ were calculated (Weber, 1990; Weir, 1990; Paetkau et al., 1995). The unweighed pair group method of cluster analysis using arithmetic means (UPGMA) was used for grouping of genotypes. Dendrograms were constructed based on Jaccard´s similarity coefficient using DARwin 5.0.158 statistical software (http://darwin.cirad.fr/darwin; Perrier and Jacquemoud-Collet, 2006).

The presence/absence binary system was also used for Principal component analysis (PCA) using the Statgraphics Centurion XV.II statistical software.

RESULTS AND DISCUSSION

Specific detection of *P. tritici-repentis*

PCR methods were successfully used for detection of many various plant pathogens, including wheat pathogens such as *Blumeria graminis*, *Fusarium* spp., *Gaeumannomyces graminis*, *Microdochium nivale* spp., *Puccinia* spp., *Rhizoctonia cerealis*, *Zymoseptoria tritici*, *Septoria nodorum* (*Parastagonospora nodorum*), *Oculimacula* spp., *Tilletia tritici* (McCartney et al., 2003). According to expanding occurrence of *P. tritici-repentis* in different areas of Europe and its difficult differentiation from *P. nodorum* a specific DNA marker for identification of this pathogen is demanded too. In 2011, Mavragani et al. reported species-specific PCR-DGGE markers for distinguishing *Pyrenophora* sp. Their PCR primer pair was able to detect six *Pyrenophora* species (*P. tritici-repentis*, *P. teres*, *P. semeniperda*, *P. japonica*, *P. graminea* and *P. avenae*), but species were distinguishable from each other using more demanding DGGE analysis.

In our work, PCR primers for specific detection of *P. tritici-repentis* in leaf and seed samples of wheat were developed. These primers are usable for standard agarose gel electrophoresis. After optimisation of PCR reaction, primer pair DTR1-F and DTR1-R was proven to be specific with a PCR product of 382 bp amplified for all *P. tritici-repentis* isolates tested. To test its specificity, DNA samples from other fungal pathogenes (*P. graminea*, *P. teres* f. *teres*, *P. teres* f. *maculata*, *Z. tritici*, *P. nodorum*, *R. secalis*, *F. graminearum*, *F. culmorum*, *F. poe* and *F. avenaceum*) and healthy wheat leaves were screened by PCR amplification. Primer pair DTR1-F and DTR1-R did not cross - react with DNA of other pathogens or healthy wheat used in this study (Figure 2a).

Figure 2b shows PCR detection of *P. tritici-repentis* by DTR1-F and DTR1-R in wheat cultivars, eleven days after artificial infection of juvenile leaves and also detection of pathogen in naturally infected wheat seed from field conditions. The specific primer pair correctly amplified diagnostic band from both - infected leaves and seeds.

According to this result, a protocol for reliable detection of *P. tritici-repentis* and its differentiation from other pathogens (including *P. nodorum*) by use of PCR was made.

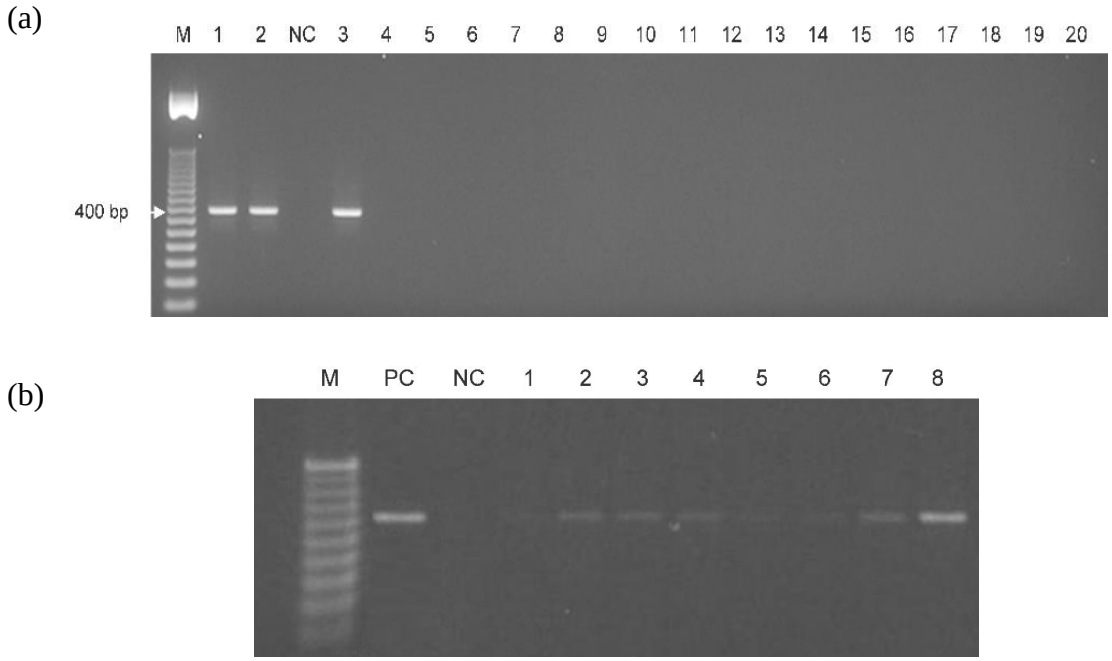

Figure 2. Agarose gel electrophoresis of PCR products after amplification with primer pair DTR1
(a): M - DNA marker; lines 1 – *P. tritici-repentis* DS1 (SK); 2 – *P. tritici-repentis* DC (CZ); NC – negative control; line 3 – P. tritici-repentis DF2 (FIN); 4 – healthy wheat leaves; 5 – *P. graminea* (SK); 6 – *P. teres* f. *teres* (AUS); 7 - *P. teres* f. *maculata* (AUS); 8 – *P. teres* f. *maculata* (CZ); 9 – *S. tritici* (CZ); 10 – *Z. tritici* (USA); 11 – *P. nodorum* (SK); 12 – *P. nodorum* (FIN); 13 – *P. nodorum* (USA); 14 – *R. secalis* (SK); 15, 16 – *R. secalis* (NOR); 17 – *F. graminearum* (SK); 18 – *F. poe* (SK); 19 – *F. avenaceum* (SK); 20 – *F. culmorum* (SK).
(b): M - DNA marker; PC – *P. tritici-repentis* positive control (SK) 382 bp; NC – negative control; lines 1-4 – wheat leaves infected with *P. tritici-repentis* (Salamouni, Glenlea, Katerwa and Coulter); lines 5-8 – naturally infected wheat seeds.

To verify if tested isolates really belong to *P. tritici-repentis* species, three isolates DC, DS1 and DF2 were chosen (one from each locality), and fragments of operons´ rDNA from these three isolates were sequenced and compared. PCR products amplified with DTR1-F and DTR1-R primers showed 98% sequence homology with DNA sequence of *Pyrenophora* (*Drechslera*) *tritici-repentis* accession number AF163060.1 (http://www.ncbi.nlm.nih.gov/nuccore/af163060.1) from which primers were designed, but the highest homology of 99% was showed with *P. tritici-repentis* accession number JX402048.1(http://www.ncbi.nlm.nih.gov/nuccore/406365250).

SSR and RAPD polymorphism within *P. tritici-repentis* isolates

The study of genetic diversity in pathogen populations is important for evaluation of pathogen capability to rapidly respond to changing environments and to overcome host resistance and fungicides (Peltonen et al., 1996). The success of local breeding programs for resistance to the disease depends to a large extent especially on the genetic variation within the pathogen population (Moreno et al., 2008). For the study of the genetic diversity in populations of various fungi, SSR markers have been used, as they are highly polymorphic between strains, co-dominant and highly reproducible compared with other markers. In this study, eight SSR primer pairs were designed for assessment of genetic diversity in the group of 24 mainly Slovak and Finnish *P. tritici-repentis* isolates. After PCR optimisation, three SSR primers (DTR6, DTR8 and DTR11) were monomorphic and five SSR primers showed polymorphism with 21 detected bands. The number of bands per primer varied from 8 (primer DTR7 with diversity index 0.545) to 2 (primers DTR9 and DTR10 with diversity index 0.079 and 0.485) (Table 2).

Gurung et al. (2013) observed similar results using 12 SSR markers and 439 isolates of *P. tritici repentis* from 5 continents. They reported 25 alleles with an average of 3 to 4 alleles per locus.

Table 2. Description of microsatellite and RAPD primers and statistical analyses based on their bands amplified in group of 24 *P. tritici-repentis* isolates
(DI = diversity index, PI = probability index, PIC = polymorphic information content)

	Primer (locus) name	Gen Bank	Core motif	Primer sequence	Annealing temp.	No. of bands	DI	PI	PIC
Microsatellite primers	DTR4	F163060	$(TC)_n$	TCAAGCTTTGCTTGGTGTTG GGCTGCCAATGATTTTAAGG	60°C	3	0.536	0.309	0.440
	DTR5	AY425485	$(CT)_n$	AGCCTATGCGACCCTAACCT AGAAGGTGTTGCTGAAATGTGA	60°C	6	0.619	0.081	0.618
	DTR6	AY083456	$(GCC)_n$	CTCGCTGCAGGATCATTCTT TAAGCACCCCTAGCCTAGCA	60°C	1	-	-	-
	DTR7	AY425482	$(ATT)_n$	AGGCCTGCGAGATACCCTAT CGCTTGATACCACCAAGTCA	60°C	8	0.545	0.184	0.545
	DTR8	AY425482	$(CA)_n$	CAGACGCCAAAACGTTTACA TTCGTCAGCTTTGGGATCTT	60°C	1	-	-	-
	DTR9	DQ919068	$(AT)_n$	AAAGTGGTATAACCCGACAGG CGTTTCAGCCACCCATTAGT	60°C	2	0.079	0.849	0.077
	DTR10	DQ919068	$(TA)_n$	TTGGAATGTCTGAAAGACTAGGAA CATGGTTACAGAACCTAGCATAAAA	59°C	2	0.485	0.383	0.368
	DTR11	AF004369	$(CG)_n$	CCGCCAACTCTTCTGAACTC CGAGCCTATAGCACCAGGTC	59°C	1	-	-	-
Average values						4.2	0.454	0.362	0.409
RAPD primers	UBC 517	Singh and Hughes (2006)		GGTCGCAGCT		16	0.910	0.001	0.909
	UBC 584			GCGGGCAGGA		23	0.934	0.000	0.934
	UBC 598			ACGGGCGCTC		19	0.906	0.001	0.905
	UBC 600			GAAGAACCGC		19	0.913	0.002	0.912
	Operon H-19			CTGACCAGCC		18	0.896	0.002	0.894
Average values						19	0.912	0.001	0.911

Similar degree of SSR polymorphism was also found by Kaye et al. (2003) in *Magnaporthe grisea* isolates, by Cardoso and Wilkinson (2008) in *Lasiodiplodia theobromae* isolates or by Scott and Chakraborty (2008) in *Fusarium pseudograminearum* isolates. Bogacki et al. (2010) described slightly higher polymorphism using 20 SSR loci in South Australian *P. teres* populations. On the other hand much higher genetic polymorphism was detected among 37 *P. tritici-repentis* isolates in Argentina by Moreno et al. (2008). They found 62 bands by use of five ISSR markers and only 2 out of 37 isolates were not differentiated.

In our study, the dendrogram created using 24 *P. tritici-repentis* isolates based on data of 5 SSR loci expressed distinction of groups with maximum and minimum similarities (Figure 3a).

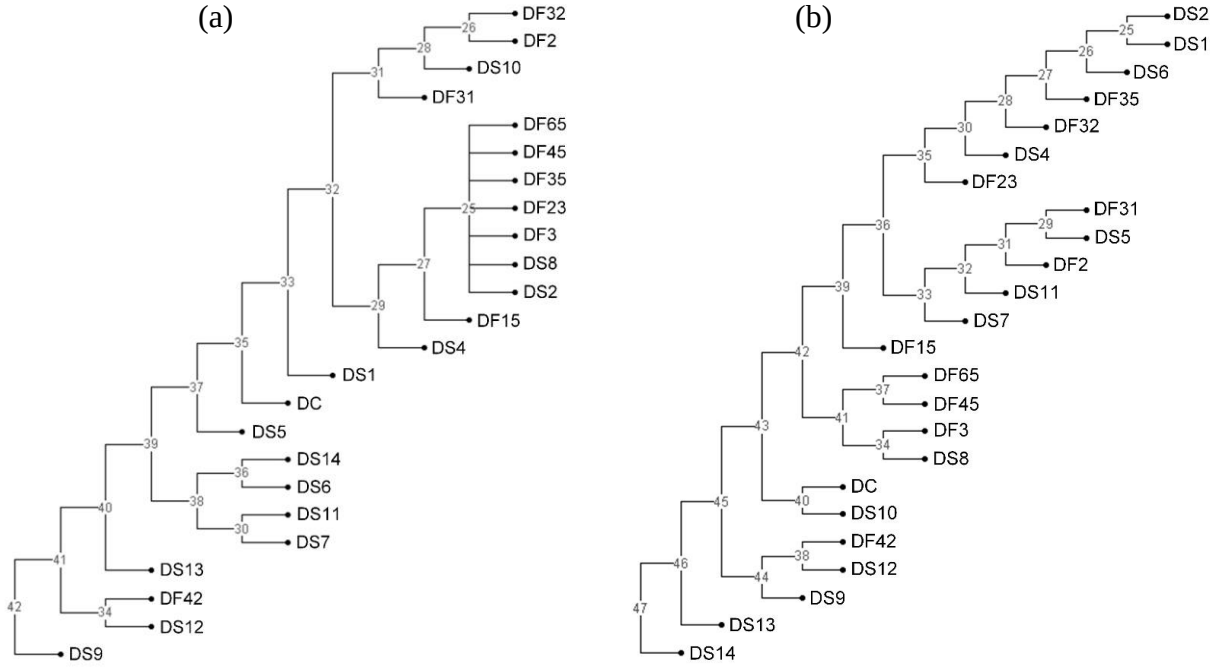

Figure 3. Relationships between 24 *P. tritici-repentis isolates* originating from Slovakia, Finland and the Czech Republic (designation in dendrograms – DS, DF and DC, respectively). Dendrograms were constructed using Unweighted pair group method with arithmetic mean generated from Jaccard distance matrix of: (a) 5 SSR loci and (b) 5 highly polymorphic RAPD primers.

There are some indications of isolates clustering into 3 groups: the first group with the most similar isolates – 4 Slovak and 9 Finnish isolates, the second group with 6 Slovak isolates and 1 Czech isolate and the third group with the most different Slovak isolates DS9, DS12, DS13 and Finnish isolate DF42. Altogether 7 isolates (2 Slovak DS2, DS8 and 5 Finnish DF3, DF23, DF35, DF45 and DF65) were not distinguished from each other and they differed from two other non-differentiated Finnish isolates (DF2 and DF32) only by one band. Since these loci did not display so high polymorphism, more SSR primers may be required to discriminate between all isolates. Principal component analysis (PCA) constructed from SSR data of 24 *P. tritici-repentis* isolates as well as cluster analysis showed similar grouping between Slovak and Finnish isolates (Figure 4a).

By use of five published highly polymorphic RAPD primers in group of 24 *P. tritici-repentis* isolates, much higher polymorphism was found (95 bands in total). The number of bands per primer varied from 16 (primer UBC517 with diversity index 0.910) to 23 (primers UBC584 with diversity index 0.934) (Table 2). Similar degree of RAPD polymorphism was found by Santos et al. (2002) between twelve *P. tritici-repentis* isolates obtained from different locations in Brazil. They found 45 bands by use of 9 RAPD markers. In the dendrogram constructed by UPGMA analysis of RAPD data (Figure 3b) similar grouping of the most different isolates DS14, DS13, DS9, DS12 and DF42 can be observed and this result is similar to SSR data analysis. By use of RAPD markers only two Slovak isolates (DS1 and DS2) from the same locality (Maly Saris) and from the same year (2005) were not differentiated. It proves the high potential of RAPD markers for evaluations of *P. tritici-repentis* genetic diversity, but the disadvantage of these markers is lower reproducibility and higher subjectivity in evaluation of RAPD profile. Principal component analysis created using RAPD data showed higher variability between isolates than PCA analysis from SSR data. Two incurred groups of isolates were distinguishable from each other (Figure 4b).

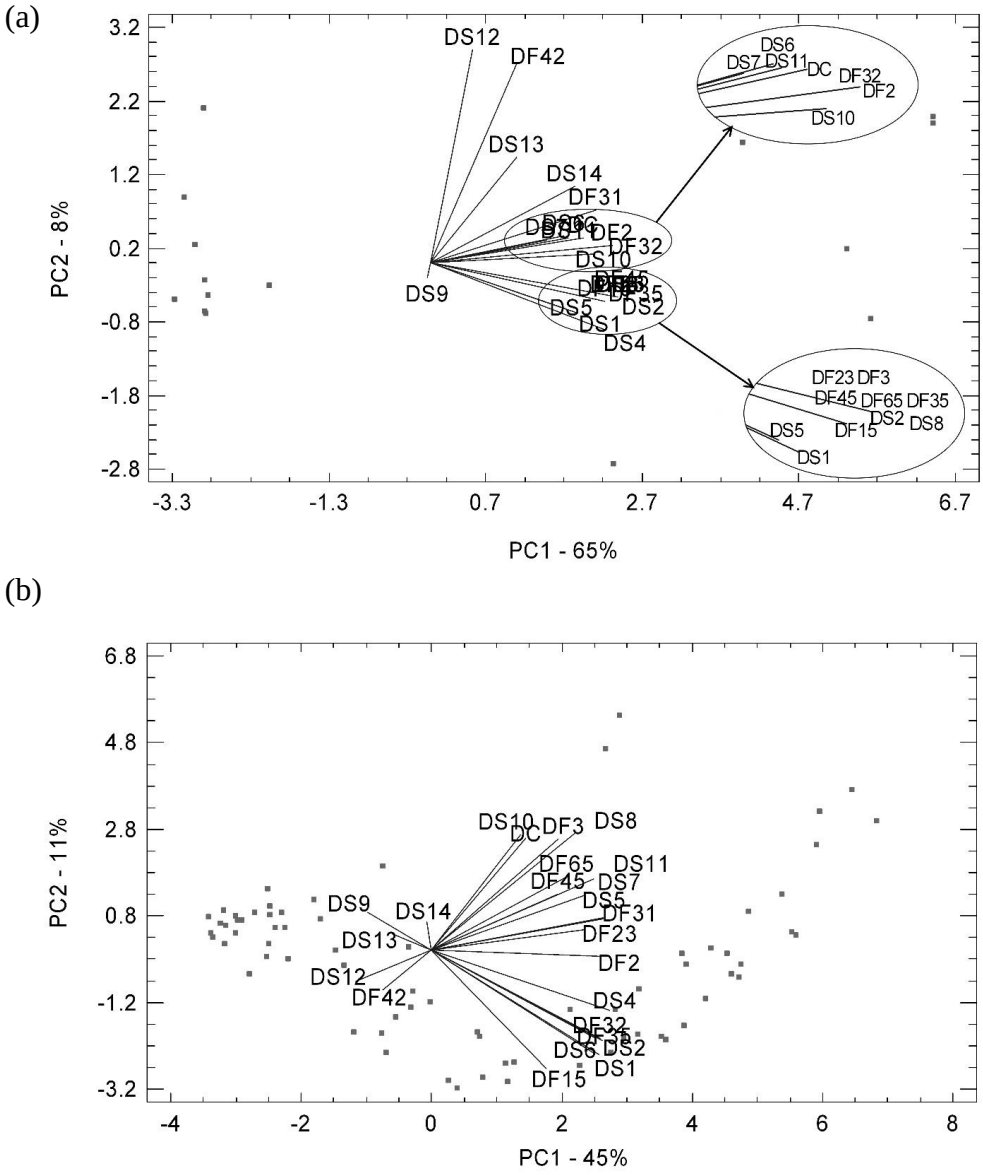

Figure 4. Two-dimensional PCA biplots constructed using 24 *P. tritici-repentis* isolates originating from Slovakia, Finland and the Czech Republic (designation in biplots - DS, DF and DC, respectively)

(a) Biplot based on data of 5 SSR loci and 21 detected alleles. The first and second principal component explained 65% and 8% (respectively) of the variability in the data with total of 73% of the variability.

(b) Biplot based on data of 5 highly polymorphic RAPD primers and 95 detected PCR bands. The first and second principal component explained 45% and 11% (respectively) of the variability in the data with total of 56% of the variability. Biplots display both the observation and the variables on a single plot. The point symbols correspond to the observation and the ends of the solid lines correspond to the variables.

High levels of genetic polymorphism among *P. tritici-repentis* isolates has been found with the use of RAPD, AFLP and ISSR markers, but often without correlation with geographic origin, toxin production, pathogenicity or race classification of the isolates (Santos et al., 2002; Friesen et al., 2005; Singh and Hughes, 2006; Iram and Ahmad, 2007; Leišová et al., 2008; Moreno et al., 2008). Similarly high level of genetic and genotypic diversity was found by AFLP in *R. collo-cygni* (Leisova-Svobodova et al., 2012). Also in this work, by cluster and PCA analyses of SSR and RAPD data, association between genetic diversity of the isolates and the area from which the isolates were collected was not detected. The isolates from the same region appeared in different groups and the most similar isolates were from different geographic regions. The reason for

high genetic variability between isolates independent of the race structure or geographic origin can be the occurrence of sexual reproduction in nature and long-distance dispersal of inoculum of this pathogen (Singh and Hughes, 2006), as its spores can travel 10-200 km (De Wolf et al., 1998) and this pathogen is seed borne also and so fungal inoculum can travel long distances by seed transport. Only recently Leišová-Svobodová et al. (2010) found significant correlation between presence or absence of two SSAP and five IRAP markers and the presence or absence of the *Ptr Tox A* gene, which is considered to be the main pathogenicity factor of this fungus. However, they showed also that the variability of isolates established by retrotransposon analysis cannot be explained by geographic origin.

CONCLUSIONS

Based on our results a protocol for reliable PCR detection of *P. tritici-repentis* in leaf and seed samples of wheat and for its distinguishing from other pathogens by use of specific primer pair DTR1-F and DTR1-R was made. This protocol can be useful in production of healthy food as well as in early and precise identification and differentiation of this pathogen in plant protection processes. Our study of intraspecific variability in group of *P. tritici-repentis* isolates mainly from Slovakia and Finland did not show any relation to geographic origin, but proved the suitability of SSR and RAPD markers for genetic diversity studies of this pathogen. Knowledge of the pathogen genetic diversity helps in the development of a successful disease management, especially in the development of resistant cultivars, effective fungicides and biological control agents.

Acknowledgements

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