Fusarium – molecules maketh the mould?

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Research on *Fusarium* diversity is now at a watershed. The availability of a range of molecular methodologies has the potential to enable us to answer some long-standing questions about the genus. Molecular methods are also likely to influence greatly the future development of *Fusarium* research. This contribution reviews the application of molecular techniques in *Fusarium* from a taxonomic perspective. In particular it makes the plea that molecular studies should not develop in isolation, but should be closely integrated into other areas of research.

Keywords: taxonomy, RAPD, SSR-PCR, rDNA ITS, rDNA IGS.

Following the introduction of the generic name *Fusarium* by Link in 1809, *Fusarium* taxonomy has developed through several phases. The first phase lasted about 100 years during which, as in many other groups of fungi, *Fusarium* species were delineated primarily according to the host plant on which they were collected. Then, during the latter part of the 1800's a new technology was adopted - growth of strains in pure culture *in vitro* at first on natural substrata, then on gelatin and later on agar media (Ainsworth, 1976). *In vitro* cultivation revolutionised fungal research and led to a second phase of *Fusarium* taxonomy. During the first third of this century Wollenweber and other colleagues discarded the old host-based approach and developed a classification based on comparative studies of isolates grown under controlled conditions in pure culture on agar and natural substrata. Taxa were delineated using a combination of phenotypic traits, including colony morphology, conidial characteristics, pigmentation (i.e. secondary metabolite production) and in some cases host substrate. This approach has remained the basis of all *Fusarium* classification systems to date and, 60 years after publication of *„Die Fusarien"* (Wollenweber & Reinking, 1935), remains the routine method of identification of species in the genus.

However, phenotype-based taxonomy has been problematic in *Fusarium,* resulting in several classification systems with widely differing species concepts, e.g. Wollenweber & Reinking (1935) and Snyder & Hansen (1945). An underlying cause of these contrasting systems has been the phenotypic plasticity exhibited by *Fusarium* cul-

tures and also their tendency to degenerate and lose their „wild type" characteristics. When comparing isolates, the observed variation in phenotypes may be due to several possible causes: 1. they are different taxa, 2. they reflect genetic variation within a single taxon, 3. phenotypic plasticity, 4. cultural degeneration, 5. one or both may be mixed/contaminated cultures. Attempting to distinguish between these possibilities is one of the key practical problems of *Fusarium* taxonomy. Culture plasticity and instability may be minimized by using carefully standardised incubation conditions and low nutrition media, but they remain concerns. Even in carefully grown, wild-type isolates, the traditional morphological characters available are relatively limited and have probably led us to underestimate the diversity present in the genus. This has been particularly apparent in taxa such as *F. oxysporum* Schlecht., where the range of biological activities is not matched by morphological differentiation.

Molecular techniques are already having a significant impact on mycological research (e.g. Bruns & al., 1991; Metzenberg, 1991; Kohn, 1992; Samuels & Seifert, 1995). Perhaps the most important stimulus to molecular research so far has been the exploitation of DNA polymerases for *in vitro* amplification of DNA, i.e. the polymerase chain reaction (PCR). PCR-based technology has revolutionised DNA research by making it widely accessible, rather than the domain of the few, as was previously the case. In general terms, PCR is having a *qualitative* impact by allowing molecular researchers to generate new forms of information that were previously prohibitively difficult to obtain. Secondly, PCR has a *quantitative* effect by increasing the number of studies generating DNA-based data. Thirdly, PCR has increased the *range* of organisms subjected to DNA analysis, so improving the diversity of our dataset.

Since *Fusarium* is a genus of economic significance it will undoubtedly continue to attract the attention of molecular researchers. The potential for exploiting PCR-based methods in *Fusarium* research is enormous, both in applied work and systematics. In systematic studies it is important to make a clear distinction between the procedures of classification and identification. This distinction reflects quite different types of practical questions and has implications for the types of methodology most likely to be successful. Molecular methods should allow us to significantly improve our classification system for *Fusarium* and progress towards resolving some long-standing issues, including: the phylogenetic relationship between *Fusarium* and other Hypocrealean genera; the generic concept in *Fusarium;* sectional relationships; species concepts; population structure analysis and delineation of infra-specific taxa; mechanisms of evolutionary change in *Fusarium* populations; mechanisms of culture instability. Identification issues tend to be application-specific, driven by the needs of ap-

plied workers in various fields such as plant pathology, mycotoxicology or industrial mycology. Although diverse, these may be broken into three categories: identification of „abstract taxa", such as species, which contain an array of genotypes; identification of strains with specific biological properties or genotypes; fingerprinting of individual strains. Molecular methods may also allow us to circumvent the problems of phenotypic plasticity and degeneration by looking directly at the DNA.

In much the same way that pure culture triggered off the second, phenotypic phase of *Fusarium* systematics, modern techniques of DNA analysis are likely to create a new, third phase based on genotypic data. The implications of this for *Fusarium* researchers are potentially as revolutionary as the change from host-based to phenotype-based taxonomy. Based on current trends, it seems likely that in 50 years time routine identifications of *Fusarium* taxa will not be based on morphology, many identifications will primarily be by DNA analysis and at least some will be carried out by automated identification systems, as is already the case with some bacteria. The rate and type of progress made in the coming years will be driven by two main factors: 1. the availability of appropriate technology, and 2. the questions asked by researchers in the applied mycology of *Fusarium*. It seems likely that people's expectations will change as possibilities for more precise determination at the strain or population level become available. Identification of „species" may become less relevant in future if people are more interested to identify particular clones, vc groups, or strains carrying genes required for particular metabolites or pathogenicity factors.

Probably the most popular PCR technique in recent years has been RAPDs (Random Amplified Polymorphic DNA), since primer kits and experimental protocols are readily available. Several studies have reported successful use of RAPDs, often in conjunction with other techniques, to delineate and identify *Fusarium* taxa (Crowhurst & al., 1991; Wright & al., 1992; Grajal-Martin & al., 1993; Pomazi & al., 1993; Ouellet & Seifert, 1993; Assigbetse & al., 1994; Kelly & al., 1994; Manulis & al., 1994; Mes & al., 1994; Schilling & al., 1994). However, the reproducibility of RAPDs is known to be problematic (e.g. Penner & al., 1993) due to the extreme sensitivity of the technique. It remains to be proven that RAPDs will be practical for routine identification of unknown *Fusarium* isolates by labs around the world and it would seem wise for us to investigate other, more robust PCR techniques for identification purposes.

Also, if we are to exploit molecular methodologies most effectively, we need to put the various different techniques into perspective so that we know their strengths and limitations (Kohn, 1992). To do this we require more comparative studies of several methodologies, prefe-

rably studying taxa which are well-characterised and understood. This will enable us to use the most appropriate technique for the type of question we are trying to answer. The present study was aimed at comparing the results obtained from different PCR procedures, including arbitrary fingerprinting and targeted PCR. A set of 29 *Fusarium* strains were analysed, all isolated from *Triticum* (Tab. 1). Of these, 18 were *Fusarium culmorum* (W. G. Sm.) Sacc. isolates, selected to represent population diversity at several levels, with strains coming from the same district in UK in the same year, different districts in the same year, the same district in different years and from different continents. The time span of their isolations was 57 years (1929–1986). *F. culmorum* was chosen for study because it is an uncontroversial, well understood species of economic significance in plant pathology and mycotoxicology. For inter-species comparison, 4 strains of *F. avenaceum* (Fr.) Sacc. and 3 of *F. crookwellense* Burgess, Nelson & Toussoun were included. Three further isolates (strains 27–29) had degenerated colony and microscopic morphologies, thus preventing reliable identification. These strains were included in order to assess the ability of DNA analysis techniques to identify cultures with symptoms of phenotypic degeneration. The techniques used included fingerprinting with PCR conditions giving increased stringency using a RAPDs 10-base primer, three simple sequence repeat (SSR) primers and an anchored-SSR primer. Targeted PCR amplified the nuclear ribosomal DNA internal transcribed spacer (ITS) and intergenic spacer (IGS) regions. The rDNA PCR products obtained were digested with restriction endonucleases and separated by electrophoresis.

Materials and methods

Isolates studied

The list of isolates studied is given in Tab. 1, together with collection data and names based on their morphological characteristics. All isolates, apart from 26, were grown from lyophilised material in IMI culture collection onto SNA (Nirenberg, 1976) without filter paper.

Morphology

Morphology of isolates was examined using cultures grown on potato sucrose agar (PSA) (Booth, 1971) and on SNA + filter paper at 20 C for 10–14 d, illuminated by fluorescent lamps (daylight and near UV). Inoculum for DNA extraction was taken from SNA plates without filter paper.

Tab. 1. – List of *Fusarium* strains studied.

Isolate	IMI number	Name	Origin	Sender / Collector	Date
1	089364	*Fusarium culmorum* *	*Triticum* seedling, ? UK.	G. Watts Padwick (F209)	1934
2	089367	*Fusarium culmorum*	*Triticum* inflorescence, Alta, Canada.	G. Watts Padwick	1929
3	159025	*Fusarium culmorum*	*Triticum*, UK.	E. W .H. Ford	1971
4	270555	*Fusarium culmorum*	*Triticum*, Cambridge, UK.	J. Knibbs	1982
5	270559	*Fusarium culmorum*	*Triticum*, Cambridge, UK.	J. Knibbs	1982
6	271473	*Fusarium culmorum*	*Triticum*, Derby, UK.	J. Ibbotson (1551/6)	1982
7	271484	*Fusarium culmorum*	*Triticum*, Derby, UK.	J. Ibbotson (1552/6)	1982
8	271901	*Fusarium culmorum*	*Triticum*, Exeter, UK.	S. Wilde (725/9a)	1982
9	271903	*Fusarium culmorum*	*Triticum*, Exeter, UK.	S. Wilde (726/8a)	1982
10	271904	*Fusarium culmorum*	*Triticum*, Exeter, UK.	S. Wilde (727/3d)	1982
11	271964	*Fusarium culmorum*	*Triticum*, Kent, UK.	M. Bateson (683/61)	1982
12	272853	*Fusarium culmorum*	*Triticum*, Reading, UK.	E. Niles (2895 8/2)	1982
13	272858	*Fusarium culmorum*	*Triticum*, Reading, UK.	E. Niles (3124 2/4)	1982
14	273774	*Fusarium culmorum*	*Triticum*, Wolverhampton, UK.	D .S. Williams (2253/4/C)	1982
15	273788	*Fusarium culmorum*	*Triticum*, Wolverhampton, UK.	D .S. Williams (2708/7/F)	1982
16	309752	*Fusarium culmorum*	*Triticum*, Cambridge, UK.	P. Benedikz (F22/86/1)	1986
17	309754	*Fusarium culmorum*	*Triticum*, Cambridge, UK.	P. Benedikz (F24/86/1)	1986
18	309755	*Fusarium culmorum*	*Triticum*, Cambridge, UK.	P. Benedikz (F25/86/1)	1986
19	309760	*Fusarium culmorum*	*Triticum*, Cambridge, UK.	P. Benedikz (F30/86/1)	1986
20	137728	*Fusarium avenaceum*	*Triticum*, Washington, USA.	J. Cook	1978
21	271493	*Fusarium avenaceum*	*Triticum*, Derby, UK.	J. Ibbotson (1550/5)	1982
22	271921	*Fusarium avenaceum*	*Triticum*, Exeter, UK.	S. Wilde (726/2a)	1982
23	272869	*Fusarium avenaceum*	*Triticum*, Reading, UK.	E. Niles (726/2a)	1982
24	318869	*Fusarium crookwellense*	*Triticum* seed, Ontario, Canada.	R. M. Clear (F315)	1987
25	327774	*Fusarium crookwellense*	*Triticum* spike, Rome, Italy.	V. Balmas, P. Del Seccone	1988
26	366152	*Fusarium crookwellense*	*Triticum*, Harpenden, UK.	P. Jennings, Fc/1	1995
27	272901	*Fusarium* sp. *	*Triticum*, Reading, UK.	E. Niles (3354 2/1a)	1982
28	273780	*Fusarium* sp. *	*Triticum*, Wolverhampton, UK.	D. S. Williams (2577/3/A)	1982
29	309749	*Fusarium* sp. *	*Triticum*, Cambridge, UK.	P. Benedikz (F19/86/1)	1986

* – denotes strains with degenerated morphology.

DNA extraction

DNA was extracted using the method of Cenis (1992), except that strains were grown on GYM liquid medium and isopropanol precipitation was extended overnight in order to maximise extraction. GYM (glucose yeast medium) contained: $NH_4H_2PO_4$ 1 g, KCl 0.2 g, $MgSO_4.7H_2O$ 0.2 g, glucose 10 g, yeast extract (Beta Lab, East Molesey, UK) 5 g, Cu solution 1 ml, Zn solution 1 ml, distilled water 1 l. Cu solution contained 0.005 g $CuSO_4.5H_2O$ per l and Zn solution 0.01 g $ZnSO_4.7H_2O$ per l distilled water.

PCR amplification

PCR was carried out using an MJ Research Inc. PTC-100 thermal cycler. Each reaction (total volume 25 µl) contained dNTP's 200 µM each, primer(s) 0.2 µM, Super Tth DNA polymerase (HT Biotechnology Ltd, UK) 0.25 U (0.2X manufacturers recommended concentration) in Tth reaction buffer, sample DNA ca. 10 ng. The reaction buffer contained 1.5 mM $MgCl_2$. For RAPDs, additional $MgCl_2$ was added to give 3 mM final concentration.

RAPDs

An arbitrary 10-mer RAPDs primer #71 (5´-CGGCTTGGGT-3´) was used with a more stringent annealing temperature (52 C) than normally used for RAPDs. Cycle conditions: 95 C for 5 mins, 35 C for 1 min; then 35 cycles of 72 C for 2 mins, 94 C for 30 sec, 52 C for 1 min; followed by final extension at 72 C for 7 mins.

Simple sequence repeat (SSR) PCR

SSR primers 5´-$(CAT)_5$-3´, 5´-$(CAG)_5$-3´, 5´-$(TCC)_5$-3´ and an anchored-SSR primer (Zietkiewicz & al., 1994) 5´-$(CA)_8$RG-3´ were used singly, with 1.5 mM $MgCl_2$. PCR cycle conditions: 95 C for 5 mins, 35 C for 1 min; then 35 cycles of 72 C for 2 mins, 94 C for 30 sec, either 40, 45 or 58 C for 1 min; followed by final extension at 72 C for 7 mins.

Nuclear ribosomal DNA ITS / IGS restriction fragment patterns

Primers ITS1 (5´-TCCGTAGGTGAACCTGCGG-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) were used to amplify the nuclear ribosomal RNA gene ITS1 and ITS2 regions and the intervening 5.8S segment (White & al., 1990). Cycle conditions: 95 C for 3 mins; then 25 cycles of 95 C for 1 min, 40 C for 30 sec, 72 C 2 mins; followed by final extension at 72 C for 10 mins. The whole IGS region was amplified using primers 3´28S (5´-CTGAACGCCTCTAAGTCAG-3´) and CNS1 (5'-GAGACAAGCATATGACTAC-3') using the same cycle conditions as for ITS amplification, except annealing at 52 C. Also, part of the IGS region adjacent to the 3´ end of the large subunit was amplified

with primers CNL12 (5′-CTGAACGCCTCTAAGTCAG-3′) and U: 49–67 (5′-AATACAAGCACGCCGACAC-3′) with cycle conditions: 94 C for 2 mins; then 12 cycles of 95 C for 35 sec, 58 C for 55 sec, 72 C for 45 sec; then 12 cycles of the same but extending at 72 C for 2 mins; then 10 cycles of the same but 72 C for 3 mins; followed by final extension at 72 C for 5 mins. ITS amplification product was digested with restriction endonucleases *Bcl* I, *Bgi* I, *Bgi* II, Bsc I, *Dra* I, *EcoR* I, *EcoR* V, *Hae* III, *Hind* III, *Hinf* I, *Msp* I, *Pst* I, *Pvu* II, *Sac* II, *Sca* I, *Sma* I, *Taq* I and *Xba* I. IGS amplification products were digested with *EcoR* I, *Hae* III, *Hinf* I, *Msp* I and *Taq* I. Total reaction volume was 10 µl (comprising PCR product 5 µl, enzyme 0.1 µl, enzyme buffer (10 X concentration) 1 µl, H_2O 3.9 µl) for 16 h at 37 C, or 8 h at 65 C for *Taq* I.

Electrophoresis

20 µl of PCR product were mixed with 5 µl of gel loading dye (EDTA 100 mM, sucrose 40%, bromophenol blue 0.05%) and loaded onto 1.5% SeaKem LE agarose (FMC BioProducts, ME, USA) gels in 1X TAE buffer. These were run in a 20 cm horizontal gel tank at 75 V, 500 mA, for 2-2.5 h and stained in ethidium bromide. GibcoBRL 1 Kb DNA ladder was use as size markers. ITS and IGS RFLP gels used 3% Metaphor agarose (FMC BioProducts) in 1 X TAE buffer and were loaded with 10 µl digest plus 5 µl of gel loading dye. These were run in a 30 cm horizontal gel tank at 100 V, 500 mA for 3.5–4 h and stained in ethidium bromide. GibcoBRL 100 base pair DNA ladder was used as size markers. All amplifications and digestions were repeated at least twice.

Results

Morphology

Most isolates had colony and microscopic morphologies typical of their named species, but isolate 1 showed some signs of degeneration, with weak pigmentation on PSA. Degenerated isolates 27–29 had been previously identified as *F. graminearum* Schwabe. Isolates 27 and 28 formed polyblastic conidiogenous cells indicating that they could not be *F. graminearum,* but belonged to section *Arthrosporiella sensu* Booth (1971). Their most likely identities were either *F. avenaceum* or *F. sporotrichioides* Sherb. Isolate 29 most closely resembled *F. graminearum,* but had weak pigmentation and irregular spores.

Increased stringency RAPDs

Welsh & McClelland (1990) described arbitrary primed PCR with two initial cycles of low stringency annealing, followed by subsequent cycles at higher annealing temperatures. However, it has become commonplace to carry out RAPDs at low annealing temperature (35 C) for

all cycles. When used with 35 C annealing and 3 mM $MgCl_2$, RAPDs primer #71 gives complex band patterns with a wide range of fungi and is routinely used at IMI as part of a strain fingerprinting programme (data not shown). With an initial cycle at 35 C followed by a more stringent annealing temperature of 52 C, simpler banding patterns are obtained, which have improved reproducibility in repeat amplifications. It can be seen in Fig. 1 that *F. culmorum* isolates shared a common banding pattern, but that some strains lacked a subset of bands. Repeat amplifications gave consistent banding patterns from each strain. *F. avenaceum* isolates 20–23 gave a different, distinct banding pattern, with similarities between isolate 20, from USA in 1978, and isolates 21–23 from UK in 1982. The three *F. crookwellense* isolates 24–26 all gave different banding patterns. Degenerated strain 27 gave a band pattern like *F. avenaceum,* whereas strains 28 and 29 gave different, distinct patterns.

Simple Sequence Repeat PCR

SSR primer $(CAT)_5$ yielded a smear at the top of the gels, with no distinct bands visible, but Primers $(CAG)_5$ and $(TCC)_5$ gave distinct banding patterns (Fig. 2). Both primers yielded one uniformly present strong band and several, variable weak bands for *F. culmorum* isolates, with a different pattern for *F. avenaceum.* These differences were observed at both 45 C and 58 C annealing, but in repeat amplifications the weak bands were sometimes not visible. *F. avenaceum* strain 22 gave a different band pattern from the other strains of that species with primer $(CAG)_5$ in repeat amplifications and at different annealing temperatures. This distinction was not evident with primer $(TCC)_5$, nor RAPDs primer #71. As with RAPD primer #71, the *F. crookwellense* isolates gave different patterns, but with 24 and 25 being most similar to each other. Degenerated isolate 27 gave bands most similar to those of *F. avenaceum.* The putative *F. graminearum* strain 29 gave bands similar to those of the *F. culmorum* pattern with primer $(CAG)_5$, but differed from *F. culmorum* with primer $(TCC)_5$. The anchored-SSR primer yielded a similar picture, with uniform banding amongst *F. culmorum* isolates and a distinct pattern for *F. avenaceum. F. crookwellense* isolates 24–25 and putative *F. graminearum* strain 29 gave a similar banding pattern to that of *F. culmorum.* Degenerated strain 27 gave the same banding pattern as *F. avenaceum.* Strain 28 also shared bands with *F. avenaceum,* but had an extra major band.

Restriction digestion of rDNA ITS amplification product

The ribosomal RNA gene ITS regions were amplified using primers ITS1 and ITS4 in order to generate a PCR product including both ITS

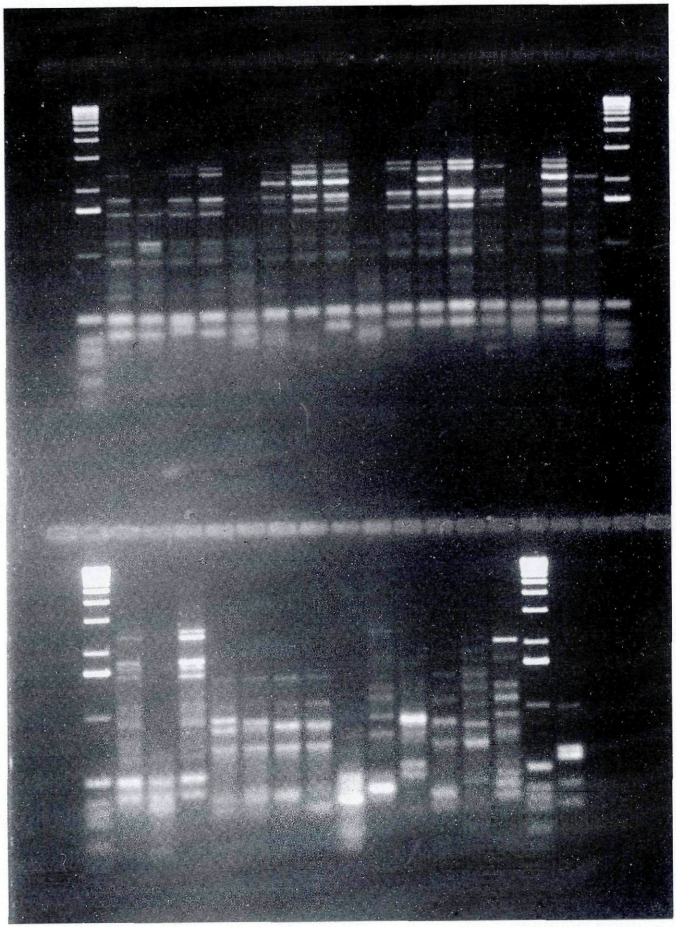

Fig. 1. *Fusarium* isolates with RAPD primer #71 at 52 C annealing. Top lanes (left/right): lane 1. DNA size marker; lanes 2–17. *F. culmorum* isolates 1–16; lane 18. DNA size marker. Bottom lanes (left/right): lane 1. DNA size marker; lanes 2–4. *F. culmorum* isolates 17–19; lanes 5–8. *F. avenaceum* isolates 20–23; lanes 9–11. *F. crookwellense* isolates 24–26; lanes 12–14. degenerated *Fusarium* sp. isolates 27–29; lane 15. DNA size marker.

Tab. 2. – Band patterns and their estimated restriction fragment sizes obtained from rDNA ITS digestions. Band patterns (A–D) and their component fragment sizes (base pairs).

Enzyme	A	B	C	D
Taq I	215, 140, 90, 60	240, 210, 60	210, 100, 90, 60	-
Hae III	390, 120, 90	380, 120, 90	370, 120, 90	350, 120, 90
EcoR I	310, 280	320, 290	-	-
Pst I	390, 190	440, 140	-	-
Msp I	320, 260	320, 210	-	-
Hinf I	280 (double?), 50	290, 280, 50	-	-

Fragments smaller than 50 bp were not resolved. No digestion of *F. culmorum* ITS occurred with *Bcl* I, *Bgi* I, *Bgi* II, *Bsc* I, *Dra* I, *EcoR* V, *Hind* III, *Pvu* II, *Sac* II, *Sca* I, *Sma* I and *Xba* I.

regions and the intervening 5.8S segment. Amplification resulted in a single band of 550–600 bp, which was uniform in size amongst the *F. culmorum* isolates studied. There was some evidence of slight variation in size between the other species, but probably less than 50 bp. Digestion with restriction enzymes resulted in the fragments as shown in Tab. 2. The patterns for each isolate are shown in Tab. 3. With the restriction enzymes used it was possible to differentiate between *F. culmorum* and *F. avenaceum*, but not all strains of *F. culmorum* and *F. crookwellense*. Putative *F. graminearum* strain 29 was more similar to *F. culmorum* and *F. crookwellense* than to *F. avenaceum*. Degenerated strain 27 gave exactly the same digestion pattern as *F. avenaceum*. Strain 28 was most similar to *F. avenaceum*, but was distinguishable by its digestion patterns with *Hae* III and *Msp* I.

Restriction digestion of rDNA IGS amplification product

The ribosomal RNA gene IGS region was amplified with two sets of primers. Primers 3´28S and CNS1 amplified the whole IGS region, from the 3´ end of the 28S subunit to the 5´ end of the 18S subunit. These primers yielded a PCR product of 2300 to 2700 bp, the size of product varying between strains by several hundred bp (Tab. 4). Band sizes were consistent within the strains of *F. culmorum* and *F. avenaceum* tested, but varied between the three strains of *F. crookwellense*. Primers CNL12 and U:49–67 amplified from a position near the 3´ end of the 28S subunit to a site some way within the IGS region. Again, these primers yielded different sized PCR product in the different taxa (Tab. 4). This was digested with restriction enzymes *Taq* I, *Hae* III, *Msp* I, *EcoR* I and *Hinf* I, giving fragment patterns as shown in Tab. 5 (Figs. 3 and 4). The patterns for the strains tested are summarised in Tab. 6. These patterns showed consistency within taxa and

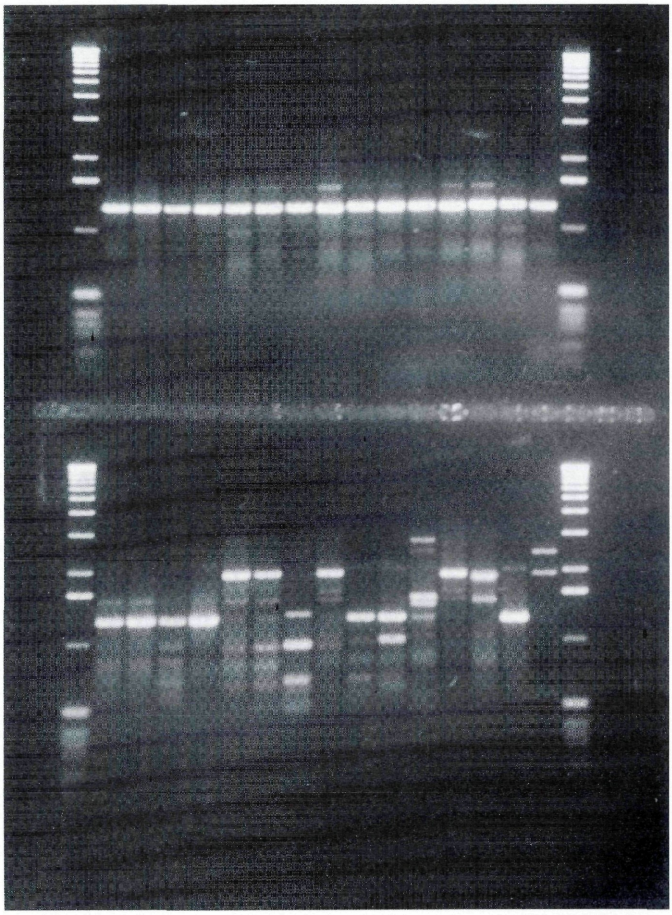

Fig. 2. – *Fusarium* isolates with Simple Sequence Repeat primer (CAG)5 at 45 C annealing. Top lanes (left/right) lane 1. DNA size marker; lanes 2–16. *F. culmorum* isolates 1–15; lane 17. DNA size marker. Bottom lanes (left/right): lane 1. DNA size marker; lanes 2–5. *F. culmorum* isolates 16–19; lanes 6–9. *F. avenaceum* isolates 20–23; lanes 10–12. *F. crookwellense* isolates 24–26; lanes 13–15. degenerated *Fusarium* sp. isolates 27–29; lane 16. – DNA control; lane 17. DNA size marker.

Tab. 3. – Restriction digest patterns of rDNA ITS PCR product digested with six restriction enzymes.

Isolate	Name	*Taq* I	*Hae* III	*EcoR* I	*Pst* I	*Msp* I	*Hinf* I
1	*F. culmorum*	A	B	A	A	U	A
2	*F. culmorum*	A	B	A	A	U	A
3	*F. culmorum*	A	C	A	A	U	A
4	*F. culmorum*	A	C	A	A	U	A
5	*F. culmorum*	A	C	A	A	U	A
6	*F. culmorum*	A	B	A	A	U	A
7	*F. culmorum*	A	C	A	A	U	A
8	*F. culmorum*	A	C	A	A	U	A
9	*F. culmorum*	A	B	A	A	U	A
10	*F. culmorum*	A	B	A	A	U	A
11	*F. culmorum*	A	B	A	A	U	A
12	*F. culmorum*	A	B	A	A	U	A
13	*F. culmorum*	A	B	A	A	U	A
14	*F. culmorum*	A	B	A	A	U	A
15	*F. culmorum*	A	C	A	A	U	A
16	*F. culmorum*	A	C	A	A	U	A
17	*F. culmorum*	A	C	A	A	U	A
18	*F. culmorum*	A	B	A	A	U	A
19	*F. culmorum*	A	C	A	A	U	A
20	*F. avenaceum*	B	A	B	B	A	B
21	*F. avenaceum*	B	A	B	B	A	B
22	*F. avenaceum*	B	A	B	B	A	B
23	*F. avenaceum*	B	A	B	B	A	B
24	*F. crookwellense*	A	C	A	A	U	A
25	*F. crookwellense*	A	C	A	A	U	A
26	*F. crookwellense*	C	C	A	A	U	A
27	*Fusarium* sp.	B	A	B	B	A	B
28	*Fusarium* sp.	B	C	A	B	B	B
29	*Fusarium* sp.	A	D	A	A	U	A

Patterns are defined in Tab. 2. U = uncut.

Tab. 4. – Estimated size of IGS amplification product (base pairs) obtained from *Fusarium* isolates using primer pairs 3'28S / CNS1 and CNL12 / U: 49–67.

Isolate	Name	3'28S / CNS1	CNL12 / U:49-67
1	*F. culmorum**	2300	490
18	*F. culmorum*	2300	490
19	*F. culmorum*	2300	490
20	*F. avenaceum*	2500	950
21	*F. avenaceum*	2500	950
22	*F. avenaceum*	2500	950
24	*F. crookwellense*	2500	600
25	*F. crookwellense*	2450	600
26	*F. crookwellense*	2300	FA
27	*Fusarium* sp.*	2500	950
28	*Fusarium* sp.*	2700	1100
29	*Fusarium* sp.*	2300	FA

* strains with degenerated phenotype. FA = failed amplifications.

Tab. 5. – Band patterns and their estimated restriction fragment sizes obtained from digestions of rDNA IGS PCR product from primers CNL12 and U: 49–67. Band patterns (A–D) and their component fragment sizes (base pairs).

Enzyme	A	B	C	D
Taq I	200, 150, 115	230, 170, 150, 115, 90, 80	200, 150, 140, 115	380, 150, 140, 115, 90
Hae III	410, 80	460, 130, 80	540, 80	460, 240, 80
Msp I	350, 300, 195, 90	350, 320, 300, 90	–	–
Hinf I	190, 110, 100, 90	610, 120, 95, 90, 50	220, 190, 110, 90	750, 120, 95, 90, 50

Fragments smaller than 50 bp were not resolved. No digestion occurred with *EcoR* I.

Tab. 6. – Restriction digest patterns of rDNA IGS PCR product from primers CNL12 and U: 49–67, digested with five restriction enzymes.

Isolate	Name	*Taq* I	*Hae* III	*EcoR* I	*Msp* I	*Hinf* I
1	*F. culmorum*	A	A	U	U	A
18	*F. culmorum*	A	A	U	U	A
19	*F. culmorum*	A	A	U	U	A
20	*F. avenaceum*	B	B	U	A	B
21	*F. avenaceum*	B	B	U	A	B
22	*F. avenaceum*	B	B	U	A	B
24	*F. crookwellense*	C	C	U	U	C
25	*F. crookwellense*	C	C	U	U	C
27	*Fusarium* sp.	B	B	U	A	B
28	*Fusarium* sp.	D	D	U	B	D

Patterns are defined in Tab. 5. U = uncut.

were able to distinguish between the species included. The fragment patterns of *F. culmorum* and *F. crookwellense* were similar, and suggested that there may have been a deletion from the *F. culmorum* IGS of about 100 bp. Degenerated strain 27 again gave the same digestion pattern as *F. avenaceum,* suggesting that this is its identity. Degenerated strain 28 had a similar fragment pattern to *F. avenaceum,* but its IGS product was longer than the latter by about 150 bp. Further strains need to be studied, but these results suggest that IGS restriction digests may be used to differentiate and identify *Fusarium* species, even in isolates with phenotypic degeneration. The IGS region appeared to contain significant deletion/insertion differences between taxa, as well as base substitutions.

Discussion

It is probably no exaggeration to say that we have entered a new phase of *Fusarium* research. We have to accept that molecular methods are going to become increasingly important in *Fusarium* systematics, as in other economically important genera. This is likely to profoundly change our understanding of *Fusarium* and the ways in which we will work with it in future. DNA analysis has the potential to solve some of the problems that have hindered the phenotypic phase of *Fusarium* taxonomy, especially phenotypic plasticity and cultural degeneration.

Taking the results from the various techniques used in this study, it can be seen that the different methods broadly concurred with each other, but with differing levels of clarity, resolution and repeatability. The results support the findings of previous studies using other tech-

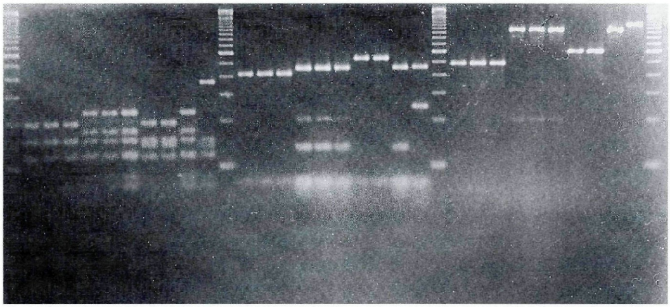

Fig. 3. – Restriction patterns from rDNA IGS region amplified with primers CNL12 and U: 49–67. Lane (left/right) 1 100 bp DNA size marker (smallest band 100 bp, bright band 600 bp); lanes 2–11 *Taq* I digested: lanes 2–4. *F. culmorum* isolates 1, 18, 19; lanes 5–7. *F. avenaceum* isolates 20–22; lanes 8–9. *F. crookwellense* isolates 24–25; lanes 10–11. degenerated *Fusarium* isolates 27–28; lane 12. 100 bp DNA size marker; lanes 13–22: same isolates digested with *Hae* III; lane 23. 100 bp DNA size marker; lanes 24–33: same isolates, undigested DNA; lane 34. 100 bp DNA size marker.

niques (Nicholson & al., 1993; Koopmann & al., 1994; Schilling & al., 1994), that there is relative homogeneity within *F. culmorum*.

RAPDs 10-mer #71 used at 52 C annealing yielded band patterns differentiating at both the strain and species level. *F. culmorum* isolates shared a common banding pattern, but with individual strains varying by lacking one or more of these bands. This variation between

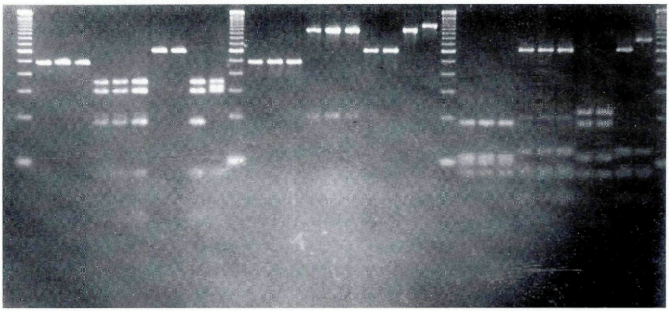

Fig. 4. – Restriction patterns from rDNA IGS region amplified with primers CNL12 and U: 49–67. Lane (left/right) 1 100 bp DNA size marker (smallest band 100 bp, bright band 600 bp); lanes 2–11 *Msp* I digested: lanes 2–4. *F. culmorum* isolates 1, 18, 19; lanes 5–7. *F. avenaceum* isolates 20–22; lanes 8–9. *F. crookwellense* isolates 24–25; lanes 10–11. degenerated *Fusarium* isolates 27–28; lane 12. 100 bp DNA size marker; lanes 13–22: same isolates digested with *EcoR* I (but uncut); lane 23. 100 bp DNA size marker; lanes 24–33: same isolates, digested with *Hinf* I; lane 34. 100 bp DNA size marker.

isolates did not correlate with district or year of collection. Isolate 2, from Canada in 1929, shared a similar pattern to strains isolated in UK during the 1980's, suggesting stability over time and between geographical sources. This primer was able to differentiate *F. avenaceum*, but not *F. crookwellense*. Arbitrary-primed PCR, such as RAPD, has advantages such as not needing to have prior DNA sequence data for your test organisms, primer kits are commercially available and it is easy to generate large volumes of data. However, despite the widespread usage and apparent success of RAPDs in *Fusarium* research, it could be argued that it is not an appropriate technique for many taxonomic studies. RAPDs is usually carried out with short (10-mer) primers, with annealing at around 35 C and with elevated magnesium ion concentrations, so resulting in low amplification stringency. It is known that RAPDs banding patterns are highly sensitive to the type of polymerase used, polymerase concentration, sample DNA concentration, primer concentration, magnesium concentration, thermal cycling parameters and DNA impurities (Caetano-Anollés, 1993; Ellsworth & al., 1993; Muralidharan & Wakeland, 1993; Penner & al., 1993; Tommerup & al., 1995). Only by meticulous standardisation of all these factors can reproducible banding patterns be obtained. RAPDs therefore suffers from analogous problems to those that we have experienced with phenotypic taxonomy, i.e. plasticity and the need for standardised conditions, except that RAPDs is even more sensitive and difficult to reproduce. Also, unless RAPDs bands are probed or sequence characterised, it cannot be assumed that co-migrating bands are actually the same product. Assumptions of relatedness may be acceptable in studies of populations of closely related strains where identical or very similar band profiles are obtained, but if strains give different band profiles no conclusions can be drawn about their relatedness. It remains to be seen whether such techniques will be sufficiently reliable and reproducible to be used for routine identification of unknown isolates from diverse sources, by workers in labs spread around the world.

Simple sequence repeat primers $(CAG)_5$, $(TCC)_5$ and $(CA)_8RG$ gave distinct banding patterns resembling those typically obtained with RAPDs fingerprinting, with a combination of strong and weak bands. It is possible that the major bands represent targeted inter-repeat amplification products, whilst the weak bands were arbitrary products of the type formed in RAPDs (Weising & al., 1995). In most cases similar banding patterns were obtained at 45 C and at 58 C, which is higher than the thoeretical melting temperature of the primers (50 C) according to the Wallace rule. Similar results were reported by Weising & al. (1995). This suggests that SSR-PCR with these primers is less sensitive to temperature conditions than RAPDs and may make the technique more robust between laboratories. However, anomalous

banding patterns were sometimes observed. For example, *F. avenaceum* strain 22 gave a different band pattern from the other strains of that species with primer $(CAG)_5$. One possible explanation could be chromosomal rearrangement, which is reported to occur in Fusaria (Miao, 1990; Fekete & al., 1993; Kim & al., 1993; Migheli & al., 1993; Boehm & al., 1994; Nagy & Hornok, 1994; Nazareth & Bruschi, 1994, Nagy & al., 1995), and which might be expected to change the pattern of dispersed repeats. SSR primer $(CAT)_5$ yielded a smear at the top of the gels, suggesting either that CAT repeat DNA was present very abundantly, or else present as a large localised region in which primers annealed in many positions. Since CAT repeats are reported to be highly abundant in at least some fungi (e.g. Wildeman & al., 1986; DeScenzo & Harrington, 1994), the former may be the most likely explanation. Overall, SSR-PCR gave results similar to those obtained with RAPDs primer #71 at increased stringency. If such primers were to be used for identification purposes several criteria would need to apply: 1. a combination of several primers should be used; 2. PCR conditions would need to be carefully standardised as with RAPDs; 3. PCR conditions should be optimised for each primer used.

Specifically targeted PCR aimed at rDNA spacer regions, followed by restriction digestion, gave more clear cut differentiation between the taxa studied and would be expected to be robust and repeatable for identification purposes. This is a significant advantage over arbitrary PCR and it would seem a better course for us to explore PCR methods using targeted primers, rather than arbitrary PCR, for taxonomic studies above the population level in *Fusarium*. Ribosomal RNA genes are rich sources of data and have the advantage of being studied in a wide range of different organisms. Sequence analysis of rDNA is likely to provide valuable information for the development of classification of *Fusarium* at several taxonomic ranks (Gaudet & al., 1989; Peterson, 1991; O'Donnell, 1992). However, it would be interesting to compare results of rDNA variation with other parts of the genome, including expressed genes (Donaldson & al., 1995), regions not subject to concerted evolution and to apparently neutral regions such as pseudogenes. Identification of *Fusarium* taxa by restriction endonuclease digestion of amplified spacer regions appears to be a real possibility, even for phenotypically degenerated strains. rDNA ITS regions were found to vary only slightly in length in the isolates here studied, whereas the IGS had both base substitutions and sizable deletions/insertions. Whereas this size variation would cause alignment problems in sequencing studies, it may be advantageous for identification purposes using restriction digestions. Further work is in progress to investigate this potential.

An important issue that must be addressed is how to integrate molecular data with our existing information systems. We have a res-

ponsibility to make the fruits of our improved understanding arising from molecular research accessible to the whole of the biological community. Most people identifying Fusaria at present use a microscope. Today the cost of a PCR machine and electrophoresis system is similar to that of a research microscope. It is not difficult to imagine that the people using their microscopes today will, in five years or so, use a microscope and PCR system for *Fusarium* identification on a routine basis. Maybe in 5–10 years some will use PCR alone. However, DNA sequencing equipment is today considerably more expensive and it seems much less likely that it will come to be in widespread use unless there is a radical change in the technology to make it cheaper. It would be irresponsible for us to develop an identification system for *Fusarium* that depends upon having access to expensive technology and which would therefore prohibit use by many people, especially in developing countries. As we use DNA analysis to gain more sophisticated insights into *Fusarium* variation, and develop our classification system accordingly, we must then relate this insight back in a form that will be appropriate to the needs of the eventual end-users of the information. This may involve identifying diagnostic phenotypic traits, if possible, or else developing low-tech DNA based procedures. In the future it may be possible to buy off-the-shelf kits to identify, for example, Fusaria on wheat in temperate regions, or fungal pathogens on rice seed. Meanwhile we should bear in mind the needs of the whole mycological community, not just an affluent minority.

Finally, there is the difficult question of species concepts. We need to acknowledge that there is not, and never will be, a single definition of what a species is in *Fusarium*. This is not due to a failure on the part of taxonomists, but simply an acceptance of biological reality. Biological systems are complex and dynamic and cannot be reduced to a series of uniform-sized pigeon holes. The best we can do is to keep updating our understanding on the basis of new data. At one extreme we can adopt a pragmatic, even cynical, view that species are just „whatever groups of collections that it helps people to delineate". Most biologists may accept that concepts of higher taxa, including genera and sections, are arbitrary (e.g. Booth, 1978), but the idea implicit of species is that they somehow reflect the natural, functional biological diversity of living populations. Molecular data will undoubtedly contribute greatly to our understanding of *Fusarium* diversity, but it will not automatically define species boundaries. We will always have to interpret patterns of diversity in their biological context, incorporating what we know about the ecology, population genetics, incompatibility systems, life cycle, dispersal mechanisms, host/parasite relationships, etc.

The title of this contribution „Molecules maketh the mould" is derived from an old English saying, attributed to William of Wykeham

(1324–1404), that „Manners maketh the man", meaning that one should judge a person not by their title, but by what they do. Similarly, we should not look at *Fusarium* simply as a set of DNA sequences, but should always bear in mind its wider biological context.

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References

Ainsworth, G. C. (1976). Introduction to the history of mycology. – Cambridge University Press, Cambridge, UK, 359 pp.

Assigbetse, K. B., D. Fernandez, M. P. Dubois & J.-P. Geiger (1994). Differentiation of *Fusarium oxysporum* f.sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. – Phytopathology 84: 622–626.

Boehm, E. W. A., R. C. Ploetz & H. C. Kistler (1994). Statistical analysis of electrophoretic karyotype variation among vegetative compatibility groups of *Fusarium oxysporum* f.sp. *cubense*. – Mol. Plant-Microbe Interact. 7: 196–207.

Booth, C. (1971). The genus *Fusarium*. – CMI, Kew, UK, 237 pp.

—— (1978). Do you believe in genera? – Trans. Brit. Mycol. Soc. 71: 1–9.

Bruns, T. D., T. J. White & J. W. Taylor (1991). Fungal molecular systematics. – Annu. Rev. Ecol. Syst. 22: 525–564.

Caetano-Anollés, G. (1993). Amplifying DNA with arbitrary oligonucleotide primers. – PCR Meth. Applic. 3: 85–94.

Cenis, J. L. (1992). Rapid extraction of fungal DNA for PCR amplification. – Nucl. Acids Res. 20: 2380.

Crowhurst, R. N., B. T. Hawthorne, E. H. A. Rikkerink & M. D. Templeton (1991). Differentiation of *Fusarium solani* f.sp. *cucurbitae* races 1 and 2 by random amplification of polymorphic DNA. – Curr. Genet. 20: 391–396.

DeScenzo, R. A. & T. C. Harrington (1994). Use of $(CAT)_5$ as a DNA fingerprinting probe for fungi. – Phytopathology 84: 534–540.

Donaldson, G. C., L. A. Ball, P. E. Axelrood & N. L. Glass (1995). Primer sets developed to amplify conserved genes from filamentous ascomycetes are useful in differentiating *Fusarium* species associated with conifers. – Appl. Environm. Microbiol. 61: 1331–1340.

Ellsworth, D. L., K. D. Rittenhouse & R. L. Honeycutt (1993). Artifactual variation in randomly amplified polymorphic DNA banding patterns. – BioTechniques 14: 214–216.

Fekete, C., R. Nagy, A. J. M. Debets & L. Hornok (1993). Electrophoretic karyotypes and gene mapping in eight species of the *Fusarium* sections *Arthrosporiella* and *Sporotrichiella*. – Curr. Genet. 24: 500–504.

Gaudet, J., J. Julien, J. F. Lafay & Y. Brygoo (1989). Phylogeny of some *Fusarium* species determined by large-subunit rRNA sequence comparison. – Mol. Biol. Evol. 6: 227–242.

Grajal-Martin, M. J., C. J. Simon & F. J. Muehlbauer (1993). Use of random amplified polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f.sp. *pisi*. – Phytopathology 83: 612–614.

Kelly, A., A. R. Alcalá-Jiménez, B. W. Bainbridge, J. B. Heale, E. Pérez-Artéz & R. M. Jiménez-Díaz (1994). The use of amplified fragment length polymorphisms (AFLPs) of genomic DNA for the characterization of isolates of *Fusarium oxysporum* f.sp. *ciceris*. – In: A. Schots & al. (eds.). Modern assays for plant pathogenic fungi. Identification, detection and quantification. CAB INTERNATIONAL, Wallingford, UK: 75–82.

Kim, D. H., R. D. Martyn & C. W. Magill (1993). Chromosomal polymorphism in *Fusarium oxysporum* f.sp. *niveum*. – Phytopathology 83: 1209–1216.

Kohn, L.M. (1992). Developing new characters for fungal systematics: an experimental approach for determining the rank of resolution. – Mycologia 84: 139–153.

Koopmann, B., P. Karlovsky & G. Wolf (1994). Differentiation between *Fusarium culmorum* and *Fusarium graminearum* by RFLP and with species specific DNA probes. – In: A. Schots & al. (eds.). Modern assays for plant pathogenic fungi. Identification, detection and quantification. CAB INTERNATIONAL, Wallingford, UK: 37–46.

Manulis, S., N. Kogan, M. Reuven & Y. Ben-Yephet (1994). Use of the RAPD technique for identification of *Fusarium oxysporum* f.sp. *dianthi* from carnation. – Phytopathology 84: 98–101.

Mes, J. J., J. van Doorn, E. J. A Roebroeck & P. M. Boonekamp (1994). Detection and identification of *Fusarium oxysporum* f.sp. *gladioli* by RFLP and RAPD analysis. – In: A. Schots & al. (eds.). Modern assays for plant pathogenic fungi. Identification, detection and quantification. CAB INTERNATIONAL, Wallingford, UK: 63–68.

Metzenberg, R. L. (1991). Benefactor's lecture: The impact of molecular biology on mycology. – Mycol. Res. 95: 9–13.

Miao, V. P. W. (1990). Using karyotype variability to investigate the origins and relatedness of isolates of *Fusarium oxysporum* f.sp. *cubense*. – In: R. C. Ploetz (ed.). *Fusarium* wilt of banana. APS Press, St. Paul, MN, USA: 55–62.

Migheli, Q., T. Berio & M. L. Gullino (1993). Electrophoretic karyotypes of *Fusarium* spp. – Exper. Mycol. 17: 329–337.

Muralidharan, K. & E. K. Wakeland (1993). Concentration of primer and template qualitatively affects products in random-amplified polymorphic DNA PCR. – BioTechniques 14: 362–364.

Nagy, R. & L. Hornok (1994). Electrophoretic karyotype differences between two subspecies of *Fusarium acuminatum*. – Mycologia 86: 203–208.

——, É. Táborhegyi, A. Witter & L. Hornok (1995). Minichromosomes in *Fusarium sporotrichioides* are mosaics of dispersed repeats and unique sequences. – Microbiol. 141: 713–719.

Nazareth, S. W. & C. V. Bruschi (1994). Electrophoretic karyotype of *Fusarium solani*. – Microbiol. 140: 1373–1375.

Nicholson, P., P. Jenkinson, H. N. Rezanoor & D. W. Parry (1993). Restriction fragment length polymorphism analysis of variation in *Fusarium* species causing ear blight of cereals. – Plant Pathol. 42: 905–914.

Nirenberg, H. (1976). Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion *Liseola*. – Mitteil. Biol. Bundesanstalt Land- und Forstwirtschaft, Berlin-Dahlem 169: 1–117.

O'Donnell, K. (1992). Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum (Gibberella pulicaris)*. – Curr. Genet. 22: 213–220.

Ouellet, Th. & K. A. Seifert (1993). Genetic characterization of *Fusarium graminearum* strains using RAPD and PCR amplification. – Phytopathology 83: 1003–1007.

Penner, G. A., A. Bush, R. Wise, W. Kim, L. Domier, K. Kasha, A. Laroche, G. Scoles, S. J. Molnar & G. Fedak (1993). Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. – PCR Meth. Appl. 2: 341–345.

Peterson, S. W. (1991). Phylogenetic analysis of *Fusarium* species using ribosomal RNA sequence comparisons. – Phytopathology 81: 1051–1054.

Pomazi, A., M. Pesti & L. Hornok (1993). Identification of *Fusarium oxysporum* f.sp. *pisi* populations in Hungary by hyphae anastomosis and DNA polymorphism. – Novenyvedelem 29: 218–224.

Samuels, G. J. & K. A. Seifert (1995). The impact of molecular characters on systematics of filamentous ascomycetes. – Annu. Rev. Phytopathol. 33: 37–67.

Schilling, A. G., E. M. Möller & H. H. Geiger (1994). RAPDs of *Fusarium culmorum* and *F. graminearum:* application for genotyping and species identification. – In: A. Schots & al. (eds.). Modern assays for plant pathogenic fungi. Identification, detection and quantification. CAB INTERNATIONAL, Wallingford, UK: 47–56.

Snyder, W. C. & H. N. Hansen (1945). The species concept in *Fusarium* with reference to *Discolor* and other sections. – American J. Bot. 32: 657–666.

Tommerup, I. C., J. E. Barton & P. A. O'Brien (1995). Reliability of RAPD fingerprinting of three basidiomycete fungi, *Laccaria, Hydnangium* and *Rhizoctonia*. – Mycol. Res. 99: 179–186.

Weising, K., R. G. Atkinson & R. C. Gardner (1995). Genomic fingerprinting by microsatellite-primed PCR: a critical evaluation. – PCR Meth. Appl. 4: 249–255.

Welsh, J. & M. McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. – Nucl. Acids Res. 18: 7213–7218.

White, T. J., T. Bruns, S. Lee & J. Taylor (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: M.A. Innis & al. (eds.). PCR Protocols: A Guide to Methods and Applications. Academic Press, London: 315–322.

Wildeman, A. G., I. Rasquinha & R. N. Nazar (1986). A „CAT" family of repetitive DNA sequences in *Saccharomyces cerevisiae*. – J. Biol. Chem. 261: 13401–13403.

Wollenweber, H. W. & O. A. Reinking (1935). Die Fusarien, ihre Beschreibung, Schadwirkung und Bekämpfung. – Paul Parey, Berlin, 355 pp.

Wright, G. K., I. Pascoe, R. van Heeswijck, D. Guest & S. Wimalajeewa (1992). Characterization of isolates of *Fusarium oxysporum* from carnations in Victoria. – Acta Horticult. No. 307: 65–72.

Zietkiewicz, E., A. Rafalski & D. Labuda (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. – Genomics 20: 176–183.

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