Distinguishing Isolates of Cereal Stem Disease Pathogens of the Genus *Tapesia* from Isolates of *Fusarium* and *Rhizoctonia* using the RAPD Method

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Abstract

VEJL P., SKUPINOVÁ S., POLIŠENSKÁ I., VÁŇOVÁ M. (2000): **Distinguishing isolates of cereal stem disease pathogens of the genus *Tapesia* from isolates of *Fusarium* and *Rhizoctonia* using the RAPD method**. Plant Protect. Sci., **36**: 132–140.

The RAPD method (Random Amplification of Polymorphous DNA) was used to distinguish *Tapesia acuformis* and *T. yallundae* isolates from other pathogens of wheat stem base diseases (*Fusarium avenaceum*, *F. culmorum*, *F. nivale*, *Rhizoctonia cerealis* and *R. solani*). Isolates of the fungi originated from infected wheat plants (*Triticum aestivum* L.) collected at various locations in the Czech Republic. Three decameric oligonucleotide-primers were selected as producing genotype-specific RAPD products. These markers distinguish all isolates of the above taxons. The intra-species genetic variability of these species also was studied. The values of Dice's coefficients of similarity suggest that isolates of *Tapesia yallundae* showed a higher degree of intra-species variability than those of *T. acuformis*. A high degree of intra-species variability was identified also in *Fusarium avenaceum*.

Key words: DNA; polymerase chain reaction (PCR); random amplified polymorphic DNA (RAPD); markers; fingerprinting; genetic similarity

The effectiveness of the production of common wheat (*Triticum aestivum* L.) is influenced by a number of factors, of which a significant one is the plants' health (BENADA *et al.* 1981). This study presents a method of using genetic markers based on DNA polymorphism to identify isolates of selected significant fungal diseases on *Triticum* species which affect the roots, root caps, stem bases and stems. The objective was to unambiguously distinguish, by RAPD markers, between R and W pathotypes of the causal agents of the stem base diseases of wheat species *Pseudocercosporella herpotrichoides* (and *Tapesia acuformis* and *T. yallundae*, respectively), obtained from infected plants in the Czech Republic.

Pseudocercosporella herpotrichoides (FRON) DEIGHTON is the pathogen of genuine stem-breaking of wheat stems. Infected plants have at the base area of their stems elliptic grey-brown to yellow-brown spots with a darkly bordered transition to healthy tissue. These symptoms are caused by a mycelium which, during late spring, forms slightly curved conidia (BOTH & WALLER 1973). Under *in vitro* conditions, it is possible to induce sporulation by this fungus on potato-dextrose agar (CHANG & TYLER 1964). Two significant pathotypes have been identified in *P. herpotrichoides* on the basis of their sensitivity to fungicides. Pathotype R had a lower sensitivity to triazole fungicides, while pathotype W showed higher sensitivity. According to current genetic and taxonomic studies, the R pathotype is regarded as a separate species named *Tapesia acuformis* (DYER *et al.* 1996), and the W pathotype corresponds to the species *T. yallundae* (WALLWORK 1987; ROBBERTSE *et al.* 1995).

From the economic point of view, *T. acuformis* and *T. yallundae* belong to the most dangerous diseases of today's cereal crops. Their occurrence is caused by a high proportion of cereals on cultivated agricultural land, and incorrect rotation of crops (PETR 1983; WIESE 1987). Apart from measures of an agro-technical character, effective treatment consists in shortening the stems by preparations based on CCC [(2-chloroethyl) trimethylammonium chloride], or a chemical protection using effective substances such as benomyl, thiophanatemethyl and thiabendazol (WIESE 1987). Within common wheat there are no genotypes that are completely resistant against this disease; they differ only in various degrees of tolerance. The genus *Aegilops* could be a source of resistance against *T. acuformis* and *T. yallundae* (WIESE 1987).

Fusarium culmorum, *F. avenaceum* and *F. nivale* are considered to be the pathogens (causal agents) of fusarial rotting of the stem bases. Warm and dry weather favours the development of *F. culmorum* and *F. avenaceum*, whereas humid and cold seasons are optimal for *F. nivale*. Fusarium is spread especially by infected seed (WARREN & KOMMEDAHL 1973; WIESE 1987).

Other fungi causing symptoms similar to those by *T. acuformis* and *T. yallundae*, are members of the genus *Rhizoctonia*. Parasitic on roots of young plants is *R. solani* (WELLER *et al.* 1986); *R. cerealis* causes pointed brown spots on the stem basis which gradually become necrotic. In a severe attack, the stems break in the area affected by the necrosis (BURPE 1980). The fungi survive in the soil in the form of sclerotia even several years (CLARKSON & GRIFFIN 1977; LIPPS & HERR 1982).

Standard identification tests make use of the specific sensitivity of pathogenic fungi to fungicides in the culture medium, and morphologic microscopic tests are currently being substituted by highly sensitive techniques of genetic markers. These markers can be based on the principle of isoenzyme spectra (JULIAN & LUCAS 1990; MOREAU & MARAITE 1996) or on polymorphism of nucleic acids. DNA analyses can be based on the principle of hybridisation of DNA from the pathogen with a specific marked probe (NICHOLSON *et al.* 1994). The variability of *Pseudocercosporella herpotrichoides* mitochondrial DNA pathotypes was studied, using the RFLP (Restrictive Fragments Longitudinal Polymorphism) technique, by NICHOLSON *et al.* (1993) and TAKEUCHI and KUNINAGA (1996).

Among other DNA marking techniques there is a procedure based on DNA amplification – polymerase chain reaction, making use of two (PCR) or one (RAPD) primer. The invention of thermally-stable DNA polymerisation and the subsequent automation of the process of *in vitro* DNA amplification, allowed rapid development of PCR markers, routine amplification of a single DNA molecule and detection of a single copy of a gene (WILLIAMS *et al.* 1990; INNIS & GELFAND 1990). The obtained DNA fragments are separated by electrophoresis or on polyacrylamid gel (SAMBROOK *et al.* 1989).

Among the first to use the PCR method to distinguish *T. acuformis* from *T. yallundae* were POUPARD *et al.* (1993) and GAC *et al.* (1996) who monitored the variability of DNA sequence for the ribosomal ITS (Internal Transcribed Sequences) area. Specific RAPD markers were used for identification and at the same time quantification of *T. acuformis* and *T. yallundae* by NICHOLSON *et al.* (1997). Genetic studies of *Fusarium* species on the basis of PCR were conducted for example by SCHILLING *et al.* (1996), who distinguished *F. graminareum* and *F. culmorum*. Variability within *F. poae* was studied by RAPD polymorphism by KERÉNYI *et al.* (1997). Detection of *F. avenaceum* by means of polymerising chain reaction was published also by TURNER *et al.* (1998). Variability of the genus *Rhizoctonia* was studied by use of DNA markers also for example by NICHOLSON and PARRY (1996).

MATERIAL AND METHODS

Origin of Isolates: Isolates of *Tapesia yallundae*, *T. acuformis*, *Fusarium avenaceum*, *F. culmorum*, *F. nivale*, *Rhizoctonia solani* and *R. cerealis* were derived from infected plants from various locaties in the Czech Republic (Table 1).

Table 1. Isolates of fungal diseases of stem bases of wheat (*Triticum aestivum* L.)

Designation	Species	Location	Designation	Species	Location
1	*Tapesia yallundae*	Josefinka 6	21	*Rhizoctonia solani*	Kroměříž 1
2	*Tapesia yallundae*	KM1C(6)	22	*Rhizoctonia solani*	Chlumec nad Cidlinou 2
3	*Tapesia yallundae*	Litoměřice 15	23	*Rhizoctonia cerealis*	Kroměříž 3
4	*Tapesia yallundae*	Říčany 7	24	*Rhizoctonia cerealis*	Chlumec nad Cidlinou 4
5	*Tapesia yallundae*	Znojmo 1	25	*Fusarium avenaceum*	Holice
6	*Tapesia yallundae*	Znojmo 9	26	*Fusarium avenaceum*	Říčany
7	*Tapesia yallundae*	Kroměříž F-10	27	*Fusarium avenaceum*	Holice
8	*Tapesia yallundae*	Josefinka 1	28	*Fusarium avenaceum*	Pelhřimov
9	*Tapesia yallundae*	Nový Jičín 15	29	*Fusarium avenaceum*	Litomyšl
10	*Tapesia yallundae*	Kroměříž F-18	30	*Fusarium avenaceum*	Vranov nad Dyjí
11	*Tapesia acuformis*	Velká Chýška 14	31	*Fusarium avenaceum*	Vyškov II
12	*Tapesia acuformis*	Polná 25	32	*Fusarium avenaceum*	Chlumec nad Cidlinou
13	*Tapesia acuformis*	Velká Bitýška 20	33	*Fusarium avenaceum*	Velká Chýška
14	*Tapesia acuformis*	Říčany 1	34	*Fusarium avenaceum*	Velká Chýška
15	*Tapesia acuformis*	Velká Chýška 9	35	*Fusarium culmorum*	Holice
16	*Tapesia acuformis*	Pelhřimov 12	36	*Fusarium culmorum*	Holice
17	*Tapesia acuformis*	Polná 2	37	*Fusarium culmorum*	Chlumec nad Cidlinou
18	*Tapesia acuformis*	Říčany 6	38	*Fusarium nivale*	Holice
19	*Tapesia acuformis*	Jihlava 5	39	*Fusarium nivale*	Znojmo
20	*Tapesia acuformis*	Velká Chýška 10			

Isolation and Culture of Fungi: Pieces of stems of infected plants 1.5–2 cm long were surface-sterilised by 1% sodium hypochloride over 5 min. After thorough rinsing by sterile distilled water, the pieces were dried in the sterile environment of a flow box (BATEMAN 1993). The part of stem containing mycelium was divided into a number of segments and these placed in separate Petri dishes with potato-dextrose agar and incubated in the dark at a constant temperature of 20°C.

Isolation of DNA: For isolating DNA, a modified method by SAGHAI-MAROOF et al. (1984) was used. DNA was isolated from the mycelium grown in a Petri dish. The mycelium was fixed by liquid nitrogen, and homogenised in a sterilised polypropylene centrifugal test tube by a glass stick under continuous cooling by liquid nitrogen. CTAB extraction buffer (modified by SAGHAI-MAROOF *et al.* 1984) was used. For degradation of RNA was used RNAsis A. DNA concentration was determined by UV spectrophotometry. The isolated DNA was diluted to a 20 ng/ml concentration. The DNA's quality – high molecularity – was verified using electrophoresis.

DNA Amplification: The RAPD method was used for DNA amplification. The RAPD reaction of 25 µl volume took place in a Cyclogene thermocycler (Techne, Great Britain). Eighty decameric primers were tested, using sets made by Genset (France), Operon (USA) and BioVendor (Czech Republic). The RAPD composition was as follows: Template DNA 10.0 ng/25 µl, primer 25.0 ng/25 µl, recombinant *Taq* polymerase (MBI Fermentas, Lithuania) 0.7 U/25 µl, dNTP 200 µM, $MgCl_2$ 2.5 mM, Tris-HCl (pH 8.8) 10 mM, KCl 50 mM, Nonidet P40 0.08%. The reaction mix was covered by 30 ml mineral oil. The following temperature and time profile was used for the amplification: 1 cycle (94.0°C/240 s), 40 cycles (94°C/30 s, 36.0°C/45 s, 72.0°C/90 s), 1 cycle (72.0°C/300 s). After completing the amplification, the samples were stored in a thermocycler at 4.0°C.

Electrophoretic Separation of RAPD Markers: 10 µl of RAPD product was used for the separation, collected from under the oil level without previous purification. Loading buffer with ficoll, bromphenol blue and xylene cyanole blue was used according to SAMBROOK *et al.* 1989. RAPD markers were separated on a Sub Cell (Bio Rad, USA) horizontal electrophoresis in 1.5% agarose gel at constant voltage of 100 V (3.3 V per 1 cm clearance between electrodes) over 150 minutes. TBE buffer was used for the electrophoresis. For visualisation, SYBR® Gold staining (Molecular Probes, USA) was used. After the separation was completed, the gels were placed in staining solution and left to incubate under gentle shaking for 1 hr. Visualisation was achieved by using UV transilluminator. Digital camera CAMEDIA C-1400L (Olympus, Japan) with EP H7 adapter (Polaroid, Great Britain) and Wratten 22 and Wratten 2A (Kodak, USA) gelatine UV filters were used to produce photographic documentation. The electrophoreogram was digitised with a resolution of 1.4 million pixels.

Determining Dice's Coefficients of Similarity and Construction of Dendrograms: The computer program GelManager for Windows Version 1.5. (®P.J.H. Jackman 1991–1994, BioSystematica, Great Britain) was used to evaluate and determine statistical characteristics of the PCR markers. Only polymorphous RAPD bands were used which were subsequently processed as binary data. Mutual similarity was characterised by means of the "present-absent" relationship of the band, regardless of the actual value of its optical density. The evaluation of similarity itself was done by means of Dice's coefficients of similarity (JACKMAN 1994):

$$S = \frac{2m}{a+b} \times 100\ [\%]$$

S – Dice's coefficient of similarity [%]
m – number of identical bands between two compared electrophoretic profiles (a, b)
a – total number of bands in electrophoretic profile a
b – total number of bands in electrophoretic profile b

The computer program arranged the similarity coefficients in the matrices which served as input data for the construction of dendrograms on the basis of UPGMA (Unweighted Pair Group Method using Averages) cluster analysis.

RESULTS

Tapesia acuformis developed colonies of light-brown to pink colour, while *T. yallundae* formed black-green colonies. Fungi of the genus *Fusarium* were identified by the fast growth of their colonies and by typical conidia.

The selected method of isolation produced high-molecular DNA in sufficient quantity, suitable for subsequent amplification. Tests using electrophoresis confirmed that the isolated DNA was free of fragments of degraded DNA and RNA molecules. The DNA yield from one fungal colony of about 3 cm diameter varied from 12 µg to 20 µg. The purity of the isolated DNA, expressed as the ratio of absorption A_{260}/A_{280}, was within the range of 1.8–1.9.

Table 2. Sequence of primers, providing genotype-specific markers

Designation	Sequence	Number of basis	CG [%]	T_m [°C]	Kit
A	5'-AGG GCC GTC T-3'	10	70.0	22.15	Operon (USA)
B	5'-AGC GTC CTC C-3'	10	70.0	17.18	Operon (USA)
C	5'-GTC AGG GCA A-3'	10	60.0	13.98	Operon (USA)

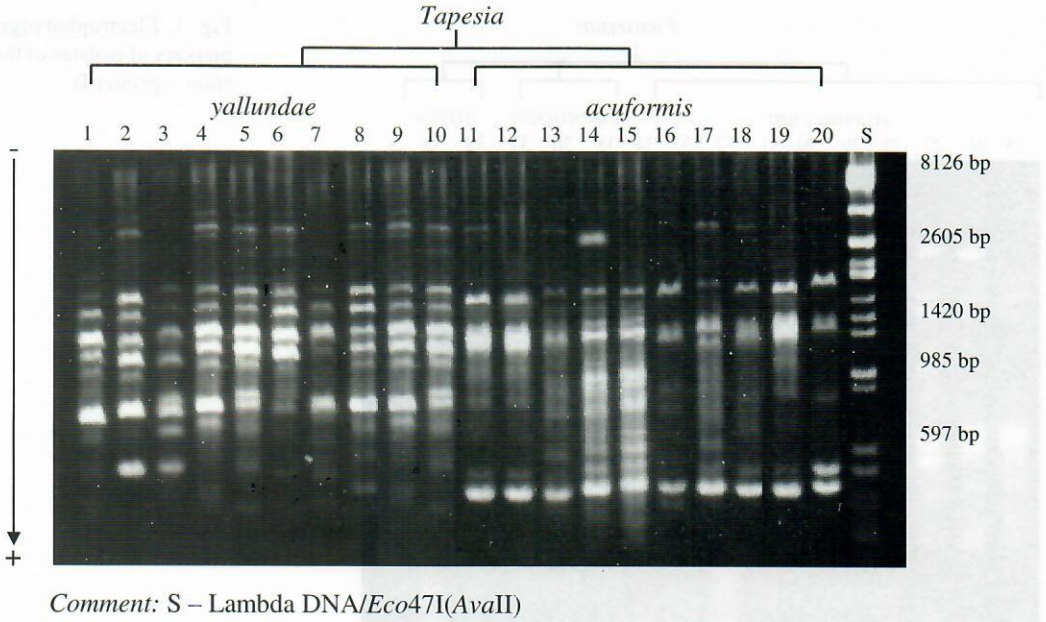

Fig. 1. Electrophoreogram of RAPD markers of isolates of the genus *Tapesia* – primer C

Of the total number of 80 tested decameric primers, only three oligonucleotides provided genotype-specific RAPD bands which were not affected by the occurrence of unspecific amplifications. The characteristics and marking of selected primers are presented in Table 2. These primers are applicable for study of genetic variability of the genera *Tapesia*, *Fusarium* and *Rhizoctonia*. Figs. 1–3 illustrate selected digitised electrophoreograms, characterising the isolates of *Tapesia*, *Fusarium* and *Rhizoctonia*. Optimal quality of pictures taken by the CAMEDIA C-1400L camera was achieved by direct digitisation of the electrophoreograms.

For statistical processing – determining Dice's coefficients of similarity, only genotype-specific bands were used. An overview of these specific bands and their approximate sizes (bp) are presented in Table 3. Binary data,

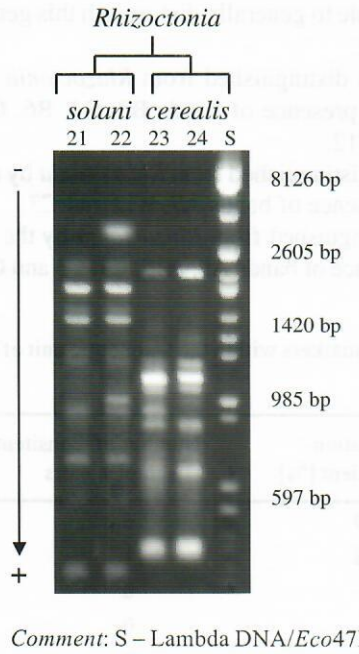

Fig. 2. Electrophoreogram of RAPD markers of isolates of the genus *Rhizoctonia* – primer B

Table 3. Overview, marking and approximate size of the detected polymorphous RAPD bands when using primers A, B, C

Primer A		Primer B		Primer C	
Band	Size [bp]	Band	Size [bp]	Band	Size [bp]
A1	5000	B1	4000	C1	2800
A2	3700	B2	2700	C2	2700
A3	2800	B3	2600	C3	2500
A4	2600	B4	2200	C4	2300
A5	2300	B5	2100	C5	2050
A6	1900	B6	1990	C6	1750
A7	1800	B7	1550	C7	1550
A8	1700	B8	1450	C8	1400
A9	1500	B9	1400	C9	1200
A10	1300	B10	1250	C10	1150
A11	1100	B11	1200	C11	1050
A12	990	B12	1100	C12	950
A13	800	B13	1000	C13	900
A14	700	B14	980	C14	800
A15	400	B15	970	C15	750
		B16	940	C16	600
		B17	900	C17	450
		B18	750		
		B19	700		

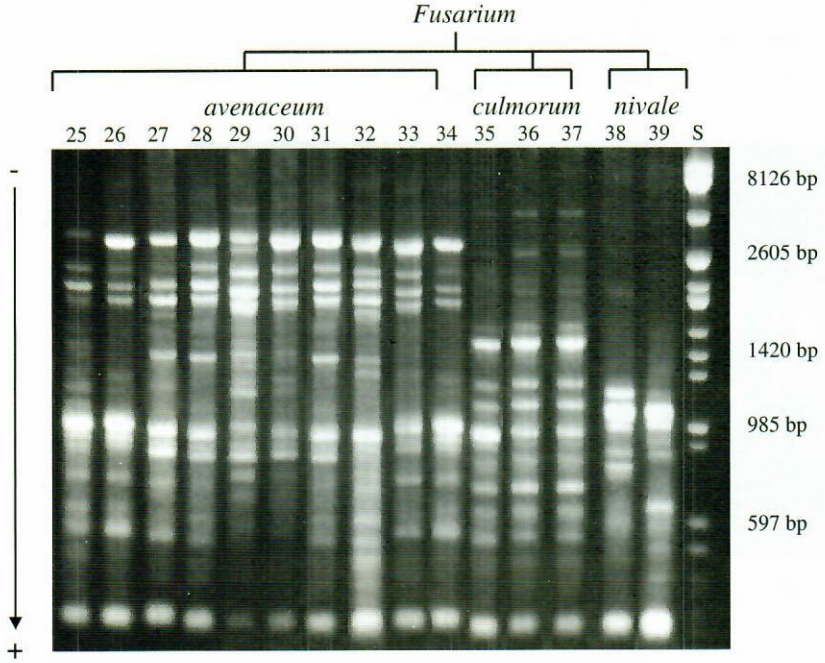

Fig. 3. Electrophoreogram of RAPD markers of isolates of the genus *Fusarium* – primer B

Comment: S – Lambda DNA/*Eco*47I(*Ava*II)

which are schematically presented in Fig. 4, served as input data to the GelManager for Windows computer program. The obtained Dice's coefficients of similarity are shown in Table 4.

For isolates of each of the analysed taxons specific RAPD markers were selected. Results of the identification – "fingerprinting" – of isolates of the genera *Tapesia*, *Fusarium* and *Rhizoctonia* can be summarised into the following points:

- *Tapesia acuformis* is distinguished from other taxons by the simultaneous presence of A7 and C2 bands.
- *T. yallundae* isolates were distinguished from *T. acuformis* isolates by the simultaneous presence of bands A3, A5, A13, B3, C6, C8, C10 and C13.
- For *T. acuformis* isolates on the other hand, the bands A7, B4, B5, B6 and C17 were specific. These bands were not present in *T. yallundae* isolates.
- For the genus *Rhizoctonia* was characteristic the simultaneous presence of bands A4, A5 and B5.
- *R. solani* was unambiguously identified by the simultaneous presence of bands A12, B7, B8, B10, B15, B18, C10, C11 and C15.
- *R. cerealis* was unambiguously identified by the simultaneous presence of bands A8, A11, A13, B13, B16, B19 and C7.
- The genus *Fusarium* was distinguished from *Tapesia* by the presence of C11 bands and the simultaneous absence of band C7.
- No common marker was found for the genus *Fusarium* which would enable to generally distinguish this genus from *Rhizoctonia*.
- *F. avenaceum* was distinguished from *Rhizoctonia* by the simultaneous presence of bands B3, B4, B6, C1, C4, C8, C9 and C12.
- *F. culmorum* was distinguished from *Rhizoctonia* by the simultaneous presence of bands A7, B12 and C3.
- *F. nivale* was distinguished from *Rhizoctonia* by the simultaneous presence of bands A9, A15, C1, C3 and C5.

Table 4. Characteristics of Dice's coefficients of similarity, obtained by means of RAPD markers within the taxonomic unit of the species

Genus	Species	Number of analysed samples	Average Dice's coeffitient [%]	Variation coeffitient [%]	Number of consitent samples pairs
Tapesia	*yallundae*	10	93.9	3.9	6
	acuformis	10	95.5	3.8	11
Rhizoctonia	*solani*	2	90.0	–	0
	cerealis	2	87.0	–	0
Fusarium	*avenaceum*	10	48.2	65.0	0
	culmorum	3	100.0	0.0	3
	nivale	2	84.0	–	0

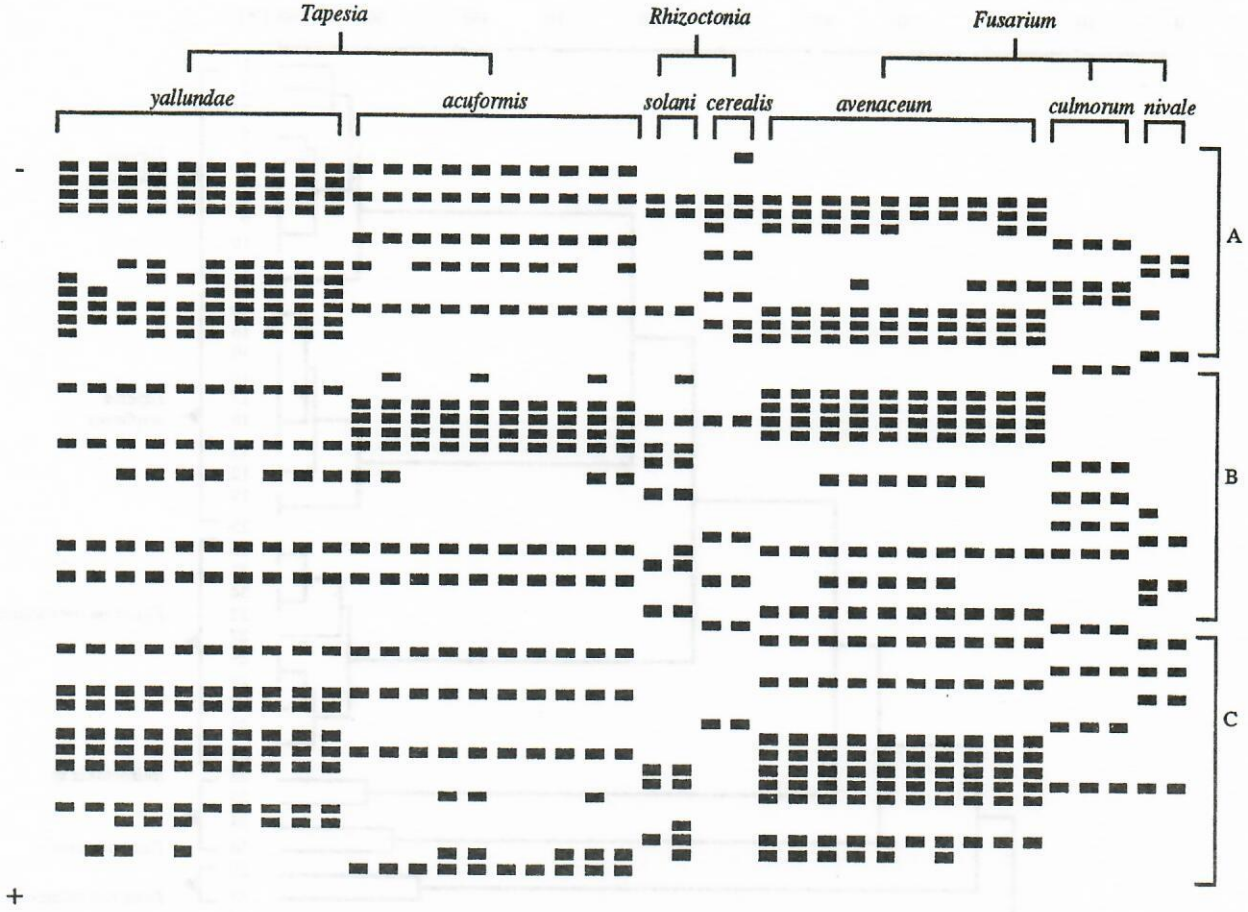

Fig. 4. Overview of detected polymorphic RAPD bands (primers A, B, C)

From the overview of Dice's coefficients of similarity (Table 4) the extent of genetic similarity within the analysed species was determined. These coefficients were used to construct a dendrogram which is presented in Fig. 5. It shows clearly that the genus *Tapesia* can be divided into two branches that correspond exactly with the taxonometric classification of *Tapesia acuformis* and *T. yallundae*, respectively.

Table 4, which evaluates statistically the obtained Dice's coefficients of similarity, suggests that the analysed isolates of *T. acuformis* are less variable than those of *T. yallundae*. Within the species *T. acuformis* 11 pairs of isolates with an identical RAPD profile were found. Interesting are also the results for the genus *Fusarium* presented in the dendrogram in Fig. 5. Isolates of different species, which are difficult to distinguish by standard methods, were grouped in clusters corresponding to their expected taxonomic classification. Within the genus *Fusarium* the species *F. avenaceum* had the highest number of isolates (10) and showed high intra-species variability; not a single pair of isolates with identical RAPD profiles was found.

DISCUSSION

Methods based on polymerase chain reaction have been used by several authors to identify fungal diseases. In this study, the RAPD method was used to distinguish *Tapesia acuformis* (10 isolates) and *T. yallundae* (10) from isolates of other organisms causing cereal stem diseases – *Fusarium avenaceum* (10), *F. culmorum* (3), *F. nivale* (2), *Rhizoctonia cerealis* (2) and *R. solani* (2 isolates). This method unambiguously distinguished these taxons from each other.

Isolation of DNA: The suggested modification of the CTAB method to isolate genome DNA (SAGHAI-MAROOF *et al.* 1984) proved to be suitable for obtaining high-molecular DNA. A similar method of isolating DNA from the genus *Tapesia*, making use of extracting buffer with CTAB, was used among others by NICHOLSON *et al.* (1997). Homogenising the mycelium in liquid nitrogen was used for *Tapesia* for example by NICHOLSON *et al.* (1994). Isolates of the genus *Fusarium*, cultured in liquid medium, were used for isolating DNA by TURNER *et al.* (1998). Enzymatic degradation of RNA as done in this study was not mentioned by any of the cited authors. Nor did they mention the yield and quality of the isolated DNA.

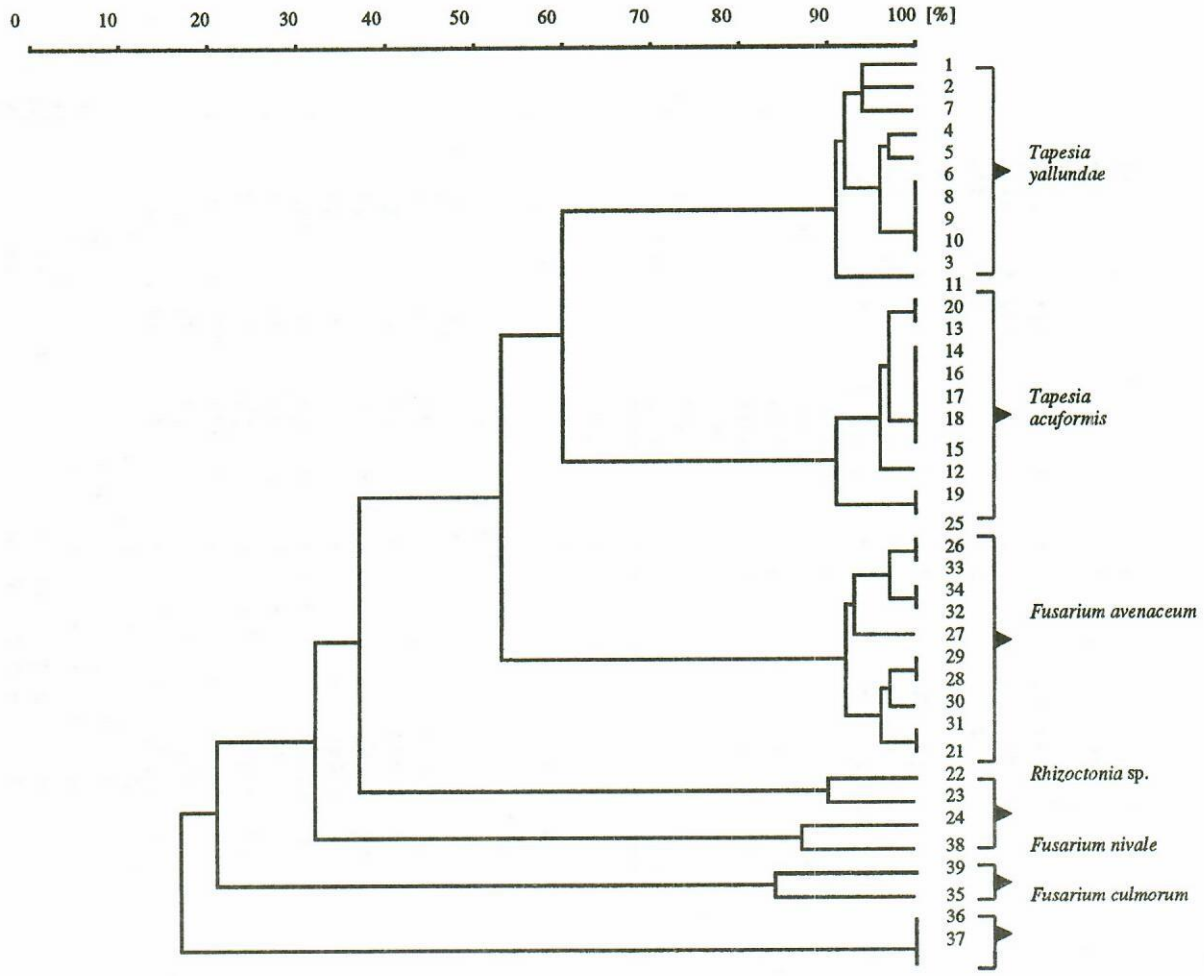

Fig. 5. Dendrogram of the analysed taxons, compiled on the basis of Dice's coefficients of similarity

In our study, the values of absorption ratio A_{260}/A_{280} varied within the interval 1.8 to 1.9, indicating very pure DNA. The yield of DNA from one fungal colony on agar made approximately 1000 amplifications possible.

Amplification of DNA – RAPD: *Tapesia acuformis* and *T. yallundae* have been characterised by means of RAPD markers by NICHOLSON *et al.* (1997). He also used a set of decameric oligonucleotides from Operon (USA) to select suitable primers. Unlike in that study, the DNA amplification described in this paper took place in a reaction mix of half the volume (25 µl). Concentration of the template DNA and the *Taq* polymerase were similar. The concentration of $MgCl_2$ (2.5 mM) used in our RAPD reactions was higher than that used by NICHOLSON *et al.* (1997). The increase of Mg^{2+} ions had the positive effect of reducing the occurrence of unspecific amplifications and increasing the ratio of RAPD bands with a higher number of base pairs. The annealing temperature of 36°C used by us was the same as that mentioned for e.g. by NICHOLSON *et al.* (1997). KERÉNYI *et al.* (1997) do not describe in detail the chemical composition of the RAPD reaction. For amplification, these authors also used decameric oligonucleotides, and achieved higher specificity of the amplifications by using an annealing temperature of 38°C.

Electrophoresis Separation of Markers and Digitisation of Electrophoreograms: RAPD markers were separated by horizontal electrophoresis with 1.5% agar gel in TBE buffer. NICHOLSON *et al.* (1997) also used agar gels to separate RAPD markers of the genus *Tapesia*. However, they did not mention the condition for separation and gel concentration. Classification of RAPD markers of the genus *Fusarium* in agar gel has also been described by KERÉNYI *et al.* (1997). All cited authors used a standard procedure - ethidium bromide - to stain the RAPD fragments. In this study, however, a highly sensitive staining, SYBR® Gold (Molecular Probes, USA), was used for visualisation on the electrophoreograms. Comparison experiments have confirmed that this staining is capable of detecting RAPD bands of low DNA concentration which cannot be visualised with ethidium bromide.

Direct digitisation of the electrophoreograms by the camera enabled to obtain high-quality photographic documentation. Such methods of photographic documentation have not been described by any of the other authors.

Data Analysis – Calculation of Dice's Coefficients of Similarity and Construction of Dendrograms: Statistical processing of the data by means of various types of coefficients of similarity have been mentioned by a number of authors. For instance TAKEUCHI and KUNINAGA (1996) used similarity coefficients by NEI and LI (1979) to express similarity of mitochondrial DNA markers in thre genus *Tapesia*. Until now. Dice's coefficients of similarity have not been used to express the extent of genetic variability in the genera *Tapesia*, *Fusarium* and *Rhizoctonia*. Input data for the GelManager for Windows computer program were binary data schematically illustrated in Fig. 4. This graphic method of entering input data, which has been described in detail by VEJL (1998), enables simultaneous evaluation of a higher number of RAPD markers. Expressing genetic distances by dendrograms has been used by a number of authors. The method to construct dendrograms from the results of cluster analysis (UPGMA) was used for result evaluation in this publication, and has also been described by KERÉNYI *et al.* (1997), TAKEUCHI and KUNINAGA (1996) and TURNER *et al.* (1998).

Distinguishing Isolates of the Genera Tapesia, Fusarium and Rhizoctonia from each other: *The isolates of Tapesia acuformis, T. yallundae, Fusarium avenaceum, F. culmorum, F. nivale, Rhizoctonia cerealis* and *R. solani* used in these experiments were taxonomically identified by standard culture and morphological tests. These methods of identification have been described for instance by WALLWORK (1987), WIESE (1987), ROBBERTSE *et al.* (1995), DYER *et al.* (1996) and POLIŠENSKÁ (1998). Genetic differences between the isolates, determined by such tests, were confirmed by the RAPD method. A similar unambiguous differentiation of both species of *Tapesia* by PCR markers was also defined by NICHOLSON *et al.* (1997). But variability between isolates of the genus *Tapesia* originating from different locations has not been studied before. The high variability of RAPD bands within *F. avenaceum* shown by us was also found by TURNER *et al.* (1998). We found no intra-species variability of RAPD markers in *F. culmorum*, while in *F. nivale*, *R. cerealis* and *R. solani* there were no identical pairs of RAPD profiles. This result can be explained by the low number of isolates of these taxons.

To conclude, this study has confirmed that the PCR technique represents an easy and quick method of unambiguous detection of isolates of fungi of the genera *Tapesia*, *Fusarium* and *Rhizoctonia*. The usefulness of the selected decameric oligonucleotides which provided specific RAPD markers will be verified in follow-up experiments, to determine whether they are capable to detect these fungal pathogens directly on the infected part of the wheat plant.

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Souhrn

VEJL P., SKUPINOVÁ S., POLIŠENSKÁ I., VÁŇOVÁ M. (2000): **Odlišení izolátů původců chorob stébel obilovin rodu *Tapesia* od izolátů rodů *Fusarium* a *Rhizoctonia* metodou RAPD**. Plant Protect. Sci., **36**: 132–140.

Metoda RAPD (náhodná amplifikace polymorfní DNA) byla použita pro odlišení izolátů *Tapesia acuformis* a *Tapesia yallundae* od izolátů ostatních původců chorob pat stébel pšenice (*Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium nivale*, *Rhizoctonia cerealis* a *Rhizoctonia solani*). Izoláty těchto patogenních hub byly odvozeny z infikovaných rostlin pšenice (*Triticum aestivum* L.) pocházejících z různých lokalit České republiky. Byly vybrány tři dekamerické oligonukleotidy – primery, které poskytovaly genotypově specifické RAPD produkty. Současně byla u těchto druhů studována vnitrodruhová genetická variabilita. Rozsah variability byl charakterizován hodnotami Diceho podobnostních koeficientů. Grafické vyjádření podobnosti RAPD bylo zpracováno na základě shlukové analýzy. Pro veškeré statistické vyhodnocení byl použit program GelManager for Windows. Z hodnot Diceho podobnostních koeficientů vyplývá, že izoláty druhu *Tapesia yallundae* vykazovaly vyšší stupeň vnitrodruhové variability oproti izolátům *Tapesia acuformis*. Vysoký stupeň vnitrodruhové variability byl rovněž nalezen u druhu *Fusarium avenaceum*.

Klíčová slova: DNA; polymerázová řetězová reakce; náhodná amplifikace polymorfní DNA; markery; „fingerprinting"; genetická podobnost

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