Universally primed polymerase chain reaction analysis of *Fusarium avenaceum* isolated from wheat and barley in Finland

Tapani Yli-Mattila

Laboratory of Plant Physiology and Plant Molecular Biology, Department of Biology, FIN-20014 University of Turku, Finland, e-mail: tymat@utu.fi

Nina V. Mironenko

All-Russian Plant Protection Institute, Laboratory of Plant Immunity to the Pests, St. Petersburg 189620, Russia

Irina A. Alekhina

Komarov Botanical Institute, Laboratory of Fungal Biochemistry, St. Petersburg 197376, Russia

Asko Hannukkala

Institute of Plant Protection, Agricultural Research Centre of Finland, FIN-31600 Jokioinen, Finland

Sergey A. Bulat

Petersburg Nuclear Physics Institute (PNPI), Department of Molecular and Radiation Biophysics, Gatchina 188350, Russia

Twenty-two *Fusarium avenaceum* isolates from Finnish wheat and barley were analysed using the chain reaction with universal primers (UP-PCR). Each isolate could be distinguished from others by UP-PCR products on polyacrylamide gels. The isolates tested were clustered into two main groups and further into several subgroups by UP-PCR profiles and phylogenetic analyses. The phylogenetic relationships of these groups are discussed. No clear correlation was found between the groups and host plant preference or the geographic origin of *F. avenaceum* isolates. Pathogenicity tests showed differences between *F. avenaceum* isolates, but two isolates, one from wheat and the other from barley, were the most aggressive in wheat and barley. This fungus, usually known as a weak pathogen of cereals and other crops, has thus probably not evolved in respect to its ability to damage wheat or barley.

Key words: *Gibberella avenacea*, identification, genotyping, parsimony analysis, UP-PCR

Introduction

Fusarium avenaceum (Fr.) Sacc. can be regarded as a weak pathogen. Under conditions unfavourable to its hosts, it may cause damping off, root rot, stalk rot and/or fruit rot (Gerlach and Nirenberg 1982). The sexual state (teleomorph) of *F. avenaceum*, called *Gibberella avenacea* (Booth 1971), is quite rare in nature (Booth and Spooner 1984) and has never been reported from Finland.

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In Finland, *Fusarium avenaceum* is a common inhabitant of living and dead organic substrates (Ylimäki and Jamalainen 1986). It is frequently found on cereal grain, where it causes seedling blight and has potential for mycotoxin production (Ylimäki 1981). Together with other *Fusarium* species, *F. avenaceum* is associated with foot and root rot diseases of all cereals grown in Finland (Mäkelä and Parikka 1980). It is considered less destructive than *F. culmorum*, but a wide range of variation in pathogenicity between isolates has been reported by Uoti (1976). *F. avenaceum* has also caused considerable storage losses in potato (Seppänen 1981a).

Like those of *F. oxysporum*, *F. avenaceum* isolates are very difficult to distinguish from each other on the basis of morphological or physiological characters. *F. avenaceum* isolates have been grouped into three main types by isozyme analysis (Yli-Mattila et al. 1996). Restriction fragment length polymorphism (RFLP) (Nicholson et al. 1993) and random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analyses (Yli-Mattila et al. 1996, Yli-Mattila and Hyvönen 1996) yielded a higher resolution than isozyme analysis, making it possible to distinguish nearly all *F. avenaceum* isolates from one another.

In the present study the less familiar polymerase chain reaction with universal primers (Universally Primed Polymerase Chain Reaction – UP-PCR) was used (Bulat et al. 1992, Bulat et al. 1994, Naumov et al. 1997). The main difference between UP-PCR and RAPD-PCR (Williams et al. 1990) and arbitrarily primed PCR (AP-PCR) (Welsh and McClelland 1990) is that the single primers of UP-PCR (ca 16 nucleotides) consist of random 3' end and "natural" minisatellite-like sequences (which can be found in any genome) at the 5' end, which along with a specific PCR protocol using Tsp polymerase as well as high ramping thermal cycler, makes it less sensitive to reaction conditions (minor changes in annealing temperature, Mg^{2+} concentration etc.) than AP- PCR and RAPD-PCR. The primer extention at the 5' end by minisatellite-like sequence makes the primer hybridization more

stable. UP-PCR banding patterns are fully reproducible, even between different cyclers, provided that exactly the same protocol including ramping rate is used (S.A. Bulat, unpublished data).

The purpose of the present study was to assess identification and phylogenetic relationships in *F. avenaceum* isolated from wheat and barley in Finland. The UP-PCR technique was employed to test the correlation between the genome structure of the isolates and their host preference. Such a correlation has previously been demonstrated for the fungus *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana*), which is also a pathogen of wheat and barley and of which the sexual stage is rare in nature (Bulat and Mironenko 1993). Another objective was to compare the isolate relationships obtained here with those obtained previously by RAPD-PCR analysis (Yli-Mattila et al. 1996, Yli-Mattila and Hyvönen 1996). In addition, the species uniqueness of *F. avenaceum* at the genome level as compared with other *Fusarium* fungi was studied by means of cross dot blot hybridization of UP-PCR products. Preliminary results of UP-PCR analysis have been presented (Yli-Mattila et al. 1997).

Material and methods

Fungal isolates, growth conditions and DNA extraction

Twenty-three isolates of *Fusarium avenaceum* and six isolates from other *Fusarium* species were isolated from barley, wheat or oats collected in different locations in Finland (Table 1, Fig. 1) as described by Yli-Mattila et al. (1996, 1997). Each isolate was grown for 4–7 days on a cellophane membrane on the surface of potato-dextrose agar at 25°C. DNA was extracted from fresh mycelium with a chloroform/octanol mixture (Yli-Mattila et al. 1997) to eliminate proteins, lipids and polysaccharides, which are potential inhibitors of PCR reaction.

Table 1. List of *Fusarium* isolates. Map numbers refer to the Figure 1.

Species Code (Isolate	No. No.*)	Host (isolated from)	Geographic origin (map number)	Year of isolation
F. avenaceum				
15	(93015)	barley (stem base)	Apukka (1)	1992
28	(93014)	barley (stem base)	Apukka (1)	1992
51	(92003)	barley (root)	Honkajoki (2)	1986
40	(92004)	barley (root)	Honkajoki (2)	1986
14	(92016)	barley (root)	Kihnio (3)	1986
25	(92006)	barley (root)	Nousiainen (4)	1986
17	(92013)	barley (stem base)	Kankaanpää (5)	1986
19	(92020)	barley (root)	Rautalampi (7)	1986
20	(92024)	barley (root)	Harjavalta (8)	1986
47	(92026)	barley (root)	Leppävirta (9)	1986
50	(92009)	barley (root)	Parkano (10)	1986
37	(92014)	wheat (root)	Janakkala (11)	1986
38	(92015)	wheat (root)	Nummi (12)	1986
39	(93084)	wheat (stem base)	Pälkäne (6)	1992
46	(93071)	wheat (stem base)	Pälkäne (6)	1992
26	(93095)	wheat (stem base)	Kokemäki (13)	1992
27	(93093)	wheat (stem base)	Kokemäki (13)	1992
41	(93088)	wheat (stem base)	Kokemäki (13)	1992
42	(93096)	wheat (stem base)	Kokemäki (13)	1992
45	(93094)	wheat (stem base)	Kokemäki (13)	1992
21	(92005)	wheat (root)	Vihti (14)	1986
23	(92007)	wheat (root)	Karjaa (15)	1986
43	(93101)	oats (stem base)	Kokemäki (13)	1992
F. graminearum				
2	(92029)	barley (root)	Espoo (16)	1986
F. culmorum				
35	(93004)	oats (stem base)	Pälkäne (6)	1992
F. equiseti				
9	(92011)	barley (root)	Kruunupyy (19)	1986
F. redolens				
11	(93152)	barley (root)	Strömfors (18)	1986
F. oxysporum				
33	(93138)	barley (root)	Espoo (16)	1986
F. poae				
53	(93146)	barley (root)	Siuntio (17)	1985

*Isolate numbers are stock numbers of isolates in the collection of the Agricultural Research Centre of Finland.

Pathogenicity testing

All *F. avenaceum* isolates except isolate 50 were studied for pathogenicity. They were transferred to 9 cm plastic petri dishes on potato dextrose agar (PDA, Difco) and incubated for 2 weeks at 18–20°C under continuous light to enhance spore production.

The pathogenicity of *F. avenaceum* isolates to potato tubers was tested on cv. Bintje. The tuber material was certified basic seed that had been multiplied from meristem culture for two

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Fig. 1. Geographical origin of *Fusarium* fungi collected in Finland. Map numbers refer to those given in Table 1.

Pathogenicity to barley and wheat seedlings was studied in a greenhouse test on health-inspected commercial certified seed of barley cv. Pokko and wheat cv. Tapio. Twenty-five seeds were sown in 0.5–l plastic pots in sterile sand. The seeds were inoculated by pouring 10 ml of spore suspension of *F. avenaceum* (100 000 spores/ml) onto them, after which they were covered with a 2-cm layer of sterile sand. Four replicates of each isolate were inoculated and pots were organized by randomized block design in the greenhouse. Tests on wheat were carried out twice. The plants were grown for 4 weeks at 18–20°C under 12-h light periods and were then removed carefully from the sand. Stem bases were rated into four categories: 0= healthy, 1= stem lesions, 2= totally brown stem bases and 3= dead plants. To compare the pathogenicity of *F. avenaceum* isolates to wheat, barley and potato the different disease ratings of the cereals and potato were scaled to 0–100. The highest average disease score, 100 was given to the isolate causing the most severe disease symptoms. The average disease scores of other isolates were expressed as percentages of the highest rating.

A simple regression model for pathogenicity of *F. avenaceum* isolates to barley, wheat and potato was calculated using the SAS GLM procedure (Littel et al. 1991).

generations in the greenhouse and for two generations in the field. It was health tested against major viral, bacterial and fungal pathogens. Tubers uniform in shape within the size fraction of 40–45 mm were selected for the test. They were washed under tap water and their surface was sterilized with 10% natriumhypochlorite (NaOHCl) for 10 min. The tubers were then washed with sterile water and dried for 12 h.

Five tubers per isolate were inoculated using the method described by Seppänen (1981b). Each set of five tubers was placed in a separate plastic box, where they were incubated for 3 weeks in darkness at 6–8°C. After inoculation they were cut, and the depth and width of the rotten tissue were measured. The tuber test was repeated twice for each isolate.

UP-PCR amplification

UP-PCR was performed using the thermal cycler TC-1000M (PNPI, St. Petersburg, Russia) for 30 cycles as described by Yli-Mattila et al. (1997). The rate of temperature change was about 4°C s^{-1}. The sequences of universal primers designed and synthesized in PNPI were as follows:

0.3–2 (16 mer) 5'-TGAGGACAACGGTTCC-3' (Bulat et al. 1992)

AA2M2 (16 mer) 5'-GAGCGACCCAGAGCGG-3' (this work)

HE 45 (16 mer) 5'-GTAAAACGAGGCCAGT-3' (this work)

Of these, 0.3–2 and AA2M2 primers were used to generate UP-PCR products to be analysed

Table 2. Binary character matrix of *F. avenaceum* isolates analysed by UP-PCR with two universal primers.

OTU	Primer AA2M2 Ch=30 + (2)**		Primer 0.3–2 Ch=28 + (7)**	
A*	000000000000000000000000000000000		000000000000000000000000000000000	
M			H G F E D C B	
38	0100000000001011110101100000111	(01)	1010110111101000101100000001	(0000001)
50	0100000000001011110101100000111	(01)	1010110010100100100101100000001	(0000110)
17	0000010000010111101011000000111	(01)	1010101111100100101101001001	(0000000)
19	0100011000010111101011000000111	(00)	0010101111101000101001001001	(0001000)
37	0011000000000100110010001111001	(01)	1001010000000101000111001010	(0000000)
45	0011000000000100010001000111001	(01)	1001101000010101000110001010	(0000000)
23	0011000000000000110000001110 00	(01)	1001101000000111000011111010	(0000000)
20	0011000000000001100000011000 0	(01)	1001001000000111000011111010	(1000000)
26	0011000000000001100000011000 0	(01)	0001101000000111010011001110	(0000000)
39	0000001010101000110110011100 11	(01)	1001101000000101000010101010	(0100000)
27	0000001010101000100101011001 1	(01)	1101110001000101000001011010	(0000000)
25	0000001010101000100101011001 1	(01)	1101110000011010000000011010	(0000000)
40	0000001010001000100101011001 1	(01)	1101101000010101000000101110	(0000000)
14	0000000101000100010000001110 01	(01)	1001101001000101000011011010	(0000000)
15	0000000101000001100000011100 1	(11)	1101110001000101010001001010	(0000000)
47	0000000101000100010000001100 11	(01)	0101101000000010000110011010	(0000000)
21	1000001010101000111110011100 11	(01)	1001101001000101010111011010	(0000000)
28	1000100011001100010000001100 00	(01)	1100101000000101000001001110	(0010000)
46	0000000101000100010010001110 01	(01)	1001101000000101000011011110	(0000000)
41	0000000101000100110010001110 01	(01)	1101101000000101000001011110	(0000000)
42	0000000101000100110010001110 01	(01)	1001101000000101000011001100	(0000000)
51	0000100001000100010000001100 00	(01)	1001101000000101000111001010	(0000000)

OTU – Operative Taxonomic Unit (isolate); A* – Artificial Ancestor; Ch – character (only phylogenetically significant and autapomorphic characters shown);
**Number of autapomorphic characters; M – position of molecular weight markers (B – 0.25 kb; C – 0.34 kb; D – 0.45 kb; E – 0.47 kb; F – 0.51 kb; G – 0.80, H –1.16 kb) of Fig. 3.

by gel electrophoresis. The HE 45 primer was used only in dot blot hybridization experiments.

Several amounts (0.1–1.0 µl) of template DNA were tested in UP-PCR and run on 1.7% agarose (Sigma A-7431) gel at 150 V with cooled TBE buffer to be sure of the reliability of electrophoretic banding profiles. In all cases it was possible to obtain reproducible results on agarose gel by choosing appropriate amounts of template DNA and primer. The efficiency of DNA amplification was also estimated on agarose gel, and equal amounts of the amplification products with the sharpest bands were run on 6% polyacrylamide gel (thickness 0.8 mm, length 20 cm) at 160 V for 10–14 h with cold (ca 12°C) TBE buffer in order to obtain better resolution than

with agarose gel. Both agarose and polyacrylamide gels were stained with ethidium bromide and photographed in UV light.

Dot blot hybridization analysis

Amplification products of three *F. avenaceum* isolates (17, 28 and 14), which represented the two main groups of RAPD-PCR (Yli-Mattila et al. 1996, Yli-Mattila and Hyvönen 1996) profiles, were compared with those of *F. graminearum* (isolate 2), *F. culmorum* (isolate 35), *F. equiseti* (isolate 9), *F. redolens* (isolate 11), *F. oxysporum* (isolate 33) and *F. poae* (isolate 53) and also with isolate 43, which, according to

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17   28   14   43   53   33   11   9   35   2

Fig. 2. Dot blot hybridization of UP-PCR products generated with primers HE 45 (A) and AA2M2 (B) in *F. avenaceum* (isolates 17, 28, 14 and 43), *F. poae* (isolate 53), *F. oxysporum* (isolate 33), *F. redolens* (isolate 11), *F. equiseti* (isolate 9), *F. culmorum* (isolate 35) and *F. graminearum* (isolate 2). Dotted samples were hybridized with labelled total UP-PCR products generated with primers HE 45 (A) or AA2M2 (B) for isolate 28.

previous RAPD-PCR and isozyme analyses (Yli-Mattila et al. 1996, Yli-Mattila and Hyvönen 1996), is only distantly related to other *F. avenaceum* isolates.

Total UP-PCR products from all these isolates generated with primers HE 45 and AA2M2 were dotted onto nylon filters (Hybond N⁺, Amersham) according to the manufacturer's instructions. The filter-bound fixed PCR products from these isolates were hybridized with the corresponding amplification products of *F. avenaceum* isolate 28, labelled with (alpha-^{32}P) dCTP (Izotop, St. Petersburg), at 68°C for up to 16 h, as described by Sambrook et al. (1989). The filters were washed and exposed to x-ray film as described by Yli-Mattila et al. (1997).

Data analysis

The negatives of the polyacrylamide gels were scanned using an original scanning device (V. Zenin, Institute of Cytology, St. Petersburg, Russia) and the images obtained were processed with original image analysis software (N. Klopov, V. Patzekin, PNPI, Russia). High-resolution image analysis allowed us to study all bands in the gel. In this way we could detect weak bands, which are not clearly visible in the photo in Fig. 3. The sizes of the bands were determined according to

the peaks of the densitograms of the UP-PCR profiles. All bands detected in image analysis were recorded in a binary matrix irrespective of their intensity.

The data produced by the image analysis (the binary matrix shown in Table 2 and the Dice distance matrix derived from it using the program of N. Klopov, PNPI, Russia) were analysed by the Wagner parsimony (branch and bound algorithm), Neighbor-Joining (NJ) and unweighted pair group method with arithmetic mean (UPGMA) methods of PHYLIP 3.5 (Felsenstein 1993). These analyses included only phylogenetically informative characters, which were present in at least two isolates. To root phylogenetic trees, an Artificial Ancestor (A) taxon (Hennig 1966, Pavlinov 1989) with all characters "0" was invoked.

The reliability of the phylogenetic trees was explored by a bootstrapping procedure using the PHYLIP package. This procedure was adapted for parsimonious trees using SEQBOOT to generate 3 x 100 different data sets (jumble options 5, 81 and 97), which were analysed by the branch and bound method followed by the CONSENSE program. For the UPGMA and NJ methods the same 300 binary data sets were converted to Dice distance ones before analysis by the NEIGHBOR program followed by CONSENSE.

In addition to the branch and bound algorithm, Maximum parsimony analysis was performed with the heuristic search mode of PAUP 3.1.1 by random addition and tree bisection-reconnection (TBR) swapping and by collapsing zero-length branches (Swofford 1993).

Results

Dot blot hybridization analysis of *Fusarium* isolates

F. avenaceum isolates 17, 28 and 14, which were chosen as representatives of all isolates by UP-PCR profiles, showed a strong positive signal
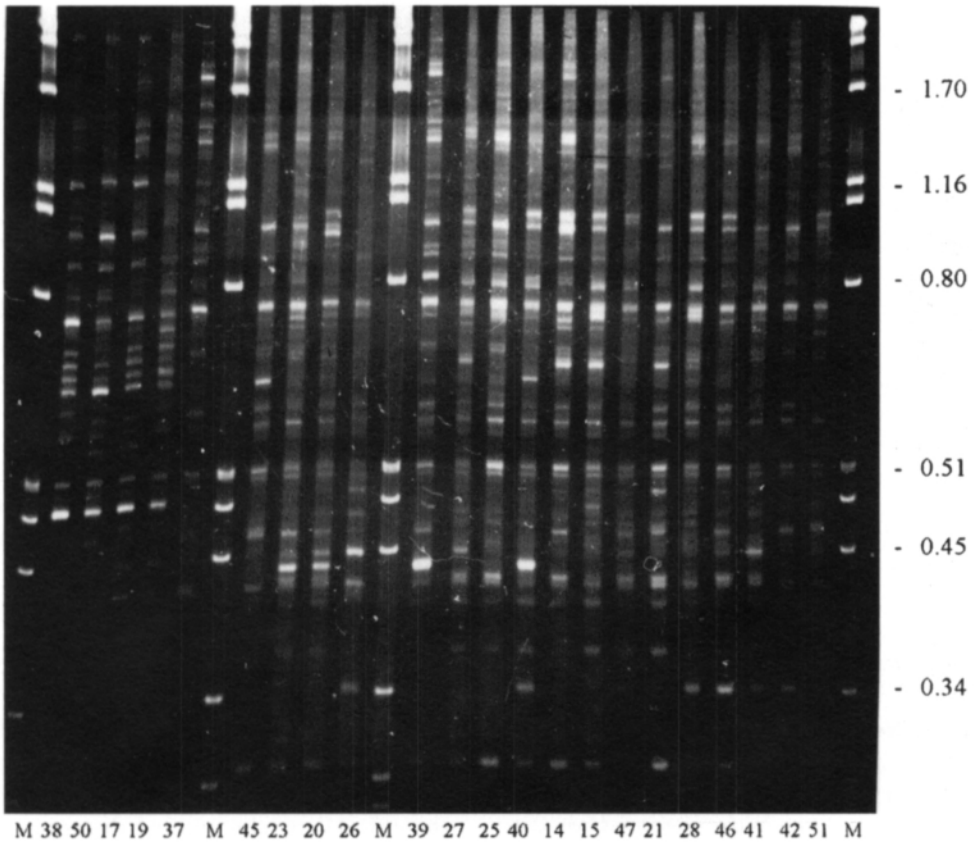
Fig. 3. UP-PCR banding profiles of *Fusarium avenaceum* isolates with primer 0.3–2. Molecular weight markers (M) – lambda DNA digested by Pst1. The sizes (kb) of six molecular weight markers are indicated (modified from the proceedings paper of T. Yli-Mattila et al. 1997).

when UP-PCR products of isolate 28, generated with two UP-PCR primers, were used as a label, whereas amplified DNA from other *Fusarium* species showed no hybridization (Fig. 2). The hybridization signal from isolate 43 was somewhat weaker than that from other *F. avenaceum* isolates.

UP-PCR analysis of *F. avenaceum* isolates

All *F. avenaceum* isolates collected from wheat and barley could be distinguished from each other by the UP-PCR technique using two primers (Fig. 3, Table 2). The primers produced UP-PCR

profiles consisting of 7–16 phylogenetic and autapomorphic bands per primer (Table 2). The sizes of bands ranged from 200 to 2000 bp (Fig. 3).

The isolates studied were not derived from single spores, but the clonal origin of four selected isolates of *F. avenaceum* was confirmed by UP-PCR analysis. Single-spore colonies were isolated from original isolates 20, 28, 39 and 51, which were characterized by the most distinguishable UP-PCR patterns on agarose gel. UP-PCR products from two single-spore colonies of each of these isolates, together with the original DNA sample, were analysed by polyacrylamide gel electrophoresis. No intrastrain differences were found, and all four isolates could be dis-
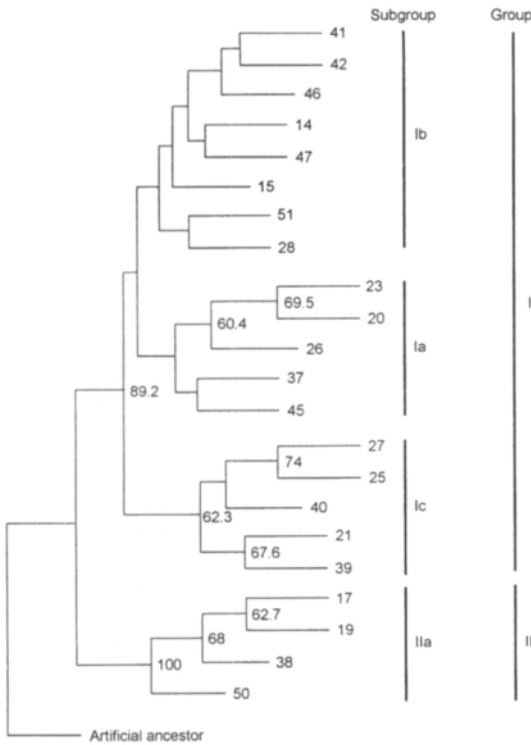
Fig. 4. Phylogenetic relationships among *F. avenaceum* isolates as obtained by Wagner parsimony method of PHYLIP 3.5. Majority rule and strict Wagner consensus tree. Only bootstrap values greater than 50% are shown.

including the smallest ones, for each data set. Neither the consensus tree topology nor the bootstrap values actually differed for the two settings (100 and 1000). In addition, the topologies of the Neigbor-Joining and UPGMA consensus trees made of 300 trees (results not shown) were the same as in the Wagner parsimony consensus tree (Fig. 4), allowing us to use these 7672 trees in calculating bootstrap values.

In the Wagner parsimony consensus tree (Fig. 4) of the UP-PCR matrix (Table 2) *F. avenaceum* isolates could be divided into two main groups with bootstrap values higher than 89%. Group II consisted of isolates 17, 19, 38 and 50. These two groups are also clearly visible in Fig. 3, although group I is fairly heterogeneous. In addition, group I could be divided into three subgroups, of which Ic (isolates 27, 25, 40, 21 and 39) was supported by a bootstrap value higher than 50%.

The 50% majority rule consensus tree obtained by the heuristic search mode of PAUP 3.1.1 showed a topology identical to the Wagner tree of PHYLIP 3.5 (Fig. 4), except for slight changes in the large subgroup Ib (results not shown). All parsimony and distance methods highlighted subgroup IIa as the most distantly related.

tinguished from each other (results not shown). Since the isolation methods were the same in all *F. avenaceum* isolates, the rest of the isolates were also probably genetically homogeneous. This is important, because a mixture of genotypes can give confused UP-PCR profiles.

Phylogenetic relationships among *F. avenaceum* isolates

In each of the 300 data sets analysed by the branch and bound method, the search broke off when the upper limit was set at 100 or 1000 (the "how many" option), but we succeeded in obtaining 7672 trees characterized by quite different CIs (= consistency index, Felsenstein 1993),

Pathogenicity

The relative pathogenicity of *F. avenaceum* isolates in wheat was between 42 and 100, in barley 16–100 and in potato 63–100 (Table 3). The isolates least pathogenic in wheat were 51 and 25 and the most pathogenic 38, 17, 47 and 14. In barley the least pathogenic isolates were 40, 25 and 51 and the most pathogenic 38 and 17; in potato the least pathogenic isolates were 27, 45 and 21 and the most pathogenic 17, 51 and 38.

The best correlation was found between pathogenicity to wheat and barley (pathogenicity to barley = 11.3 + 0.55x pathogenicity to wheat; R-square 0.33, F-value 18.42 and p 0.00012). There was no statistically significant correlation between pathogenicity to barley and potato

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Table 3. Relative pathogenicity of *F. avenaceum* isolates to barley, wheat and potato.

Isolate	Phylogenetic group	Wheat	Barley	Potato
37	Ia	74	83	81
26	Ia	82	84	68
45	Ia	80	85	65
20	Ia	89	85	74
23	Ia	87	89	68
51	Ib	42	26	95
47	Ib	95	83	82
41	Ib	87	84	84
46	Ib	75	84	88
15	Ib	84	86	72
42	Ib	71	87	84
14	Ib	91	88	70
27	Ic	84	83	81
40	Id	88	16	77
25	Ic	64	21	70
27	Ic	83	86	63
21	Ic	85	87	67
39	Ic	90	88	88
19	IIa	81	80	79
17	IIa	96	90	100
38	IIa	100	100	89

(R-square 0.07, F-value 3.09, p 0.087) or wheat and potato (R-square 0.12, F-value 5.27, p 0.027), but isolates 17 and 38 were among the 2–3 most pathogenic isolates in all three crops.

Discussion

All isolates of *F. avenaceum* could be separated into two main groups and distinguished from one another by UP-PCR analysis, which produces more characters per primer than does RAPD-PCR (Bulat et al. 1995, Yli-Mattila et al. 1996, Yli-Mattila and Hyvönen 1996). Image analysis of polyacrylamide gel negatives enabled us to clearly separate bands from each other and to compare bands of equal size more accurately than was possible in the previous RAPD-PCR work (Yli-Mattila et al. 1996, Yli-Mattila and

Hyvönen 1996), in which only visual analysis of photos from agarose gel was used.

The UP-PCR products from other *Fusarium* fungi were completely nonhomologous with those from *F. avenaceum* in the dot blot hybridization analysis, and so we could not infer the phylogeny of *F. avenaceum* isolates from those of other *Fusarium* species by comparing UP-PCR banding profiles. Therefore, in *F. avenaceum*, phylogenetic analyses were performed only on isolates whose UP-PCR products gave a strong hybridization signal showing that these isolates produced homologous bands and indicating that they belonged to the same species (Bulat and Mironenko 1992, Bulat et al. 1995). On these criteria isolate 43 did not belong to *F. avenaceum* and was excluded from UP-PCR analysis.

The phylogenetic analyses of UP-PCR products run on polyacrylamide gel revealed two quite different groups: a large one (I), comprising the majority of isolates, and a smaller one (II), with isolates differing greatly in genome structure from the others. The parsimonious trees obtained for the same *F. avenaceum* isolates with UP-PCR and RAPD data (Yli-Mattila and Hyvönen 1996) were found to be almost the same at main group level. In the RAPD-PCR analysis, group II also included isolate 37 instead of 38, which constituted its own RAPD group. At subgroup level, the differences between UP-PCR and RAPD-PCR trees were much greater. The resolution and the number of branches with a bootstrap value higher than 50% were superior in the UP-PCR parsimony tree. However, there were still branches in the UP-PCR parsimony tree with a bootstrap value lower than 50%, which may have been due to the lack of a hierarchic signal within the main groups of isolates collected from a comparatively small area, as was suggested by Yli-Mattila and Hyvönen (1996).

The differences between the trees for UP-PCR and RAPD-PCR data are probably due to the fact that the polyacrylamide gel used in the UP-PCR analysis had a better resolution than the agarose gel used in RAPD analysis. Differences in primer sequences and in protocols cannot,

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however, be excluded. There were also some differences in the algorithms used for phylogeny inference. We are now studying PCR products generated with the same RAPD-PCR primers in a polyacrylamide gel in the hope that we shall be able to compare UP- and RAPD-primer based data with greater accuracy in the future.

The reliability and suitability of RAPD-PCR results for phylogenetic analyses have been questioned, especially above the intraspecific level, due to the uncertain homology of the bands (e.g. Rieseberg 1996), their non-codominant inheritance, asymmetrical transformation features and possible GC priming bias, which altogether make current models of parsimony inappropriate (Backeljeau et al. 1995). In UP-PCR the phylogenetic analysis is performed only at taxon level, where the amplification products are cross-homologous, the primers are not GC-rich and the better resolution obtained by polyacrylamide gel causes fewer errors in finding homologous bands; the two other features of bands (non-codominant inheritance and asymmetrical transformation), however, remain. UP-PCR data may thus be more appropriate for parsimony analysis than RAPD-PCR data. In addition, the parsimony tree of the present study was supported by NJ and UPGMA trees, and previous studies dealing with different fungi (S.A. Bulat, personal communication) show that nearly all bands of UP-PCR profiles are independent of each other and derived from numerous locations dispersed throughout the genome. It should also be noted that it is practically impossible to test all bands in a profile on cross homology by Southern hybridization and that the findings of some studies of phylogenetic relationships based on RAPD-PCR or AP-PCR data are in accordance with morphological (Millan et al. 1996) and isozyme (Castagnone-Sereno et al. 1994) results and the history of strains (Canzian et al. 1995).

The use of an artificial ancestor (Hennig 1966, Pavlinov 1989), which is one kind of ingroup comparison, has been criticized (Watrous and Wheeler 1981). It nevertheless offered the only way to calculate all the bootstrap values and still obtain a reasonable tree, since PHYLIP's CONSENSE program produces trees of different topology when different taxa are allocated as an outgroup. We furthermore found that the Artificial Ancestor created does not affect the branching order of other taxa, since its position has been defined so as to coincide with the midpoint root in a tree.

According to the hybridization analysis performed here and the previous results of RAPD-PCR and isozyme studies (Yli-Mattila et al. 1996), isolate 43 is more distantly related to other *F. avenaceum* strains than any other strain under study. Further molecular and morphological studies are required to clarify the status of this isolate and the isolates of group II which differed from all other isolates of *F. avenaceum*. The latest morphological studies (H. Nirenberg, personal communication) have confirmed that the isolates 28 and 37 of group I and the isolate 17 of group II really do belong to *F. avenaceum*, but isolate 43 was identified as *F. tricinctum*. RFLP and sequence analyses of ribosomal DNA in *F. avenaceum* isolates are now in progress.

No clear correlation was found between isolate clustering and their host plant preference or geographic origin, except for two subgroups detected in all trees inferred. In one group (IIa) three of the four isolates (50, 17 and 19) were from barley and in another (Ia) four of the five isolates (45, 37, 23 and 26) were from wheat. Of the five isolates collected from one field at Kokemäki, isolates 41 and 42 were clustered to subgroup Ib and isolates 45 and 26 to subgroup Ia. The lack of a clear correlation between *F. avenaceum* groups and geographic origin and the host habits of isolates is in agreement with RAPD-PCR and isozyme analyses of the same isolates (Yli-Mattila et al. 1996). This fungus, known as a weak pathogen of cereals and other crops, thus apparently exists under field conditions in the form of numerous vegetative clones and very probably does not evolve in its ability to damage wheat and barley.

Pathogenicity testing of *F. avenaceum* isolates on wheat and barley shows no clear correlation between the *F. avenaceum* isolate grouping and its pathogenicity. Moreover, the most

pathogenic isolates against potato (17 and 51) were positioned quite far from each other in the phylogenetic trees. However, the same two isolates (38 and 17) were among the most pathogenic isolates in wheat and barley, which suggests that the strains of *F. avenaceum* have not specialized for wheat or barley. In addition, it can be noted that these two most pathogenic isolates against wheat and barley belong to the same phylogenetic group (IIa) and that they were also among the most pathogenic isolates against potato.

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SELOSTUS

Vehnästä ja ohrasta eristettyjen F. avenaceum -punahomekantojen analysointi UP-PCR-menetelmällä

Tapani Yli-Mattila, Nina V. Mironenko, Irina A. Alekhina, Asko Hannukkala ja Sergey A. Bulat
Turun yliopisto, All-Russian Plant Protection Institute, Komarov Botanical Institute, Maatalouden tutkimuskeskus ja Petersburg Nuclear Physics Institute

Suomalaisia vehnästä ja ohrasta eristettyjä punahomekantoja *(Fusarium avenaceum)* tutkittiin UP-PCR (Universally Primed Polymerase Chain Reaction) -menetelmällä. *F. avenaceum* -kannat voitiin jakaa erilaisilla feneettisillä ja fylogeneettisillä tietokoneohjelmilla kahteen pääryhmään, jotka vielä jakautuivat useaan alaryhmään. Fylogeneettisten ryhmien ja kantojen maantieteellisen jakauman välillä ei voitu havaita selvää riippuvuutta. Kannat erosivat toisistaan patogeenisuustesteissä, mutta samat ohrasta ja vehnästä eristetyt isolaatit olivat patogeenisimpiä sekä vehnällä että ohralla. Tulosten perusteella vaikuttaa siltä, että *F. avenaceum*, jonka tiedetään olevan suhteellisen heikko patogeeni viljakasveilla, ei ilmeisesti ole erikoistunut kumpaankaan isäntäkasviin.