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Genetic variability of central European isolates of the *Fusarium graminearum* species complex

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Abstract

The main causative agents of *Fusarium* head blight in central Europe are *Fusarium graminearum* and *F. culmorum*. We examined the mycotoxin producing ability, aggressiveness and molecular variability of *F. graminearum* isolates. Altogether twenty-six Hungarian, three Austrian isolates and representatives of eight species identified in the *F. graminearum* species complex were involved in this study. Mycotoxin producing abilities of the isolates were tested by GC-MS and HPLC. The central European isolates were found to belong to chemotype I (producing deoxynivalenol). Most isolates produced more 15-acetyl-deoxynivalenol than 3-acetyl-deoxynivalenol suggesting that they belong to chemotype Ib. All *F. graminearum* isolates were found to be highly pathogenic in *in vitro* aggressiveness tests. Phylogenetic analysis of random amplified polymorphic DNA profiles, and restriction profiles of the intergenic spacer region of the ribosomal RNA gene cluster of the isolates allowed clustering of the central European isolates into 17 and 16 haplotypes, respectively. When RAPD and IGS-RFLP data were combined, almost every single central European *F. graminearum* isolate could be differentiated (27/29 haplotypes). Sequence analysis of a putative reductase gene of some isolates was also performed. Based on molecular data, the majority of the central European isolates belonged to *F. graminearum sensu stricto* characteristic to the northern hemisphere, with the exception of one Hungarian isolate, which was not related to any known species of the *F. graminearum* species complex based on sequence data. The taxonomic assignment of two other Hungarian isolates, previously suggested as belonging to *F. boothii* based on mitochondrial DNA restriction profiles, was supported by sequence analysis.

Introduction

Fusarium head blight (FHB) is economically one of the most important fungal diseases of wheat throughout the world. The main causative agents of this disease are members of section *Discolor* of the *Fusarium* genus. Aggressiveness tests proved that isolates of *Fusarium graminearum*, *F. culmorum* and *F. avenaceum* are the most aggressive to wheat among Fusaria in Hungary (Mesterházy, 1985). In Hungary, *Fusarium* head blight reached epidemic levels in several years during the last decade, causing dramatic yield losses (Varga et al., 2004). However, contamination of wheat by the mycotoxins produced by *F. graminearum* and *F. culmorum* is the most serious effect of FHB, since these mycotoxins are harmful both to humans and animals. *Fusarium graminearum* isolates produce a range of trichothecenes including deoxynivalenol (DON) and its derivatives, nivalenol (NIV) and 4-acetyl-nivalenol (fusarenone X), and the oestrogenic mycotoxin zearalenone. Trichot-

hecenes are potent inhibitors of eukaryotic protein synthesis, and cause food refusal, diarrhoea, vomiting and dermatitis in animals (Goswami and Kistler, 2004). Studies have also suggested that trichothecenes are involved in plant pathogenesis (Mesterházy, 2002; Proctor et al., 2002).

Fusarium graminearum sensu lato is a monophyletic species complex consisting of at least nine phylogenetic species, some of which are localized in particular geographical regions (O'Donnell et al., 2000, 2004; Ward et al., 2002). These species have been formally named, with *F. graminearum sensu stricto* retained for the species most commonly associated with *Fusarium* head blight worldwide. Another species of this complex, *F. asiaticum* is also known to be responsible for *Fusarium* head blight in some parts of Asia (O'Donnell et al., 2004). However, only limited data are available regarding the assignation of Hungarian isolates of *F. graminearum sensu lato*. Previously we examined the genetic variability of *F. culmorum* using a variety of techniques (Tóth et al., 2004). In this study, our aim was to examine the genetic variability of central European isolates of the *F. graminearum* species complex using phenotypic and genotypic methods. In this paper, the name *F. graminearum* for *F. graminearum sensu stricto* in the sense of O'Donnell et al. (2004) was used wherever possible. For the species complex, *F. graminearum sensu lato* or *F. graminearum* species complex were used, while the different species names were applied for the different lineages/species identified by O'Donnell et al. (2004). Species names and lineage numbers are shown in Table 1.

Materials and methods

Phenotypic methods

Altogether 38 *Fusarium* isolates including 26 Hungarian, three Austrian isolates, representatives of the eight species of the *F. graminearum* species complex identified by O'Donnell et al. (2000, 2004) and one *F. pseudograminearum* isolate were examined (Table 1). The Hungarian isolates were collected by Á. Mesterházy, while the Austrian isolates were kindly provided by Prof. P. Ruckenbauer (IFA-Tulln, Austria). The isolates were inoculated to sterilized rice medium (Szécsi and Bartók, 1995) and grown for 21 days at 25 °C. After incubation, rice cultures were freeze-dried and 5 g were extracted in centrifuge tubes (30 ml) with 20 ml acetonitrile-water (84:16, v/v) on a vertical shaker for 2 h. After centrifugation at $5000\times g$ for 10 min, 2 ml of the crude extracts were cleaned by solid-phase extraction using extraction columns filled with 0.5 g neutral alumina/activated charcoal (20:1, m/m). The eluates were evaporated to dryness, redissolved in 100 μl of pyridine and derivatised with 100 μl of silylating mixture (BSA/TMSI/TMCS, 3/3/2, v/v) at 65 °C for 30 min. Samples (1 μl) were analyzed by GC–MS (HP 5890 Series II GC Plus gas chromatograph, HP 5989 B mass spectrometer). Temperatures of split injector, GC–MS interface, MS EI (70 eV) ion source and quadrupole mass analyzer were 280, 290, 200 and 100 °C, respectively. For separation of derivatised trichothecenes an HP Ultra 2 capillary column (12 m$\times$0.2 mm$\times$0.33 μm) was applied with a temperature programme of 235 °C 5 min, then 10 °C/min heating rate to 295 °C and finally held for 10 min on the same temperature. Selected ion monitoring (SIM) was applied to detect the molecular and characteristic fragment ions of trichothecenes. For zearalenone production, the rice cultures were pre-incubated at 25 °C for 14 days, then held at 15 °C for 7 days. Zearalenone content of the crude rice culture extracts were analyzed after membrane filtration by HP 1090 Series II liquid chromatograph equipped with an Agilent 1100 fluorescence detector. Five μl samples were analyzed using an HPLC column (200$\times$2.1 mm) filled with Hypersil ODS 5 μm packing and a binary gradient of water-acetonitrile (from 30 to 100 % acetonitrile in 5 min) at a flow rate of 0.45 ml min^{-1}. Excitation and emission wavelengths of fluorescence detector were 236 and 445 nm respectively. Quantitative evaluation of GC-MS and HPLC/FLD analyses were performed by using 6 points external standard calibration. *In vitro* aggressiveness tests were carried out according to Mesterházy (1985). The wheat cultivars used were Várkony (moderately resistant) and 1933 (Ttj-RC103; susceptible).

Genotypic methods

The *Fusarium* isolates were inoculated into potato dextrose broth (Sigma) and incubated on a rotary shaker at 200 rpm for 5 days at 28 °C. Total

Table 1. Origin, mycotoxin production and aggressiveness of the isolates examined

Species Isolate	Substrate	Origin[c]	Chemotype	Mycotoxin production[d] (mg kg^{-1})				Aggressiveness[e]	RAPD IGS-RFLP haplotypes	
				DON	3AcDON	15AcDON	ZEA			
F. graminearum										
Fg21[a]	Wheat ear	H, Szeged	Ia	11471.2	1060.6	241.6	9031.5	0.00	1	1′
Fg26	Wheat stem	H, Szeged	I	368.5	n.d.	68.1	4399.9	0.60	8	1′
Fg9	Wheat stem	H, Szeged	Ib	464.8	5.3	78.9	4771.9	0.10	2	7′
Fg39	Wheat stem	H, Szeged	Ib	859.5	12.1	105.9	2217.4	21.08	1	6′
Fg6	Wheat stem	H, Szeged	I	625.6	n.d.	90.2	12270.6	1.54	2	2′
Fg38	Wheat stem	H, Szeged	Ib	507.7	7.3	94.5	2124.1	0.60	6	2′
Fg16	Wheat stem	H, Szeged	Ib	874.6	19.6	120.6	n.d.	3.00	6	1′
Fg128	Wheat stem	H, Szeged	Ib	316.4	4.7	67.9	3840.9	5.56	7	1′
Fg8	Wheat stem	H, Szeged	Ib	2116.0	9.5	101.7	24055.0	0.98	6	1′
Fg19	Wheat stem	H, Szeged	Ib	997.3	15.4	104.0	2222.4	0.00	1	5′
Fg30	Wheat ear	H, Szeged	Ib	201.3	5.4	83.9	1468.6	3.31	1	15′
Fg20	Wheat ear	H, Szeged	I	54.7	n.d.	25.0	2064.8	7.31	13	15′
Fg17	Wheat stem	H, Szeged	Ib	418.0	6.1	79.1	3597.7	2.40	2	8′
Fg18	Wheat stem	H, Szeged	Ib	874.9	9.8	122.7	1992.4	1.04	2	8′
Fg35	Wheat ear	H, Szeged	I	15952.8	1238.5	1593.6	7050.8	13.27	10	12′
Fg10	Wheat stem	H, Szeged	Ib	8957.1	60.9	413.9	16228.2	21.54	16	4′
Fg31	Wheat ear	H, Szeged	Ia	7300.8	260.5	126.4	18694.6	18.42	4	8′
Fg129	Wheat stem	H, Szeged	Ib	7401.9	45.7	192.0	4197.3	0.10	9	3′
Fg27	Wheat stem	H, Szeged	Ib	310.0	5.7	59.9	2437.4	22.42	3	8′
Fg402	Wheat ear	H, Szeged	I	n.a.	n.a.	n.a.	n.a.	18.22	1	16′
Fg14502	Wheat seed	H, Kiszombor	Ib	1990.7	48.1	135.8	20139.2	1.52	5	1′
FgV2	Wheat seed	H, Martonvásár	Ib	7164.9	191.8	435.0	16018.3	0.38	12	14′
Fg12377	Maize seed	H, Vésztő	Ib	13627.6	281.1	519.1	18760.7	0.00	11	13′
FgES11	Wheat seed	H, Eszterág	Ib	1092.9	16.3	633.3	6673.5	3.33	1	10′
FgHF010	Wheat ear	H, Debrecen	I	n.d.	n.d.	n.d.	7222.0	16.48	1	9′
FgHF012	Wheat ear	H, Ipolydamásd	Ib	496.6	7.1	36.0	337.9	5.35	17	11′
Fg22	Wheat seed	A	Ib	7079.2	382.4	455.6	1458.7	13.83	15	1′
Fg40	Wheat seed	A	Ib	465.1	11.9	74.5	7397.3	1.54	14	1′
Fg44	Wheat seed	A	Ib	4590.8	28.3	211.7	2444.2	0.00	14	7′
Fg28	Wheat stem	H, Bekes	I	n.a				n.a		
Fg29	Wheat stem	H, Bekes	I	n.a				n.a		
F. austroamericanum (lineage 1)										
NRRL 28585[b]	Grape	VEN	II[f]	n.a.				2.21		
F. meridionale (lineage 2)										
NRRL 28436	Orange	NCL	II	n.a.				22.19		
F. boothii (lineage 3)										
NRRL 29020	Maize	ZA	Ib	n.a.				1.04		

Table 1. Continued

Species Isolate	Substrate	Origin[c]	Chemo type	Mycotoxin production[d] (mg kg^{-1})				Aggressi- veness[e]	RAPD IGS-RFLP haplotypes
				DON	3AcDON	15AcDON	ZEA		
F. mesoamericanum (lineage 4)									
NRRL 29148	*Hedera helix*	USA	Ia	n.a.				16.71	
F. acaciae-mearnsii (lineage 5)									
NRRL 26752	*Acacia* sp	ZA	unknown	n.a.				3.56	
F. asiaticum (lineage 6)									
NRRL 26156	Wheat	CHN	Ia	n.a.				22.54	
F. graminearum (lineage 7)									
NRRL 13383	Maize	IRN	II	n.a.				4.79	
F. cortaderiae (lineage 8)									
NRRL 29306	Wheat	AUS	II	n.a.				5.46	
F. pseudograminearum									
NRRL 28069	Wheat stem base	MA	Ia	n.a.				0.56	

[a]Fg: Collection of Á. Mesterházy, Szeged, Hungary.
[b]NRRL: Agricultural Research Service Culture Collection, Peoria, IL.
[c]H-Hungary, A-Austria, USA-United States of America, AUS-Australia, MA-Morocco, ZA-South Africa, VEN–Venezuela, IRN–Iran, CHN–China, NCL–New-Caledonia.
[d]DON, deoxynivalenol; NIV, nivalenol; 3AcDON, 3-acetyl-deoynivalenol; 15AcDON, 15-acetyl-deoynivalenol; n.a.: not analyzed.
[e]Percentage of the plants surviving the treatment (average of 8 measurements); n.d.: not detected
[f]Type isolates of the different *F. graminearum* clade lineages were not tested for mycotoxin production, their chemotype assignments came from Ward et al. (2002).

nucleic acids were isolated from the lyophilized mycelia of the strains according to standard protocols (Leach et al., 1986). RAPD analyses were carried out with Operon (Operon Technologies, Inc., Alameda, CA; www.operon.com) arbitrary decamer primers used in pairwise combinations according to standard procedures (Rinyu et al., 1995). The primer combinations used were: OPA01-OPZ03, OPA04-OPH10, OPA05-OPP19, OPB02-OPM09, OPB05-OPH11, OPB06-OPM14, OPB08-OPL13, OPC16-OPO04, OPC18-OPJ19, OPE17-OPG16, OPF10-OPL14, OPG11-OPN13, OPG19-OPW14, OPH13-OPN05, OPJ15-OPY01, OPK16-OPY19, OPM18-OPO12, OPP18-OPY14, OPR15-OPY03, OPW17-OPY02. All RAPD analyses were repeated at least three times, and only bands which appeared in all tests were scored. The intergenic spacer region (IGS) of the rRNA gene cluster was amplified as described previously (Mishra et al., 2002), and digested with *Hae*III, *Eco*RI, *Hpa*II, *Rsa*I, *Sau*3AI and *Alu*I restriction enzymes (Carter et al., 2002). The resulting fragments were separated in agarose gel and visualized under UV light. A putative reductase gene fragment was amplified and sequenced according to O'Donnell et al. (2000). DNA fragments were purified from the excised agarose blocks using Genelute spin columns (Supelco). Direct sequencing of the fragments was performed on ABI 373A DNA sequencer (Applied Biosystems Inc.) using dye dideoxy terminator reaction chemistry. Sequences were determined from both strands. Sequences of part of the putative reductase gene of the examined isolates were deposited into the GenBank database under accession numbers AY786340-AY786351. Previously published sequences of the putative reductase gene of species of the *F. graminearum* species complex and *F. pseudograminearum* were also included in the analysis (O'Donnell et al., 2004). To identify the mating type genes present in the *Fusarium* isolates, PCR primers designed by Kerényi et al. (2004) were used as described previously (Tóth et al., 2004).

Data analysis

Phylogenetic analyses of RAPD, IGS-RFLP and sequence data were carried out using the PHYLIP software package (Felsenstein, 1995). A *F. pseudograminearum* isolate was used as outgroup in these analyses. The binary matrices of RAPD and IGS-RFLP data were converted to distance matrices using PhylTools (Buntjer, 1997). Sequence alignments were performed using CLUSTAL-X (Thompson et al., 1997) and improved manually. Evolutionary distances between the sequences were calculated by Kimura's formula (Kimura, 1980) using the programme DNADIST. Phylogenetic trees were prepared by the neighbour-joining method (Saitou and Nei, 1987) using the programme NEIGHBOR of the PHYLIP package. Maximum likelihood analysis of sequence data was carried out using the programme DNAML of the package. Bootstrap values (Felsenstein, 1985) were calculated from 1000 replications of the bootstrap procedure using programmes SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

Results

Mycotoxin production and in vitro aggressiveness tests

Mycotoxin producing abilities of the isolates were examined by GC–MS and HPLC analysis. The mycotoxins analysed were DON, 3-acetyl-DON (3AcDON), 15-acetyl-DON (15AcDON), NIV and zearalenone. Most isolates produced zearalenone (Table 1). All Hungarian isolates produce DON and consequently belong to chemotype I (Table 1). The majority of the isolates produced both 3AcDON and 15AcDON derivatives; however, many of them produced 15AcDON in larger quantities and consequently belong to chemotype Ib (Miller et al., 1991). Huge differences were observed among DON producing abilities of the isolates (54–16,000 mg kg^{-1}). None of the central European isolates produced NIV or fusarenone X. Aggressiveness tests were carried out according to the method of Mesterházy (1985). Most *F. graminearum* isolates were found to be highly pathogenic towards wheat *in vitro* tests (Table 1).

Molecular studies

RAPD analyses were performed using 40 different random primers. The *F. graminearum* isolates were highly variable with most primers (data not shown). For preparing the distance matrix, the

presence or absence of 262 RAPD fragments were scored. Phylogenetic analysis using the neighbour-joining technique allowed clustering of the central European isolates into 17 different haplotypes (Table 1). No correlation was observed between mycotoxin chemotype or geographic origin, and RAPD profiles of the isolates tested. The molecular variability of the isolates was also assessed using IGS-RFLP analysis. For preparing the distance matrix, the presence or absence of 52 RFLP fragments was scored. Phylogenetic analysis using the neighbour-joining technique differentiated the central European isolates into 16 different haplotypes based on their IGS-RFLP profiles (Table 1). No correlation was observed between mycotoxin chemotype, RAPD profiles, geographic origin and IGS-RFLP profiles of the isolates tested. When RAPD and IGS-RFLP data were combined (314 fragments; Figure 1), almost every single central European *F. graminearum* isolate could be differentiated from each other (27/29 haplotypes). The examined Austrian isolates formed a distinct branch on the tree. Representatives of the eight species of *F. graminearum sensu*

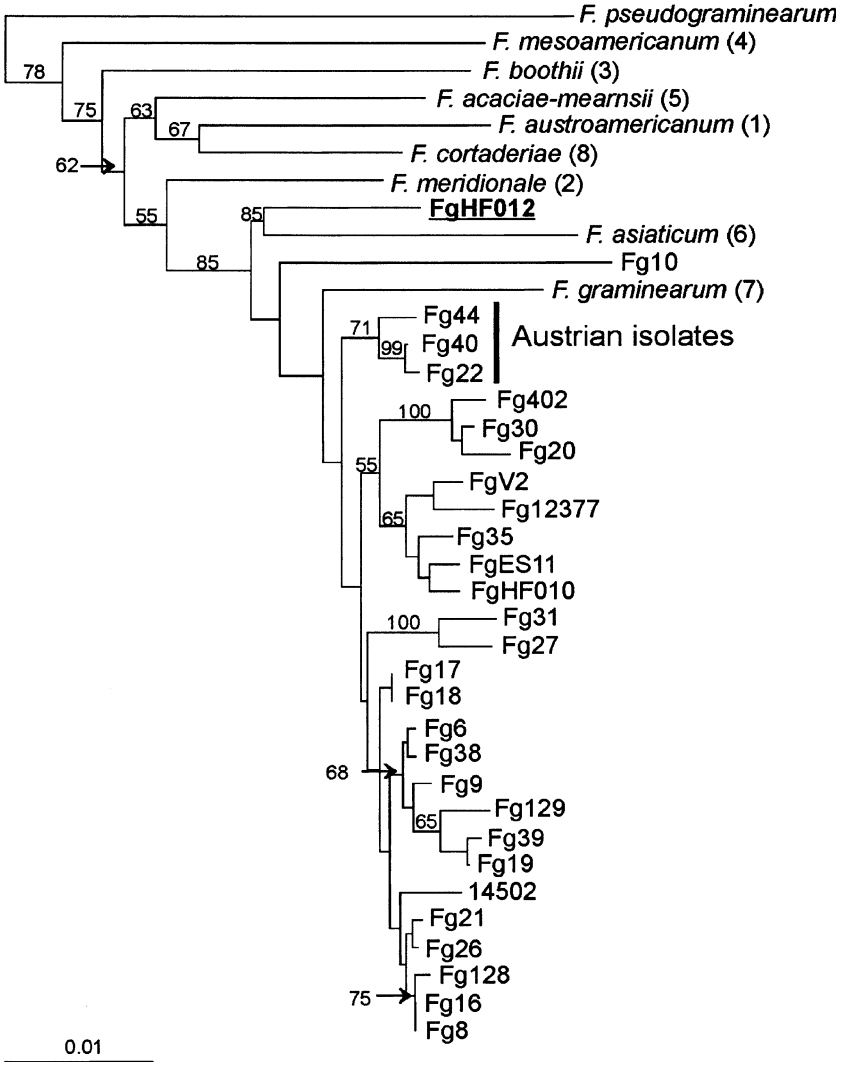

Figure 1. Evolutionary relationships among *F. graminearum* isolates based on IGS-RFLP and RAPD data. Austrian isolates are labelled. The atypical isolate FgHF012 is underlined. Bootstrap values >50% are shown. Numbers in brackets indicate lineage numbers according to O'Donnell et al. (2000).

lato exhibited unique RAPD and IGS-RFLP profiles. The Hungarian isolates were found to be most closely related to an isolate representing *F. graminearum sensu stricto* (lineage 7 according to O'Donnell et al., 2000) characteristic for the Pan-Northern hemisphere, with the exception of one isolate (FgHF012) which was most closely related to an isolate representing *F. asiaticum* (lineage 6; O'Donnell et al., 2000). Mating type gene homologs were also looked for in the isolates. All isolates carried both mating type idiomorphs. Sequences of a putative reductase gene were also determined in selected isolates. Altogether 341 nucleotides of a putative reductase gene fragment were used in the phylogenetic analysis (other parts of the sequenced region aligned ambiguously, and were omitted from the analysis). Within this region, 16 parsimony informative sites were found. A neighbour-joining tree of sequence data is presented in Figure 2. Maximum likelihood analysis resulted in a tree with very similar topology. Most central European isolates formed a well-defined clade with *F. graminearum sensu stricto* isolates. Isolate FgHF012 formed a distinct branch outside of this clade, indicating that it belonged to another species of *F. graminearum sensu lato*. Two Hungarian isolates of *F. graminearum sensu lato* (Fg28 and Fg29) previously suggested as belonging to *F. boothii* (lineage 3) based on mitochondrial DNA RFLP data (Láday et al., 2004) formed a well-defined clade together with *F. boothii* isolates based on sequence data (Figure 2).

Discussion

In this study, the genetic variability of central European *F. graminearum sensu lato* isolates was investigated. Two chemotypes have previously been described in *F. graminearum sensu lato* (Miller et al., 1991; Szécsi and Bartók, 1995). Isolates of chemotype I produced DON and/or its acetylated derivatives, while chemotype II isolates produced NIV and/or fusarenone X. All Hungarian isolates belonged to chemotype I. None of the isolates produced both DON and NIV, although such isolates have been identified previously in several surveys (Sugiura et al., 1990; Szécsi and Bartók, 1995). Most isolates produced both 3AcDON and 15AcDON, similar to the observations of Sugiura et al. (1990) and Ward et al. (2002). The predominant acetyl-DON isomer was 15AcDON, so most isolates belonged to chemotype Ib. It was previously observed that the chemotypes Ia and Ib have some geographical separation. However, in a more recent study, Gale et al. (2003) found some isolates belonging to either chemotypes Ia and II among 86 isolates from across the USA. In Europe, all examined Norwegian isolates belonged to chemotype Ia (Langseth et al., 2001). In contrast, the vast majority of British *F. graminearum* isolates belonged to chemotype Ib, although isolates belonging to chemotypes Ia and II have also been observed (Jennings et al., 2004). In Hungary, Szécsi and Bartók (1995) found both chemotype Ib and II isolates. In other studies, isolates belonging to chemotype Ia were found to be predominant in Japan, China, Argentina, Norway and Nepal, while chemotype Ib was predominant in the USA, Uruguay, New Zealand, Great Britain and Hungary (Lauren et al., 1992; Pineiro et al., 1996; Moon et al., 1999; Broggi and Molto, 2001; Carter et al., 2002). Consequently, there is no clear geographic structuring in the distribution of these chemotypes. Further studies are needed to clarify the role of other parameters (for example host, species, temperature, soil type) in the above mentioned distribution of Ia and Ib chemotypes.

In aggressiveness tests, most isolates were highly pathogenic to wheat in the seedling stage. Interestingly, *F. graminearum* isolates were found to be more aggressive in the *in vitro* tests compared to *F. culmorum* isolates determined previously (Tóth et al., 2004). The aggressiveness of chemotype I isolates to wheat seedlings was in general higher than that of isolates belonging to chemotype II. This observation is in line with the hypothesis that DON is a more effective virulence factor on wheat than NIV (Eudes et al., 1997; Miedaner and Reinbrecht, 2001). Indeed, Blaney and Dodman (2002) found that *F. graminearum* isolates from wheat and barley were DON producers, while those from maize predominately produced NIV. Desjardins et al. (2004) also found that DON production confers a selective advantage to *F. graminearum* on wheat.

The population structure of *F. graminearum* is poorly understood. Studies by O'Donnell and his co-workers clarified that the *F. graminearum* species complex consists of at least nine lineages/species (O'Donnell et al., 2000, 2004; Ward et al.,

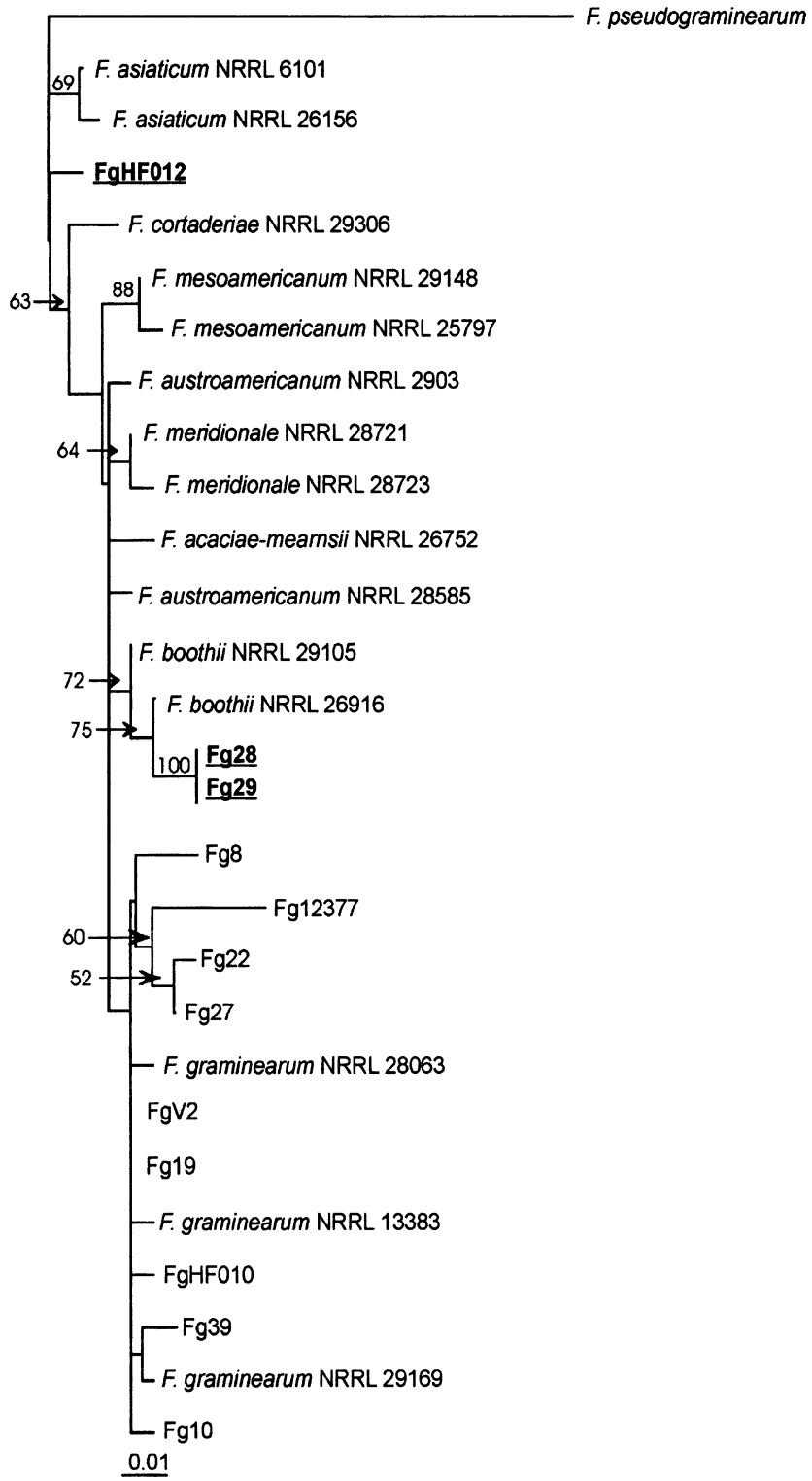

Figure 2. Evolutionary relationships among isolates of the *F. graminearum* species complex based on phylogenetic analysis of partial putative reductase gene sequences. The atypical Hungarian isolates which do not belong to the *F. graminearum sensu stricto* (lineage 7) are underlined. Bootstrap values > 50% are shown.

2002). These species could also be identified based on nuclear and mitochondrial DNA RFLPs (Gale et al., 2002; Láday et al., 2004). It was suggested that the existence of such lineages may be due to longstanding geographic isolation or other reproductive barriers. According to O'Donnell et al. (2000), the most basal (oldest) phylogenetic species of the *F. graminearum* species complex can be found in the southern hemisphere.

Most population-level studies of *F. graminearum* have been carried out in north America. Walker et al. (2001) demonstrated a high level of genotypic and phenotypic diversity in populations of *F. graminearum* in north Carolina. The analysis of 72 isolates from Canada by RAPD showed that all isolates were genetically distinct; 90.56% of the genetic variability was explained by within-region variation (Dusabenyagasani et al., 1999). Fernando et al. (2003) confirmed that diversity between Canadian isolates was more closely related to their aggressiveness and toxin production than to geographic location or to the host from which the pathogen was isolated. Zeller et al. (2003) used AFLP analysis to determine genetic diversity in *F. graminearum sensu stricto* from Kansas and north Dakota, and found genotypically and phenotypically diverse populations that could be viewed as subpopulations throughout the USA. Although previous studies indicated that a homogeneous randomly mating population is present in north America, Zeller et al. (2004) observed that the genetic and geographic distance among populations correlated well indicating that they are not merely subpopulations. Ouellet and Seifert (1993), on the other hand, characterized *F. graminearum* isolates from Canada using RAPD and PCR-RFLP and demonstrated a relatively low amount of genetic diversity.

The variability of *F. graminearum* has also been examined (although less extensively) in other parts of the world. Miedaner et al. (2001) found high molecular genetic diversity and variation in aggressiveness in *F. graminearum* populations originating from Europe. Gale et al. (2002) examined the variability of Chinese *F. graminearum sensu lato* isolates, and found that all isolates belonged to *F. asiaticum*, and it was suggested that diverse subpopulations belonged to a single homogeneous population. Gagkaeva and Yli-Mattila (2004) examined the variability of Russian *F. graminearum sensu lato* isolates and detected higher levels of genetic diversity in Asian isolates than in European ones.

We examined the genetic variability of central European *F. graminearum sensu lato* isolates by RAPD and IGS-RFLP analyses, and by sequence analysis of part of a putative reductase gene. Seventeen and 16 haplotypes could be identified among the central European isolates by RAPD and IGS-RFLP analyses, respectively. A combined analysis of RAPD and IGS-RFLP data differentiated 27 different haplotypes among the 29 isolates (Figure 1). The variability observed was much higher than that found in *F. culmorum* isolates previously (Tóth et al., 2004). The genetic diversity observed was close to that detected by Miedaner et al. (2001), who observed 53 haplotypes among the examined 70 *F. graminearum sensu lato* isolates from south-western Germany. However, they could distinguish only 8 haplotypes among the 28 examined Hungarian *F. graminearum* isolates coming from the wheat cv. Délibáb (Miedaner et al., 2001). No correlation was observed between mycotoxin chemotype or geographic origin, and RAPD profiles of the isolates tested.

Representatives of the eight species of the *F. graminearum* species complex exhibited unique RAPD and IGS-RFLP profiles. Most Hungarian isolates were found to be most closely related to an isolate representing *F. graminearum sensu stricto* characteristic for the Pan-Northern hemisphere, with the exception of a Hungarian isolate (FgHF012) which was more closely related to an isolate representing *F. asiaticum* than to *F graminearum sensu stricto* (Figure 1). Sequence data also indicated that this isolate does not belong to *F. graminearum sensu stricto* (Figure 2). *Fusarium graminearum* and *F. asiaticum* were suggested to be the most recently evolved among species of *F. graminearum sensu lato*, and both species are predominant in the northern Hemisphere (O'Donnell et al., 2004). It is well known that although these lineages are geographically separated, some of them can occur in regions where other species are predominant. For example, *F. graminearum sensu stricto* is the predominant species in north America, but representatives of *F. mesoamericanum* have also been identified (O'Donnell et al., 2004). Isolates of *Fusarium asiaticum* were found in several Asiatic countries including China, Korea, Nepal, and Brazil

(O'Donnell et al., 2004). Recently, Láday et al. (2004) identified two Hungarian isolates (Fg28 and Fg29) with mitochondrial DNA profiles similar to that of *F. boothii* which was originally observed in Africa, but latterly found in other continents (O'Donnell et al., 2004). Our sequence data support their conclusions (Figure 2). The lack of strict correlation between sequence data and geographic origin of the isolates could be due to dispersal of ascospores by wind, rain or by insects (Shields 1999), or to man-made dispersal of different genotypes as suggested earlier (O'Donnell et al., 2000).

When RAPD and IGS-RFLP data were combined (Figure 1), almost every single central European *F. graminearum* isolate could be differentiated from each other (27/29 haplotypes). Such a lack of strict correlation between trees based on different data sets indicates that recombination took place in the examined population due to outcrossing (Taylor et al., 1999). However more sophisticated tests should be carried out to clarify the population structure of *F. graminearum sensu stricto* in Hungary. If isolates from divergent populations interbreed, there is the potential for the production of new genotypes that carry novel combinations of genes for pathogenicity, host range or toxin production (Schardl and Craven, 2003). Indeed, such hybridization and recombination events among *F. graminearum* lineages have been detected by O'Donnell et al. (2000). Therefore, detection of additional isolates belonging to species other than *F. graminearum sensu stricto* of the *F. graminearum* species complex in central Europe is especially important in the future. Further work is in progress to compare the pathogenicity of the isolates belonging to different lineages to European wheat genotypes.

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