Diversity of Oat Crown Rust (*Puccinia coronata* f.sp. *avenae*) Isolates Detected by Virulence and AFLP Analyses

Hana KLENOVÁ-JIRÁKOVÁ[1], Leona LEIŠOVÁ-SVOBODOVÁ[2], Alena HANZALOVÁ[2] and Ladislav KUČERA[2]

[1]Ministry of Environment of the Czech Republic, Prague, Czech Republic;
[2]Division of Plant Genetics, Breeding and Product Quality, Crop Research Institute, Prague-Ruzyně, Czech Republic

Abstract

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Forty *Puccinia coronata* f.sp. *avenae* isolates from several European countries and Israel were tested for virulence to 18 differential oat lines. Amplified Fragment Length Polymorphism (AFLP) analysis was used to evaluate diversity among the studied isolates. Twenty-nine different pathotypes were identified. The prevailing occurrence of pathotypes with a limited number of virulence genes may indicate that oat cultivars grown in Europe possess the limited number of resistance genes. A total of 501 AFLP polymorphic fragments were scored in the studied isolates using twelve primer combinations. All isolates had the unique AFLP molecular pattern. The genetic similarity of isolates from Serbia. Austria and from the Czech Republic indicates that oat crown rust urediniospores may often migrate to particular areas. The number of virulence genes in isolates also seems to play an important role in the clustering. Most isolates possessing a lower number of virulence genes (0–4) were grouped into two clusters, whereas another cluster was composed of a majority of isolates with 4–7 virulence genes. A significant correlation relationship of 0.187 (P = 0.007) was found between AFLP and virulence/avirulence genes based on distance matrices.

Keywords: *Puccinia coronata* f.sp. *avenae*; oat crown rust; oat; virulence; AFLP; diversity

Crown rust is caused by the basidiomycete fungus *Puccinia coronata* Cda. f.sp. *avenae* P. Syd.et Syd. The pathogen can reduce either grain yield (Frey *et al.* 1973) or grain quality traits (Šebesta *et al.* 1972; Simons & Browning 1961). This obligate biotrophic fungus occurs worldwide wherever cultivated or wild oat species occur (Simons 1985). in Europe including the Czech Republic, in the Middle East (Šebesta *et al*. 2003), North and South Africa (Niekerk *et al*. 2001; Šebesta *et al*. 2003), Canada (Chong & Zegeye 2004), and South America (Leonard & Martinelli 2005), etc.

Oat crown rust has a life cycle involving sexual and asexual reproduction associated with host alternation, in which five different types of spores are generated (Váňa 1996). The disease is spread by urediniospores which are dispersed by wind over long distances, similarly like another cereal rust species, e.g. *Puccinia striiformis* on wheat (Brown & Hovmøller 2002). In the Czech Republic, oat is cultivated as a spring crop. The pathogen survives winter only on a small scale on species of wild oats or by means of an alternate host, *Rhamnus cathartica* L. It is assumed that the main source of

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the inoculum to infect cultivated oats comes from urediniospores which are dispersed throughout the area by means of prevailing winds (Šebesta *et al.* 1999a).

Genetic resistance is the most effective method for controlling the disease (Harder & Haber 1992). Studies of genetic interactions between pathogens and host plants were first made by Flor (1956). He postulated a gene-for-gene hypothesis. The application of this hypothesis enables to determine the approximate number of resistance and virulence genes and their combinations (Šebesta 1991). Numerous studies of physiological specialisation proved that oat crown rust populations are extremely diverse (Chong & Zegeye 2004), consisting of many pathotypes with appropriate virulence to commonly used resistance genes.

The knowledge of genetic diversity of plant pathogens on a DNA level is considered to be valuable for population studies. However. the genetic backgrounds of oat crown rust populations on a DNA level have not been studied as extensively as other cereal rust species. Brake *et al*. (2001) analysed genetic variability in a small set of Australian isolates of *P. coronata* f.sp. *avenae* using DAF (DNA Amplification Fingerprinting) markers. They found two sub-populations within the isolates. which probably resulted from exotic introduction of genetically distinct pathogen isolates. Dracatos *et al*. (2006) developed EST-SSR markers for the *P. coronata* f.sp. *lolii* P. Syd. *et* Syd., which is also utilisable for closely related fungal species (*Puccinia* spp.). Molecular analyses of other rust species populations have already been done (Kolmer 2001; Hovmøller *et al*. 2002; Justesen *et al*. 2002).

The main objectives of our contribution were (*i*) to assess the physiological specialisation of *P. coronata* f.sp. *avenae* isolates of different geographical origins collected from 1999 to 2005 using analyses of virulence, (*ii*) to investigate the genetic variation of these isolates by means of AFLP analysis, (*iii*) to determine if there exists a correlation between genetic variability, as detected by AFLP, and the physiological specialisation of the oat crown rust isolates.

MATERIAL AND METHODS

Fungal isolates. Thirty-seven samples of *P. coronata* f.sp. *avenae* collected in 1999–2000 and 2004–2005 were used. These samples were collected from trials or commercial fields in Austria, Belarus, Czech Republic, Estonia, Hungary, Israel, Serbia, and Sweden. One monopustule isolate was made from each sample. The monopustule isolates were multiplied on the highly susceptible oat cv. Neklan to obtain a sufficient amount of urediniospores for the analysis. Urediniospore yields obtained from three different samples were subdivided into two replications so that a total of forty isolates of *P. coronata* f.sp. *avenae* were prepared. The designation of all isolates is listed in Table 1.

Phytopathological analysis. The physiological specialization of *P. coronata* f.sp. *avenae* isolates was evaluated on 18 single gene oat lines (*Avena sativa*) with seedling resistance to *P. coronata* f.sp. *avenae, Pc*38, *Pc*39, *Pc*40, *Pc*45, *Pc*46, *Pc*48, *Pc*50, *Pc*51, *Pc*52, *Pc*54, *Pc*56, *Pc*58, *Pc*59, *Pc*62, *Pc*64, *Pc*68, *Pc*94, and *Pc*96. The highly susceptible oat cv. Neklan was used as a control of successful infection in the tests. The analyses of virulence were carried out according to Klenová and Šebesta (2006). The designation of *P. coronata* f.sp. *avenae* pathotypes was carried out by a four-letter code for the virulence combinations according to Chong *et al*. (2000).

Virulence/avirulence data from all *Pca* isolates were converted into a binary matrix (the value of 1 for virulent reaction or 0 for avirulent reaction) which was used to construct a similarity matrix between all pairs of *Pca* isolates. A dendrogram was constructed using the unweighted pair-group method with arithmetic averages (UPGMA) of cluster analysis in Statistica for Windows (StatSoft, Inc., Prague, Czech Republic).

DNA extraction. Urediniospores were harvested from infected oat leaves into sterile glass tubes using a glass cyclone collector connected to a suction-pump. Approximately 80–100 mg of freeze-dried urediniospores of each *P. coronata* f.sp. *avenae* isolate was used for DNA extraction, which was carried out either immediately after harvest or after the spores were frozen to prevent the presence of adventitious microorganisms in the samples. DNA was extracted from urediospores according to the modified protocol of Leišová *et al.* (2005) using an extraction buffer (0.35M sorbitol, 0.1M Tris, 5mM ethylenediaminetetraacetic acid (EDTA), pH 7.5) or a lysis buffer (2M NaCl, 0.2M Tris, 50mM EDTA, 2% cetyltetramethylammonium bromide (CTAB), pH 7.5). Proteins and polysaccharides

Table 1 Virulence specialisation, geographic origin, and year of sampling of *Puccinia coronata* f.sp. *avenae* isolates

Ineffective oat resistance genes to *Puccinia coronata* f.sp. *avenae*	Pathotype designation	*Pc* isolate designation	Geographic origin	Year of sampling	Clusters	
					virulence	DNA
	BBBB	*Pca*12	CZ	2004	2	3
	BBBB	*Pca*23	YU	2000	2	3
*Pc*64	BBBC	*Pca*29	CZ	2004	2	5
*Pc*38	BLBB	*Pca*28	CZ	2005	2	3
*Pc*38	BLBB	*Pca*39	SWE	1999	2	2
*Pc*45	GBBB	*Pca*6	YU	2000	2	3
*Pc*38, *Pc*56	BLBG	*Pca*34**	AUT	2000	2	3
*Pc*38, *Pc*56	BLBG	*Pca*36	AUT	2000	2	3
*Pc*38, *Pc*56	BLBG	*Pca*37**	AUT	2000	2	3
*Pc*38, *Pc*56	BLBG	*Pca*15*	CZ	2004	2	3
*Pc*38, *Pc*56	BLBG	*Pca*20*	CZ	2004	2	3
*Pc*38, *Pc*56	BLBG	*Pca*27	CZ	2005	2	3
*Pc*38, *Pc*56	BLBG	*Pca*26	YU	2004	2	5
*Pc*38, *Pc*64	BLBC	*Pca*33	AUT	2000	2	3
*Pc*38, *Pc*62	BLBD	*Pca*16	CZ	2005	2	3
*Pc*38, *Pc*45	BLBB	*Pca*10	SWE	1999	2	3
*Pc*40, *Pc*64	LBBC	*Pca*11	CZ	2005	2	1
*Pc*40, *Pc*64	LBBC	*Pca*8	SWE	2000	2	4
*Pc*45, *Pc*56	GBBG	*Pca*38	EST	2000	2	3
*Pc*40, *Pc*56	LBBG	*Pca*4	SWE	1999	2	4
*Pc*38, *Pc*40, *Pc*68	LMBB	*Pca*32	AUT	2000	2	3
*Pc*38, *Pc*45, *Pc*56	GLBG	*Pca*40	CZ	2000	2	3
*Pc*40, *Pc*45, *Pc*56	QBBG	*Pca*25	CZ	2004	2	5
*Pc*38, *Pc*45, *Pc*62	GLBD	*Pca*17	HU	2005	3	3
*Pc*40, *Pc*45, *Pc*54	QBBL	*Pca*31	ISR	2004	2	5
*Pc*40, *Pc*45, *Pc*46, *Pc*64	SBBC	*Pca*14	EST	2004	4	4
*Pc*40, *Pc*45, *Pc*46, *Pc*51	SBLB	*Pca*30	EST	2005	4	5
*Pc*39, *Pc*40, *Pc*45, *Pc*46	SGBB	*Pca*1	ISR	2005	4	4
*Pc*38, *Pc*39, *Pc*40, *Pc*45, *Pc*94	QQBB-94	*Pca*19	CZ	2005	3	3
*Pc*38, *Pc*40, *Pc*45, *Pc*50, *Pc*94	QLBQ-94	*Pca*2	ISR	2000	3	3
*Pc*38, *Pc*40, *Pc*45, *Pc*54, *Pc*64, *Pc*94	QLBM-94	*Pca*35	YU	2000	3	3
*Pc*40, *Pc*45, *Pc*46, *Pc*51, *Pc*54, *Pc*64	SBLM	*Pca*21***	EST	2004	4	4
*Pc*40, *Pc*45, *Pc*46, *Pc*51, *Pc*54, *Pc*64	SBLM	*Pca*13***	EST	2004	4	4
*Pc*40, *Pc*45, *Pc*46, *Pc*51, *Pc*64, *Pc*96	SBLC-96	*Pca*7	YU	2004	4	4
*Pc*40, *Pc*45, *Pc*46, *Pc*51, *Pc*54, *Pc*58, *Pc*64	SBNM	*Pca*5	CZ	2004	4	3
*Pc*38, *Pc*40, *Pc*45, *Pc*46, *Pc*51, *Pc*54, *Pc*64	SLLM	*Pca*24	CZ	2004	4	3
*Pc*40, *Pc*45, *Pc*46, *Pc*51, *Pc*54, *Pc*56, *Pc*64	SBLR	*Pca*3	CZ	2005	4	4
*Pc*40, *Pc*45, *Pc*46, *Pc*51, *Pc*54, *Pc*58, *Pc*64	TBNM	*Pca*9	YU	2004	4	4
*Pc*38, *Pc*39, *Pc*40, *Pc*45, *Pc*46, *Pc*48, *Pc*50, *Pc*54, *Pc*58, *Pc*59, *Pc*64	TSFM	*Pca*18	ISR	2004	1	4
*Pc*38, *Pc*39, *Pc*40, *Pc*45, *Pc*46, *Pc*48, *Pc*50, *Pc*54, *Pc*58, *Pc*59, *Pc*64	TSFM	*Pca*22	ISR	2004	1	4

*Pca*1 – *Pca*40 designation of *Puccinia coronata* f.sp. *avenae* isolates; *, **, ***designation of three pairs of isolates originating from three different *Puccinia coronata* f.sp. *avenae* samples used as the controls of reproducibility; AUT: Austria, CZ: Czech Republic, EST: Estonia, HU: Hungary, ISR: Israel, SWE: Sweden, YU: Serbia; Four-letter code – designation of virulence combination according to the nomenclature system for *Puccinia coronata* f.sp. *avenae* (Chong *et al.* 2000)

were removed using a mixture of chloroform and isoamylalcool (24:1) and 5% solution of CTAB with a minimum of 0.5M NaCl concentration. DNA was precipitated by one volume of absolute ethanol and diluted in an appropriate volume of the $TE_{0.1}$ buffer. DNA was run in a 0.8% agarose gel (60V) to verify the quality and the concentration. The λ *Hind*III (Fermentas. Vilnius, Lithuania) size standard was used.

AFLP analysis. AFLP analysis was carried out according to the AFLP™ Plant Mapping protocol (Applied Biosystems, Foster City, USA). The restriction endonucleases *Mse*I and *Eco*RI (Vos *et al*. 1995) were used for restriction and ligation. *Mse*I primers and fluorescently marked *Eco*RI primers were used for selective amplification. The reaction was performed as a multiplex PcR in the reaction mixture of 10 µl [0.2mM dNTPs, 1µM *Mse*I primer, 3 × 0.5µM *Eco*RI primers, 1 U *Taq* polymerase (Qiagen GmbH, Hilden, Germany). 1 × buffer with 10mM $MgCl_2$ and 1 µl diluted (1:20) preselective amplification reaction] in an ABI PRISM 7700 cycler (Applied Biosystems, Foster City, USA). The amplification products were separated by capillary electrophoresis in ABI PRISM 310 (Perkin Elmer Applied Biosystems, Foster City, USA). Rox500 (PN 401734, Applied Biosystems, Foster City, USA) was used as the internal size standard. Chromatograms were processed by the software Genescan and Genotyper (Applied Biosystems, Foster City, USA).

Polymorphic AFLP markers were scored as binary characters (1 – presence, 0 – absence) for each isolate; the monomorphic markers across all *P. coronata* f.sp. *avenae* isolates were not included. A dendrogram was constructed using the neighbour-joining (NJ) method of cluster analysis in Statistica for Windows (StatSoft, Inc., Prague, Czech Republic).

The AFLP data were further analysed using a set of programs in PHYLIP. the PHYLogeny Inference Package v3.6. (Felsenstein 1989, 2004). Phylogenetic trees were constructed using the neighbour-joining method. Consensus tree program, version 3.63, was used for consensus tree construction.

The correlations between AFLP and virulence/avirulence genes based on distance matrices were analysed by the Mantel test with 100 000 permutations (Mantel 1967). The calculation was carried out using zt-win software (Bonnet & Van der Peer 2002).

The genetic diversity index (DI) based on the Gini-Simpson index (Gini 1912) was calculated according to the formula: $DI = 1 - \Sigma p_i^2$, where p_i is the frequency of the i^{th} allele.

RESULTS

Phytopathological analysis

Forty isolates of *P. coronata* f.sp. *avenae* were the subjects of analyses of virulence. Each isolate was tested at least twice on a set of 18 differentials. Their virulence specialisations are listed in Table 1. Twenty-nine different pathotypes were recognised among the isolates possessing 0–11 virulence genes. Virulence patterns were different in all areas where the isolates were collected. Twenty-three pathotypes were identified in individual cases, five pathotypes were recorded twice, and the pathotype BLBG (virulent to the resistance genes *Pc*38 and *Pc*56) was identified seven times within a set of isolates.

Using the UPGMA method of cluster analysis of virulence data. the isolates were differentiated into four virulence clusters based on a 0.2 level of linkage distance. The clusters differ from each other in a number of virulence genes and their combinations (Figure 1). The first cluster covers two isolates from Israel with eleven virulence genes (Table 1).

The second largest cluster involves 24 isolates. each possessing from zero to three different virulence genes. The isolates are virulent to resistance genes *Pc*38 (14 isolates), *Pc*56 (11 isolates), *Pc*40 and *Pc*45 (by six isolates). Four isolates are virulent to *Pc*64. Rare virulence to the resistance genes *Pc*54 and *Pc*62 was found in two single isolates. No virulence to any of the 18 differentials was found in two isolates. The isolates included in this cluster originated mostly from Austria, the Czech Republic, Serbia, and Sweden but there are also two single isolates from Estonia and Israel.

The third cluster comprises four isolates possessing from three to six virulence genes. All isolates are virulent to the resistance genes *Pc*38 and *Pc*45; all but one isolate are virulent to *Pc*40 and *Pc*94. Every single isolate in the cluster is virulent to some of the resistance genes that are considered to be highly effective; *Pc*39, *Pc*50, *Pc*54, and *Pc*62. The isolates are geographically heterogeneous.

The last cluster comprises ten isolates possessing from four to seven virulence genes. The isolates are uniformly virulent to the resistance genes *Pc*40,

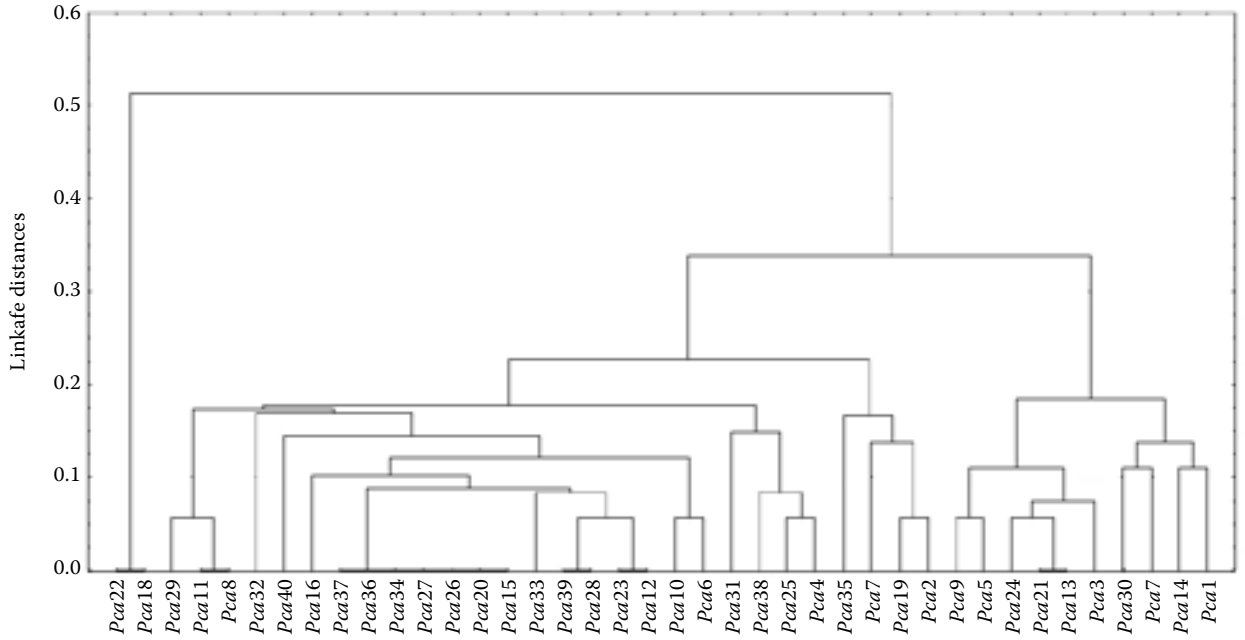

Figure 1. Dendrogram based on the data of virulence combinations of *Puccinia coronata* f.sp. *avenae* isolates

*Pc*45, and *Pc*46. Furthermore, resistance genes *Pc*51, *Pc*54, and *Pc*64 were frequently overcome by the isolates included in this cluster. Rare virulence to the resistance genes *Pc*39, *Pc*58, and *Pc*96 was identified in three single isolates; one isolate was virulent to two highly effective resistance genes *Pc*50 and *Pc*58, which is a unique finding. The isolates were collected in Estonia, the Czech Republic, Serbia, and Israel.

AFLP analysis

Forty-two primer combinations were initially screened for their capacity to reveal polymorphism among two *P. coronata* f.sp. *avenae* isolates. On the basis of the screening, twelve primer combinations which produced polymorphic and well readable fragments were selected for further analysis with all fungal isolates. For each primer combination. 13-58 DNA fragments, ranging in size from 50 bp to 495 bp, were detected. A total of 501 AFLP polymorphic fragments were scored with all the isolates; this corresponds to an average of 41 polymorphic bands per primer combination. The number of polymorphic alleles and the average DIs per primer combination are listed in Table 2.

The neighbour-joining clustering analysis showed five clusters based on a 0.17 level of linkage distance (Figure 2). The dendrogram consists of two clusters covering single isolates and three robust clusters. The first cluster consists of the isolate with two virulence genes which originated from the Czech Republic; the second cluster is represented by the Swedish isolate with one virulence gene only.

Table 2 Multiplexed AFLP primer combinations used in this study

MseI	EcoRI-fam EcoRI-ned EcoRI-joe	Number of polmorphic alleles	Average DI
CAT	TC	38	0.254
	TA	23	0.200
	AT	45	0.243
CTC	TC	17	0.256
	TA	13	0.293
	AT	48	0.255
CTT	TC	53	0.292
	AT	52	0.285
	AA	55	0.299
CAA	TC	58	0.228
	TT	50	0.277
	TA	49	0.278

DI – Diversity Index

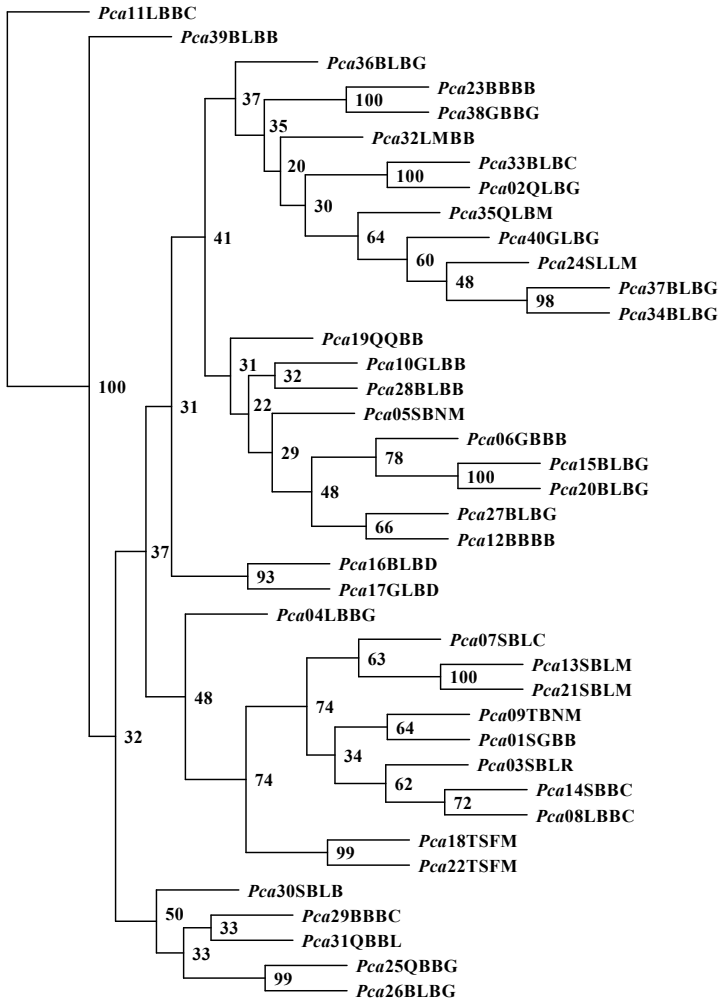

Figure 2. Neighbour-joining phylogenetic tree of genetic diversity of *Puccinia coronata* f.sp. *avenae* as detected by AFLP analysis. Phylogenetic trees were constructed using the neighbour-joining method and Consensus tree program. The numbers on branches indicate the number of times the partition of the isolates into the two sets which are separated by that branch occurred among the trees, out of 100 trees

The third largest cluster comprises 22 isolates possessing from zero to seven virulence genes. The most frequent virulence patterns, with up to three virulence genes within the isolates, were observed in this cluster. Most isolates were collected in the countries which are in close geographical proximity: Austria, Hungary, and the Czech Republic. The fourth cluster involves 11 isolates, harbouring from 4 to 11 virulence genes except for two isolates with two virulence genes only. The majority of isolates in this cluster were collected in Israel, Estonia, Serbia, and Sweden. The last cluster comprises five geographically heterogeneous isolates of different virulence patterns, with up to four virulence genes.

Genetic diversity within the group of isolates with the same virulence specialization was shown. as exemplified in the group of isolates BLBG, i.e. *Pca*15 (*Pca*20), *Pca*26, *Pca*27, *Pca*34 (*Pca*37), and *Pca*36. These isolates are grouped in the third cluster with the exception of the isolate *Pca*26, which is included in the first cluster. Similarly, the isolates *Pca*11 and *Pca*8 (both designated LBBC) or *Pca*28 and *Pca*39 (both designated BLBB) are included in different clusters.

A significant correlation of 0.187 ($P = 0.007$) was found between AFLP and virulence/avirulence genes based on distance matrices.

DISCUSSION

In the case of phytopathological analysis, physiological specialisation surveys serve to estimate the relative prevalence and distribution of virulence phenotypes and to detect shifts toward the virulence to resistance genes being used in oat breeding programs (Chong & Zegeye 2004). Forty *P. coronata* f.sp. *avenae* isolates collected in six European countries and Israel were included in our study. In total 29 different pathotypes were found among the isolates and it shows a high variability

of virulence patterns in all the monitored areas and years even though the set of studied isolates was relatively small.

Our results confirm numerous studies of *P. coronata* f.sp. *avenae* virulence showing the extreme diversity of pathogen populations, consisting of many virulence phenotypes (Simons 1985; Harder & Haber. 1992; Chong 2000; Leonard & Martinelli 2005). The majority of pathotypes in our analysis were identified only in individual cases (Table 1). However. pathotypes harbouring fewer virulence genes occurred more frequently. The pathotype BLBG (two virulence genes, i.e. *Pc*38 and *Pc*56) was identified repeatedly in three geographically close countries including the Czech Republic in the years 2000, 2004, and 2005. Similarly, BBBB pathotypes (no virulence genes), BLBB (one virulence gene,i.e. *Pc*38) or LBBC (two virulence genes. i.e. *Pc*40, *Pc*64) were identified twice in the set. According to Šebesta (1970) the dominance of pathotypes with low numbers of virulence genes might be caused by the prevailing cultivation of susceptible oat cultivars. In such cases, the race specific resistance could not be asserted as a selective factor. For instance, all oat cultivars with the exception of cv. Vok were evaluated as susceptible to *P. coronata* f.sp. *avenae* in the Czech Republic (Klenová 2007).

However, several pathotypes with four virulence genes and more were also found (Table 1). The pathotypes with the highest number of virulence genes were obtained from Israel, a centre of diversity for *Avena sterilis* L., the progenitor of cultivated oat (Leggett & Thomas 1995). Oat crown rust populations have shown to be highly polymorphic for virulence corresponding to the race-specific resistances found in *A. sterilis* accessions from Israel and other countries of the Mediterranean region (Leonard *et al*. 2004).

AFLP has already been proved to be a suitable technique for the molecular analysis of rust pathogen (Kolmer 2001). In the light of the oat crown rust extreme variability in virulence, a high variability on a molecular level could be assumed. However, the level of variation detected by AFLP may deviate from the real level of variation at the nucleotide level (Brown 1996). According to Justesen *et al*. (2002), a potential loss of restriction site may result in the loss of two fragments that leads to overestimation of the amount of variation. In contrast, two polymorphism loci may be recorded as one locus under certain circumstances. Therefore, high-grade AFLP products and careful assessment of AFLP patterns are required.

The study proved that in some cases the oat crown rust isolates that were closely related or identical for virulence patterns could be distinguished by the AFLP technique. All the isolates in the test had unique AFLP patterns. Similarly, Kolmer (2001) distinguished all the isolates of *P. triticina* using the AFLP technique to detect molecular polymorphism. In our study, three pairs of isolates which served as controls of the correctness of the analysis showed only very small differences, which could be caused either by the heterocaryotic urediniospores (Váňa 1996) or simply by unintentional contaminations with respect to growth conditions of biotrophic fungi (Justesen *et al*. 2002).

The DNA analysis of all 40 oat crown rust isolates did not show a striking distinction according to their origin, the isolates collected in different countries were mostly clustered together. However, most isolates grouped in the third cluster were sampled in geographically close areas, i.e. in the Czech Republic, Austria, and Serbia. Such a similarity of genetic background may indicate that urediniospores often migrate to particular areas. The occurrence of the migration pathways of spores was already proved by Hovmøller *et al*. (2002), who studied the migration of *P. striiformis* f.sp. *tritici* in North-West Europe. The common transfer of oat crown rust spores from South-East Europe to Central Europe by wind was already predicted by Šebesta *et al*. (1999b), who performed a European survey of *P. coronata* f.sp. *avenae* isolates using phytopathological analyses.

The number of virulence genes in the isolates seems to play also an important role in the clustering. Most isolates harbouring a lower number of virulence genes (0–4) are grouped into the third and fifth cluster. whereas the fourth cluster contains a majority of isolates with 4–7 virulence genes.

Furthermore, the virulence patterns of most isolates in the third cluster were related, which could indicate the prevailing asexual propagation in some areas; mutations or somatic recombination were already proved (Zimmer *et al*. 1963; Bartoš *et al*. 1969).

The isolates *Pca*11 and *Pca*39, which represent individual clusters, reveal neither unique virulence patterns nor other specific recordable traits that could give reasons for its distinct AFLP pattern. Therefore, it can be assumed that other effects may affect the genetic background of the oat crown

rust isolates. According to Abu-El Samen *et al.* (2003), virulence/avirulence loci represent only a small portion of the total genetic variations that might exist among different races within a plant pathogen population.

A low but highly significant correlation was found between AFLP and virulence/avirulence genes based on distance matrices. The results are in accordance with several studies in which comparisons of phytopathological as well as molecular polymorphisms were made (Chen *et al.* 1993; Brake *et al.* 2001). According to Brake *et a*l. (2001) the low correlation between phytopathological and molecular variability may be a reflection of the host-directed selection of virulence. Better knowledge of resistance genes present in oat cultivars is necessary to confirm the hypothesis.

In conclusion, the utilisation of the AFLP technique together with phytopathological analysis provides useful information about the diversity of *P. coronata* f.sp. *avenae* isolates. Although the set of isolates in the present study was relatively small, it may indicate the migration of oat crown rust spores from South-East Europe to Central Europe. Both sexual and asexual modes of reproduction could result in a very high diversity of oat crown rust populations on a phytopathological as well as on a molecular level. The prevailing occurrence of the pathotypes with small numbers of virulence genes may indicate that oat cultivars grown in Europe possess a small number of resistance genes, if any.

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Corresponding author:

Mgr. Leona Svobodová-Leišová, Ph.D., Výzkumný ústav rostlinné výroby, v.v.i., Odbor genetiky, šlechtění a kvality produkce, oddělení molekulární biologie, 161 06 Praha 6-Ruzyně, Česká republika
tel. + 420 233 022 425, e-mail: leisova@vurv.cz