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Differentiation of *Fusarium subglutinans* f. sp. *pini* by Histone Gene Sequence Data

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***Fusarium subglutinans* f. sp. *pini* (= *F. circinatum*) is a pathogen of pine and is one of eight mating populations (i.e., biological species) in the *Gibberella fujikuroi* species complex. This species complex includes *F. thapsinum*, *F. moniliforme* (= *F. verticillioides*), *F. nygamai*, and *F. proliferatum*, as well as *F. subglutinans* associated with sugarcane, maize, mango, and pineapple. Differentiating these forms of *F. subglutinans* usually requires pathogenicity tests, which are often time-consuming and inconclusive. Our objective was to develop a technique to differentiate isolates of *F. subglutinans* f. sp. *pini* from other isolates identified as *F. subglutinans*. We sequenced the histone H3 gene from a representative set of *Fusarium* isolates. The H3 gene sequence was conserved and contained two introns in all the isolates studied. From both the intron and the exon sequence data, we developed a PCR-restriction fragment length polymorphism technique that reliably distinguishes *F. subglutinans* f. sp. *pini* from the other biological species in the *G. fujikuroi* species complex.**

Fusarium subglutinans f. sp. *pini* is an important pathogen of pine that causes pitch canker in mature trees (6, 13) and root rot and damping-off in seedlings (2, 34). This fungus can be spread by both infected seedlings and seed (1, 28). The management of *F. subglutinans* f. sp. *pini* would be greatly improved if a quick screening method were available for seed and nursery stock.

F. subglutinans f. sp. *pini* represents one of eight mating populations (i.e., biological species) in the *Gibberella fujikuroi* species complex (6, 23). Three of these mating populations, B, E, and H (*F. subglutinans* f. sp. *pini*), have *F. subglutinans* anamorphs (5, 14, 19, 20). Strains of *Fusarium* isolated from pineapple (*F. subglutinans* f. sp. *ananas*) and mango, for which a teleomorph is not known, also have *F. subglutinans* anamorphs (27, 32, 33).

Distinguishing *F. subglutinans* f. sp. *pini* from the other species in *Fusarium* section *Liseola* usually requires pathogenicity tests or sexual crosses with known tester strains (6, 7, 35). These assays are time-consuming and labor-intensive and do not always yield clear-cut answers. Molecular tools such as random amplification of polymorphic DNA (RAPD) (9, 35, 36), mitochondrial restriction fragment length polymorphisms (RFLP) (7), and ribosomal DNA (rDNA) internal transcribed spacer (ITS1 and ITS2) sequences (25, 37) have been tested for their efficacy in differentiating *F. subglutinans* f. sp. *pini* isolates from other isolates of *F. subglutinans*. Because of the technical difficulties associated with mitochondrial RFLP and the low repeatability of RAPD data, we do not consider these techniques useful for diagnostic purposes. Two different copies of the ITS2 region were identified in the same isolate within some of the species in *Fusarium* section *Liseola* (25, 37), and a reliable diagnostic technique based on these sequences could not be developed. Alternative regions such as the histone and β-tubulin genes might be used more effectively.

O'Donnell et al. (26) used the DNA sequences of the nuclear rDNA large subunit, mitochondrial small subunit, and β-tubulin to develop a phylogeny that includes 36 taxa in the *G. fujikuroi* species complex. These sequences may potentially be useful for diagnostic purposes, but we began our study prior to publication of the phylogeny of O'Donnell et al. (26).

We used an alternative region of the genome, the histone H3 gene, to distinguish *F. subglutinans* f. sp. *pini* isolates from other isolates of *F. subglutinans*. Histone genes encode histone proteins, which are the major constituents of chromatin (16, 21). Four histone proteins, H2A, H2B, H3, and H4, make up the nucleosomal core (17). The gene encoding the H3 protein is well conserved, especially at the amino acid level (12, 31), and the presence of introns enhances its value in taxonomic and phylogenetic studies of closely related organisms (8, 38). Although the histone H4 gene also has these characteristics, it is generally too highly conserved to be suitable for evolutionary studies (30).

Our objectives in this study were (i) to sequence the histone H3 gene from various strains in the *G. fujikuroi* species complex, (ii) to compare the relationships thus determined with those established by use of other sequences, and (iii) to develop a PCR-RFLP procedure based on the histone H3 gene sequence for the routine identification of *F. subglutinans* f. sp. *pini*.

MATERIALS AND METHODS

Fungal isolates. All isolates were maintained on 2% (wt/vol) malt extract agar (Biolab Diagnostics Ltd., Fedlife Park, Midrand, South Africa) in the culture collections of the Forestry and Agricultural Biotechnology Institute at the University of Pretoria, Pretoria, South Africa, and the Medical Research Council, Tygerberg, South Africa. We examined 42 *Fusarium* isolates, including *F. subglutinans* f. sp. *pini*, pathogenic to pine; *F. subglutinans* f. sp. *ananas*, pathogenic to pineapple; *F. subglutinans* isolates associated with maize and mango; and the mating type tester strains from all eight mating populations in the *G. fujikuroi* species complex (Table 1). To test the efficacy of the PCR-RFLP technique for use as a species diagnostic technique (see below), we tested 60 strains of the H mating population identified by Britz et al. (5) and 80 strains representing

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TABLE 1. Host and origin of the different *Fusarium* isolates from the *G. fujikuroi* (Sawada) Wollenw. species complex used in this study

Mating population	Species[a]	Isolate(s)[b]	Host and/or origin	Source	GenBank accession no.
A	*F. moniliforme* Sheldon	MRC 6191, KSU 0999, PEN-M3703	Maize, United States	J. F. Leslie	AF150859
A	*F. moniliforme*	MRC 6155, KSU 0149, PEN3125	Maize, United States	J. F. Leslie	AF150858
B	*F. subglutinans* (Wollenw. and Reinking) Nelson, Toussoun, and Marasas	MRC 6524, KSU 3852, PEN-M6865	Laboratory cross	J. F. Leslie	AF150861
B	*F. subglutinans*	MRC 6525, KSU 3853, PEN-M6866	Laboratory cross	J. F. Leslie	AF150860
C	*F. proliferatum* (Matsushima) Nirenberg	MRC 6570, KSU 4921	Rice, Taiwan	J. F. Leslie	AF150873
C	*F. proliferatum*	MRC 6571, KSU 4922	Rice, Taiwan	J. F. Leslie	AF150872
D	*F. proliferatum*	MRC 6568, KSU 4853	Laboratory cross	J. F. Leslie	AF150871
D	*F. proliferatum*	MRC 6569, KSU 4854	Laboratory cross	J. F. Leslie	AF150870
E	*F. subglutinans*	MRC 6483, KSU 0990, PEN-M3696	Maize, United States	J. F. Leslie	AF150845
E	*F. subglutinans*	MRC 6512, KSU 2192, PEN-M3693	Maize, United States	J. F. Leslie	AF150844
F	*F. thapsinum* Klittich et al.	MRC 6536, KSU 4092	Laboratory cross	J. F. Leslie	AF150857
F	*F. thapsinum*	MRC 6537, KSU 4093	Laboratory cross	J. F. Leslie	AF150856
G	*F. nygamai* Burgess and Trimboli	MRC 7548, KSU 5111	Laboratory cross	J. F. Leslie	AF150854
G	*F. nygamai*	MRC 7549, KSU 5112	Laboratory cross	J. F. Leslie	AF150855
H	*F. subglutinans* f. sp. *pini* Correll et al.	MRC 6209, BBA 69854	Pine, South Africa	A. Viljoen	AF150846
H	*F. subglutinans* f. sp. *pini*	MRC 6211	Pine, South Africa	A. Viljoen	AF150847
H	*F. subglutinans* f. sp. *pini*	MRC 6213	Pine, South Africa	A. Viljoen	AF150849
H	*F. subglutinans* f. sp. *pini*	MRC 6228, PEN-M1290	Pine, United States	P. E. Nelson	AF150850
H	*F. subglutinans* f. sp. *pini*	MRC 7437, FL 103	Pine, United States	T. R. Gordon	AF150848
H	*F. subglutinans* f. sp. *pini*	MRC 7438	Pine, United States	A. Viljoen	AF150851
H	*F. subglutinans* f. sp. *pini*	MRC 7439, FL 15	Pine, United States	T. R. Gordon	AF150852
H	*F. subglutinans* f. sp. *pini*	MRC 7440, FSP 9	Pine, United States	T. R. Gordon	AF150853
	F. subglutinans	MRC 2730	Mango, South Africa	W. F. O. Marasas	AF150865
	F. subglutinans	MRC 3477	Mango, South Africa	W. F. O. Marasas	AF150868
	F. subglutinans	MRC 3478	Mango, South Africa	W. F. O. Marasas	AF150869
	F. subglutinans	MRC 3479	Mango, South Africa	W. F. O. Marasas	AF150867
	F. subglutinans	MRC 7034	Mango, United States	W. F. O. Marasas	AF150864
	F. subglutinans	MRC 7035	Mango, United States	W. F. O. Marasas	AF150866
	F. subglutinans	MRC 7037	Mango, United States	W. F. O. Marasas	AF150863
	F. subglutinans	MRC 7038	Mango, United States	W. F. O. Marasas	AF150862
E	*F. subglutinans*	MRC 115	Maize, South Africa	W. F. O. Marasas	AF150843
E	*F. subglutinans*	MRC 620	Maize, South Africa	W. F. O. Marasas	AF150842
E	*F. subglutinans*	MRC 714	Maize, South Africa	W. F. O. Marasas	AF150841
E	*F. subglutinans*	MRC 756	Maize, South Africa	W. F. O. Marasas	AF150839
E	*F. subglutinans*	MRC 837	Maize, South Africa	W. F. O. Marasas	AF150840
E	*F. subglutinans*	MRC 1077	Maize, South Africa	W. F. O. Marasas	AF150837
E	*F. subglutinans*	MRC 1084	Maize, South Africa	W. F. O. Marasas	AF150838
	F. subglutinans f. sp. *ananas* Ventura, Zambolim, and Gilb.	MRC 6782	Pineapple, Brazil	J. A. Ventura	AF150834
	F. subglutinans f. sp. *ananas*	MRC 6783	Pineapple, Brazil	J. A. Ventura	AF150833
	F. subglutinans f. sp. *ananas*	MRC 6784	Pineapple, Brazil	J. A. Ventura	AF150836
	F. subglutinans f. sp. *ananas*	MRC 6785	Pineapple, Brazil	J. A. Ventura	AF150835
	F. oxysporum Schlecht. emend. Snyd. and Hans.	MRC 6212	Pine, South Africa	A. Viljoen	AF150832

[a] Synonyms for *F. moniliforme*, *F. subglutinans* f. sp. *pini*, and *F. subglutinans* f. sp. *ananas* are *F. verticillioides* Gerlach and Nirenberg (11), *F. circinatum* Nirenberg and O'Donnell (24), and *F. guttiforme* Nirenberg and O'Donnell (24), respectively. The proposed synonyms for *F. subglutinans* from mating population B and *F. proliferatum* from mating population C are *F. sacchari* O'Donnell and Cigelnik (25) and *F. fujikuroi* Gerlach and Nirenberg (11), respectively.

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populations A to F identified by Yan et al. (39). These strains were reassorted and then encoded so that the assays were done in a blind manner.

DNA isolation. Flasks containing 100 ml of malt extract broth (2% [wt/vol]) (Biolab) were inoculated with 1-ml spore suspensions (>1,000 spores/ml). After 2 weeks of static incubation at room temperature (20 to 25°C), mycelium was harvested by filtration through no. 1 filter paper (Whatman BioSystems Ltd., Maidstone, Kent, United Kingdom). Harvested fungal tissue was ground to a powder in liquid nitrogen with a mortar and pestle and homogenized in extraction buffer containing 5% (wt/vol) CTAB (*N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide), 1.4 M NaCl, 0.2% (vol/vol) 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), and 1% (wt/vol) polyvinylpyrrolidone. This homogenate was incubated at 60°C for 1 h and centrifuged (16,000 × *g*) at room temperature. We performed phenol-isoamyl alcohol-chloroform (25:1:24) extractions and removed residual phenol with an additional chloroform extraction. Nucleic acids

were precipitated by adding 0.1 volume of 3 M sodium acetate (pH 5.2) and 0.6 volume of 2-propanol, followed by incubation at 4°C overnight. Precipitated DNA was centrifuged (16,000 × *g*), washed with 70% ethanol, and resuspended in deionized water. This protocol is a variation of the one developed by Murray and Thompson (22).

PCR amplification. PCR amplification was performed as described by Glass and Donaldson (12) with primers H3-1a (5′-ACTAAGCAGACCGCCCGCAG G-3′) and H3-1b (5′-GCGGGCGAGCTGGATGTCCTT-3′). These primers were constructed to flank at least one intron and amplify approximately 450 bp of the *Neurospora crassa* histone H3 gene. Each PCR mixture contained 1 mM deoxynucleotide triphosphates (0.25 mM each), 2.5 mM $MgCl_2$, 0.2 μM H3-1a, 0.2 μM H3-1b, 0.25 ng of DNA per μl, 0.05 U of Super-Therm DNA polymerase [Southern Cross Biotechnology (Pty.) Ltd., Cape Town, South Africa] per μl, and 1× Super-Therm reaction buffer. PCR mixtures were overlaid with mineral oil, and reactions were performed on an Omnigene thermocycler (Hybaid, Middlesex, United Kingdom) with an initial denaturation step of 1 min at 92°C. This step was followed by 30 cycles of denaturation at 92°C (1 min), annealing at 68°C (1 min), and elongation at 72°C (1 min). A final extension was performed at 72°C for 5 min.

DNA sequencing. PCR products were purified with a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Histone H3 gene fragments from the 42 *Fusarium* isolates included in this study, were sequenced (see Table 1 for GenBank accession numbers) in both directions with primers H3-1a and H3-1b. Reactions were performed on an ABI PRISM 377 automated DNA sequencer with an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, United Kingdom).

Sequence Navigator version 1.0.1 (Perkin-Elmer Applied BioSystems, Inc., Foster City, Calif.) was used for translation of DNA sequences to amino acid sequences. DNA sequences were aligned manually by inserting gaps, and phylogenetic analyses were performed with PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b1 (29). Each gap was treated as a fifth character (NEWSTATE) in heuristic searches, with tree-bisection-reconnection branch swapping and MUL TREES (saving of all optimal trees) effective. Bootstrap analyses were based on 1,000 replications. *Fusarium oxysporum* (MRC 6212) was used as an outgroup.

Sexual compatibility tests. The seven *F. subglutinans* isolates recovered from maize in South Africa (Table 1) were crossed with mating population E tester strains and with one another in all possible pairwise combinations (5, 18). Crosses were scored as positive when ascospores were observed exuding from perithecia.

PCR-RFLP technique. Amplified DNA was digested with two restriction enzymes, *Cfo*I and *Dde*I (Boehringer Mannheim South Africa Pty. Ltd.). Digestions were performed consecutively by adding 5 U of *Cfo*I to 15 μl of unpurified PCR product (3); after 3 h of incubation at 37°C, 5 U of *Dde*I was added and the sodium chloride concentration was adjusted to 100 mM. These digestion reaction mixtures were then incubated at 37°C for an additional 5 h. We resolved PCR-RFLP profiles on 3% (wt/vol) agarose gels (Promega Corporation, Madison, Wis.; molecular biology-grade agarose) containing ethidium bromide (0.2 μg/ml). Electrophoresis was performed at 3 V/cm (room temperature) with 0.5× electrophoresis buffer containing 4.5 mM Tris, 4.5 mM boric acid, and 1 mM EDTA (pH 8.0). Nucleic acids were visualized with a UV transilluminator (302 nm).

Verification of technique. To test the efficacy of the PCR-RFLP technique described here, histone H3 gene PCR products from 60 strains representing mating population H and 80 strains representing mating populations A to F were amplified, digested, and electrophoresed as described above. We compared the resulting PCR-RFLP profiles to those generated from the representatives of the *G. fujikuroi* species complex.

RESULTS

DNA sequencing. The *Fusarium* histone H3 gene fragments ranged from 519 to 527 bp in length and contained two introns (intron 1 and intron 2) whose positions within the sequences were conserved. Intron 1 was 83 bp long for strains from mating population H, *F. oxysporum*, and *F. subglutinans* f. sp. *ananas*; 81 bp long for mating populations C and D and *F. subglutinans* isolated from mango; 85 bp long for mating populations A and G; 82 bp long for mating populations E and F and *F. subglutinans* isolated from maize; and 77 bp long for mating population B. Intron 2 was 57 bp long for all of the isolates, except for *F. oxysporum*, for which it was 58 bp long.

The coding regions of the *Fusarium* histone H3 genes were highly conserved, and we observed no deletions or insertions. We detected no differences in amino acid sequence, and coding sequence variation within the *Fusarium* genes was generally limited to the third position within the codon. The *Fusarium* histone H3 amino acid sequence differed from that of *N. crassa* (GenBank accession no. CAA25761) only at position 91 (A→L) (38), whereas that of *Aspergillus nidulans* (GenBank accession no. CAA39154) differed at two positions, 29 and 99 (both S→A) (10). *N. crassa* has a single intron at the same position as *Fusarium* intron 2, but its sequence was quite different from that of intron 2.

Phylogenetic analysis with PAUP 4.0b1 generated a single most-parsimonious tree from 469 bp of aligned DNA sequence (Fig. 1). This tree was comprised of two distinct clades. Clade 1 included isolates from mating populations H and E as well as isolates of *F. subglutinans* f. sp. *ananas* and *F. subglutinans* isolates from maize. The bootstrap value for this clade indicated 96% unity. Clade 2 included isolates from mating populations A, B, C, D, F, and G as well as *F. subglutinans* isolates from mango. The support for the unity of this clade was 70%.

Two subgroups made up clade 1 (Fig. 1). The first subgroup included *F. subglutinans* f. sp. *ananas*. The second subgroup included *F. subglutinans* f. sp. *pini* and isolates from mating population E, clustering together with 96% certainty. Clade 2 was subdivided into two smaller subgroups, one of which included isolates from mating populations B, C, and D as well as the *F. subglutinans* isolates from mango, with 87% support. The second subgroup in clade 2 contained isolates from mating populations A, F, and G, with 71% support.

Sexual compatibility tests. Three of the *F. subglutinans* isolates associated with maize (MRC 1077, MRC 837, and MRC 714) were sexually compatible with one of the mating type tester strains for mating population E (MRC 6483). The remaining four isolates did not cross with one another or either of the tester strains.

PCR-RFLP technique. PCR-RFLP analysis of the amplified histone H3 gene products with *Dde*I and *Cfo*I enabled us to distinguish *F. subglutinans* f. sp. *pini* from the rest of the isolates included in this study (Fig. 2). Unique PCR-RFLP profiles were generated for each group included in this study, except for mating populations C and D, mating population G, and *F. subglutinans* isolated from mango. From the restriction enzyme profiles, we constructed restriction maps for all host-specific groups of *F. subglutinans* as well as *F. moniliforme*, *F. proliferatum*, *F. thapsinum*, and *F. nygamai* (Fig. 3).

Verification of technique. All 60 mating population H strains were positively identified as *F. subglutinans* f. sp. *pini* in a blind test of the PCR-RFLP technique. We identified none of the strains from the collection of Yan et al. (39) as *F. subglutinans* f. sp. *pini*, and the expected profiles were generated for each of their representatives of mating populations A, B, E, and F. The blind test of 140 samples was 100% successful, providing 95% confidence that the error rate for this test is less than 2%.

DISCUSSION

In this study, we were able to distinguish *F. subglutinans* f. sp. *pini* (mating population H) from *F. subglutinans* isolates associated with mango, maize (mating population E), sugarcane (mating population B), and pineapple and *F. moniliforme* (mating population A), *F. proliferatum* (mating populations C and D), *F. thapsinum* (mating population F) and *F. nygamai* (mating population G). The PCR-RFLP technique has been used successfully by the Tree Pathology Co-operative Programme diagnostic clinic to identify isolates of *F. subglutinans* f. sp. *pini* for the last year. Seven outbreaks of root rot in South African nurseries have been correctly diagnosed as being caused by *F. subglutinans* f. sp. *pini* (4). We thus have confidence that this technique is robust and can be used with a high degree of certainty.

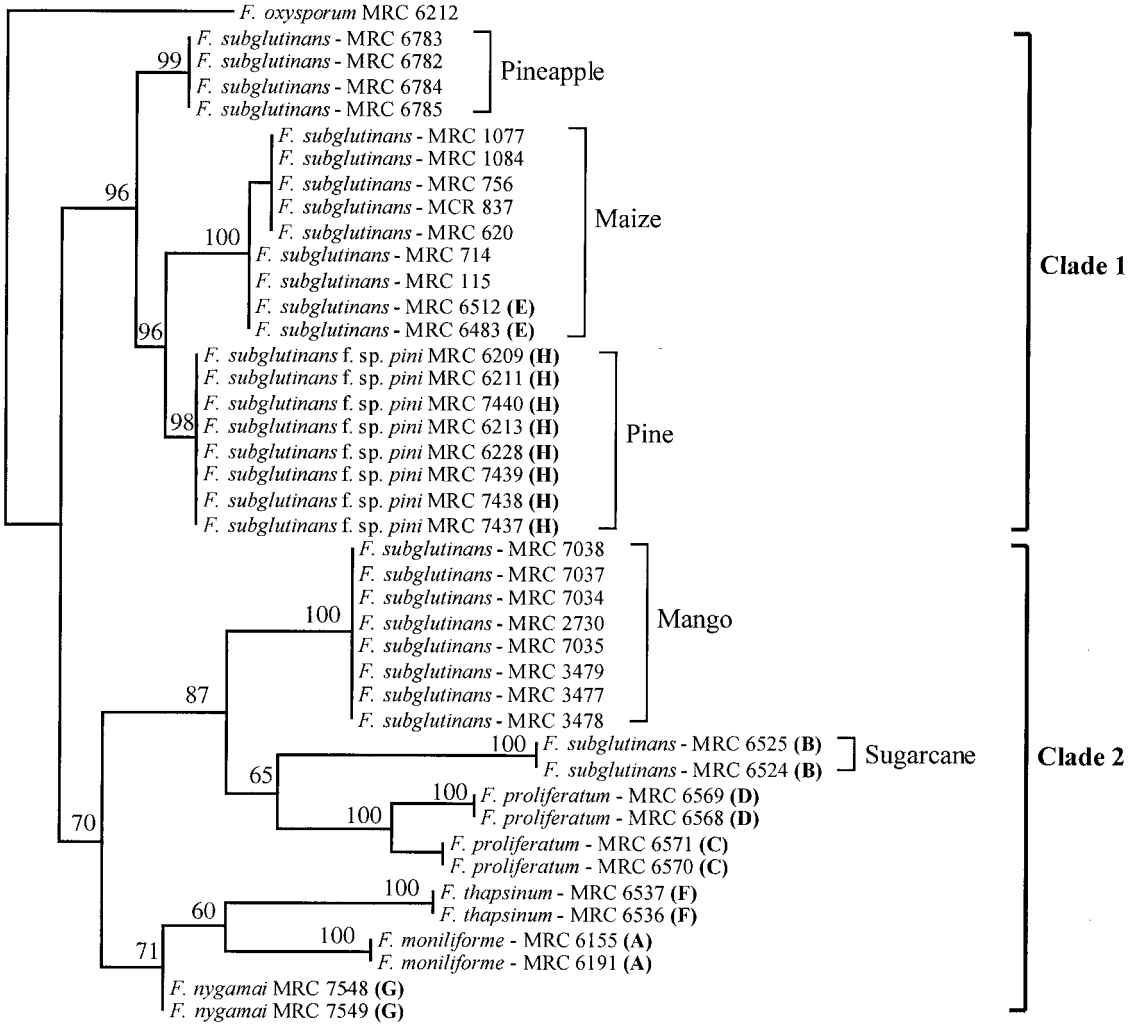

FIG. 1. Phylogram generated with histone H3 gene sequence data from the isolates included in this study by use of PAUP 4.0b1. Bootstrap values based on 1,000 replications are indicated as percentages. Bold letters in parentheses refer to the *G. fujikuroi* mating populations. This dendrogram is rooted to *F. oxysporum* MRC 6212. The length of the tree was 201 steps, and the values for homoplasy index and the retention index were 0.24 and 0.94, respectively.

Phylogenetic analyses with the *Fusarium* histone H3 gene sequence data generated a phylogram (Fig. 1) that was similar to those produced by O'Donnell et al. (26). The results presented here and those based on β-tubulin and mitochondrial small-subunit DNA sequences (26) are similar to those obtained with isozymes (15) in two aspects. First, mating populations C and D form a closely related group in all cases. Second, mating population E is phylogenetically distinct from mating populations A, B, C, D, F, and G.

There are, however, two major differences between DNA-based phylogenies and those based on isozymes. With isozymes, Huss et al. (15) showed mating populations C and D to be most closely related to mating population G. The DNA-based phylogenies (26; this study), however, indicate that mating population G is most closely related to mating populations A and F and that these three mating populations form a distinct cluster separate from both mating populations C and D. Also, in contrast to the results from the isozyme study (15) both DNA-based phylogenies (26; this study) indicate that mating populations C and D are most closely related to mating population B.

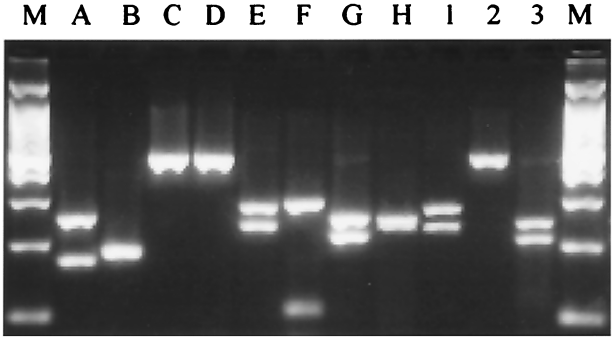

FIG. 2. PCR-RFLP profiles generated by digestion of *Fusarium* histone H3 gene amplification products with *Dde*I and *Cfo*I. Electrophoresis were performed on 3% agarose gels at 3 V/cm. Lanes M, 100-bp ladder (1,500, 1,000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp); lane A, mating population A; lane B, mating population B (*F. subglutinans* associated with sugarcane); lane C, mating population C; lane D, mating population D; lane E, mating population E (*F. subglutinans* associated with maize); lane F, mating population F; lane G, mating population G; lane H, *F. subglutinans* f. sp. *pini* (mating population H); lane 1, *F. subglutinans* from maize; lane 2, *F. subglutinans* from pineapple (*F. subglutinans* f. sp. *ananas*); lane 3, *F. subglutinans* from mango.

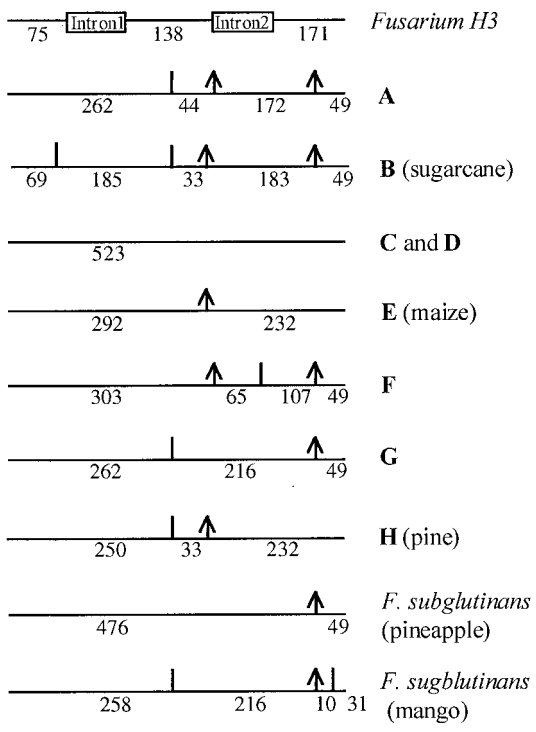

FIG. 3. Restriction maps of the histone H3 genes from the different isolates of *Fusarium*, generated with restriction enzymes *Dde*I and *Cfo*I. The *Fusarium* introns are indicated as boxes, and the exons are indicated as horizontal lines. Bold letters refer to the *G. fujikuroi* mating populations. An arrow indicates a *Cfo*I restriction site, and a vertical line indicates a *Dde*I restriction site. Exon and fragment sizes are indicated in base pairs.

F. subglutinans f. sp. *pini* has previously been reported to belong to mating population B (29), but our results and those presented by Britz et al. (5) and O'Donnell et al. (26) suggest otherwise. Nirenberg and O'Donnell (24) elevated this fungus to species level and provided the name *F. circinatum* (teleomorph = *G. circinata*) for it. Although our results are consistent with those of O'Donnell et al. (26) and support the placement of *F. subglutinans* f. sp. *pini* in a distinct taxon, the distinguishing morphological characters reported by Nirenberg and O'Donnell (24) appear to be inadequate to make definite identifications of the fungus (5).

F. subglutinans f. sp. *pini*, *F. subglutinans* f. sp. *ananas*, mating population E, and *F. subglutinans* isolated from maize are closely related to each other and are included in clade 1. Although some of the *F. subglutinans* isolates from maize and those belonging to mating population E appeared in two separate but closely related groups, this separation was caused by only two nucleotide base-pair differences. Since some individuals from both of these groups could cross with one of the mating type E tester strains, we do not believe that the second cluster of isolates from maize represents a separate mating population. The overall appearance of clade 1 corresponds to that of the so-called American clade described by O'Donnell et al. (26). This similarity suggests an equivalence of *F. subglutinans* f. sp. *pini* and *F. circinatum* as well as of *F. subglutinans* f. sp. *ananas* and *F. guttiforme*.

The two subgroups that constitute clade 2 in our study correspond to the African and Asian clades of O'Donnell et al. (26). The African clade includes mating populations A, F, and G, whereas the Asian clade includes mating populations B, C, and D. The latter clade also includes *F. subglutinans* isolates associated with mango, which are phylogenetically separate from *F. subglutinans* isolates associated with maize, pineapple, and pine but phylogenetically more closely related to *F. subglutinans* from mating population B (Fig. 1).

The results of this study and those of O'Donnell et al. (26) have identified a number of conserved genes that are useful for phylogenetic and taxonomic studies among species of *Fusarium*. The H3 gene, as well as the β-tubulin gene, allows for a higher degree of resolution than rDNA ITS1 and ITS2. Species previously considered too closely related for separation into distinct groups can now be separated based on histone or β-tubulin gene sequence. Moreover, rapid identification of fungi such as the pitch canker pathogen is now possible with a PCR-RFLP technique based on the histone H3 gene sequence.

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